

Development and evaluation of a medicated chewing gum containing *Sceletium tortuosum*

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ABSTRACT

Sceletium tortuosum is one of the most promising medicinal plant species endemic to South Africa. It belongs to the *Mesembryanthemaceae* family and is traditionally used for treatment of anxiety and depression. Recent studies have shown that it is safe to use and that minimal to no side effects present in most patients. Four major alkaloids (mesembrenone, mesembrine, mesembranol and mesembrenol) have been identified in this species and are responsible for the pharmacological action.

Medicated chewing gum (MCG) is used globally not only for its confectionary role, but also as a delivery system. MCG is defined by the European Pharmacopoeia (EP) as a “solid dose preparation with a base consisting mainly of gum that is intended to be chewed but not to be swallowed, providing a slow steady state release of the medicine contained”. MCG is well described in scientific literature although no information could be found on MCG containing *S. tortuosum*. The purpose of this study is to develop and evaluate a medicated chewing gum containing *S. tortuosum* crude extract.

Various MCG formulations were prepared containing the active ingredient (i.e. *S. tortuosum* crude extract containing mesembrine alkaloids), Cafosa®'s Health In Gum (HIG) base powders (i.e. HIG 01, 03 or 04 base powder), sweetener, flavouring agent and a lubricant (0% or 0.5% magnesium stearate) using a full factorial design. The flow and compressibility properties of *S. tortuosum* extract powder, the HIG base powders and the different formulations were analysed utilising the SeDeM Expert Diagram System. Formulations were tableted by means of direct compression on a Korsch® single tablet press with a 12 mm diameter flat punch to form individual MCG units. Each of the different MCG units was formulated in such a way as to contain a theoretical value of 6.38 mg of *S. tortuosum* crude extract that is equivalent to 50 mg raw *S. tortuosum* plant material, which represents the estimated quantity to be chewed as a single dose by indigenous people.

The optimal MCG formulation achieved successful Parameter Index (PI), Parameter Profile Index (PPI) and Good Compressibility Index (GCI) values of 0.75, 7.16 and 6.81, respectively, indicating that the S0.5HIG01 is the most appropriate for direct compression according to the SeDeM Expert Diagram System. Additionally, index values indicated that overall *S. tortuosum* extract MCG formulations comprising magnesium stearate are deemed more appropriate for direct compression. The SeDeM Expert Diagram System could be successfully applied to directly compressible MCG formulations.

The MCG formulations produced from the factorial design were evaluated in terms of physico-chemical properties and dissolution studies in order to obtain optimised MCG formulations. Prepared MCG units of the different formulations were evaluated in terms of morphology, mass variation, crushing strength, diameter, thickness, tensile strength, friability and pharmaceutical availability. Scanning electron microscopy (SEM) was used to evaluate the morphology of the *S. tortuosum* crude extract, the HIG base powders as well as the different MCG units.

The physico-chemical properties as well as the dissolution studies of the formulations containing magnesium stearate indicated improved values when compared to formulations that contained no magnesium stearate. Therefore, in this study, different MCG units containing *S. tortuosum* crude extract with different base powders in their formulations were developed with the potential to be manufactured and implemented in the pharmaceutical industry.

KEYWORDS: *S. tortuosum*; Mesembrine alkaloids; Direct compression; Medicated chewing gum (MCG); Cafosa®'s Health In Gum bases; SeDeM Expert Diagram System; Trans-mucosal delivery

UITTREKSEL

Sceletium tortuosum is een van die mees belowende medisinale plantspesies endemies tot Suid-Afrika. Dit behoort aan die *Mesembryanthemaceae* familie en word tradisioneel gebruik vir die behandeling van angs en depressie. Onlangse studies het aangedui dat dit veilig is vir menslike gebruik, met minimum tot geen nuwe-effekte. Vier hoof-alkaloïede (mesembrenoon, mesembrien, mesembranol en mesembrenol) is geïdentifiseer in hierdie plantspesie en is verantwoordelik vir die farmakologiese aktiwiteit.

Medisinale kougom (MK) word wêreldwyd nie net vir konfeksionêre doeleindes gebruik nie, maar ook as 'n afleweringstelsel vir medikasie. Medisinale kougom word deur die Europese Pharmacopoeia (EP) gedefinieer as 'n soliede doseervorm waarvan die basis hoofsaaklik uit 'n kougom bestaan wat bedoel is om gekou te word sonder dat dit ingesluk word om sodoende 'n gekontroleerde, vertraagde vrystelling van die aktiewe bestanddeel wat in die kougom teenwoordig is, te bewerkstellig. Alhoewel medisinale kougom omvattend beskryf word in bestaande wetenskaplike literatuur, is daar tans geen literatuur beskikbaar in verband met *S. tortuosum*-bevattende medisinale kougom nie. Die doel van hierdie studie is om medisinale kougom wat *S. tortuosum* ru-ekstrak bevat te ontwikkel en te evalueer.

Verskeie medisinale kougomformulerings is berei wat die aktiewe bestanddeel (naamlik *S. tortuosum* ru-ekstrak wat mesembrine alkaloïede bevat), Cafosa® se "Health In Gum (HIG)" basis poeiers (naamlik HIG 01, 03 of 04), 'n versoeter, 'n smaakmiddel en 'n smeermiddel (naamlik 0% of 0.5% magnesiumstearaat) bevat. Deur gebruik te maak van die SeDeM Deskundige Diagram-sisteem (SeDeM Expert Diagram System) is 'n poeiergedeelte van die *S. tortuosum* ru-ekstrak, die HIG-basisse asook die verskeie formulerings gebruik vir die evaluering van die betrokke poeiermengsels se vloe- en saampersbaarheidseienskappe. Laasgenoemde poeiermengsels is direk saamgepers op 'n Korsch® enkel-tabletpers met 12 mm deursnee stempels om individuele tablette of MKs saam te pers. Elke MK-eenheid is geformuleer om 6.38 mg *S. tortuosum* ru-ekstrak te bevat wat ekwivalent is aan 50 mg rou *S. tortuosum* plantmateriaal, wat die beraamde hoeveelheid is wat deur 'n inheemse persoon as 'n enkele dosis geneem word.

Die optimale MK-formulering het volgens die SeDeM Deskundige Diagram-sisteem geslaagde Parameter-indeks (PI), Parameter Profiel-indeks (PPI) en Goeie Saampersbaarheids-indeks (GCI) waardes behaal van 0.75, 7.16 en 6.81, onderskeidelik, wat aandui dat die S0.5HIG01 formule die mees geskikte formule vir direkte samepersing is. Die indeks waardes het ook aangedui dat die *S. tortuosum*-bevattende formulerings wat wel

magnesiumstearaat bevat, meer geskik is vir direkte samepersing as formulerings wat geen magnesiumstearaat bevat nie. Die SeDeM Deskundige Diagram-sisteem is suksesvol toegepas in die formulering van direk saampersbare *S. Tortuosum*-bevattende MK-eenhede.

Die MK-formulerings is geëvalueer ten opsigte van hul fisies-chemiese eienskappe deur die implementering van 'n volledige faktoriaalontwerp asook dissolusie studies om die optimale formulering te identifiseer wat optimale MK-eenhede sal lewer. MK-eenhede van verskeie formulerings is geëvalueer in terme van morfologie, massavariasie, breeksterkte, deursnee, dikte, trekvastheid, brosheid en farmaseutiese beskikbaarheid van die aktiewe bestanddeel. Skandeerelektronmikroskopie (SEM) is gebruik om die morfologie van die *S. tortuosum* ru-ekstrak-poeier, die "HIG" basisse asook die verskillende MK-eenhede te evalueer.

Die fisies-chemiese eienskappe asook die dissolusie-resultate van die formulerings wat magnesiumstearaat bevat, het beter waardes getoon in vergelyking met die formulerings wat geen magnesiumstearaat bevat het nie. Dus is daar in hierdie studie geslaag om verskillende MK-formulerings wat *S. tortuosum* ru-ekstrak en verskillende basisse bevat te ontwerp wat die potensiaal besit om in die farmaseutiese bedryf geïmplementeer en vervaardig te kan word.

SLEUTELWOORDE: *S. tortuosum*; Mesembrien alkaloïede; Direkte samepersing; Medisinale kougom (MK); Cafosa®'s "Health In Gum" basisse; SeDeM Deskundige Diagram-sisteem; Trans-mukosale aflewering

Chapter 1:

INTRODUCTION, PROBLEM STATEMENT, AIM AND OBJECTIVES

1.1 INTRODUCTION

1.1.1 *Sceletium tortuosum*

Sceletium tortuosum (L.) N. E. Br (Mesembryanthemaceae) also referred to as “kougoed” or “kanna” is a plant endemic to South Africa, which grows in the Karoo regions of the Western, Eastern and Northern Cape provinces of South Africa (Patnala & Kanfer, 2009). It is a traditional masticatory herbal medicine, which was originally utilised as a sedative by the Khoi and San people (Scott & Hewett, 2008; Smith *et al.*, 1996; Waterhouse *et al.*, 1979). Early settlers in the Cape region noticed the plant’s appetite and thirst suppressant effects, while other traditional medicinal uses included treatment of major depression, anxiety, insomnia, bulimia nervosa, digestive problems, obsessive-compulsive disorders and analgesic effects against toothache and stomach pains (Shikanga, Hamman *et al.*, 2012; Smith *et al.*, 1996).

Although the fermented stems and leaves are most commonly chewed, it has been consumed as a tea; taken as a tincture, elixir or infusion, but it has also been smoked and has occasionally been used as a snuff (Forbes, 1986; Jacobsen, 1960; Pappe, 1868; Smith *et al.*, 1996; Van Wyk & Wink, 2004). Fresh leaves are directly applied to a painful tooth or a drop of fresh leaf juice is placed on the tongue in order to relieve the pain (Smith *et al.*, 1998). Currently, various commercial *S. tortuosum* products are available such as conventional tablets, capsules, teas, sprays, extracts and tinctures (Harvey *et al.*, 2011; Smith *et al.*, 1996; Terburg *et al.*, 2013).

The psychoactive properties of this plant are attributed to four major alkaloids, namely mesembrine, mesembrenone, mesembrenol, and mesembranol, which are collectively known as the mesembrine alkaloids. The potential of these alkaloids to treat central nervous system disorders has been attributed to their ability to act as serotonin reuptake inhibitors, thereby contributing to regulating the neurochemical balance in the brain (Harvey *et al.*, 2011; Shikanga, Hamman *et al.*, 2012). Phosphodiesterase (PDE4) inhibition has also been reported for

especially mesembrenone (Harvey, *et al.*, 2010). Strong *in vivo* experimental evidence exists that PDE4 inhibitors can reverse depression, relieve anxiety and advance cognition (O'Donnell & Zhang, 2004; Rutten *et al.*, 2006). Therefore, the alkaloid, mesembrenone, can act as a dual serotonin reuptake inhibitor and PDE4 inhibitor, whereas mesembrine alkaloids can act as highly selective serotonin reuptake inhibitors.

1.1.2 Medicated chewing gum

Medicated chewing gum is defined as a solid, single oral dosage form that releases a drug(s) through the mechanical action of chewing. It consists of a gum base, which is intended for chewing and not for swallowing; and it provides a relatively slow and sometimes prolonged release of the active(s) that are incorporated in the core mass (Chaudhary & Shahiwala, 2012; Ughade *et al.*, 2012).

Medicated chewing gum is attracting a lot of attention of formulation scientists because this dosage form provides added patient benefits and also provides new competitive technological and marketing advantages (Goel *et al.*, 2008; Iijima *et al.*, 2004; Paradkar, *et al.*, 2015; Rømer, 1994; Shete *et al.*, 2015). During mastication, the drug in the medicated chewing gum is released from a solid polymeric mass into the saliva. It can thus either be absorbed through the oral mucosa directly into the jugular veins, where it surpasses metabolism in the gastrointestinal tract and liver; or it can be swallowed where it is then absorbed from the gastro-intestinal tract. Other advantages and applications of medicated chewing gums in general include increased patient compliance, especially in children or patients with swallowing disorders, the product can be taken anywhere at any time and does not require liquids, dry mouth is counteracted, prolonged direct contact of the dissolved active ingredient with the stomach wall and the risk of irritation is reduced (Belmar & Ribé, 2013; Pagare *et al.*, 2012; Ughade *et al.*, 2012).

Medicated chewing gum consists of a neutral, insoluble and tasteless masticatory gum-base and several non-masticatory excipients such as fillers, softeners, sweeteners, flavouring and texture regulating agents. Due to the complex formulation and manufacture of medicated chewing gums, it has been restricted to a particular niche of pharmaceutical products including treatment of motion sickness as well as for nicotine replacement. Conventional manufacturing techniques included hot mixing (or melting) and extrusion or freezing, grinding and tableting that require specialised equipment. The relatively large temperature changes required during these processes renders it unsuitable for many thermolabile drugs. Other limitations when utilising these conventional methods include non-uniformity of the drug dose, the lack of a precise

shape, form and weight, moisture content can cause problems, careful monitoring and management of humidity during production is essential and difficult (Belmar & Ribé, 2013; Kvist *et al.*, 1999; Maggi *et al.*, 2005; Ughade *et al.*, 2012).

Directly compressible excipients have become available that enable relatively easy and cost-effective manufacture and production of medicated chewing gums for pharmaceutical applications. Furthermore, higher levels of active ingredients can be included, while the lower moisture content improves shelf-life of active molecules and standard tablet presses can be used to manufacture quality medicated chewing gum products utilising only dry powder excipients (Belmar & Ribé, 2013; Kvist *et al.*, 1999; Maggi *et al.*, 2005; Ughade *et al.*, 2012).

1.1.3 SeDeM Expert Diagram System

The SeDeM Expert Diagram System (Pérez-Lozano *et al.*, 2006; Suñé-Negre *et al.*, 2005; Suñé-Negre *et al.*, 2008) is a galenic technique which is applied in tablet-preformulation studies to analyse the suitability of ingredients and excipients, in powder form, for direct compression. This system identifies physical powder properties of substances (active ingredients and excipients) that need to be improved in order to produce a formulation that can be prepared by means of direct compression. The information obtained by applying SeDeM indicates the degree to which the substances can be successfully compressed by means of direct compression. The SeDeM Expert Diagram System is also a valuable tool for studying the reproducibility of the process (Aguilar-Díaz *et al.*, 2009; Khan *et al.*, 2014; Pérez-Lozano *et al.*, 2006; Singh & Kumar, 2012; Suñé-Negre *et al.*, 2005; Suñé-Negre *et al.*, 2008).

The SeDeM Expert Diagram System describes the profile of excipients and active ingredients in powder form with respect to their appropriateness for direct compression. This profile designates whether a powder can be successfully compressed by means of direct compression or whether it needs to be adjusted with more suitable and/or additional excipients. It can also be used to calculate the amount of excipients with certain characteristics required for correction of a particular property to ensure the suitability of the final blend for direct compression. Moreover, the SeDeM Expert Diagram System could be perceived as a Quality by Design (ICH Q8) tool (Aguilar-Díaz *et al.*, 2009; Khan *et al.*, 2014; Saurí *et al.*, 2014; Singh & Kumar, 2012; Suñé-Negre *et al.*, 2008).

SeDeM utilises 12 powder tests to determine if a powder is appropriate for direct compression (Figure 1.1). These tests are grouped into five incidence factors on the basis of the physical characteristics of the powder and the functionality of the drug namely the dimension, compressibility, flowability/powder flow, lubricity/stability and the lubricity/dosage. Pharmacopoeial powder test methodologies are employed to determine the 12 parameters. Numerical values of the powder parameters are placed on a scale from 0 to 10, considering 5 as the minimum acceptable value. When all the radius values are 10, the SeDeM diagram forms a restricted regular polygon, drawn by linking the radius values with linear segments (Aguilar-Díaz *et al.*, 2009; García-Montoya *et al.*, 2010; Pérez-Lozano *et al.*, 2006; Suñé-Negre *et al.*, 2005). Characteristics of the formulation ingredients in terms of each parameter that determines if the formulation is suitable for direct compression are plotted to form a polygon diagram (Figure 1.1).

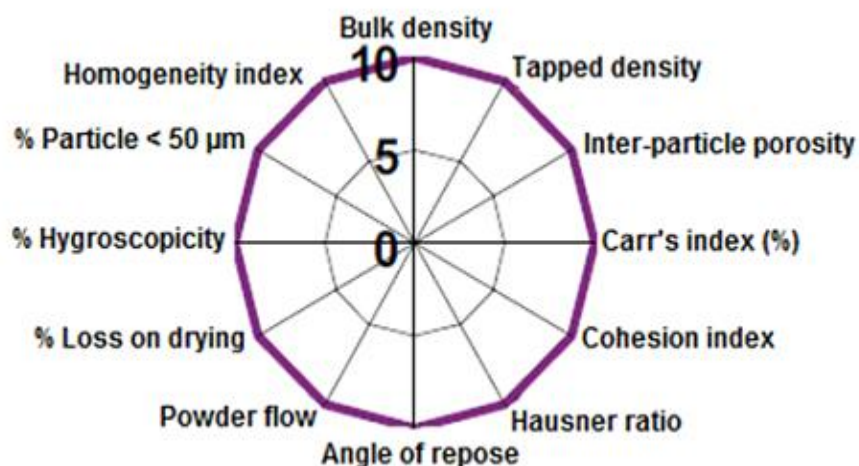


Figure 1.1: Illustration of the SeDeM diagram with 12 parameters (Suñé-Negre *et al.*, 2014)

1.2 RESEARCH PROBLEM

Most of the current available commercial products containing extracts of *S. tortuosum* are solid or liquid oral dosage forms that are intended to be swallowed for absorption from the gastro-intestinal tract (Harvey *et al.*, 2011; Smith *et al.*, 1996; Terburg *et al.*, 2013). However, the stability of the active components of this plant, especially the mesembrine alkaloids, in the gastro-intestinal tract juices has not yet been studied. This medicinal plant (*S. tortuosum*) has originally been chewed and the pharmacologically active phytoconstituents thereof are most probably absorbed from the oral cavity across the oral mucosa (i.e. buccal and sublingual

epithelium) avoiding the first pass effect during this manner of drug intake. *In vitro* data indicated prominent diffusion of the active components (i.e. mesembrine alkaloids) across buccal and sublingual membranes (Shikanga, Hamman *et al.*, 2012).

To the knowledge of the researcher, no dosage form or product has yet been developed that represent the same way of delivering *S. tortuosum* plant material, extracts or alkaloids as it was traditionally done (i.e. by mastication or chewing while kept in the oral cavity). The research problem that needs to be addressed in this study is whether it is possible to develop/formulate a medicated chewing gum that contains and effectively releases the active ingredients of *S. tortuosum* (i.e. mesembrine alkaloids) when chewed. A sub-problem is to determine which gum base excipient is the most suitable for direct compression of a chewing gum by applying the SeDeM expert diagram system.

1.3 AIM AND OBJECTIVES

The aim of this study is to develop and evaluate a directly compressible medicated chewing gum containing *S. tortuosum* crude extract. Furthermore, the SeDeM Expert Diagram System is applied to determine which excipients are suitable for manufacture of the chewing gum by means of direct compression.

The main objectives are therefore to:

- Prepare and characterise a crude extract from *S. tortuosum* plant material and chemically characterise the extract by means of liquid chromatography-mass spectrometry (LC-MS) analysis and to analyse the dissolution samples by means of high-performance liquid chromatography (HPLC).
- Evaluate the flowability and compressibility of different gum bases by means of the SeDeM Expert Diagram System to determine which excipients are suitable for manufacture of a direct compressible chewing gum containing *S. tortuosum* crude extract.
- Manufacture medicated chewing gum formulations (utilising a full factorial design) with different gum bases containing a crude extract from *S. tortuosum* by means of direct compression; and to evaluate these products in terms of physical properties (e.g. hardness, friability, dimensions). Furthermore, to re-formulate the chewing gum preparations by adding additional excipients based on the SeDeM predictions.

- Conduct dissolution tests on selected directly compressed medicated chewing gum formulations containing *S. tortuosum* crude extract with a specialised dissolution apparatus for chewing gum preparations.

Chapter 2: LITERATURE STUDY

2.1 INTRODUCTION

Sceletium tortuosum is one of the most promising medicinal plant species endemic to South Africa in terms of pharmaceutical development. Eight *Sceletium* species have been recognised, which include *S. emarcidum*, *S. crassicaule*, *S. exalatum*, *S. expansum*, *S. strictum*, *S. rigidum*, *S. tortuosum* and *S. varians* (Gerbaulet, 1996). *S. tortuosum* belongs to the *Mesembryanthemaceae* family (Smith *et al.*, 1996; Klak, *et al.*, 2007). During the past few years, *S. tortuosum* was seriously scrutinised to determine the value of its possible health benefits and also to recognise the pharmacological action of some of its phytochemicals (i.e. the mesembrine alkaloids). The earliest notes referring to this plant date back to the seventeenth century during an expedition that was conducted by Governor Van der Stel in 1685. The value of the plant's medicinal properties was soon noticed by the early settlers in the Cape region which led to further examination of the plant (Smith *et al.*, 1996).

Sceletium tortuosum has been used for medicinal purposes by the Bushmen and Khoi-Khoi people for centuries (Scott & Hewett, 2008; Smith *et al.*, 1996; Waterhouse *et al.*, 1979). Recent studies have shown that it is safe to use this plant's alkaloids and that minimal to no side effects present in most patients (Nell *et al.*, 2013; Dimpfel *et al.*, 2016; Gericke & van Wyk, 2001). This plant is ideal for the treatment of anxiety and depression, a condition that is increasingly apparent in society as it counteracts the symptoms through its pharmacological mechanisms (Coetzee *et al.*, 2016; Harvey *et al.*, 2011; Shikanga, Viljoen *et al.*, 2012). Improved memory and learning skills are also associated with the alkaloids in this plant species. Traditionally, the plant material was chewed by indigenous people only after it was fermented. The released alkaloids were most probably absorbed through the mucous membranes of the cheek (buccal) and the mucous membranes that are located under the tongue (sub-lingual) from where the remaining traces were swallowed and absorbed in the gastrointestinal tract (Shikanga, Hamman *et al.*, 2012).

Medicated chewing gum is a reasonable unknown dosage form that is used to administer a variety of medicines. There are several ways to manufacture chewing gum, but the most practical and relatively inexpensive way for it to be produced is by means of a compression

process where a mixture of powders is compressed directly with a force large enough to produce a chewing gum “tablet” (Kvist *et al.*, 1999; Biradar *et al.*, 2005; Pagare, *et al.*, 2012; Morjaria *et al.*, 2004).

The SeDeM Expert Diagram System is a galenic technique for identifying flaws that may occur in the ingredients of a directly compressible formulation. The SeDeM Expert Diagram System predicts the suitability of powder(s) for direct compression and also predicts to what extent the properties of the powder(s) should be altered to become directly compressible (Aguilar-Díaz *et al.*, 2009; García-Montoya *et al.*, 2010; Khan *et al.*, 2014).

2.2 THE MEDICINAL PLANT “*SCELETIUM TORTUOSUM*”

2.2.1 History and location

The ethnomedical practise of plants in Southern Africa was firstly explored by the San or also known as Bushmen people around 20,000 years ago (Deacon, 1983). The Khoi-Khoi, on the other hand, practised ethnomedical usage of plants only for the last approximately 2,000 years (Soodyal, 2003). To the indigenous people, knowledge of medicinal plants was a matter of survival as it was their only conventional method of healing or treating a specific medical condition. According to Wilson *et al.* (2002), the first ethnomedical records of collection wof *Sceletium* plants in South Africa was south of Springbok on the 20th day of October 1685 during Cape Governor Simon van der Stel’s expedition to the Copper Mountains during the years 1685-1686. The species was most probably of *Sceletium expansum* (L.) L. Bolus. The journals that were found from this expedition are most probably the earliest records of ethnomedical studies done on a variety of plant species including the *Sceletium* species (Scott & Hewett, 2008).

Sceletium tortuosum is located mostly in the south-western areas of South Africa (the Karoo region) which include the Western, Eastern and Northern Cape provinces (Patnala & Kanfer, 2009). Initially, the fermented stems and leaves of *S. tortuosum* were chewed by indigenous people. As time went by, it was also ingested as an elixir or infusion, and also smoked and snuffed (Smith *et al.*, 1996; Smith *et al.*, 1998).

2.2.2 Mechanism of pharmacological action and uses

S. tortuosum was originally used by the indigenous people as a mood-altering substance. The cause of the anxiolytic-narcotic effects have been associated with the presence of four major alkaloids (Smith *et al.*, 1996; Smith *et al.*, 1998), which are collectively known as the mesembrine alkaloids. The four major mesembrine alkaloids are mesembrine, mesembrenone, mesembrenol and mesembranol. Although every alkaloid possess some activity in itself, the combination as it is found in *S. tortuosum* plants, works best to obtain the desired pharmacological effect. The mesembrine alkaloids possesses the ability to inhibit uptake of certain mono-amines (Harvey *et al.*, 2011; Shikanga, Viljoen *et al.*, 2012) and are responsible for the anxiolytic–narcotic activity found in the *Sceletium* species (Smith *et al.*, 1996; Smith *et al.*, 1998).

According to a study conducted by Harvey *et al.* (2011) on the binding and inhibiting effects of *S. tortuosum*, binding of the alkaloids was noticed on all 77 receptors and ion binding sites. The binding sites that were significantly affected (>80% inhibition of binding) were the following receptors: gamma-aminobutyric acid (GABA), 5-hydroxytryptamine (5HT) also known as serotonin transporters, δ_2 -opioid, μ -opioid and the cholecystokinin-1 receptors. Prostaglandin E2 receptors were inhibited by 77%, whereas all the other receptors were inhibited below 70%. Potent and concentration dependent effects were found at the 5HT transporter after a concentration-dependency study was performed. For inhibition on other binding sites, however, significantly higher concentrations were required (Harvey *et al.*, 2011).

In brief, there were two main pharmacological actions identified so far for the alkaloids of *S. tortuosum*, namely:

- The inhibition of serotonin re-uptake by blocking the transporter where serotonin is reabsorbed (Harvey *et al.*, 2011; Shikanga, Viljoen *et al.*, 2012), and
- The inhibition of the phosphodiesterase enzyme (PDE4) (Coetzee *et al.*, 2016).

2.2.2.1 Serotonin reuptake inhibition

Serotonin, also known as 5-HT is a monoamine neurotransmitter that assists with a person's wellbeing and happiness. Serotonin is mainly localised in three areas: The nerve pathways, the gastro-intestinal tract and on the blood platelets. Serotonin localised in the nerve pathways, originates in the raphe nuclei area in the midbrain, the pons and medulla; and from there it branches out into the central nervous system (CNS) from where it is led to the cerebral cortex

and through the brainstem to the peripheral nervous system inside the spinal cord. Blocking the 5-HT receptors will result in an increased 5-HT hormone level (Palladino, 2013).

Serotonin can also be absorbed from food and plays a major role in the gastro-intestinal tract as it controls gastrointestinal motility and chloride secretion through interneurons and motor neurons (Palladino, 2013). Most of the serotonin is stored in the enterochromaffin (EC) cells of the intestine after it is absorbed from the gastro-intestinal tract (Nozawa *et al.*, 2009; Palladino, 2013). A noticeable, but far smaller amount of serotonin is found in the blood-platelets in the blood (De Clerck *et al.*, 1984).

According to a study done by Harvey *et al.* (2011), *S. tortuosum* extract had noticeable blocking effects on the GABA, 5HT-transporters, δ 2-opioid, μ -opioid and the cholecystokinin-1 (or –A) receptors. The effect of the 5HT-transporter receptor was furthermore found to be concentration dependent. Higher concentrations of the extract were needed to inhibit the other sites although they were also found to be concentration dependent. The study also showed that the mesembrine alkaloid was most effective on binding to the 5HT transporter as it was 20 times more potent compared to the mesembrenone alkaloid; and 87 times more active compared to the mesembrenol alkaloid (Harvey *et al.*, 2011). A limited effect was noted against Noradrenaline uptake (IC_{50} -10 μ m), but no effect was found on dopamine uptake at a concentration of 10 μ m (Gericke & Viljoen, 2008).

The single isolated alkaloid, mesembrine, showed potent inhibitory effects on the serotonin reuptake (Gericke & van Wyk, 2001) with an IC_{50} value (the concentration of an inhibitor where the response or binding is reduced by half) of 4.3 μ g/mL and an affinity (K_i) value (indicator of how potent an inhibitor is) of 1.4 nM towards this transporter/receptor (Harvey *et al.*, 2011). The serotonin inhibitory effects were confirmed with synthetic mesembrine, which had an IC_{50} of 29 nM against serotonin uptake (Gericke & Viljoen, 2008).

2.2.2.2 Phosphodiesterase (PDE4) enzyme inhibition

Phosphodiesterase (PDE4) is an enzyme that reduces cyclic adenosine monophosphate (cAMP), a molecule present in neurons to help with signalling when it is stimulated. Cyclic adenosine monophosphate activates the cAMP-response-element-binding (CREB) protein which helps with the growth and elasticity of the synapses. Inhibiting PDE4 results in increasing CREB stimulation and improving mental health as cAMP's concentration is increasing progressively (Benito & Barco, 2010).

Phosphodiesterase inhibitors are commonly used in treating a variety of medical conditions related mainly to the central nervous system. Diseases that are being treated with PDE4 inhibitors include anxiety disorders and Alzheimer's disease (Smith *et al.*, 2009), schizophrenia and dementia (Maxwell *et al.*, 2004; Kanen *et al.*, 2007), multiple sclerosis (Dinter, 2000), Parkinson's disease (García-Osta *et al.*, 2012), Huntington's disease, stroke and conditions such as asthma, rheumatoid arthritis, as well as chronic obstructive pulmonary disease (Dyke & Montana, 2002; Halene & Siegel, 2007; Schudt *et al.*, 2011).

Inhibition of PDE4 improves cognitive function as well as long-term memory (Barad *et al.*, 1998); it furthermore promotes wakefulness (Lelkes *et al.*, 1998), acts as a neuroprotective (Block *et al.*, 2001; Chen *et al.*, 2007); and suppresses inflammatory effects (Teixeira *et al.*, 1997). Therefore, a recent application is that PDE4 inhibitors are used in the treatment regime of a variety of inflammatory diseases (Schudt *et al.*, 2011).

Inhibition of the PDE4 enzyme has been reported after *S. tortuosum* was tested *in vitro*, with an IC₅₀ of 8.5 µg/mL (Harvey *et al.*, 2010; Harvey *et al.*, 2011). Inhibition of the PDE4 enzyme was found to be concentration dependent (Harvey *et al.*, 2011). The most active enzyme inhibiting activity on PDE4 (B type) was obtained with the alkaloid mesembrenone, which is 17 times more active on this specific binding site compared to mesembrine and 34 times more active compared to the mesembrenol alkaloid (Harvey *et al.*, 2011).

Inhibition of PDE4 activity was also shown by the presence of the mesembrine alkaloid and its synthetic analogues (Napoleitano *et al.*, 2001). The mesembrine synthetic analogues were found to be more potent inhibitors than mesembrine itself (IC₅₀-29 µM); with IC₅₀'s of 0.1-1 µM. The synthetic analogues tested in this particular study inhibited the PDE4 activity completely and the PDE3 activity was reduced by 88%. All other enzymes were inhibited on a much lower scale. Concentration dependency of PDE3 and -4 was also determined; and results confirmed that PDE4 (IC₅₀-8.5 µg/ml) was inhibited on a larger scale compared to PDE3 (IC₅₀- 274 µg/ml) (Harvey *et al.*, 2011).

2.2.3 Previous studies conducted

A study was conducted by Loria *et al.* (2014) where dependency and obsession were researched by monitoring habit forming activities in a number of male Sprague Dawley rats (175-200 grams) that were genetically modified to be depressed, anxious, abuse liable and nociceptive. Their behavioural responses were observed in the following tests: forced

swimming, elevated plus, rotarod, hot plate and conditioned place preference. Results showed that *S. tortuosum* had anti-depressant properties; however a state of ataxia (neurological dysfunction of motor coordination affecting balance, movement, speech and sight) was noted. No evidence of habit forming features was found in this study (Loria *et al.*, 2014; Mariotti *et al.*, 2005).

In another study, neurocognitive effects were monitored in 21 healthy adults aged 54.6 years $\pm$ 6.0 years and a male:female ratio of 9:12. Daily administration of Zembrin[®] (25 mg extract of *S. tortuosum*) or a placebo tablet was given to each subject for a period of 3 weeks. A series of neuropsychological tests were done and results showed a distinct improvement of cognitive set flexibility and executive function compared to the group that was provided with the placebo tablet. Improvement in sleeping patterns and general mood were also noted. Furthermore, Zembrin[®] enhanced the PDE-4-cAMP-CREB process which may have the potential for treating early Alzheimer's dementia (Chiu *et al.*, 2014).

Zembrin[®] was administered orally in three different strengths (i.e. 2.5, 5.0 and 10.0 mg/kg), where after electropharmacograms were collected from 17 adult Fischer rats to monitor the spectral power for 8 frequency ranges. The electropharmacograms of Zembrin[®] were compared to electropharmacograms of other herbal extracts. It was found that a synthetic compound used as a cognitive enhancer, namely Citocoline, had a similar electropharmacogram as Zembrin[®]. Similarities were also found between Zembrin[®] and Rolipram[®], a synthetic PDE4 inhibitor. These results furthermore suggested that the activity of Zembrin[®] is dose dependent; Zembrin[®] has potential to enhance cognitive function, to act as an anti-depressant and may act as an analgesic agent (Dimpfel *et al.*, 2016).

State anxiety, which is due to an increased activity in a certain part of the brain (amygdala), is a condition where a patient experiences an anxious feeling in a response to a stressful situation, but this condition is not related to chronic anxiety. Reduced cognitive function is noted in patients suffering from state anxiety (Bishop *et al.*, 2007). When 25 mg *S. tortuosum* extract was given to state anxiety patients prior to a perceptual-load task which can trigger the condition, a reduction of activity in the amygdala was noted in comparison to patients receiving a placebo tablet (Terburg *et al.*, 2013).

On another occasion, 90 male Wistar rats (300 grams) were given either 5 mg/kg or 20 mg/kg of *S. tortuosum* extract orally for a period 17 days. Their behaviour was assessed after 50% of the

rats were exposed to stress for a period of 1 hr every day for the last three days of admitting *S. tortuosum*. The rats were sacrificed 24 h after the last session of stress exposure and their blood was collected for analysis. A regulated decrease in anxiety was noted by performing elevated maze tests on the last day. A decrease of corticosterone, a hormone that is secreted when stress is experienced, was also noted. However, increased Interleukin (IL)-1 β levels obtained in the study may contradict the claim that *S. tortuosum* has inhibiting effects on the serotonin reuptake mechanism. (IL)-1 β is known to induce corticosterone production via induction of the corticosterone releasing hormone. (IL)-1 β is also known to stimulate serotonin uptake. This obtained increased Interleukin (IL)-1 β levels together with observed increased levels of C-reactive protein and prostaglandin E(2) noted in this study suggested intolerance to the treatment according to Smith (2011). A suppressive effect on the T-helper 1 immune function, also known as the cell mediated immune response, was noted according to a decrease in IL-2 levels and an increased level of T-helper 2 (humoral) anti-inflammatory cytokine IL-10. These levels were detected in subjects independent of stress that was treated on a higher dose of *S. tortuosum* extract. Suppression of the T-helper 1-immune function may result in a tolerance for *S. tortuosum* over time. Increased levels of IL-10 have been implicated to hypersensitivity and intolerance in food. The article concluded that the rats that received a dosage of 5 mg/kg/day had positive effects in handling psychological stress. However, both of the 5 mg/kg/day and 20 mg/kg/day dosages caused an inflammatory effect and varying degrees of T-helper 1 immune suppression (Smith, 2011).

2.2.4 *In vivo* studies: safety and toxicology

Hypnotic effects were noticeable when an alkaloid mixture of *S. tortuosum* was injected into the skin of a frog. Additional tests showed more drastic effects on the frog. Initially, the respiratory rate increased; and uneasiness and increased moisture of the skin were noted. After 10-12 min a slower respiratory rate was noted and the movement of the frog was impaired. Complete recovery took 4-8 h (Meiring, 1895).

In another study, no toxicological evidence was obtained after *S. tortuosum* was given to rats for a period of two weeks. Dosage was gradually increased up to the *no observable adverse effect limit* (NOAEL) of 5 000 mg/kg, which is 1 800 times higher than the recommended intake of 25 mg/day. Another test group in the same study that was given 900 mg/kg daily for a period of 90 days, also failed to show any haematological, clinical or historical toxicological effects (Murbach *et al.*, 2014). These results were confirmed by a study conducted by Smith (2011)

who furthermore concluded that the alkaloids present in the *S. tortuosum* extract depicted positive effects on psychological stress (Smith, 2011).

Research conducted in 2002 by Hirabayashi *et al.* (2002) found that no toxic effects were presented after dry powdered *S. tortuosum* plant material were given orally twice a day to 7 beagle dogs with diagnosed dementia for a period of 6 days. The same dosage was given to cats with a stress tendency once a day for 7 days as well. A typical symptom of dementia is nocturnal barking and meowing. Beneficial effects were observed in both cats and the dogs that were diagnosed with dementia and their barking and meowing decreased significantly or in some cases stopped completely (Hirabayashi *et al.*, 2002; Hirabayashi *et al.*, 2004). These findings were later confirmed in 2005 in a comprehensive study on dogs by Hirabayashi *et al.* (2005) where 31 dogs and 2 cats, all diagnosed with dementia, were tested.

Cytotoxic tests were conducted on mesembrenone utilising three cell lines. Two different human cell lines were used namely, human lymphoid neoplasm (Molt 4) cells and human hepatoma (HepG 2) cells, as well as one murine alveolar non-tumoral fibroblasts (LMTK) cell line. The evaluation of cytotoxic activity of the mesembrenone alkaloid and vincristine sulphate was measured by applying 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, also known as the MTT test. Cytotoxicity were shown on the Molt 4 cells with an ED₅₀ value of 0.6 µg/ml while no cytotoxicity were found on the HepG 2 cells as the ED₅₀ value was >50 µg/ml, which was the cut-off value. The LMTK cells showed an ED₅₀ value of 10 µg/ml. Specificity was shown by the mesembrenone alkaloid against Molt 4 cells if it is compared to LMTK cells (Weniger *et al.*, 1995).

Anxiolytic effects were reported when human subjects took a *S. tortuosum* preparation (Gericke & van Wyk, 2001). Standardised *S. tortuosum* extracts have also been given once a day for a three-month period to 37 healthy adults in two different dose strengths, namely 8 mg and 25 mg in order to determine if any adverse effects occurred. A placebo group was also included. Variables that were tested comprised a physical examination, a 12-lead electro-diagram (ECG), laboratory assessments (biochemistry, haematology and urine analysis), as well as noting any adverse effects that emerged. There were no significant differences between the three groups in any area of examination. Improvement in sleep and handling stress were documented in the diaries of patients in the two test groups that received *S. tortuosum*. Therefore, it could be concluded that both of the *S. tortuosum* extract doses (8 mg and 25 mg) were well tolerated in human subjects (Dimpfel *et al.*, 2016; Nell *et al.*, 2013).

2.2.5 Products currently on the market

An increasing number of products containing *S. tortuosum* can be found on the market today. This may not only be due to the increased demand in the market for natural food supplements, but also the fact that these alkaloids can be used to counter-act an increased occurrence of lowered mood, stress, anxiety and depression in a contemporary world (Harvey *et al.*, 2011; Shikanga, Viljoen *et al.*, 2012).

Available products are typically in the form of tablets, teas, sprays, tinctures or capsules (Harvey *et al.*, 2011; Smith *et al.*, 1996; Terburg, 2013). In these products, there are different concentrations of dried and fine herbal material present that fall between 50 to 200 mg per dosing unit. Although the total concentration of *S. tortuosum* alkaloids in each batch differs, the concentration per unit never exceeds 2% of the plant material's dry weight. Therefore, it can be concluded that the amount of alkaloids available per single dose falls within the limits of 1-4 mg (Van Wyk & Wink, 2004; Gericke & Viljoen, 2008). Examples of a few *S. tortuosum* containing products that are currently on the market are discussed below.

2.2.5.1 Elev 8[®] tablets

Elev 8[®] tablets (Figure 2.1) contain 25 mg of Zembrin[®] per tablet. This product is approved for sale and is registered by the South African Medicines Control Council (MCC) as complementary medicine. This product has been available in most pharmacies since September 2012 (Health24, 2015).



Figure 2.1: Elev 8[®] tablets in their original packaging (Brunel, 2015)

2.2.5.2 *Sceletium tortuosum* herbs

These herbs (Figure 2.2) are produced and sold by a company called *Medico Herbs*[®]. An amount of 910 grams of dried *S. tortuosum* herbs is available in a pure smokers cut (Medico Herbs, 2009).



Figure 2.2: Dried *Sceletium tortuosum* plant material purchasable in South Africa (Medico Herbs, 2009)

2.2.5.3 *Sceletium tortuosum* vegecaps

This product (Figure 2.3) claims to contain 100 mg of *S. tortuosum* extract per capsule and is made of organic materials. It is advised that one capsule must be taken once or twice a day or as prescribed by a healthcare practitioner (Faithful to Nature, 2016).



Figure 2.3: *Sceletium tortuosum* vegecaps in original packaging (Faithful to Nature, 2016)

2.2.5.4 *Sceletium tortuosum* raw refined powder, capsules and oral spray

Medico Herbs® are the manufacturers of products shown in Figure 2.4. The *S. tortuosum* raw refined powder is sold at quantities of 50 grams and 100 grams. Maximum intake of the powder should not exceed 200 milligrams per day. The *S. tortuosum* capsule product contains thirty capsules, each containing 100 milligrams of extract. It is recommended that one capsule be taken daily. The oral spray consists of a liquid that contains the *S. tortuosum* extract. Three sprays can be sprayed into the mouth as and when needed. If the spray is used three times a day, one bottle will last over a month (Medico Herbs, 2009).



Figure 2.4: *Sceletium tortuosum* raw refined plant material powder, capsules and oral spray (Medico Herbs, 2009)

2.2.5.5 A variety of *Sceletium tortuosum* products

Sceletium South Africa® is a company that has a whole series of products each containing *S. tortuosum* plant material. These products (Figure 2.5) are sold nationally as well as internationally (Sceletium South Africa, 2016).



Figure 2.5: *Sceletium tortuosum* products as advertised (*Sceletium South Africa, 2016*)

2.3 MEDICATED CHEWING GUM

2.3.1 Introduction

Chewing gum is mostly known for the confectionery role it plays in today's society, but the value that chewing gum possesses to be used as a trans-mucosal drug-delivery system should not be overlooked. Medicated chewing gum (MCG) is defined by the European Pharmacopoeia (EP) as a “solid dose preparation with a base consisting mainly of gum that is intended to be chewed but not to be swallowed, providing a slow steady state release of the medicine contained” (Gadhavi *et al.*, 2011).

The first commercial available chewing gum was manufactured in 1848, marketed in the US and was called “State of Maine Pure Spruce Gum”. The first patent for the manufacturing of chewing gum was filed in 1869 by Mr. W.F. Semple of Ohio (U.S. Patent No. 98,304) (Heema & Stuti, 2011). “Aspergum” was the first MCG product manufactured and launched in 1928; and it contained acetylsalicylic acid to treat headaches (Gadhavi *et al.*, 2011).

Chewing gum generally consists of a gum base, which is water insoluble and other ingredients which usually are water soluble (Pagare *et al.*, 2012). MCGs can be used as a local treatment or as a systemic delivery system by direct absorption of drug released into the bloodstream through the buccal and sublingual mucosa (Madhav *et al.*, 2009; Aslani & Rafiei, 2012; Gadhavi *et al.*, 2011).

Formulation of MCGs utilises different excipients (Table 2.1) and usually include the active ingredient(s), gum base, filler(s), softeners, emulsifiers, flavouring agents and sweeteners (Surana, 2010). MCGs are designed so that the drug is released by the chewing action inside the mouth, where after it is absorbed sublingually and buccally either for a local effect or a systemic effect through circulation after absorption through the capillaries in the mucous membranes (Patel *et al.*, 2011; Madhav *et al.*, 2009).

Table 2.1: *List of water soluble and water insoluble components of medicated chewing gum (Pagare et al., 2012)*

	Component:	Description or function:	Examples:
Water insoluble	Plasticisers (3-20% of total weight of gum base)	Regulates cohesiveness of the product. Two types are known: Natural and Synthetic.	Natural: Rosin Esters Synthetic: Terpene Resins derived from α -pinene and/or d-limonene.
	Elastomers (40-70% of total weight of gum base)	Elasticity and gummy texture is provided.	Natural rubbers: Latex Natural gums: Jelutong, Lechi Caspi, perillo and Chicle.
	Fillers or texturisers (2-60% of total weight of gum base)	Creates a reasonable size gum, improves chewability and provides texture.	Magnesium carbonate, calcium carbonate, Ground Limestone, Magnesium and Aluminium silicate, Clay, Alumina, talc, Titanium Oxide and mono/ di/ tri Calcium phosphate.
Water soluble	Softeners and emulsifiers	Optimisation of chewability and the feel of the gum in the mouth.	Glycerin, Tallow, lecithin, fatty acids like Stearic acid, Oleic acid, Palmitic acid and Linoleic acid, and mono/ di/ tri glycerides.
	Colourants and whiteners	Provides a preferred colour to the gum.	FD and C type dyes and lakes. Fruit and vegetable extracts. Titanium Dioxide.
	Sweeteners (50-65% of total weight of gum base)	Provides a sweeter taste. Aqueous sweeteners retain moisture, blend ingredients together and are used as softeners. Bulk sweeteners	Aqueous sweeteners: Sorbitol, Corn syrups, hydrogenated starch hydrolysates. Bulk sweeteners: Sugar components include Sucrose, Dextrose, Maltose, Fructose, Dextrin, Galactose, corn syrup. Sugarless components include Sorbitol, Manitol, Xylitol, hydrogenated starch hydrolysate.
	Bulking agents	Used if low calorie chewing gum is required.	Indigestible dextrin, Polydextrose, Inulin. Guar gum hydrolysate, Fructooligosaccharides and Oligofructose.
	Flavouring agents	Improves flavour in chewing gum.	Essential oils: Citrus oil, peppermint oil, Spearmint oil, fruit essences, Mint oil, Clove oil and oil of wintergreen.
	Active component/ ingredient (0.5-30% of final gum weight)	Present in core and/or coating	

The rate of drug absorption is influenced by a number of factors. Physical properties of the active ingredient, such as the particle size and chemical properties, which include ionisation, stability in the presence of enzymes of the gastro-intestinal tract and the lipophilicity of the active ingredient determine the rate of absorption. A small, lipophilic and enzymatically stable active ingredient that is unionised will be absorbed at a faster rate. An ingredient that is soluble in saliva will be released out of the gum base within 10 to 15 min of chewing where an ingredient that is soluble in lipids will initially dissolve into the gum base; therefore release of the active ingredient occurs at a significantly slower rate (Pagare *et al.*, 2012). Complete release of the active ingredient in MCGs takes between 20 to 30 min. Drug release as well as the trans-mucosal absorption is influenced by the amount of saliva produced and the of chewing frequency (Rowe, 2003). Medication that is not absorbed sublingually or by means of the buccal route is resolved in the mucosa and swallowed. Further absorption then takes place in the digestive tract (Morjaria *et al.*, 2004; Aslani & Rafiei, 2012). MCGs are used all over the world as a controlled drug delivery system. A list of various MCGs that are available can be seen in Tabel 2.2.

The advantages of MCG as a dosage form over traditional drug delivery systems include (Kvist *et al.*, 1999; Biradar *et al.*, 2005; Pagare *et al.*, 2012; Morjaria *et al.*, 2004):

- It can be taken at any time of the day with high discretion as it does not require water to swallow.
- It is beneficial for patients that have problems with swallowing.
- It has a relatively quick onset of action as the substance is absorbed directly into the systemic circulation.
- Increased bioavailability is attained.
- It is a highly acceptable dosage form for children.
- MCGs counteract dry mouth.
- Fewer side effects occur compared to conventional oral dosage forms since the gum base does not cause any discomfort because it does not reach the stomach. The amount of substance that is swallowed with saliva and that reaches the stomach is small and it reaches the stomach at a systematic rate.
- Decrease in toxicity has been observed.
- Enterohepatic circulation will be avoided and the first-pass metabolism will not occur.
- It creates a relaxing and tension easing sensation.
- MCGs can freshen breath.

- Ear discomfort during flying is reduced.
- Snack cravings are satisfied.
- MCGs can contribute to clean teeth after meals.

Table 2.2: *List of MCGs marketed worldwide as well as their active substance, the aim of treatment and the location where it is commercially available (Pagare et al., 2012)*

Trade Mark	Active substance	Aim	Commercially available
Aspergum	Aspirin	Pain relief	North America
Nicorette	Nicotine	Smoking cessation	Worldwide
Nicotinelle	Nicotine	Smoking cessation	Western Europe, Australia
Trawell	Dimenhydrinate	Travel illness	Italy, Switzerland
Superpep	Dimenhydrinate	Travel illness	Germany, Switzerland
Chooz	Calcium carbonate	Stomach & neutralization	USA
Endykay Vit C	Vitamin C	General health	Middle East, UK
Stamil Vit C	Vitamin C	General health	Australia
Source Vit C	Vitamin C	General health	Australia
Brain	DHA* & CCE**	Enhanced brain activity	Japan
Stay Alert	Caffeine	Alertness	USA
Café Coffee	Caffeine	Alertness	Japan
Buzz Gum	Guarana	Alertness	UK
Go Gum	Guarana	Alertness	Australia
Chroma Slim	CR***	Diet	USA
Fluorette	Fluoride	Cariostatic	USA
Vitaflo CHX	Chlorhexidine	Tooth decay prevention	USA
Travvel	Dimenhydrinate	Motion sickness	USA, Australia
V6	Xylitol	Treatment or prevention of dental caries	UK

* DHA - Docosahexaenoic acid

**CCE – Capacitative Calcium Entry

***CR - Chromium

Limitations of MCG as a dosage form, on the other hand, include (Surana, 2010; Aslani & Rafiei, 2012; Pagare *et al.*, 2012):

- Risk of an overdose increases as it is easier to consume more than the prescribed amount in a short period;
- Sorbitol in MCGs can cause flatulence and diarrhoea;
- Additives can cause problems (cinnamon can cause ulcers and liquorice can cause hypertension);
- The concentration of the active ingredient reduces when it is diluted with saliva and not all active ingredients can be formulated into MCG;
- Not all of the medicine is compatible with the GI juices and therefore a substantial part of the medication dose can be destroyed if swallowed;
- The action of chewing a gum on a regular basis can cause stress on the jaw joints' cartilage which can cause discomfort for life. Excessive chewing can create chronic tightness in two of the eight muscles that are being used when chewing. This can cause chronic intermittent headaches due to pressure on the nerves;
- Certain types of gums adhere more to certain dentures and fillers;
- Certain substances can stain teeth; and
- Certain substances taste unpalatable and therefore can't be formulated into MCGs.

2.3.2 Methods of production of medicated chewing gum

2.3.2.1 Fusion

In this conventional method, the components of the gum base are melted and put into a kettle mixer where other components (e.g. sweeteners, syrups, active ingredients and other excipients) are added at a specific time. The mixture is sent through a series of rollers to produce a thin, wide ribbon which is lightly coated with a fine sugar powder to avoid sticking to the equipment and to add flavour. The gum is cooled in a temperature controlled room for 48 h to set properly where after it is cut into desirable sizes and packaged for sale. Limitations to this method of production for MCG's include limited use of thermolabile drugs; it complicates accuracy and uniformity of drug doses; a precise shape, form or weight cannot be attained and moisture content can cause problems (Ughade *et al.*, 2012; Morjaria *et al.*, 2004).

A nicotine chewing gum formulation produced by means of the fusion method usually contains the active ingredient, glycerine, liquid glucose, sugars, various sweeteners (e.g. aspartame,

stevia, liquorice or sodium saccharin), several taste-masking agents (e.g. zinc acetate, sodium acetate or sodium chloride) and a flavouring agent (Aslani & Rafiei, 2012). An example of a formulation can be seen in Table 2.3.

Table 2.3: *Formulation of a nicotine medicated chewing gum product by utilising the fusion method (Aslani & Rafiei, 2012)*

Ingredients	% w/w
Active ingredient (nicotine)	0.2
Gum base (Elvasti)	14.5
Sugar	64.1
Liquid glucose	18.1
Glycerol	1.9
Flavouring agent (banana)	1.2

2.3.2.2 Cooling, grinding and tableting

With this method, the chewing gum base is refrigerated at a temperature of -15°C. The cooling process is mediated with certain cooling agents. These agents include certain additives that include anti-caking and grinding agents, liquid nitrogen and hydrocarbon slush (from solid carbon dioxide) that facilitate the cooling process (Ughade *et al.*, 2012).

Anti-caking agents, such as precipitated silicon dioxide, are mixed with the gum base prior to the grinding procedure and help to prevent agglomeration of the chewing gum particles. Grinding agents, such as alkaline metal phosphate, alkaline earth metal phosphate and maltodextrin, prevents the gum base from sticking to the grinding apparatus and is used in concentrations of 2-8% of the total weight. The use of grinding agents are limited not only due to their alkalinity, which may be incompatible with acidic ionisable therapeutic agents, but the particles tend to remain in the mixture and are present in the chewing gum. This can cause problems from a safety and therapeutic point of view. Hydrocarbon slush lowers the temperature of the chewing gum base to a temperature of -78.5°C. Hydrocarbon slush sublimates on warming the gum mixture, does not have any adverse reactions such as adhesion to the apparatus, does not affect the chewing gum base as it is not absorbed and it never leaves any residue that can be harmful or undesired. Hydrocarbon slush is therefore the preferred cooling agent. When the desired temperature is obtained, the chewing gum base is cold enough to remain brittle throughout the grinding procedure. As soon as the gum base powder

has been removed from the cooler temperature, other ingredients such as sweeteners and binders can be added to the powder. Any conventional compression process, e.g. punching, can be used to compress this powder mixture (Ughade *et al.*, 2012).

2.3.2.3 Direct compression

A free flowing, directly compressible chewing gum excipient is used and mixed with the flavouring agents and the active ingredient, after which the mixture is directly compressed to form a MCG. A conventional tablet press can be used, which enables rapid manufacturing and low cost development. According to Pagare *et al.* (2012), chewing gums produced by means of direct compression are harder; and it crumbles when it is being crushed between the teeth during mastication, which enables the medication to be released at a faster rate compared to the above mentioned methods. Limitations associated with melting and freezing, which are present in the other methods of production, can be overcome with direct compression. The manufacturing process complies with the Food Chemicals Codex specifications as well as the Food and Drug Administration (FDA) as it is manufactured under Current Good Manufacturing Practice (CGMP) regulations (Ughade *et al.*, 2012). Table 2.3 presents a formulation used for a MCG produced by direct compression.

Table 2.4: Formulation of directly compressible nicotine MCGs (Morjaria *et al.*, 2004)

Ingredient	% w/w
Pharmagum® (S/M)	84.8
Nicotine Polacrilex (active ingredient)	2.2
Magnesium stearate	2.0
Sorbitol	8.0
Sodium carbonate	3.0

Cafosa® has developed a directly compressible gum base powder material that is available on the market for the manufacturing of MCG. This preformulated powder is available in a variety of mixtures containing different amounts of excipients in each, which are included based on the exact properties that are required in the final product. The excipients in this base are mainly a mixture of gum base with sorbitol, xylitol or mannitol, as well as plasticisers and anticaking agents (Morjaria *et al.*, 2004). Three different gum bases produced by Cafosa® as well as the flavouring agent, their characteristics and evaluations can be seen in Annexure A. The

proposed handling procedure of the Health in Gum bases by Cafosa® can be seen in Annexure B.

As a conclusion of the different methods of production, the fusion process is used for most of the MCGs manufactured today (Morjaria *et al.*, 2004). Heating at the beginning of this method of production creates a lot of restrictions in terms of what medication can be used in the manufacturing of MCG with this specific method as many active ingredients are thermally labile. Heat restrictions can be excluded by making use of directly compressible combinations or powders that turn into a chewing gum. Through this technique, a larger variety of medicinal chewing gum containing different active ingredients can be manufactured in a fast and inexpensive way (Morjaria *et al.*, 2004).

2.3.3 Release of medication from medicated chewing gum formulations and absorption through the oral epithelium

Most of the active ingredient contained in a MCG is released into the saliva as the gum is broken up during the chewing process; and is absorbed either through the buccal and sublingual membranes or from the gastrointestinal tract (GIT) after it is swallowed. Drug transport across buccal and sublingual membranes follows simple Fickian diffusion. Passive diffusion occurs in accordance with the pH partition theory (Pagare *et al.*, 2012). Permeation of substances across the buccal mucosa follows transcellular and paracellular routes as illustrated in Figure 2.6.

Absorption of drugs from the oral cavity is achieved through two main phases. The first phase is the process where the active ingredient(s) (such as alkaloids) is released into the oral cavity (Figure 2.7). The active ingredient(s) are absorbed via passive absorption through the lining of the oral cavity mucosa, also called the buccal epithelium (Figure 2.8) into the blood, which is the second phase (Patel *et al.*, 2011).

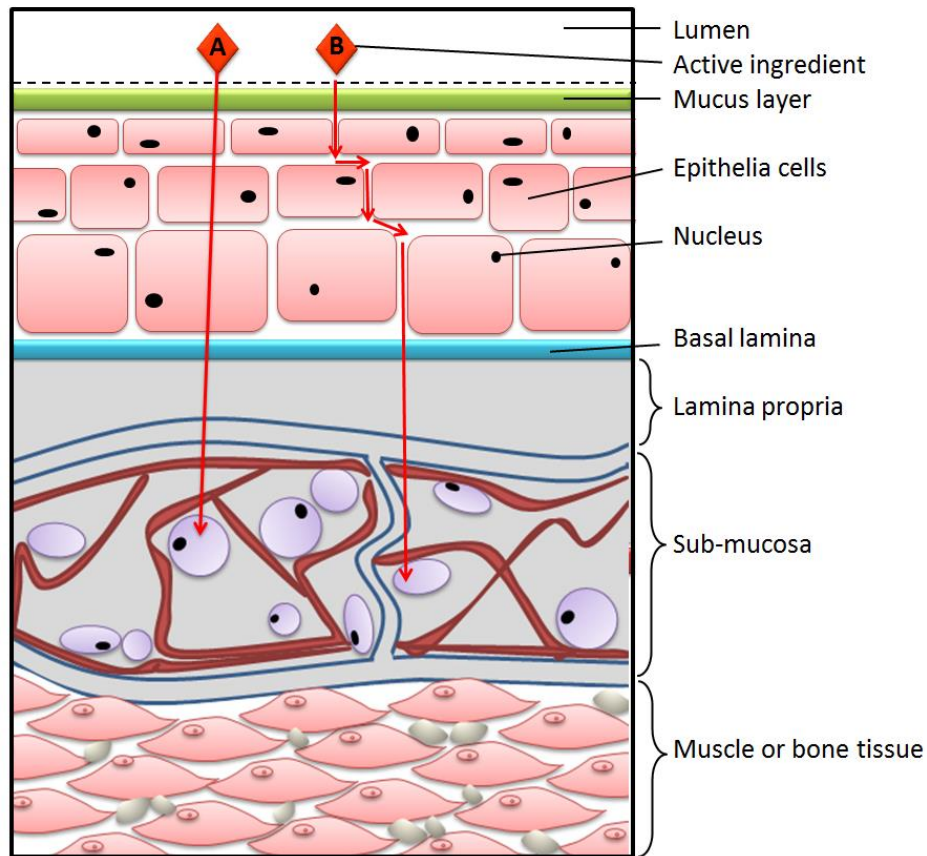


Figure 2.6: A schematic representation of (A) Transcellular diffusion and (B) Paracellular diffusion across the oral cavity mucosal epithelium

The oral cavity consists of the lips; cheeks; tongue; soft and hard palate; and the floor of the mouth. Three types of oral mucosa have been identified in the oral cavity: the lining mucosa, (buccal epithelium); sublingual mucosa; and specialised mucosa. Lining mucosa is located in the outer vestibule as well as in the sublingual region. Specialised mucosa is found on the dorsal surface of the tongue while the masticatory mucosa is located in the upper parts of the mouth (hard palate) as well as in the gums or gingiva (Patel *et al.*, 2011).

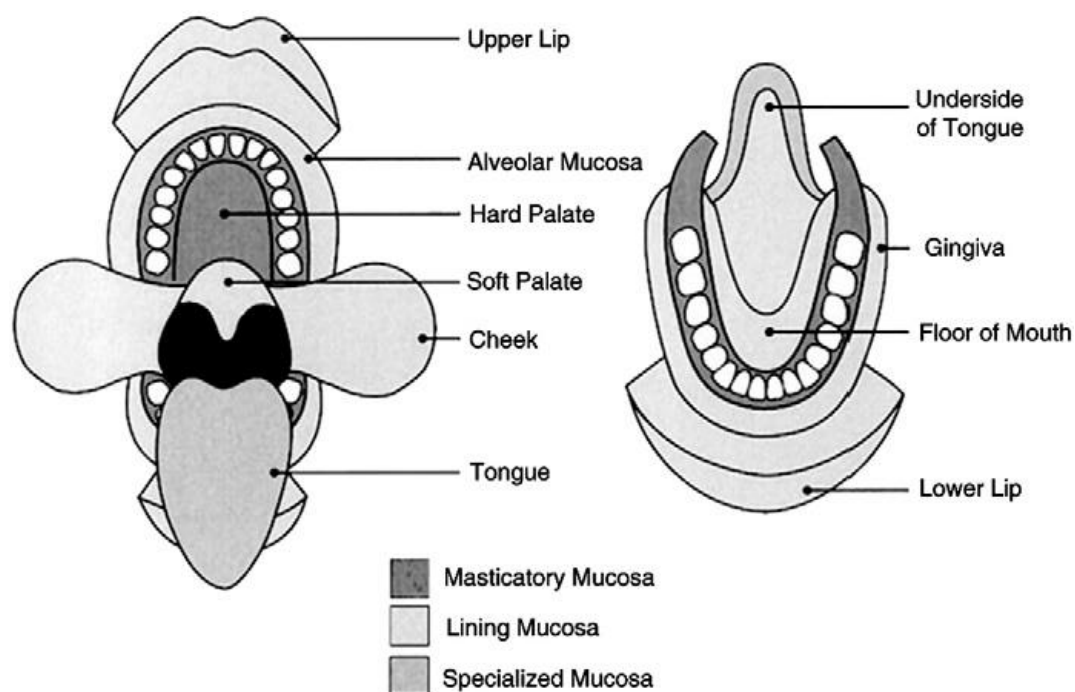


Figure 2.7: Schematic representation of the outer vestibule cavity (Patel *et al.*, 2011)

Oral mucosa consists of three layers (Figure 2.8), namely, the oral epithelium, the lamina propria and the sub-mucosa (Pagare *et al.*, 2012). The oral epithelium composes of multiple layers of basal cells, which show different stages of matured cells. These basal cells possess the ability to divide through the process of mitosis and multiply to maintain a constant layer of cells at the surface of the tissue (Rossi *et al.*, 2005). The oral epithelium is non-keratinised that is flexible to some extent, whereas keratinised epithelium (e.g. the skin) is dehydrated, chemically resistant and tough (Pagare *et al.*, 2012). Furthermore, it is relatively common that oral cavity mucosa lack tight junctions. Therefore, the epithelium is endowed with loose intercellular links which include gap junctions, desmosomes and hemi-desmosomes (Rossi *et al.*, 2005). The main function of the oral epithelium is to act as a shield to protect the layers of tissue underneath against fluid loss and harmful agents that may be present in the oral cavity (Patel *et al.*, 2011).

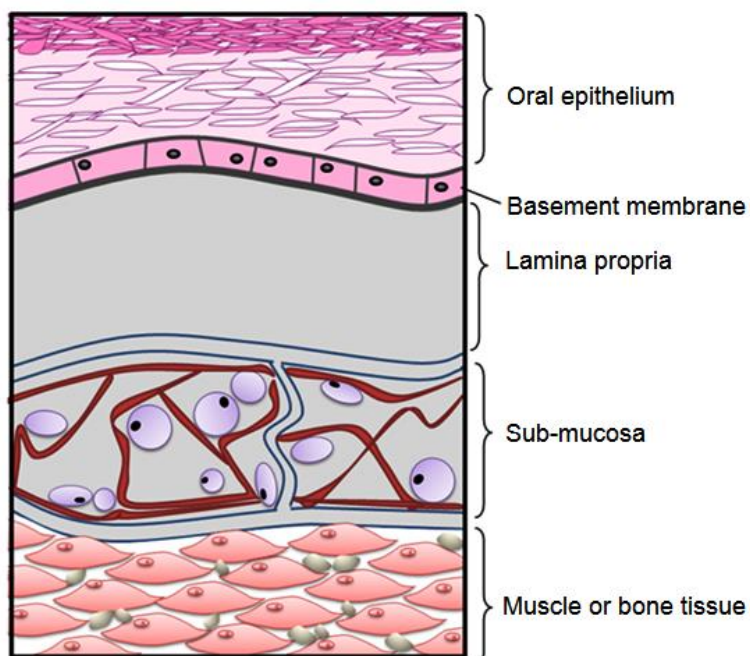


Figure 2.8: Schematic representation of the different layers of buccal mucosa

The lamina propria and sub-mucosa consisting of collagen elastic fibre and other cellular components carry nerve fibres and blood capillaries that serve the mucosa. The abundance of blood vessels in this layer makes it possible for substances such as drug molecules or alkaloids to enter the systemic circulation via the oral cavity (Pagare *et al.*, 2012).

A total amount of three pairs, or six salivary glands, are located in the human mouth at the parotid, the sub-maxillary (also submandibular) and the sublingual areas; as can be seen in Figure 2.9. Whenever the chewing action is executed, the salivary glands are stimulated and start to secrete saliva. Approximately 0.5 to 2 l of saliva is produced by these six glands per day. Advantages of saliva regarding medication or substance use include:

- Improved permeation through a moist surface compared to a dry surface (e.g. where a drug is absorbed through the skin); and
- Medication or substances that are administered in a solid dosage form is in need of a dissolving medium, such as saliva, before it can be absorbed across the oral mucosa (Pagare *et al.*, 2012).

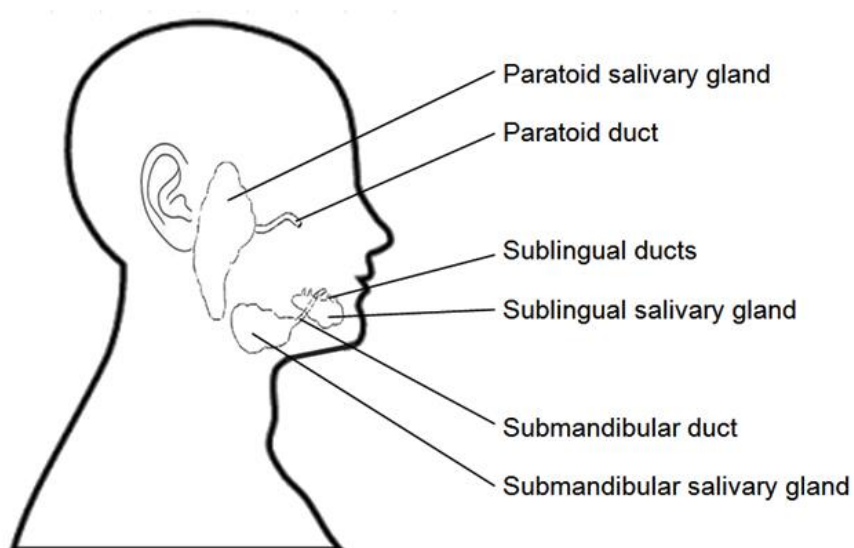


Figure 2.9: *Schematic representation of the parotid, sub-maxillary and sublingual salivary glands*

The factors that can affect mucosal delivery of drugs can be classified into three categories, namely:

- Membrane factors such as the thickness and permeability of the membrane that can affect the amount and the rate at which the active ingredient reaches the systemic circulation.;
- Environmental factors, such as:
 - Saliva: the consistency/viscosity as well as pH is important in passive diffusion. Saliva consists of 99% water and has a pH of 6.5 to 7.5,
 - Salivary glands: Excessive washout of the active ingredient may occur if the delivery system is not placed close to a salivary duct. However, higher local drug concentrations can be achieved if it is placed next to an opening of a salivary duct,
 - Chewing time and chewing rate: 60 chews per minute is an average chewing rate and MCG should preferably be chewed for a period of between 20 - 30 min, otherwise a part of the active ingredient dose can be lost,
 - Aqueous solubility of the drug: If the active ingredient is relatively good water soluble, a higher percentage of the substance will be released per time unit,
 - Percentage drug: The percentage of drug in the MCG as unit can affect the release thereof due to concentration gradient effects, but it is usually not significantly,

- Contact time: The duration of contact between the MCG and the walls of the oral cavity will determine the magnitude of the effect that will be stimulated,
- Physiochemical properties of active ingredient: substances that are relatively highly soluble in saliva will be released at a relatively high rate as soon as it makes contact with the saliva, where lipid soluble substances will be released slower,
- Inter individual variability: Every person chews at a slightly different rate and for a different length of time. The European Pharmacopoeia suggests proper release of substances should occur at a chewing rate of 60 chews per minute (EP, 2016),
- Formulation factors where the formulation can affect the release of an active substance from a MCG. According to the European Pharmacopoeia (2016), if the lipophilic part of the gum formulation is increased, the release rate will decrease.

2.3.4 Evaluation of medicated chewing gums

MCG as dosage form needs to be evaluated to determine the quality of the product and also to determine how the gum performs when it undergoes a series of tests. Quality determination tests according to the European Pharmacopoeia (2016) includes product identification, assay and also tests for the uniformity of mass, the uniformity of dosage units in terms of visual inspection, and the uniformity of content in terms of active substance (Gajendran *et al.*, 2010). Further optional tests of MCGs quality include crushing strength, tensile strength, friability, texture analysis, evaluation of flavours and sweeteners, tests for coating, impurities, product feel and consistency, residual solvents, water content and degradation products. Performance testing includes *in vitro* (dissolution) studies that determine the quantity of active components released over time from the MCG while mechanically “chewed” (Viljoen *et al.*, 2013; Gajendran *et al.*, 2010).

During dissolution studies, the mastication process must be simulated for accurate dissolution results as only a fraction of the total amount of active ingredient will be released by using conventional dissolution methods. A few devices are used to imitate the mastication process (Christrup & Møeller, 1986; Rider *et al.*, 1992; Kvist *et al.*, 1999). The European Pharmacopoeia published a monograph in 2000 describing “a suitable apparatus for studying the *in vitro* release of drug substances from MCG.” This compendial apparatus is called the European Pharmacopoeia chewing apparatus (EP, 2016). This apparatus (Figure 2.10) consists of a chewing chamber, two horizontal pistons and one vertical piston that represents

the tongue; and keeps the gum in the correct position for the two horizontal pistons to execute the mastication process (Pagare *et al.*, 2012).

A study conducted by Morjaria *et al.* (2004) showed that improved release of active ingredients could be obtained from directly compressible MCG compared to other conventional chewing gums that were manufactured using the melting method. The release of active ingredients from the MCG's were analysed using the European Pharmacopoeia chewing apparatus. Other studies (Jensen *et al.*, 1988; Christrup & Møller, 1986; Christrup *et al.*, 1988; Faraj *et al.*, 2007; Pedersen & Rassing, 1990; Pedersen & Rassing, 1991) were carried out using this apparatus from the European Pharmacopoeia and results indicated that the methodology is viable and reproducible (Pagare *et al.*, 2012).

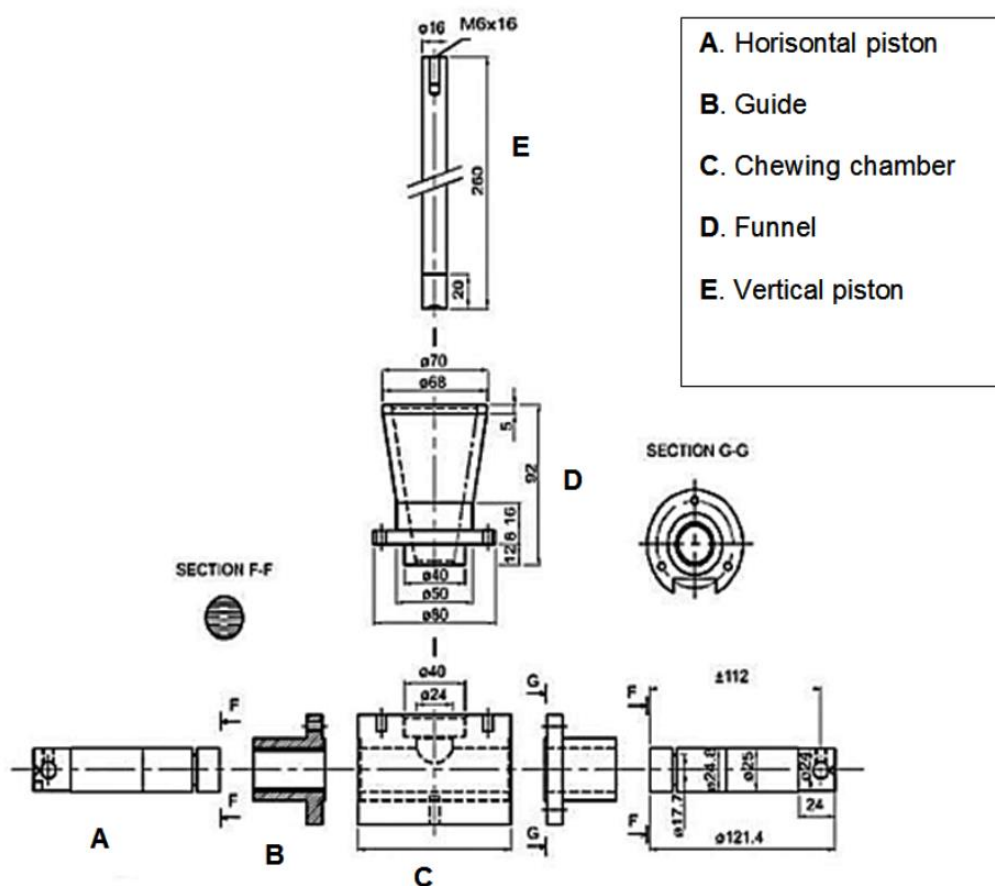


Figure 2.10: Construction of the European Pharmacopoeia apparatus (compendial) for testing the dissolution of chewing gum (adapted from Pagare *et al.*, 2012)

A non-compendial chewing apparatus (Figure 2.11) that was used by Kvist *et al.* (1999) consisted of six individual chewing modules. The test cells of this module may be filled with 20 -

70 ml liquid e.g. artificial saliva, while the upper and lower pistons simulate the chewing action. The temperature can be adjusted between 25 - 45°C, and the chew frequency can also be set to a value between 12 - 120 strokes per minute. Setting the correct temperature is extremely important as the consistency of the chewing gum changes rapidly along with the temperature. The insoluble part of the chewing gum remains intact throughout the whole study. It was noted that if any part of the chewing gum for some reason did not undergo the mastication process, release of the active ingredient was delayed.

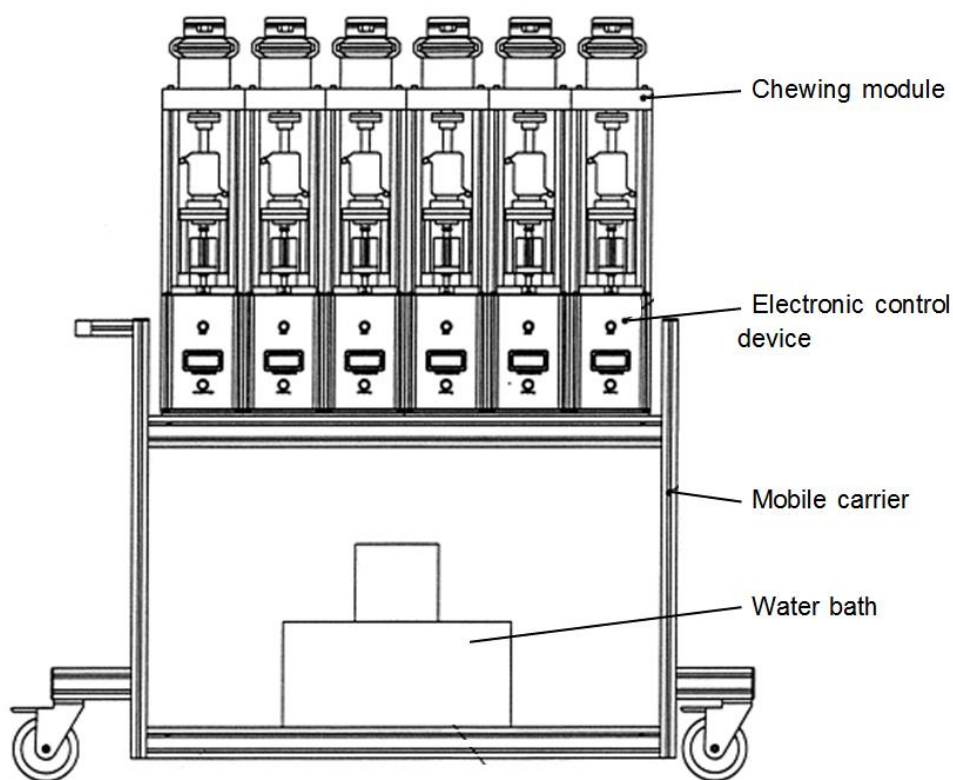


Figure 2.11: A drawing of a non-compendial chewing apparatus for dissolution studies of medicated chewing gums with six chewing modules (adapted from Kvist *et al.*, 1999)

A single chewing module of the non-compendial chewing apparatus used for dissolution studies of MCGs in this study is shown in Figure 2.12 (Kvist *et al.*, 1999). In accordance with the general guidelines for any dissolution apparatus, visual inspection of the mastication process is possible as the chewing module is completely transparent (Cantor, 1981).

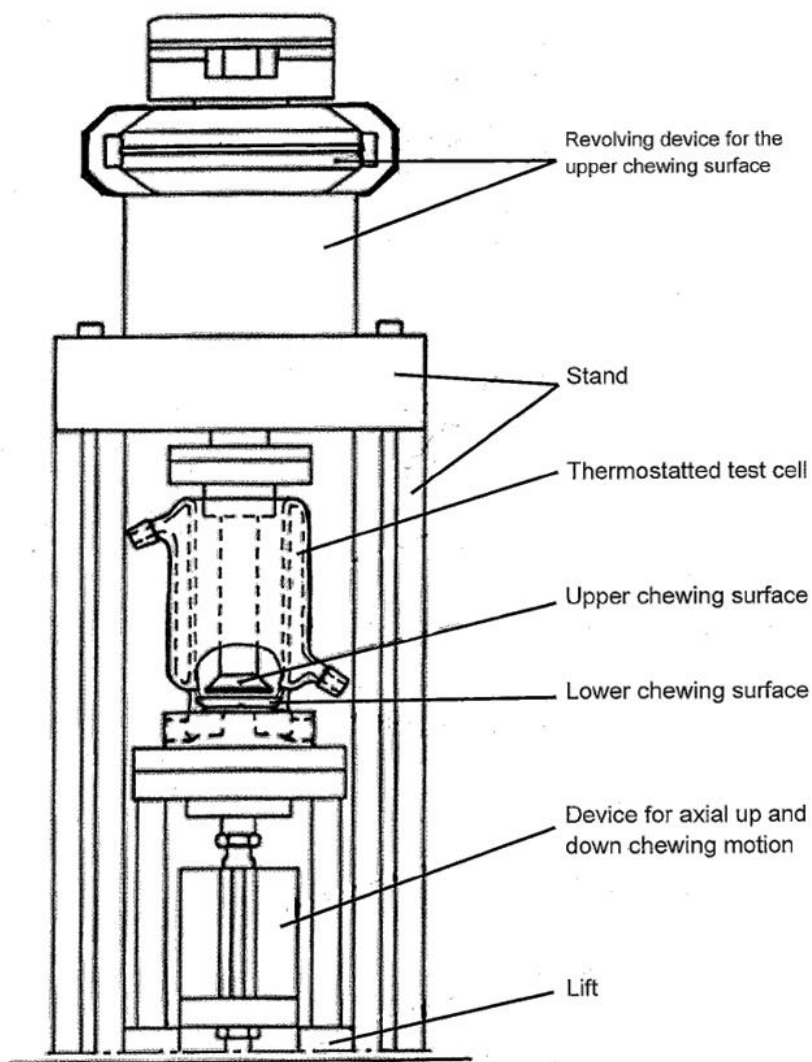


Figure 2.12: Illustration of a single chewing module (adapted from Kvist *et al.*, 1999)

2.4 SEDEM EXPERT DIAGRAM SYSTEM

2.4.1 Introduction

The SeDeM Expert Diagram System, also known as “Sediment Delivery Model”, can be used in preformulation studies to help predict whether a certain powder that is tested is suitable for direct compression. A number of twelve powder flow parameters have been selected to be determined by means of experiments and indices. Incidence factors are then calculated by using certain equations (Bhavsar *et al.*, 2015; Pérez-Lozano *et al.*, 2006; Suñé-Negre *et al.*, 2005; Suñé-Negre *et al.*, 2008). The twelve parameters must be fulfilled by the powder formulation for it to be directly compressible. Afterwards all twelve parameter values are

converted by several SeDeM specific equations to obtain a value between one and ten. These values are represented visually on a polygon diagram in order to more easily compare the differences between the different powders tested. Areas where improvement of different parameters in the formulation of the powder is necessary are also identified. This system provides three index values namely the Parameter Index (PI), Parameter profile Index (PPI) and the Good Compressibility Index (GCI) that can be determined from the twelve processed parameter values (Khan *et al.*, 2014).

2.4.2 Parameters examined by the SeDeM method

The twelve parameters of the SeDeM Expert Diagram System used to determine whether a powder is directly compressible, are based on powder flow properties and other physical tests, which include the following (Bhavsar *et al.*, 2015; Khan *et al.*, 2014; Suñé-Negre *et al.*, 2013):

- Bulk density (Da): It is the aerated density of the powder before being tapped. The value refers to the ratio of the total mass of the powder to the bulk volume of the powder. The bulk density is influenced by the powder's particle size distribution, the cohesiveness and shape of the particles;
- Tapped density (Dc): It is the density of the powder after being tapped. The value refers to the ratio of the total mass of the powder to the tapped volume of the powder;
- Interparticle porosity (Ie): It is defined as the pore space located between the grains/particles of the powder;
- Carr's index (IC): An indication (%) of the propensity of the powder that is going to be compressed;
- Cohesion index (Icd): It is the highest possible compaction force that can be used to produce the final product (e.g. tablet or medicated chewing gum). This is measured in newton (N);
- Hausner ratio (IH): It is the ratio of the tapped density of the powder to the aerated bulk density of the powder;
- Angle of repose (α): It is the maximum angle that is possible to form between the surface of the powder pile in respect to the horizontal surface on which the pile is formed. All of the powder samples must be poured from a predetermined height;
- Powder flow (t''): It is used to determine the vertical flow properties of divided solids under specific conditions. It is determined by measuring the time (measured in seconds and tenths of a second) for a powder sample to flow through a 10 mm orifice;

- Loss on drying (%HR): It is a test designed to measure the amount of water that is present in the powder sample after it was exposed to a drying chamber under specific conditions;
- Hygroscopicity (%H): It is the percentage weight that is gained after the powder was left in a humidifying chamber under specific conditions and for a predetermined period;
- Particle size (%Pf): It is the percentage of particles in the powder sample that is smaller than 50 μm . This test is done by passing the powder through a specific sieve size for a predetermined time period; and the
- Homogeneity index (I_H): A mathematical equation used to calculate the uniformity of the powder particles in a powder sample after it was passed through a sieve (Bhavsar *et al.*, 2015).

The above mentioned parameters are used to calculate five incidence factors based on the physical characteristics of the powders and the performance of the active compound:

- Dimensional factor: This factor includes the Bulk density (D_a) and Tapped density (D_c) test results. These parameters affect the powder's ability to pile up. This affects the final size of the tablets. The values of these two tests are also used in the calculation of other mathematical indices and to determine the compression parameter;
- Compressibility factor: This factor includes the Interparticle porosity (I_e), Carr's index (I_C), and Cohesion index (I_{cd}) tests. These parameters affect the compressibility of the powder;
- Flowability/Powder Flow factor: This factor includes the Hausner ratio (I_H), Angle of repose (α) and the Powder flow (t'') tests. These parameters influence the flowability of the powder when subjected to the direct compression process;
- Lubricity/Stability factor: This factor includes the Loss on drying (%HR) and Hygroscopicity (%H) tests. The future stability and lubricity of the powder is influenced by this factor; and the
- Lubricity/Dosage: This factor includes the Particle size (%Pf) and the Homogeneity Index (I_H) tests. This factor affects the tablet's dosage and the lubricity of the powder (Bhavsar *et al.*, 2015; Suñé-Negre *et al.*, 2013).

When all the parameters are established as required by the SeDeM system, the experimental values (V) are converted to SeDeM diagram radius (r) values with the equations that can be seen in Table 2.5.

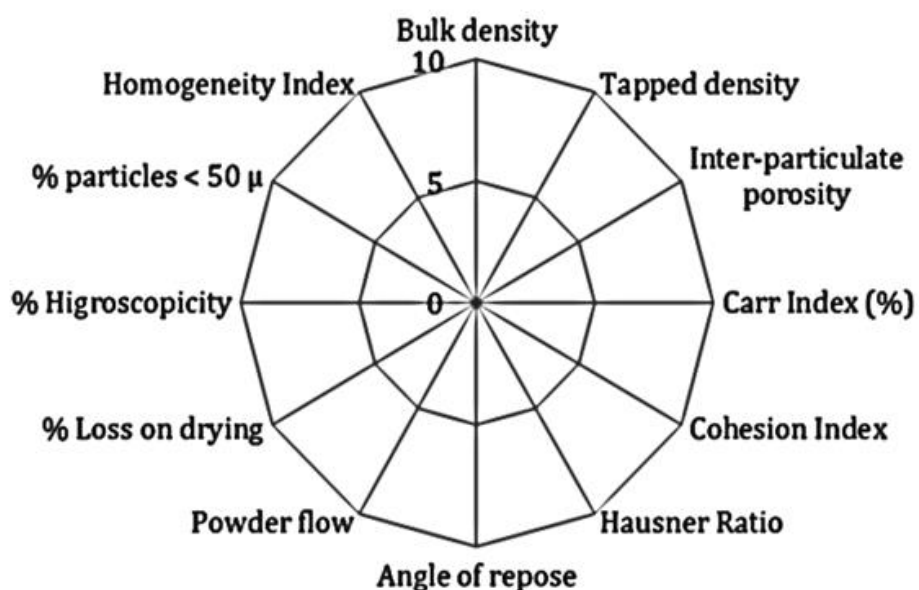
Table 2.5: Description of the incidence factors that is part of the SeDeM Expert Diagram System (Bhavsar et al., 2015; Khan et al., 2014)

Incidence Factor	Parameter	Symbol	Unit	SeDeM radius conversion equations
Dimension	Bulk density	Da	g/ml	10 V
	Tapped density	Dc	g/ml	10 V
Compressibility	Interparticle porosity	le	-	10 V/1.2
	Carr's index	IC	%	V/5
	Cohesion index	lcd	N	V/20
Flowability/ Powder flow	Hausner ratio	IH	-	(30-10 V)/2
	Angle of repose	α	-	10-(V/5)
	Powder flow	t''	S	10-(V/2)
Lubricity/ stability	Loss on drying	%HR	%	10-V
	Hygroscopicity	%H	%	10-(V/2)
Lubricity/ dosage	Particle size smaller than 50 μm	%Pf	%	10-(V/%)
	Homogeneity index	I θ	-	500V

The acceptable values for the incidence factors proposed by the SeDeM Expert Diagram System and the ranges of the radius (r) values for each of the incidence factors can be seen in Table 2.6. The SeDeM Expert Diagram System indicates the characteristics of the powder being tested to give an indication of whether the formulation will be directly compressible or not. Suppose all the radius values of the twelve parameters of the SeDeM system meet the standard values that are predetermined by the SeDeM system completely, thus having a radius (r) value of 10, the SeDeM diagram takes on the form of a dodecagon. A graphical representation of a blank SeDeM diagram can be seen in Figure 2.13 (Bhavsar et al., 2015).

Table 2.6: *Limit values for incidence factors that are considered acceptable by the SeDeM Expert Diagram System and the ranges of the radius values (r) (Khan et al., 2014)*

Incidence Factor	Parameter	Limit (V)	Radius (r)
Dimension	Bulk density	0-1 (g/ml)	0-10
	Tapped density	0-1 (g/ml)	0-10
Compressibility	Interparticle porosity	0-1.2	0-10
	Carr's index	0-50 (%)	0-10
	Cohesion index	0-200 (N)	0-10
Flowability/Powder flow	Hausner ratio	3-1	0-10
	Angle of repose	50-0 (°)	0-10
	Powder flow	20-0 (s)	0-10
Lubricity/Stability	Loss on drying	10-0 (%)	0-10
	Hygroscopicity	20-0 (%)	0-10
Lubricity/Dosage	Particle size smaller than 50 μm	50-0 (%)	0-10
	Homogeneity index	0-0.02	0-10

**Figure 2.13:** *A representation of a dodecagon shape diagram based on the twelve parameters of the SeDeM Expert Diagram System (Bhavsar et al., 2015)*

2.4.3 Acceptable limits for the SeDeM indices

In order to determine whether the powder is appropriate for direct compression, the Parameter, Parameter Profile, and the Good Compressibility Indexes are calculated on the basis of the results obtained from the twelve SeDeM Expert Diagram System parameters with the following equations (Bhavsar *et al.*, 2015):

Parameter Index (PI):

$$PI = \frac{\text{no of } P \geq 5}{\text{no of } Pt} \quad [2.1]$$

Where No of $P \geq 5$ indicate the number of parameters with a value that is equal or higher than 5; and No of Pt indicate the total amount of parameters studies.

Parameter Profile Index (PPI):

$$PPI = r \quad [2.2]$$

Where r is the value (average) of all parameters.

Good Compressibility Index (GCI):

$$GCI = PPI \times f \quad [2.3]$$

Where f is the reliability factor (dodecagon/ circle area). For twelve parameters, the f value is 0.952 (Bhavsar *et al.*, 2015).

The acceptability limits of the PPI and GCI indexes correspond to a value of 5, where qualification for direct compression is any value equal to or more than 5 (Bhavsar *et al.*, 2015).

2.4.4 Application of the SeDeM Expert Diagram System

The SeDeM Expert Diagram System can be used in six different applications, which are discussed briefly in the following sections:

- To establish whether an active ingredient is suitable for direct compression. Through subjecting the active substance in powder form to the twelve parameters and formulations set by the SeDeM Expert Diagram System, a SeDeM diagram is constructed, which provides a visual indication of the properties of the substance. The index values provide the final indication of whether the substance meets the requirements to be acceptable in terms of the direct compression process;

- To determine the amount of excipient(s) that must be added to an active ingredient, which is unsuitable for direct compression. This is done by adding excipients so that the powder mixture will meet the correct requirements to become suitable for direct compression. The following mathematical equation can be applied using the parameters obtained from the SeDeM Expert Diagram System that will identify shortcomings and the corrective measures to allow direct compression.

$$CP = 100 - \left\{ \frac{RE - R}{RE - RP} \right\} \times 100 \quad [2.4]$$

Where CP is the percentage (%) of corrective excipient; RE is the compressibility of the corrective excipient; R is the compressibility to be obtained in the powder mixture and RP is the compressibility of the active ingredient that needs to be corrected;

- For quality control of the batches of a single active ingredient that is going to be used to produce a product by means of the direct compression method. The application of this method comes down to determine whether the active ingredients of different batches meet the same characteristics. By using this method, the reproducibility of the product can be verified;
- To differentiate between active substances in the same chemical family. The application is based on the fact that active ingredients demonstrate unique physical properties even though they belong to the same chemical family;
- To differentiate between excipients belonging to the same functional group. The application can also demonstrate differentiation between excipients that fall into the same functional class, for example diluents; and
- To produce orally disintegrating tablets by utilising a new SeDeM model: SeDeM-ODT (orally disintegrating tablets) by direct compression. The SeDeM-ODT model provides an additional index, namely the index of good compressibility and bucodispersibility (IGCB Index). The IGCB index is obtained by means of the SeDeM Expert Diagram System method and is determined by not only five incidence factors as usual, but six incidence factors. The index gives an indication of whether the directly compressed tablets are suitable for usage as bucodispersible tablets. Bucodispersible tablets disintegrate in less than three minutes (Bhavsar *et al.*, 2015).

2.5 SUMMARY

The plant *S. tortuosum* was traditionally chewed by indigenous people who first used the plant for medicinal purposes. *S. tortuosum* is known for its pharmacological ability to inhibit both serotonin re-uptake and the phosphodiesterase enzyme (PDE4). Previous studies proved that this plant species possess pharmacological effects in the following areas: acting as an anti-depressant; improved cognitive set flexibility and executive function; improved sleeping patterns; reduction in state anxiety; and a decreased effect in general anxiety. *In vivo* studies indicated that hypnotic effects were noticeable when injected into the skin of a frog. No toxicological evidence was obtained when tested on any animal subject. Products containing *S. tortuosum* extract (tablets, teas, sprays, tinctures or capsules) are commercially available, mostly for the treatment of depression, anxiety and stress. There is however no product available that is appropriate for effective trans-mucosal absorption.

Medicated chewing gum (MCG) is a product that is increasingly becoming popular worldwide for its ability to deliver an active substance trans-mucosal. There are several MCG products available globally that are used for medicinal purposes. Manufacturing of MCGs are accomplished through a fusion method; a cooling, grinding and tableting method; or by means of direct compression. Release of the active substance from MCGs occurs during the chewing process. The active substance is then absorbed through the buccal and sublingual membranes; or from the GIT after it was swallowed. Transport across buccal and sublingual membranes follows simple Fickian diffusion; and to a lesser extend passive diffusion, that occurs in accordance with the pH partition theory. Permeation of substances across the buccal mucosa follows transcellular and paracellular routes.

Evaluation of the physicochemical properties is important to determine the quality of MCGs. These evaluation experiments generally include the following tests: product identification, assay, uniformity of mass, uniformity of dosage units, uniformity of content in terms of active substance, crushing strength, tensile strength, friability, texture analysis, evaluation of flavours and sweeteners, tests for coating, impurities, product feel and consistency, residual solvents, water content, degradation products, and *in vitro* (dissolution) studies. During dissolution studies, the mastication process must be simulated for accurate dissolution results as only a fraction of the total amount of active ingredient will be released by using conventional dissolution methods.

The SeDeM Expert Diagram System can be utilised during preformulation experiments in order to assist in predicting whether a certain powder that is tested is suitable for direct compression. A number of twelve parameters are determined by means of experiments and indices. Incidence factors are then calculated using specific equations. Subsequently, all twelve parameter values are converted through several SeDeM Expert Diagram System equations to obtain a value between one and ten. These values can then be visually demonstrated on a polygon in order to compare the differences between the different powders tested. Areas where improvement of different parameters in the formulation of the powder is deemed necessary can now also be identified. This system furthermore provides three index values, namely the Parameter Index (PI), Parameter Profile Index (PPI) and the Good Compressibility Index (GCI) that are determined from the twelve processed parameter values. The acceptability limits of the PPI and GCI correspond to a value of 5, where qualification for direct compression is any value equivalent to or more than 5.

Chapter 3: MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter provides details regarding the materials, equipment and methods used to conduct the experiments of this research project. This study focused on delivering active chemical compounds (i.e. mesembrine alkaloids) in *S. tortuosum* extract in the traditional way, which is through the oral mucosa and therefore medicated chewing gums (MCG) were developed as delivery systems.

The SeDeM Expert Diagram System was used to obtain a directly compressible formulation for production of the MCG's by means of compression on a tablet press. In order to formulate, prepare and evaluate different MCG formulations, a series of experiments were conducted. Experiments included preparation of a crude extract from *S. tortuosum* plant material and chemical characterisation of the extract through measurement of the mesembrine alkaloid contents by means of Ultra-high Performance Liquid Chromatographic (UPLC) analysis linked to a Mass Spectrometer (MS). Additionally, SeDeM analysis of the *S. tortuosum* extract powder; SeDeM analysis of a series of Cafosa®'s "Health in Gum" base materials; and SeDeM analysis of MCG formulations were conducted.

A full factorial design was subsequently utilised in order to identify different formulation compositions. These MCG formulations were tableted by means of direct compression; and their physical properties consequently evaluated in order to determine the optimal MCG formulation containing *S. tortuosum* extract powder.

3.2 MATERIALS

The materials that were utilised in this study are listed in Table 3.1. All materials used during this study were of analytical grade.

Table 3.1: *List of materials*

Material	Batch number	Manufacturer
Health In Gum 01 base	L110216114	CAFOSA® GUM, S.A.U. Barcelona
Health In Gum 03 base	L250216114	CAFOSA® GUM, S.A.U. Barcelona
Health In Gum 04 base	L180616114	CAFOSA® GUM, S.A.U. Barcelona
<i>S. tortuosum</i> plant materials and extracts		Provided by Prof Alvaro Viljoen (TUT)
Pruv®	30005105	Penwest, Church House, 48 Church Street, Reigate Surrey, England
ESPRAY® SD Cherry NI HH NK	07.03.2008008SD	Frutarom, S.A. (Pty)Ltd, Po Box 4449, Honeydew
Xylitol	MKBQ8024V	SIGMA-ALDRICH Chemie GmbH, Kappelweg 1, D-91625 Schnelldorf, Germany
D-mannitol	WXBB6836V	SIGMA-ALDRICH Chemie GmbH, Kappelweg 1, D-91625 Schnelldorf, Germany
D-Sorbitol	MKBT0975V	SIGMA-ALDRICH Chemie GmbH, Kappelweg 1, D-91625 Schnelldorf, Germany

3.3 PREPARATION AND CHARACTERISATION OF *SCELETIUM TORTUOSUM* EXTRACT

The aerial parts of the naturally occurring low-mesembrine chemotype of *S. tortuosum* were air-dried prior to extraction. All of the dried plant materials were submitted to a grinding procedure utilising a Retch MM400® ball mill (MCL, Rheinische Straße 36 42781, Haan, Germany) in order to produce a fine powder.

Following grinding, 20 g of the dried, refined plant material was properly mixed with 100 ml of the extraction solvent, i.e. distilled water. The mixture was placed in a Eumax® digital ultrasonic cleaner (Labotec, UD200SH-6L, Johannesburg, South Africa) at a temperature of 37°C for a period of 10 min where after it was stirred for another 10 min on a Lasec® hotplate stirrer (Benchmark Scientific Inc., Indonesia) at approximately 25°C. The mixture was centrifuged for 5 min with the SIGMA® 3-16 KL centrifuge (SIGMA® 3-16 KL Laboratory Centrifuges, D-37520

Osterode am Harz, Germany) at 5 500 rpm (at approximately 25°C). The supernatant was collected and filtered through Whatman no. 4 filter paper (Macherey-Nagel, Separations, Randburg, South Africa).

The filtered solution was frozen in a freezer (Forma Scientific, Inc., Box 649 Marietta, Ohio 45750, USA) at -86°C for a period of 4 h where after it was lyophilised using a Virtis SP[®] Scientific Sentry 2.0 freeze-dryer (United Scientific[®] 115 Pty. Ltd., Johannesburg, South Africa) for a period of 72 h (Shikanga, Viljoen *et al.*, 2012). The remnants were collected and refined with a mortar and pestle until a fine brown powder, i.e. the extract that contained the mesembrine alkaloids, was obtained. This extraction procedure was repeated another four times in order to obtain the total extract quantity from 100 g of the plant material. The yield was calculated using Equation 3.1:

$$\% \text{Yield} = \frac{\text{Amount of powder after extraction (g)}}{\text{Amount plant material used (g)}} \quad [3.1]$$

The quantity of the four key mesembrine-alkaloids namely mesembrenol, mesembranol, mesembrine and mesembrenone (Gericke & Viljoen, 2008) present in the alkaloid water-base extract was quantified by means of Liquid Chromatography-Mass Spectrometry (LC-MS) analysis against validated analytical reference compounds (Harvey *et al.*, 2011; Jeffs *et al.*, 1969; Shikanga, Viljoen *et al.*, 2012). The method that was followed for the LC-MS analysis of *S. tortuosum* extract powder can be described as follows:

For the sample preparation, a mass of 50 mg of the powdered extract was weighed and mixed with 10 ml methanol. This mixture was sonicated for 15 min where after it was filtered through a syringe filter with a membrane size of 0.2 µm. The filtrated sample was collected for LC-MS injection.

The Ultra-high Performance Liquid Chromatographic (UPLC) analysis was performed on a Waters Acquity UPLC system with a Photodiode Array Detector (PDA) (Waters, Milford, MA, USA). Separation via UPLC was achieved on an Acquity UPLC Ethylene-Bridged-Hybrid (BEH) C₁₈ column (150 mm x 2.1 mm i.d., 1.7 µm particle size, Waters) and the temperature was maintained at 30°C. The mobile phase consisted of two solvents namely solvent A: 0.1% ammonium hydroxide in water, and solvent B: 90% acetonitrile (ACN). The flow rate was 0.3 ml/min. A graduate elution was conducted as follows: 80% A: 20% B to 60% A: 40% B in 2 min. It was then changed to 50% A: 50% B in 4.5 min and changed back to the initial ratio in

0.2 min. The total running time was 8.5 min. The compound was injected into the mobile phase with an injection volume of 1.0 μ l (full-loop injection). Collected data were processed by chromatographic software Masslynx 4.1 and tabulated. The quantitative aspect of the method was validated by determining the linearity, recovering the limit of detection (LOD) and the limit of quantification (LOQ).

3.4 POWDER EVALUATION AS PER SeDeM EXPERT DIAGRAM SYSTEM

Evaluation of the mesembrine alkaloid containing extract powder; selected gum bases; and different MCG formulations, was conducted according to the parameter tests of the SeDeM Expert Diagram System (i.e. bulk density (Da), tapped density (Dc), inter-particle porosity (Ie), Carr's index (IC), cohesion index (Icd), Hausner ratio (IH), angle of repose (α), powder flow (t''), loss on drying (%HR), hygroscopicity (%H), particle size (%Pf), and the homogeneity index ($I\theta$)). Results obtained from the parameters are processed using the equations presented in Table 3.2 (Suñé-Negre *et al.*, 2014).

Table 3.2: *List of Abbreviations, Units, Limits and Radius values of the different parameters in each factor*

Incidence	Parameter	Symbol	Acceptable ranges	Equation for conversion to radius values
Dimension	Bulk density Tapped density	Da Dt	0–1 g/ml 0–1 g/ml	$Da = m/V_a$ $Dc = m/V_c$
Compressibility	Inter-particle porosity Carr's index Cohesion index	Ie IC Icd	0–1.1 0–50% 0–200 N	$Ie = Dc - Da/Dc \times Da$ $IC = ((Dc - Da)/Dc) 100$ Experimentally determined
Flowability	Hausner ratio Angle of repose Powder flow	IH α t''	3–1 50–0° 20–0 s	$IH = Dc/Da$ Experimentally determined Experimentally determined
Lubricity/Stability	Loss on drying Hygroscopicity	%HR %H	20–0% 0–50%	Experimentally determined Experimentally determined
Lubricity/Dosage	Particles < 50 μ m Homogeneity index	%Pf $I\theta$	50–0 0–2 $\times 10^{-2}$	Experimentally determined $I\theta = F_m/(100 + \Delta F_{mn}^a)$

Results obtained are converted to radius values in order to obtain an irregular shape polygon with a maximum radius of 10. This polygon provides a graphical representation of the advantages and disadvantages of each pharmaceutical powder. When these polygons are overlaid, collective characteristics are highlighted. The SeDeM Expert Diagram System furthermore affords an indication of whether a specific powder is suitable for direct compression or not; if a radius value of 5 or lower is obtained, the powder shows a limitation for the specific parameter. This system can also predict the amount of excipient required to compensate for insufficient drug characteristics, thus preformulation time and experiments are deduced.

The data obtained was used to quantitatively determine the suitability of each gum base for direct compression into MCG preparations as well as to obtain the most appropriate combination of ingredients for an optimal MCG formulation (Pérez-Lozano *et al.*, 2006; Suñé-Negre *et al.*, 2005; Suñé-Negre *et al.*, 2008). The parameter values that were obtained from evaluating the different powders according to the SeDeM method were grouped into five factors, which are described in the following sections.

3.4.1 Dimensional factor

The dimensional factor included two parameters, namely: bulk and tapped densities. The bulk (prior to tapping) and tapped (post tapping) volumes of three powder samples (50 g) of *S. tortuosum* extract; one of the selected gum bases; or one of the different MCG formulations (powder mixture formulations according to the factorial design) were determined by placing the samples individually in 250 ml measuring cylinders on the Tap Density Tester (Erweka® SVM 121/221, Germany). The machine was operated for 3 min at 200 taps per min. The bulk (Da) and tapped (Dc) densities (g.ml⁻¹) were calculated utilising the following equations:

$$Da = \frac{m}{V_a} \quad [3.2]$$

$$Dc = \frac{m}{V_c} \quad [3.3]$$

Where Da is the bulk density, m is the mass of the powder and V_a the initial or bulk volume of the powders. Dc is the tapped density and V_c the final or tapped volume of the powders (USP-NF, 2016).

3.4.2 Compressibility factor

Inter-particle porosity, Carr's index (%) and the cohesion index (Icd) are grouped to represent the compressibility factor. In order to calculate the inter-particle porosity and Carr's index, the calculated values of the bulk (Da) and tapped (Dc) densities were used. The inter particle porosity (Ie) was calculated using Equation 3.4; whereas Carr's index was determined as a percentage (%) using Equation 3.5 (Suñé-Negre *et al.*, 2011; USP-NF, 2016).

$$I_e = \frac{D_c - D_a}{D_c \times D_a} \quad [3.4]$$

$$CI = \left(\frac{D_c - D_a}{D_c} \right) \times 100 \quad [3.5]$$

The cohesion index (Icd) was experimentally determined by compressing the powder on a Korsch® XP1 single station tablet press (Korsch®, Germany) at the highest possible compaction force. The hardness (N) of the MCG produced from each powder mixture was noted and the mean values were calculated. First, the raw powder was tested and because the powder was easily compressible, it was not necessary to add any other excipients. The SeDeM Expert Diagram System recommends that if a powder mixture cannot be tableted, a lubricant powder mixture should be added, i.e. 2.36% w/w talc, 0.14% w/w Aerosil® 200, and 1.00% w/w magnesium stearate, to improve the compressibility (Suñé-Negre *et al.*, 2011).

3.4.3 Flowability/powder flow factor

The flowability/powder flow factor includes the Hausner ratio (HR), angle of repose (α) and powder flow (t'') parameters (Suñé-Negre *et al.*, 2011). The Hausner ratio is calculated as the ratio of the tapped density (Dc) to the bulk density (Da); and was calculated utilising the following equation (USP-NF, 2016):

$$HR = \frac{D_c}{D_a} \quad [3.6]$$

Angle of repose (α) was determined for each powder by measuring 50 g of the specific powder mixture and pouring it into a stainless steel funnel fitted with a shutter (10 mm diameter orifice) applying the fixed-height method (Lavoie *et al.*, 2002). The powder was allowed to flow through the funnel by removing the shutter so that a heap was formed on a solid surface. To calculate the angle of repose, the subsequent equation was used (USP-NF, 2016):

$$\tan \alpha = \frac{h}{r} \quad [3.7]$$

Where h is the height of the powder heap and r the radius of the powder heap (USP-NF, 2016).

Powder flow (t'') or flow rate was calculated by measuring the time it takes for a 50 g powder sample to freely flow through an orifice (10 mm) of a hopper once the shutter is opened. In this study the mean flow rate was calculated according to weight with Equation 3.8 (BP, 2016; Lavoie *et al.*, 2002).

$$t'' = \frac{m}{t} \quad [3.8]$$

Where t'' is the powder flow measured in g.s^{-1} ; m is the mass (g) and t is the time (s) it takes the powder to fully flow through the orifice (BP, 2016).

3.4.4 Lubricity/stability factor

Loss on drying (LOD) is designed to measure the amount of water and volatile matters in a powder sample when the sample is dried under specified conditions. In brief, a powder sample was placed in a drying oven which was set at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until a constant weight was obtained (USP-NF, 2016).

In order to determine the hygroscopicity (%H), a 1 g sample of each powder mixture was kept in a humidifying chamber at a relative humidity of 76% ($\pm 2\%$) and a temperature of $22^{\circ}\text{C} (\pm 2^{\circ}\text{C})$ for 24 h. The percentage (%) increase in sample weight was calculated using Equation 3.9 (Suñé-Negre *et al.*, 2011).

$$\%H = \left(\frac{m_e - m_0}{m_e} \right) \times 100 \quad [3.9]$$

Where m_0 is the mass (g) of the powder sample prior to exposure to the set conditions and m_e is the mass (g) of the powder sample after exposure.

3.4.5 Lubricity/dosage factor

Particle size analyses of the powders were conducted by means of laser diffraction utilising a Malvern® Mastersizer-2000 (Malvern Instruments Ltd®, UK) equipped with a Hydro 2000 SM dispersion unit. The percentage of particles measuring smaller than 50 µm (%Pf) was determined.

In order to calculate the homogeneity index ($I\theta$); data from the particle size analysis was used. These results were fractioned into the following ranges: > 0.335 mm; 0.212 – 0.335 mm; 0.100 – 0.212 mm; 0.05 – 0.100 mm; and < 0.05 mm. The percentage (%) particles in each range were calculated. Equation 3.10 was applied to the data obtained (Suñé-Negre *et al.*, 2008).

$$I\theta = \frac{F_m}{100 + (d_m - d_{m-1})F_{m-1} + (d_{m+1} - d_m)F_{m+1} + (d_m - d_{m-2})F_{m-2} + \dots + (d_m - d_{m-n})F_{m-n} + (d_{m+n} - d_m)F_{m+n}} \quad [3.10]$$

Where F_m = % particles in the majority range; F_{m-1} = %particles in the range immediately below the majority range; F_{m+1} = %particles in the range immediately above the majority range; n = order number of the fraction studied under a series, with respect to the major fraction; d_m = mean diameter of the particles in the major fraction; d_{m-1} = mean diameter of the particles in the fraction of the range immediately below the majority range; d_{m+1} = mean diameter of the particles in the fraction of the range immediately above the majority range.

3.4.6 Determination of acceptable limit values for each parameter

The numeric values for each SeDeM diagram parameter were converted to radius values (r) that were plotted on a polygon diagram. The r values indicate the characteristics of the product and whether or not the powder is suitable for direct compression (the r value should be > 5.00 to indicate suitability for direct compression). The following indices (Equations 3.11 – 3.15) were calculated based on the SeDeM Expert Diagram System (Aguilar-Díaz *et al.*, 2009; Aguilar-Díaz *et al.*, 2012; Pérez-Lozano *et al.*, 2006; Suñé-Negre *et al.*, 2005; Suñé-Negre *et al.*, 2008; Suñé-Negre *et al.*, 2011):

$$\text{Parameter index (PI)} = \frac{\text{No. } P \geq 5}{\text{No. Pt}} \quad [3.11]$$

Where No. $P \geq 5$ represents the parameters with values equal to or more than 5 and No. P_t represents the total number of parameters. Acceptability limit corresponds to a score of 5 or higher.

$$\text{Parameter profile index (PPI)} = \text{mean } r \geq 5 \text{ of all parameters} \quad [3.12]$$

Where mean r is the mean value of the parameters calculated. The acceptable limit corresponds to a score of 5 or higher.

$$\text{Good compressibility index (GCI)} = \text{PPI} \times f \quad [3.13]$$

Where f is the reliability factor and was calculated as follows:

$$f = \frac{\text{Polygon area}}{\text{circle area}} \quad [3.14]$$

The acceptability limit was calculated by:

$$\text{GCI} = \text{PPI} \times f > 5 \quad [3.15]$$

3.4.7 Calculating the amount of excipient required to adjust a deficient parameter

The parameter that needs to be corrected can be adjusted by calculating the type and minimum amount of excipient required for the specific active component to be subjected to the direct compression process. In order to select the most effective excipient, as well as the lowest possible concentration required to correct the compressibility deficiencies, Equation 3.16, that considers a minimum radius value of >5 , can be applied (Sauri *et al.*, 2014; Suñé-Negre *et al.*, 2013).

$$\text{CP} = 100 - \left(\frac{\text{RE} - \text{R}}{\text{RE} - \text{RP}} \times 100 \right) \quad [3.16]$$

Where, CP is the percentage of corrective base (minimum amount to be added); RE, is the mean-incidence radius value (compressibility) of the corrective excipient; R, is the mean-incidence radius value to be obtained in the blend; and RP, is the mean-incidence radius value (i.e. compressibility) of the *S. tortuosum* extract to be corrected.

3.5 FORMULATION OF MEDICATED CHEWING GUMS

3.5.1 Factorial design

The composition of the different MCG formulations containing *S. tortuosum* extract was determined by means of a full factorial design. Factorial design is a statistical method that is used to determine how numerous factors in a formulation affect and interacts with each other. Employing a factorial design condenses the number of experiments that has to be utilised when multiple factors are being studied at the same time (de Kock, 2005).

The effect of different variables (i.e. MCG base type and lubricant concentration) on the characteristics of the MCG units was investigated. One active ingredient, which was *S. tortuosum* extract, was combined with three CAFOSA®'s HIG chewing gum base types (i.e. HIG 01, HIG 03 and HIG 04), the sweetener (i.e. D-Sorbitol 20% w/w) and two different levels (absence and presence) of the lubricant (i.e. magnesium stearate). Abbreviations were assigned to each factor investigated and are presented in Table 3.3.

Table 3.3: *Formulation factors, variables with abbreviations and levels investigated in this study*

Factor	Variable	Abbreviation	Level
Lubricant (Magnesium stearate)	Concentration (0% w/w and 0.5% w/w)	0 or 0.5	2
Base types	Health In Gum 01 (77.862% w/w) Health In Gum 03 (77.862% w/w) Health In Gum 04 (77.862% w/w)	HIG 01 HIG 03 HIG 04	3

Table 3.4 shows the factorial design with the different MCG formulations that were prepared. For example, the formulation “S0.5HIG01” represents MCG units containing 0.638% w/w *S. tortuosum* extract, 0.5% w/w magnesium stearate and 77.862% w/w gum base type 01. Another example, the formulation “S0HIG03” represents MCG units containing 0.638% w/w *S. tortuosum* extract, 0% w/w magnesium stearate and 77.862% w/w gum base type 03.

Table 3.4: *Factorial design for medicated chewing gum formulations with lubricant concentration and type of gum base as variables*

		Health In Gum powder bases		
		Health In Gum 01	Health In Gum 03	Health In Gum 04
Lubricant: Magnesium stearate	0%	S0HIG01	S0HIG03	S0HIG04
	0.5%	S0.5HIG01	S0.5HIG03	S0.5HIG04

3.5.2 Preparation of powder mixtures and medicated chewing gums

The powder mixtures as determined by the factorial design were prepared with a Turbula® mixer (T2C, W.A. Bachofen AG Maschinenfabrik, Bâle, Switzerland) by mixing the predetermined quantities of the extract, one of the selected gum bases as well as the excipients for 7 min at 69 rpm in a honey jar, sealed with Parafilm®. Each medicated chewing gum contained approximately 25 mg *S. tortuosum* extract powder. After mixing the different powder mixtures, they were sealed with Parafilm® in glass containers and stored for at least 24 h prior to tableting.

The six MCG formulations were prepared by means of direct compression using a Korsch® XP1 single station tablet press and 12 mm punches. All of the specific tableting settings were determined by the powder properties of the various mixtures. The mass of the MCG tablets were set to be approximately 1.00 gram each.

3.6 EVALUATION OF MEDICATED CHEWING GUMS

The prepared MCG formulations were evaluated in terms of morphology, mass variation, crushing strength, diameter, thickness, tensile strength, friability and pharmaceutical availability. All tests were conducted in triplicate where after a mean value was calculated.

3.6.1 Morphology

Morphology of the surface and internal structures of MCG's was evaluated by mounting units of the different formulations on a metal disc with a silicon adhesive. The MCG's were coated in an Eiko® ion coater (model IB-2, Eiko engineering, Japan) using a gold and palladium (66:34) mixture under a vacuum of 1.5 torr and scanning electron micrographs were taken using a FEI Quanta® 250 Environmental Scanning Electron Microscope with a Field Emission Gun (FEI, Netherlands). Micrographs of the external surfaces and internal structures were taken at a magnification of 130X and 1000X, respectively (Chinyemba, 2012).

3.6.2 Mass variation

Mass variation was conducted on 20 randomly selected MCG units from each formulation, which were individually dusted with an art brush to eliminate any excess powder. Each unit was weighed on an analytical balance (Precisa®, Zurich, Switzerland). The average weight, standard deviation (RSD) and the percentage relative standard deviation (%RSD) were calculated and evaluated (BP, 2016; Viljoen *et al.*, 2013).

3.6.3 Crushing strength, diameter, thickness and tensile strength

Crushing strength, diameter and thickness were determined with a Pharma Test® (model PTB-311, Switzerland) tablet test unit at a rate of 0.1 cm.min⁻¹. A randomly selected sample of 10 MCG units from each formulation was diametrically compressed between the platens of the diametric-compression tester and the crushing strength (N) was recorded. The tensile strength value was calculated using the following equation:

$$T = \frac{2P}{\pi Dt} \quad [3.17]$$

Where T is the tensile strength (N.mm⁻²), P is the crushing strength or hardness (N), D is the diameter (mm) and t is the thickness (mm) (BP, 2016; Viljoen *et al.*, 2013).

3.6.4 Friability

Friability was determined using a friabilator (Erweka® GmbH, Heusenstamm, Germany) for a duration of 4 min at 25 rpm. Ten MCG units were dusted and weighted before and after rotation. Friability for each formulation was done in triplicate in order to calculate a mean value. Equation 3.18 was utilised to calculate the percentage friability (%F).

$$\%F = \left(\frac{W_1 - W_2}{W_1} \right) \times 100 \quad [3.18]$$

Where W₁ is the total weight of dusted MCG before rotation, W₂ is the total weight of dusted MCGs after completion of the rotation (BP, 2016; Viljoen *et al.*, 2013).

3.6.5 Dissolution

Drug release from the MCGs was done according to the British Pharmacopoeia (2016) using a Chewing Gum Tesing DRT-1 apparatus (Erweka® DRT-1, Ottost. 20, 63150 Heusenstamm,

Germany). A photograph of a multiple module Chewing Gum Testing (DRT-6) apparatus is shown in Figure 3.1. The following settings and conditions were fixed as default values:

- Temperature of the test medium (i.e. artificial saliva): $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$;
- Volume of thermostatted test cell: 40 ml;
- Chewing frequency: $40 \text{ strokes} \cdot \text{min}^{-1}$;
- Distance between the upper and lower jaws: 1.6 mm;
- Twisting angle: 20° and
- Total chewing time: 45 min.



Figure 3.1: Schematic representation of a Chewing Gum Testing DRT-6 apparatus (TLS, 2015)

The volume of the thermostatted test cell or chewing chamber was filled with 40 ml artificial saliva and left to equilibrate at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. One of the selected MCG units of a specific formulation was inserted into the single module chewing apparatus and was subjected to *in vitro* chewing for 45 min. A 2 ml single endpoint sample withdrawal was conducted after exposure to the mechanical chewing; it was filtered through a $10 \mu\text{m}$ porous membrane filter (053112-SSK,

PINFIL10S-DK, Cannula filters, Telford, Pennsylvania, USA) and transferred to HPLC-vials for analysis. This was done in triplicate for each MCG formulation and the samples were subsequently analysed by means of HPLC to determine the quantity of alkaloids released (BP, 2016; Gajendran *et al.*, 2010; Kvist *et al.*, 1999).

3.6.5.1 Preparation of artificial saliva

One litre of artificial saliva (Table 3.5) was prepared using the method described by Morjaria *et al.* (2004). All of the constituents were dissolved in 900 ml distilled water in a 1 000 ml volumetric flask. After the pH was set to 6.7, the solution was made up to volume and it was stirred for approximately 10 min in order to allow the chemicals to completely dissolve. The pH of the solution was adjusted to 6.7 with either 0.5 M hydrochloric acid or 0.5 M sodium hydroxide, utilising a Mettler Toledo®, Five Easy FF20 pH meter (Mettler-Toledo AG®, 8603 Schwerzenbach, Switzerland).

Table 3.5: *Artificial saliva formulation (Morjaria et al., 2004)*

Component	Quantity (mmol/L ⁻¹)
KH ₂ PO ₄	2.50
Na ₂ HPO ₄	2.40
KHCO ₃	15.00
NaCl	10.00
MgCl ₂	1.50
CaCl ₂	1.50
Citric Acid	0.15
pH is adjusted with NaOH or HCL to 6.7	

3.6.5.2 Ultra Performance Liquid Chromatographic analysis of artificial saliva samples

Chromatography is an analytical method that is used to separate specific molecules in a mixture depending on their molecular composition and structure. This analysis involves two phases: The stationary phase, which is either a solid particle or a liquid supported on a solid, and a mobile phase such as a liquid or a gas. As the mobile phase is passed through the column with a pump, the analyte is carried with the mobile phase i.e. the molecules of the analyte move along with the mobile phase through the stationary phase. Different separation rates are achieved due to certain molecules that own different affinities towards the stationary phase.

The stronger the affinity of the component in the sample towards the stationary phase, the slower the movement of the component will be. This causes different components to have different separation rates. The different retention times are recorded by a detector for each molecule investigated (Kupiec, 2004).

UPLC analysis was performed on a Waters Acquity UPLC system with a Photodiode Array Detector (PDA) (Waters, Milford, MA, USA). Separation via UPLC was achieved on an Acquity UPLC Ethylene-Bridged-Hybrid (BEH) C₁₈ column (150 mm x 2.1 mm i.d., 1.7 µm particle size, Waters) and the temperature was maintained at 30°C. The mobile phase consisted of two solvents, solvent A: 0.1% ammonium hydroxide in water, and solvent B: 90% acetonitrile (ACN). The flow rate was set at 0.3 ml/min. A gradient elution was as follows: 80% A: 20% B to 60% A: 40% B in 2 min. It was then changed to 50 A: 50% B in 4.5 min and changed back to the initial ratio in 0.2 min. The total running time was 8.5 min. The compound was injected into the mobile phase with an injection volume of 1.0 µl (full-loop injection). Collected data were processed by chromatographic software Masslynx 4.1. The quantitative aspect of the method was validated by determining the linearity, recovering the limit of detection (LoD) and the limit of quantification (LoQ).

3.6.6 Statistical analysis of data

The SeDeM Expert Diagram System, the homogeneity index, the standard deviation (RSD) and the percentage standard deviation (%RSD) were calculated using Microsoft® Excel™ 2010 for Windows™ (Microsoft® Corporation, Seattle, Washington, USA). Where necessary, further analysis of data was statistically analysed by means of a one way analysis of variance (ANOVA) at a confidence level of 5% to indicate significant differences ($p < 0.05$).

Chapter 4:

RESULTS AND DISCUSSION

4.1 INTRODUCTION

This chapter provides details regarding the results that were obtained, processed and interpreted. In summary, a water extract of *S. tortuosum* plant material was prepared whereafter the yield was calculated and the amount of mesembrine alkaloids was quantified by means of LC-MS. The SeDeM Expert Diagram System was subsequently applied to the extract powder as well as Cafosa®'s Health in Gum bases in order to generate profiles of their physico-chemical properties. These profiles were used to predict the addition of suitable amounts of a corrective excipient to produce MCG tablets by means of direct compression.

The MCG powder formulations determined by a full factorial design were also subjected to the SeDeM Expert Diagram System scrutiny before compaction into MCG units. Further changes were made to the formulations based on the data provided by the SeDeM results. The final MCG units were evaluated in terms of morphology, mass variation, crushing strength, diameter, thickness, tensile strength, friability, and pharmaceutical availability (dissolution).

4.2 YIELD AND CHEMICAL CHARACTERISATION OF *SCELETIUM TORTUOSUM* EXTRACT

A dark-brown hygroscopic powder (Figure 4.1) was obtained after extraction of *S. tortuosum* plant material. The yield of the prepared extract was determined prior to the quantification of the alkaloids present in the *S. tortuosum* extract. First, the yield was calculated on a smaller batch of 20 g dried *S. tortuosum* plant material to determine if the extraction method was capable of producing sufficient extract (i.e. an acceptable yield of > 10%).



Figure 4.1 Photograph of *Sceletium tortuosum* extract powder

The first and smaller (20 g) batch's yield was calculated as 13.20% (Table 4.1). It was evident that the extraction method that was followed was successful as the amount of the *S. tortuosum* extract powder (2.64 g) extracted from 20 g dried plant material was acceptable. The second and larger batch of 80 g dried *S. tortuosum* plant material was extracted and an amount of 10.12 g extract was obtained. The yield was calculated as 12.65% (Table 4.1) for this second batch of extract. Subsequently the percentage yield for the total extracted material was calculated and a value of 12.75% extract was obtained from a total 100 g of dried *S. tortuosum* plant material that was used.

Table 4.1: Summary of *Sceletium tortuosum* extracts and percentage yield

Batch	Plant material (g)	Extract (g)	Calculation	Yield (%)
1	20 g	2.6394 g	$\left(\frac{2.6394 \text{ g}}{20 \text{ g}}\right) \times 100$	13.20
2	80 g	10.1200 g	$\left(\frac{10.1200 \text{ g}}{80 \text{ g}}\right) \times 100$	12.65
Total:	100 g	12.7594 g	$\left(\frac{12.7594 \text{ g}}{100 \text{ g}}\right) \times 100$	12.75

The quantity of the four mesembrine alkaloids namely mesembrenol, mesembranol, mesembrenone and mesembrine present in the water-base *S. tortuosum* extract was quantified by means of LC-MS analysis against validated analytical reference compounds (Table 4.2). The LC-MS chromatograms of the extract are shown in Figure 4.2.

Table 4.2: Quantities ($\mu\text{g}/\text{mg}$, $n=2$) and percentage weight per weight (% w/w) of the four alkaloids detected in the *Sceletium tortuosum* extract

Alkaloid	Amount of alkaloids ($\mu\text{g}/\text{mg}$)	Percentage alkaloids (% w/w) in extract	Percentage alkaloids (% w/w) in total alkaloid content
Mesembrenol	0.000	0.000	0.000
Mesembranol	0.580	0.058	5.200
Mesembrenone	4.220	0.422	37.700
Mesembrine	6.400	0.640	57.100
Total	11.200	1.120	100.000

In a study conducted by Shikanga, Viljoen *et al.* (2012) it was concluded that of the three extraction methods used (i.e. water, MeOH and acid-base), the MeOH extract presented with the highest yield ($8.70 \pm 0.10\%$ dry weight of plant material). Although the acid-base extract presented with a lower yield ($0.44 \pm 0.01\%$ dry weight of plant material), it produced the highest alkaloid content. The water extract depicted a yield of $6.41 \pm 0.06\%$ of the dry plant material weight and the concentrations of the alkaloids present in the dry plant material were 0.529 mg/g mesembrenone, 0.460 mg/g mesembrenol, 0.234 mg/g mesembrine and 0.214 mg/g mesembranol, respectively. As evident from Table 4.2, only three of the four alkaloids are present in the water extract prepared in this study with a total amount of $11.20 \mu\text{g}/\text{mg}$ alkaloids in the extract powder, but a higher quantity of mesembrenone and mesembrine were obtained compared to the previous study. Qualitative studies done by Shikanga, Viljoen *et al.* (2012) identified the mesembrine alkaloid to be the main alkaloid constituent present in most of the evaluated commercial products, whereas mesembrenol along with mesembrine and mesembrenone are used as marker compounds to monitor the quality of raw materials containing *S. tortuosum* extract or plant materials.

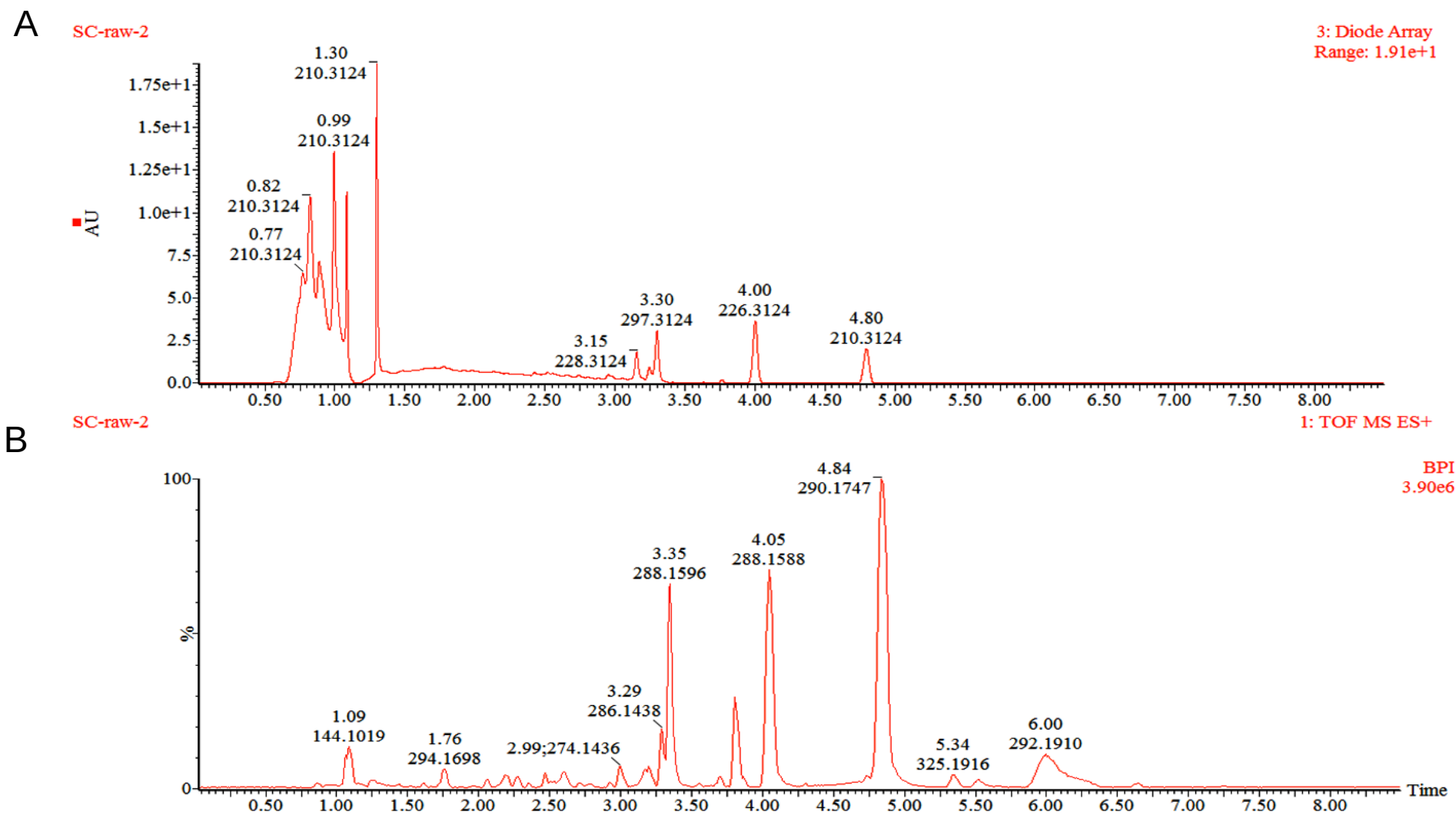


Figure 4.2: (A) UPLC chromatogram and (B) MS chromatogram of the *Sceletium tortuosum* extract

The mesembrine alkaloid is present in the highest concentration of all four the alkaloids in the water extract prepared in this study with a value of 6.40 µg/mg. As mentioned in Chapter 2, the mesembrine alkaloid possesses the ability to inhibit the uptake of certain mono-amines (i.e. GABA, 5HT, δ₂-opioid, µ-opioid and the cholecystokinin-1 receptors) more drastically than the other alkaloids. Mesembrine is 20 times more potent than mesembrenone and 87 times more active compared to mesembrenol (Harvey *et al.*, 2011; Shikanga, Viljoen *et al.*, 2012). The relative high concentration of the mesembrine alkaloid is therefore important as the 5 HT- and PDE4-inhibition, which is responsible for the anxiolytic–narcotic activity found in the *Sceletium* species, depends on the presence of this alkaloid (Smith *et al.*, 1996; Smith *et al.*, 1998).

Mesembrenone is present in the second highest concentration (4.22 µg/mg) in the extract. As discussed in Chapter 2, mesembrenone possesses the ability to inhibit the PDE4 enzyme, which is mainly associated with mental wellbeing that includes improvement of cognitive function, long-term memory, wakefulness, neuro-protection and inflammatory suppression effects (Barad *et al.*, 1998; Block *et al.*, 2001; Chen *et al.*, 2007; Lelkes *et al.*, 1998). The mesembrenone alkaloid is shown to be 17 times more active on this specific binding site (PDE4) compared to mesembrine; and 34 times more active compared to the mesembrenol alkaloid (Harvey *et al.*, 2011).

4.3 EVALUATION OF THE POWDER CHARACTERISTICS

4.3.1 Introduction

Direct compression has some limitations, especially considering the excipients utilised for this production method. The excipients need to compensate for poor flowability as well as inadequate compression properties that are frequently characteristic of active pharmaceutical ingredients. These characteristics may limit the quantity of the drug that can be incorporated into a direct compression tablet formulation (Jivraj *et al.*, 2000). Moreover, the time and cost implicated in order to experiment with drug and excipient combinations to determine the correct or optimised tablet formulation are considered challenges for this method of tablet production. The SeDeM Expert Diagram System was specifically developed to decrease both the number of experiments as well as the time necessary to render an acceptable direct compression tablet formulation. This system specifies which of the drug powder properties need to be adjusted in order to assist the successful formulation and manufacture of tablets by means of direct compression (Suñé-Negre *et al.*, 2014; Suñé-Negre *et al.*, 2011; Suñé-Negre *et al.*, 2008; Pérez-Lozano *et al.*, 2006).

The SeDeM Expert Diagram System generates a unique profile for each excipient and drug by means of generally utilised powder assessment techniques that are widely accepted within the pharmaceutical industry. These unique profiles can be used to predict suitable excipient to drug combinations as well as ratios for direct compression tablets. The SeDeM Expert Diagram System combines quantitative and experimental results from 12 parameters to conclude the particular properties of each powder.

The acceptability for direct compression can be determined through calculation of the Parameter Index (PI), the Parameter Profile Index (PPI), the Good Compressibility Index (GCI) and the reliability factor (f). The PI was calculated by dividing the number of radii larger or equal to 5 by the amount of factors tested. PI values equal or higher than 0.5 are considered satisfactory. Moreover, PPI is a value equal to the average value of all the parameters, where a value of more than 5 is deemed acceptable. The reliability factor (f) is determined by dividing the polygon area by the circle area of the polygon. Utilising an infinite number of parameters will provide an f value of 1, whereas using 12 factors contributes an f value of 0.952 and using just 8 factors decreases reliability to 0.9. The GCI value is obtained by multiplying the f value with the PPI value. A GCI value higher than 5 is considered adequate for direct compression. This study used all 12 factors as described by Suñé-Negre *et al.* (2013).

4.3.2 Powder flow properties of *Sceletium tortuosum* extract powder and the Health In Gum base powders

The flowability scale prerequisites of any substance in powder form are shown in Table 4.3, which was adapted from the British Pharmacopoeia (2016). The performance of the flow property range from extremely poor to excellent according to their scores achieved. For example, while outstanding flow properties will be achieved if the powder has an angle of repose of less than 30°, extremely poor flow properties will have an angle of repose higher than 66°. A powder obtaining a Carr's index of between 1-10% and a Hausner ratio smaller than 1.11 will predict excellent flow properties (BP, 2016).

Table 4.4 provides the flow properties of the *S. tortuosum* extract powder and the HIG 01, 03 or 04 base powders prior to the addition of any excipients. According to Table 4.3 and as evident from Table 4.4, the *S. tortuosum* extract powder depicted fair powder flow as indicated by the Carr's index (15.94%) and Hausner ratio (1.19) values. The angle of repose value (20.3°) indicated excellent powder flow and therefore contributed to an acceptable flowability scale of the extract powder. The flow rate of the *S. tortuosum* extract powder

was, however, significantly lower ($p < 0.05$) compared to the HIG bases, indicating relatively poor flowability.

Table 4.3: *Flowability scale prerequisites*

Flow property	Carr's index (%)	Hausner ratio	Angle of repose (α)
Excellent	1-10	1.00-1.11	25-30
Good	11-15	1.12-1.18	31-35
Fair	16-20	1.19-1.25	36-40
Passable	21-25	1.26-1.34	41-45
Poor	26-31	1.35-1.45	46-55
Very poor	32-37	1.46-1.59	56-65
Extremely poor	> 38	> 1.60	> 66

Table 4.4: *Flow properties of *Sceletium tortuosum* extract powder, the Health In Gum base powders 01, 03 and 04 prior to the addition of the extract (Percentage relative standard deviation in parenthesis)*

Test	Formulations			
	<i>S. tortuosum</i> extract	HIG 01	HIG 03	HIG 04
Bulk density (g/cm ³)	0.79 (1.93)	0.68 (0.85)	0.68 (0.00)	0.65 (0.89)
Tapped density (g/cm ³)	0.94 (0.00)	0.90 (12.78)	0.90 (1.29)	0.86 (0.00)
Hausner ratio	1.19 (2.12)	1.32 (1.15)	1.32 (1.31)	1.33 (0.87)
Carr's Index (%)	15.94 (10.41)	24.35 (3.48)	24.16 (3.99)	24.81 (2.70)
Flow rate (g/s)	4.06 (2.84)	9.2 (1.25)	9.06 (1.27)	8.90 (1.29)
Angle of repose (α)	20.30 (4.98)	19.12 (4.10)	19.80 (2.55)	20.00 (2.92)

Although the *S. tortuosum* extract powder depicted fair flowability, the hygroscopicity property of this extract powder influenced the powder flow profile drastically. The extract powder could only be handled for a short period where after it became sticky due to the absorption of moisture from the atmosphere. The extract powder could therefore only be handled when the atmospheric humidity was relatively low, which was on sunny days.

Upon examination of the morphology of the *S. tortuosum* extract powder, it was clear that this powder not only depicted a relatively wide particle size distribution, but also presented particles of irregular shape and surfaces (Figure 4.3). Particle sizes varied from approximately 43.80 μm to 1.193 mm. The relatively poor or sub-optimal flowability properties could therefore be attributed to uneven particle shape and size; as well as a relatively large particle size distribution in addition to the large amount of fairly small particles (i.e. particles < 50 μm) observed in this extract powder.

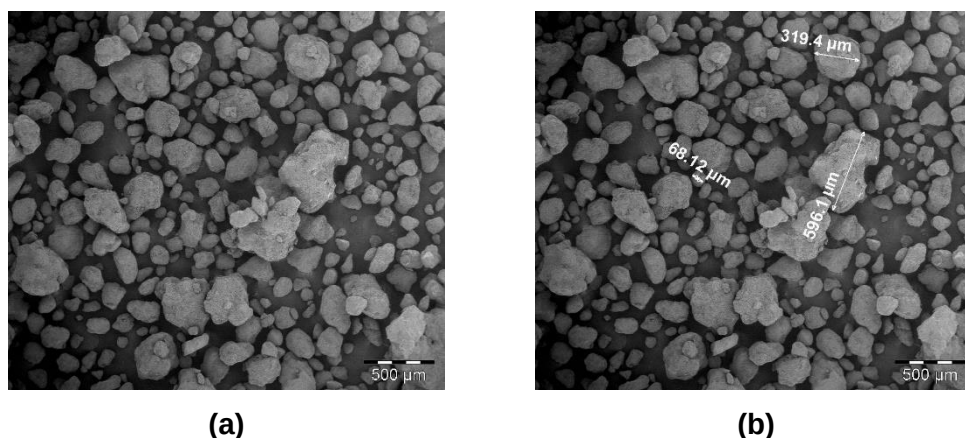


Figure 4.3: (a) SEM micrograph of *Sceletium tortuosum* extract powder at a 95X magnification; and (b) SEM micrograph of *Sceletium tortuosum* extract powder at a 95X magnification including random size measurements of individual particles

Considering the results obtained for the HIG base powders (Table 4.4), it was clear that these three powders did not differ radically from each in terms of flowability as the data for these powders showed comparable values. All of the HIG base powders depicted passable Carr's index and Hausner ratio values, whereas the angle of repose for all three HIG base powders displayed excellent flowability. Furthermore, studying the micrographs (Figure 4.4) taken of the three HIG base powders, it was evident that these powders depicted a more spherical (round) shape with a more uniform particle size and size distribution (33.39–810.0 μm) compared to the *S. tortuosum* extract powder, indicating why the flowability of these powders was improved. Moreover, the surfaces of these particles are also smoother than, and not as irregular as the particles of the *S. tortuosum* extract powder.

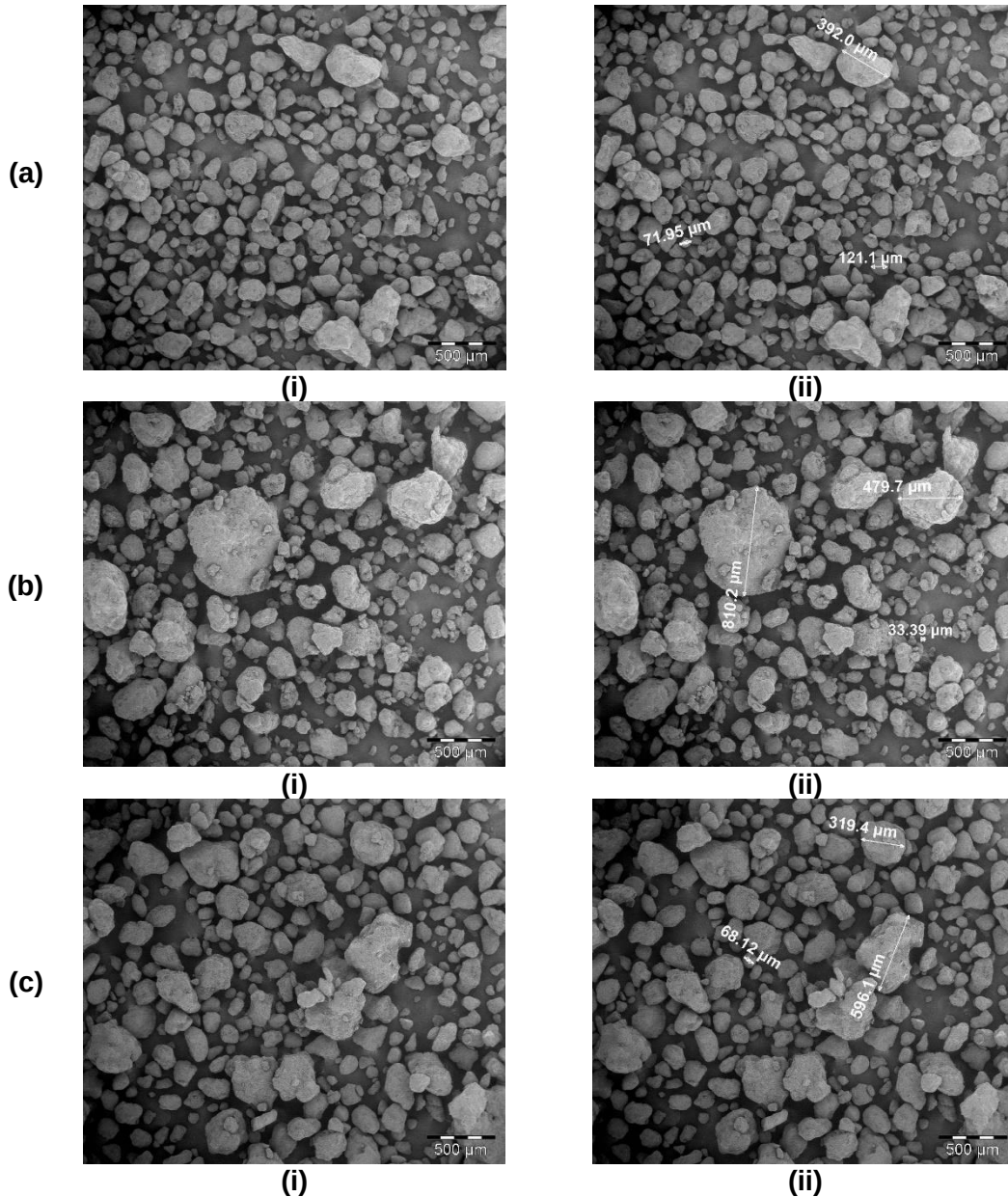


Figure 4.4: SEM micrographs of Health In Gum 01 base powder displayed in row **(a)**; Health In Gum 03 base powder in row **(b)**; and Health In Gum 04 base powder in row **(c)**. SEM micrographs of the random size measurements of individual particles of the different bases are displayed in column **(i)** and **(ii)** at a magnification of 95X

HIG 01 and 04 base powders seem to be more homogeneous in particle size (particle sizes of both powders are between 68.12 µm and 596.1 µm) as well as particle shape (more round compared to HIG powder 03). Particles from HIG 03 base powder, on the other hand, appear more coarse and uneven. It is therefore an indication that HIG 01 and 04 base powders will probably display enhanced flowability compared to the HIG 03 base powder. The SeDeM Expert Diagram System was, consequently, again utilised to differentiate more distinctively between these base powders.

4.3.3 Evaluation of *Sceletium tortuosum* extract powder as per SeDeM Expert Diagram System

The SeDeM Expert Diagram System was applied to a sample of 50 g *S. tortuosum* extract powder, which seemed to have a non-uniform particle size distribution when visually inspected. The 12 parameters tested were subsequently processed in order to obtain the radius values (r), which are displayed in Table 4.5. These values obtained were then utilised to construct a polygon or SeDeM diagram for the *S. tortuosum* extract powder. To ensure an accurate visual representation of the SeDeM diagram, the “r” value for hygroscopicity, which displays a negative value, was altered to the minimum value of 0 as seen in Figure 4.5.

Table 4.5: *Evaluation of Sceletium tortuosum extract powder as per SeDeM Expert Diagram System (Percentage relative standard deviation in parenthesis)*

Parameter	Limits (V)	Results		
		Experimental values (V)	Converted experimental value (r)	Factor and average values
Bulk Density (Da)	0–1 (g/ml)	0.79 (1.93)	7.90	Dimension 8.65
Tapped Density (Dc)	0–1 (g/ml)	0.94 (0.00)	9.40	
Inter-particle Porosity (Ie)	0–1.2	0.20 (12.79)	1.67	Compressibility 2.58
Carr's Index (IC)	0–50 (%)	15.94 (10.41)	3.19	
Cohesion Index (Icd)	0–200 (N)	58.00 (14.73)	2.90	
Hausner Ratio (IH)	3–1	1.19 (2.12)	6.03	Flowability 6.65
Angle Of Repose (α)	50–0 (°)	20.30 (4.97)	5.94	
Powder Flow (t'')	20–0 (g/s)	4.06 (2.84)	7.97	
Loss on Drying (%HR)	10–0 (%)	6.30 (9.01)	3.70	Lubricity/ Stability -3.61
Hygroscopicity (%H)	20–0 (%)	41.85 (16.00)	-6.28	
Particles < 50 μm (%Pf)	50–0 (%)	0.95 (0.35)	9.81	Lubricity/ Dosage 6.50
Homogeneity Index (Iθ)	0–2 $\times 10^{-2}$	0.67 (0.59)	3.19	

Overall, six of the twelve parameters acquired are higher than the minimum required value of 5, and are therefore in the acceptable range for direct compression according to the SeDeM Expert Diagram System. However, the other six parameters (Hygroscopicity >>> Inter-particle Porosity > Cohesion Index > Carr's Index = Homogeneity Index > Loss on Drying) have values below 5 and need to be improved in order to be able to prepare MCGs by means of direct compression. Additional homogeneity index reports can be seen in Annexure C. In Table 4.5, values printed in bold represent unacceptable values according to the limits set by the SeDeM Expert Diagram System. From the results attained, the lubricity/stability incidence depicted the lowest value of -3.61, followed by the compressibility incidence with a value of 2.58.

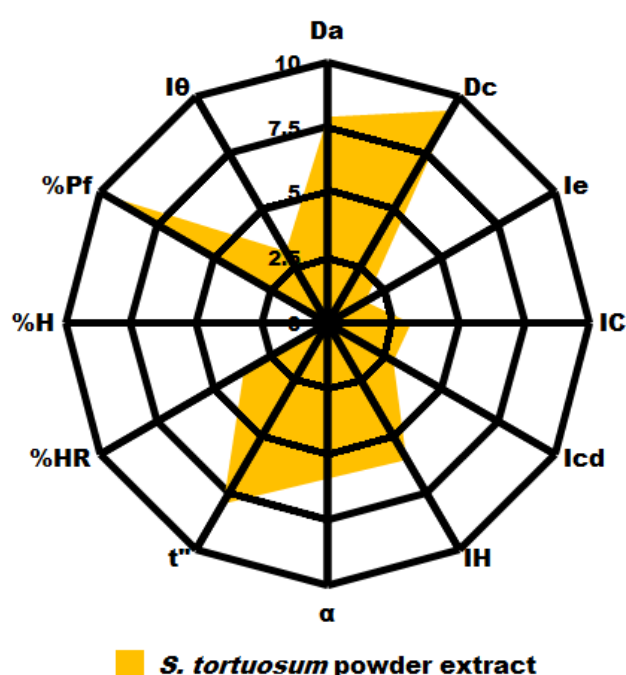


Figure 4.5: SeDeM diagram for *Sceletium tortuosum* extract powder

The experimental value of the inter-particle porosity (0.20) of the extract powder is within the correct limit of 0–1.2; however, the converted experimental value (1.67) is below the required value of 5. This is probably due to the natural packing formation of the uneven particle sizes of the extract powder (Figure 4.3) that allows for relatively more spaces between these particles than is preferred. The irregular particle sizes of the *S. tortuosum* extract powder were furthermore confirmed by means of the homogeneity index for this powder that was calculated from the data obtained from the particle size and particle size distribution analysis utilising the Malvern® Mastersizer-2000. The homogeneity index is used to analyse the uniformity of particles in a sample volume (Kataria *et. al.*, 2012). In Annexure D, the results indicating a lack in uniformity when considering particle size distribution of the extract

powder (especially %RSD values) can be viewed in more detail. Particle size analysis depicted a relatively large variation of between 45–1 200 μm . The average particle size was estimated as 428.76 μm (%RSD = 15.635; $n = 9$) and the span as 1.818 (%RSD = 3.623; $n = 9$). The converted experimental value for the homogeneity index of 3.19 is less than optimal according to the SeDeM Expert Diagram System, therefore confirming relative large variation in particle sizes.

As previously stated, the Carr's index and Hausner ratio of the extract powder indicated fair flowability, however, according to the SeDeM Expert Diagram System, when the Carr's index value was converted to the experimental value of 3.19; it was clearly below the preferred limit of 5. The Hausner ratio, angle of repose, and powder flow parameters on the other hand, did adhere to the SeDeM Expert Diagram System limits.

Considering the cohesion index, it was undeniable that the *S. tortuosum* extract powder depicted unacceptable compressibility characteristics as tablets compressed from the extract powder illustrated a relatively low crushing strength of only 58 N at the highest possible compression force. The converted experimental value for this parameter is only 2.90 indicating poor compressibility features.

Experimental values of 6.30% and 41.85% for the loss on drying and the hygroscopicity parameters, correspondingly, were calculated; and the converted values for these parameters were estimated as 3.70 and -6.28, respectively. These converted values are clearly below the acceptable value of 5 and indicated that the *S. tortuosum* extract powder is highly hygroscopic. This observation is supported by the fact that although the extract powder was stored in an air-tight container and sealed with Parafilm®, it still managed to absorb water or moisture from the surrounding environment; and the extract powder depicted a sticky appearance when the atmospheric humidity was relatively high (during overcast and rainy days). Care should thus be taken when storing the *S. tortuosum* extract powder for long periods of time; and the quality of the extract powder should be monitored in order to detect if any hydrolysis occurred.

The lubricity/stability index, represented by the loss on drying (%HR) and hygroscopicity index (%H) parameters, denoted a good indication of future stability as well as the ability to lubricate the tablet press. Due to the fact that the extract was very sticky when handled, it made sense that the stability will be affected as the loss on drying and hygroscopicity parameters reached experimental values of only 3.70% and -6.28%, respectively. The compressibility index on the other hand is signified by the inter-particle porosity, Carr's index, and cohesion index parameters. Failing to comply with the standards of the compressibility

index results suggested an inability of the extract powder to be directly compressed at the suggested hardness for tablets as stated by the BP (2016), which is between 80-120 N (Bhavsar *et al.*, 2015; Suñé-Negre *et al.*, 2013).

The PI, PPI and GCI were subsequently calculated and results showed that the *S. tortuosum* extract powder is not suitable for direct compression as the PPI and GCI index values were not within the acceptable range of 5-10, with values of 4.49 and 4.64, respectively (Table 4.6). The PI value for *S. tortuosum* is considered satisfactory as a value of 0.5 or higher is acceptable as previously discussed. Furthermore, there are six parameters of this powder that have room for improvement.

Table 4.6: *Index values and acceptability for Sceletium tortuosum extract powder as per SeDeM Expert Diagram System*

Index	Value	Acceptability
Parameter Index (PI)	0.50	Acceptable
Parametric profile Index (PPI)	4.49	Not acceptable
Good Compression Index (GCI)	4.64	Not acceptable

4.3.4 Evaluation of Health In Gum base powders as per SeDeM Expert Diagram System

The SeDeM Expert Diagram System was applied to three MCG, HIG base powders, numbered 01, 03 and 04, prior to addition of any excipients. These bases powders all have an off-white colour; and when visually inspected also seemed to have a non-uniform particle size distribution. The 12 parameters were evaluated for each HIG base powder and the radius values were calculated from the results. Results obtained for the individual parameters and factors for each of the HIG base powders are presented in Tables 4.7 and 4.8, respectively. Again, the values printed in bold in Table 4.7 represent unacceptable values according to the limits set by the SeDeM Expert Diagram System. A polygon for all three of the HIG base powders was subsequently constructed and can be observed in Figure 4.6.

Table 4.7: *Experimental values (V) and converted experimental values (r) for Health In Gum base powders as per SeDeM Expert Diagram System (Percentage relative standard deviation in parenthesis)*

Parameter	Health In Gum Bases (HIG)					
	HIG 01		HIG 03		HIG 04	
	"V"	"r"	"V"	"r"	"V"	"r"
Bulk Density (Da)	0.68 (0.84)	6.80	0.68 (0.00)	6.80	0.65 (0.89)	6.50
Tapped Density (Dc)	0.90 (12.78)	9.00	0.90 (1.29)	9.00	0.86 (0.00)	8.60
Inter-particle Porosity (Ie)	0.36 (3.24)	3.00	0.36 (3.23)	3.00	0.38 (3.62)	3.17
Carr's Index (IC)	24.35 (3.48)	4.87	24.16 (3.99)	4.83	24.81 (2.70)	4.96
Cohesion Index (Icd)	283 (3.72)	14.15	292 (1.57)	14.67	261 (10.29)	13.05
Hausner Ratio (IH)	1.32 (1.15)	5.60	1.32 (1.31)	5.60	1.33 (0.87)	5.57
Angle Of Repose (α)	19.12 (4.10)	6.14	19.80 (2.55)	6.04	20.00 (2.92)	6.00
Powder Flow (t'')	9.20 (1.25)	5.40	9.06 (1.27)	5.47	8.90 (1.29)	5.55
Loss on Drying (%HR)	0.95 (0.00)	9.05	3.10 (17.27)	6.90	1.89 (0.00)	8.11
Hygroscopicity (%H)	18.02 (7.44)	0.99	5.05 (27.09)	7.48	15.67 (9.75)	2.17
Particles < 50 μm (%Pf)	0.13 (5.66)	9.98	0.15 (0.00)	9.97	0.21 (0.00)	9.96
Homogeneity Index (Iθ)	0.87 (1.87)	4.36	0.96 (4.17)	4.79	0.97 (0.44)	4.85

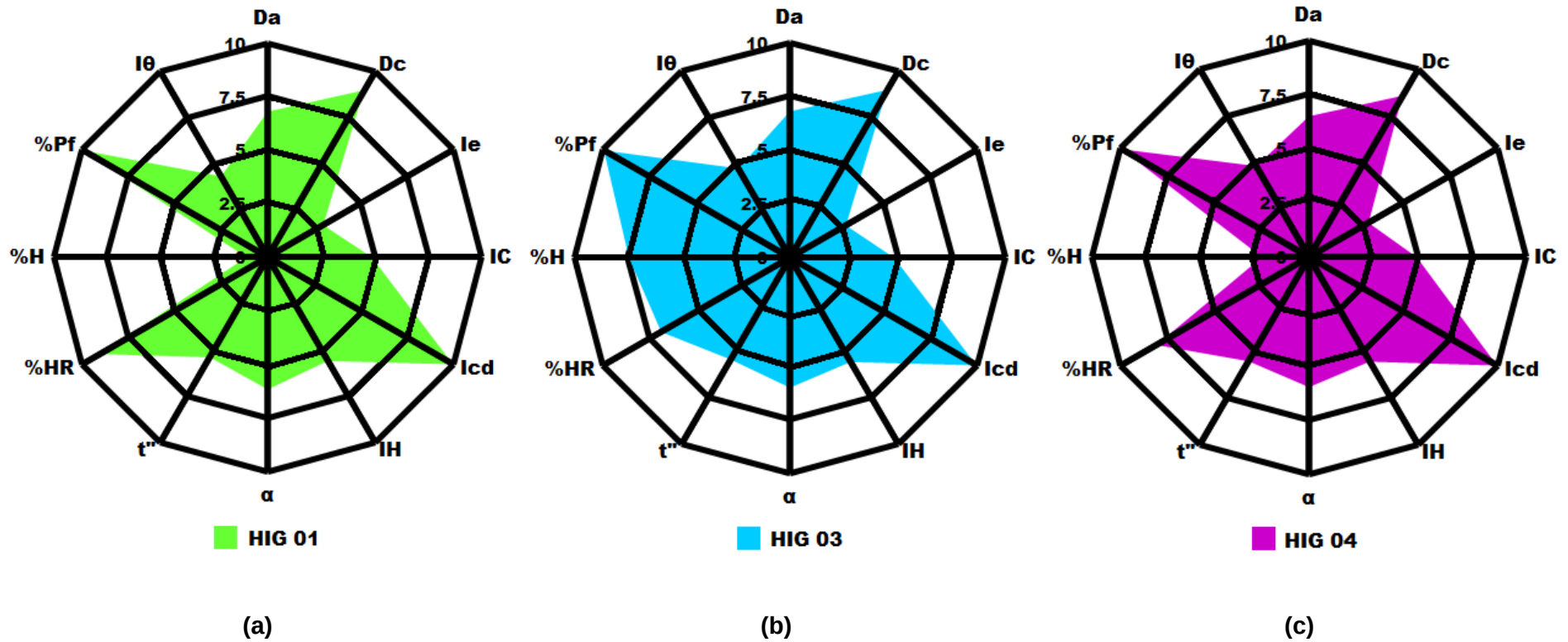


Figure 4.6: SeDeM diagrams for (a) Health In Gum 01 base powder; (b) Health In Gum 03 base powder; and (c) Health In Gum 04 base powder

As evident from the results achieved, the HIG base powders did not show considerable differences between each other for most of the parameters tested. Relative differences could, however, be detected between the loss on drying and hygroscopicity indexes. Moreover, the SeDeM Expert Diagram System analysis revealed that none of the three HIG base powders depicted acceptable values for the inter-particle porosity; Carr's index; or homogeneity index parameters. HIG 01 and HIG 04 base powders also did not display acceptable values for the hygroscopicity parameter. The following rank order for the overall unacceptable values could be established: Carr's index > homogeneity index > inter-particle porosity > hygroscopicity. In general it seems as though the HIG 03 base powder are less hygroscopic and will probably depict increased flowability and be more directly compressible compared to the other two base powders. However, considering the angle of repose and powder flow parameters of all three HIG bases, no clear difference in flowability could be detected between these three base powders. Results obtained for all of the HIG base powders are nonetheless within the acceptable limits (> 5) for these parameters. The polygon profiles of the HIG base powders appear relatively similar, especially for the HIG 01 and HIG 04 base powders. The HIG 03 base powder only differs more profoundly at the hygroscopicity point and to a lesser extent at the loss on drying point, showing a relatively improved or higher value for hygroscopicity and a slightly lower value for loss on drying.

From Table 4.7 it is observed that the experimental values of the inter-particle porosity parameter for all of the base powders are within the correct limit of 0–1.2; however, none of the converted experimental values fall within the acceptable range of higher than a value of 5. As with the *S. tortuosum* extract powder, these results are possibly due to the natural packing formation of uneven sized particles of the three base powders. The variation in particle sizes was not clearly observable in Figure 4.4, nonetheless, analysis of the particle sizes and size distributions of the three base powders (Annexure D) revealed a relatively wide span for each of the base powders, namely 1.485 (%RSD = 10.688; n = 12), 1.762 (%RSD = 14.133; n = 12), and 1.557 (%RSD = 14.062; n = 12) for HIG 01, 03 and 04 base powders, respectively. Variation between samples was furthermore relatively large considering the %RSD in brackets, indicating non-uniform particle size distributions. The average particle sizes were estimated as 371.100 µm (%RSD = 7.875; n = 12) for HIG 01 base powder; 360.955 µm (%RSD = 3.708; n = 12) for HIG 03 base powder; and 377.255 µm (%RSD = 1.699; n = 12) for HIG 04 base powder. Thus, the average particle sizes of the different base powders did not vary considerably, demonstrating that the base powders depicted relatively similar average particle

sizes. Particle size variation within each base powder was, however, confirmed by the converted experimental values of the homogeneity index for the different base powders, which were calculated as 4.36, 4.79, and 4.85 for HIG 01, 03 and 04 base powders, respectively.

Considering the Carr's index, Hausner ratio and cohesion index values for all three HIG base powders, only the Carr's index values did not adhere to the acceptable limits set by the SeDeM Expert Diagram System, rendering these base powders more compressible compared to the *S. tortuosum* extract powder. Experimental values of the Carr's index parameter for HIG 01, 03 and 04 base powders were between 24.16% and 24.81%, which is classified as "passable" by the flowability scale prerequisites (Table 4.3). The converted experimental values were calculated as 4.87, 4.83 and 4.96 respectively, which are relatively close to the set limit of 5. Upon compression of the HIG base powders, maximum crushing strength values of higher than 250 N were obtained for all of the base powders.

The results also produced exceptional converted experimental values for the cohesion index parameter, which are higher than the set standard by Suñé-Negre *et al.* (2014). In order to produce a visual representation within the SeDeM diagram, these values are converted to the maximum value of 10 (Figure 4.6). Exceeding the cohesion index's maximum limit is not a concern as it is an indication of good compressibility of all of the HIG base powders.

As stated previously, the HIG base powders did not depict considerable differences between each other in terms of the tested parameters, except between the loss on drying and hygroscopicity parameters. When evaluating and interpreting the properties of powders, the composition of the mixture is an important aspect to consider. The composition of CAFOSA®'s HIG base powders is tabulated in Table 4.8.

Studying the different compositions, it is clear that the HIG 03 base contains no plasticiser and it comprises a significant percentage isomalt, which is a polyol derived from sucrose. Plasticisers are known to increase the flexibility, workability or the ability to stretch of the rubber agent in the base of chewing gum. It is also incorporated to prevent the chewing gum from crumbling during prolonged storage (Lawton, 2004). Research showed that plasticisers are mostly resinous materials; and examples include waxes, hydrogenated rosin esters, paracoumarones (Louis, 1941), glyceryltriacetates (Triacetin), PEG-600, oleic acid and tributyl citrate (Mehta & Trivedi, 2012). A study by Lawton (2004) concluded that specific types of plasticisers pose different effects on the water absorption of MCGs at different relative humidity conditions where changes

in ultimate tensile strength were used as guidance. Chewing gum containing hygroscopic plasticisers exhibited more pronounced decreases in ultimate tensile strength (Lawton, 2004). Therefore, it can be assumed that the type and amount of plasticiser present in a MCG will affect the loss on drying and hygroscopicity to some extent; and will thus also affect the tensile strength results and cohesion index (Bergo *et al.*, 2013). Unfortunately, the manufacturers of CAFOSA®'s HIG bases did not declare the type of plasticiser incorporated.

Table 4.8: *Composition of Health In Gum 01, 03 and 04 base powders*

Ingredient		Concentration present in HIG 01 base (%)	Concentration present in HIG 03 base (%)	Concentration present in HIG 04 base (%)
Gum base		22-26	33-37	28-32
Plasticiser		Max. 2		Max. 2
Anticaking agent (E-551)		Max. 2	Max. 2	Max. 2
Antioxidant (BHT)		Max. 260 ppm	Max. 370 ppm	Max. 320 ppm
Isomalt			Up to 100	
Sweetener	Xylitol	8-12		8-12
	Sorbitol	Up to 100	5-9	
	Mannitol			8-12

Both the HIG 01 and 04 base powders displayed relatively higher converted experimental values for the loss on drying parameter as both these bases contain a plasticiser that may increase the moisture content, but the moisture in the MCG is probably present as physically absorbed water within the particles or as strongly bound chemisorbed water. Upon drying, the moisture will thus not easily be removed from the MCG. On the other hand, the moisture existent in the HIG 03 base powder is possibly present as absorbed monolayers or multi-layers on the particle surfaces; or as condensed water on the surface that can easily dissipate upon drying (Viljoen *et al.*, 2013), thus rendering a lower converted value for loss on drying. Furthermore, the addition of a plasticiser will influence the hygroscopicity parameter negatively as the presence of this excipient will increase the moisture content and “stickiness” of the powder. The HIG 03 base powder depicted an experimental value of 5.05 (%RSD = 27.09) and an acceptable converted value of 7.48 whereas the HIG 01 and 04 base powders displayed unacceptable experimental values of 18.02 (%RSD = 7.44) and 15.67 (%RSD = 9.75), respectively. This resulted in undesirable converted values of 0.99 and 2.17, correspondingly.

However, due to the fact that MCGs are mechanically “broken up” by means of the chewing process and do not necessitate disintegration in order to release the active ingredient, the unacceptable hygroscopicity index values for the HIG 01 and 04 base powders are not expected to be detrimental.

The HIG 03 base powder contains a high isomalt concentration, which is a non-carcinogenic polyol derived from sucrose that is utilised as a taste masking agent. Isomalt is manufactured in a two-step production process rendering it an approximate equimolecular mixture which is mechanically and chemically stable. Based on its stable chemical structure, it does not react with other compounds, for example amino acids, to form Maillard reaction products. It furthermore has very low hygroscopicity, providing optimal protection, even for moisture sensitive active ingredients. Isomalt has shown to have excellent flowability and powder characteristics. Agglomerated isomalt particles depict rough surfaces and cavities within where the active ingredient can be incorporated in high concentrations without influencing flowability or increasing segregation (Quodbach *et al.*, 2012). Thus, the incorporation of isomalt into HIG 03 base powder may have contributed to the lowered hygroscopicity values and increased flowability of this base powder.

Furthermore, the artificial sweetener that is included in the HIG bases differs. Xylitol is present in the HIG 01 base powder; sorbitol is present in the HIG 01, 03 and 04 base powders; and mannitol is present in the HIG 04 base powder. Xylitol differs from sorbitol and mannitol on their chemical compositions. In a study done on the physical properties of whey protein isolate films with xylitol amongst others, it was found that increased levels of xylitol had no effect on permeability, moisture content or glass transition; but a decreased percentage of elongation, tensile strength and elastic modulus was noted (Shaw *et al.*, 2002). Sorbitol and mannitol are six-carbon, straight-chain polyhydric alcohols and possess six hydroxyl groups. The differences in these two sweeteners are in the location of a single hydroxyl group on the second carbon atom and, however, this difference seems insignificant, the dissimilarity may have a powerful influence. The most outstanding difference between these isomers exists in their hygroscopicity (Le & Mulderrig, 2001). Sorbitol is hygroscopic (Le & Mulderrig, 2001, Jivraj *et al.*, 2000) and tends to increase the moisture content thus causing a decrease in tensile strength (Shaw *et al.*, 2002). Sorbitol is more likely to maintain equilibrium in the surrounding environment as it provides improved moisture control. Protection of the product containing sorbitol is due to the slower rate of change in moisture content, maintaining the quality of the product and extending shelf-life (Le & Mulderrig, 2001). Limitations of sorbitol are that its hygroscopic nature can

accelerate the degradation of moisture sensitive compounds and hardening of the tablets over time as the sorbitol recrystallizes (Jivraj *et al.*, 2000). Although sorbitol tends to decrease tensile strength when incorporated into a formulation, the HIG 03 base presented the highest tensile strength with a cohesion index parameter value of 292 N. This can be attributed to the fact that the HIG 03 base contains no plasticiser, which is known to cause decreases in tensile strength as the concentration of the plasticiser is increased (Bergo *et al.*, 2013).

Mannitol is a non-hygroscopic molecule (Le & Mulderrig, 2001), which tends to form granules (Bethea, 2015). Because of mannitol's inertness and stability against moisture, it is mostly used as a pharmaceutical and nutritional tablet excipient (Le & Mulderrig, 2001). It can also be used as a sweetening agent in formulations containing moisture sensitive components (Wade & Weller, 1994). Its cooling effect in the mouth makes it a useful excipient for lozenges, chewable tablets (Wade & Weller, 1994) and thus making it also fit for MCGs.

The sweeteners present in HIG 01 and 04 base powders are non-hygroscopic which indicates an underlying reason for the high hygroscopicity parameter values (Le & Mulderrig, 2001; Shaw *et al.*, 2002). Another possible reason for the high moisture content of the HIG 03 base powder is the presence of sorbitol which has hygroscopic properties and tends to increase the moisture content in a formulation (Shaw *et al.*, 2002).

Contradictory, sorbitol that is included in the HIG 03 base powder will increase the moisture content of this base which may lead to a decrease in tensile strength. The HIG 03 base powder has a cohesion index parameter value of 292 N which is higher than the values obtained for the HIG 01 and 04 base powders (283 N and 261 N, respectively). Since the HIG 03 base powder contains no plasticiser, an increase in ultimate tensile strength was expected as an increased concentration of a hygroscopic plasticiser in the base predicts a decrease in ultimate tensile strength and vice versa (Bergo *et al.*, 2013).

In summary of the information regarding the sweeteners; when xylitol is incorporated in a formulation, it will most likely decrease the tensile strength, which will affect the compressibility of the mixture. Sorbitol will cause a decrease in tensile strength and increase moisture content that will affect the hygroscopicity and loss on drying parameters; which influence the future stability and lubricity of the product. Mannitol is probably the most stable sweetener molecule to incorporate into a formulation as it is a non-hygroscopic molecule that will increase the

hygroscopicity and loss on drying parameters, causing increased stability and lubricity over time.

From Table 4.9 it is furthermore clear that all of the HIG base powders adhered to the set limits for all of the SeDeM factor values. Thus, according to the SeDeM Expert Diagram System, these bases can be directly compressed into tablets that will adhere to the standards set by the Pharmacopoeia (BP, 2016; EP, 2016). Overall, the dimension and lubricity/dosage factors seemed the most ideal factors of the HIG base powders having the highest general values. Flowability of the bases depicted the lowest values, which may indicate that mass variation may be an aspect that needs to be monitored for all three bases.

Table 4.9: *Factors and values for Health In Gum base powders as obtained by means of the SeDeM Expert Diagram System*

Factors	HIG 01	HIG 03	HIG 04
Dimension	7.90	7.90	7.55
Compressibility	7.34	7.48	6.04
Flowability	5.73	5.70	5.71
Lubricity/Stability	7.17	7.19	5.14
Lubricity/Dosage	9.99	7.38	7.40

The PI, PPI and GCI were consequently calculated and the results are displayed in Table 4.10. All of these values are within the set limits of the SeDeM Expert Diagram System. Generally, results obtained for these index values showed that the HIG 03 base will perform best when directly compressed, closely followed by the HIG 01 base. The System predicted that no significant modifications to the content of the three base powders are necessary as their respective GCI values are > 5.

Table 4.10: *Index values of Health In Gum 01, 03 and 04 base powders*

Index	HIG 01	HIG 03	HIG 04
PI	0.67	0.75	0.67
PPI	6.61	7.03	6.29
GCI	6.30	6.70	5.98

4.3.5 Comparing SeDeM diagrams of the *Sceletium tortuosum* extract powder and the Health In Gum base powders

As explained previously, the SeDeM Expert Diagram System facilitates the characterisation of powdered substances and predicts any required amendments in order to become directly compressible. Mixing of the active ingredient (in this study it is *S. tortuosum* extract) with an excipient to obtain a satisfactory compressibility parameter (> 5) will increase the deficient compressibility parameters (< 5) correcting the deficiency of the mixture according to the SeDeM Expert Diagram System (Sauri *et al.*, 2014; Suñé-Negre *et al.*, 2008).

As discussed in Section 4.3.3, the *S. tortuosum* extract powder depicted lower average values than required for the compressibility (2.58) and lubricity/dosage (-3.61) incidence factors. Insufficient experimental values (< 5) were obtained for the inter-particle porosity (1.67), Carr's index (3.19%), the cohesion index (2.90 N), loss on drying (3.70%), hygroscopicity (-6.28%) and the homogeneity index (3.19). Therefore, the *S. tortuosum* extract are considered not suitable for direct compression. The HIG bases depicted experimental values that were deemed acceptable (> 5) especially in areas where improvement is required for the *S. tortuosum* extract powder, and consequently, the SeDeM Expert Diagram System predicts that all three the HIG bases will increase the values of the *S. tortuosum* extract producing a powder mixture that will increase the deficit values of *S. tortuosum* extract making it more suitable for tableting by means of direct compression.

Figure 4.7 shows superimposed SeDeM diagrams of the *S. tortuosum* extract and the HIG base powders. By superposing the SeDeM diagrams of the *S. tortuosum* extract with each of the HIG bases, the degree of similarity or differences between the two powders can be used to determine the suitability for direct compression. In column (i) of Figure 4.7, the polygon of *S. tortuosum* extract powder is superimposed on the polygon of the specific HIG base powder, whereas in column (ii), the polygon of the HIG base powder is superimposed on the polygon of the *S. tortuosum* extract powder.

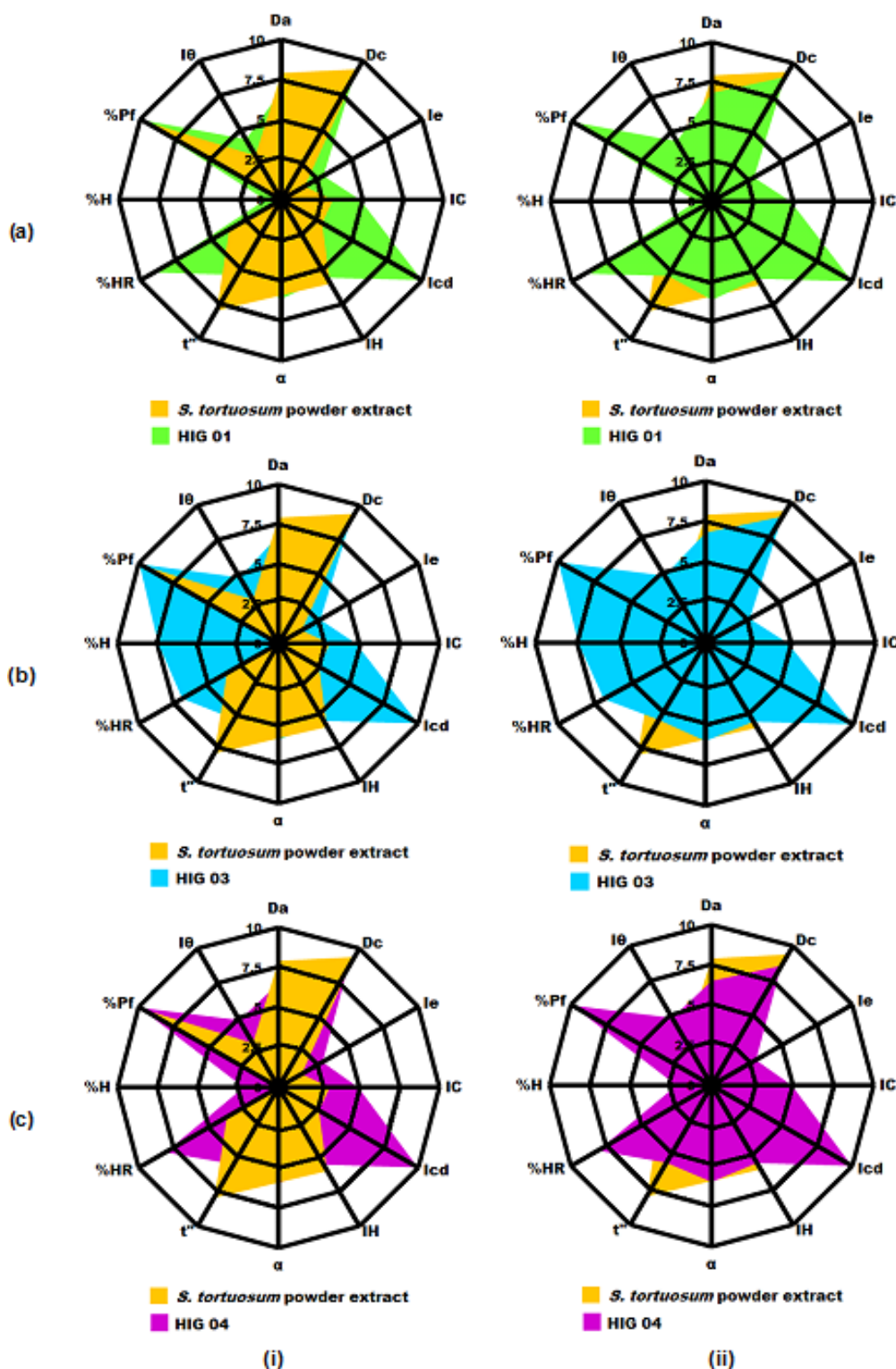


Figure 4.7: Superimposed SeDeM diagrams of *Sceletium tortuosum* extract powder (yellow) with **(a)** Health In Gum 01 (green); **(b)** Health In Gum 03 (blue); and **(c)** Health In Gum 04 (purple). In column **(i)** the polygon of *Sceletium tortuosum* extract powder is overlaid on the polygon of the specific Health In Gum base powder, whereas in column **(ii)**, the polygon of the Health In Gum base powder is overlaid on the polygon of the *Sceletium tortuosum* extract powder

It is clear from Figure 4.7 that some of the parameters of the *S. tortuosum* extract will be difficult to correct by means of adding one of the selected bases. For example, it is predicted by the SeDeM Expert Diagram System that the hygroscopicity parameter will not be increased by adding HIG 01 to the *S. tortuosum* extract, whereas adding HIG 03 will probably drastically increase this parameter. As stated beforehand, the compressibility and lubricity/stability (which consists of the loss on drying and hygroscopicity parameters) factors for the *S. tortuosum* extract depicted detrimental values rendering the extract not directly compressible and indicating that the extract is more hygroscopic than optimal, contains more moisture and might therefore be more easily degraded. However, the lubricity/stability factor can be controlled by other means than adding excipients. Hygroscopicity for example can be controlled by storing the extract in air tight containers and ensuring that production of MCGs is done under controlled conditions (i.e. temperature and humidity). Moreover, MCGs do not require disintegration characteristics as it is mechanically broken down in the oral cavity, thus, the fact that the extract may become slightly “sticky”, is not a major concern during release from the dosage form. Flowability may however be affected by the higher moisture concentration and the addition of a lubricant, for example magnesium stearate, should be considered.

Compressibility, on the other hand, can be adjusted as mentioned through utilising the SeDeM Expert Diagram System by calculating the type and minimum amount of base required for the *S. tortuosum* extract to be subjected to direct compression equipment. Thus, in order to select the most effective HIG base, as well as the lowest possible concentration required to correct the deficiencies, and more specific, the compressibility, the mathematical equation of the SeDeM Expert Diagram System (Equation 3.6) that considers a minimum radius value (R) of > 5 , was applied (Sauri *et al.*, 2014; Suñé-Negre *et al.*, 2013). Table 4.11 lists the results obtained.

Table 4.11: Predicted minimum Health In Gum base powder concentration required to be mixed with the *Sceletium tortuosum* extract to achieve direct compression

HIG base	HIG 01	HIG 03	HIG 04
RE	7.34	7.48	6.04
RP	2.58	2.58	2.58
R	5.00	5.00	5.00
% HIG base	50.84	49.39	69.94

In summary, all of the HIG bases are able to increase the compressibility factor to an acceptable value when incorporated in a specific concentration. No significant difference in the base concentration needed in order to obtain an acceptable directly compressible MCG formulation could be found between HIG 01 and HIG 03 base powders. According to the SeDeM Expert Diagram System, a minimum concentration of approximately 50% of HIG 01 and HIG 03 base powders is needed, rendering these two bases the most suitable to correct the deficit (compressibility) of the *S. tortuosum* extract powder when comparing the three HIG bases. It is furthermore predicted by the SeDeM Expert Diagram System that almost 70% of HIG 04 base powder is required. Thus, according to these results, it will not be possible to include a relatively high concentration (>30%) of *S. tortuosum* extract powder into a MCG consisting of the HIG 04 base powder. Moreover, Figure 4.7 shows the prediction of the SeDeM Expert Diagram System more clearly. Here it is evident that the compressibility incidence will be increased regardless of the type of HIG base powder utilised. No precise distinction could be observed from this figure for the HIG bases. However, the HIG 04 base powder depicted a slightly lower increase in the cohesion index parameter compared to the other bases. Interestingly, all three bases were able to slightly increase the homogeneity index to almost the same extent, whereas more pronounced differences between the influence of the three HIG bases on the loss on drying and hygroscopicity parameters can be seen. According to the SeDeM Expert Diagram System predictions, the HIG 01 base powder will probably have a more pronounced effect on the loss on drying parameter, followed by the HIG 04 base powder. However, hygroscopicity will probably not be improved utilising the HIG 01 base powder, but the HIG 03 base powder will almost certainly increase the hygroscopicity parameter more notably.

4.3.6 Preparation and characterisation of medicated chewing gum powder mixtures

As described in Chapter 3, the composition of the different MCG formulations containing *S. tortuosum* extract powder was determined by means of a full factorial design. From the powder characterisation results as well as the SeDeM Expert Diagram System predictions obtained, it was contemplated to include the amount of magnesium stearate (0 – 0.5%) and the type of HIG base powders (HIG 01, 03 or 04) as variables into the factorial design (Table 3.3).

Throughout the powder characterising and SeDeM parameter studies, it was observed that sticking occurred during compaction of the MCG units that contained no lubricant in the formulation. The MCG units significantly adhered to the tableting punches and were only able

to be compressed manually as most MCGs needed to be physically removed. The SeDeM Expert Diagram System furthermore proposed the addition of a lubrication mixture (talc, magnesium stearate and silicon dioxide) if the powder mixture causes abrasiveness. The addition of magnesium stearate in these formulations would also decrease the stickiness of the MCG units during the direct compression process. This is because magnesium stearate forms a hydrophobic layer around the powder particles and thereby decreases the stickiness (Uzunović & Vranić, 2007). It was therefore decided to include magnesium stearate concentration as a variable in the factorial design.

According to the CP values calculated in the previous section, the SeDeM Expert Diagram System proposed that a MCG comprising *S. tortuosum* extract powder should consist of a minimum of approximately 50% of either HIG 01 or 03 base powder; or 70% of HIG 04 base powder, in order to obtain a workable, directly compressible formulation. It was therefore decided to formulate MCGs weighing 1 000 mg where each MCG contains 25 mg *S. tortuosum* extract (2.5% w/w) in order to be able to compare the data obtained from the factorial design. Furthermore, a commercial product, Elev8™, comprising 25 mg *S. tortuosum* extract, is already available on the market as oral, film coated tablets.

The same abbreviations for the different formulations are used as described in Chapter 3, Section 3.5.1 (Factorial design). The best formulations were identified based on the following responses: mass variation (lower is favoured), tensile strength (higher is preferred) and friability (lower is favoured). Important to note is that disintegration was considered as an unimportant aspect because MCGs are manually broken down during the chewing process. Dissolution studies were only carried out on three formulations that were considered most ideal according to their physical characteristics.

4.3.6.1 Evaluation of the flow properties of medicated chewing gum powder mixtures

Table 4.12 exhibits the different MCG powder formulations that were directly compressed, which were based on the factorial design. The *S. tortuosum* extract powder concentration in a single MCG was based on the yield (12.75%) and the amount incorporated into a commercial product (Elev8™). Elev8™ film coated tablets contain 25 mg Zembrin®, which is the patented name for the *S. tortuosum* extract that is equivalent to 50 mg dry plant material (Murbach *et al.*, 2014). Therefore, according to the yield of the water extract obtained in this study, one MCG unit should contain 6.38 mg *S. tortuosum* extract powder, which is equivalent to 50 mg dry plant

material. The equivalent of the dry plant material was used since dry plant material is traditionally chewed. In addition, a lower dose of the extract is required in the MCG compared to that of the oral tablet due to the absence of the first-pass effect and absence of luminal degradation enzymes.

Table 4.12: *Medicated chewing gum formulations*

Ingredient	Concentration (%)
<i>S. tortuosum</i> extract	0.638
HIG 01, 03 or 04 base powder	Ad 100
Magnesium stearate	0 or 0.5
D-sorbitol	20
Cherry flavourant	1.5

The flow properties of the different MCG powder mixtures are summarised in Table 4.13. From these results it is clear that all of the powder mixtures depicted relatively enhanced flowability compared to the *S. tortuosum* extract powder and individual HIG base powders alone. Overall, only the flow rate of the mixtures is comparatively slower. A functionality of magnesium stearate is to lower the inter-particle forces between the particles in the powder acting as a glidant and anti-adherent (Carter, 2001). This effect can be seen in the overall flowability results as the MCG powder mixtures containing 0.5% w/w magnesium stearate attained higher tapped density values compared to the formulations containing no magnesium stearate. Interestingly, MCG powder mixtures containing no magnesium stearate depicted excellent Hausner ratios, Carr's index values, as well as angle of repose values; whereas MCG powder mixtures comprising magnesium stearate portrayed excellent angle of repose values, good to excellent Carr's index values, and only good Hausner ratio values. No significant difference in the flow rate could be established between MCG powder mixtures containing magnesium stearate and those containing none. The addition of magnesium stearate into the MCG powder mixtures therefore affected the flowability, however, its main role should be to reduce adherence of the particles to the tablet press. Furthermore, no distinctive conclusion concerning flowability could be drawn between the MCG powder mixtures containing different HIG base powders. It seems as though powder mixtures that consisted of HIG 04 base powder might have slightly increased flowability compared to the powder mixtures containing the other two HIG base powders (i.e. HIG01 and 03 base powders).

Table 4.13: *Flow properties of the different medicated chewing gum powder mixtures (Percentage relative standard deviation in parenthesis)*

Test	MCG powder mixtures					
	S0HIG01	S0HIG03	S0HIG04	S0.5HIG01	S0.5HIG03	S0.5HIG04
Bulk density (g/cm³)	0.74 (1.35)	0.73 (1.57)	0.68 (0.83)	0.77 (0.75)	0.74 (2.07)	0.73 (1.57)
Tapped density (g/cm³)	0.78 (0.74)	0.78 (0.74)	0.70 (0.83)	0.86 (0.00)	0.82 (0.71)	0.81 (0)
Hausner ratio	1.05 (2.54)	1.07 (1.43)	1.02 (1.13)	1.12 (0.51)	1.11 (1.80)	1.10 (2.09)
Carr's Index (%)	4.71 (41.14)	6.38 (20.07)	2.39 (34.35)	10.86 (6.17)	9.80 (13.11)	9.46 (15.07)
Flow rate (g/s)	3.40 (0.00)	3.60 (55.56)	3.80 (0.00)	3.00 (3.77)	3.46 (3.33)	3.40 (0.00)
Angle of repose (α)	12.12 (0.64)	14.07 (4.92)	14.57 (3.67)	15.99 (3.83)	15.11 (3.54)	15.10 (6.12)

4.3.6.2 Evaluation of medicated chewing gum powder mixtures as per SeDeM Expert Diagram System

The SeDeM Expert Diagram System was applied to six MCG formulations (powder mixtures) to evaluate their suitability for direct compression. Twelve parameters were tested and the results were used to calculate the radius values. Figures 4.8 and 4.9 show the superimposed SeDeM diagrams of the *S. tortuosum* extract powder and the HIG base powders. Results obtained for the parameters and factors for the MCG formulations are presented in Tables 4.14 and 4.15, respectively. The values in bold represent unacceptable values obtained according to the SeDeM Expert Diagram System. SeDeM diagrams were subsequently constructed.

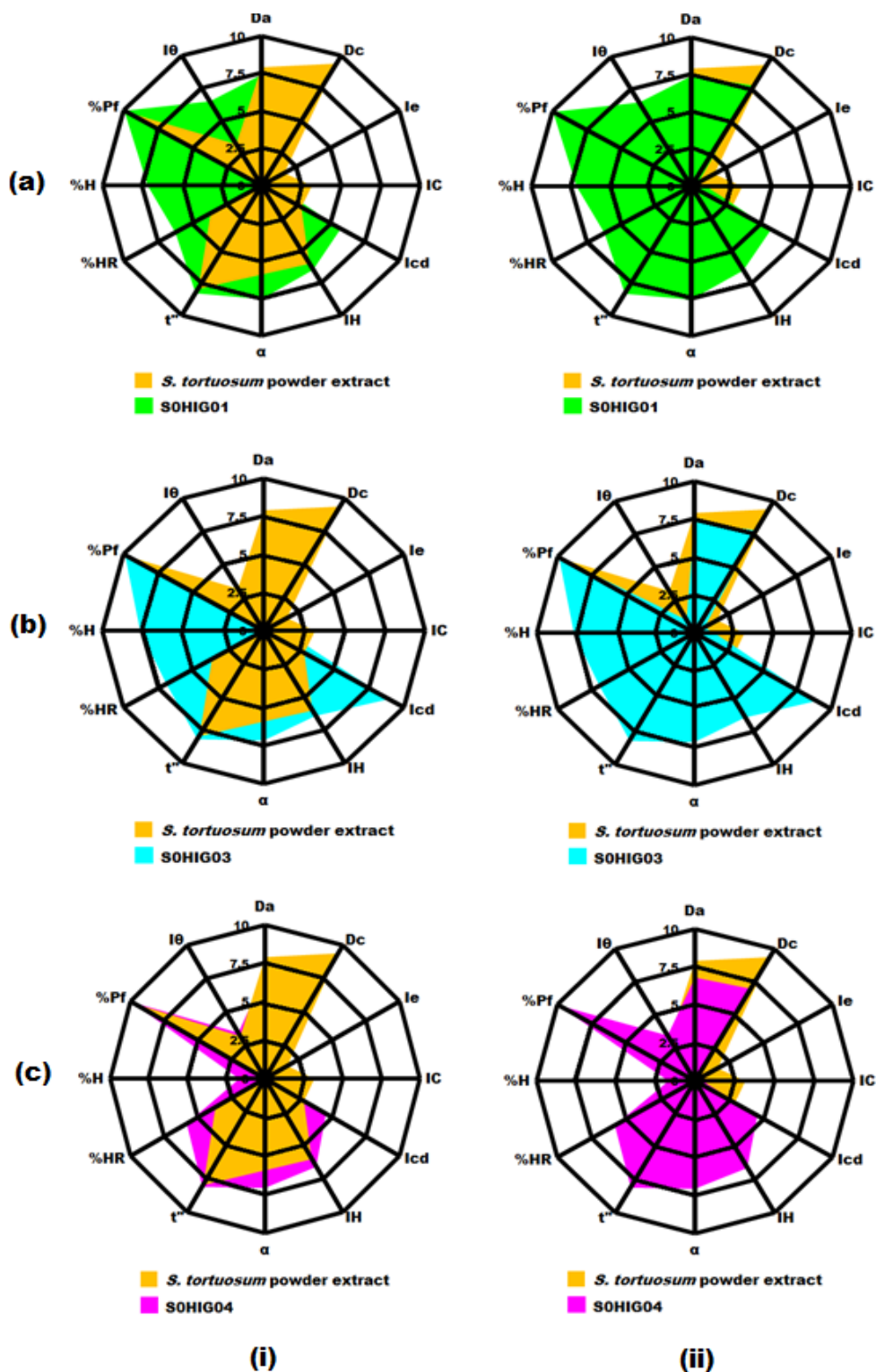


Figure 4.8: Superimposed SeDeM diagrams of *Sceletium tortuosum* extract powder (yellow) on formulations: **(a)** S0HIG 01 (green); **(b)** S0HIG 03 (blue); and **(c)** S0HIG 04 (purple). In column **(i)** the polygon of *Sceletium tortuosum* extract powder is overlaid on the polygon of the specific formulation, whereas in column **(ii)**, the polygon of the formulation is overlaid on the polygon of the *Sceletium tortuosum* extract powder

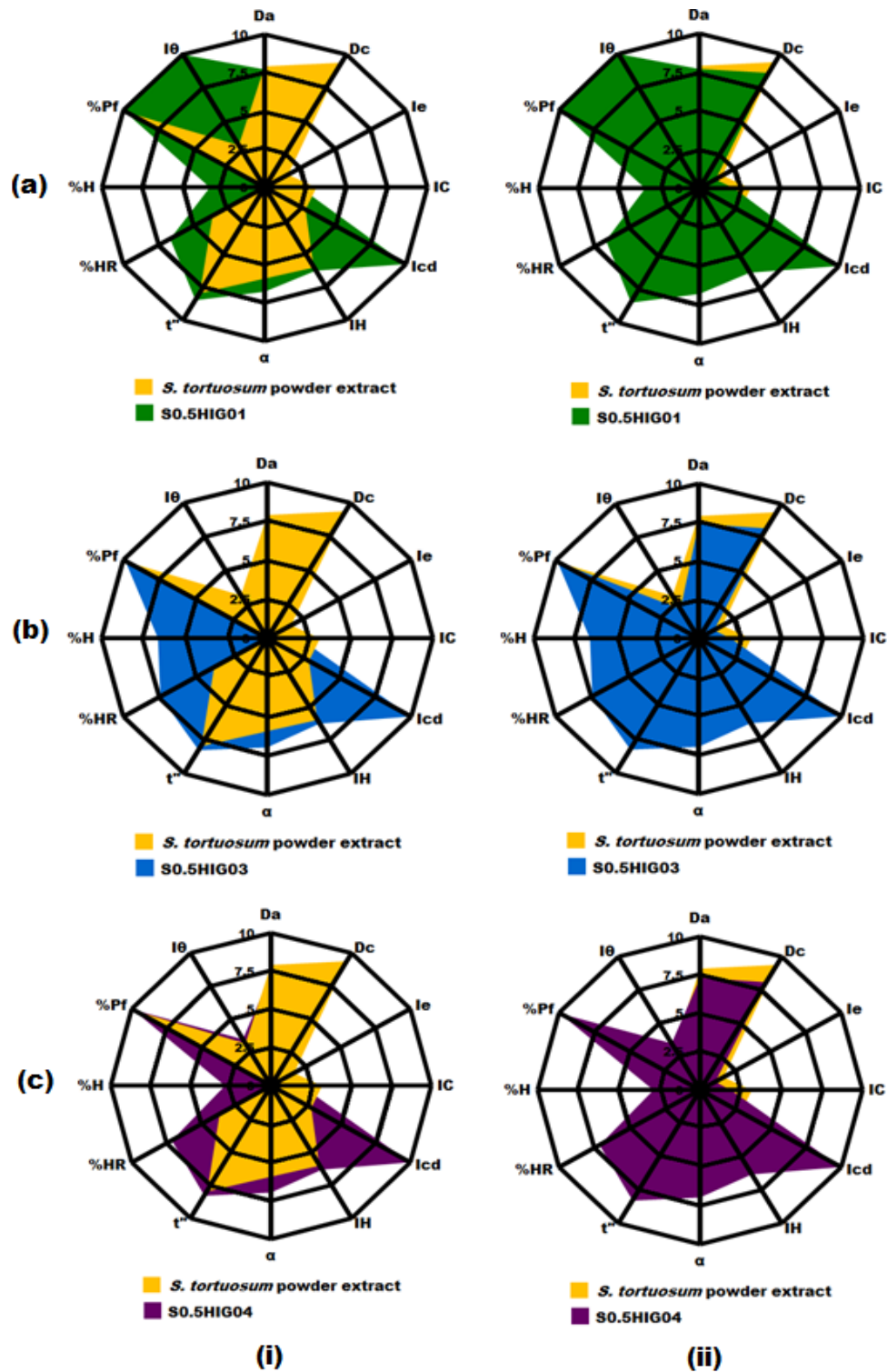


Figure 4.9: Superimposed SeDeM diagrams of *Sceletium tortuosum* extract powder (yellow) on formulations: **(a)** S0.5HIG 01 (green); **(b)** S0.5HIG 03 (blue); and **(c)** S0.5HIG 04 (purple). In column **(i)** the polygon of *Sceletium tortuosum* extract powder is overlaid on the polygon of the specific formulation, whereas in column **(ii)**, the polygon of the formulation is overlaid on the polygon of the *Sceletium tortuosum* extract powder

By superimposing the SeDeM diagrams of the *S. tortuosum* extract powder with each of the different formulations, the degree of similarity or difference between the two components can be used to determine the suitability for direct compression. In column (i) of Figures 4.8 and 4.9, the polygon of *S. tortuosum* extract powder is superimposed on the polygon of the specific MCG formulation described underneath each diagram, whereas in column (ii), the polygon of the corresponding MCG formulation is superimposed on the polygon of the *S. tortuosum* extract powder.

As evident from the results achieved (Tables 4.14 and 4.15), differences could be seen in formulations S0HIG01, S0HIG03 and S0HIG04 regarding the cohesion, loss on drying, hygroscopicity and homogeneity indices. Differences in formulations S0.5HIG01, S0.5HIG03 and S0.5HIG04 existed in terms of the loss on drying, hygroscopicity and the homogeneity indices. Furthermore, the SeDeM Expert Diagram System analysis revealed that none of the formulations depicted acceptable values for the inter-particle porosity; and Carr's index parameters. Formulation S0HIG03 also did not display an acceptable value for the loss on drying, although the values increased when magnesium stearate was added in formulation S0.5HIG03. The hygroscopicity and homogeneity index parameters tend to be problematic in formulations containing the HIG 03 and 04 base powders. The following rank order for the overall unacceptable values could be established: homogeneity index > loss on drying > hygroscopicity > Carr's index > inter-particle porosity. As discussed previously, the HIG 03 base powder is less hygroscopic and will probably improve the flowability and compressibility of the formulations compared to the formulations containing the HIG 01 and 04 base powders. However, no clear differences could be seen in the flowability factors when comparing the Hausner ratio, angle of repose and powder flow parameters of the six formulations. As for the compressibility factor, the inter-particle porosity, the Carr's index parameters, and especially the cohesion index parameter improved in the formulations which contained the lubricant, magnesium stearate. The parameter values acquired for all of the six formulations failed the requirements set by the SeDeM Expert Diagram System regarding the inter-particle porosity and Carr's index parameters, however, the cohesion index values were within the acceptable limits (> 5). The polygon profiles for these formulations are relatively similar, differing mainly at two parameters, namely the homogeneity index, where formulations containing HIG 01 base powder reached higher values; and the hygroscopicity parameter, where formulations containing HIG 01 and 03 base powders also depicted higher values, except for formulation

S0HIG01, where the hygroscopicity parameter differs more profoundly compared to the S0.5HIG01 formulation.

Table 4.14: *Experimental values (V) and converted experimental values (r) of medicated chewing gum formulations without magnesium stearate (Percentage relative standard deviation in parenthesis)*

Parameter	Formulations					
	S0HIG01		S0HIG03		S0HIG04	
	“V”	“r”	“V”	“r”	“V”	“r”
Bulk Density (Da)	0.74 (1.35)	7.40	0.73 (1.57)	7.30	0.68 (0.83)	6.80
Tapped Density (Dc)	0.78 (0.74)	7.80	0.78 (0.74)	7.80	0.70 (0.83)	7.00
Inter-particle Porosity (Ie)	0.06 (48.24)	0.50	0.09 (22.22)	0.75	0.03 (34.64)	0.25
Carr's Index (IC)	4.71 (41.14)	0.94	6.38 (20.07)	1.28	2.39 (34.35)	0.48
Cohesion Index (Icd)	115.30 (8.01)	5.77	179.00 (7.82)	8.95	91.00 (2.20)	4.55
Hausner Ratio (IH)	1.05 (2.54)	6.50	1.07 (1.43)	6.43	1.02 (1.13)	6.60
Angle Of Repose (α)	12.12 (0.64)	7.58	14.07 (4.92)	7.19	14.57 (3.67)	7.09
Powder Flow (t’')	3.40 (0.00)	8.30	3.60 (55.56)	8.20	3.80 (0.00)	8.10
Loss on Drying (%HR)	3.73 (7.91)	6.27	3.04 (2.05)	6.96	4.16 (27.99)	5.84
Hygroscopicity (%H)	5.65 (15.74)	7.175	4.69 (66.03)	7.66	16.65 (8.35)	1.68
Particles < 50 μm (%Pf)	0.06 (0.00)	9.988	0.40 (0.00)	9.92	0.14 (0.00)	9.97
Homogeneity Index (Iθ)	1.30 (16.10)	6.50	0.20 (24.16)	1.00	0.69 (8.35)	3.45

Table 4.15: *Experimental values (V) and converted experimental values (r) of medicated chewing gum formulations containing 0.5% w/w magnesium stearate (Percentage relative standard deviation in parenthesis)*

Parameter	Formulations					
	S0.5HIG01		S0.5HIG03		S0.5HIG04	
	"V"	"r"	"V"	"r"	"V"	"r"
Bulk Density (Da)	0.77 (0.75)	7.70	0.74 (2.07)	7.40	0.73 (1.57)	7.30
Tapped Density (Dc)	0.86 (0.00)	8.60	0.82 (0.71)	8.20	0.81 (0.00)	8.10
Inter-particle Porosity (Ie)	0.14 (4.03)	1.17	0.13 (15.38)	1.08	0.13 (13.32)	1.08
Carr's Index (IC)	10.86 (6.17)	2.17	9.80 (13.11)	1.96	9.46 (15.07)	1.89
Cohesion Index (Icd)	292.30 (1.54)	14.62	344.67 (1.31)	16.73	267.00 (2.62)	13.35
Hausner Ratio (IH)	1.12 (0.51)	6.27	1.11 (1.80)	6.30	1.10 (2.09)	6.33
Angle Of Repose (α)	15.99 (3.83)	6.80	15.11 (3.54)	6.98	15.10 (6.12)	6.98
Powder Flow (t'')	3.00 (3.77)	8.50	3.46 (3.33)	8.27	3.40 (0.00)	8.30
Loss on Drying (%HR)	3.29 (8.27)	6.71	2.55 (23.45)	7.45	2.85 (10.52)	7.15
Hygroscopicity (%H)	13.25 (17.49)	3.38	6.81 (22.79)	6.60	14.25 (19.58)	2.88
Particles < 50 μm (%Pf)	0.16 (13.69)	9.97	0.55 (15.43)	9.89	0.21 (3.45)	9.96
Homogeneity Index (Iθ)	2.00 (2.55)	10.00	0.44 (33.73)	2.20	0.70 (8.88)	3.51

Formulation S0HIG01 performed relatively well as it exhibited only two parameters below the required value (< 5), which included the inter-particle porosity with a relatively low value of 0.06 (%RSD = 48.24) and the Carr's index value of 4.71% (%RSD = 41.14). These two parameters affected the compressibility to the extent where the value for the compressibility incidence factor of the formulation was a mere 2.40 as depicted in Table 4.16. The *S. tortuosum* extract powder (compressibility of 2.58), the hygroscopic properties of the plasticiser (Bergo *et al.*, 2013) and the artificial sweetener contributed in decreasing the tensile strength (Shaw *et al.*, 2002) and also contributed to a relatively low compressibility incidence factor. Formulation S0HIG03 and S0HIG04 had four parameters that exhibited unacceptable values, influencing the inter-particle porosity (0.09, %RSD = 22.22), Carr's index (6.38, %RSD = 20.07), loss on drying (3.04, %RSD = 2.05) and homogeneity index (0.2, %RSD = 24.16). The relative large %RSD of each parameter indicates that relatively high variation occurred within the replications of these parameters.

Table 4.16: Factor values of the medicated chewing gum formulations

Factors	S0HIG01	S0HIG03	S0HIG04	S0.5HIG01	S0.5HIG03	S0.5HIG04
Dimension	7.60	7.55	6.90	8.15	7.80	7.7
Compressibility	2.40	3.66	1.75	5.98	6.59	5.44
Flowability	7.46	7.27	7.26	7.19	6.30	7.20
Lubricity/ Stability	6.72	7.31	3.76	5.04	7.02	5.01
Lubricity/ Dosage	8.24	5.46	6.71	9.98	6.05	6.74

According to an article by Jivraj *et al.* (2000), excipients that are undergoing direct compression are required to have a high bulk density. It is also stated that particle size has relatively little effect on the compactibility of the powder mixture. The dimensional incidence factors for all the formulations are higher than the required limit (> 5) set by the SeDeM Expert Diagram System and are therefore deemed suitable for direct compression. This can also be seen on the SeDeM diagrams (Figures 4.8 and 4.9) as all of the bulk- and tapped density values are clearly above a value of 5.

According to the evaluation as per SeDeM Expert Diagram System all of the values for the inter-particle porosity and the Carr's index parameters were not only below the required value of 5 for all of the formulations, but the values were lower than the value obtained for the *S. tortuosum*

extract powder. Inter-particle porosity is an indication of insufficient spaces located between the particles of a powder, which may negatively influence the compressibility of that powder. The limit for the inter-particle porosity value (Chapter 2) must be between 0-1.2, where an experimental score of 1.2 would represent a perfect converted value of 10 on the polygon (Bhavsar *et al.*, 2015; Khan *et al.*, 2014; Suñé-Negre *et al.*, 2013). As mentioned before, *S. tortuosum* extract powder attained an inter-particle porosity of 0.20 (%RSD = 12.79) and although the formulations that contain magnesium stearate attained slightly higher values (Table 4.15) than the formulations containing no magnesium stearate; these values are still considerably lower than that of the *S. tortuosum* extract powder.

Analysis of the particle sizes and the particle size distributions of the formulations (Annexure D) revealed a relatively wide span for each of the formulations. The average particle sizes were estimated as 451.076 μm (%RSD = 4.863; $n = 12$); 221.887 μm (%RSD = 2.163; $n = 12$) and 343.635 μm (%RSD = 4.722; $n = 12$) for formulations S0HIG01, S0HIG03 and S0HIG04, respectively; and 470.098 μm (%RSD = 5.465; $n = 12$); 311.970 μm (%RSD = 7.209; $n = 12$) and 344.345 μm (%RSD = 1.522; $n = 12$) for formulations S0.5HIG01, S0.5HIG03 and S0.5HIG04, correspondingly. The particle sizes of the formulations that contain no magnesium stearate varied more drastically whereas formulations comprising magnesium stearate depicted a slightly smaller variation in average particle size distribution.

Considering the compressibility factor, the Carr's index of formulations S0HIG01, S0HIG03 and S0HIG04 attained values that indicate that the propensity of the powder being compressed (%) is lower than required. The data indicates that the compressibility factor improved when magnesium stearate was included in the formulations. The compressibility factor consists of the inter-particle porosity, cohesion index and Carr's index parameters. These parameters will therefore influence the compressibility of the powder utilised for direct compression. The inter-particle porosity parameter increased as the inclusion of magnesium stearate reduced the amount of pores located between the grains. Furthermore, the inclusion of magnesium stearate which has a relatively small particle size increased the tapped density of the different MCG formulations, which invariantly improved the Carr's index values. The cohesion index values also improved for these formulations as an even higher compaction force could be utilised to manufacture MCGs by means of direct compression. As exhibited in Table 4.16, the compressibility factor was the only factor which attained values that was lower than 5 for the formulations containing no magnesium stearate.

The flowability factor of the formulations includes the Hausner ratio, angle of repose and the powder flow parameters. According to the standards set by the BP (2016) powder flowability was excellent for all, but one of the six formulations. Formulation S0.5HIG01 depicted a “good” value according to the standards set by the BP (2016). The angle of repose values categorised all of the formulations in the class that have excellent flow properties.

Formulations S0HIG01 and S0HIG03 depicted a relatively high lubricity/stability factor value. On the other hand, formulation S0HIG04 attained a lower lubricity/stability factor value, thus failing to comply with the SeDeM Expert Diagram System requirement. The hygroscopicity of formulations S0.5HIG01 and S0.5HIG04 also failed to comply with the SeDeM Expert Diagram System requirement. This can be attributed to the distinctly lower hygroscopicity parameters obtained for these formulations, which can be linked to the effect of the plasticiser that probably increased hygroscopicity in these formulations. A degree of hygroscopicity was expected in all of the formulations as they contain D-sorbitol to improve the taste, which increased the hygroscopicity parameter values and decreased the loss on drying parameter values. Formulation S0HIG04 absorbed the most moisture (i.e. exhibited the highest hygroscopicity) of all the formulations.

Magnesium stearate forms a hydrophobic layer around particles, which results in an increased repelling effect on water molecules; thereby prohibiting water from entering the MCG units (Uzunović & Vranić, 2007). The formation of a hydrophobic layer is generally problematic for the disintegration process in tablets, which is not applicable to the dosage form developed in this study (i.e. MCGs) as disintegration is not a prerequisite due to the mechanical process of chewing. A hygroscopic plasticiser will also lead to decrease the tensile strength of the MCGs by decreasing the cohesion index parameter. Formulations S0HIG01, S0HIG04, S0.5HIG01 and S0.5HIG04 are seemingly softer compared to formulations S0HIG03 and S0.5HIG04.

The lubricity/dosage factor reached acceptable values higher than 5. Formulation S0HIG03 attained the lowest value of 5.46. As also observed from the SeDeM diagrams (Figure 4.8, images b-ii, c-ii), the homogeneity index was problematic in formulations S0HIG03, S0HIG04, S0.5HIG03 and S0.5HIG04; however, this did not decrease the incidence factor to the extent where it failed to comply with the requirements set by the SeDeM Expert Diagram System. The homogeneity index of the formulations containing HIG 03 and 04 base powders indicated once again, that these formulations have a relatively large particle size distribution.

Table 4.17 presents the PPI and GCI values for all the different MCG formulations investigated in this study. It can be seen that five of the six formulations were within acceptable limits as they all have values higher than 5 (Bhavsar *et al.*, 2015). Formulation S0HIG04 attained a compressibility index value of 4.90 (bold) causing the formulation to fail the standards set by the SeDeM Expert Diagram System. Although the three formulations S0HIG01, S0HIG03 and S0HIG04 displayed GCI values of 6.19, 6.08 and 5.16, respectively, thus passing the SeDeM Expert Diagram System requirements for direct compression; larger MCG batches could not be compressed from these formulations as previously explained (the MCG units exhibited sticking to the upper punch). This was the only challenge experienced, which led to the addition of a lubricant to the MCG formulations in order to overcome this problem. The formulations are ranked based on their PPI and GCI values as follow: S0.5HIG01 > S0.5HIG03 > S0.5HIG04 > S0HIG01 > S0HIG03 > S0HIG04.

Table 4.17: Index values for the medicated chewing gum formulations

Index	S0HIG01	S0HIG03	S0HIG04	S0.5HIG01	S0.5HIG03	S0.5HIG04
PI	0.83	0.75	0.58	0.75	0.75	0.67
PPI	6.23	6.12	5.15	7.16	6.92	6.40
GCI	5.93	5.83	4.90	6.81	6.59	6.10

Comparison of the SeDeM diagrams of the *S. tortuosum* extract powder and the MCG formulations predicted that the formulations will influence the *S. tortuosum* extract powder positively; and the powder mixtures will be more appropriate for tableting by means of direct compression. Formulation S0.5HIG01 reflected the highest PPI and GCI values, indicating that this formulation is the most appropriate for direct compression according to the SeDeM Expert Diagram System. Additionally, index values indicated that overall *S. tortuosum* extract MCG formulations comprising magnesium stearate are also deemed more appropriate for direct compression.

4.4 FORMULATION OF MEDICATED CHEWING GUMS

The aim of this study is to develop a MCG formulation containing 6.38 mg *S. tortuosum* extract in a single MCG unit that is directly compressible. The MCG units manufactured from this formulation should also adhere to the official criteria of the Pharmacopoeias for physical properties of solid oral dosage forms such as tablets. This section focuses on the properties of

the different MCG units that were directly compressed by utilising a full factorial design in order to investigate the effect that the inclusion of magnesium stearate and HIG base type has on the properties of the MCG units. The addition of other excipients was deemed unnecessary as the manufacturers of the HIG base powders stated that these bases already contain a variety of excipients that are unique to each formulation (Table 4.8) so that it can be directly compressed into MCG tablets. Therefore, as seen in Table 4.12, the formulations evaluated consisted of the *S. tortuosum* extract powder; a sweetener; a flavourant; either HIG 01, 03 or 04 base powders; as well as no or 0.5% w/w magnesium stearate. The MCG units of the different formulations were subsequently evaluated in terms of morphology, physical tablet tests and dissolution end points. All tests conducted were done in triplicate and according to the British Pharmacopoeia (2016) standards.

4.4.1 Morphology

Scanning electron microscopy (SEM) images of the different tableted MCG formulations were taken at 130X and 1 000X magnifications. The individual MCG units were cut in half to clarify what effect the compression forces had on the internal structure of the MCG units during compression. Figures 4.10 and 4.11 display how deformation of the powder particles emerged during compression. Due to the fact that no visual differences in the deformation of the MCG formulations with and without magnesium stearate could be observed, only SEM images of the formulations comprising magnesium stearate are shown here.

From Figure 4.10, a degree of plastic deformation can be observed for the particles of all three HIG base powders. The S0.5HIG04 formulation seems to possess a smoother outer surface indicating a higher degree of plastic deformation. Observing the higher magnification SEM images of the outer surfaces (Figure 4.10b), it seems as though the S0.5HIG04 formulation produced MCG units that depicted increased particle binding due to a higher degree of deformation. Contradictory to this, the S0.5HIG01 and S0.5HIG03 formulations portrayed less plastic deformation with additional cracks and more individual particles still visible after compression. Moreover, from these SEM images, it appears as though the S0.5HIG03 might have compressed slightly more effectively compared to the S0.5HIG01 formulation as the SEM image at the higher magnification reveals less individual particles.

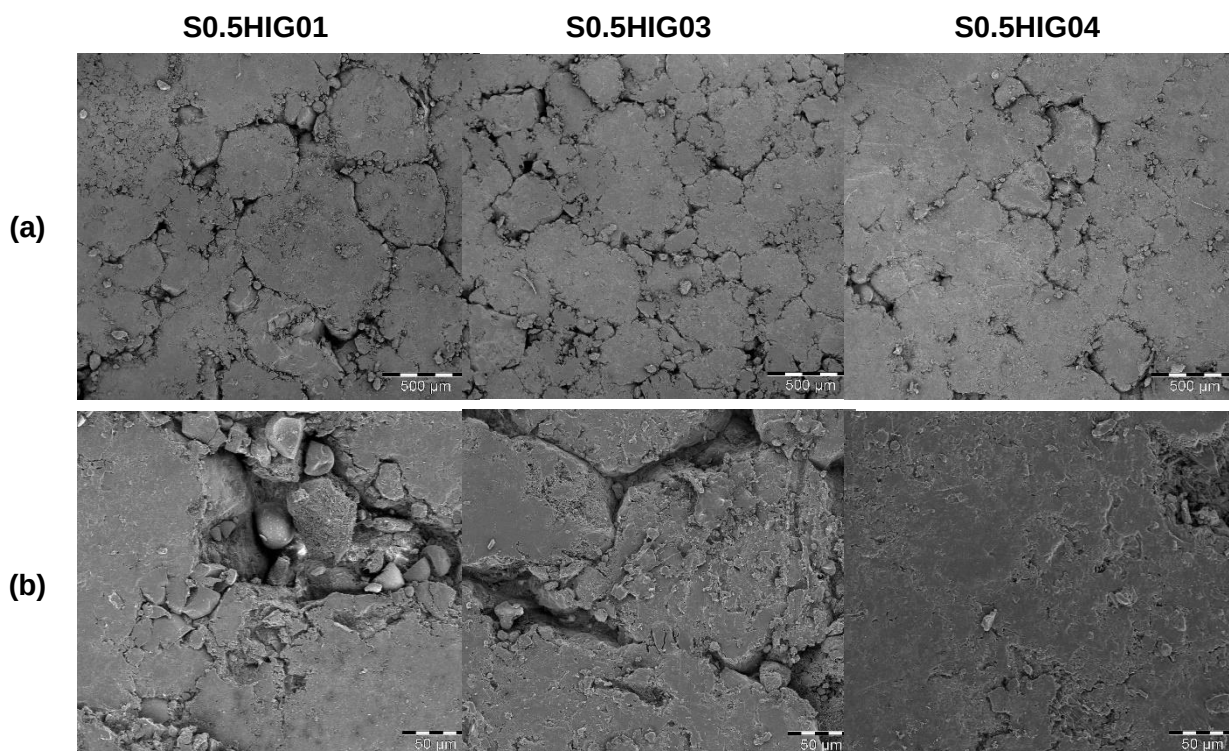


Figure 4.10: SEM micrographs of the outer surfaces the individual S0.5HIG01; S0.5HIG03; and S0.5HIG04 medicated chewing gum units. Rows (a) and (b) display magnifications at 130X and 1 000X, respectively

Figure 4.11 highlights the internal structures of the different MCG formulations after direct compression. The internal structures of the S0.5HIG01 formulation seem more uneven with a few individual particles still noticeable, thus, indicating less plastic deformation. The S0.5HIG03 and S0.5HIG04 formulations seem to possess more cohesive particles, which may be indicative of a higher degree of binding during direct compression.

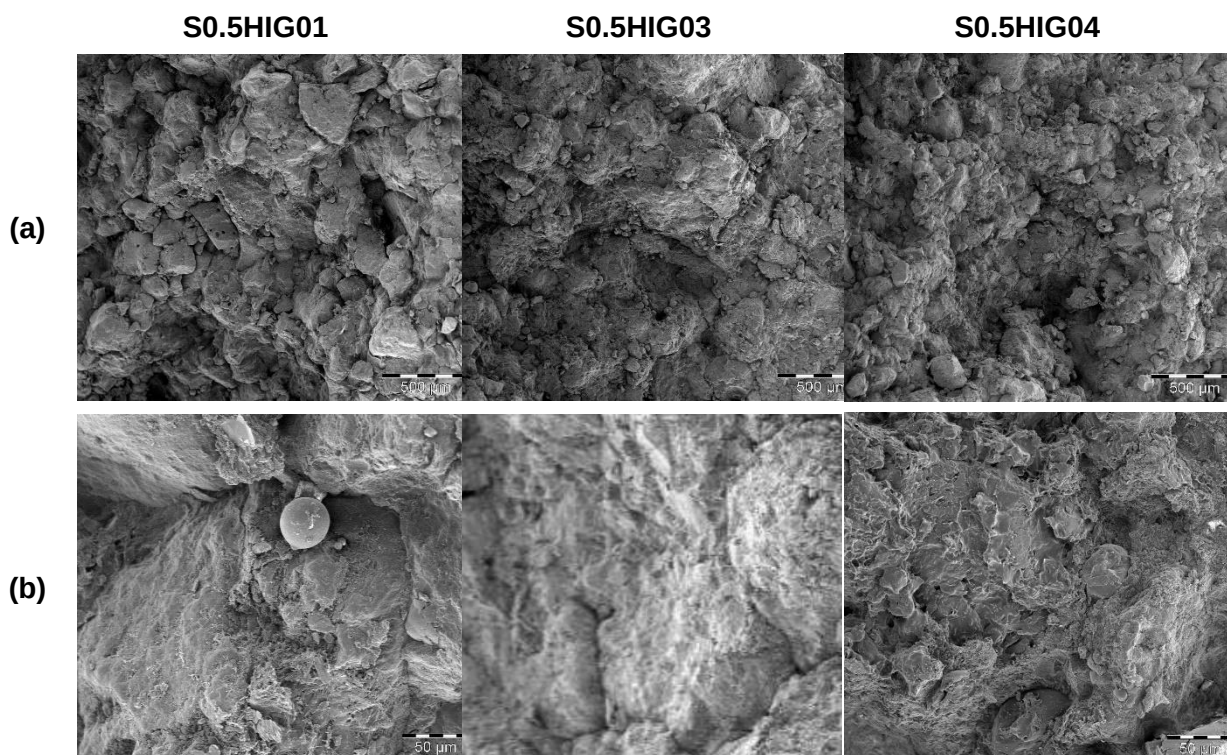


Figure 4.11: SEM micrographs of the internal structures of the individual S0.5HIG01; S0.5HIG03; and S0.5HIG04 medicated chewing gum units after it was cut in half. Rows (a) and (b) display magnifications at 130X and 1 000X, respectively

4.4.2 Evaluation of the physical properties of the different medicated chewing gum units

After evaluation of the powder properties of the six MCG formulations, the powder mixtures were tableted into MCG units by means of direct compression using a Korsch® XP1 single station tablet press and 12 mm punches. The tablet mass of each MCG unit was set to be approximately 1.00 gram and the fill volume was kept constant between the different formulations. The physical properties of the MCG units prepared from the different formulations as per full factorial design were evaluated in terms of mass variation, crushing strength, diameter, thickness, tensile strength, and friability. Table 4.18 provides the average values obtained for the physical property tests as well as the percentage relative standard deviation in parenthesis. Values in bold are considered most optimal.

Table 4.18: *Physical test results for the different medicated chewing gum formulations (Percentage relative standard deviation in parenthesis)*

	Mass variation (g)	Crushing strength (N)	Diameter (mm)	Thickness (mm)	Average tensile strength (N.mm⁻²)	Friability (%)
S0HIG01	0.862 (12.019)	108.670 (16.601)	11.940 (0.052)	8.410 (3.501)	0.689 (3.175)	0.043 (41.655)
S0HIG03	0.848 (10.751)	179.130 (7.818)	11.920 (0.234)	8.280 (12.451)	1.155 (7.233)	0.034 (21.649)
S0HIG04	0.852 (11.084)	91.410 (2.203)	11.980 (0.455)	8.150 (0.104)	0.596 (2.199)	0.028 (32.797)
S0.5HIG01	1.039 (1.185)	192.300 (2.344)	11.960 (0.161)	7.720 (2.429)	1.326 (2.149)	0.037 (41.659)
S0.5HIG03	1.024 (0.630)	217.670 (1.164)	11.950 (0.091)	7.610 (0.457)	1.524 (0.738)	0.000 (0.000)
S0.5HIG04	0.973 (1.017)	185.670 (1.648)	11.960 (0.127)	7.450 (1.201)	1.130 (0.775)	0.000 (0.000)

From the results obtained it is clear that the formulations containing no magnesium stearate produced MCG tablets of approximately 850 mg with a relatively high %RSD (average of 11.28%) compared to the formulations containing 0.5% w/w magnesium stearate, which produced MCG units of higher mass (approximately 1 g) and lower %RSD. This indicates that the inclusion of magnesium stearate improved the flowability of the different MCG formulations into the tablet die, which resulted in the manufacturing of more reproducible MCG units when considering weight. Furthermore, the problems experienced with cohesion of the MCG formulations (without magnesium stearate) to the punches may have caused increases in mass variation between the MCG units of the specific formulation. The magnesium stearate was able to lower the inter-particle forces as it serves as a lubricant and glidant, which increased not only the flowability of the particles into the tablet die, but it also lowered cohesion to the tablet punches (Carter, 2001, Jivraj *et al.*, 2000).

Comparing the crushing strength values, it was interesting to note that the formulations comprising magnesium stearate illustrated higher crushing strength values and comparably %RSD values (approximately 198.55 N and 1.72%, respectively) compared to MCG formulations without the addition of magnesium stearate (approximately 126.40 N and 8.87%, respectively). Furthermore, the average tensile strength values followed the same trend as the crushing strength values, as these values are calculated from the average diameter, thickness and crushing strength values obtained. Remarkably, thickness values obtained for the different MCG formulations were more meaningfully affected by the addition of magnesium stearate than the diameter. Formulations containing magnesium stearate depicted reasonably lower thickness values, rendering them more compactly compressed (Carter, 2001) as their proportional weight values are higher. This is probably due to the ability of magnesium stearate to reduce inter-particle forces and improve the flowability of the powder particles of the formulations into openings between other particles during compression (Carter, 2001).

Formulations S0HIG01, S0HIG03 and S0HIG04 showed a relatively higher percentage friability (approximately 0.035%) compared to the S0.5HIG01, S0.5HIG03 and S0.5HIG04 formulations that depicted an average percentage friability of nearly 0.012%. All of the formulations did however still adhere to the official criteria of the British Pharmacopoeia (2016), which states that only a maximum weight loss of 1% is deemed acceptable during friability testing. A summary of the average values for each level of the different variables of each response included in the factorial design are presented in Table 4.19.

Table 4.19: *Average responses calculated from the physical properties measured for each variable at each level of the medicated chewing gum formulations. Values considered most optimal are highlighted in grey and bold text*

Variable and level		Mass variation (%RSD)	Crushing strength (N)	Tensile strength (N.mm ⁻²)	Friability (%)
Base type	HIG 01	6.602	150.485	1.008	0.040
	HIG 03	5.691	198.400	1.340	0.017
	HIG 04	6.051	138.540	0.863	0.014
Magnesium stearate concentration	0%	11.285	126.403	0.813	0.035
	0.5%	0.944	198.547	1.327	0.012

From these values attained it was clear that the inclusion of magnesium stearate played a more noticeable role in the physical properties of the MCG formulations, especially considering the mass variation, crushing strength and tensile strength values (grey highlighted areas in table). MCG formulations consisting of magnesium stearate were more uniform in weight, harder and stronger compared to MCG formulations containing no lubricant. With the friability values it was less clear which variable influenced this physical property most. The type of base incorporated was considered a factor of lesser importance as the variation between the average values obtained for most of the responses measured were not that prominent. Inclusion of magnesium stearate in at least 0.5% w/w is therefore considered necessary in order to obtain a workable MCG formulation that will not adhere to tablet tooling and which will meet the official criteria for the physical properties of tablets. It could further be concluded that when all of the physical property responses are considered, the HIG 03 base powder produced MCG units that proved more optimal as these tablets were more uniform in weight, harder and stronger compared to the other HIG base powders. As explained earlier, isomalt present in a relatively high concentration in the HIG 03 base powder has excellent flowability and powder characteristics (Quodbach *et al.*, 2012). Consequently, the incorporation of isomalt into HIG 03 may have contributed to the increased flowability of the MCG formulations containing HIG 03; and thus indirectly increased mass uniformity and compressibility of the MCG units. Furthermore, Lawton (2004) concluded that chewing gum containing hygroscopic plasticisers exhibited more pronounced decreases in ultimate tensile strength. The MCG formulations comprising the HIG 03 base powder, which does not include a plasticiser in its content, portrayed increased values in tensile strength; and the MCG formulations containing either HIG 01 or HIG 04 base powders exhibited decreased tensile strength values, thus confirming results obtained by Lawton (2004). Overall, when all the responses are considered, formulation S0.5HIG03 performed the best of all the formulations investigated. All of the MCG formulations, however, did adhere to the criteria for acceptable physical properties.

4.4.3 *In-vitro* dissolution studies

The release of an active pharmaceutical substance from any dosage form is a very important step for both drug absorption and bioavailability (Maggi *et al.*, 2005). Three MCG units from formulations S0.5HIG01, S0.5HIG03 and S0.5HIG04 were tested; and as explained beforehand, a single point dissolution study was conducted for each MCG formulation. Artificial saliva (Table 4.20) was prepared and used as dissolution medium. Samples were withdrawn in triplicate from a 40 ml thermostatted test cell after a time period of 45 min elapsed.

According to a study done by Chaudhary *et al.* (2010), there are different factors affecting the release of active substances from MCGs. These factors include person-to-person variability, physiochemical properties of the drug and the formulation factors.

Table 4.20: *Calculations from quantity to mass for artificial saliva used in the dissolution test*

Component	Quantity (mmol/L ⁻¹)	Molecular mass (g/mol)	Calculation	Mass (g)
KH₂PO₄	2.50	136.09	136.09*2.50/1000	0.340
Na₂HPO₄	2.40	358.14	358.14*2.40/1000	0.859
KHCO₃	15.00	100.12	100.12*15.0/1000	1.500
NaCl	10.00	58.44	58.44*10.0/1000	0.584
MgCl₂	1.50	203.30	203.30*1.50/1000	0.305
CaCl₂	1.50	110.99	110.99*1.50/1000	0.167
Citric Acid	0.15	210.14	210.14*0.15/1000	0.032

Person-to-person variability includes the unique chewing force, frequency and chewing time that may differ between patients. Physiochemical properties of the active compound include its molecular mass; ionised or non-ionised form; lipophilicity or hydrophilicity; stability to salivary enzymes; and its solubility in salivary fluids. For example, an active compound that is saliva soluble (i.e. water-soluble) will be released more rapidly compared to an active compound that is lipid-soluble which must first diffuse through the gum base where after it is released into the salivary fluid. Formulation factors that play a role in the release of the active substance include the composition, the type and amount of gum base, solubilising agents and softening agents. For instance, in one study the effect of gum base mass on the release of the active compound was investigated using salicylamide as a representative drug. Salicylamide incorporated into a MCG with a large percentage gum base released a significant lower percentage of the active component compared to salicylamide incorporated into a MCG with a lower percentage gum base, which released approximately double the salicylamide amount (Chaudhary *et al.*, 2010; Christrup & Moeller, 1986). In another study where the release of nicotine from custom made MCGs was compared to the release of nicotine from Nicorette® MCGs supports the statement made by Chaudhary *et al.* (2010). The results showed that the nicotine gum formulation released a concentration of 93.2% nicotine after 30 min of chewing and 95% of its nominal amount, whereas Nicorette® released only 50-60% of its nicotine content (Rudolph *et al.*, 2016).

A United States of America (USA) patent application suggested that within the first 2 min after the start of the dissolution test, a release rate of 10% w/w or more of the total content in the MCG must be obtained per min (Axelsson *et al.*, 2007). In another USA patent application that was published in 2014, values of between 31.5% and 48% of bupropion was detected in a dissolution test done on MCGs after 45 min of mechanical chewing (Fanda, *et al.*, 2014). The dissolution results obtained in this study are shown in Table 4.21.

Table 4.21: *Dissolution results of two alkaloids present in 40 ml of the artificial saliva (μg) and the percentage released quantities (%). (Percentage relative standard deviation in parenthesis)*

Formulations	Mesembrenone ($\mu\text{g}/40\text{ml}$)	Mesembrenone (%)	Mesembrine ($\mu\text{g}/40\text{ml}$)	Mesembrine (%)
S0.5HIG01	14.667	54.475 (28.386)	20.000	48.981 (10.004)
S0.5HIG03	8.000	29.713 (0.000)	10.000	24.491 (0.000)
S0.5HIG04	12.667	47.047 (36.464)	20.000	48.981 (20.000)

Rendering the quantitative data regarding the alkaloid content of the *S. tortuosum* extract powder that is summarised earlier in the chapter (Table 4.2), the *S. tortuosum* water extract contained 0.058% w/w of the mesembranol alkaloid, 0.422% w/w mesembrenone and 0.640% w/w of the mesembrine alkaloid. No mesembrenol was detected in the *S. tortuosum* extract powder. According to the dissolution test results, none of the MCG formulations displayed any release of the mesembrenol and mesembranol alkaloids. Though mesembrenol was not present in the extract, the mesembranol alkaloid was most probably present in such a small concentration that it was below the limit of detection.

The following rank order for the successful release of alkaloids from the formulations could be established: S0.5HIG01 > S0.5HIG04 >> S0.5HIG03. According to the results summarised in Table 4.21, formulation S0.5HIG01 released the highest amount of the mesembrine and mesembrenone alkaloids. Formulation S0.5HIG04 followed with the same amount of the mesembrine alkaloid released and a slightly lower value for the mesembrenone alkaloid compared to formulation S0.5HIG1. Formulation S0.5HIG03 depicted the lowest amounts for both of the alkaloids released with the amount of mesembrine alkaloid being inferior between

the two alkaloids. Taking the %RSD (shown in brackets) into consideration, it can be concluded that formulation S0.5HIG03 has a more repeatable release of alkaloids as the three samples withdrawn contained the exact same concentration of alkaloids (%RSD = 0.000). The following rank order when considering the accuracy of the samples according to the %RSD could be established: S0.5HIG03 >>> S0.5HIG01 >> S0.5HIG04.

A possible explanation for the difference in dissolution values obtained can be explained by the difference in composition of the HIG base powders present in the formulations. The composition of the HIG base powders prominently determines the physico-chemical properties that will in turn influence the dissolution of the *S. tortuosum* extract powder from the MCG units. For example, the HIG 03 base powder contains an additional sweetener, isomalt, which has a low grade of hygroscopicity (Quodbach *et al.*, 2012), which means that the particles will probably be less wettable by the artificial saliva compared to the particles of the HIG 01 and 04 base powders, which can contribute to reduce the release of the extract powder from the MCG unit. Although the mechanical action overcomes the disintegration process, the hygroscopicity of the different components in the formulation plays a major role as it controls the release of the alkaloids into the surrounding fluids. Bhoi *et al.* (2014) observed that an increase in the concentration of a plasticiser and a decrease in the concentration of a gum base may increase drug release from the formulation. In this study, formulations S0.5HIG01 and S0.5HIG04 contained a plasticiser where formulation S0.5HIG03 contained no plasticiser. Correspondingly to this finding in the previous study, formulation S0.5HIG01 and S0.5HIG04 demonstrated increased dissolution values in comparison to formulation S0.5HIG03, which released a lower concentration *S. tortuosum* extract. Although magnesium stearate was added at the lowest possible concentration, it may still affect the release of alkaloids into the artificial saliva because of the hydrophobic character of magnesium stearate (Carter, 2001).

4.5 CONCLUSION

A dark-brown hygroscopic powder was produced after a water extract of *S. tortuosum* plant material was prepared and the yield calculated. The extraction method was deemed successful as it was capable of producing sufficient extract. A yield of 12.85% extract was obtained from 100 g dried *S. tortuosum* plant material (i.e. an acceptable yield of > 10%). Next, the amount of mesembrine alkaloids was quantified by means of LC-MS against validated analytical reference compounds. Only three of the four alkaloids, namely mesembranol (5.200% w/w), mesembrenone (37.700% w/w) and mesembrine (57.100% w/w), could be quantified in this

study (total amount of 11.20 µg/mg). Higher quantities of mesembrenone and mesembrine were, however obtained compared to a study conducted by Shikanga, Viljoen *et al.* (2012).

In order to manufacture directly compressible, conventional MCG units that will adhere to the set standards of the Pharmacopoeias for physical properties and release the active compound in a sufficient concentration, powder flow behaviour and compressibility of the active ingredient should be considered. These parameters were therefore tested in this study. The SeDeM Expert Diagram System was specifically developed to reduce both the number of experiments as well as the time required to produce an acceptable direct compression tablet formulation. Therefore, the SeDeM Expert Diagram System was applied to the extract powder, Cafosa®'s Health in Gum bases, as well as the different powder formulations determined by means of a full factorial design in order to generate profiles of their physico-chemical properties. These profiles were then used to predict the addition of suitable amounts of a corrective excipient to produce MCG units by means of direct compression.

Results showed that the *S. tortuosum* extract powder consists of particles of irregular shape and surfaces and it has a relatively wide particle size distribution. These characteristics contributed to the extract powder presenting with only fair flow properties, though its flow rate indicated poor flowability. The hygroscopicity property of this extract powder furthermore influenced the powder flow profile drastically; and it was concluded that the extract powder could only be handled for a short period where after it became sticky. Thus, the atmospheric humidity will play an important role during the manufacturing process when *S. tortuosum* extract powder is to be directly compressed into MCG units. On the other hand, all three HIG base powders depicted more spherical particles with a more uniform particle size and size distribution; and their flowability was enhanced compared to that of the *S. tortuosum* extract powder. The HIG 01 and 04 base powders seemed more homogeneous in particle size and shape; whereas the HIG 03 base powder appeared more coarse and uneven which is indicative of reduced flowability compared to the other HIG base powders.

Failing to comply with the compressibility index standards set by the SeDeM Expert Diagram System; results suggested an inability of the *S. tortuosum* extract powder to be directly compressed at the suggested hardness for tablets as stated by the BP (2016). Additionally, the PI, PPI and GCI were calculated and these results showed that the *S. tortuosum* extract powder is not suitable for direct compression as the PPI and GCI values were not within the acceptable range of 5-10. Conversely, all of the HIG base powders adhered to the set limits for all of the

SeDeM factor values. Thus, according to the SeDeM Expert Diagram System, these base powders can be directly compressed into tablets that will adhere to the standards set by the Pharmacopoeia. Flowability of the base powders, however, depicted the lowest values, which may indicate that mass variation may be an aspect that needs to be monitored for all three base powders. The PI, PPI and GCI values calculated for all of the base powders are within the set limits of the SeDeM Expert Diagram System. It was predicted by this system that the HIG 03 base powder will perform best when directly compressed, closely followed by the HIG 01 base powder. The System furthermore predicted that no significant modifications to the content of the three base powders are necessary as their respective GCI values are > 5 .

The SeDeM Expert Diagram System also projected that all of the HIG base powders are able to increase the compressibility factor to an acceptable value when incorporated in a specific concentration in the MCG units. No significant difference in the base concentration needed to obtain an acceptable directly compressible MCG formulation could be found between HIG 01 and 03 base powders. According to the SeDeM Expert Diagram System, a minimum concentration of approximately 50% of HIG 01 and 03 base powder is needed, rendering these two base powders the most suitable to correct the compressibility of the *S. tortuosum* extract powder when comparing the three HIG base powders. Almost 70% of HIG 04 base powder will however be required if this base powder is to be utilised for direct compression of *S. tortuosum* extract powder into MCG units. Moreover, it will not be possible to include a *S. tortuosum* extract powder concentration higher than 30% into a MCG consisting of the HIG 04 base powder. Hygroscopicity will probably not be improved utilising the HIG 01 base powder, but the HIG 03 base powder will almost certainly increase the hygroscopicity parameter more notably.

Comparison of the SeDeM diagrams for the *S. tortuosum* extract powder and the MCG formulations predicted that the formulations will positively influence the compressibility of the *S. tortuosum* extract powder. The powder mixtures will be more appropriate for tableting by means of direct compression. Formulation S0.5HIG01 depicted the highest PPI (7.42) and GCI (7.06) values, predicting that this formulation is the most appropriate for direct compression. It was furthermore shown that overall the inclusion of magnesium stearate into *S. tortuosum* extract MCG formulations are considered necessary.

Subsequently, utilising a full factorial design, the formulations containing *S. tortuosum* extract powder and one of the HIG base powders, were tableted into MCG units by means of direct compression and the physical properties tested. Results obtained showed that the inclusion of

magnesium stearate into the MCG formulations was indeed considered necessary. This variable played a more noticeable role in the physical properties of the MCG formulations, especially considering the mass variation, crushing strength and tensile strength values. MCG formulations comprising magnesium stearate were more uniform in weight, harder and stronger. The type of base powder incorporated was considered a factor of lesser importance as the responses measured did not vary noticeably. It could further be concluded that when all of the physical property responses are considered, the HIG 03 base powder produced MCG units that were more uniform in weight, harder and stronger compared to the other HIG base powders. All of the MCG formulations, however, did adhere to the criteria for acceptable physical properties.

Even though MCG units produced from formulation S0.5HIG03 portrayed the best physical characteristics, formulation S0.5HIG01 could also easily be compressed into MCG units that portrayed good physical characteristics; and in addition this formulation released the highest alkaloid concentration during the dissolution test. Therefore, overall, formulation S0.5HIG01 performed best. It was expected that formulation S0.5HIG03 would display the most favourable organoleptic properties when comparing the three MCG formulations containing magnesium stearate, because it contains isomalt, an additional taste masking agent (Quodbach *et al.*, 2012). However, in view of the physical characterisation of the powder mixtures and MCG units, as well as the dissolution tests conducted; formulation S0.5HIG03 performed second best according to the SeDeM Expert Diagram System standards; rendering it also suitable for tableting by means of direct compression. It depicted the most suitable physical characteristics after direct compression; but release of the alkaloids from the MCG units during the mastication process was the lowest of the three formulations investigated.

According to the manufacturers of CAFOSA®'s HIG base powders the HIG 03 base powder was developed for tableting by means of direct compression and the HIG 01 and 04 base powders should be utilised for manufacturing by means of hot melting. This study, however, has shown that HIG 01 and 04 base powders can also be applied to direct compression as there is no considerable difference when the properties of formulations S0.5HIG01, S0.5HIG03 and S0.5HIG04 are compared. Results obtained from the experiments done in this study support the concluding statement:

Formulation S0.5HIG01 is most suited for manufacturing MCG containing *S. tortuosum* extract powder by means of direct compression according to the SeDeM Expert Diagram System outcomes as well as the results obtained from the physical characterisation and dissolution studies of the MCG units. Furthermore, only mesembrenone and mesembrine alkaloids were

released from the MCG units regardless the formulation constituents. Pharmacological effects obtained from *S. tortuosum* are caused by these two alkaloids. As discussed previously (Chapter 2) the mesembrenone alkaloid is mainly responsible for the PDE4 enzyme inhibition and the mesembrine alkaloid is primarily responsible for 5HT-4 inhibition. Although the mesembranol and mesembrenol alkaloids do have some activity, the pharmacological effects are not dependent on the presences of these two alkaloids (Harvey *et al.*, 2011; Shikanga, Viljoen *et al.*, 2012).

Chapter 5:

SUMMARY AND FUTURE PROSPECTS

5.1 SUMMARY

The aim of this study is to develop and evaluate directly compressible medicated chewing gums containing *S. tortuosum* crude extract.

The following objectives set, were met:

- Extract from *S. tortuosum* plant material was prepared and an acceptable yield of 12.85% was obtained from 100 g dried *S. tortuosum* plant material. The extract was characterised on the basis of their chemical composition by means of LC-MS. Quantitative data of the extract sample indicated that the extract contained three of the four mesembrine-alkaloids determined (i.e. 5.20% w/w mesembranol, 37.70% w/w mesembrenone and 57.10% w/w mesembrine). Mesembrenol was absent in the prepared extract powder.
- Evaluation of the flowability and compressibility of the *S. tortuosum* extract powder, Cafosa[®]'s Health in Gum base powders as well as the different formulations, determined by means of a full factorial design, were determined utilising the SeDeM Expert Diagram System to generate profiles of their physico-chemical properties. The three HIG base powders depicted enhanced flowability compared to that of the *S. tortuosum* extract powder. Ultimately, the SeDeM Expert Diagram System indicated that the *S. tortuosum* extract failed to comply with the standards set regarding the extract's compressibility index. The extract powder furthermore depicted unacceptable PPI and GCI values, which predict that it will most likely be impossible to directly compress *S. tortuosum* extract powder into MCG units. Conversely, the HIG base powders adhered to the standards set by the SeDeM Expert Diagram System, thus indicating its suitability for tableting by means of direct compression. Considering the PI, PPI and GCI values of the three HIG base powders, it was predicted (and subsequently verified) that the HIG 03 base powder will perform best when directly compressed, closely followed by the HIG 01 base powder.
- Medicated chewing gum containing *S. tortuosum* crude extract (Figure 5.1) were successfully developed by means of direct compression utilising a Korsch[®] single tablet press. The SeDeM Expert Diagram System predicted that the HIG base powders would

improve the compressibility factor of the *S. tortuosum* extract powder to an acceptable value. This prediction was verified as MCG formulations were manufactured which presented with compressibility values within an acceptable range (< 5). Cafosa®'s HIG 01, 03 and 04 base powders were used in different formulations that formed part of a full factorial design that was utilised to determine the different MCG formulations containing *S. tortuosum* crude extract. Inclusion of different excipients was kept to a minimum. The inclusion of magnesium stearate (0.5% w/w) was necessary in order to alter the problematic property (i.e. stickiness) of the *S. tortuosum* extract powder in the various formulations.



Figure 5.1 *Final product: Medicated chewing gum containing *Sceletium tortuosum* crude extract*

- Evaluation of the different MCG formulations in terms of the physical properties as well as analysis by the SeDeM Expert Diagram System indicated that formulation S0.5HIG01 is most suited for development of MCG units by means of direct compression as it depicted the highest PPI (7.42) and GCI (7.06) values.
- Evaluation of the MCG units in terms of morphology, mass variation, crushing strength, diameter, thickness, tensile strength, friability and pharmaceutical availability were conducted. Although results indicated that all of the formulations are deemed successful and met the official criteria of the Pharmacopoeias (BP, 2016; EP, 2016), formulation S0.5HIG03 depicted values that indicated that this formulation produced MCG units that are more uniform in weight, harder and stronger compared to the other HIG base powder formulations.
- Dissolution studies were performed on selected directly compressed MCG units of formulations containing *S. tortuosum* extract powder. The dissolution studies were done

according to the BP (2016) using a specialised Chewing Gum Testing DRT-1 apparatus. Formulation S0.5HIG01 was deemed to be the most successful as it released a mesembrine alkaloid concentration of 48.981% and a mesembrenone alkaloid concentration of 54.475%.

5.2 FUTURE PROSPECTS

After completion of this study, the following propositions for future investigations are advised:

- Examine the effect of each of the different excipients in the formulations to determine how each excipient affects the release of the active ingredient from the MCG formulations to possibly increase the amount of alkaloids released during the dissolution process,
- Perform an assay test to determine whether the theoretical concentration of the *S. tortuosum* alkaloids is present in the MCG units of the different formulations. These results can then be used to determine whether a fraction of the *S. tortuosum* alkaloids are trapped in the HIG base powders by ascertaining the amount of *S. tortuosum* alkaloids present by means of HPLC,
- Evaluate the compatibility of the crude extract and mesembrine alkaloids with the directly compressible excipients by means of differential scanning calorimetry (DSC) and microcalorimetry,
- Develop, analyse and optimise a possible coating for the MCG units,
- Investigate the effect of standard ICH test conditions on the stability of optimised directly compressed *S. tortuosum* medicated chewing gum formulations in order to estimate shelf-life,
- Improve other inadequacies pointed out by the SeDeM Expert Diagram System through the possible addition of other excipients,
- Execution of clinical trials in order to determine the correct and optimal flavoring profile and to investigate buccal and sublingual absorption to determine the quantity of *S. tortuosum* alkaloids that will be bioavailable after trans-mucosal absorption,
- Consider additional manufacturing methods of production of MCGs (i.e. hot melting) and evaluate the different MCGs through comparing physico-chemical properties.

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ANNEXURE A

Excipients characteristics and evaluations

- A.1 Cafosa®'s Health In Gum base 01
- A.2 Cafosa®'s Health In Gum base 03
- A.3 Cafosa®'s Health In Gum base 04
- A.4 Frutarom's Cherry flavouring agent

A.1 Cafosa®'s Health In Gum base 01



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CAF-R4-06-PLA-03-G60
Rev 2 / Feb. 10

CERTIFICATE OF ANALYSIS AND METHOD OF TESTING**PRODUCT: HEALTH IN GUM PWD-01**

LOT No	PRODUCTION DATE	BEST BEFORE DATE
L110216114	NOV 2014	FEB 2016
CHARACTERISTICS	SPECIFICATIONS	VALUES
Aspect	Granulate	Pass
Colour	White to yellowish	Pass
Heavy Metals	less than 10 ppm	< 10
Arsenic	less than 1,5 ppm	< 1
Lead	less than 1,5 ppm	< 1
Antioxidant (Butylated hydroxytoluene)	max. 0,026 %	Passes test
Total plate count	max 10 ³ cfu/g	Pass
European Pharmacopoeia 5.1 cat3, 2.6.12		
Yeast / Mould	max 10 ² cfu/g	Pass
European Pharmacopoeia 5.1 cat3, 2.6.12		
Coliforms	Negative / absence in 1 g	Negative
European Pharmacopoeia 5.1 cat3, 2.6.13		
Salmonella	Negative / absence in 10 g	Negative
European Pharmacopoeia 5.1 cat3, 2.6.13		
Staphylococcus Aureus	< 10 cfu/g	Negative
European Pharmacopoeia 5.1 cat3, 2.6.13		
Particle size distribution	99 % through 1000 micron	Pass
Ash content	2,5 - 4,8 %	3,8 %
Compressibility Index	≤10 %	7,0 %

The above mentioned batch/es may contain traces of soybean lecithin.

All materials used comply with US FDA regulations and are in accordance with the Food Chemicals Codex Specifications.

This product complies with the Regulation (EC) 1829/2003 of the European Parliament and of the Council on genetically modified food and feed and Regulation (EC) 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from them and amending Directive 2001/18/EC, and it can be considered non-GMO to all intents and purposes.

Robert Aguilera
Quality Control Manager



A.2 Cafosa®'s Health In Gum base 03



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CAF-R4-06-PLA-03-G60
Rev. 2 / Feb. 10

CERTIFICATE OF ANALYSIS AND METHOD OF TESTING

PRODUCT: HEALTH IN GUM PWD-03

LOT No	PRODUCTION DATE	BEST BEFORE DATE	
L250216114	NOV 2014	FEB 2016	
CHARACTERISTICS	SPECIFICATIONS	VALUES	
Aspect	Granulate	Pass	
Colour	White to yellowish	Pass	
Heavy Metals	less than 10 ppm	< 10	
Arsenic	less than 1,5 ppm	< 0,1	
Lead	less than 1,5 ppm	< 0,1	
Antioxidant (Butylated hydroxytoluene)	max. 0,037 %	Passes test	
Total plate count	3 x 10 ³ cfu/g	Pass	
European Pharmacopoeia 5.1 cat3, 2.6.12			
Yeast / Mould	3 x 10 ² cfu/g	Pass	
European Pharmacopoeia 5.1 cat3, 2.6.12			
Coliforms	Negative / absence in 1 g	Negative	
European Pharmacopoeia 5.1 cat3, 2.6.13			
Salmonella	Negative / absence in 10 g	Negative	
European Pharmacopoeia 5.1 cat3, 2.6.13			
Staphylococcus Aureus	< 10 ufc/g	Negative	
European Pharmacopoeia 5.1 cat3, 2.6.13			
Particle size distribution	99 % through 1000 micron	Pass	
Ash content	2,3 – 5,0 %	3,5 %	
Compressibility Index	≤ 10 %	7,1 %	

The above mentioned batch/es may contain traces of soybean lecithin

All materials used comply with US FDA regulations and are in accordance with the Food Chemicals Codex Specifications.

This product complies with the Regulation (EC) 1829/2003 of the European Parliament and of the Council on genetically modified food and feed and Regulation (EC) 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from them and amending Directive 2001/18/EC, and it can be considered non-GMO to all intents and purposes.

Robert Aguilar
Quality Control Manager



A.3 Cafosa®'s Health In Gum base 04



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CAF-R4-06-PLA-03-G60
Rev. 2 / Feb. 10

CERTIFICATE OF ANALYSIS AND METHOD OF TESTING

PRODUCT: HEALTH IN GUM PWD-04

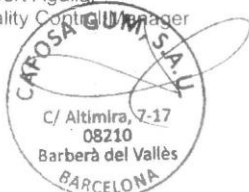
LOT No	PRODUCTION DATE	BEST BEFORE DATE	
L180616114	MAR 2015	JUN 2016	
CHARACTERISTICS	SPECIFICATIONS	VALUES	
Aspect	Granulate	Pass	
Colour	White to yellowish	Pass	
Heavy Metals	less than 10 ppm	< 10	
Arsenic	less than 1,5 ppm	< 1	
Lead	less than 1,5 ppm	< 1	
Antioxidant (Butylated hydroxytoluene)	max. 0,032 %	Passes test	
Total plate count	max 10 ³ cfu/g	Pass	
European Pharmacopoeia 5.1 cat3, 2.6.12			
Yeast / Mould	max 10 ² cfu/g	Pass	
European Pharmacopoeia 5.1 cat3, 2.6.12			
Coliforms	Negative / absence in 1 g	Negative	
European Pharmacopoeia 5.1 cat3, 2.6.13			
Salmonella	Negative / absence in 10 g	Negative	
European Pharmacopoeia 5.1 cat3, 2.6.13			
Staphylococcus Aureus	< 10 cfu/g	Negative	
European Pharmacopoeia 5.1 cat3, 2.6.13			
Particle size distribution	99 % through 1000 micron	Pass	
Ash content	2,6 - 5,1 %	4,4 %	
Compressibility Index	≤10 %	5,7 %	

The above mentioned batch/es may contain traces of soybean lecithin.

All materials used comply with US FDA regulations and are in accordance with the Food Chemicals Codex Specifications.

This product complies with the Regulation (EC) 1829/2003 of the European Parliament and of the Council on genetically modified food and feed and Regulation (EC) 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from them and amending Directive 2001/18/EC, and it can be considered non-GMO to all intents and purposes.

Robert Aguilar
Quality Control Manager



A.4 Frutarom's Cherry flavouring agent

FSA-QA-4-012



Rev : 0

Product Specification					
1.0					
PRODUCT NAME	ESPRAY® SD CHERRY N1HH NK		DATE	28/01/2015	
PRODUCT CODE	07.03.2008008SD		ISSUE NUMBER	002	
DESCRIPTION	A white to off-white powder with a fruity, typical cherry character		SUPERSEDES	001	
			PAGES	3	
			SPECIFICATION STATUS	INITIAL	
2.0					
SPECIFICATION		LOWER	UPPER	UNIT	
CHEMICAL Specifications are generated on the information from laboratory standard samples, final specifications are issued subject to average results of five manufactured batches.	CHEMICAL				
	pH	3.000	5.500	mV	METTLER
	SPECIFIC GRAVITY (20°C)				METTLER DE 40
	REFRACTIVE INDEX (20°C)				METTLER RE 40
	SALT (NaCl)			%	METTLER DL 50
	MOISTURE	0.000	6.000	%	METTLER DL 50
	ACID (CITRIC)			%	METTLER DL 50
	TDS (BRIX)			°B	METTLER RE 40
	PHYSICAL				
	APPEARANCE	A free flowing powder			
	COLOUR	White to off-white			
	FLAVOUR / ODOUR	Fruity, typical cherry character			
	SIEVE ANALYSIS	100% passes through 1500 microns			
	MICRO*				
		GMP LIMIT			
TMA = Total Microbial Activity	10 000	<	cfu/g	SANS 4833	
COLIFORMS	100	<	cfu/g	SANS 4832	
YEAST & MOULD	100	<	cfu/g	SANS 7954	
OTHER					
	THEORETICAL SODIUM (Na)		VALUE	UOM	
			8.00	mg/100g	
* Microbiological Analysis is not conducted on every batch unless per prior agreement. Specification based on Frutarom's GMP limits and verified from time to time across a range of products.					
3.0					
TYPICAL PROPERTIES	SOLUBILITY	N/A			
	FLASH POINT	N/A			
	LEGAL STATUS	NATURE IDENTICAL			
4.0					
SUITABILITY FOR DIET	VEGETARIAN	YES			
	VEGAN	YES			
	MUSLIM	YES			
	JEWISH	NO			
RELIGIOUS STATUS	HALAAL COMPLIANT	YES			
	HALAAL CERTIFIED	YES			
	KOSHER COMPLIANT	NO			
	KOSHER CERTIFIED	NO			
5.0					
GENETIC SENSITIVITY	GMO STATUS	NON GMO			
6.0					
INGREDIENT DECLARATION					
RAW MATERIAL INGREDIENT LIST					
*Note: Ingredients present at less than 10ppm (<0.001%) have been omitted.					
NO.	RAW MATERIALS (Descending Order)	E NUMBER			
1	MALTODEXTRIN (MAIZE STARCH, ALPHA AMYLASE)				
2	FLAVOURING NATURE IDENTICAL				
3	STARCH - MODIFIED MAIZE	E1450			

7.0

FOOD ALLERGEN INFORMATION	
*Note: Allergens containing materials present at less than 10ppm (<0.001%) have been omitted.	
DIRECT*	RAW MATERIALS
NO	MILK AND PRODUCTS THEREOF
NO	EGGS AND PRODUCTS THEREOF
NO	SOYBEANS AND PRODUCTS THEREOF
NO	CEREALS CONTAINING GLUTEN AND PRODUCTS THEREOF
NO	FISH AND PRODUCTS THEREOF
NO	CRUSTACEANS AND PRODUCTS THEREOF
NO	NUTS AND PRODUCTS THEREOF
NO	PEANUTS AND PRODUCTS THEREOF
NO	SESAME SEEDS AND PRODUCTS THEREOF
NO	SULPHUR DIOXIDE AND SULPHITES AND PRODUCTS THEREOF
NO	CELERY AND PRODUCTS THEREOF
NO	LUPIN AND PRODUCTS THEREOF
NO	MUSTARD AND PRODUCTS THEREOF
NO	MOLLUSCS AND PRODUCTS THEREOF

*DIRECT ALLERGENS: THESE ARE ALLERGENS THAT ARE IN THE FORMULATION.

AN ALLERGEN CONTROL PROGRAM IS IN PLACE.

THIS PRODUCT IS MANUFACTURED IN A FACILITY THAT USES INGREDIENTS THAT MAY CONTAIN MILK, EGGS, SOYBEANS, CEREALS CONTAINING GLUTEN, SESAME SEEDS, SULPHUR DIOXIDE, CELERY, MUSTARD; AND PRODUCTS THEREOF.

8.0

FOOD SENSITIVITY INFORMATION	
*Note: Ingredients present at less than 10ppm (<0.001%) have been omitted.	
DIRECT	
NO	ANTIOXIDANTS
NO	AZO COLOURS
NO	BEEF
NO	BENZOIC ACID & PHB (E210-E219)
NO	CARROT
NO	CHICKEN
NO	CINNAMON
NO	COCOA
NO	COCONUT
NO	CORIANDER
NO	DL METHIONINE (NOT RECOMMENDED FOR USE IN INFANT FOODS)
NO	GARLIC
NO	GINGER
NO	GLUTAMATE
NO	GLUTEN
NO	HONEY
NO	LACTOSE
NO	LEGUMES
YES	MAIZE
NO	ONION
NO	PENICILLIN - CHEESE BLUE
NO	PHENYLALANINE FROM ASPARTAME
NO	PORK
NO	PRESERVATIVES
NO	SULPHITES
NO	TARTRAZINE
NO	UMBELLIFERAE
NO	VANILLIN
NO	YEAST

9.0

SHELF LIFE	12 months from D.O.M when storage conditions are followed
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10.0

TRANSPORT	COOL, DARK, DRY CONDITIONS.
	KEEP OUT OF DIRECT SUNLIGHT.
	KEEP CONTAINERS SEALED AT ALL TIMES.

11.0

STORAGE	COOL, DARK, DRY WAREHOUSE CONDITIONS.
	KEEP OUT OF DIRECT SUNLIGHT.
	KEEP CONTAINERS SEALED AT ALL TIMES.

12.0

GENERAL PACKAGING	ALL PACKAGING IS LABELLED WITH THE PRODUCT NAME, PRODUCT CODE, NETT & TARE WEIGHT, ALLERGENS, MANUFACTURE DATE AND BEST BEFORE DATE.
	BATCH NUMBERS INDICATED ON EACH PACK TO CROSS REFERENCE RAW MATERIAL TRACEABILITY.
	WHERE POSSIBLE UN SPECIFICATION PACKAGING IS USED.
	POWDER PACKAGING: POLYETHYLENE BAGS WITH BLUE POLYETHYLENE LINERS. POLYETHYLENE BAGS IN DOUBLE WALL BOARD BOXES. BAGS ARE MANUFACTURED FROM VIRGIN MATERIAL.
	LIQUID PACKAGING: FLUORINATED HDPE CONTAINERS. PACKAGING IS MANUFACTURED FROM VIRGIN MATERIAL.

13.0

OTHER REQUIREMENTS	FOR FOOD INDUSTRY USE ONLY. NOT FOR DIRECT CONSUMPTION.
	PRODUCT IS FREE FROM UNWANTED FOREIGN HAZARDS AND CHEMICAL CONTAMINANTS.
	CERTIFICATE OF ANALYSIS (COA) WILL BE SENT WITH EACH DELIVERY.

THIS IS A SYSTEM GENERATED DOCUMENT. NO SIGNATURE IS REQUIRED.

ANNEXURE B

Proposed handling procedure

B.1 Health In Gum bases produced by Cafosa®

B.1 Health In Gum bases produced by Cafosa®

**Handling procedure for Health in Gum®**

1. Break the lumps, if any, with a vibrating sieve (1mm). Remove any piece that you cannot break manually
2. In the selected blender, add the powder gum, the active and any other ingredient in powder form (flavour in powder form, intensive sweeteners, colours...) following the suggested formulation enclosed. Mixing time will depend on the type of mixer and the batch size. Gentle mixing would need more time but it tends to be better than intensive mixing.
3. Add the liquid flavour so that it distributes homogeneously into the blend (for instance, spraying). If you cannot add the liquid to the mixer, you should need to make a premix between the flavour and some silicon dioxide.
4. After spraying, add between 0,5% of Silicon Dioxide with high oil absorption (for instance, Sipernat 50S from Evonik for food products or Syloid 244FP from Grace for pharma grade needings) and mix until you get a dry and free flowing powder.
5. Add lubricant (2% on the case of metallic stearates) into the blender and mix until homogeneity.
6. Sieve again, as some ingredients may agglomerate (liquid flavour and silicon dioxide).
7. Before adding the final mix to the pressing machine, add a small quantity of the selected lubricant to the tablet press and make run the machine slowly enough time for the lubricant to fill all the matrices and punches. This will reduce the initial stickiness found when the metal parts are clean.
8. Start compressing. Standard speeds are between 30k and 40k tablets·h⁻¹. Adjust pressure and weight, with final tablet hardness between 7 and 10kg.



Remarks:

- Silicon Dioxide can also be added before liquid flavour if wanted.
- Liquid Flavour should have Triacetin or oil as solvent to be chewing gum compatible.
- Make sure that the product does not heat up during the process, it will lose fluidity and become sticky (limit temperature: 27°C).
- For optimum textures, total content of Silicon Dioxide should not exceed 2%.
- Note that different pressing forces will have different effects on the tablet texture.
- Storage conditions: keep away from excessive heat in a dry cool area, in absence of direct natural or artificial light. Optimum conditions: 20°C - 50% RH.
- Please talk to your contact person at Cafosa for any question you might have.

Basic Troubleshooting

Problem	Solutions
✗ Powder sticking to punches	✓ Increase lubricant dose or ✓ Increase glidant dose or ✓ Cool the mix to 10-15°C, before compression
✗ Crumbliness of gum piece	✓ Add liquid flavour or ✓ Adjust tableting pressure or ✓ Increase Health in Gum amount in formulation
✗ Tablet too hard	✓ Adjust tableting pressure ✓ Increase liquid flavour content
✗ Coarse texture on gum	✓ Adjust glidant content or ✓ Reduce lubricant
✗ Bad taste of the API	✓ Adjust flavour combinations or ✓ Use masking technologies or ✓ Adjust intensive sweeteners
✗ Capping	✓ Adjust pressure or ✓ Increase liquid flavour level



Suggested Formulation

	HiG PWD-01	HiG PWD-03	HiG PWD-04
Health in Gum®	92,75 %	91,45 %	91,70 %
Powder Flavour	2,00 %	3,00 %	3,00 %
Encapsulated Flavour	2,00 %	2,00 %	2,00 %
Lubricant	2,00 %	2,00 %	1,50 %
Silicon Dioxide	0,55 %	0,90 %	0,70 %
Liquid Flavour	0,55 %	0,90 %	0,70 %
Intensive Sweeteners	0,20 %	0,20 %	0,20 %

These are placebo formulas. The active ingredient should be added into the formula by decreasing the equivalent Health in Gum® powder percentage.

These formulations may require some adjustment depending upon desired chewing characteristics, specific chewing gum raw materials used, available equipment and process conditions. Our Advisory Service Team is at your service for further information.

*This information is **CONFIDENTIAL**.*

Barcelona, January 2015

ANNEXURE C

Homogeneity index calculations

The homogeneity index tests were done in duplicate in order to calculate relative standard deviation (%RSD) and to assure accuracy. Therefore, Sample 1 or 2 are indicated after the following tests:

- C.1 *S. tortuosum* powder extract
- C.2 Health In Gum base 01
- C.3 Health In Gum base 03
- C.4 Health In Gum base 04
- C.5 Formulation S0HIG01
- C.6 Formulation S0HIG03
- C.7 Formulation S0HIG04
- C.8 Formulation S0.5HIG01
- C.9 Formulation S0.5HIG03
- C.10 Formulation S0.5HIG04

C.1 *S. tortuosum* powder extract (Sample 1)

Homogeneity index *S. tortuosum* no 1
Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0.06	
1180	1.33	
1000	3.61	
850	6.73	
710	8.48	
600	10.54	
500	9.58	
425	8.82	
355	7.87	57.02
300	6.91	
250	4.99	
212	4.08	15.98
180	3.9	
150	3.51	
125	2.94	
106	2.69	13.04
90	2.64	
75	2.1	
63	1.65	
53	1.2	7.59
45	0.95	
38	0	
0	0	0.95
Total	94.58	94.58

Sieve size	Average %	STD DEV	%RSD
> 355µm	57.02	0	0
355µm - 212µm	15.98	0	0
212µm - 100µm	13.04	0	0
100µm - 50µm	7.59	0	0
< 50µm	0.95	0	0
Total	94.58		

Homogeneity index I ₀			
Excipient Name			
Sieve size	AVG Diam	% in Frac	Difference in Diameter
355-500	427	57.02 Fm	dm 0
212-355	283	15.98 Fm-1	dm-(dm-1) 144
100-212	156	13.04 Fm-2	dm-(dm-2) 271
50-100	75	7.59 Fm-3	dm-(dm-3) 352
0-50	25	0.95 Fm-4	dm-(dm-4) 402

Excipient Name
I₀ 0.006344
%< 50µm 0.95

C.1 *S. tortuosum* powder extract (Sample 2)Homogeneity index *S. tortuosum* no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0	
1180	0.69	
1000	3.35	
850	6.44	
710	8.41	
600	10.69	
500	9.85	
425	10.2	
355	8.2	57.83
300	7.18	
250	5.12	
212	4.11	16.41
180	3.87	
150	3.44	
125	2.89	
106	2.64	12.84
90	2.61	
75	2.08	
63	1.63	
53	1.19	7.51
45	0.94	
38	0	
0	0	0.94
Total	95.53	95.53

Sieve size	Average %	STD DEV	%RSD
> 355µm	57.83	0	0
355µm - 212µm	16.41	0	0
212µm - 106µm	12.84	0	0
106µm - 53µm	7.51	0	0
< 50µm	0.94	0	0
Total	95.53		

Homogeneity index I ₀			
Excipient Name			
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter
355-500	427	57.425 Fm	dm
212-355	283	16.195 Fm-1	dm-(dm-1)
100-212	156	12.94 Fm-2	dm-(dm-2)
50-100	75	7.55 Fm-3	dm-(dm-3)
0-50	25	0.945 Fm-4	dm-(dm-4)

Excipient Name

I₀ 0.006397 $\times 10^{-2}$ ∴ 0,6397.

%< 50µm 0.945

C.2 Health In Gum base 01 (Sample 1)

Homogeneity index HIG base 01 no 1

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000		
1700		
1400		
1180	0.43	
1000	2.28	
850	5	
710	7.41	
600	10.74	
500	11.3	
425	13.26	
355	11.97	62.39
300	11.4	
250	8.35	
212	6.27	26.02
180	4.82	
150	2.98	
125	1.56	
106	0.87	10.23
90	0.52	
75	0.28	
63	0.19	
53	0.14	1.13
45	0.12	
38	0	
0	0	0.12
Total	99.89	99.89

Sieve size	Average %	STD DEV	%RSD
> 355µm	62.39	0	0
355µm - 212µm	26.02	0	0
212µm - 106µm	10.23	0	0
106µm - 53µm	1.13	0	0
< 50µm	0.12	0	0
Total	99.89		

Homogeneity index IØ

Excipient Name			
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter
355-500	427	62.39 Fm	dm
212-355	283	26.02 Fm-1	dm-(dm-1)
100-212	156	10.23 Fm-2	dm-(dm-2)
50-100	75	1.13 Fm-3	dm-(dm-3)
0-50	25	0.12 Fm-4	dm-(dm-4)

Excipient Name

IØ 0.008831

%< 50µm 0.12

C.2 Health In Gum base 01 (Sample 2)

Homogeneity index HIG base 01 no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000		
1700	0	
1400	0	
1180	0.34	
1000	2	
850	4.64	
710	7.05	
600	10.44	
500	11.17	
425	13.3	
355	12.16	61.1
300	11.7	
250	8.63	
212	6.5	26.83
180	4.99	
150	3.07	
125	1.59	
106	0.88	10.53
90	0.52	
75	0.28	
63	0.2	
53	0.16	1.16
45	0.14	
38	0	
0	0	0.14
Total	99.76	99.76

Sieve size	Average %	STD DEV	%RSD
> 355µm	61.1	0	0
355µm - 212µm	26.83	0	0
212µm - 106µm	10.53	0	0
106µm - 53µm	1.16	0	0
< 50µm	0.14	0	0
Total	99.76		

Homogeneity index Iθ		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter	
355-500	427	61.745 Fm	dm	0
212-355	283	26.425 Fm-1	dm-(dm-1)	144
100-212	156	10.38 Fm-2	dm-(dm-2)	271
50-100	75	1.145 Fm-3	dm-(dm-3)	352
0-50	25	0.13 Fm-4	dm-(dm-4)	402

Excipient Name

Iθ 0.008607

%< 50µm 0.13

C.3 Health In Gum base 03 (Sample 1)

Homogeneity index HIG base 03 no 1

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0.06	
1700	0.48	
1400	1.31	
1180	2.56	
1000	4.09	
850	6.56	
710	8.01	
600	10.32	
500	10.11	
425	11.43	
355	10.21	65.08
300	9.84	
250	7.4	
212	5.74	22.98
180	4.56	
150	2.89	
125	1.5	
106	0.79	9.74
90	0.4	
75	0.18	
63	0.13	
53	0.13	0.84
45	0.15	
38	0	
0	0	0.15
Total	98.85	98.79

Sieve size	Average %	STD DEV	%RSD
> 355µm	65.08	0	0
355µm - 212µm	22.98	0	0
212µm - 106µm	9.74	0	0
100µm - 50µm	0.84	0	0
< 50µm	0.15	0	0
Total	98.79		

Homogeneity index iθ		Excipient Name	
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter
355-500	427	65.08 Fm	dm
212-355	283	22.98 Fm-1	dm-(dm-1)
100-212	156	9.74 Fm-2	dm-(dm-2)
50-100	75	0.84 Fm-3	dm-(dm-3)
0-50	25	0.15 Fm-4	dm-(dm-4)

Excipient Name

iθ 0.010161

%< 50µm 0.15

C.3 Health In Gum base 03 (Sample 2)

Homogeneity index HIG base 03 no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0.05	
1400	0.5	
1180	1.9	
1000	3.53	
850	6.18	
710	7.89	
600	10.45	
500	10.44	
425	11.97	
355	10.79	63.7
300	10.44	
250	7.83	
212	6.03	24.3
180	4.73	
150	2.94	
125	1.49	
106	0.77	9.93
90	0.39	
75	0.18	
63	0.14	
53	0.14	0.85
45	0.15	
38	0	
0	0	0.15
Total	98.93	98.93

Sieve size	Average %	STD DEV	%RSD
> 355µm	63.7	0	0
355µm - 212µm	24.3	0	0
212µm - 106µm	9.93	0	0
100µm - 50µm	0.85	0	0
< 50µm	0.15	0	0
Total	98.93		

Homogeneity index Iθ		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter	
355-500	427	63.7 Fm	dm	0
212-355	283	24.3 Fm-1	dm-(dm-1)	144
100-212	156	9.93 Fm-2	dm-(dm-2)	271
50-100	75	0.85 Fm-3	dm-(dm-3)	352
0-50	25	0.15 Fm-4	dm-(dm-4)	402

Excipient Name

Iθ 0.009579

%< 50µm 0.15

C.4 Health In Gum base 04 (Sample 1)

Homogeneity index HIG base 04 no1

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0	
1180	0.52	
1000	3.08	
850	6.57	
710	8.9	
600	11.75	
500	11.36	
425	12.44	
355	10.64	65.26
300	9.76	
250	6.98	
212	5.21	21.95
180	4.06	
150	2.62	
125	1.51	
106	0.99	9.18
90	0.73	
75	0.49	
63	0.36	
53	0.26	1.84
45	0.21	
38	0	
0	0	0.21
Total	98.44	98.44

Sieve size	Average %	STD DEV	%RSD
> 355µm	65.26	0	0
355µm - 212µm	21.95	0	0
212µm - 106µm	9.18	0	0
100µm - 50µm	1.84	0	0
< 50µm	0.21	0	0
Total	98.44		

Homogeneity index Iθ			
Excipient Name			
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter
355-500	427	65.26 Fm	dm
212-355	283	21.95 Fm-1	dm-(dm-1)
100-212	156	9.18 Fm-2	dm-(dm-2)
50-100	75	1.84 Fm-3	dm-(dm-3)
0-50	25	0.21 Fm-4	dm-(dm-4)

Excipient Name
Iθ 0.01007
%< 50µm 0.21

C.4 Health In Gum base 04 (Sample 2)

Homogeneity index HIG base 04 no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0	
1180	0.08	
1000	1.98	
850	5.86	
710	8.58	
600	11.79	
500	11.64	
425	12.87	
355	11.05	63.85
300	10.14	
250	7.26	
212	5.42	22.82
180	4.23	
150	2.75	
125	1.59	
106	1.04	9.61
90	0.77	
75	0.51	
63	0.37	
53	0.27	1.92
45	0.21	
38	0	
0	0	0.21
Total	98.41	98.41

Sieve size	Average %	STD DEV	%RSD
> 355µm	63.85	0	0
355µm - 212	22.82	0	0
212µm - 106	9.61	0	0
100µm - 50	1.92	0	0
< 50µm	0.21	0	0
Total	98.41		

Homogeneity index Iθ			
Excipient Name			
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter
355-500	427	63.85 Fm	dm
212-355	283	22.82 Fm-1	dm-(dm-1)
100-212	156	9.61 Fm-2	dm-(dm-2)
50-100	75	1.92 Fm-3	dm-(dm-3)
0-50	25	0.21 Fm-4	dm-(dm-4)

Excipient Name
Iθ 0.009458
%< 50µm 0.21

C.5 Formulation S0HIG01 (Sample 1)

Homogeneity index Formula 1: HIG 01 no 1

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0.53	
1700	1.65	
1400	2.82	
1180	4.33	
1000	5.93	
850	8.44	
710	8.31	
600	10.95	
500	9.86	
425	10.3	
355	8.5	63.12
300	7.63	
250	5.43	
212	4.12	17.18
180	3.37	
150	2.38	
125	1.53	
106	1.09	8.37
90	0.83	
75	0.5	
63	0.29	
53	0.14	1.76
45	0.06	
38	0	
0	0	0.06
Total	98.99	90.49

Sieve size	Average %	STD DEV	%RSD
> 355µm	63.12	0	0
355µm - 212µm	17.18	0	0
212µm - 106µm	8.37	0	0
100µm - 50µm	1.76	0	0
< 50µm	0.06	0	0
Total	90.49		

Homogeneity index Iθ			
Excipient Name			
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter
355-500	427	63.12 Fm	dm
212-355	283	17.18 Fm-1	dm-(dm-1)
100-212	156	8.37 Fm-2	dm-(dm-2)
50-100	75	1.76 Fm-3	dm-(dm-3)
0-50	25	0.06 Fm-4	dm-(dm-4)

Excipient Name
Iθ 0.011506
%< 50µm 0.06

C.5 Formulation S0HIG01 (Sample 2)

Homogeneity index Formula 1: HIG 01 no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0.57	
1700	1.79	
1400	3.07	
1180	4.69	
1000	6.39	
850	8.97	
710	9.73	
600	11.23	
500	9.9	
425	10.09	
355	8.14	74
300	7.14	
250	5	
212	3.75	15.89
180	3.06	
150	2.19	
125	1.43	
106	1.04	7.72
90	0.81	
75	0.5	
63	0.29	
53	0.14	1.74
45	0.06	
38	0	
0	0	0.06
Total	99.98	99.41

Sieve size	Average %	STD DEV	%RSD
> 355µm	74	0	0
355µm - 212µm	15.89	0	0
212µm - 106µm	7.72	0	0
106µm - 53µm	1.74	0	0
< 50µm	0.06	0	0
Total	99.41		

Homogeneity index I0		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter	
355-500	427	74 Fm	dm	0
212-355	283	15.89 Fm-1	dm-(dm-1)	144
100-212	156	7.72 Fm-2	dm-(dm-2)	271
50-100	75	1.74 Fm-3	dm-(dm-3)	352
0-50	25	0.06 Fm-4	dm-(dm-4)	402

Excipient Name

I0 0.014462

%< 50µm 0.06

C.6 Formulation S0HIG03 (Sample 1)

Homogeneity index Formula 1: HIG 03 no 1
 Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0	
1180	0	
1000	0.32	
850	1.82	
710	3.26	
600	5.29	
500	6.13	
425	8.01	
355	8.3	24.83
300	9.46	
250	8.69	
212	8.48	26.63
180	8.99	
150	8.17	
125	6.4	
106	5.23	28.79
90	4.41	
75	2.87	
63	1.74	
53	0.88	9.9
45	0.4	
38	0	
0	0	0.4
Total	98.85	90.55

Sieve size	Average %	STD DEV	%RSD
> 355µm	24.83	0	0
355µm - 212µm	26.63	0	0
212µm - 106µm	28.79	0	0
100µm - 50µm	9.9	0	0
< 50µm	0.4	0	0
Total	90.55		

Homogeneity index Iθ		Excipient Name		
Sieve sizes	AVG Diam	% in Frac	Difference in Diameter	
355-500	427	24.83 Fm	dm	0
212-355	283	26.63 Fm-1	dm-{dm-1}	144
100-212	156	28.79 Fm-2	dm-{dm-2}	271
50-100	75	9.9 Fm-3	dm-{dm-3}	352
0-50	25	0.4 Fm-4	dm-{dm-4}	402

Excipient Name
 Iθ 0.001614
 %< 50µm 0.4

C.6 Formulation S0HIG03 (Sample 2)

Homogeneity index Formula 1: HIG 03 no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0	
1180	0.07	
1000	0.62	
850	2.19	
710	3.51	
600	5.45	
500	6.18	
425	8	
355	8.26	34.28
300	9.42	
250	8.63	
212	8.4	26.45
180	8.86	
150	7.99	
125	6.22	
106	5.06	28.13
90	4.24	
75	2.76	
63	1.67	
53	0.85	9.52
45	0.4	
38	0	
0	0	0.4
Total	98.78	98.78

Sieve size	Average %	STD DEV	%RSD
> 355µm	34.28	0	0
355µm - 212µm	26.45	0	0
212µm - 106µm	28.13	0	0
100µm - 50µm	9.52	0	0
< 50µm	0.4	0	0
Total	98.78		

Homogeneity index Iθ		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter	
355-500	427	34.28 Fm	dm	0
212-355	283	26.45 Fm-1	dm-(dm-1)	144
100-212	156	28.13 Fm-2	dm-(dm-2)	271
50-100	75	9.52 Fm-3	dm-(dm-3)	352
0-50	25	0.4 Fm-4	dm-(dm-4)	402

Excipient Name

Iθ 0.002279

%< 50µm 0.4

C.7 Formulation S0HIG04 (Sample 1)

Homogeneity index Formula 1: HIG 04 no 1
 Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0.04	
1700	0.44	
1400	1.26	
1180	2.48	
1000	3.9	
850	6.12	
710	7.36	
600	9.45	
500	9.32	
425	10.67	
355	9.67	51.04
300	9.49	
250	7.31	
212	5.89	22.69
180	5.01	
150	3.57	
125	2.24	
106	1.52	12.34
90	1.09	
75	0.63	
63	0.37	
53	0.21	2.3
45	0.14	
38	0	
0	0	0.14
Total	98.18	88.51

Sieve size	Average %	STD DEV	%RSD
> 355µm	51.04	0	0
355µm - 212µm	22.69	0	0
212µm - 106µm	12.34	0	0
100µm - 50µm	2.3	0	0
< 50µm	0.14	0	0
Total	88.51		

Homogeneity index I0		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter	
355-500	427	51.04 Fm	dm	0
212-355	283	22.69 Fm-1	dm-(dm-1)	144
100-212	156	12.34 Fm-2	dm-(dm-2)	271
50-100	75	2.3 Fm-3	dm-(dm-3)	352
0-50	25	0.14 Fm-4	dm-(dm-4)	402

Excipient Name
 I0 0.006736
 %< 50µm 0.14

C.7 Formulation S0HIG04 (Sample 2)

Homogeneity index Formula 1: HIG 04 no 2
 Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0.07	
1180	1.05	
1000	2.71	
850	5.28	
710	7.13	
600	9.75	
500	9.89	
425	11.44	
355	10.38	57.7
300	10.16	
250	7.81	
212	6.31	24.28
180	5.39	
150	3.88	
125	2.45	
106	1.67	13.39
90	1.2	
75	0.69	
63	0.39	
53	0.21	2.49
45	0.14	
38	0	
0	0	0.14
Total	98	98

Sieve size	Average %	STD DEV	%RSD
> 355µm	57.7	0	0
355µm - 212µm	24.28	0	0
212µm - 106µm	13.39	0	0
100µm - 50µm	2.49	0	0
< 50µm	0.14	0	0
Total	98		

Homogeneity index Iθ		Excipient Name			
Sieve sizes	AVG Diams	% in Frac		Difference in Diameter	
355-500	427	57.7	Fm	dm	0
212-355	283	24.28	Fm-1	dm-(dm-1)	144
100-212	156	13.39	Fm-2	dm-(dm-2)	271
50-100	75	2.49	Fm-3	dm-(dm-3)	352
0-50	25	0.14	Fm-4	dm-(dm-4)	402

Excipient Name
 Iθ 0.007073
 %< 50µm 0.14

C.8 Formulation S0.5HIG01 (Sample 1)

Homogeneity index Formula 2: HIG 01 no 1
 Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0.29	
1700	1.36	
1400	2.93	
1180	4.96	
1000	7.02	
850	9.98	
710	10.85	
600	12.56	
500	11.1	
425	11.28	
355	8.9	72.33
300	7.37	
250	4.6	
212	2.85	14.82
180	1.69	
150	0.73	
125	0.27	
106	0.16	2.85
90	0.19	
75	0.22	
63	0.23	
53	0.18	0.82
45	0.14	
38	0	
0	0	0.14
Total	99.86	90.96

Sieve size	Average %	STD DEV	%RSD
> 355µm	72.33	0	0
355µm - 212µm	14.82	0	0
212µm - 106µm	2.85	0	0
100µm - 50µm	0.82	0	0
< 50µm	0.14	0	0
Total	90.96		

Homogeneity index Iθ		Excipient Name			
Sieve sizes	AVG Diams	% in Frac		Difference in Diameter	
355-500	427	72.33	Fm	dm	0
212-355	283	14.82	Fm-1	dm-(dm-1)	144
100-212	156	2.85	Fm-2	dm-(dm-2)	271
50-100	75	0.82	Fm-3	dm-(dm-3)	352
0-50	25	0.14	Fm-4	dm-(dm-4)	402

Excipient Name
 Iθ 0.021582
 %< 50µm 0.14

C.8 Formulation S0.5HIG01 (Sample 2)

Homogeneity index Formula 2: HIG 01 no 2
Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0.02	
1400	0.9	
1180	2.97	
1000	5.41	
850	8.97	
710	10.8	
600	13.37	
500	12.3	
425	12.77	
355	10.15	77.66
300	8.37	
250	5.14	
212	3.12	16.63
180	1.79	
150	0.73	
125	0.27	
106	0.18	2.97
90	0.24	
75	0.28	
63	0.28	
53	0.23	1.03
45	0.17	
38	0	
0	0	0.17
Total	98.46	98.46

Sieve size	Average %	STD DEV	%RSD
> 355µm	77.66	0	0
355µm - 212µm	16.63	0	0
212µm - 106µm	2.97	0	0
100µm - 50µm	1.03	0	0
< 50µm	0.17	0	0
Total	98.46		

Homogeneity index Iθ		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac		Difference in Diameter
355-500	427	77.66	Fm	dm
212-355	283	16.63	Fm-1	dm-(dm-1)
100-212	156	2.97	Fm-2	dm-(dm-2)
50-100	75	1.03	Fm-3	dm-(dm-3)
0-50	25	0.17	Fm-4	dm-(dm-4)

Excipient Name
Iθ 0.020818
%< 50µm 0.17

C.9 Formulation S0.5HIG03 (Sample 1)

Homogeneity index Formula 2: HIG 03 no 1

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0.06	
1180	0.78	
1000	2.02	
850	3.99	
710	5.52	
600	7.77	
500	8.17	
425	9.81	
355	9.3	38.12
300	9.58	
250	7.84	
212	6.81	24.23
180	6.43	
150	5.29	
125	3.91	
106	3.16	18.79
90	2.76	
75	1.98	
63	1.41	
53	0.91	7.06
45	0.61	
38	0	
0	0	0.61
Total	98.11	88.81

Sieve size	Average %	STD DEV	%RSD
> 355µm	38.12	0	0
355µm - 212µm	24.23	0	0
212µm - 106µm	18.79	0	0
106µm - 53µm	7.06	0	0
< 50µm	0.61	0	0
Total	88.81		

Homogeneity index Iθ		Excipient Name			
Sieve sizes	AVG Diameter	% in Frac		Difference in Diameter	
355-500	427	38.12	Fm	dm	0
212-355	283	24.23	Fm-1	dm-(dm-1)	144
100-212	156	18.79	Fm-2	dm-(dm-2)	271
50-100	75	7.06	Fm-3	dm-(dm-3)	352
0-50	25	0.61	Fm-4	dm-(dm-4)	402

Excipient Name
Iθ 0.00334
%< 50µm 0.61

C.9 Formulation S0.5HIG03 (Sample 2)

Homogeneity index Formula 2: HIG 03 no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0.1	
1400	1.23	
1180	2.6	
1000	4.09	
850	6.1	
710	6.85	
600	8.26	
500	7.81	
425	8.82	
355	8.12	53.98
300	8.32	
250	6.88	
212	6.06	21.26
180	5.78	
150	4.75	
125	3.47	
106	2.74	16.74
90	2.33	
75	1.62	
63	1.13	
53	0.72	5.8
45	0.49	
38	0	
0	0	0.49
Total	98.27	98.27

Sieve size	Average %	STD DEV	%RSD
> 355µm	53.98	0	0
355µm - 212µm	21.26	0	0
212µm - 106µm	16.74	0	0
100µm - 50µm	5.8	0	0
< 50µm	0.49	0	0
Total	98.27		

Homogeneity index Iθ		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac		Difference in Diameter
355-500	427	53.98	Fm	dm
212-355	283	21.26	Fm-1	dm-{dm-1}
100-212	156	16.74	Fm-2	dm-{dm-2}
50-100	75	5.8	Fm-3	dm-{dm-3}
0-50	25	0.49	Fm-4	dm-{dm-4}

Excipient Name

Iθ 0.005432

%< 50µm 0.49

C.10 Formulation S0.5HIG04 (Sample 1)

Homogeneity index Formula 2: HIG 04 no 1
 Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0	
1180	0.36	
1000	2.24	
850	5.15	
710	7.48	
600	10.56	
500	10.82	
425	12.42	
355	11	49.03
300	10.35	
250	7.57	
212	5.76	23.68
180	4.63	
150	3.16	
125	1.95	
106	1.35	11.09
90	1.03	
75	0.66	
63	0.44	
53	0.28	2.41
45	0.2	
38	0	
0	0	0.2
Total	97.41	86.41

Sieve size	Average %	STD DEV	%RSD
> 355µm	49.03	0	0
355µm - 212µm	23.68	0	0
212µm - 106µm	11.09	0	0
100µm - 50µm	2.41	0	0
< 50µm	0.2	0	0
Total	86.41		

Homogeneity index I0		Excipient Name		
Sieve sizes	AVG Diameter	% in Frac	Difference in Diameter	
355-500	427	49.03 Fm	dm	0
212-355	283	23.68 Fm-1	dm-(dm-1)	144
100-212	156	11.09 Fm-2	dm-(dm-2)	271
50-100	75	2.41 Fm-3	dm-(dm-3)	352
0-50	25	0.2 Fm-4	dm-(dm-4)	402

Excipient Name
 I0 0.006586
 %< 50µm 0.2

C.10 Formulation S0.5HIG04 (Sample 2)

Homogeneity index Formula 2: HIG 04 no 2

Sieve sizes 0.355mm, 0.212mm, 0.100mm, 0.05mm

Sieve size	Vol % on sieve	
2000	0	
1700	0	
1400	0	
1180	0.35	
1000	2.11	
850	4.81	
710	7.04	
600	10.06	
500	10.46	
425	12.2	
355	11.02	58.05
300	10.58	
250	7.88	
212	6.11	24.57
180	4.97	
150	3.39	
125	2.07	
106	1.4	11.83
90	1.04	
75	0.65	
63	0.43	
53	0.28	2.4
45	0.21	
38	0	
0	0	0.21
Total	97.06	97.06

Sieve size	Average %	STD DEV	%RSD
> 355µm	58.05	0	0
355µm - 212µm	24.57	0	0
212µm - 106µm	11.83	0	0
100µm - 50µm	2.4	0	0
< 50µm	0.21	0	0
Total	97.06		

Homogeneity index Iθ		Excipient Name			
Sieve sizes	AVG Diameter	% in Frac		Difference in Diameter	
355-500	427	58.05	Fm	dm	0
212-355	283	24.57	Fm-1	dm-(dm-1)	144
100-212	156	11.83	Fm-2	dm-(dm-2)	271
50-100	75	2.4	Fm-3	dm-(dm-3)	352
0-50	25	0.21	Fm-4	dm-(dm-4)	402

Excipient Name

Iθ 0.007468

%< 50µm 0.21

ANNEXURE D

Malvern particle size distribution reports

The particle size distribution tests were done in triplicate with three samples in order to assure accuracy. Therefore, Sample 1, 2 or 3 are indicated after the following tests:

- D.1 *S. tortuosum* powder extract
- D.2 Health In Gum base 01
- D.3 Health In Gum base 03
- D.4 Health In Gum base 04
- D.5 Formulation S0HIG01
- D.6 Formulation S0HIG03
- D.7 Formulation S0HIG04
- D.8 Formulation S0.5HIG01
- D.9 Formulation S0.5HIG03
- D.10 Formulation S0.5HIG04

D.1 *S. tortuosum* powder extract (Sample 1)

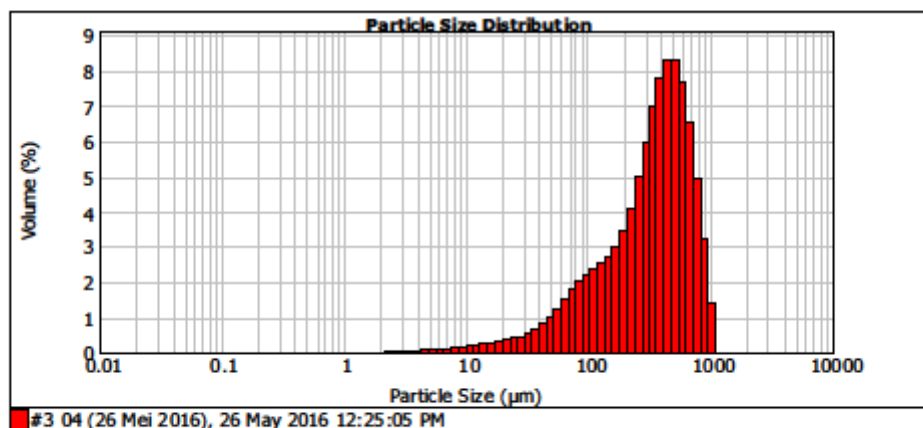
MASTERSIZER

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: #3 04 (26 Mei 2016)	SOP Name: Sonia van der Walt (Gum base)	Measured: 26 May 2016 12:25:05 PM	
Sample Source & type: Supplier = Sonja	Measured by: Jan Steenkamp	Analysed: 26 May 2016 12:25:06 PM	
Sample bulk lot ref: #3 04 (Sample 2)	Result Source: Measurement		
Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 12.26 %
Dispersant Name: Cyclohexane	Dispersant Rt: 1.426	Weighted Residual: 2.278 %	Result Emulation: Off
Concentration: 0.2242 %Vol	Span : 1.829	Uniformity: 0.564	Result units: Volume
Specific Surface Area: 0.0479 m²/g	Surface Weighted Mean D[3,2]: 125.271 um	Vol. Weighted Mean D[4,3]: 376.283 um	Density: 1.000 g/cm³

d(0.1): 73.249 um d(0.5): 352.465 um d(0.9): 717.825 um

Mesh No	Aperture μ m	Volume In %	Vol Below %	Mesh No	Aperture μ m	Volume In %	Vol Below %	Mesh No	Aperture μ m	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	9.85	70.43	120	125	2.89	18.44
10	1700	0.00	100.00	36	425	10.20	60.58	150	106	2.64	15.56
12	1400	0.00	100.00	44	355	8.20	50.38	170	90	2.61	12.91
14	1180	0.00	100.00	52	300	5.12	42.18	200	75	2.08	10.31
16	1000	0.69	99.31	60	250	5.12	34.99	240	63	1.63	8.23
18	850	6.44	95.97	72	212	4.11	29.87	300	53	1.19	6.60
22	710	8.41	89.53	85	180	3.87	25.76	350	45	0.94	5.41
25	600	10.69	81.12	100	150	3.44	21.89	400	38		4.47
30	500		70.43	120	125		18.44				



Operator notes:

D.1 S. tortuosum powder extract (Sample 2)



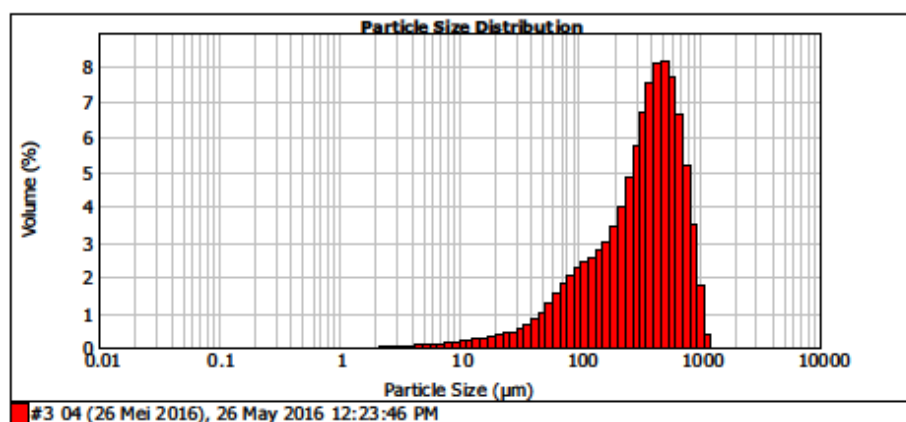
MASTERSIZER



Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: #3 04 (26 Mei 2016)	SOP Name: Sonja van der Walt (Gum base)	Measured: 26 May 2016 12:23:46 PM	
Sample Source & type: Supplier = Sonja	Measured by: Jan Steenekamp	Analyzed: 26 May 2016 12:23:48 PM	
Sample bulk lot ref: #3 04 (Sample 2)	Result Source: Measurement		
Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obecuration: 12.35 %
Dispersant Name: Cyclohexane	Dispersant RI: 1.426	Weighted Residual: 2.248 %	Result Emulation: Off
Concentration: 0.2273 %Vol	Span : 1.870	Uniformity: 0.58	Result units: Volume
Specific Surface Area: 0.0476 m ² /g	Surface Weighted Mean D[3,2]: 125.993 um	Vol. Weighted Mean D[4,3]: 383.787 um	Density: 1.000 g/cm ³
d[0.1]: 73.144 um	d[0.5]: 356.051 um	d[0.9]: 739.069 um	

Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	9.58	69.25	120	125	2.94	18.60
10	1700	0.00	100.00	36	425	9.82	59.67	150	106	2.69	15.66
12	1400	0.06	100.00	44	355	7.87	49.85	170	90	2.64	12.97
14	1180	1.33	99.94	52	300	6.91	41.98	200	75	2.10	10.33
16	1000	3.61	96.61	60	250	4.99	35.07	240	63	1.65	8.23
18	850	6.73	95.00	72	212	4.08	30.09	300	53	1.20	6.58
22	710	8.48	88.27	85	180	3.90	26.01	350	45	0.95	5.39
25	600	10.54	79.79	100	150	3.51	22.11	400	38		4.44
30	500		69.25	120	125		18.60				



Operator notes:

D.1 S. tortuosum powder extract (Sample 3)



MASTERSIZER



Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name:
#3 04 (26 Mei 2016)
Sample Source & type:
Supplier = Sonja
Sample bulk lot ref:
#3 04 (Sample 3)

SOP Name:
Sonja van der Walt (Gum base)
Measured by:
Jan Steenkamp
Result Source:
Measurement

Measured:
26 May 2016 12:31:52 PM
Analysed:
26 May 2016 12:31:53 PM

Particle Name:
Titanium Dioxide
Particle RI:
2.741
Dispersant Name:
Cyclohexane

Accessory Name:
Hydro 2000SM (A)
Absorption:
0.1
Dispersant RI:
1.426

Analysis model:
General purpose
Size range:
0.020 to 2000.000 μm
Weighted Residual:
2.324 %

Sensitivity:
Enhanced
Obscuration:
12.49 %
Result Emulation:
Off

Concentration:
0.2900 % Vol

Span :
1.725

Uniformity:
0.521

Result units:
Volume

Specific Surface Area:
0.0381 m^2/g

Surface Weighted Mean D[3,2]:
157.594 μm

Vol. Weighted Mean D[4,3]:
428.290 μm

Density:
1.000 g/cm^3

d(0.1): 91.215 μm

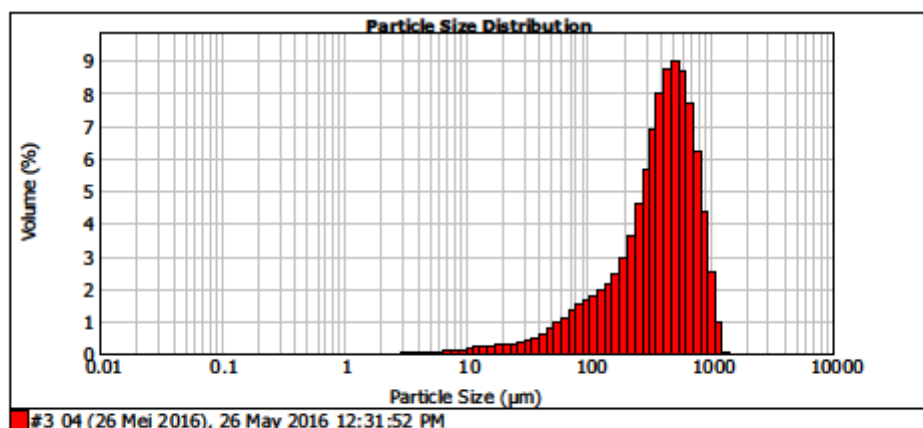
d(0.5): 404.784 μm

d(0.9): 789.557 μm

Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000	0.00	100.00
10	1700	0.00	100.00
12	1400	0.00	100.00
14	1180	0.26	99.74
16	1000	2.21	97.53
18	850	4.59	92.94
22	710	8.03	84.91
25	600	9.74	75.17
30	500	11.78	63.39

Mesh No	Aperture μm	Volume In %	Vol Below %
30	500	10.43	63.39
36	425	10.38	52.97
44	355	8.02	42.99
52	300	6.71	34.57
60	250	4.55	27.86
72	212	3.48	23.31
85	180	3.12	19.83
100	150	2.68	16.71
120	125		14.03

Mesh No	Aperture μm	Volume In %	Vol Below %
120	125	2.20	14.03
150	106	1.99	11.83
170	90	1.94	9.84
200	75	1.54	7.90
240	63	1.22	6.36
300	53	0.90	5.14
350	45	0.72	4.24
400	38		3.52



Operator notes:

D.2 Health In Gum base 01 (Sample 1)



MASTERSIZER 2000

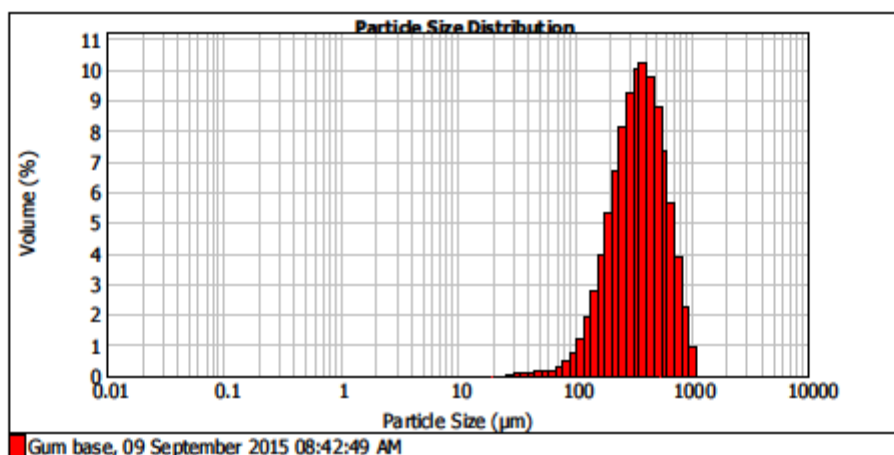
Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Sonia van der Walt (Gum base)	Measured: 09 September 2015 08:42:49 AM
Sample Source & type: Supplier	Measured by: Jan Steenekamp	Analysed: 09 September 2015 08:42:50 AM
Sample bulk lot ref: Sample 01-1 (S1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 10.38 %
Dispersant Name: Alcohol	Dispersant RI: 1.320	Weighted Residual: 1.388 %	Result Emulation: Off
Concentration: 0.4538 %Vol	Span : 1.397	Uniformity: 0.431	Result units: Volume
Specific Surface Area: 0.0205 m ² /g	Surface Weighted Mean D[3,2]: 292.110 um	Vol. Weighted Mean D[4,3]: 392.358 um	Density: 1.000 g/cm ³

d(0.1): 170.798 um d(0.5): 356.948 um d(0.9): 669.439 um

Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	11.30	74.15	120	125	1.56	3.81
10	1700	0.00	100.00	36	425	13.26	62.85	150	106	0.87	2.25
12	1400	0.00	100.00	44	355	11.97	49.60	170	90	0.52	1.38
14	1180	0.43	100.00	52	300	11.40	37.63	200	75	0.28	0.86
16	1000	2.28	99.57	60	250	8.35	26.23	240	63	0.19	0.57
18	850	5.00	97.29	72	212	6.27	17.87	300	53	0.14	0.39
22	710	7.41	92.30	85	180	4.82	11.61	350	45	0.12	0.25
25	600	10.74	84.89	100	150	2.98	6.79	400	38		0.13
30	500		74.15	120	125		3.81				



Operator notes:

D.2 Health In Gum base 01 (Sample 2)



MASTERSIZER 2000

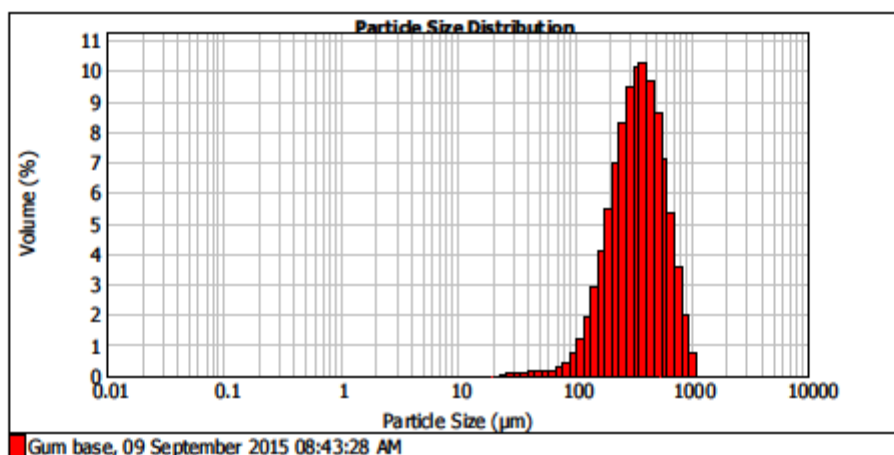
Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Sonia van der Walt (Gum base)	Measured: 09 September 2015 08:43:28 AM
Sample Source & type: Supplier	Measured by: Jan Steenekamp	Analysed: 09 September 2015 08:43:29 AM
Sample bulk lot ref: Sample 01-1 (S1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 10.54 %
Dispersant Name: Alcohol	Dispersant RI: 1.320	Weighted Residual: 1.420 %	Result Emulation: Off
Concentration: 0.4500 %Vol	Span : 1.391	Uniformity: 0.428	Result units: Volume
Specific Surface Area: 0.0211 m ² /g	Surface Weighted Mean D[3,2]: 284.978 um	Vol. Weighted Mean D[4,3]: 384.822 um	Density: 1.000 g/cm ³

d(0.1): 168.572 um d(0.5): 350.029 um d(0.9): 655.600 um

Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	11.17	75.52	120	125	1.59	3.99
10	1700	0.00	100.00	36	425	13.30	64.35	150	106	0.88	2.40
12	1400	0.00	100.00	44	355	12.16	51.05	170	90	0.52	1.52
14	1180	0.34	100.00	52	300	11.70	38.89	200	75	0.28	1.01
16	1000	2.00	99.66	60	250	8.63	27.18	240	63	0.20	0.72
18	850	4.64	97.66	72	212	6.50	18.56	300	53	0.16	0.53
22	710	7.05	93.01	85	180	4.99	12.06	350	45	0.14	0.37
25	600	10.44	85.96	100	150	3.07	3.99	400	38		0.23
30	500		75.52	120	125		3.99				



Operator notes:

D.2 Health In Gum base 01 (Sample 3)



MASTERSIZER 2000

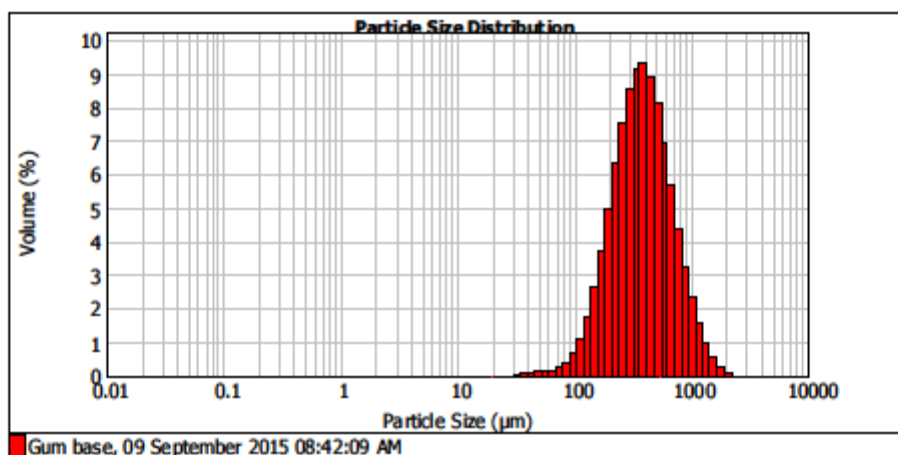
Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Sonia van der Walt (Gum base)	Measured: 09 September 2015 08:42:09 AM
Sample Source & type: Supplier	Measured by: Jan Steenekamp	Analysed: 09 September 2015 08:42:11 AM
Sample bulk lot ref: Sample D1-1 (S1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 10.29 %
Dispersant Name: Alcohol	Dispersant RI: 1.320	Weighted Residual: 1.465 %	Result Emulation: Off
Concentration: 0.4775 %Vol	Span : 1.669	Uniformity: 0.526	Result units: Volume
Specific Surface Area: 0.0193 m ² /g	Surface Weighted Mean D[3,2]: 310.091 um	Vol. Weighted Mean D[4,3]: 444.376 um	Density: 1.000 g/cm ³

d(0.1): 175.405 um d(0.5): 375.889 um d(0.9): 802.800 um

Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %
8	2000	0.24	100.00	30	500	10.32	68.56	120	125	1.45	3.42
10	1700	0.80	99.76	36	425	12.11	58.25	150	106	0.80	1.96
12	1400	1.44	98.32	44	355	11.02	46.14	170	90	0.47	1.16
14	1180	2.36	95.96	52	300	10.60	35.12	200	75	0.25	0.69
16	1000	3.55	92.41	60	250	7.83	24.52	240	63	0.16	0.44
18	850	5.73	86.68	72	212	5.91	16.68	300	53	0.12	0.28
22	710	7.31	79.37	85	180	4.55	10.77	350	45	0.10	0.15
25	600	9.99	69.38	100	150	2.80	3.42	400	38	0.10	0.05
30	500			120	125						



Operator notes:

D.3 Health In Gum base 03 (Sample 1)



Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

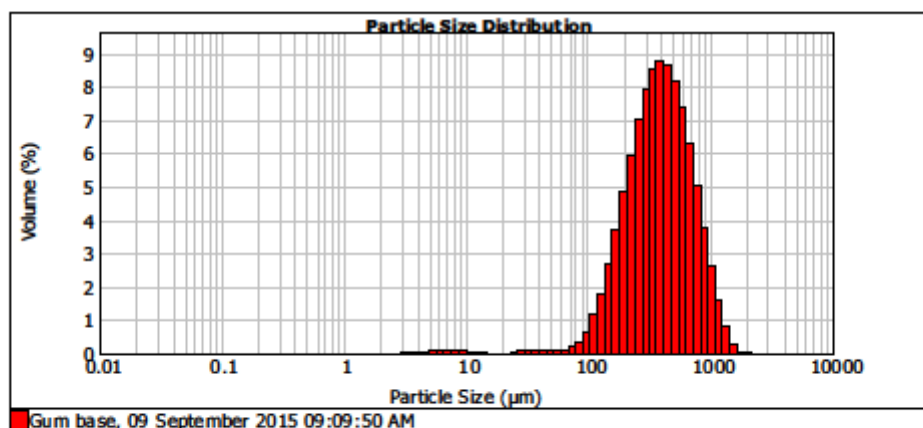
Sample Name: Gum base	SOP Name: Sonja van der Walt (Gum base)	Measured: 09 September 2015 09:09:50 AM
Sample Source & type: Supplier	Measured by: Jan Steenkamp	Analysed: 09 September 2015 09:09:51 AM
Sample bulk lot ref: Sample 03-1 (B1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obecuration: 12.63 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 0.937 %	Result Emulation: Off

Concentration: 0.3958 %Vol	Span : 1.674	Uniformity: 0.525	Result units: Volume
Specific Surface Area: 0.0282 m ² /g	Surface Weighted Mean D[3,2]: 212.510 um	Vol. Weighted Mean D[4,3]: 444.582 um	Density: 1.000 g/cm ³

d[0.1]: 168.363 um d[0.5]: 383.703 um d[0.9]: 810.819 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.06	100.00	30	500	66.62	66.62	120	125	1.50	4.44
10	1700	0.48	99.94	36	425	10.11	56.51	150	106	0.79	2.94
12	1400	1.31	99.46	44	355	11.43	45.08	170	90	0.40	2.16
14	1180	2.56	98.16	52	300	10.21	34.87	200	75	0.18	1.75
16	1000	4.09	95.60	60	250	7.40	25.03	240	63	0.13	1.57
18	850	6.56	91.51	72	212	5.74	17.63	300	53	0.13	1.45
22	710	8.01	84.95	85	180	4.56	11.89	350	45	0.13	1.32
25	600	10.32	76.95	100	150	2.89	7.33	400	38	0.15	1.17
30	500		66.62	120	125		4.44				



Operator notes:

Malvern Instruments Ltd.
Malvern, UK
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Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 9 Sep 2015.msa
Record Number: 15
2015/12/01 12:36:01 PM

D.3 Health In Gum base 03 (Sample 2)

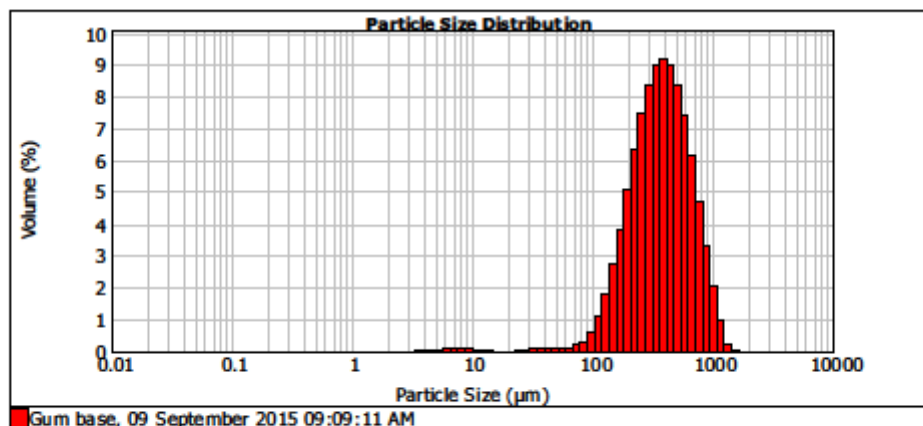


Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Sonja van der Walt (Gum base)	Measured: 09 September 2015 09:09:11 AM	
Sample Source & type: Supplier	Measured by: Jan Steenkamp	Analysed: 09 September 2015 09:09:12 AM	
Sample bulk lot ref: Sample 03-1 (B1)	Result Source: Measurement		
Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 μm	Obscuration: 12.42 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 0.942 %	Result Emulation: Off
Concentration: 0.4031 %Vol	Span : 1.570	Uniformity: 0.489	Result units: Volume
Specific Surface Area: 0.0274 m^2/g	Surface Weighted Mean D[3,2]: 219.300 μm	Vol. Weighted Mean D[4,3]: 420.186 μm	Density: 1.000 g/cm^3

d(0.1): 168.145 μm d(0.5): 370.877 μm d(0.9): 750.322 μm

Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	69.51	69.51	120	125	1.49	4.33
10	1700	0.00	100.00	36	425	10.44	59.07	150	106	0.77	2.84
12	1400	0.05	99.95	44	355	11.97	47.10	170	90	0.39	2.07
14	1180	1.90	99.45	52	300	10.44	36.31	200	75	0.18	1.69
16	1000	3.53	97.55	60	250	7.83	25.87	240	63	0.14	1.51
18	850	6.18	94.03	72	212	6.03	18.04	300	53	0.14	1.38
22	710	7.89	87.85	85	180	4.73	12.01	350	45	0.15	1.24
25	600	10.45	79.96	100	150	2.94	7.27	400	38		1.09
30	500		69.51	120	125		4.33				



Operator notes:

D.3 Health In Gum base 03 (Sample 3)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

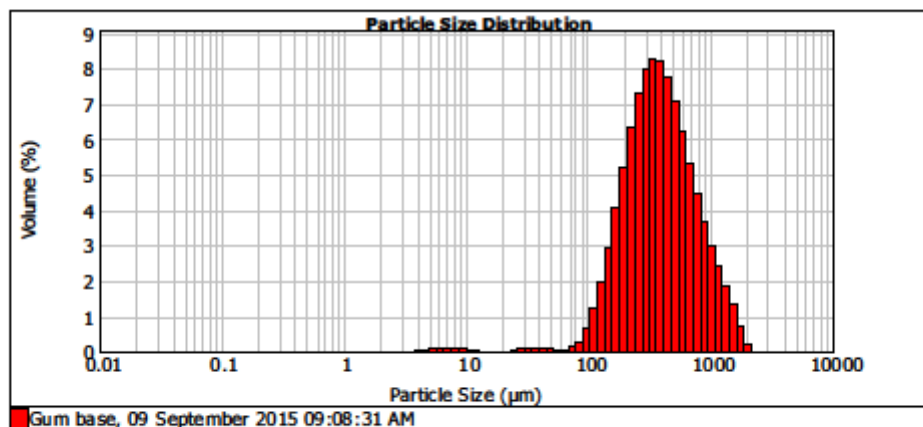
Sample Name: Gum base	SOP Name: Sonja van der Walt (Gum base)	Measured: 09 September 2015 09:08:31 AM
Sample Source & type: Supplier	Measured by: Jan Steenekamp	Analysed: 09 September 2015 09:08:32 AM
Sample bulk lot ref: Sample 03-1 (S1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 11.79 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 0.914 %	Result Emulation: Off

Concentration: 0.4194 %Vol	Span : 2.044	Uniformity: 0.628	Result units: Volume
Specific Surface Area: 0.025 m ² /g	Surface Weighted Mean D[3,2]: 240.046 um	Vol. Weighted Mean D[4,3]: 476.080 um	Density: 1.000 g/cm ³

d(0.1): 167.609 um d(0.5): 377.186 um d(0.9): 938.736 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.74	100.00	30	500	8.98	66.03	120	125	1.58	4.07
10	1700	1.89	99.26	36	425	10.68	57.04	150	106	0.78	2.49
12	1400	2.56	97.37	44	355	10.03	46.36	170	90	0.35	1.71
14	1180	3.31	94.81	52	300	10.09	36.33	200	75	0.12	1.36
16	1000	4.13	91.49	60	250	7.84	28.23	240	63	0.09	1.24
18	850	5.79	87.36	72	212	6.21	18.40	300	53	0.11	1.15
22	710	6.74	81.58	85	180	4.98	12.19	350	45	0.14	1.05
25	600	8.81	74.83	100	150	3.13	7.21	400	38		0.91
30	500		66.03	120	125		4.07				



Operator notes:

Malvern Instruments Ltd.
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Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007518

File name: 9 Sep 2015.msa
Record Number: 13
2016/12/01 12:34:52 PM

D.4 Health In Gum base 04 (Sample 1)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name:
Health In Gum (HIG) Number 4

Sample Source & type:
Supplier

Sample bulk lot ref:
050515114 (Sample 1)

SOP Name:
Hydro 2000 SM (RIIP - organic)

Measured by:
Jan Steenekamp

Result Source:
Measurement

Measured:
03 June 2015 02:50:18 PM

Analysed:
03 June 2015 02:50:19 PM

Particle Name:
Titanium Dioxide

Particle RI:
2.741

Dispersant Name:
Alcohol

Accessory Name:
Hydro 2000SM (A)

Absorption:
0.1

Dispersant RI:
1.320

Analysis model:
General purpose

Size range:
0.020 to 2000.000 μm

Weighted Residual:
2.560 %

Sensitivity:
Enhanced

Obscuration:
4.57 %

Result Emulation:
Off

Concentration:
0.1512 %Vol

Span :
1.453

Uniformity:
0.452

Result units:
Volume

Specific Surface Area:
0.026 m^2/g

Surface Weighted Mean D[3,2]:
230.824 μm

Vol. Weighted Mean D[4,3]:
410.510 μm

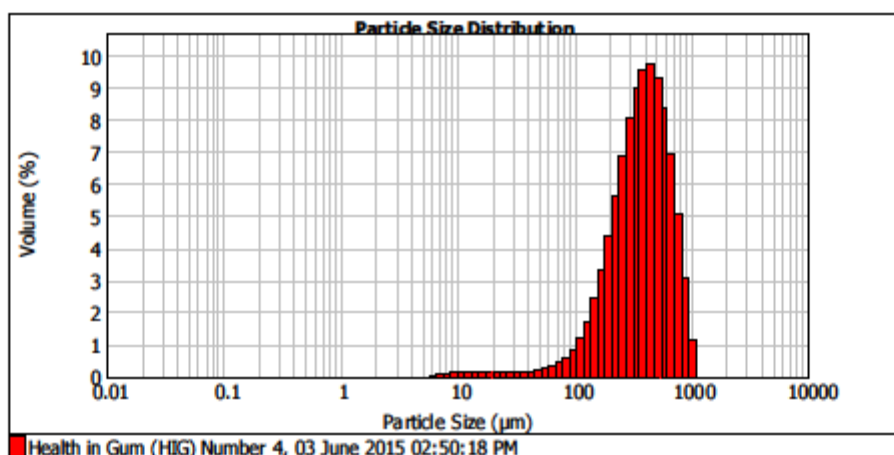
Density:
1.000 g/cm^3

d(0.1): 160.303 μm

d(0.5): 380.091 μm

d(0.9): 712.740 μm

Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	11.36	69.17	120	125	1.51	6.09
10	1700	0.00	100.00	36	425	12.44	57.81	150	106	0.99	4.58
12	1400	0.00	100.00	44	355	10.64	45.36	170	90	0.73	3.59
14	1180	0.52	100.00	52	300	9.76	34.72	200	75	0.49	2.86
16	1000	3.08	99.48	60	250	6.98	24.97	240	63	0.36	2.37
18	850	6.57	96.40	72	212	5.21	17.98	300	53	0.26	2.00
22	710	8.90	89.83	85	180	4.06	12.77	350	45	0.21	1.74
25	600	11.75	80.92	100	150	2.62	8.72	400	38		1.53
30	500		69.17	120	125		6.09				



Operator notes:

D.4 Health In Gum base 04 (Sample 2)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name:
Health In Gum (HIG) Number 4

SOP Name:
Hydro 2000 SM (RIIP - organic)

Measured:
03 June 2015 02:50:57 PM

Sample Source & type:
Supplier

Measured by:
Jan Steenkamp

Analysed:
03 June 2015 02:50:58 PM

Sample bulk lot ref:
050515114 (Sample 1)

Result Source:
Measurement

Particle Name:
Titanium Dioxide

Accessory Name:
Hydro 2000SM (A)

Analysis model:
General purpose

Sensitivity:
Enhanced

Particle RI:
2.741

Absorption:
0.1

Size range:
0.020 to 2000.000 um

Obscuration:
4.46 %

Dispersant Name:
Alcohol

Dispersant RI:
1.320

Weighted Residual:
2.523 %

Result Emulation:
Off

Concentration:
0.1441 %Vol

Span :
1.410

Uniformity:
0.438

Result units:
Volume

Specific Surface Area:
0.0266 m²/g

Surface Weighted Mean D[3,2]:
225.757 um

Vol. Weighted Mean D[4,3]:
395.264 um

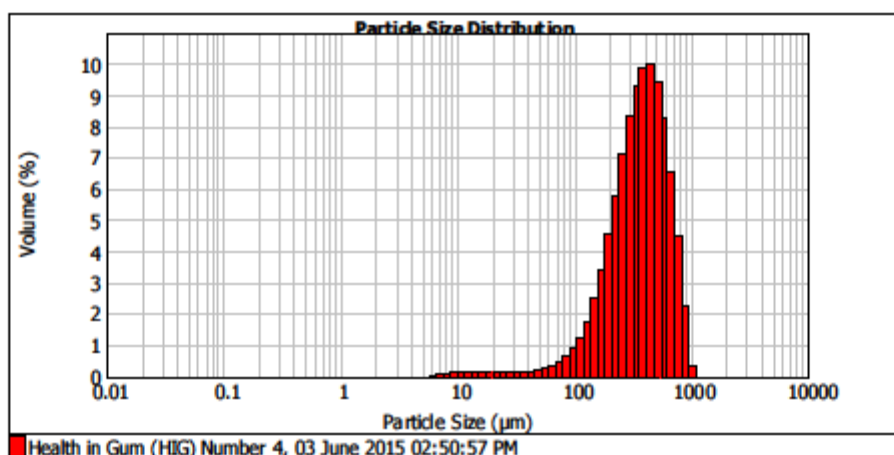
Density:
1.000 g/cm³

d(0.1): 157.031 um

d(0.5): 369.559 um

d(0.9): 677.962 um

Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	11.64	71.70	120	125	1.59	6.35
10	1700	0.00	100.00	36	425	12.87	60.06	150	106	1.04	4.76
12	1400	0.00	100.00	44	355	10.14	47.19	170	90	0.77	3.72
14	1180	0.00	100.00	52	300	7.26	36.14	200	75	0.51	2.95
16	1000	1.98	99.92	60	250	5.42	26.00	240	63	0.37	2.44
18	850	5.86	97.93	72	212	4.23	18.74	300	53	0.27	2.06
22	710	8.58	92.07	85	180	2.75	13.32	350	45	0.21	1.80
25	600	11.79	83.49	100	150		9.10	400	38		1.58
30	500		71.70	120	125		6.35				



Operator notes:

D.4 Health In Gum base 04 (Sample 3)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name:
Health In Gum (HIG) Number 4

SOP Name:
Hydro 2000 SM (RIIP - organic)

Measured:
03 June 2015 02:54:59 PM

Sample Source & type:
Supplier

Measured by:
Jan Steenekamp

Analysed:
03 June 2015 02:55:00 PM

Sample bulk lot ref:
050515114 (Sample 1)

Result Source:
Measurement

Particle Name:
Titanium Dioxide

Accessory Name:
Hydro 2000SM (A)

Analysis model:
General purpose

Sensitivity:
Enhanced

Particle RI:
2.741

Absorption:
0.1

Size range:
0.020 to 2000.000 um

Obscuration:
19.13 %

Dispersant Name:
Alcohol

Dispersant RI:
1.320

Weighted Residual:
1.870 %

Result Emulation:
Off

Concentration:
0.4718 %Vol

Span :
1.809

Uniformity:
0.563

Result units:
Volume

Specific Surface Area:
0.0375 m²/g

Surface Weighted Mean D[3,2]:
159.879 um

Vol. Weighted Mean D[4,3]:
331.239 um

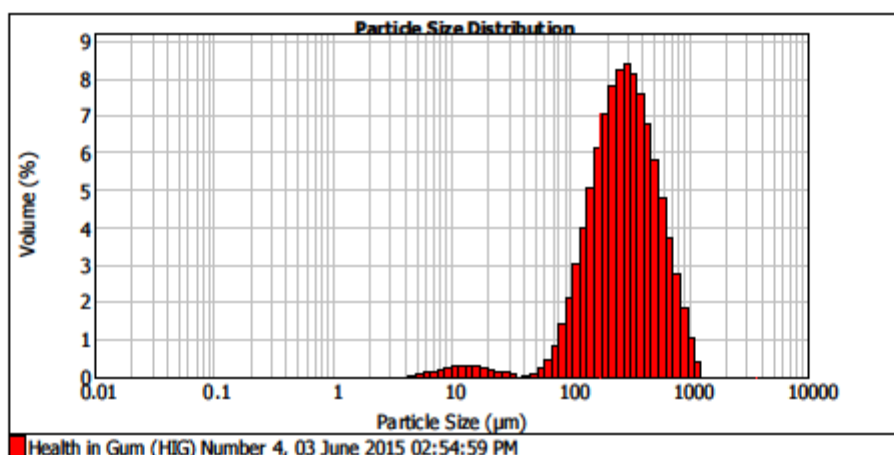
Density:
1.000 g/cm³

d(0.1): 117.291 um

d(0.5): 280.805 um

d(0.9): 625.275 um

Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %	Mesh No	Aperture um	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	7.73	81.63	120	125	3.78	11.62
10	1700	0.00	100.00	36	425	9.88	73.90	150	106	2.50	7.84
12	1400	0.00	100.00	44	355	10.01	64.02	170	90	1.63	5.34
14	1180	0.10	99.90	52	300	10.99	54.01	200	75	0.78	3.71
16	1000	0.88	99.03	60	250	9.42	43.02	240	63	0.32	2.94
18	850	1.91	97.12	72	212	8.35	33.60	300	53	0.09	2.62
22	710	3.58	93.54	85	180	7.73	25.24	350	45	0.00	2.53
25	600	4.88	88.66	100	150	5.89	17.51	400	38		
30	500	7.03	81.63	120	125		11.62				



Operator notes: rpm adjusted to 1500

D.5 Formulation SOHIG01 (Sample 1)



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Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

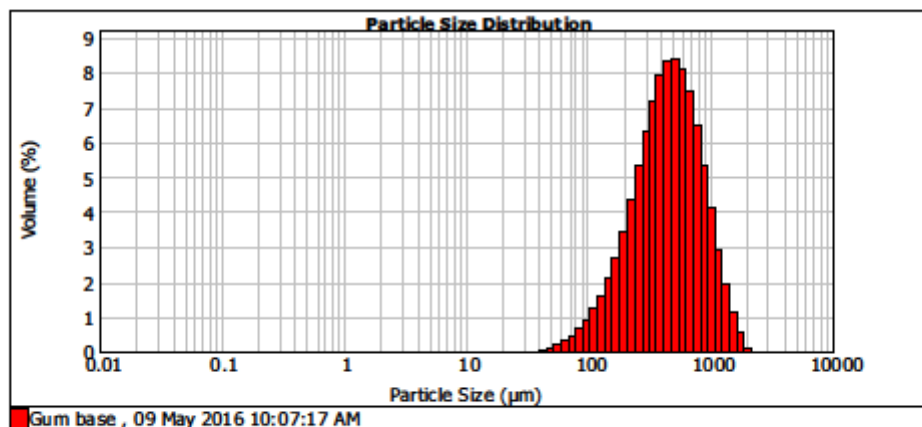
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:07:17 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:07:18 AM
Sample bulk lot ref: 201 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 14.85 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 1.899 %	Result Emulation: Off

Concentration: 0.7732 %Vol	Span : 1.768	Uniformity: 0.549	Result units: Volume
Specific Surface Area: 0.0177 m ² /g	Surface Weighted Mean D[3,2]: 339.463 um	Vol. Weighted Mean D[4,3]: 526.840 um	Density: 1.000 g/cm ³

d(0.1): 178.413 um d(0.5): 452.849 um d(0.9): 979.028 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.53	100.00	30	500	9.86	56.04	120	125	1.53	4.44
10	1700	1.65	99.47	36	425	10.30	46.18	150	106	1.09	2.91
12	1400	2.82	97.82	44	355	8.50	35.89	170	90	0.83	1.82
14	1180	4.33	95.00	52	300	7.63	27.38	200	75	0.50	0.99
16	1000	5.93	90.68	60	250	5.43	19.75	240	63	0.29	0.49
18	850	8.44	84.74	72	212	4.12	14.31	300	53	0.14	0.20
22	710	9.31	76.30	85	180	3.37	10.19	350	45	0.06	0.06
25	600	10.95	67.00	100	150	2.38	6.82	400	38		0.00
30	500		56.04	120	125		4.44				



Operator notes:

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Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007518

File name: 9 May 2016.mea
Record Number: 2
2016/12/01 02:56:02 PM

D.5 Formulation SOHIG01 (Sample 2)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

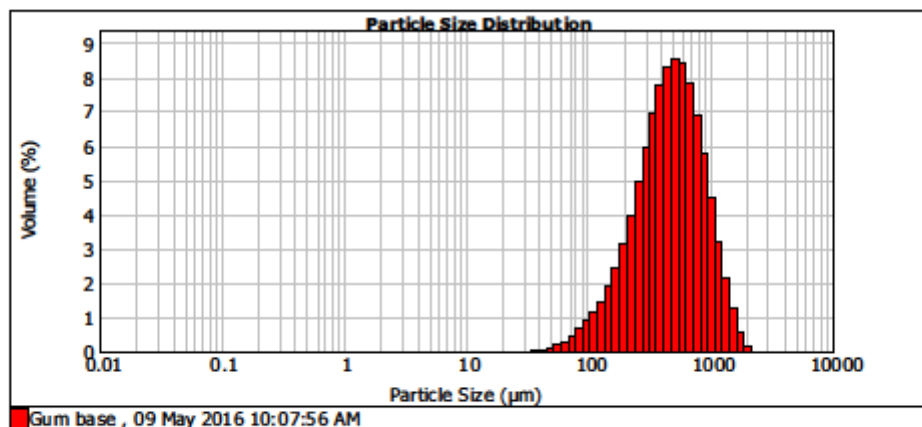
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:07:56 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:07:57 AM
Sample bulk lot ref: 201 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 μm	Obscuration: 14.88 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 1.886 %	Result Emulation: Off

Concentration: 0.7992 %Vol	Span : 1.736	Uniformity: 0.536	Result units: Volume
Specific Surface Area: 0.0171 m^2/g	Surface Weighted Mean D[3,2]: 350.079 μm	Vol. Weighted Mean D[4,3]: 543.839 μm	Density: 1.000 g/cm^3

d(0.1): 184.251 μm d(0.5): 472.076 μm d(0.9): 1003.702 μm

Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000		100.00	30	500	9.90	53.55	120	125	1.43	4.28
10	1700	0.57	99.43	36	425	9.90	43.65	150	106	1.04	2.85
12	1400	1.79	97.63	44	355	10.09	33.56	170	90	0.81	1.80
14	1180	3.07	94.57	52	300	8.14	25.42	200	75	0.50	1.00
16	1000	4.69	89.87	60	250	7.14	18.28	240	63	0.29	0.50
18	850	6.39	83.48	72	212	5.00	13.28	300	53	0.14	0.21
22	710	8.97	74.52	85	180	3.75	9.53	350	45	0.06	0.06
25	600	9.73	64.78	100	150	3.06	6.47	400	38	0.06	0.00
30	500	11.23	53.55	120	125	2.19	4.28				



Operator notes:

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Serial Number : MAL1007518

File name: 9 May 2016.msa
Record Number: 3
2016/12/01 02:56:33 PM

D.5 Formulation SOHIG01 (Sample 3)



MASTERSIZER 2000

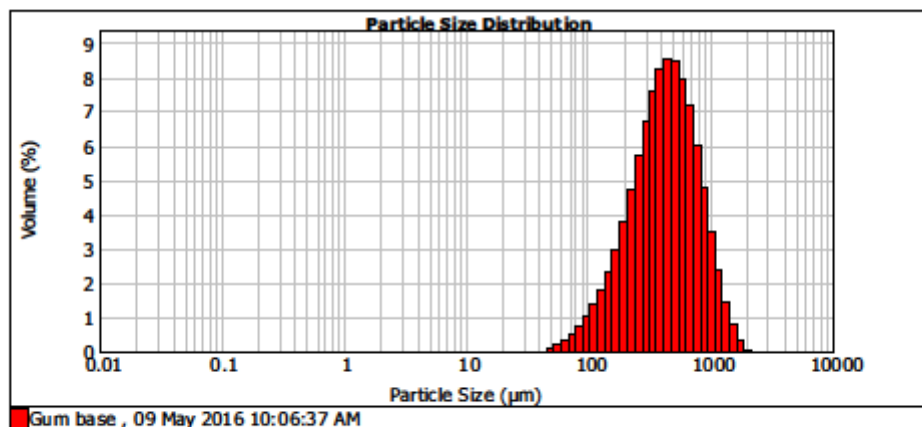
Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIP - organic)	Measured: 09 May 2016 10:06:37 AM
Sample Source & type: Supplier - Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:06:39 AM
Sample bulk lot ref: 201 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Ri: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 μm	Obscuration: 14.99 %
Dispersant Name: Alcohol	Dispersant Ri: 1.320	Weighted Residual: 1.883 %	Result Emulation: Off
Concentration: 0.7480 %Vol	Span : 1.738	Uniformity: 0.541	Result units: Volume
Specific Surface Area: 0.0185 m^2/g	Surface Weighted Mean D[3,2]: 325.023 μm	Vol. Weighted Mean D[4,3]: 496.412 μm	Density: 1.000 g/cm^3

d(0.1): 170.390 μm d(0.5): 428.303 μm d(0.9): 914.648 μm

Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000	0.33	100.00	30	500	10.08	59.60	120	125	1.73	4.84
10	1700	1.16	99.67	36	425	10.71	49.52	150	106	1.23	3.11
12	1400	2.20	98.51	44	355	8.98	38.61	170	90	0.92	1.88
14	1180	3.64	96.31	52	300	8.17	29.83	200	75	0.54	0.97
16	1000	5.27	92.67	60	250	4.52	21.67	240	63	0.30	0.43
18	850	7.85	87.40	72	212	3.74	15.78	300	53	0.13	0.13
22	710	9.01	79.55	85	180	2.68	7.51	350	45	0.00	0.00
25	600	10.94	70.54	100	150			400	38		
30	500		59.60	120	125		4.84				



Operator notes:

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Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007518

File name: 9 May 2016.mea
Record Number: 1
2016/12/01 02:55:18 PM

D.6 Formulation SOHIG03 (Sample 1)



Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

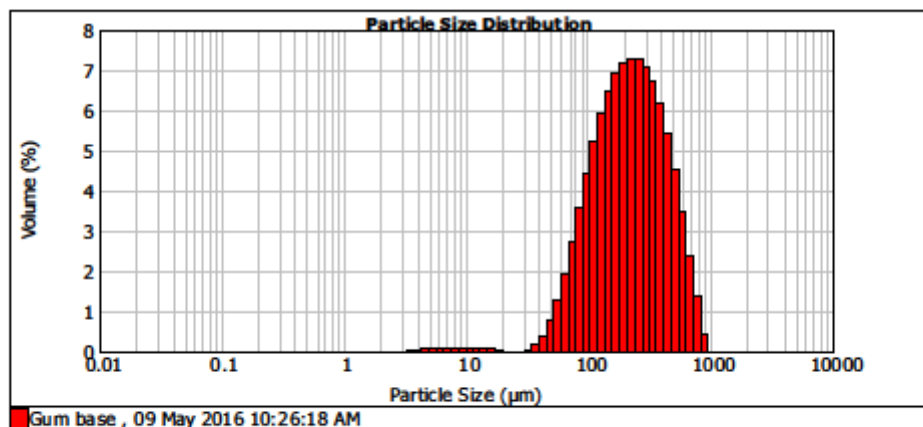
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:26:18 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenekamp	Analysed: 09 May 2016 10:26:20 AM
Sample bulk lot ref: 203 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 16.21 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 1.109 %	Result Emulation: Off

Concentration: 0.3459 %Vol	Span : 1.957	Uniformity: 0.605	Result units: Volume
Specific Surface Area: 0.0427 m ² /g	Surface Weighted Mean D[3,2]: 140.506 um	Vol. Weighted Mean D[4,3]: 261.921 um	Density: 1.000 g/cm ³

d(0.1): 85.302 um d(0.5): 217.212 um d(0.9): 510.473 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	6.13	89.31	120	125	6.40	23.08
10	1700	0.00	100.00	36	425	8.01	83.18	150	106	5.23	16.69
12	1400	0.00	100.00	44	355	8.30	75.17	170	90	4.41	11.45
14	1180	0.00	100.00	52	300	9.46	66.87	200	75	2.87	7.04
16	1000	0.32	99.68	60	250	8.69	57.41	240	63	1.74	4.17
18	850	1.82	97.86	72	212	8.48	48.72	300	53	0.88	2.43
22	710	3.26	94.60	85	180	8.99	31.25	350	45	0.40	1.55
25	600	5.29	89.31	100	150	8.17	23.08	400	38		1.15
30	500			120	125						



Operator notes:

D.6 Formulation SOHIG03 (Sample 2)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

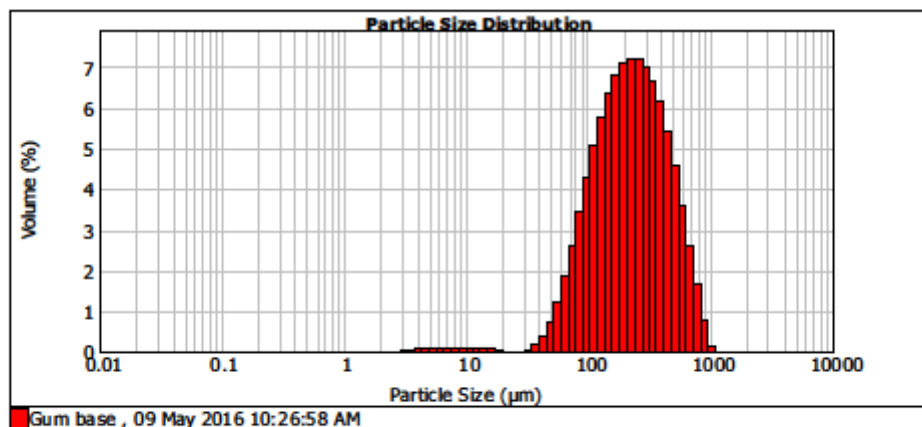
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:26:58 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:26:59 AM
Sample bulk lot ref: 203 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 15.84 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 1.144 %	Result Emulation: Off

Concentration: 0.3305 %Vol	Span : 1.996	Uniformity: 0.615	Result units: Volume
Specific Surface Area: 0.0434 m ² /g	Surface Weighted Mean D[3,2]: 138.123 um	Vol. Weighted Mean D[4,3]: 269.320 um	Density: 1.000 g/cm ³

d(0.1): 86.143 um d(0.5): 221.647 um d(0.9): 528.519 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	6.18	88.17	120	125	6.22	22.42
10	1700	0.00	100.00	36	425	8.00	81.99	150	106	4.24	16.21
12	1400	0.00	100.00	44	355	8.26	73.99	170	90	2.76	11.15
14	1180	0.07	100.00	52	300	9.42	65.73	200	75	1.67	6.91
16	1000	0.62	99.93	60	250	8.63	56.31	240	63	0.85	4.15
18	850	2.19	97.12	72	212	8.40	47.68	300	53	0.40	1.62
22	710	3.51	93.62	85	180	7.99	30.42	350	45		
25	600	5.45	88.17	100	150			400	38		
30	500			120	125						



Operator notes:

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File name: 9 Mei 2016.mea
Record Number: 9
2016/12/01 03:00:18 PM

D.6 Formulation SOHIG03 (Sample 3)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

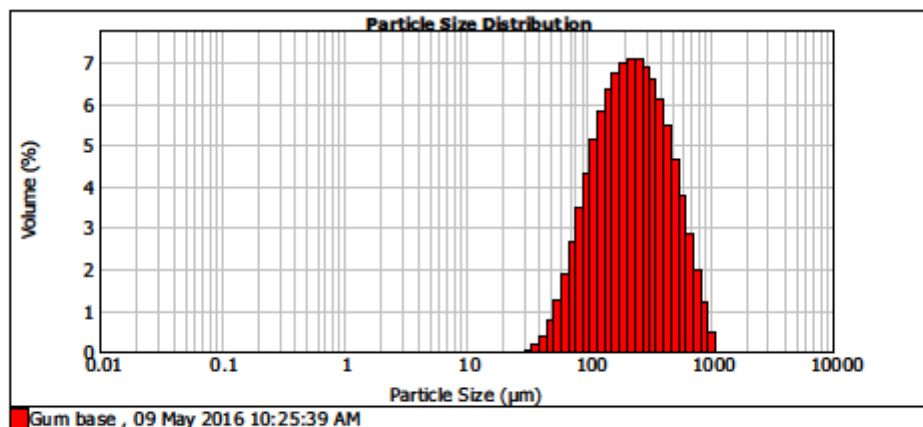
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:25:39 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:25:40 AM
Sample bulk lot ref: 203 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 16.56 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 1.195 %	Result Emulation: Off

Concentration: 0.4542 %Vol	Span : 2.052	Uniformity: 0.633	Result units: Volume
Specific Surface Area: 0.0338 m ² /g	Surface Weighted Mean D[3,2]: 177.657 um	Vol. Weighted Mean D[4,3]: 280.728 um	Density: 1.000 g/cm ³

d(0.1): 89.546 um d(0.5): 226.802 um d(0.9): 554.902 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000		100.00	30	500	6.23	86.59	120	125	6.26	21.52
10	1700	0.00	100.00	36	425	7.96	80.36	150	106	5.12	15.26
12	1400	0.00	100.00	44	355	8.15	72.40	170	90	4.32	10.14
14	1180	0.23	100.00	52	300	9.25	64.25	200	75	2.81	5.82
16	1000	1.20	99.77	60	250	8.47	55.01	240	63	1.69	3.01
18	850	2.56	98.57	72	212	8.27	46.54	300	53	0.85	1.32
22	710	3.79	96.01	85	180	8.77	38.27	350	45	0.37	0.47
25	600	5.63	92.22	100	150	7.98	29.90	400	38		0.10
30	500		86.59	120	125		21.52				



Operator notes:

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Mastersizer 2000 Ver. 5.60
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File name: 9 May 2016.mea
Record Number: 7
2016/12/01 02:58:45 PM

D.7 Formulation SOHIG04 (Sample 1)

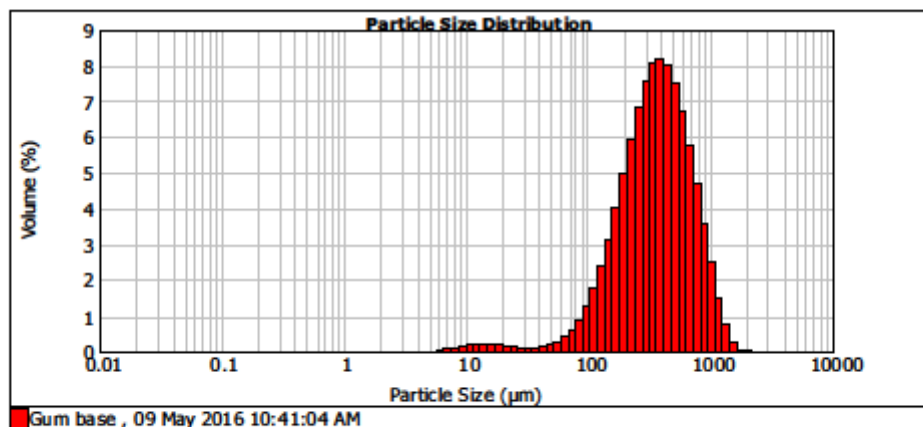


Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:41:04 AM	
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:41:06 AM	
Sample bulk lot ref: 204 (Sample 2)	Result Source: Measurement		
Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 11.73 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 2.289 %	Result Emulation: Off
Concentration: 0.3577 %Vol	Span : 1.826	Uniformity: 0.567	Result units: Volume
Specific Surface Area: 0.0284 m ² /g	Surface Weighted Mean D[3,2]: 210.927 um	Vol. Weighted Mean D[4,3]: 423.265 um	Density: 1.000 g/cm ³

d(0.1): 139.507 um d(0.5): 361.288 um d(0.9): 799.139 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.04	100.00	30	500	9.32	68.95	120	125	2.24	8.00
10	1700	0.44	99.96	36	425	10.67	59.63	150	106	1.52	5.77
12	1400	1.26	99.53	44	355	9.67	48.96	170	90	1.09	4.25
14	1180	2.48	98.26	52	300	9.49	39.29	200	75	0.63	3.16
16	1000	3.90	95.78	60	250	7.31	29.80	240	63	0.37	2.52
18	850	6.12	91.89	72	212	5.89	22.48	300	53	0.21	2.15
22	710	7.36	85.77	85	180	5.01	16.59	350	45	0.14	1.94
25	600	9.45	78.41	100	150	3.57	11.58	400	38		1.80
30	500		68.95	120	125		8.00				



Operator notes:

D.7 Formulation SOHIG04 (Sample 2)



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Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

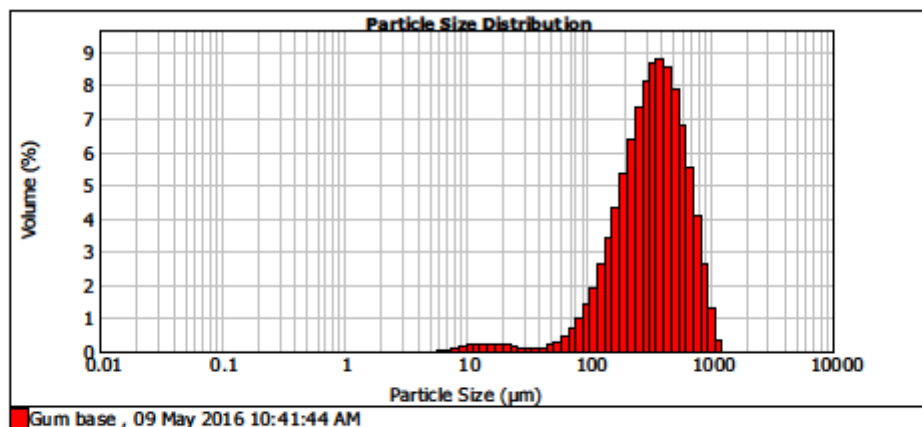
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:41:44 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:41:45 AM
Sample bulk lot ref: 204 (Sample 2)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 11.11 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 2.075 %	Result Emulation: Off

Concentration: 0.3279 %Vol	Span : 1.645	Uniformity: 0.509	Result units: Volume
Specific Surface Area: 0.0301 m ² /g	Surface Weighted Mean D[3,2]: 199.089 um	Vol. Weighted Mean D[4,3]: 381.045 um	Density: 1.000 g/cm ³

d(0.1): 133.550 um d(0.5): 340.252 um d(0.9): 693.222 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	9.89	74.00	120	125	2.45	8.75
10	1700	0.00	100.00	36	425	11.44	64.11	150	106	1.67	6.30
12	1400	0.07	100.00	44	355	10.38	52.67	170	90	1.20	4.62
14	1180	1.05	99.93	52	300	10.16	42.30	200	75	0.69	3.42
16	1000	2.71	98.88	60	250	7.81	32.14	240	63	0.39	2.73
18	850	5.28	96.17	72	212	6.31	24.33	300	53	0.21	2.34
22	710	7.13	90.89	85	180	5.39	18.02	350	45	0.14	2.12
25	600	9.75	83.75	100	150	3.88	12.63	400	38		1.98
30	500		74.00	120	125		8.75				



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File name: 9 May 2016.mea
Record Number: 18
2016/12/01 03:05:13 PM

D.7 Formulation SOHIG04 (Sample 3)



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Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

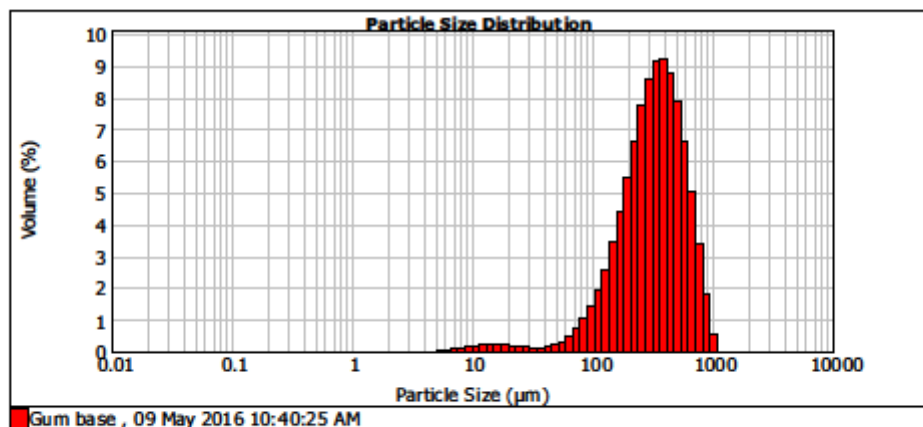
Sample Name: Gum base Sample Source & type: Supplier = Sonia vd Walt Sample bulk lot ref: 204 (Sample 2)	SOP Name: Hydro 2000 SM (RIIP - organic) Measured by: Jan Steenkamp Result Source: Measurement	Measured: 09 May 2016 10:40:25 AM Analysed: 09 May 2016 10:40:26 AM
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Particle Name: Titanium Dioxide Particle Rt: 2.741 Dispersant Name: Alcohol	Accessory Name: Hydro 2000SM (A) Absorption: 0.1 Dispersant Rt: 1.320	Analysis model: General purpose Size range: 0.020 to 2000.000 um Weighted Residual: 2.087 %	Sensitivity: Enhanced Obscuration: 12.05 % Result Emulation: Off
---	---	---	--

Concentration: 0.3399 %Vol	Span : 1.559	Uniformity: 0.484	Result units: Volume
Specific Surface Area: 0.0316 m ² /g	Surface Weighted Mean D[3,2]: 189.790 um	Vol. Weighted Mean D[4,3]: 361.918 um	Density: 1.000 g/cm ³

d(0.1): 131.301 um d(0.5): 329.367 um d(0.9): 644.846 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000		100.00	30	500	10.18	77.18	120	125	2.46	9.08
10	1700	0.00	100.00	36	425	12.02	67.00	150	106	1.69	6.62
12	1400	0.00	100.00	44	355	11.00	54.98	170	90	1.24	4.93
14	1180	0.25	100.00	52	300	10.76	43.98	200	75	0.74	3.69
16	1000	1.79	99.75	60	250	8.20	33.22	240	63	0.44	2.95
18	850	4.44	97.96	72	212	6.53	25.02	300	53	0.25	2.51
22	710	6.66	93.52	85	180	5.50	18.49	350	45	0.17	2.25
25	600	9.67	86.86	100	150	3.91	12.99	400	38		2.08
30	500		77.18	120	125		9.08				



Operator notes:

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Mastersizer 2000 Ver. 5.60
 Serial Number : MAL1007518

File name: 9 May 2016.msa
 Record Number: 16
 2016/12/01 03:04:19 PM

D.8 Formulation S0.5HIG01 (Sample 1)



MASTERSIZER 2000

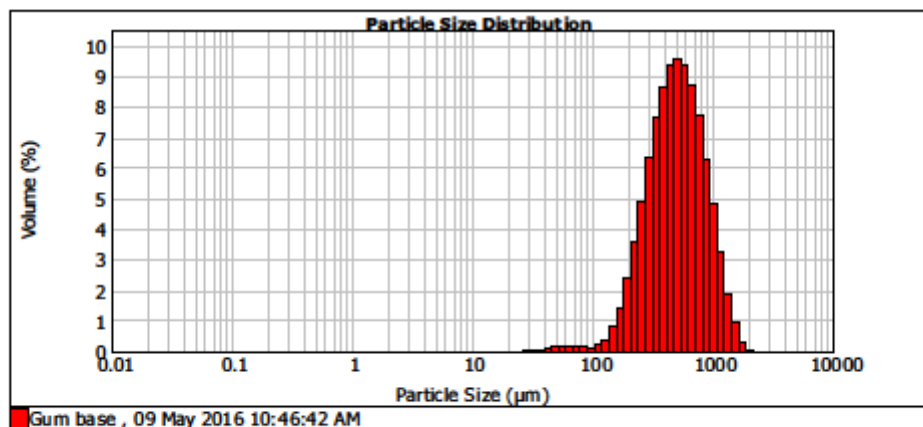
Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:46:42 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:46:43 AM
Sample bulk lot ref: 301 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 13.58 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 3.878 %	Result Emulation: Off
Concentration: 0.8268 %Vol	Span : 1.497	Uniformity: 0.469	Result units: Volume
Specific Surface Area: 0.015 m ² /g	Surface Weighted Mean D[3,2]: 399.635 um	Vol. Weighted Mean D[4,3]: 564.721 um	Density: 1.000 g/cm ³

d(0.1): 239.349 um d(0.5): 499.599 um d(0.9): 987.414 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.29	100.00	30	500	11.10	50.06	120	125	0.27	1.54
10	1700	1.36	99.71	36	425	11.28	38.96	150	106	0.16	1.27
12	1400	2.93	98.35	44	355	8.90	27.67	170	90	0.19	1.11
14	1180	4.96	95.42	52	300	7.37	18.77	200	75	0.22	0.92
16	1000	7.02	90.47	60	250	4.60	11.40	240	63	0.23	0.70
18	850	9.98	83.45	72	212	2.85	6.81	300	53	0.18	0.47
22	710	10.85	73.47	85	180	1.69	2.27	350	45	0.14	0.29
25	600	12.56	62.62	100	150	0.73	1.54	400	38		0.14
30	500		50.06	120	125						



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Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007518

File name: 9 May 2016.mea
Record Number: 20
2016/12/01 03:06:10 PM

D.8 Formulation S0.5HIG01 (Sample 2)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

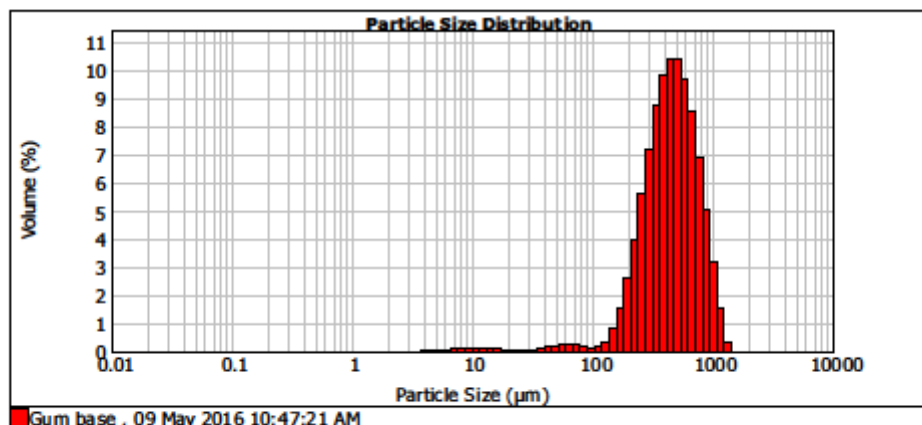
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:47:21 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:47:23 AM
Sample bulk lot ref: 301 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 12.66 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 3.555 %	Result Emulation: Off

Concentration: 0.4571 %Vol	Span : 1.357	Uniformity: 0.426	Result units: Volume
Specific Surface Area: 0.0246 m ² /g	Surface Weighted Mean D[3,2]: 243.863 um	Vol. Weighted Mean D[4,3]: 492.934 um	Density: 1.000 g/cm ³

d(0.1): 221.561 um d(0.5): 452.622 um d(0.9): 835.988 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000		100.00	30	500	12.30	57.56	120	125	0.27	3.20
10	1700	0.00	100.00	36	425	45.26		150	106	0.24	2.93
12	1400	0.02	99.98	44	355	12.77	32.49	170	90	0.18	2.75
14	1180	0.90	99.08	52	300	10.15	22.34	200	75	0.28	2.51
16	1000	2.97	96.11	60	250	8.37	13.98	240	63	0.28	2.23
18	850	5.41	90.71	72	212	5.14	8.84	300	53	0.23	1.94
22	710	8.97	81.73	85	180	3.12	5.72	350	45	0.17	1.71
25	600	10.80	70.93	100	150	1.79	3.93	400	38		1.54
30	500	13.37	57.56	120	125	0.73	3.20				



Operator notes:

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Serial Number : MAL1007518

File name: 9 May 2016.msa
Record Number: 21
2016/12/01 03:06:35 PM

D.8 Formulation S0.5HIG01 (Sample 3)



MASTERSIZER 2000

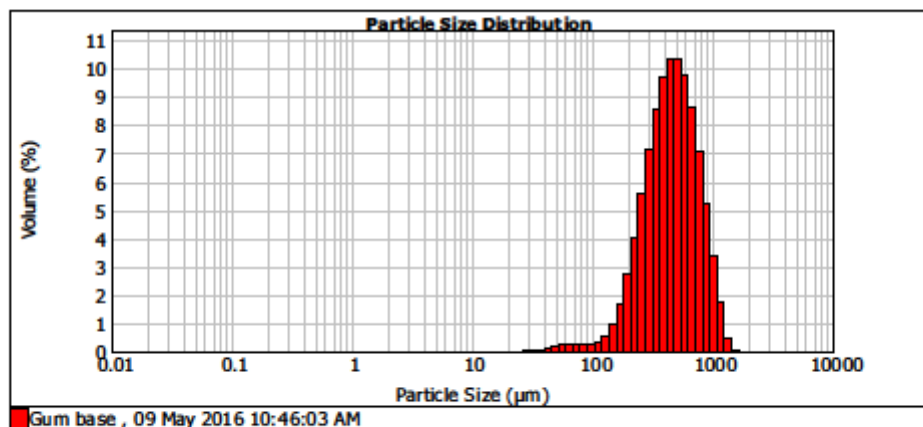
Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:46:03 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:46:04 AM
Sample bulk lot ref: 301 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 12.90 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 3.675 %	Result Emulation: Off
Concentration: 0.7183 %Vol	Span : 1.355	Uniformity: 0.422	Result units: Volume
Specific Surface Area: 0.0163 m ² /g	Surface Weighted Mean D[3,2]: 367.080 um	Vol. Weighted Mean D[4,3]: 501.420 um	Density: 1.000 g/cm ³

d(0.1): 226.089 um d(0.5): 458.074 um d(0.9): 846.863 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	12.24	56.63	120	125	0.42	2.07
10	1700	0.04	100.00	36	425	12.64	44.39	150	106	0.28	1.65
12	1400	1.05	99.96	44	355	10.03	31.75	170	90	0.30	1.37
14	1180	3.15	98.91	52	300	8.31	21.72	200	75	0.29	1.07
16	1000	5.60	95.76	60	250	5.18	13.41	240	63	0.27	0.78
18	850	9.18	90.16	72	212	3.24	8.22	300	53	0.21	0.51
22	710	10.94	80.98	85	180	1.98	4.98	350	45	0.15	0.30
25	600	13.41	70.04	100	150	0.94	3.00	400	38		0.15
30	500		56.63	120	125		2.07				



Operator notes:

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Mastersizer 2000 Ver. 5.60
Serial Number : MAL1007548

File name: 9 May 2016.mea
Record Number: 19
2016/12/01 03:05:46 PM

D.9 Formulation S0.5HIG03 (Sample 1)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

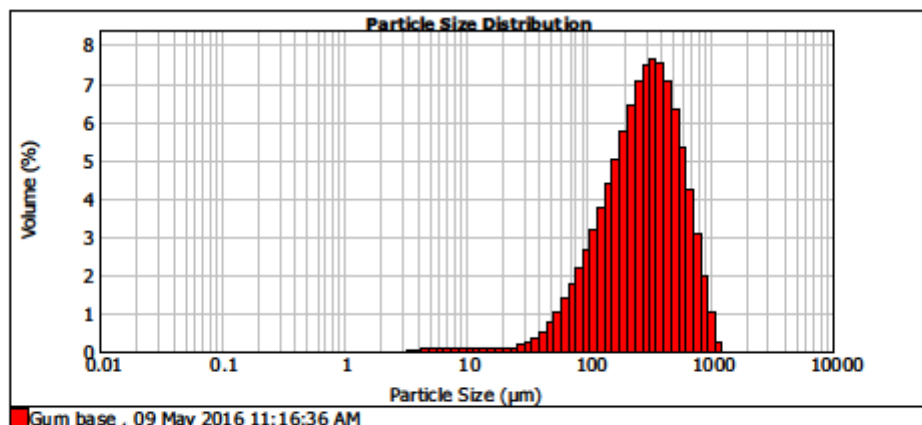
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 11:16:36 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 11:16:37 AM
Sample bulk lot ref: 303 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 μm	Obscuration: 14.12 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 2.019 %	Result Emulation: Off

Concentration: 0.3298 %Vol	Span : 1.919	Uniformity: 0.594	Result units: Volume
Specific Surface Area: 0.0384 m^2/g	Surface Weighted Mean D[3,2]: 156.075 μm	Vol. Weighted Mean D[4,3]: 331.973 μm	Density: 1.000 g/cm^3

d(0.1): 92.251 μm d(0.5): 286.024 μm d(0.9): 641.084 μm

Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	8.17	79.85	120	125	3.91	16.64
10	1700	0.00	100.00	36	425	9.81	71.68	150	106	3.16	12.73
12	1400	0.06	100.00	44	355	9.30	61.87	170	90	2.76	9.57
14	1180	0.78	99.94	52	300	9.58	52.58	200	75	1.98	6.80
16	1000	2.02	97.13	60	250	7.84	43.00	240	63	1.41	4.82
18	850	3.99	93.14	72	212	6.81	35.17	300	53	0.91	3.42
22	710	5.52	87.63	85	180	6.43	21.92	350	45	0.61	2.51
25	600	7.77	79.85	100	150	5.29	16.64	400	38		1.90
30	500			120	125						



Operator notes:

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File name: 9 May 2016.mea
Record Number: 26
2016/12/01 03:09:07 PM

D.9 Formulation S0.5HIG03 (Sample 2)



Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

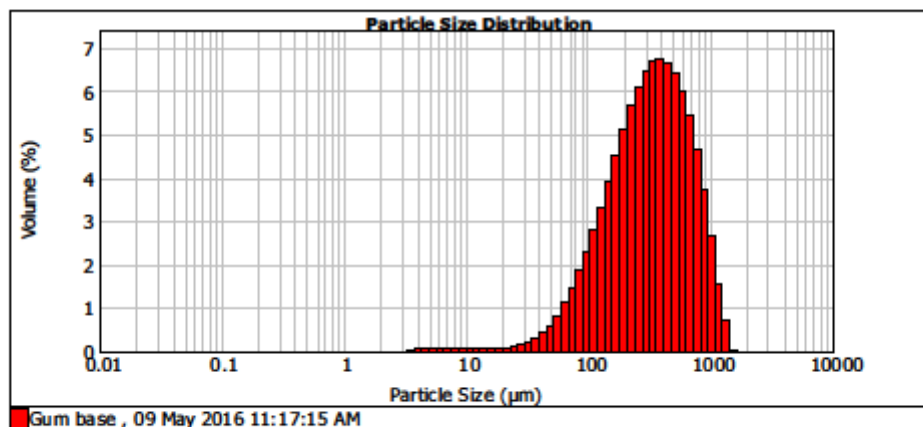
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 11:17:15 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 11:17:17 AM
Sample bulk lot ref: 303 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 14.22 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 2.866 %	Result Emulation: Off

Concentration: 0.3560 %Vol	Span : 2.135	Uniformity: 0.658	Result units: Volume
Specific Surface Area: 0.0349 m ² /g	Surface Weighted Mean D[3,2]: 171.856 um	Vol. Weighted Mean D[4,3]: 394.849 um	Density: 1.000 g/cm ³

d(0.1): 101.610 um d(0.5): 325.934 um d(0.9): 797.494 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	7.81	70.78	120	125	3.47	14.24
10	1700	0.00	100.00	36	425	8.82	62.97	150	106	2.74	10.77
12	1400	1.23	99.90	44	355	8.12	54.15	170	90	2.33	8.03
14	1180	2.60	98.68	52	300	8.32	46.04	200	75	1.62	5.70
16	1000	4.09	96.08	60	250	6.88	37.71	240	63	1.13	4.07
18	850	6.10	91.99	72	212	6.06	30.83	300	53	0.72	2.94
22	710	6.85	85.90	85	180	5.78	24.77	350	45	0.49	2.22
25	600	8.26	79.04	100	150	4.75	19.00	400	38		1.73
30	500		70.78	120	125		14.24				



Operator notes:

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Serial Number : MAL1007518

File name: 9 May 2016.mea
Record Number: 27
2016/12/01 03:09:50 PM

D.9 Formulation S0.5HIG03 (Sample 3)



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Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

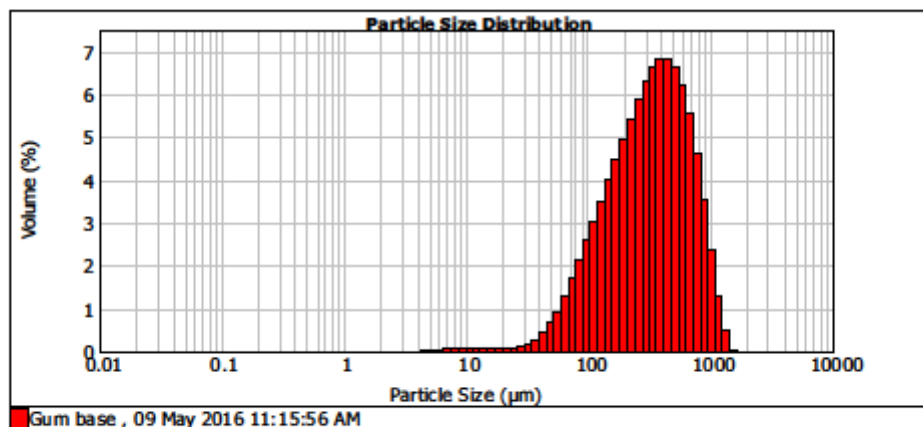
Sample Name: Gum base Sample Source & type: Supplier = Sonia vd Walt Sample bulk lot ref: 303 (Sample 1)	SOP Name: Hydro 2000 SM (RIIP - organic) Measured by: Jan Steenkamp Result Source: Measurement	Measured: 09 May 2016 11:15:56 AM Analysed: 09 May 2016 11:15:58 AM
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Particle Name: Titanium Dioxide Particle Rt: 2.741 Dispersant Name: Alcohol	Accessory Name: Hydro 2000SM (A) Absorption: 0.1 Dispersant Rt: 1.320	Analysis model: General purpose Size range: 0.020 to 2000.000 um Weighted Residual: 2.395 %	Sensitivity: Enhanced Obscuration: 15.11 % Result Emulation: Off
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Concentration: 0.4227 %Vol	Span : 2.092	Uniformity: 0.65	Result units: Volume
Specific Surface Area: 0.0325 m ² /g	Surface Weighted Mean D[3,2]: 184.620 um	Vol. Weighted Mean D[4,3]: 387.289 um	Density: 1.000 g/cm ³

d(0.1): 98.076 um d(0.5): 323.952 um d(0.9): 775.717 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000		100.00	30	500	8.02	71.28	120	125	3.74	15.28
10	1700	0.00	100.00	36	425	63.26		150	106	3.06	11.53
12	1400	0.06	99.94	44	355	7.97	54.39	170	90	2.65	8.47
14	1180	2.29	98.99	52	300	8.02	46.42	200	75	1.85	5.82
16	1000	3.85	96.70	60	250	6.59	38.40	240	63	1.27	3.97
18	850	6.03	92.85	72	212	5.87	31.81	300	53	0.78	2.70
22	710	7.00	86.82	85	180	5.75	25.95	350	45	0.50	1.92
25	600	8.54	79.82	100	150	4.92	20.20	400	38		1.42
30	500		71.28	120	125		15.28				



Operator notes:

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File name: 9 May 2016.mea
 Record Number: 25
 2016/12/01 03:08:26 PM

D.10 Formulation S0.5HIG04 (Sample 1)



MASTERSIZER 2000

Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

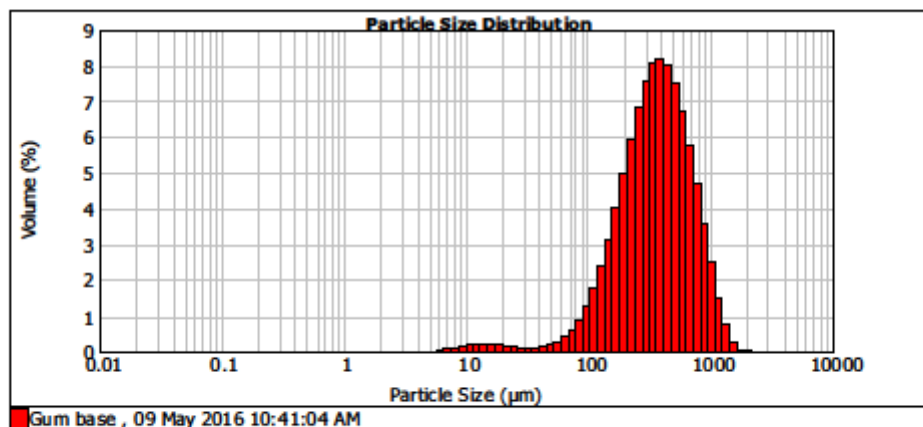
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 10:41:04 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 10:41:06 AM
Sample bulk lot ref: 204 (Sample 2)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 11.73 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 2.289 %	Result Emulation: Off

Concentration: 0.3577 %Vol	Span : 1.826	Uniformity: 0.567	Result units: Volume
Specific Surface Area: 0.0284 m ² /g	Surface Weighted Mean D[3,2]: 210.927 um	Vol. Weighted Mean D[4,3]: 423.265 um	Density: 1.000 g/cm ³

d(0.1): 139.507 um d(0.5): 361.288 um d(0.9): 799.139 um

Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %	Mesh No	Aperture µm	Volume In %	Vol Below %
8	2000	0.04	100.00	30	500	9.32	68.95	120	125	2.24	8.00
10	1700	0.44	99.96	36	425	10.67	59.63	150	106	1.52	5.77
12	1400	1.26	99.53	44	355	9.67	48.96	170	90	1.09	4.25
14	1180	2.48	98.26	52	300	9.49	39.29	200	75	0.63	3.16
16	1000	3.90	95.78	60	250	7.31	22.48	240	63	0.37	2.52
18	850	6.12	91.89	72	212	5.89	16.59	300	53	0.21	1.94
22	710	7.36	78.41	85	180	5.01	11.58	350	45	0.14	1.80
25	600	9.45	68.95	100	150	3.57	8.00	400	38		
30	500			120	125						



Operator notes:

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2016/12/01 03:04:46 PM

D.10 Formulation S0.5HIG04 (Sample 2)



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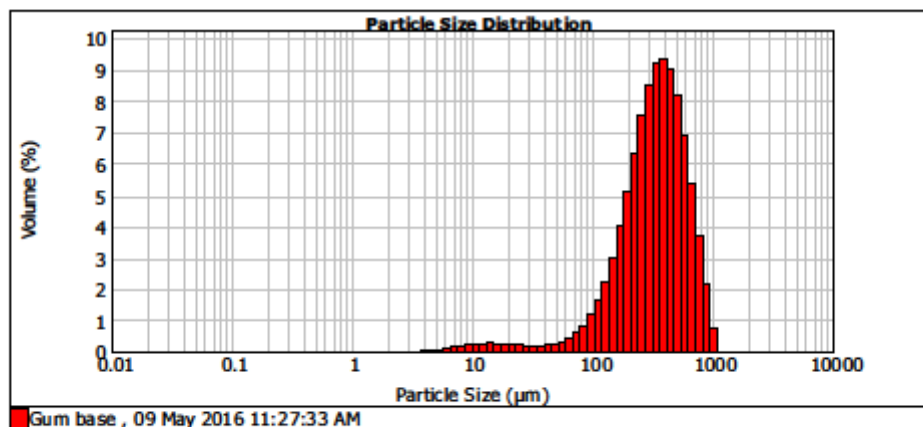
Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 11:27:33 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 11:27:34 AM
Sample bulk lot ref: 304 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 μm	Obscuration: 12.89 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 2.268 %	Result Emulation: Off
Concentration: 0.3181 %Vol	Span : 1.554	Uniformity: 0.481	Result units: Volume
Specific Surface Area: 0.036 m^2/g	Surface Weighted Mean D[3,2]: 166.599 μm	Vol. Weighted Mean D[4,3]: 370.884 μm	Density: 1.000 g/cm^3

d(0.1): 132.665 μm d(0.5): 339.589 μm d(0.9): 660.428 μm

Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	10.46	75.64	120	125	2.07	9.04
10	1700	0.00	100.00	36	425	12.20	65.18	150	106	1.40	6.97
12	1400	0.00	100.00	44	355	11.02	52.98	170	90	1.04	5.57
14	1180	0.35	100.00	52	300	10.58	41.96	200	75	0.65	4.53
16	1000	2.11	99.65	60	250	7.88	31.38	240	63	0.43	3.88
18	850	4.81	97.55	72	212	6.11	23.50	300	53	0.28	3.44
22	710	7.04	92.74	85	180	4.97	17.40	350	45	0.21	3.16
25	600	10.06	85.69	100	150	3.39	12.43	400	38		2.95
30	500		75.64	120	125		9.04				



Operator notes:

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File name: 9 May 2016.msa
Record Number: 33
2016/12/01 03:12:39 PM

D.10 Formulation S0.5HIG04 (Sample 3)



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Result: Sieve BS 410 : 1986 / (ISO 565 : 1990) Report

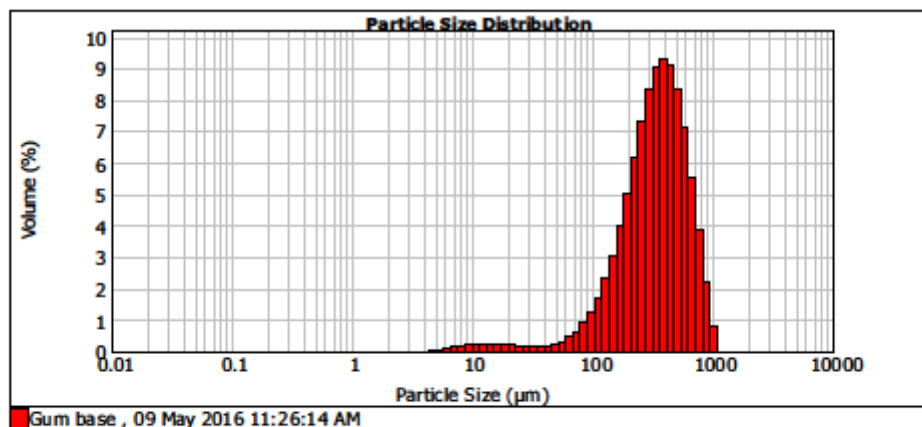
Sample Name: Gum base	SOP Name: Hydro 2000 SM (RIIP - organic)	Measured: 09 May 2016 11:26:14 AM
Sample Source & type: Supplier = Sonia vd Walt	Measured by: Jan Steenkamp	Analysed: 09 May 2016 11:26:15 AM
Sample bulk lot ref: 304 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle Rt: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 μm	Obscuration: 13.68 %
Dispersant Name: Alcohol	Dispersant Rt: 1.320	Weighted Residual: 2.524 %	Result Emulation: Off

Concentration: 0.3569 %Vol	Span : 1.558	Uniformity: 0.481	Result units: Volume
Specific Surface Area: 0.0343 m^2/g	Surface Weighted Mean D[3,2]: 174.832 μm	Vol. Weighted Mean D[4,3]: 374.509 μm	Density: 1.000 g/cm^3

d(0.1): 132.116 μm d(0.5): 343.478 μm d(0.9): 667.284 μm

Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %	Mesh No	Aperture μm	Volume In %	Vol Below %
8	2000	0.00	100.00	30	500	10.54	74.84	120	125	2.17	9.08
10	1700	0.00	100.00	36	425	12.12	64.30	150	106	1.51	6.91
12	1400	0.00	100.00	44	355	10.81	52.19	170	90	1.13	5.41
14	1180	0.36	100.00	52	300	10.29	41.38	200	75	0.70	4.28
16	1000	2.20	99.64	60	250	7.66	31.09	240	63	0.45	3.58
18	850	5.03	97.44	72	212	5.97	23.43	300	53	0.28	3.13
22	710	7.29	92.41	85	180	4.93	17.46	350	45	0.21	2.85
25	600	10.28	85.12	100	150	3.45	12.54	400	38		2.65
30	500		74.84	120	125		9.08				



Operator notes: