Use of *Aloe vera* and *Aloe marlothii* materials as excipients in beads produced by extrusion-spheronization

Patience Chinyemba

(B. Pharm)

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Supervisor

Prof. J.H. Hamman

Co-supervisors

Dr. J.H. Steenekamp

and

Prof. A.M. Viljoen

NWU

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ABSTRACT

Microcrystalline cellulose (MCC) is the most commonly used excipient in the manufacture of spherical particles or beads by extrusion spheronisation. However, the use of MCC in beads has its limitations such as prolonged release of drugs due to lack of disintegration. The aim of this study was to determine if *Aloe vera* and *Aloe marlothii* leaf materials can be used as excipients in the production of beads prepared by extrusion spheronisation. A 2^3 full factorial design was employed for optimisation and to explore the effects of the concentration of MCC, polyvinylpyrrolidone and aloe materials on the sphericity and release rate of ketoprofen. Scanning electron microscopy revealed more porous beads when aloe materials were included in the bead formulations compared to the formulation with MMC alone. The bead formulations containing aloe materials exhibited faster drug release compared to that of the formulation containing MCC alone. Dissolution data of the optimised formulations were analysed in terms of mean dissolution time (MDT) as well as fit factors (f_1 and f_2). The optimised bead formulations had dissolution profiles comparable to that of the formulation containing MCC alone at pH 1.2 and 4.5 (f_2 values > 70), but less comparable to the reference at pH 6.8 ($50 < f_2 < 65$) due to faster drug release. *Aloe vera* and *Aloe marlothii* leaf materials can be used successfully together with MCC in the production of beads by extrusion spheronisation.

Keywords: Microcrystalline cellulose (Avicel pH101); *Aloe vera*; *Aloe marlothii*; extrusion spheronisation; beads.

UITTREKSEL

Mikrokristallyne sellulose (MCC) is een van die mees algmeen gebruikte hulpstowwe in die vervaardiging van sferiese deeltjies of krale deur middel van ekstrusie sferonisasie. Die gebruik van MCC in krale het egter nadele soos stadige vrystelling van die geneesmiddel as gevolg van die gebrek aan disintegrasie van die krale. Die doel van hierdie studie was om vas te stel of blaarmateriaal vanaf *Aloe vera* en *Aloe marlothii* gebruik kan word as hulpstowwe in die vervaardiging van krale deur middel van ekstrusie sferonisasie. ‘n Vol faktoriale (2^3) ontwerp was gebruik vir optimalisering en om die effekte van die konsentrasie van MCC, polivinielpirolidoon en aalwyn op sferisiteit en vrystellingstempo van ketoprofen. Skanderings elektron mikroskopie het aangetoon dat die krale wat aloe materiale bevat meer poreus is as die kraalformulering wat slegs MCC bevat. Die kraalformulerings wat aalwynmateriale bevat het ketoprofen teen ‘n vinniger tempo vrygestel as die formulering wat MCC alleen bevat. Dissolusiedata vanaf die ge-optimaliseerde kraalformulerings was ge-analiseer in terme van gemiddelde dissolusie tyd (MDT) asook passingsfaktore (f_1 and f_2). Die ge-optimaliseerde kraalformulerings se dissolusieprofiele was vergelykbaar met die formulering wat slegs MCC bevat by pH 1.2 en 4.5 (f_2 waardes > 70), maar minder vergelykbaar by pH 6.8 ($50 < f_2 < 65$), as gevolg van ‘n vinniger geneesmiddelvrystelling. Materiaal van die blare vanaf *Aloe vera* en *Aloe marlothii* kan dus suksesvol gebruik word vir die formulering van krale wat deur middel van ekstrusie sferonisasie gemaak word.

Sleutelwoorde: Mikrokristallyne sellulose (Avicel pH101); *Aloe vera*; *Aloe marlothii*; ekstrusie sferonisasie; krale.

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CHAPTER 1

INTRODUCTION

1 INTRODUCTION

The development of spherical pellets or beads as multiple-unit drug delivery systems is increasing due to advantages such as a more predictable *in vivo* drug delivery profile and ease of preparation. Beads are uniformly dispersed in the gastrointestinal tract after oral administration, which results in maximised drug absorption, reduced peak plasma fluctuations and reduced side effects. In addition, spherical beads possess low surface area to volume ratios, exhibit good flow properties and therefore uniform packing into hard gelatine capsules. Their spherical shape makes them excellent candidates for coating as desired for aesthetic properties or controlled release of active ingredients (Koo & Heng, 2001:1383, Sinha *et al*., 2005:1).

Extrusion spheronisation is one of the most popular methods for the production of spherical pellets or beads as it produces relatively dense and homogenous beads. Furthermore, this bead preparation method has short processing times, which result in savings on production costs (Mallipeddi *et al*., 2010:53). The use of suitable excipients and fillers is essential for the successful production of beads by extrusion spheronisation (Charoenthai *et al*., 2007:2469). Different excipients from a variety of sources have been evaluated for the formation of spherical beads. Microcrystalline cellulose (MCC) is the gold standard as an extrusion spheronisation excipient based on its good binding properties that provide cohesiveness to a wetted mass. Furthermore, it is able to absorb and retain a large quantity of water thereby improving wetted powder mass plasticity and thereby enhancing spheronisation (Dukić-Ott *et al*., 2009:39).

However, MCC has distinct disadvantages such as the lack of disintegration of beads prepared from MCC resulting in the prolonged release of drugs with a lag phase (Sriamornsak *et al*., 2008:275). The incorporation of other excipients such as water-soluble fillers and disintegrants has been investigated to obtain pellet disintegration and/or faster drug release from MCC-based beads (Dukić-Ott *et al*., 2009:40).

2 MULTIPLE UNIT DOSAGE FORMS

Pharmaceutical solid formulations can be divided into two major groups namely single-unit dosage forms and multiple-unit dosage forms. Single-unit dosage forms are oral dosage forms where each unit contains one full dose of the active ingredient that is intended to be administered singularly (Gandhi *et al*., 1999:160). In multiple-unit dosage forms, the complete dose of the active pharmaceutical ingredient is divided between several subunits, thus this type of dosage form consists of a number of small discrete units each containing a fraction of the dose (Dey *et al*., 2008:1068).

Multiple-unit dosage forms present advantages over single-unit dosage forms in terms of improved efficacy and reduced toxicity. The multiple units are also referred to as pellets, spherical granules or spheroids. They are usually less than 2 mm in diameter, therefore they can leave the stomach continuously resulting in less inter and intra-subject variability. They are also freely dispersed in the gastrointestinal tract, thereby invariably maximising drug absorption, reducing plasma peak fluctuations and minimising potential side effects without appreciably lowering the drug bioavailability (Sinha *et al*., 2005:1).

3 ALOE AS A NATURAL SOURCE FOR POLYSACCHARIDES

Aloes have been used therapeutically for a long time with some medicinal properties being attributed to the leaf gel, while other pharmacological effects have been associated with the exudate (Reynolds & Dweck, 1999:3). Research has shown that the polysaccharides in *Aloevera* gel and whole leaf materials have the ability to improve drug bioavailability as they possess absorption enhancing properties (Hamman, 2008:1600).

In the Pharmaceutical industry, aloe materials have been used for the manufacture of topical products as well as tablets and capsules. *A. vera* gel has also been used in the manufacture of directly compressible matrix type tablets that showed modified release over an extended period of time (Jani *et al.,* 2007:90). Other aloe materials such as *Aloeferox* gel have also been used successfully in the production of matrix type tablets (Jambwa *et al*., 2011:439; Jambwa *et al.,* 2011:51), but not yet in the production of beads by extrusion spheronisation. Since aloe leaf materials (i.e. *A. vera, A. ferox, A. marlothii* and *A. speciosa*) have shown potential to act as drug absorption enhancers (Beneke *et al.,*2012:475; Lebitsa *et al.,* 2012:297), their inclusion into dosage forms such as beads may fulfil multiple functions such as being a filler as well as a drug absorption enhancer.

4 PROBLEM STATEMENT

Microcrystalline cellulose (MCC) is the only excipient available for use in the formation of beads by extrusion spheronisation due to its favourable properties but it has its limitations. Research has been carried out in search for other suitable excipients from renewable resources but to no avail. This study was done to evaluate the use of aloe leaves material as potential excipients in the manufacture of beads by extrusion spheronisation.

5 RESEARCH AIM AND OBJECTIVES

The aim of this study was to investigate the use of *Aloe marlothii* and *Aloe vera* leaf materials as excipients in the production of beads by extrusion spheronisation.

Based on preliminary studies, up to 30% (w/w) *A. vera* whole leaf could be incorporated into the beads, but only 10% (w/w) of *A. vera* gel, *A. marlothii* gel and whole leaf could be incorporated into the beads manufactured by means of extrusion spheronisation.

The objectives of the study were:

- To manufacture beads by extrusion spheronisation containing four different aloe leaf materials (i.e. *A. vera* gel, *A. vera* whole leaf, *A. marlothii* gel and *A. marlothii* whole leaf) in concentrations as determined by a full factorial design of experiments.
- To evaluate the beads in terms of sphericity, drug content, friability, mass variation, surface morphology and core structure by means of scanning electron microscopy and the dissolution of a model compound.
- To obtain optimised bead formulations from response contour plots based on the design of experiments using sphericity and dissolution results as responses.
- To characterise the optimised bead formulations in terms of sphericity, friability, mass variation and dissolution and to calculate the mean dissolution time and fit factors.

CHAPTER 2

BEAD FORMULATIONS BY EXTRUSION SPHERONISATION AND OPTIMISATION

1 INTRODUCTION

The discovery and development of new drugs are expensive and time consuming. An alternative way to produce new medicinal products from existing conventional dosage forms is the development of novel drug delivery systems. For example, in controlled release dosage forms the active pharmaceutical ingredient is delivered into the systemic circulation at a predetermined rate to achieve optimum therapeutic responses, prolonged efficacy and potentially decrease toxicity (Gandhi *et al*., 1999:160).

Drug release profiles can be controlled by adding certain excipients to a formulation or by further processing the dosage form by applying a coating layer that can modulate drug release. Multiple-unit dosage forms have many advantages over single-unit dosage forms such as a higher degree of homogenous dispersion in the gastrointestinal tract after oral ingestion, a reduced risk of systemic toxicity due to dose dumping and a reduced risk of high local concentrations that may irritate the gastrointestinal tract lining (Lopes *et al.,* 2006:95; Ishida *et al.,* 2008:46). Multiple-unit dosage forms contain the dose to be administered in a number of sub-units, which is then calculated by the sum of the quantity of the drug in each sub-unit (Lopes*,* 2006:93). Examples of multiple-unit dosage forms are granules, beads (also referred to as pellets) or mini-tablets in capsule systems. Although several methods are employed in the production of beads, extrusion spheronisation has gained much popularity due to its relatively low production costs and short production times (Mallipedi *et al*., 2010:53).

The use of natural polymers for pharmaceutical applications is attractive because they are readily available from renewable sources, non-toxic, inexpensive, potentially biodegradable and usually also biocompatible. However, substances from plant origin pose potential challenges such as being produced in small quantities by the plant in mixtures that are structurally complex. The plant composition may differ according to the location of the plants as well as the time of harvesting (Beneke *et al.,* 2009:2602). Some natural polymers may act as multifunctional excipients in dosage forms by fulfilling more than one function such as being a filler as well as acting as an absorption enhancer (Hamman & Steenekamp, 2012:220).

2 MULTIPLE-UNIT DRUG DELIVERY SYSTEMS

In single-unit dosage forms, each unit contains one dose of the drug intended to be administered, which allows for high drug loading and cost-effective manufacturing operations (Gandhi *et al.,* 1999:160). On the other hand, multiple-unit drug delivery systems consist of multiple small discrete units, each containing a portion of active substance. Hence, in order to deliver the total recommended dose, these subunits are filled into a sachet, encapsulated in hard-gelatine capsules or compressed into tablets (Dey *et al*., 2008:1068). In pharmaceutical preparations, the multiple units can be in the form of pellets, spherical granules, spheroids or small tablets (Gandhi *et al*., 1999:161).

Pellets or beads are spherical, free-flowing granules with a narrow size distribution, typically varying between 500 and 1500 µm in diameter depending on their application, which can serve as multi-particulate dosage forms (Dukić-Ott *et al*., 2009:38). Their applications are not only found in the pharmaceutical industry but also in the agricultural sector for the production of fertilizer and fish food as well as in the polymer industry (Vervaet *et al*., 1995:131).

Multiple-unit drug delivery systems have the following advantages over single-unit dosage forms:

- They rapidly pass through the pylorus sphincter regardless of the filling level of the stomach or the volume and density of chyme because of their relatively small size, which consequently leads to a reduction in inter- and intra-subject variability of plasma profiles,

- They are freely dispersed in the gastrointestinal tract, thereby maximizing drug absorption, contributing to reduced peak plasma fluctuations and minimizing potential side-effects without lowering the bioavailability,

- Incompatible drugs can be combined in the same dosage form by incorporating each drug in their own sub-units that can be mixed later,

- Pellets or beads with different release mechanisms can be mixed to give a new modified release profile with more reproducible blood levels,

- They are less susceptible to dose dumping in comparison to reservoir type single-unit formulations,

- They avoid high local concentrations of the active pharmaceutical ingredient, which may irritate the stomach mucosa,

- They also have technological advantages such as better flow properties, less friability, are easy to coat and provide uniformity in packing (Gandhi *et al*., 1999:161;Sinha *et al*., 2005:1;Steckel & Mindermann-Nogly, 200:107;Vervaet *et al*., 1995: 151).

3 EXTRUSION-SPHERONISATION

Several methods are used in the preparation of spherical beads, which include ionotropic gelation, solution/suspension layering, powder layering, direct pelletisation using high shear mixers, conventional or rotary fluid-bed granulators and extrusion-spheronisation (Dukić-Ott *et al*., 2009:38). Extrusion-spheronisation has become one of the most popular methods for bead preparation due to its advantages such as the production of relatively dense and homogenous beads of low surface porosity as well as short processing times with a lower number of operators required (Mallipedi *et al*., 2010:53).

Extrusion-spheronisation consists of five unit operations or production steps, which include blending of the dry powders, wet massing by addition of a liquid binder, shaping the wet mass into spaghetti-like cylinders by means of extrusion (extrudate), breaking up the extruded cylinders into short pieces which are shaped into spheres (spheronisation) and drying (see Figure 1) (Dukić-Ott *et al*., 2009:39; Sousa *et al*., 2002:91;Vervaet *et al*., 1995:131).

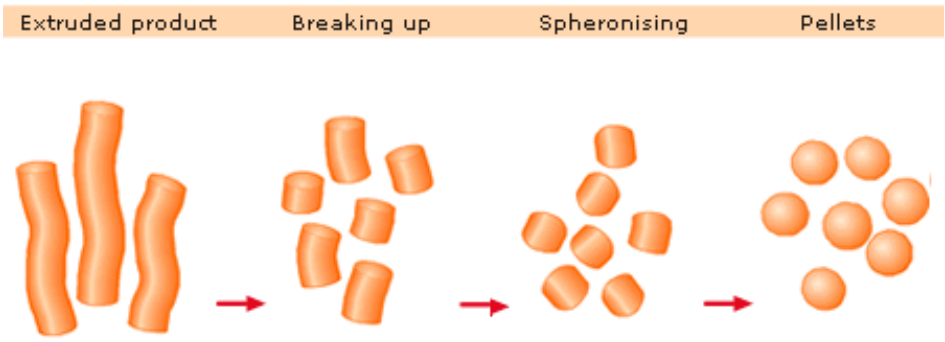


Figure 1: Schematic presentation of the steps involved in the formation of beads from extrudates by means of extrusion spheronisation (Anon, s.a.)

3.1 Factors influencing the quality of the beads

A wide range of process and formulation factors have an influence on the characteristics and final quality of the beads. Some of the characteristics that are important for final quality include the shape or sphericity of the beads, the release kinetics of the model drug from the beads, the porosity, the surface morphology and the physical strength or hardness (Vervaet *et al*., 1995:137).

3.1.1. Moisture content

The moisture content can range between limits within which beads of acceptable quality can be formed. If the moisture content is below the lower limit, a lot of dust is formed and if it is higher than the upper limit, the powder mass will be over-wetted leading to the formation of dumbbells due to the agglomeration of pellets during spheronisation (Vervaet *et al*., 1995:137). The amount of wetting or granulation liquid (e.g. water) required to produce a wet powder mass with the appropriate consistency is related to the solubility of the drug that is included in the bead formulation (Tomer *et al.,* 2001:238). A soluble drug requires more granulation fluid than an insoluble drug, which may easily result in the formation of an over-wetted mass (Vervaet *et al*., 1995:138).

3.1.2. Type of granulation or wetting liquid

The most commonly used wetting or granulation liquid for extrusion spheronisation is water, though the use of alcohol and water/alcohol mixtures has also been reported. The type of granulation liquid determines the hardness of the beads, which ultimately affects the *in vitro* drug release rate. Increasing the water content normally leads to an increase in the hardness of the beads, which correlates with a slower *in vitro* drug release rate (Milli & Schwartz, 1990:1415).

3.1.3. Physical properties of the excipient materials

The bulking agents or filling materials used in the preparation of beads may affect the product size, the sphericity and the release rate of the drug from the final beads. These differences in the physical properties of the beads do not only result from the difference in the composition of the beads but may also occur due to a difference in type of the same material (e.g. different grades of microcrystalline cellulose). A difference in the dissolution profiles of beads containing microcrystalline cellulose and those containing a blend of microcrystalline cellulose and sodium carboxymethylcellulose has been reported. The use

of similar materials from different suppliers can also lead to changes in the characteristics of the beads (Gandhi *et al*., 1999:164; O'Connor & Schwartz, 1985:1847).

Another characteristic of the raw powder materials that may have a profound influence on the extrusion characteristics of the wet mass, the size and sphericity of the resulting beads is their particle size. Beads that are manufactured with a finer grade of microcrystalline cellulose (MCC, Avicel PH101) are smaller than the beads produced using a coarser grade of MCC (i.e. Avicel PH102). The solubility of the raw material used to produce the beads influences the amount of granulation liquid needed to produce a wet mass of appropriate plasticity (Vervaet *et al*., 1995:138).

3.1.4. Type of extruder

An axial screw extruder produces a more dense material compared to a radial screw extruder. The type of extruder determines the amount of granulation liquid needed for optimum extrusion. The differences in the length-to-radius ratio of the extrusion screen used and the differences in shear rate and shear stress of each type of extruder result in a difference in the sphericity and particle size distribution of the beads (Baert *et al*., 1993:7).

3.1.5. Extrusion speed

The total output of the extrudate from the wetted powder mass is governed mainly by the extrusion speed. Surface impairments of the beads such as roughness and sharkskinning become more pronounced with increasing extrusion speed, which leads to the production of beads that have a wide particle size distribution with many fines (Vervaet *et al*., 1995:139).

3.1.6. Properties of the extrusion screen

The extrusion screen is characterised by its thickness and the diameter of the perforations. A change in any of these parameters results in a change in the final quality of the beads. The diameter of the perforations determines the size of the pellets, the larger the diameter of the perforations, the larger the diameter of the beads. The thickness of the screen is measured using the length-to-radius ratio of the screen. A screen with a lower length-to-radius ratio such as the twin-screw extruder forms rough and loosely bound extrudates. In comparison the gravity feed extruder which has a screen with a larger length-to-radius ratio produces smooth and well-bound extrudate because of the greater densification of the wet mass in the screen (Baert *et al*., 1993:12).

3.1.7. Extrusion temperature

A rise in the temperature during the extrusion cycle can alter the moisture content of the granulate due to evaporation of the granulation liquid resulting in some differences between the beads produced at the beginning of the extrusion cycle and at the end (Vervaet *et al*., 1995:140).

3.1.8. Spheronisation speed

The spheronisation speed determines the hardness, sphericity, porosity, bulk and tapped densities, friability, flow rate and surface structures of the final beads. An increase in the spheronisation speed results in an increase in the centrifugal forces, which give the granules greater interparticular impacts, thus a decrease in porosity, which subsequently leads to having a more compact harder structure that is less porous and has a smoother surface. (Bataille *et al*., 1993:665)

3.1.9. Spheronisation time

The spheronisation time has a wide variety of effects on the final quality of the beads. An increase in the spheronisation time usually results in a narrower particle size distribution, higher sphericity, change in bulk and tapped densities and change in the yield of a certain size range (Vervaet *et al*., 1995:139).

3.1.10. Spheroniser load

The spheroniser load influences important properties of the final beads such as the yield, size range, hardness and roundness of the beads. According to Chariot *et al*., (1987:1646), the yield of beads of a specific size range decreased with increased spheronisation speed at a low spheroniser load and increased with extended spheronisation time at a higher spheroniser load. An increased spheroniser load is also associated with increased hardness and decreased sphericity of the final beads.

3.1.11. Drying method

The drying method determines the hardness, porosity and texture of the surface of the beads produced by extrusion-spheronisation. For example, beads that are dried in a microwave oven have a rougher surface, are more porous and softer compared to those dried in a conventional dry heat oven (Bataille *et al*., 1993:654).

3.2 Excipients for production of beads by extrusion-spheronisation

The excipients used in extrusion-spheronisation play an important role in the production of beads with acceptable quality. Due to the nature of the extrusion-spheronisation process, not all moistened powder mixtures can be successfully extruded and spheronised. The moistened powder mixture must be a cohesive plastic mass that remains homogenous during extrusion and possesses inherent fluidity that permits flow during extrusion together with self-lubricating properties as it passes through the die. The resultant strands of extrudates must not adhere to each other and must exhibit enough plasticity to maintain the shape imposed by the die. For the extrudates to be spheronised, they must be brittle enough to break into uniform lengths on the spheroniser plate and have sufficient plasticity to be rounded into spheres by the action of the friction plate in the spheroniser (Basit *et al*., 1999:500).

Furthermore, the ideal excipient required for the production of beads by extrusion spheronisation must be insoluble in water, have large water absorption and retention capacity, binding properties, sufficiently large surface area for interaction with water and other ingredients in powder mixture and the ability to enhance drug release. Most active pharmaceutical ingredients do not have the required characteristics to produce acceptable beads, therefore, excipients that function as spheronisation aids are added to the powder mixtures. The most widely used excipient for bead production by means of extrusion spheronisation is microcrystalline cellulose (MCC) because it produces wetted powder masses with the appropriate rheological properties. MCC produces beads of good sphericity, low friability, high density and smooth surface properties. It absorbs and retains a large quantity of water that facilitates extrusion, improves wetted mass plasticity and enhances spheronisation. It does not undergo phase separation during extrusion or spheronisation and provides pellets of acceptable quality over relatively wide ranges of water content and processing parameters (Almeida Prieto *et al*., 2005:511, Sinha *et al*., 2005:1, Dukić-Ott *et al*., 2009:38).

Despite being a good excipient for bead production by extrusion-spheronisation (Mallipedi et al, 2010:53), MCC is not always the ideal excipient due to distinct disadvantages (Dukić-Ott *et al*., 2009:39). Some examples of these disadvantages include batch-to-batch variability of commercially available raw materials, heat generation during extrusion spheronisation and failure of beads to disintegrate. Its main disadvantage is the impediment of bead disintegration, which often results in incomplete drug release from the beads (Sriamornsak *et al*., 2008:275).

4 KETOPROFEN AS MODEL COMPOUND

Ketoprofen or 2-(3-benzoylphenyl)propionic acid is a non-steroidal anti-inflammatory drug (NSAID), that is widely used to alleviate pain, inflammation and stiffness caused by conditions such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis or abdominal cramps associated with menstruation. The mechanism of action of ketoprofen is associated with its ability to inhibit the biological synthesis of prostaglandins. Ketoprofen is formulated and administered as a racemic mixture of the *R* and *S* enantiomers. The S(+)-enantiomer is the only one that displays pharmacodynamic activity (Vueba *et al*., 2004:51).

Ketoprofen is taken orally; the usual dose is 50-100 mg twice daily with food. Controlled release dosage forms of ketoprofen are available that contain 200 mg active ingredient which can be administered once daily. With the conventional formulations, ketoprofen is readily absorbed from the gastrointestinal tract and the peak plasma concentration occurs within 0.5-2 h which abruptly falls to very low levels resulting in the frequent administration of the drug. Due to its relatively low bioavailability, the drug has to be administered in high doses, which increases the incidence of side effects. Ketoprofen therefore serves as a good candidate for development of modified release multi-unit dosage forms that allow once daily administrations leading to improved patient compliance and maintaining therapeutic plasma levels over extended periods of time (Uner *et al*., 2005:27, Vueba *et al*., 2004:51).

5 ALOE AS A SOURCE OF NATURAL POLYMERS

5.1 Botany of aloe

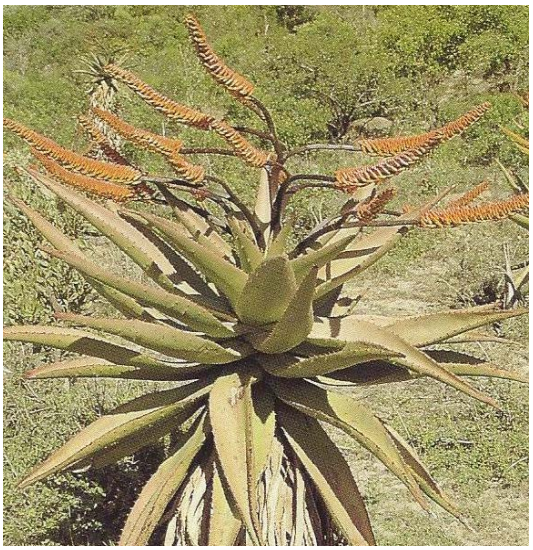
Aloe is a unique plant group that is predominantly found in Africa, which has been proven over the years to be one of the most important natural sources of biologically active compounds. The genus consists of almost 420 species, which can be further divided into 10 groups (Van Wyk & Smith, 2005:30), that are confined mainly in Southern and Eastern Africa as well as Madagascar. The term *aloe* is derived from the Arabic term *alloeh*, which means a shining bitter substance, which refers to the exudate (Dagne *et al*., 200:1055). Aloes were once traditionally grouped with lily-like plants (family Liliaceae) but have now been placed in their own family called Asphodelaceae together with their close relatives such as red-hot pokers and bulbines following recent refinements (Van Wyk & Smith, 2005:30).

Aloe plants come in different shapes and sizes, from miniature ball-shaped rosettes through robust shrubs and single-stemmed specimens to massive trees as shown in Figures 2A-D.

The two most conspicuous characteristics of aloes are their succulent leaves that are arranged in rosettes and their tall, candle-like inflorescences, which make it generally easier to distinguish from their relative species. The flowers are tubular and are born on a simple or branched inflorescence. In addition, the leaves are usually armed with fierce or soft marginal and terminal prickles that remain on the plant when they dry out. Though they are easy to recognise, aloes are often confused with a number of other plants which also occur in South Africa, these include *Agave, Kniphofia, Bulbine, Gasteria, Haworthia, Astroloba and Chortolorion* (Van wyk & Smith, 2005:8).

A

B



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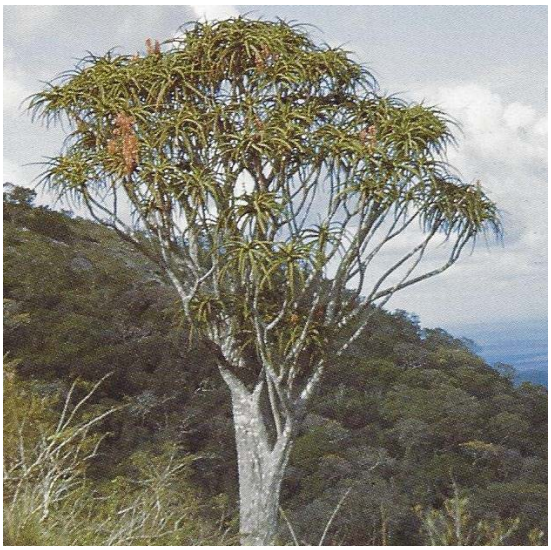


D

Figure 2: Photographs of representative species namely A) *Aloe brevifolia,* B) *Aloe plicatilis,* C) *Aloe marlothii* and D) *Aloe barberae* (Van wyk & Smith, 2005:34,40,60,244)

Except for a few, aloes are protected by environmental legislation in all provinces of South Africa, making it illegal to remove plants from their natural habitats unless one is in possession of a collection permit and has the consent of the owner. The population of aloe species has declined due to a number of factors, which include urban and industrial expansion, agricultural development, afforestation and mining activities. Aloes are perennials that can thrive for many years that do not need special growing conditions and are pre-adapted to harsh climates. Once established, they need very little after-care. However, they don't grow optimally without water as most people have been made to believe. They can indeed tolerate long periods of drought, but they thrive and flower well when adequate water is provided in the correct season (Van Wyk & Smith, 2005:18,20).Out of a large number of aloe species in South Africa only two (*Aloe vera* and *Aloe ferox*) are of commercial importance in international trade (Dagne *et al*., 2000:1055).

5.2 Uses of aloe

Aloe leaves have been used for various medicinal purposes for thousands of years. Aloe leaves yield two medicinal products, a mucilaginous gel and bitter exudate. The gel is incorporated in cosmetic products in the form of aloin as a natural skin-lightener as it is believed to be a melanin synthesis inhibitor. Shampoos, shaving and skin care products also contain aloe gel because of its moisturizing and soothing properties. The bitter exudate is mainly used as a laxative as listed in modern pharmacopoeias and a bittering agent in certain beverages (Dagne *et al*., 200:1055). It is also claimed that aloe gel can enhance immunity, improve liver function, prevent asthma, and act as an anti-inflammatory, anti-ulcer, anti-diabetes and anti-hypertensive agent. The extremely bitter juice that oozes from the small canals situated just below the surface in the green part of the leaf is used widely as a first aid treatment for burn wounds (Grace *et al.,* 2009:172)

5.3 Phytochemical composition

Over 130 phytochemicals have been identified in aloe plants that belong to different classes, including anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes and flavonoids. Aloe species can be grouped into three major chemical groups namely the flavonoid-producing species, anthrone producing species and plicataloside accumulating species (Dagne *et al*., 2000:1056).

5.4 *Aloe vera*

The leaves of *Aloe vera* (L.) Burm.f. (*Aloebarbadensis* Miller) are made up of an outer green rind, a yellow exudate just below the skin and the innermost pulp. The pulp is made up of clear, soft, moist and slippery tissue that consists of large thin-walled parenchyma cells in which water is held in the form of viscous mucilage or gel. The raw pulp of *A. vera*contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water (Hamman, 2008:1600).

It is used in the food industry for the production of health drinks and beverages, in the cosmetics industry to act as base material in the production of creams, lotions, soaps, shampoos, facial cleaners and other products. It has shown potential for wound healing, treatment of frost bites, alleviation of ulcers as well as treatment of diabetes and cancer (Reynolds & Dweck, 1999:11-18).

A. vera contains more than 75 identified chemical constituents and its therapeutic effects cannot be correlated well with any individual component but are believed to be a result of the synergistic effect of the compounds therein, though many of the medicinal effects have been attributed to the polysaccharides. Due to its absorption enhancing effects, *A. vera* gel may be employed to effectively deliver poorly absorbable drugs through the oral route of drug administration. In addition, the dried powder obtained from *A. vera* gel was successfully employed to manufacture directly compressible matrix type tablets that slowly released the model compound over an extended period of time (Jani *et al.,* 2007:90; Hamman, 2008:1600;Jambwa *et al.,* 2011:433).

Figure 3: Photograph of an *Aloe vera* plant (Uppal, 2012)

5.5 *Aloe marlothii*

Aloe marlothii Berger also known as mountain aloe is a large, single stemmed aloe that grows up to 6 m in height and is abundant on rocky north-facing slopes (Symes *et al*., 2009:2). It is found in South Africa, Botswana, Mozambique and Zimbabwe and has been used previously as a source of drug aloes. It has broad leaves that are dull green to grayish green in colour with dark brown spines along the margins and bright orange to red tubular flowers (Figure 4) that are rich in nectar providing an important source of energy for sunbirds. It's leaves have prickles along the margins as well as on the upper and lower surfaces (Bisrat *et al.,* 2000:949; O'Brien, 2005:31).

Although *Aloe marlothii* is no longer used commercially in the production of laxatives, it still plays an important role in traditional medicines. It is used to treat roundworm infections, for stomach troubles such as constipation and for hastening the weaning of children (Van der Bank *et al.,* 1995:251).

Figure 4: Photograph of an *Aloe marlothii* plant (Van Wyk & Smith, 2005:62)

6 'DESIGN OF EXPERIMENTS' AS AN INSTRUMENT TO OPTIMISE PHARMACEUTICAL FORMULATIONS

The purpose of conducting research experiments in industry and academia is to develop processes and products that are optimised for their intended use. Traditionally, optimising of a formulation or process entailed studying the influence of the corresponding composition and process variables by Changing One Single variable at a Time (COST), while keeping the rest constant (Singh *et al*., 2004: 28). However, this method does not provide a true reflection of the effects or factors and operating conditions as interactions between these factors can occur that it cannot account for. One solution is to construct a carefully selected set of experiments in which all relevant factors are varied simultaneously, which is called 'Design of Experiments' (DoE). Design of experiments or experimental design can be defined as the strategy for setting up experiments in such a manner that the information required is obtained as efficiently and precisely as possible (Lewis *et al*., 1999:2). It provides a reliable basis for decision-making thereby providing a framework for changing all the important factors systematically while requiring only a limited number of experiments. In the DoE method, more than one factor is varied at a time in situations where there is an interest in the effects of multiple input factors on output responses. With the rapidly increasing cost of experiments, it is essential that optimisation of research is done as efficiently as possible. DoE is used to ensure that the selected experiments produce the maximum amount of relevant information. This approach is capable of identifying critical factors and their interactions with a minimal number of experiments (Ray *et al*., 2009:311).

DoE can be used in the development of new products and processes, enhancement of existing products and processes, optimisation of quality and performance of a product, optimisation of an existing manufacturing procedure, screening important factors, minimisation of production costs and pollution as well as the robustness testing of products and processes (Eriksson *et al*., 2008:8).

There are three main types of problems that DOE is applicable to, which include screening, optimisation and robustness. With optimisation, the interest lies in defining which combination of important factors will result in optimal operation conditions. With robustness, the aim is to determine the sensitivity of a product or production procedure to small changes in the factor settings (Eriksson *et al*., 2008:10).

Prior to conducting any experiments, the experimenter has to specify some input conditions such as the number of factors and their ranges, the number of responses and the

experimental objective. The experiments are carried out and the results of the experiments are collected. The data is investigated using regression analysis that gives a model relating the changes in factors to the changes in responses. The model will indicate which factors are important, and how they combine in influencing the responses. The modelling results are converted into response contour plots, which are used to clarify where the best operating conditions are (Eriksson *et al*., 2008:11-14).

Areas where DoE is used in industrial research, development and production are:

- optimization of manufacturing processes,
- optimization of analytical processes,
- screening and identification of important factors,
- robustness testing of methods,
- robustness testing of products,
- formulation experiments.

The essence of DoE is to plan informative experiments, to analyse the resulting data to get a good model and from the model create meaningful maps of the system. There are three critical problems that DoE handles more effectively than COST, the first is the understanding of a system or process that is influenced by many factors. DoE allows the variation of more than one factor simultaneously thereby enabling the estimation of interactions between these factors. Secondly the systematic and unsystematic variability and finally the reliable maps of investigated system are hard to produce without a proper DoE foundation. For a response contour plot to be valid and meaningful, it is essential that the experiments be positioned in such a way as to cover as much as possible of the domain of the contour plot which is usually not the case with the COST approach. With DoE it is not only possible to sharpen the estimate by averaging but also estimate the size of noise using the standard deviation of residuals (Eriksson *et al*., 2008:16-17).

CHAPTER 3

MATERIALS AND METHODS

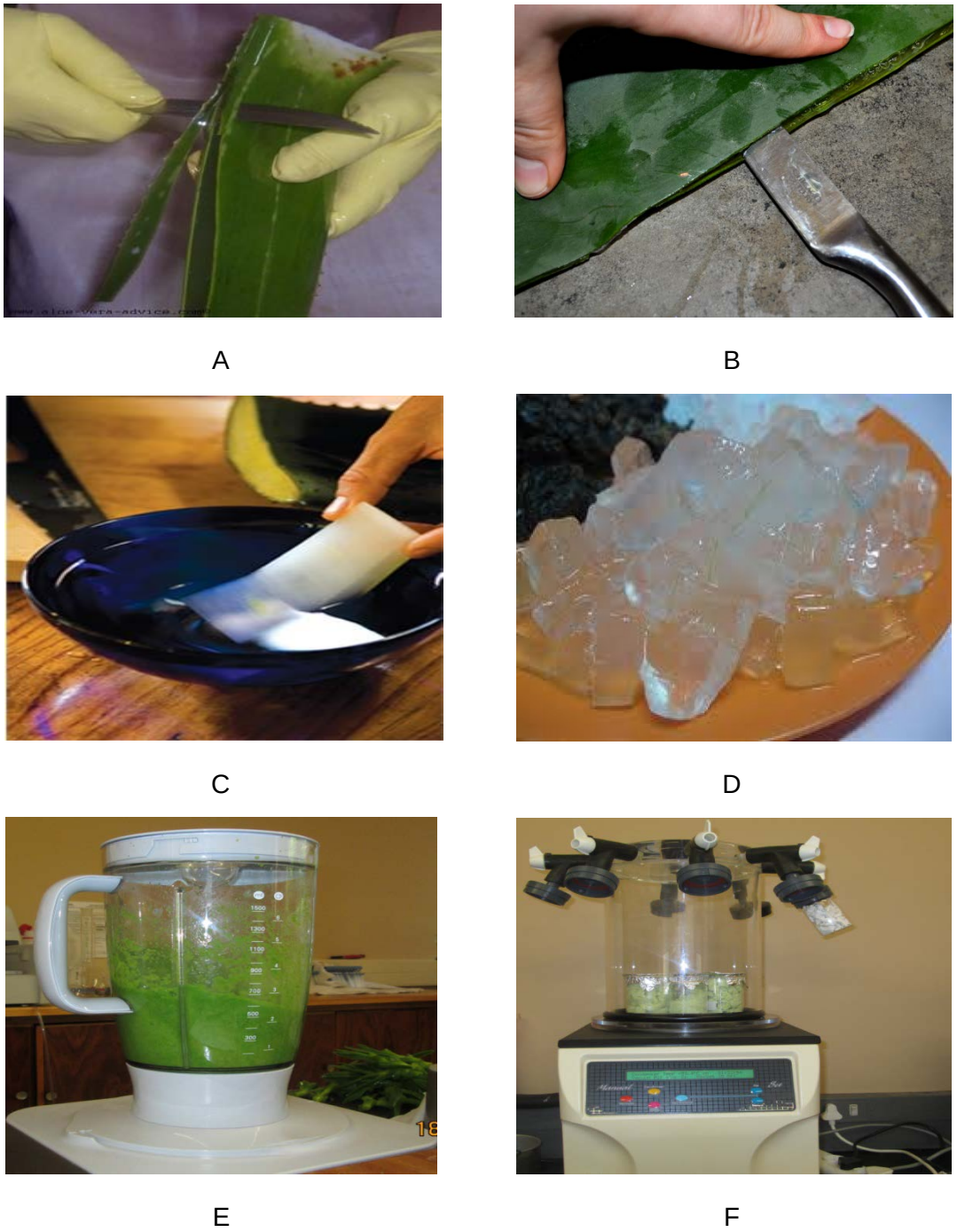
1 Materials

Aloe vera dehydrated gel (Daltonmax700®,Batch No. 700AQ11PK01) and *A. vera* whole leaf material (Daltonmax700®,Batch No. 715AQ11PK01) were generous gifts from Improve Inc (Texas, USA). The model drug ketoprofen (batch No. FP11110030043) was purchased from DB Fine Chemicals (South Africa). Vinylpyrrolidone-vinylacetate-copolymer (PVP, Kollidon VA 64 Batch No. 26460375L0) was bought from BASF (Germany). Avicel® pH 101 (microcrystalline cellulose, Batch No. 60839C) was obtained from FMC Bioplymers (Ireland). Potassium dihydrogen orthophosphate (KH_2PO_4), sodium hydroxide (NaOH) and hydrochloric acid (HCl) for the preparation of dissolution media were obtained from Merck chemicals (Pty) Ltd (South Africa). Sodium acetate and glacial acetic acid for the preparation of acetate buffer were obtained from Sigma Aldrich (Switzerland) and Labchem (Pty) Ltd (South Africa), respectively. The reagents used during HPLC analysis include acetonitrile and methanol, which were obtained from Merck Chemicals (Germany) and (South Africa), respectively.

2 Methods

2.1 Processing of *Aloe marlothii* gel and whole leaf powder

A. marlothii leaves were obtained from plants growing on a farm in the North West Province, South Africa. The leaves were removed from the plants in such a way to allow further growth, i.e. sustainable harvesting, by removing only a few leaves from the bottom of the rosette of leaves. Furthermore, the leaves were kept intact with no exposure of any inner parts of the leaves to the atmosphere. The leaves were processed to obtain gel and whole leave materials as illustrated in the pictures in Figure 5A-H. The first step entailed cutting off the extreme ends of the leaves. To obtain gel material, the skin of the leaves was removed and the fillet or pulp was cut into smaller cubes after rinsing it in cold water once. To obtain whole leaf material, the skin was also removed after which all the leave parts were rinsed and then cut into smaller cubes. The cubes were liquidised in a food processor and then frozen in a -80ºC fridge where after it was lyophilized in a Virtis freeze drier (UK).



A B C D E F

Figure 5: Pictures illustrating the steps in the processing of the *A. marlothii* leaves. A) Cutting off the edges of the leaves, B) removing the skin from the leaves, C) rinsing the pulp in water, D) pulp cut into small cubes, E) liquidising whole leaf material, F) freeze drying a sample of aloe whole leaf material

A voucher specimen of the *A. marlothii* plant is kept in the herbarium at North-West University, Potchefstroom, South Africa.

2.2 ^{1}H-NMR fingerprinting of aloe materials

A quantity of 50 mg of each of the *A. vera* materials together with 5 mg of the internal standard, nicotinic acid amide (NSA), were dissolved in 1 ml of D_2O and their ^{1}H-NMR spectra were recorded with an Avance 600 MHz NMR spectrometer (Bruker). A quantity of 30 mg of each of the *A. marlothii* materials together with a very small amount of the reference compound, 3-(Trimethylsilyl)-propionic acid-D4 (TPS), were dissolved in 1.5 ml of D_2O and their ^{1}H-NMR spectra were recorded with a 600 MHz NMR spectrometer (Bruker).

2.3 Design of experiments (DoE)

A 2^3 full factorial design was used for optimizing the bead formulations. Computer software namely MODDE 9.0™ by Umetrics from Sweden, was used to develop the design of experiments (DoE). Three centre points were added to measure experimental error and determine the reproducibility. Formulation and process variables and their ranges were acquired from preliminary experiments. After entering the variables into the software, the screening option was chosen in designing the experimental runs. The low and high levels for microcrystalline cellulose (MCC or Avicel® PH 101) ranged from 70 – 100% (w/w), *Aloe vera* whole leaf (AVWL) ranged from 0 – 30% (w/w) and *Aloe vera* gel (AVG) as well as *Aloe marlothii* gel (AMG) and whole leaf (AMWL) ranged from 0 – 10% (w/w), while the low and high levels of Vinylpyrrolidone-vinylacetate-copolymer(PVP or Kollidon VA 64) ranged from 0 - 5% (w/w). Increasing the concentration of AVWL above 30% (w/w) and of AMG, AVG and AMWL above 10% (w/w) resulted in no beads. The spheronisation time and speed were constant at 5 min and 1 500 rpm, respectively. There were ten formulations identified by MODDE 9.0 for each plant material including three centre points. The studied factors and their levels are summarised in Table 1a and b.

Table 1a: 2^3 Full factorial design of experiments for *Aloe vera* whole leaf

Factors	Levels of factors	
	-1	+1
Amount of MCC*	70% w/w of dry mix	100% w/w of dry mix
Amount of PVP*	0% w/w of dry mix	5% w/w of dry mix
Amount of AVWL*	0% w/w of dry mix	30% w/w of dry mix

*MCC = microcrystaline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer, AVWL = *Aloe vera* wholeleaf

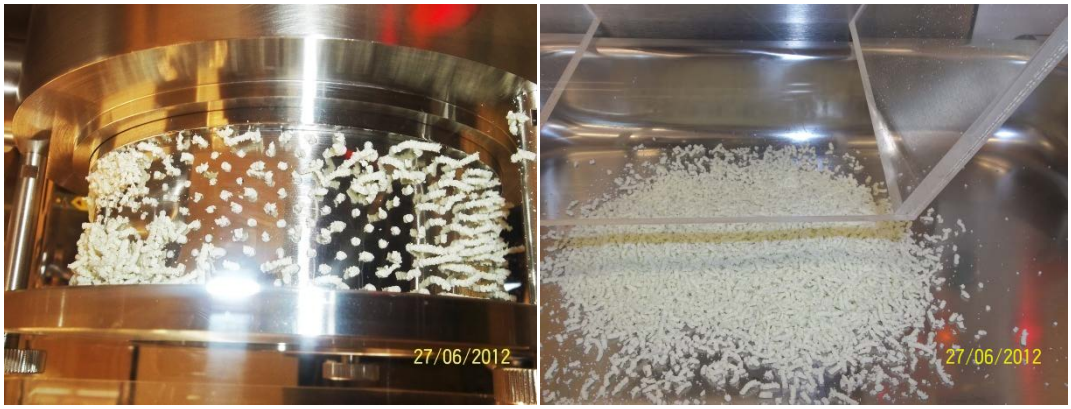
Table 1b: 2^3 Full factorial design of experiments for *Aloe vera* gel, *Aloe marlothii* gel and whole leaf

Factors	Levels of factors	
	-1	+1
Amount of MCC*	90% w/w of dry mix	100% w/w of dry mix
Amount of PVP*	0% w/w of dry mix	5% w/w of dry mix
Amount of AVG, AMG, AMWL*	0% w/w of dry mix	10% w/w of dry mix

*MCC = microcrystaline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer, AVG = *Aloe vera* gel, AMG = *Aloe marlothii* geland AMWL = *Aloe marlothii* whole leaf

2.4 Bead manufacture by extrusion spheronisation

Different masses of the ingredients for the bead formulations including aloe powders (i.e. AVG, AVWL, AMG and AMWL), MCC (Avicel® PH101) and PVP (Kollidon® VA 64) were weighed out according to the worksheet generated by the DoE. The dry powders for each formulation were mixed in a Turbula mixer (Willy, A. Bachofen, Switzerland) for 6 min. De-ionized water was added to the powder mixture of each bead formulation while blending the powder mass in a Kenwood®planetary mixer for 2 min. The resultant wetted powder mass of each bead formulation was passed through the extruder (Type 20 Caleva®, Caleva Process Solutions, England) fitted with a 2 mm extrusion screen at a speed of 25 rpm to form spaghetti-like extrudates (refer to Figure 6A and B).



A B

Figure 6: Pictures illustrating A) the extrusion of wetted powder mix through the 2 mm extrusion screen, B) extruded cylinders ready for spheronisation

The extrudates of each bead formulation was immediately introduced to the Caleva® spheroniser MBS (Caleva Process Solutions, England) to form spherical beads (refer to Figure 7A and B). The spheroniser was operated at 1500 rpm for 5 min to form the beads that were dried in a conventional oven overnight at 37 °C. The beads were filled into hard gelatine empty capsules manually.

A B

Figure 7: Pictures illustrating A) the Caleva® spheroniser and B) the formation of spherical beads from the extrudate

2.5 Bead characterization

2.5.1 Scanning Electron Microscopy

A total of six beads were chosen from each of the following formulations containing 100% (w/w) MCC, 3.75% (w/w), 7.5% (w/w) and 10% (w/w) of AVG, AMG and AMWL as well as 15% (w/w) and 30% (w/w) for AVWL. The beads were pasted onto stubs by means of double-sided carbon tape. Two of the beads were cut in half to expose the internal structure of the beads. The beads were coated in an ion coater (Eiko engineering) using gold and palladium in a ratio of 66%:34% under a vacuum of 1.5 torr. After coating, the beads were placed in a scanning electron microscope (FEI quanta FEG 250) and micrographs were taken of the external surface and internal structure of the beads at a magnification of 130X and 1000X, respectively.

2.5.2 Sphericity

The sphericity of the beads was determined using the Motic™ images analysis system. Photomicrographs (Figure 8) were taken using a camera (Moticam 2300) attached to a light microscope (Nikon SM2-1). The maximum diameter and perimeter ten beads from each

batch were determined by drawing lines across and around the beads respectively using tools from Motic images. The sphericity of the beads was calculated as "Pellips" according to the following equation (Almeida-Prieto *et al*., 2007:768, Koo & Heng, 2001:1384):

$$\text{Pellips} = \frac{p}{d_{max}} \quad (1)$$

Where p is the perimeter and d_{max} is the maximum diameter (Almeida-Prieto*et al*., 2007:768)

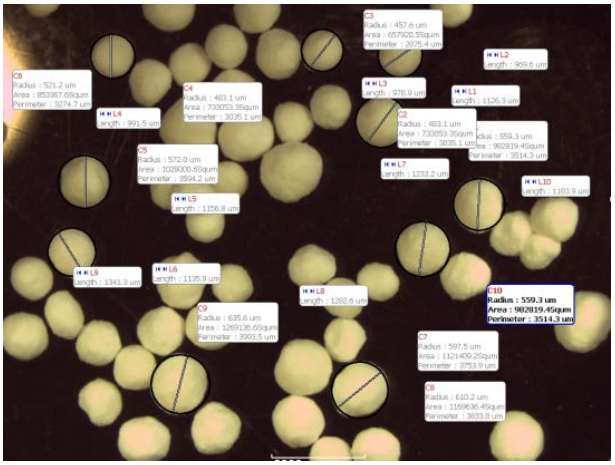


Figure 8: Photomicrograph of beads containing 3.75% w/w *A. marlothii* gel indicating the perimeter and diameter measurements (the lines across the beads represent the diameter and the lines around the beads represent the perimeter)

2.5.3 Mass variation

To determine mass variation of hard gelatine capsules (size 0) filled with beads from each formulation, 20 empty capsules were weighed individually and the average mass of empty capsules was determined. Twenty filled capsules were then selected randomly from each formulation and weighed individually. The mass of the contents was determined by subtracting the average mass of empty capsules from the mass of filled capsules. The average mass of the beads in the capsules was calculated and each capsule's content was compared to that of the average mass. The mass variation of capsules weighing more than 300 mg must not have a percentage deviation of ± 7.5% from the average (BP2012:A341).

2.5.4 Friability

Beads weighing 3 g were placed in a friability tester (Paravalux Electric motors, England) along with 25 glass beads with a diameter of 5 mm. The friability tester was operated at 25 revolutions per minute (rpm) for 4 min to give 100 revolutions in total. The beads and glass beads were placed in a 18-mesh sieve. The glass beads were removed from the sieve and

after smaller particles were allowed to pass through, the mass of the beads was weighed. Friability (F) was determined by calculating the percentage loss in mass according to the following equation:

$$F = \frac{W_1 - W_2}{W_1} \quad (2)$$

Where W_1 is the mass of the beads before the friability test and W_2 is the mass of the same beads after they were exposed to friabilating. The friability of each batch of beads was assessed in duplicate (*Mallipedi* et al., 2010:55, Lee *et al*., 2005:621).

2.6 Drug release studies

The dissolution of all the bead formulations as composed by the DoE was carried out in potassium phosphate buffer at pH 6.8, while the dissolution of the optimised bead formulations were determined at pH 1.2, 4.5 and 6.8. The buffer solutions were prepared according to the methods outlined in the following sections.

2.6.1 Preparation of 0.1 N HCl (pH 1.2)

The amount of hydrochloric acid (HCl) to be added to distilled water to make the 0.1 N HCl solution with pH 1.2 using 32% (w/v) HCl was calculated as described below.

The molarity of a 32% HClsolution:

= 320/36.5
= 8.767 M

To get the volume of HCl needed to produce 1000 ml of 0.1 N HCl:

$C_1V_1 = C_2V_2$
$8.767 \times v_1 = 0.1 \times 1000$
$V_1 = (0.1 \times 1000) / 8.767$
= 11.406 ml

Therefore 11.406 ml of 32% (w/v) HCl were added to a 1000 ml volumetric flask and made up to volume with distilled water to produce a solution of 0.1 N HCl.

2.6.2 Preparation of acetate buffer (pH 4.5)

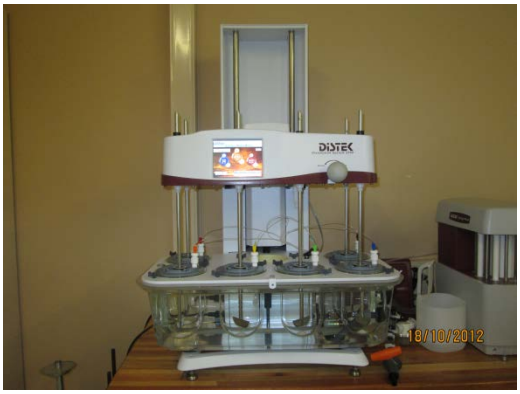
An acetate buffer with a pH of 4.5 was prepared by adapting the formula of the USP (2008:2653) for methazolamide tablets. A mass of 2.99 g sodium acetate and 1.66 ml of glacial acetic acid were added to a 1 L volumetric flask and water was added to make up the volume.

2.6.3 Preparation of potassium phosphate buffer (pH 6.8)

A potassium phosphate buffer with a pH of 6.8 was prepared according to the BP (2012:A332) formula. A volume of 112 ml of 0.2 M sodium hydroxide (NaOH) was added to 250 ml of 0.2 M potassium dihydrogen orthophosphate (KH_2PO_4) and distilled water was added to make the volume up to 1000 ml. A solution of 0.2 M KH_2PO_4 was prepared by dissolving 27.22 g of KH_2PO_4 powder in distilled water and making it up to 1000 ml with distilled water. A solution of 0.2 M NaOH was made by dissolving 8 g of NaOH powder in 1000 ml of distilled water. The pH of the buffer solution was measured and adjusted to 6.8 using 0.1 M HCl or 0.1 M NaOH.

2.6.4 Dissolution test

Dissolution studies were carried out using the USP paddle method in a six station dissolution apparatus (Distek 2500 dissolution apparatus as depicted in Figure 9A, North Brunswick, NJ, USA). The stirring rate was 50 rpm in 900 ml of buffer maintained at 37 ± 0.5 °C. Samples of 5 ml were drawn using an auto sampler (Distek evolution 4300 North Brunswick, NJ, USA) as displayed in Figure 9B at predetermined time intervals of 30, 60, 90, 120, 180, 240, 360, 480, 600, 720 and 1440 min. After the sample was withdrawn at 24 h, the stirring rate was set at 250 rpm for a further 15 min and the last sample was then collected.



A B

Figure 9: Pictures depicting A) the Distek six station dissolution apparatus and B) autosampler

2.7 Analysis of samples by High Performance Liquid Chromatography

The concentration of the dissolved drug in the dissolution samples was determined by high performance liquid chromatography (HPLC). The HPLC system parameters were as follows:

Analytical instrument:	Agilent HP1100 series equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software
Column:	Venusil XBP C18 (2), 150 x 4.6 mm, 5 µm, 100 Å pores
Mobile phase:	Water, acetonitrile and acetic acid in the ratio of 29:70:1
Flow rate:	1 ml/min
Injection volume:	50 µl
Detection:	UV at 255 nm
Retention time:	The average retention time for the analyte is 3.03 minutes
Stop time:	6 min
Solvent:	Phosphate buffer pH 7.4

2.7.1 Validation test procedure and acceptance criteria

The HPLC method was validated through determination of linearity, precision, accuracy; ruggedness and specificity. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose (International Conference on Harmonisation, 2005:1). The purpose of this validation was to determine if the HPLC method was accurate and reliable in the determination of the quantity of ketoprofen released from the beads during dissolution testing.

2.7.1.1 Preparation of ketoprofen solutions

A quantity of approximately 25 mg of ketoprofen was accurately weighed and transferred into a 100 ml volumetric flask. A volume of 5 ml of methanol was used to dissolve the ketoprofen and phosphate buffer saline (PBS, pH 7.4) was added to make up to volume.

Dilutions were made from the above solution as follows:

i) 5 ml of the solution was diluted to 50 ml with PBS pH 7.4 to render a concentration of 25 µg/ml,

ii) 5 ml of the solution in (i) were diluted to 50 ml with PBS pH 7.4 to render a concentration of 2.5 µg/ml,

iii) 5 ml of solution in (ii) were diluted to 50 ml with PBS pH 7.4 to render a concentration of 0.25 µg/ml,

iv) 5 ml of solution in (iii) were diluted to 50 ml with PBS pH 7.4 to render a concentration of 0.025 µg/ml.

A volume of 50 µl of the solutions with different concentrations was injected at different volumes into the chromatograph to construct a linear regression curve.

2.7.1.2 Linearity

The linearity of an analytical method is its capacity to elicit test results that are directly or by means of a well-defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range (USP 35 2011:880).

Calibration solutions were prepared according to the method previously described (refer to paragraph 2.7.1.1). Volumes of 12.5, 25 and 50 µl of the different concentrations were

injected in duplicate into the chromatograph. Acceptance criteria: Linear regression analysis should yield a regression coefficient (R^2) of ≥ 0.999.

2.7.1.3 Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that procedure to the true value (USP 35, 2011:878).

A ketoprofen stock solution was prepared according to the method previously described (refer to paragraph 2.7.1.1). Dilutions were made from the above solution as follows:

i) A volume of 10 ml of the standard solution was diluted to 50 ml with PBS,

ii) A volume of 10 ml of the solution in (i) was further diluted to 100 ml with PBS,

iii) A volume of 5 ml of the solution in (ii) was diluted to 100 ml with PBS.

A volume of 50 µl of the different solutions was injected three times in duplicate. Acceptance criteria: Recovery must be between 98 – 102%.

2.7.1.4 Ruggedness (robustness)

Ruggedness is also known as intermediate precision, expressed within laboratory variation as on different days or with different analysts or equipment within the same laboratory. It was done to determine the ruggedness of HPLC in the determination of ketoprofen over 24 h.

Samples were prepared as described under sample preparation (please refer to paragraph 2.7.1.1). A volume of 50 µl of the sample was injected every hour for 24 h.

Acceptance criteria: The solutions should not be used for a period longer than it takes to degrade by 2 %.

2.7.1.5 Precision

Precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. It is usually expressed as the standard deviation or relative standard deviation of a series of measurements. It can be considered at three levels: repeatability, intermediate precision and reproducibility (BP, 2012: 8).

2.7.1.5.1 Inter-day precision

Day 1: Results for the calibration solution prepared for accuracy were used.

Day 2: Samples were prepared as described under ketoprofen solution preparation (please refer to paragraph 2.7.1.1).

Day 3: Samples were prepared as described under ketoprofen solution preparation (please refer to paragraph 2.7.1.1).

Acceptance criteria: The %RSD of the analyses for inter-day precision must be less than 5 %.

2.7.1.5.2 Repeatability (intra-day precision)

Repeatability expresses the precision of the HPLC over a short interval of time under the same operating conditions. It is also termed intra-assay precision. Ketoprofen solutions were prepared as previously described (please refer to paragraph 2.7.1.1).

A volume of 50 µl was injected six times and the retention times noted.

Acceptance criteria: The peak and retention times should have a relative standard deviation (RSD) of 2 % or less.

2.7.1.6 Specificity

This is the ability of an analytical procedure to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components (USP35, 2011:879)

A sample was prepared by weighing approximately 13 mg of ketoprofen and dissolving it in 5ml of methanol. The solution was transferred to a 100 ml volumetric flask and made up to volume using buffers that were used as dissolution media i.e. HCl at pH 1.5 and phosphate buffer at pH 4.5 and 6.8. Samples were injected at volumes of 10, 20, 30, 40 and 50 µl into the chromatograph in duplicate.

2.7.1.7 Processing of dissolution profiles

The dissolution data was processed and analysed by calculating the Mean Dissolution Time (MDT) (Sousa *et al*., 2002:113), difference factor (f_1) and similarity factor (f_2) to compare the

dissolution profiles of the different formulations. MDT is a statistical moment for the cumulative dissolution process and is defined by the following equation:

$$MDT = \sum_{i=1}^{n} t_i \times M_t / M_\infty \quad (3)$$

Where M_t is the amount of the drug released at time t; t_i is the time (minutes) at the midpoint between i and i-1; M_∞ is the overall amount of the drug released (Reppas & Nicolaides, 2000:232)

The dissolution profiles containing plant material were compared to the formulation containing 100% (w/w) microcrystalline cellulose (MCC) using the difference and similarity factors (f_1 and f_2) that were calculated according to the following equations:

$$f_1 = \left[\frac{\sum_{t=1}^{n}|R_t - T_t|}{\sum_{t}^{n} R_t}\right] \quad (4)$$

$$f_2 = 50 \log\{(1 + \frac{1}{n}\sum_{t=1}^{n} W_t[R_t - T_t]^2)^{-0.5} \times 100\%\} \quad (5)$$

Where R_t is the reference assay at time point *t;* T_t is the test assay at time point *t; n* is the number of pull points and W_t is an optional weight factor (Moore & Flanner, 1996:66).

3 Optimisation of bead formulations

Optimisation of the bead formulations based on the sphericity and dissolution (MDT values) as responses was done with MODDE® software. Each response (i.e. sphericity and MDT) was applied individually as illustrated step-by-step in Figure 10A-L.

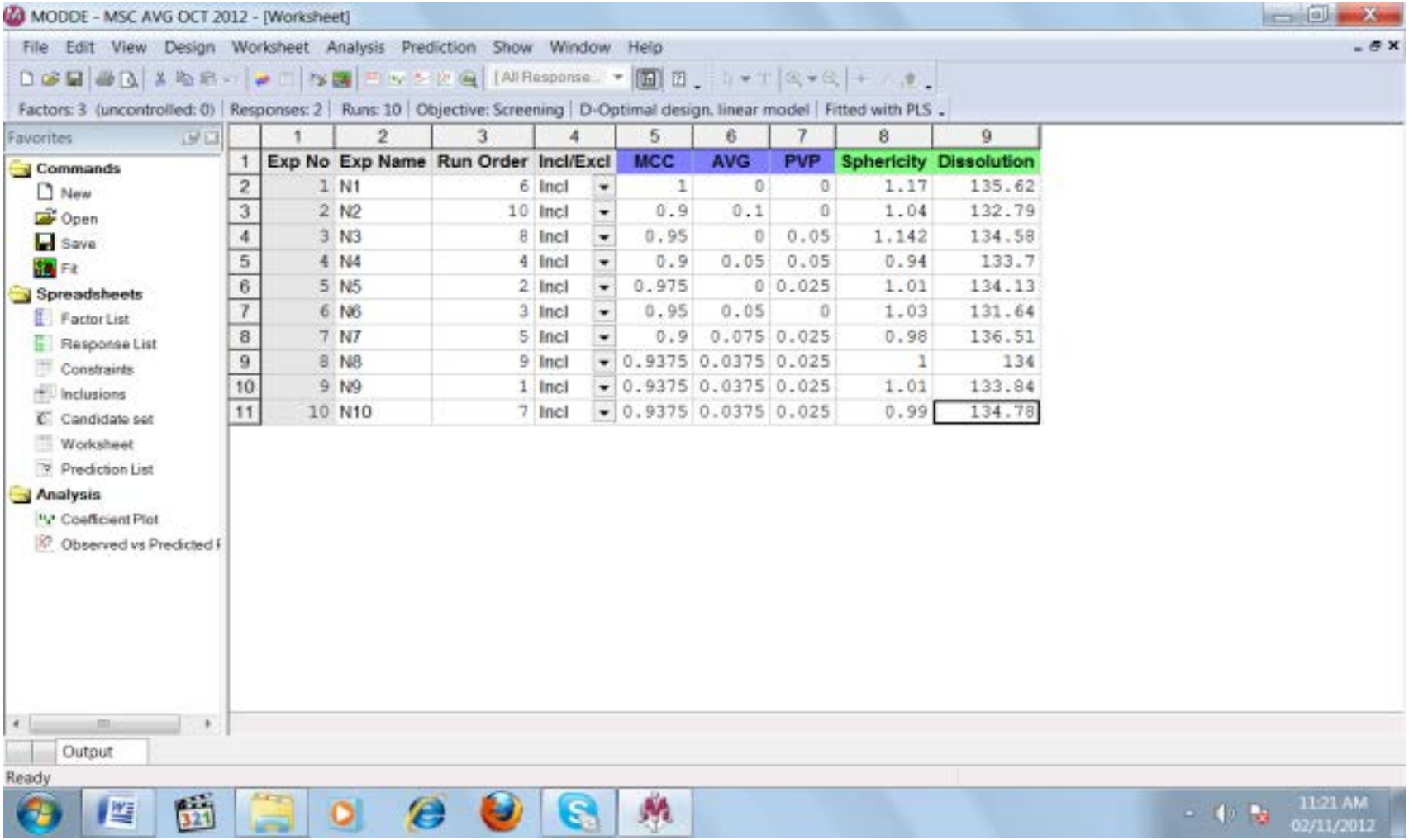
The results obtained from the sphericity and dissolution experiments for all the bead formulations were entered into the worksheet (Figure 10A). The results were processed by choosing the “Analysis” tab (Figure 10B) in the “Analysis wizard” menu, which gave the further option of selecting any response that was entered (Figure 10C). After selecting the response for analysis, the replication plot was opened (Figure 10D), which showed the variation in results for all experiments, followed by a histogram (Figure 10E) that showed the shape of the response distribution and is used to determine if a transformation is needed when the desired “bell shaped” normal distribution is not present. The next plot that was opened is the summary plot (Figure 10F), which is a summary of the basic statistics in four parameters as described below.

- **R2 (green)**: shows the model fit and 0.5 indicates a model with a low significance.
- **Q2 (blue)**: shows the estimate of the future prediction precision which should be greater than 0.1 for a significant model and greater than 0.5 for a good model.
- **Model validity (yellow)**: is a test for certain problems, e.g. a value less than 0.25 indicates the presence of outliers, an incorrect model or a transformation problem.
- **Reproducibility (light blue)**: shows the variation of the replicates compared to the overall variability.

The coefficient plot (Figure 10G) was then opened, which is a graphical presentation of the model terms, followed by a residuals N-plot (Figure 10H), which shows the residuals of a response in comparison to the normal probability of the distribution. Finally, the Observed vs. Predicted plot (Figure 10I) was opened, which displays observed values versus predicted values with the correlation coefficient. When all the analysis for one response is done, the programme gives you an option to go on to the next response.

The concentration of the different ingredients for the beads needed to produce optimum formulas for the two responses (i.e. sphericity and MDT) were averaged out so as to obtain beads with the desired sphericity and dissolution.

The actual screen displays from MODDE® for each step in the optimisation process are depicted in Figure 10A-J below.



	1	2	3	4	5	6	7	8	9
1	Exp No	Exp Name	Run Order	Incl/Excl	MCC	AVG	PVP	Sphericity	Dissolution
2	1	N1	6	Incl	1	0	0	1.17	135.62
3	2	N2	10	Incl	0.9	0.1	0	1.04	132.79
4	3	N3	8	Incl	0.95	0	0.05	1.142	134.58
5	4	N4	4	Incl	0.9	0.05	0.05	0.94	133.7
6	5	N5	2	Incl	0.975	0	0.025	1.01	134.13
7	6	N6	3	Incl	0.95	0.05	0	1.03	131.64
8	7	N7	5	Incl	0.9	0.075	0.025	0.98	136.51
9	8	N8	9	Incl	0.9375	0.0375	0.025	1	134
10	9	N9	1	Incl	0.9375	0.0375	0.025	1.01	133.84
11	10	N10	7	Incl	0.9375	0.0375	0.025	0.99	134.78

Figure 10A: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: worksheet where responses have been added

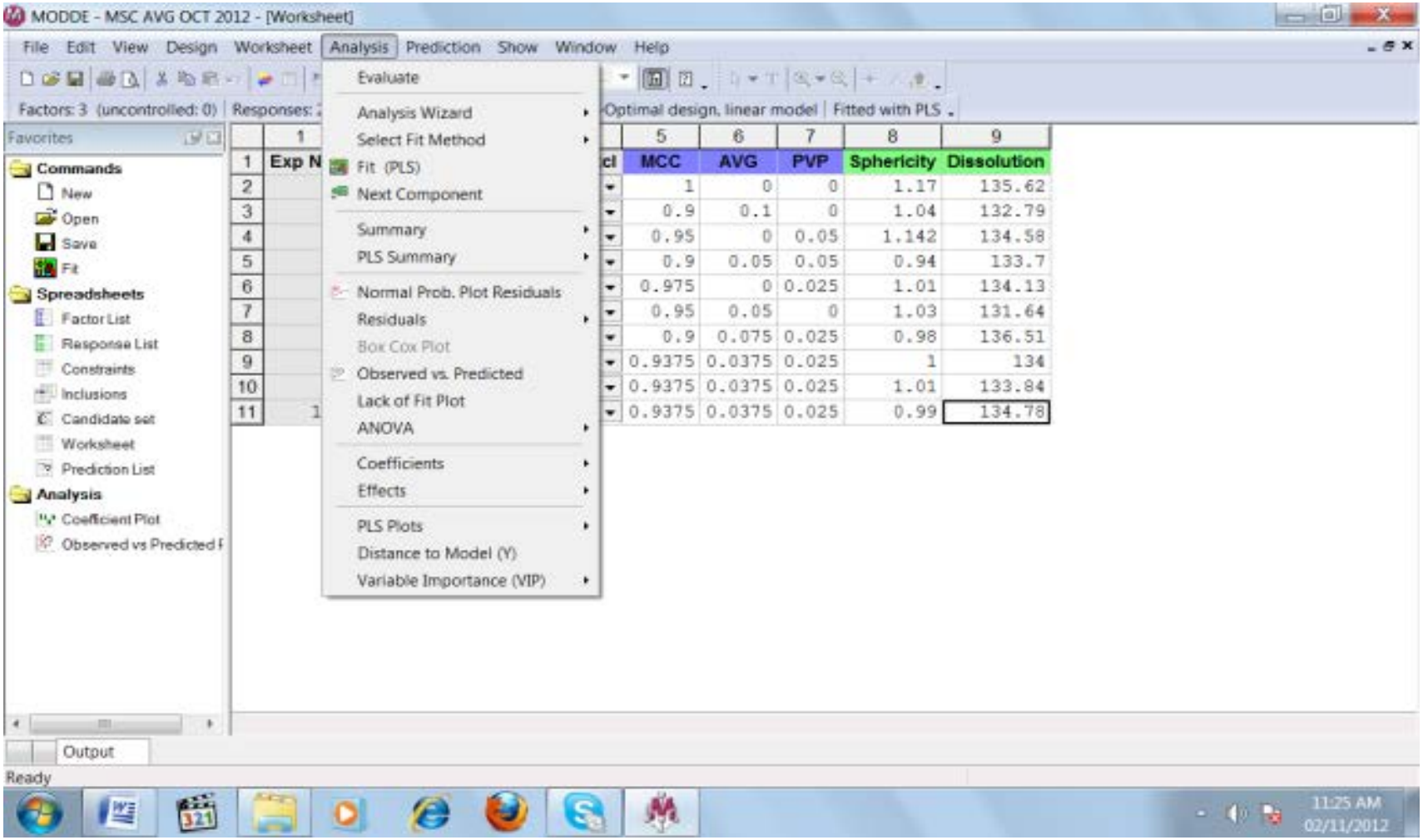
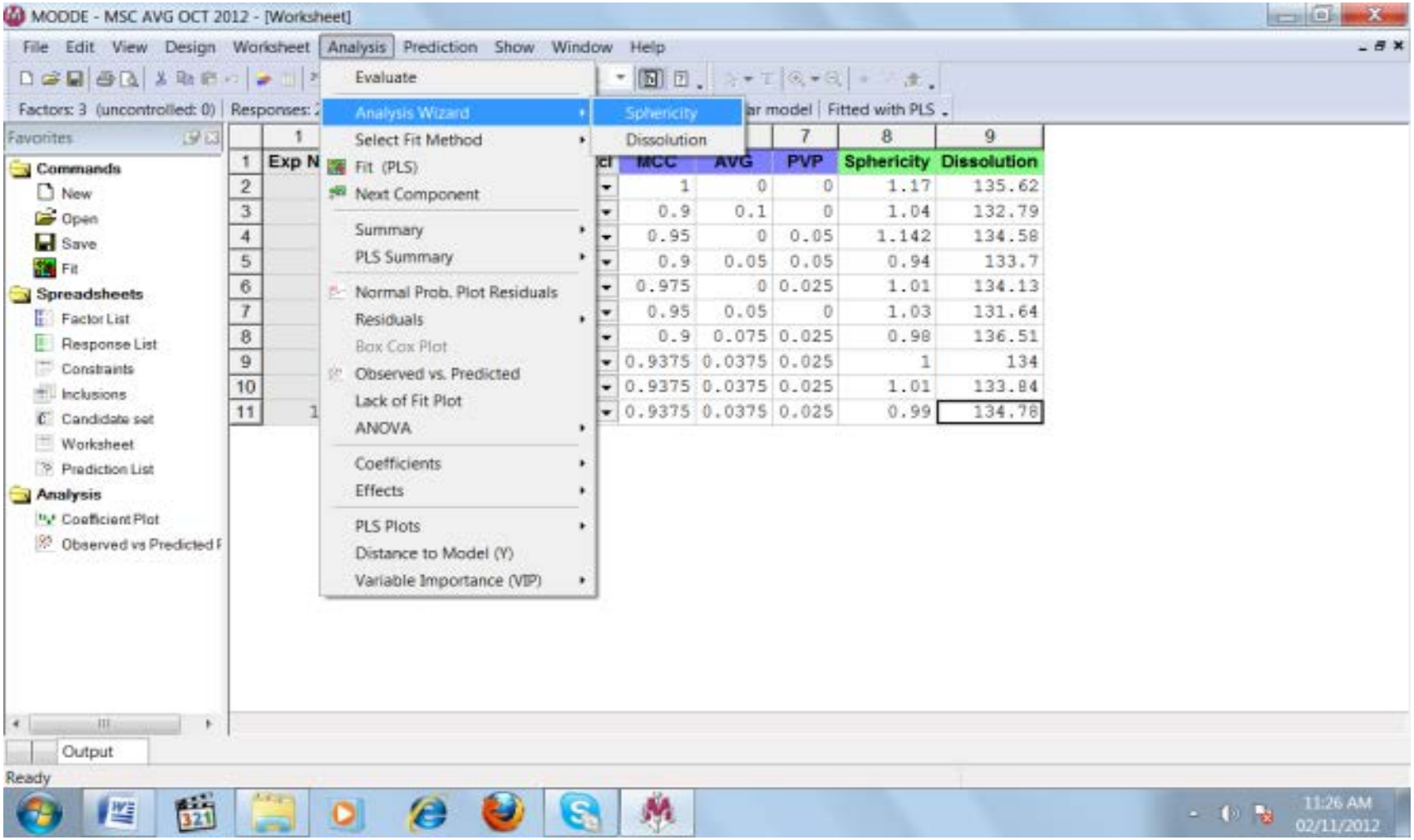


Figure 10B: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the analysis tab was selected



	1	7	8	9
1	Exp N	MCC	AVG	PVP	Sphericity	Dissolution
2		1	0	0	1.17	135.62
3		0.9	0.1	0	1.04	132.79
4		0.95	0	0.05	1.142	134.58
5		0.9	0.05	0.05	0.94	133.7
6		0.975	0	0.025	1.01	134.13
7		0.95	0.05	0	1.03	131.64
8		0.9	0.075	0.025	0.98	136.51
9		0.9375	0.0375	0.025	1	134
10		0.9375	0.0375	0.025	1.01	133.84
11	1	0.9375	0.0375	0.025	0.99	134.78

Figure 10C: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the response to be analysed was selected

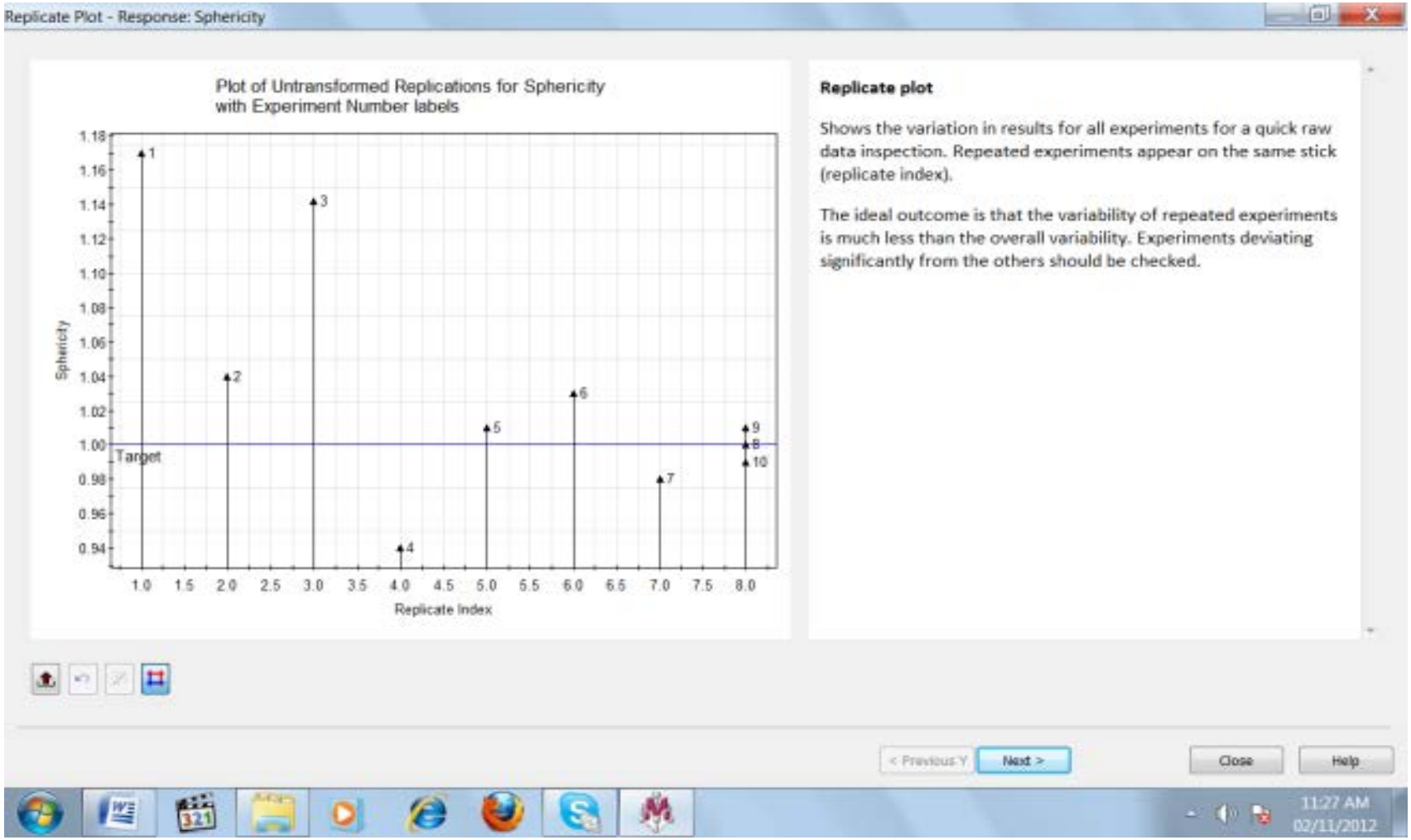


Figure 10D: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the replication plot showing the variation in results

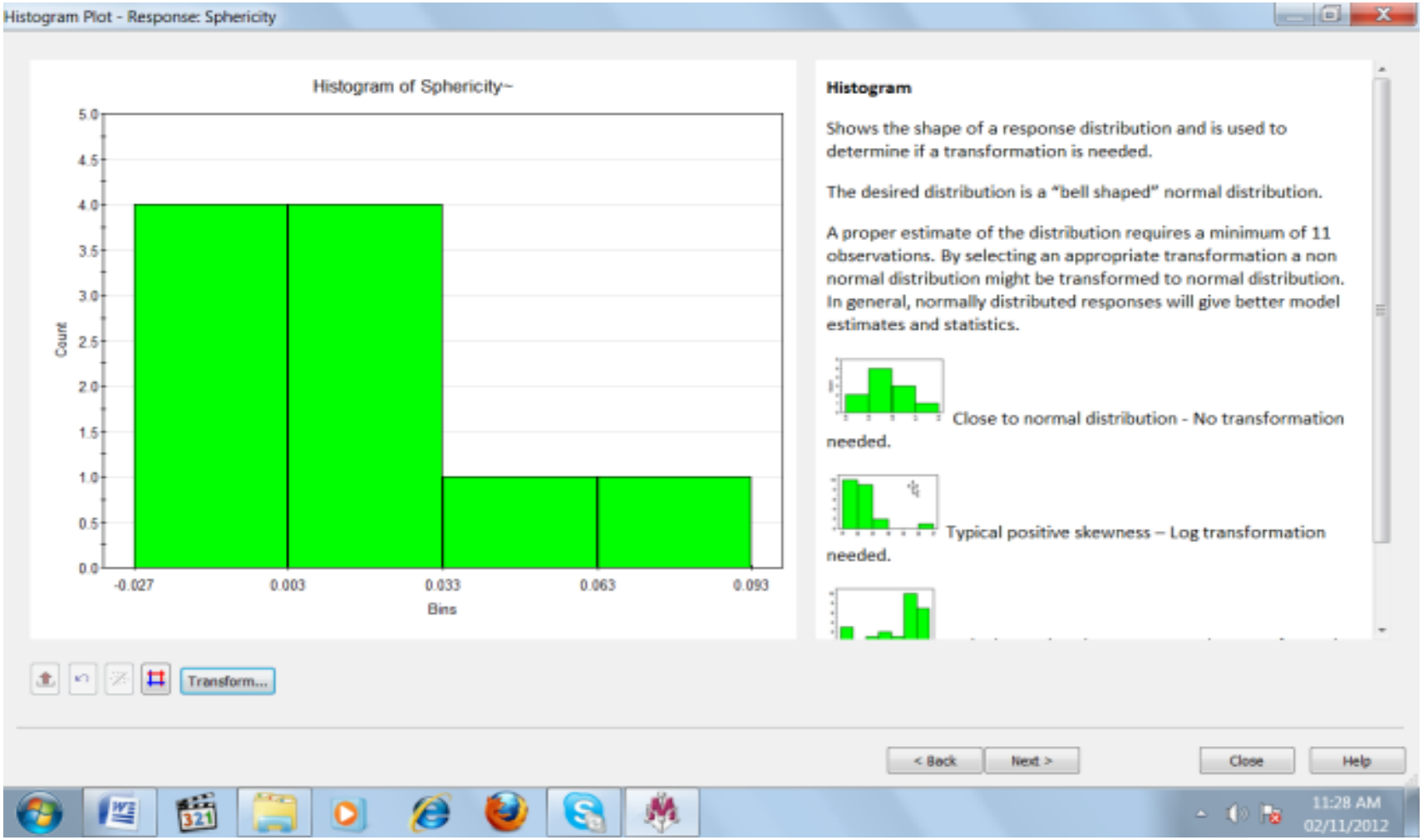


Figure 10E: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the histogram showing the shape of the response distribution

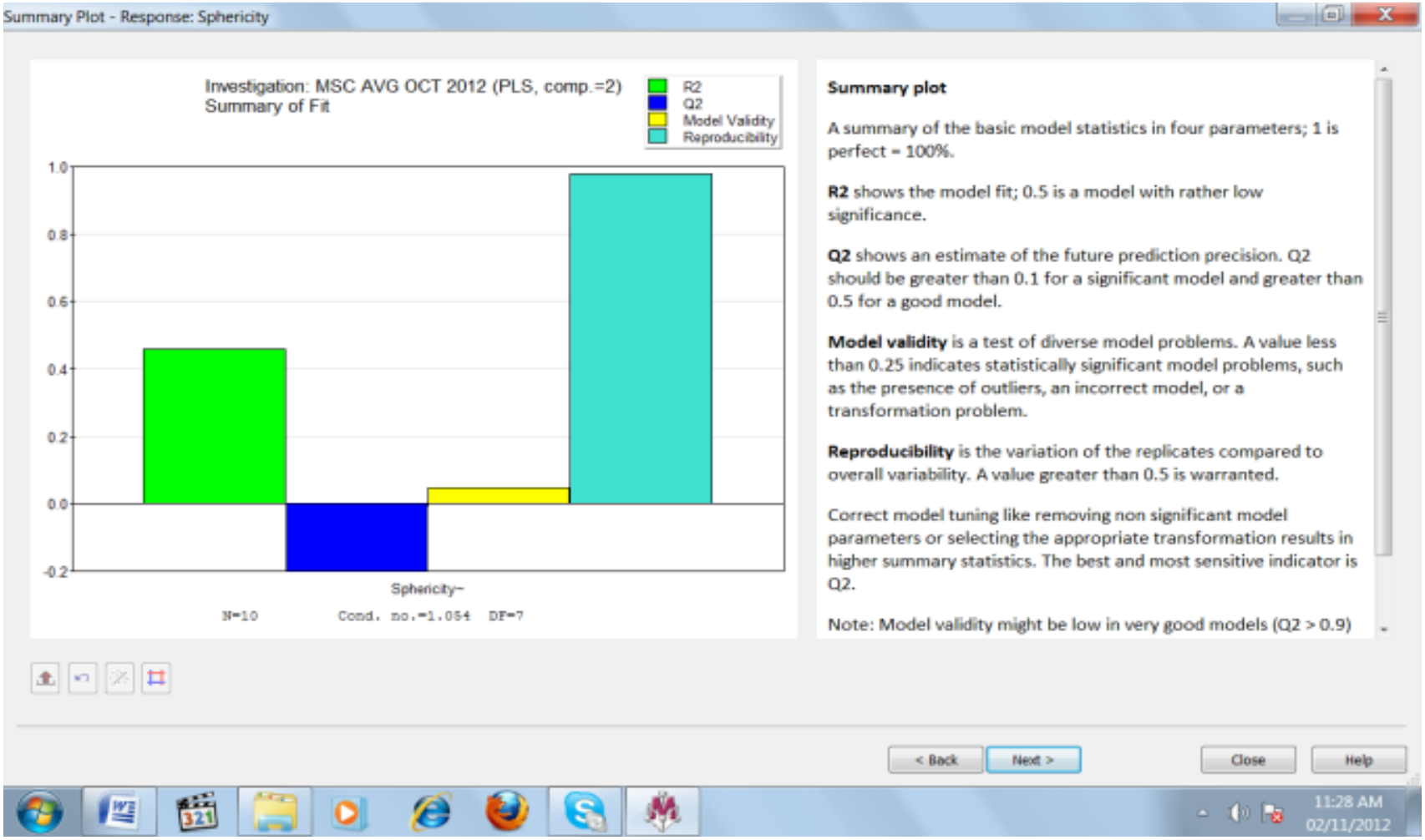


Figure 10F: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the summary plot with the basic model statistics

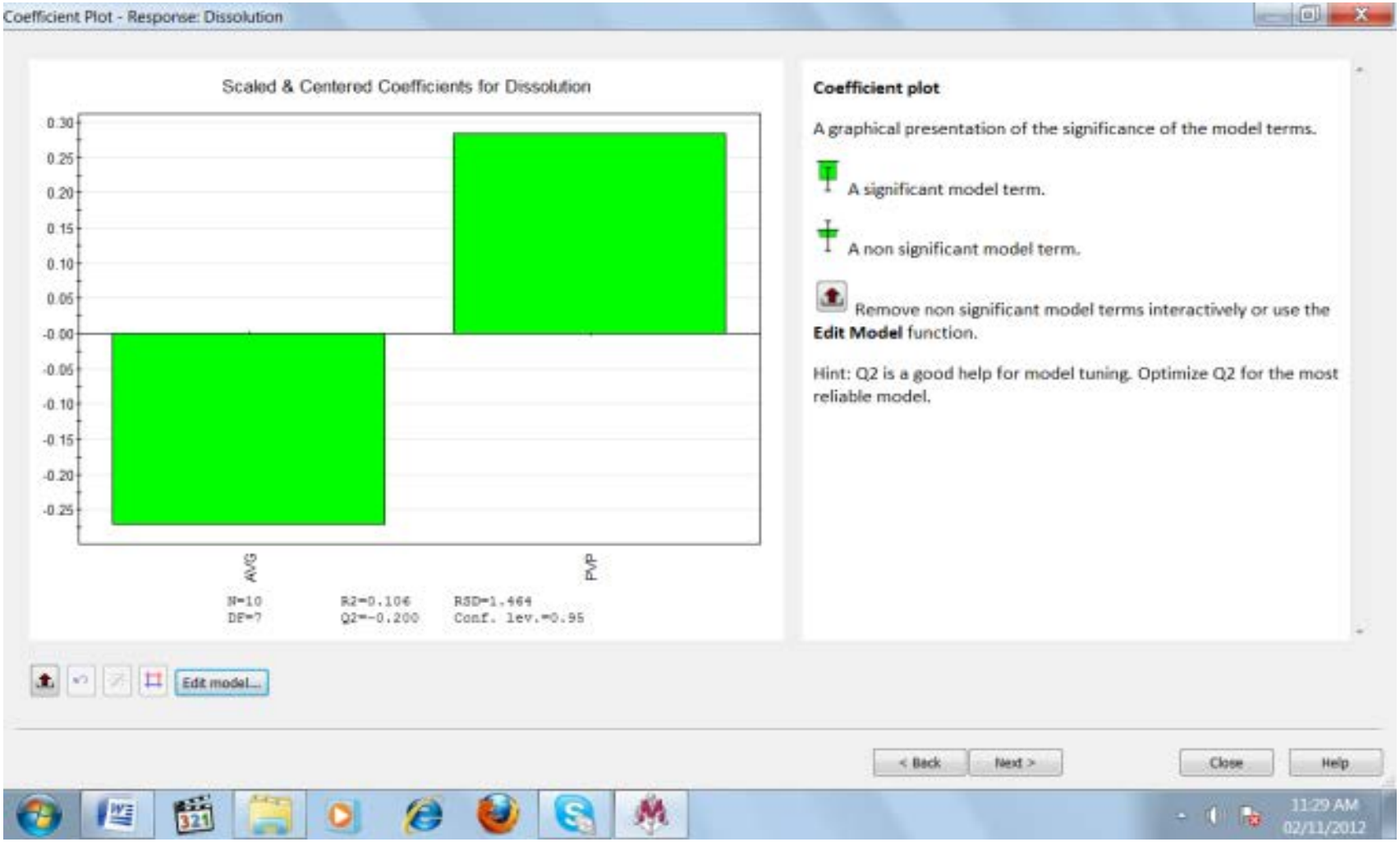


Figure 10G: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the coefficient plot

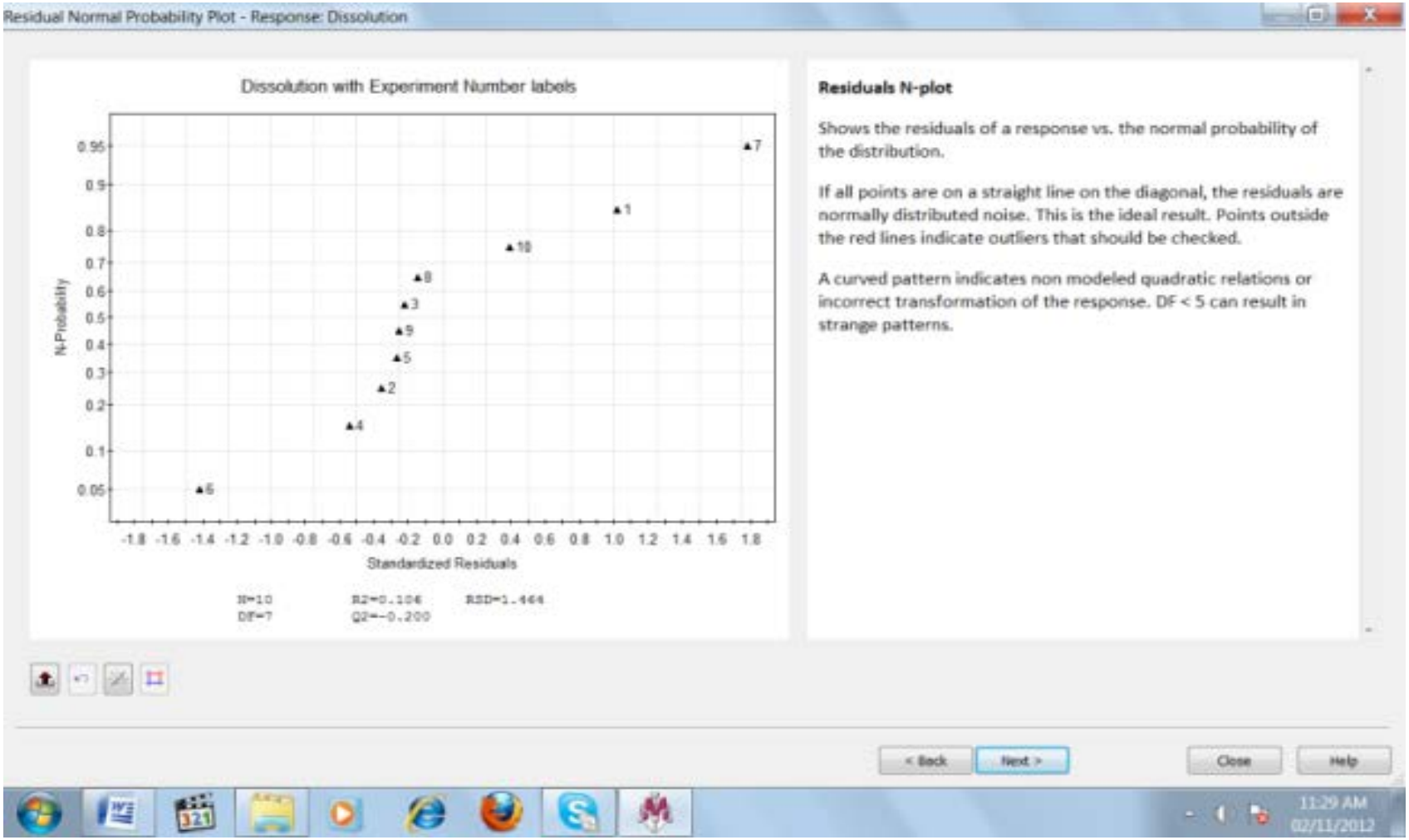


Figure 10H: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the residuals N-plot

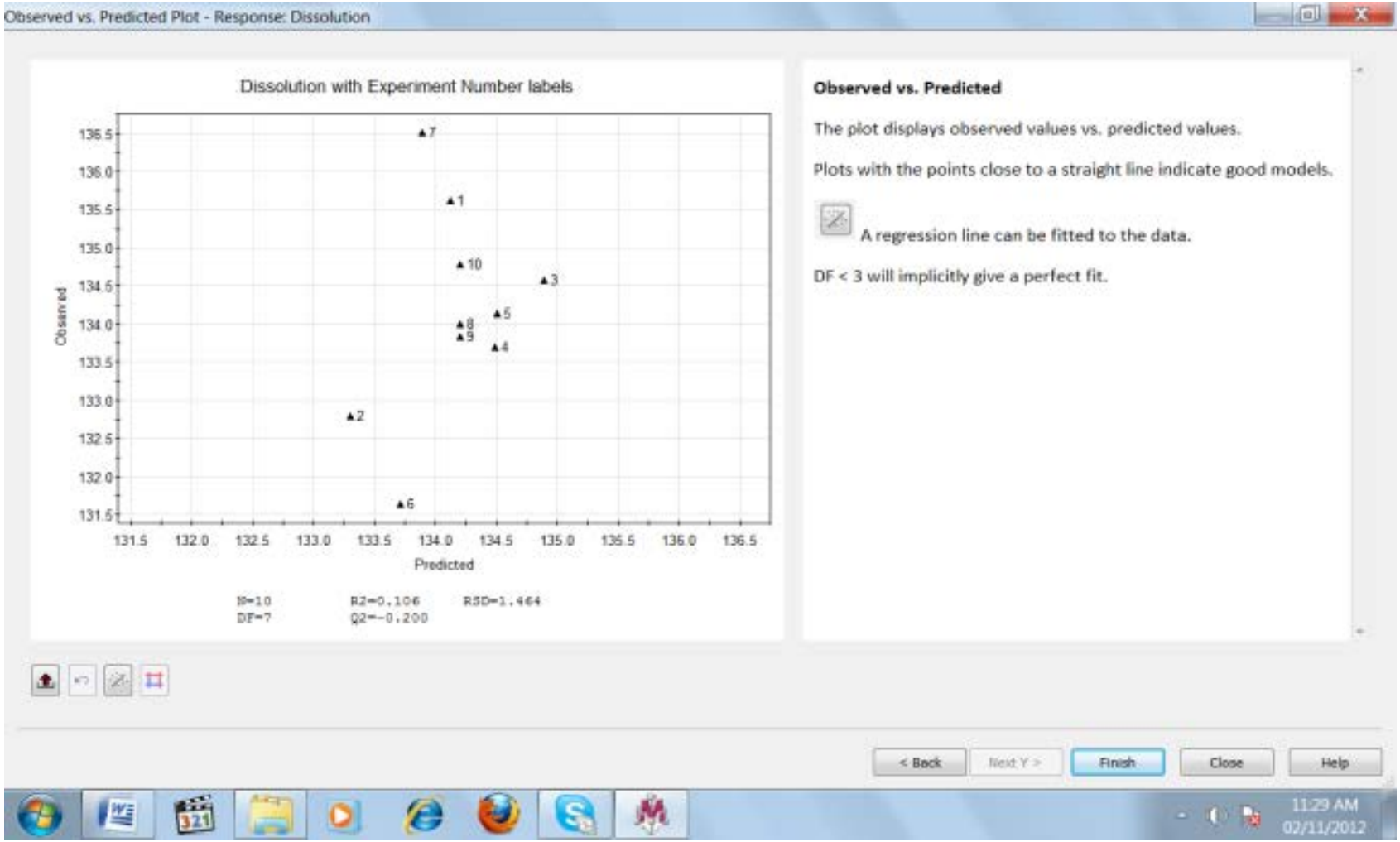


Figure 10I: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the observed vs. predicted values plot

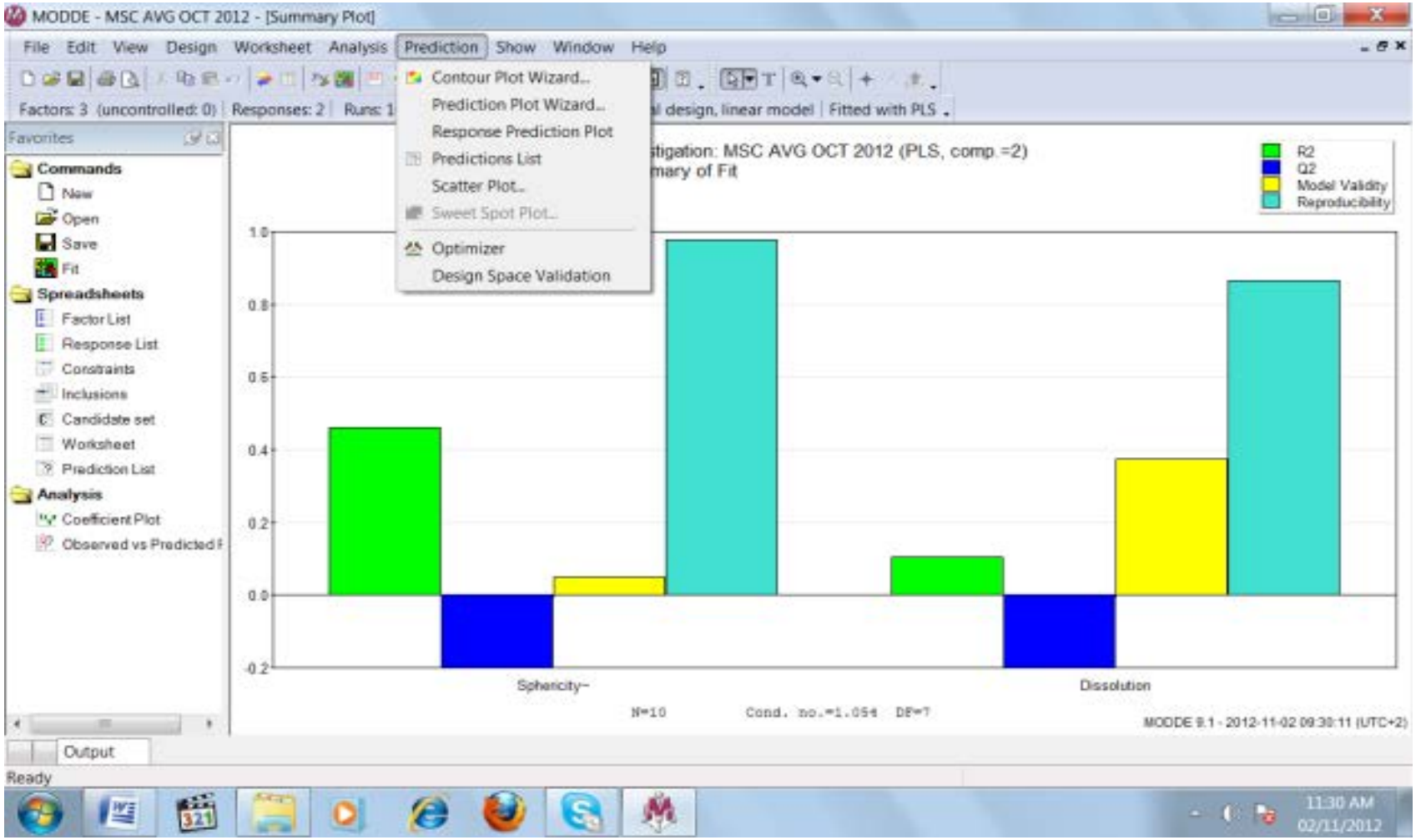


Figure 10J: Illustration of the steps followed in MODDE 9.0™ to optimise the bead formulations: the summary of fit plots

After analysis the “Prediction” (Figure 11A) tab was clicked followed by “Contour plot wizard” (Figure 11B), which produced the contour plot. On the contour plot, the mouse was moved to the desired response value and the concentrations of the different factors that must be used to produce the desired response showed at the bottom of the page as well as next to the mouse. The contour plots were opened for both sphericity and dissolution for each of the four different plant materials.

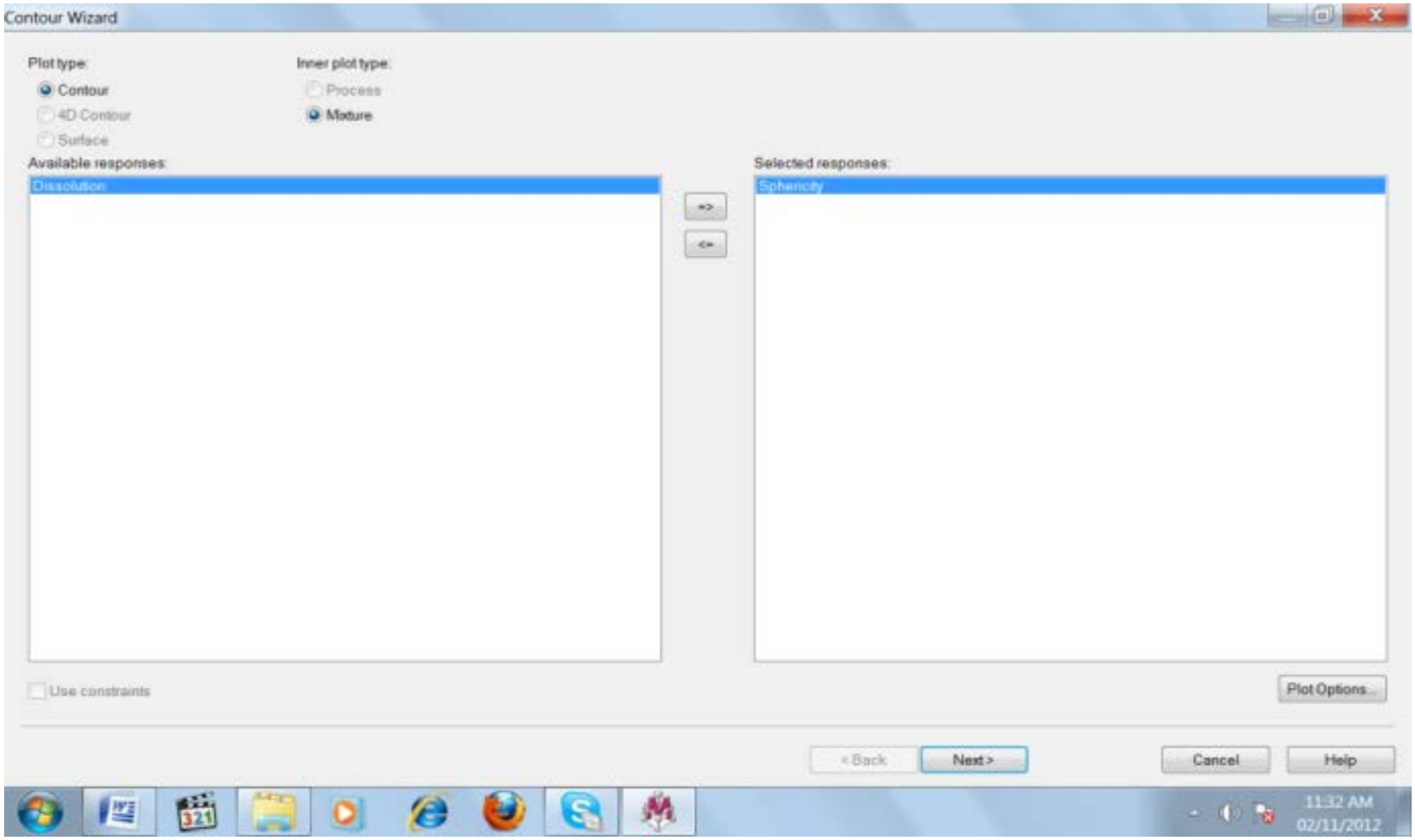


Figure 11A: Illustration from MODDE® on drawing a contour plot: contour wizard

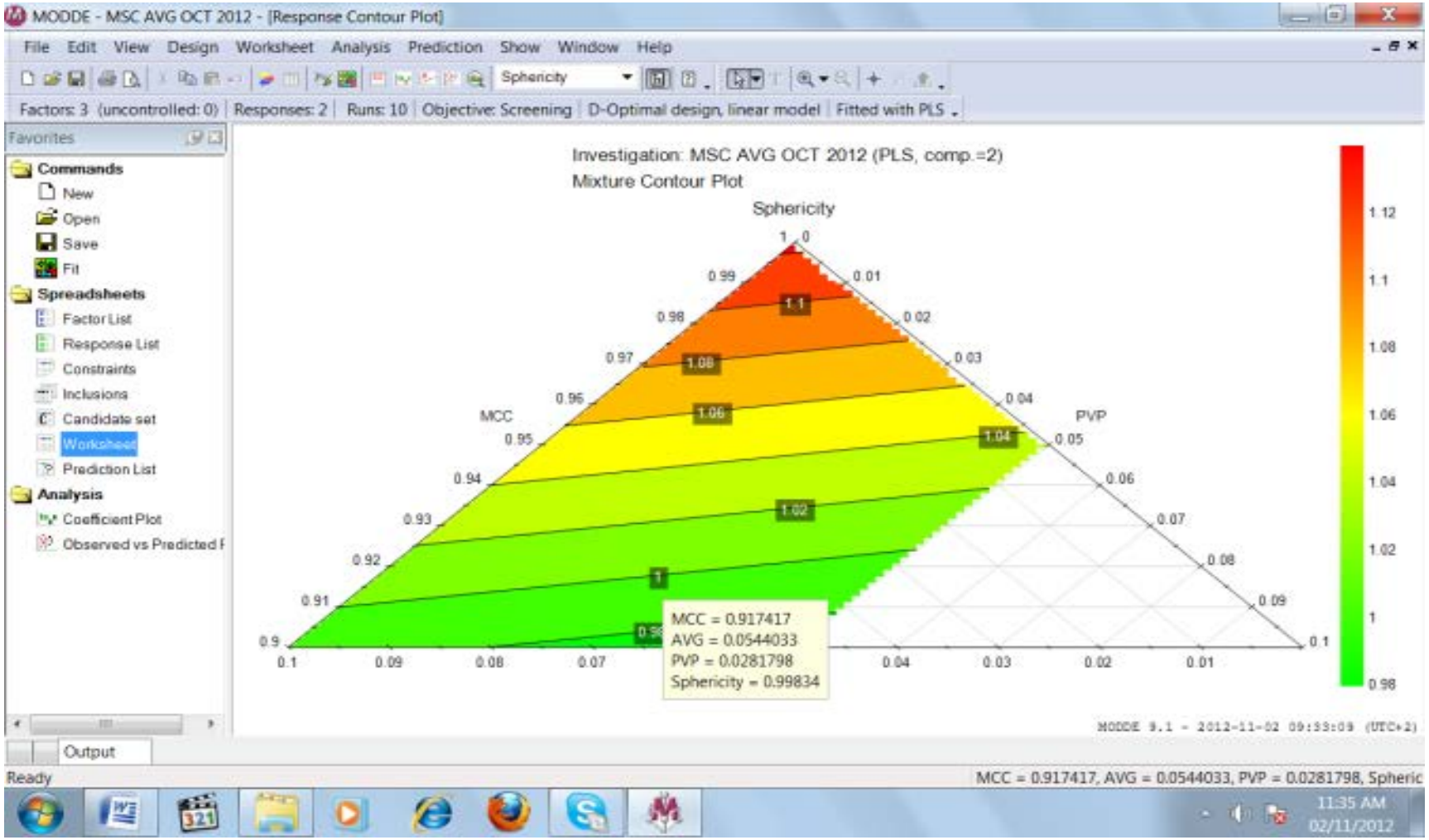


Figure 11B: Illustration from MODDE® on drawing a contour plot: the response contour plot

4 Assay of ketoprofen content in the optimised bead formulations

Two capsules were emptied of their contents into a mortar. The beads were crushed using a pestle to form a powder. A mass equivalent to theoretically containing 50 mg of ketoprofen of the powder was accurately weighed and transferred into a 100 ml volumetric flask. A volume of 60 ml of 75% (v/v) of methanol was added to the flask. The solution was placed in a hot water sonicating bath for 15 min. After sonication, the solution was made up to volume and left to stand for 5-10 min. A volume of 5 ml of the supernatant was transferred to another 100 ml volumetric flask and made up to volume using 75% (v/v) methanol. From this volumetric flask 200 µl of solution was transferred to a vial and taken to the HPLC for analysis. The test was carried out in duplicate. The amount of ketoprofen contained in the beads must be 92.5 to 107.5 % of the stated amount. The amount of ketoprofen in the samples was calculated using the calibration curve (Equation 6). The reading for AMG will be used as an example.

$$y = mx + c \quad (6)$$

The average reading was 1022, therefore:

1022 = 40.80x + 57.70
= (1022 – 57.70)/40.80
= 23.63 µl/ml

There is 23.63 µg/ml in the 200 µl as well as in the 5 ml, therefore in 100 ml:

= 23.63 x 100
= 2363 µg
= 2.363 mg

If this is 5 ml of the supernatant in 100 ml, then:

= 2.363 x 20
= 47.26 mg

% drug content:

$$= \frac{\text{Actual amount of drug in the sample}}{\text{Predicted amount of drug in the sample}} \times 100$$

$$= \frac{47.26}{50} \times 100$$

= 94.5%

CHAPTER 4

RESULTS AND DISCUSSION

1 ^{1}H-NMR characterisation of the aloe materials

The ^{1}H-NMR spectra of *A. vera*gel and whole leaf (Daltonmax 700®) as well as *A. marlothii* gel and whole leaf materials are given in Figures 12A-D.

According to the these NMR spectra, aloverose (acetylated polymannose) was present in the *A. vera*gel and whole leaf materials, but it could not be detected in the *A. marlothii*materials which is in accordance with previously published data that showed aloe species indigenous to South Africa only contain glucose and not mannose (O'Brien *et al.,* 2011:993).

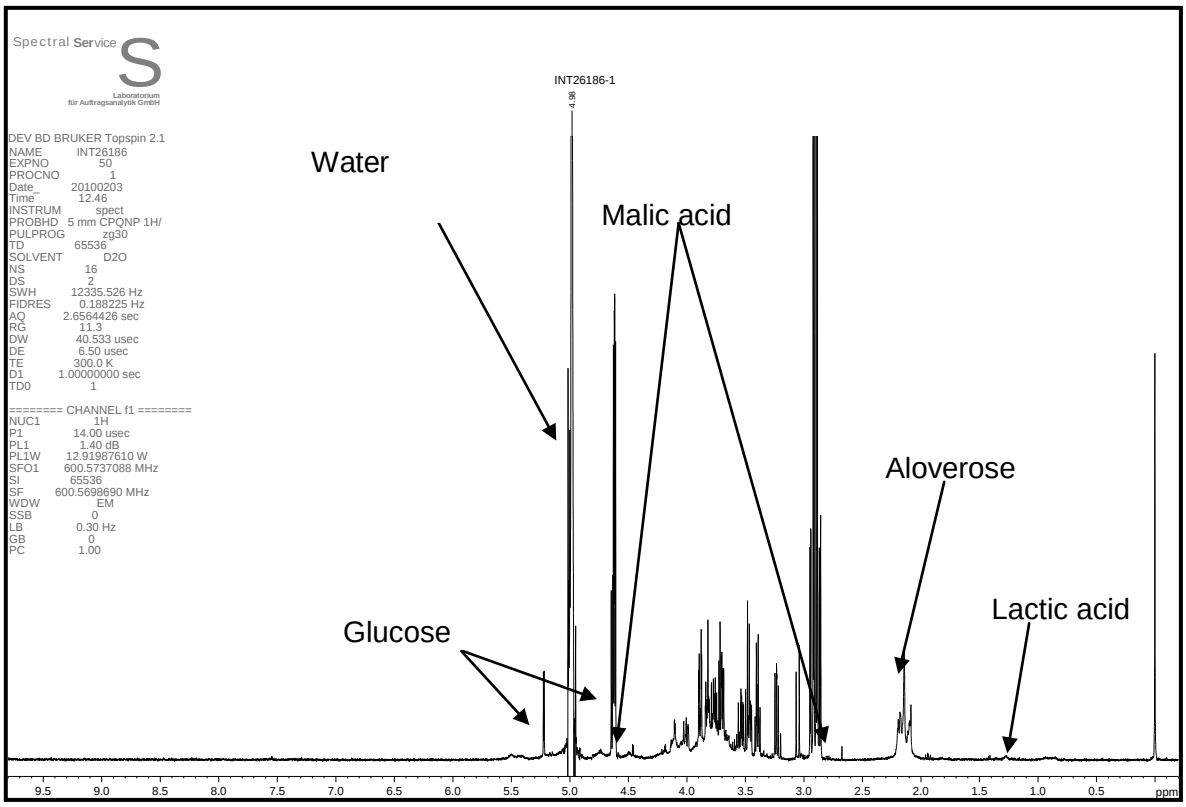


Figure 12A: The ^{1}H-NMR spectrum of *Aloe vera* dehydrated gel material (Daltonmax 700®)

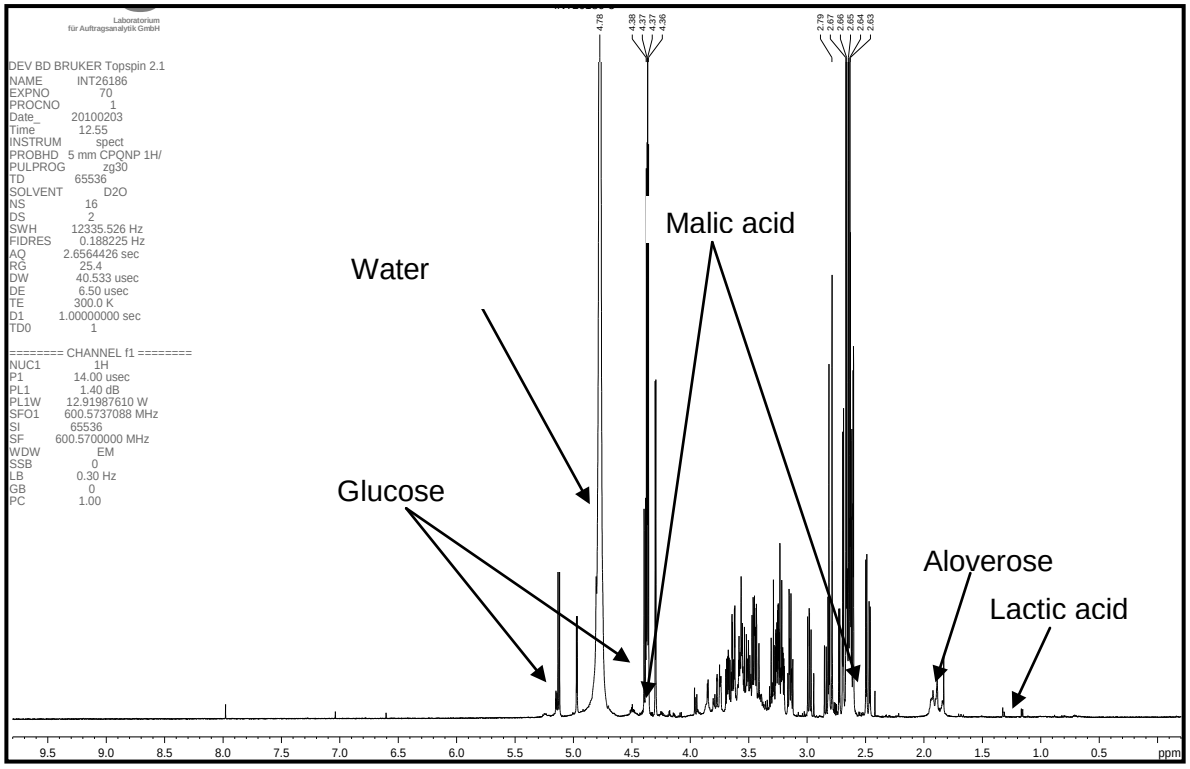


Figure 12B: The ^{1}H-NMR spectrum of *Aloe vera* whole leaf material (Daltonmax700®)

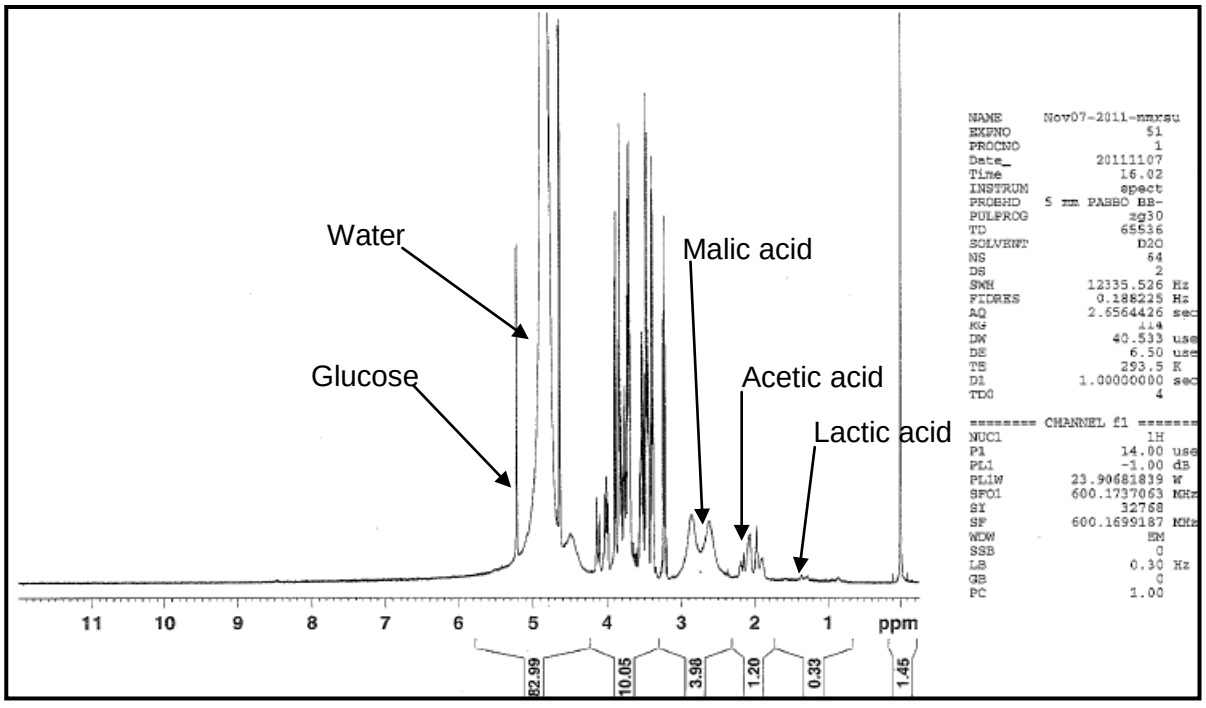


Figure 12C: The ^{1}H-NMR spectrum of *Aloe marlothii* gel material

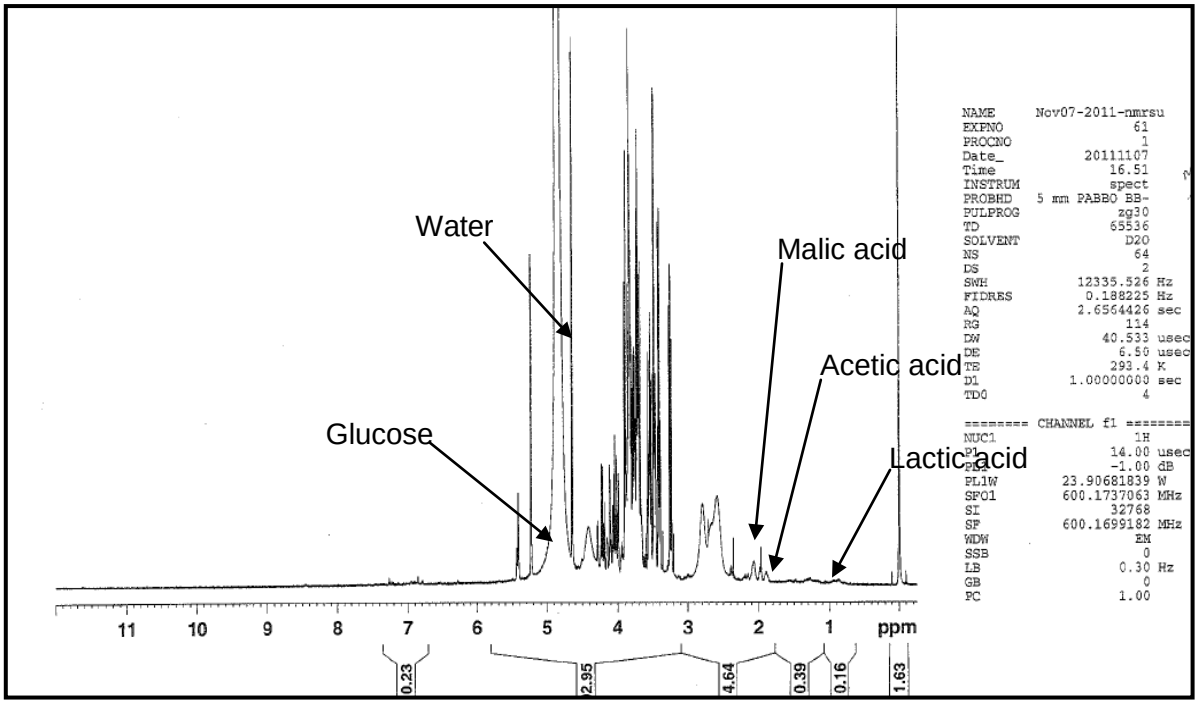


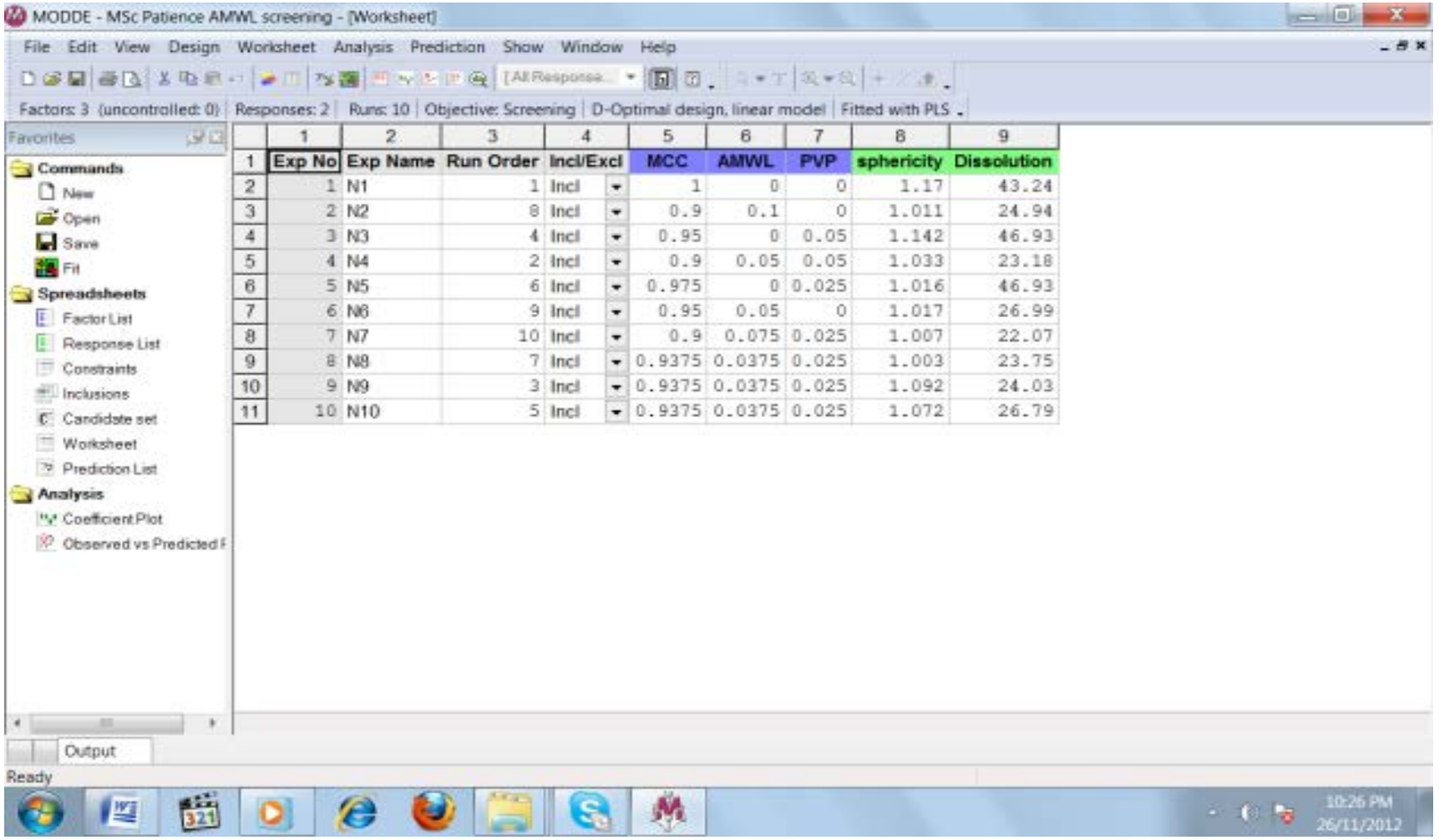
Figure 12D: The ^{1}H-NMR spectrum of *Aloe marlothii* whole leaf material

In general, fresh *A. vera* gel consists of three main components namely aloverose (partly acetylated polymannan or acemannan), glucose and malic acid, which are detectable by ^{1}H-NMR spectroscopy and serve as marker molecules for the identification of aloe gel material. The presence of iso-citric acid (or whole leaf marker) indicates that the sample contains aloe whole leaf material. High quantities of lactic acid indicate bacterial degradation of the gel material, while succinic and fumaric acid are produced by the plant's own enzymatic action. The presence of acetic acid and formic acid is caused by hydrolysis of aloverose and thermal degradation of glucose storage.

From the ^{1}H-NMR spectrum in Figure 12A, it is evident that the *A. vera* gel material used in this study contains all three main marker molecules namely aloverose, glucose and malic acid, which positively confirms its identity. *Aloe vera* whole leaf material also contains aloverose, but in a lower quantity than the gel material due to a dilution effect by the extra material from the skin of the leaf. The ^{1}H-NMR spectrum also indicates the presence of lactic acid and succinic acid in low levels, which shows that no appreciable degradation of the *A. vera* gel material has taken place (Jambwa *et al*., 2011:435). *Aloe marlothii* gel and whole leaf material did not contain any aloverose. They contained malic acid, glucose and acetic acid (Figures 12C and D).

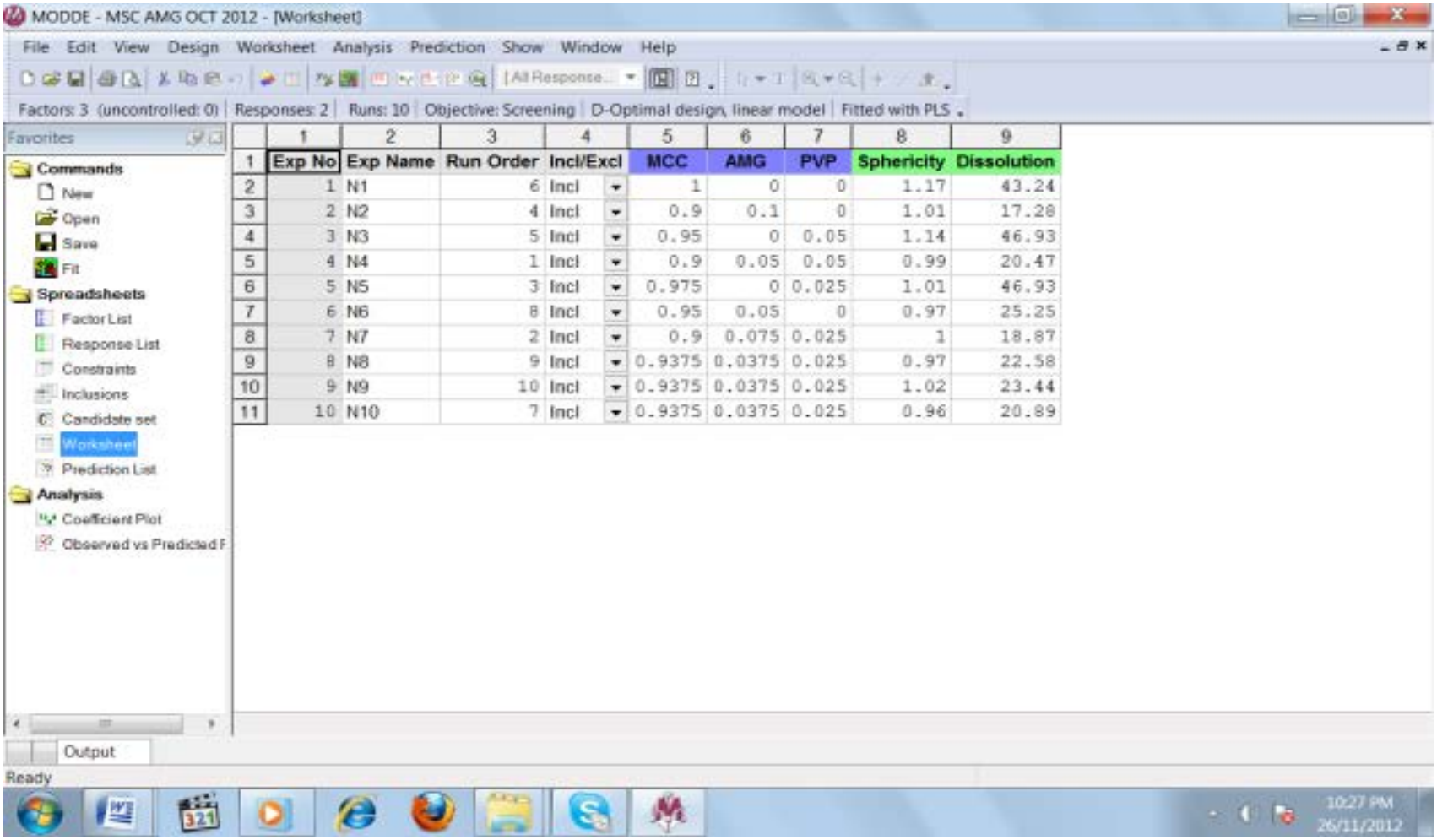
2 Bead formulations according to the design of experiments

The composition of the formulations for the beads containing the different aloe materials as determined by MODDE® software is depicted in Figure 13A-D.



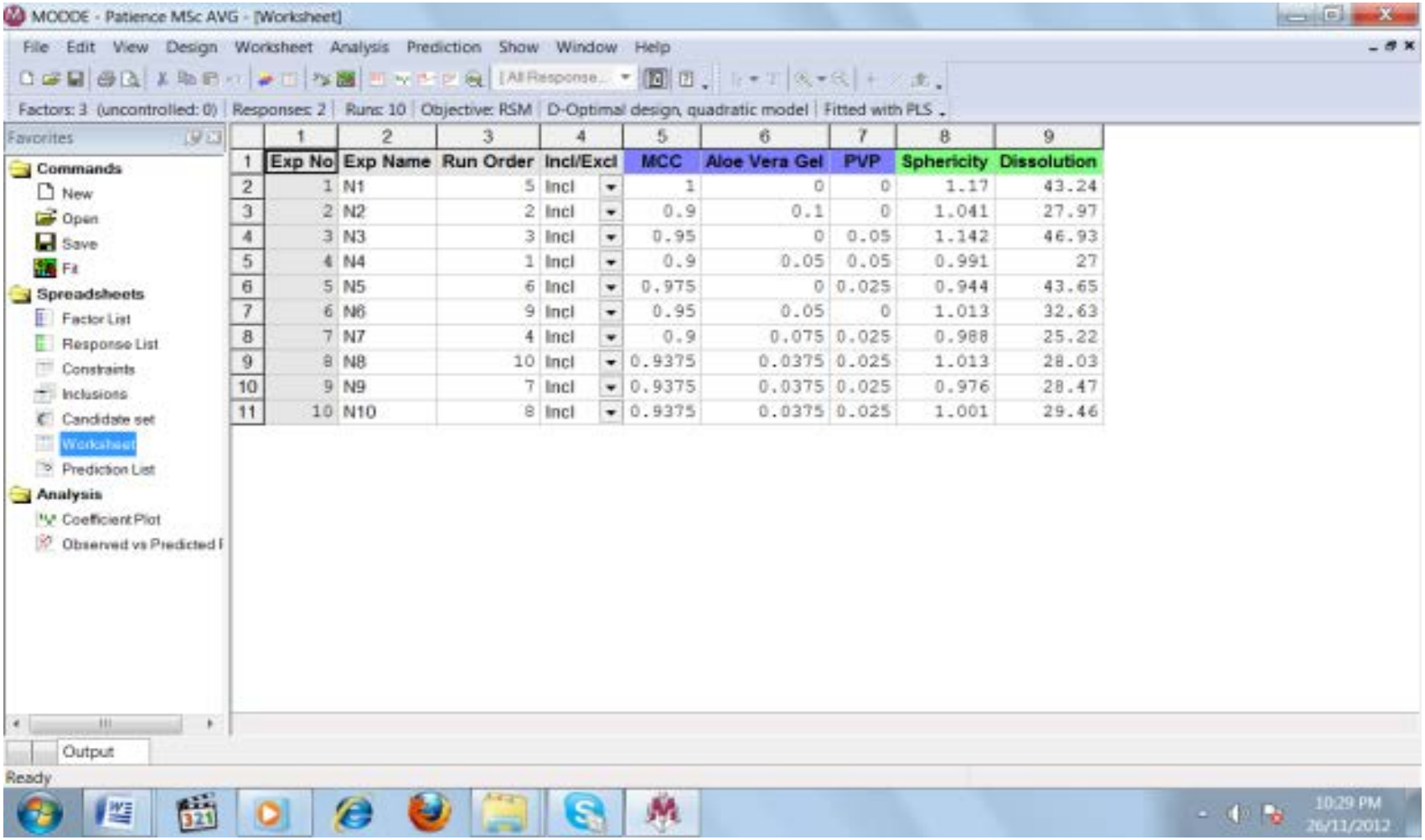
	1	2	3	4	5	6	7	8	9
1	Exp No	Exp Name	Run Order	Incl/Excl	MCC	AMWL	PVP	sphericity	Dissolution
2	1	N1	1	Incl	1	0	0	1.17	43.24
3	2	N2	8	Incl	0.9	0.1	0	1.011	24.94
4	3	N3	4	Incl	0.95	0	0.05	1.142	46.93
5	4	N4	2	Incl	0.9	0.05	0.05	1.033	23.18
6	5	N5	6	Incl	0.975	0	0.025	1.016	46.93
7	6	N6	9	Incl	0.95	0.05	0	1.017	26.99
8	7	N7	10	Incl	0.9	0.075	0.025	1.007	22.07
9	8	N8	7	Incl	0.9375	0.0375	0.025	1.003	23.75
10	9	N9	3	Incl	0.9375	0.0375	0.025	1.092	24.03
11	10	N10	5	Incl	0.9375	0.0375	0.025	1.072	26.79

Figure 13A: The composition of formulations containing *Aloe marlothii* whole leaf



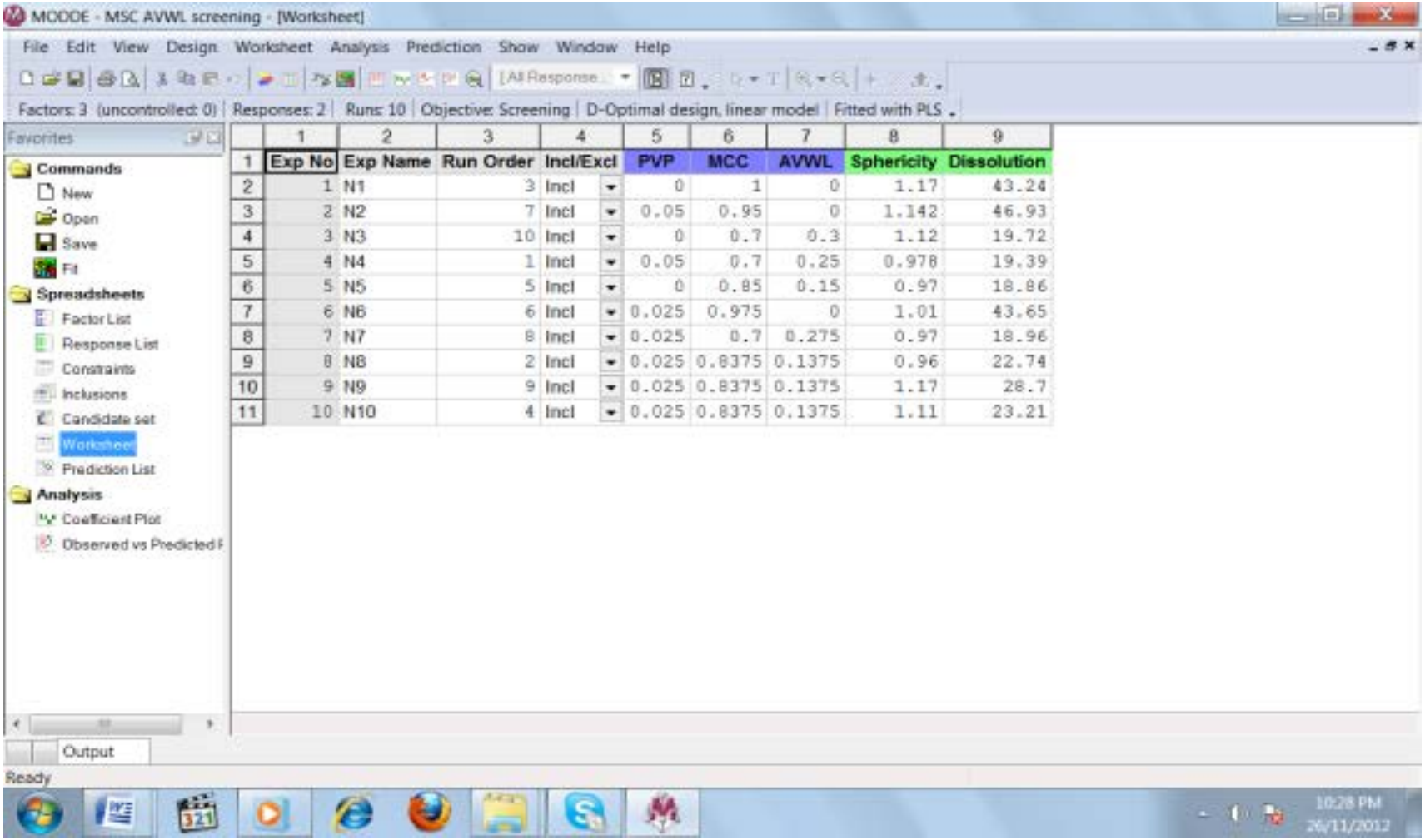
	1	2	3	4	5	6	7	8	9
1	Exp No	Exp Name	Run Order	Incl/Excl	MCC	AMG	PVP	Sphericity	Dissolution
2	1	N1	6	Incl	1	0	0	1.17	43.24
3	2	N2	4	Incl	0.9	0.1	0	1.01	17.28
4	3	N3	5	Incl	0.95	0	0.05	1.14	46.93
5	4	N4	1	Incl	0.9	0.05	0.05	0.99	20.47
6	5	N5	3	Incl	0.975	0	0.025	1.01	46.93
7	6	N6	8	Incl	0.95	0.05	0	0.97	25.25
8	7	N7	2	Incl	0.9	0.075	0.025	1	18.87
9	8	N8	9	Incl	0.9375	0.0375	0.025	0.97	22.58
10	9	N9	10	Incl	0.9375	0.0375	0.025	1.02	23.44
11	10	N10	7	Incl	0.9375	0.0375	0.025	0.96	20.89

Figure 13B: The composition of formulations containing *Aloe marlothii* gel



	1	2	3	4	5	6	7	8	9
1	Exp No	Exp Name	Run Order	Incl/Excl	MCC	Aloe Vera Gel	PVP	Sphericity	Dissolution
2	1	N1	5	Incl	1	0	0	1.17	43.24
3	2	N2	2	Incl	0.9	0.1	0	1.041	27.97
4	3	N3	3	Incl	0.95	0	0.05	1.142	46.93
5	4	N4	1	Incl	0.9	0.05	0.05	0.991	27
6	5	N5	6	Incl	0.975	0	0.025	0.944	43.65
7	6	N6	9	Incl	0.95	0.05	0	1.013	32.63
8	7	N7	4	Incl	0.9	0.075	0.025	0.988	25.22
9	8	N8	10	Incl	0.9375	0.0375	0.025	1.013	28.03
10	9	N9	7	Incl	0.9375	0.0375	0.025	0.976	28.47
11	10	N10	8	Incl	0.9375	0.0375	0.025	1.001	29.46

Figure 13C: The composition of formulations containing *Aloe vera* gel



	1	2	3	4	5	6	7	8	9
1	Exp No	Exp Name	Run Order	Incl/Excl	PVP	MCC	AVWL	Sphericity	Dissolution
2	1	N1	3	Incl	0	1	0	1.17	43.24
3	2	N2	7	Incl	0.05	0.95	0	1.142	46.93
4	3	N3	10	Incl	0	0.7	0.3	1.12	19.72
5	4	N4	1	Incl	0.05	0.7	0.25	0.978	19.39
6	5	N5	5	Incl	0	0.85	0.15	0.97	18.86
7	6	N6	6	Incl	0.025	0.975	0	1.01	43.65
8	7	N7	8	Incl	0.025	0.7	0.275	0.97	18.96
9	8	N8	2	Incl	0.025	0.8375	0.1375	0.96	22.74
10	9	N9	9	Incl	0.025	0.8375	0.1375	1.17	28.7
11	10	N10	4	Incl	0.025	0.8375	0.1375	1.11	23.21

Figure 13D: The composition of formulations containing *Aloe vera* whole leaf

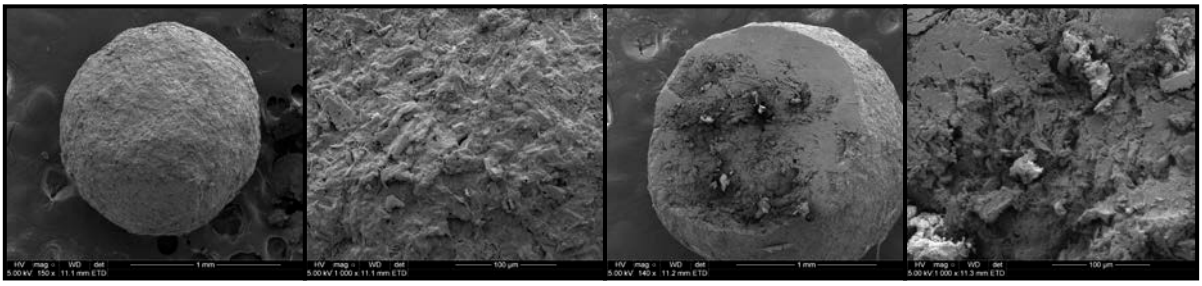
The beads were prepared according to the compositions of the formulations determined by the DOE (Figure 13A-D) and the beads were evaluated in terms of sphericity and dissolution (i.e. mean dissolution time or MDT). The sphericity and MDT results obtained from the characterization of all the bead formulations were then entered into the MODDE® worksheets in order to generate the optimised bead formulations for each aloe material. Only 30% (w/w) of AVWL and up to 10% (w/w) of AMG, AVG and AMWL could be incorporated into the beads successfully. No beads were formed beyond these concentrations.

3 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to evaluate the surface morphology and internal structure of the beads prepared by extrusion spheronisation according to the formulations generated by the DOE.

3.1 Beads containing microcrystalline cellulose only

The SEM photomicrographs of the bead formulation containing microcrystalline cellulose (MCC) only are shown in Figure 14A-D.



A B C D

Figure 14: Scanning electron micrographs of a bead containing microcrystaline cellulose only. A) External surface structure at magnification of 130X and B) 1000X, C) its internal structure at magnification 130X and D) 1000X

The beads consisting of MCC only as an excipient appear to have a dense structure with a relatively smooth surface morphology and only very few small pores are visible on the outer surface at the largest magnification (Figure 14B). Almost no pores are visible on the internal structure of this particular bead formulation. This indicates the ability of MCC to form densely structured beads by means of extrusion spheronisation, which may not easily disintegrate. Furthermore, very few pores exist through which water can enter and dissolve the drug inside the bead and these beads will probably release the encapsulated drug at a

relatively slow rate. It is therefore expected that beads consisting of MCC only will display a sustained release dissolution profile rather than an immediate burst release profile such as obtained with tablets that disintegrate relatively fast.

3.2 *Aloe marlothii* gel

The SEM photomicrographs of the bead formulations containing *A. marlothii* gel (AMG) are depicted in Figure 15A-K.

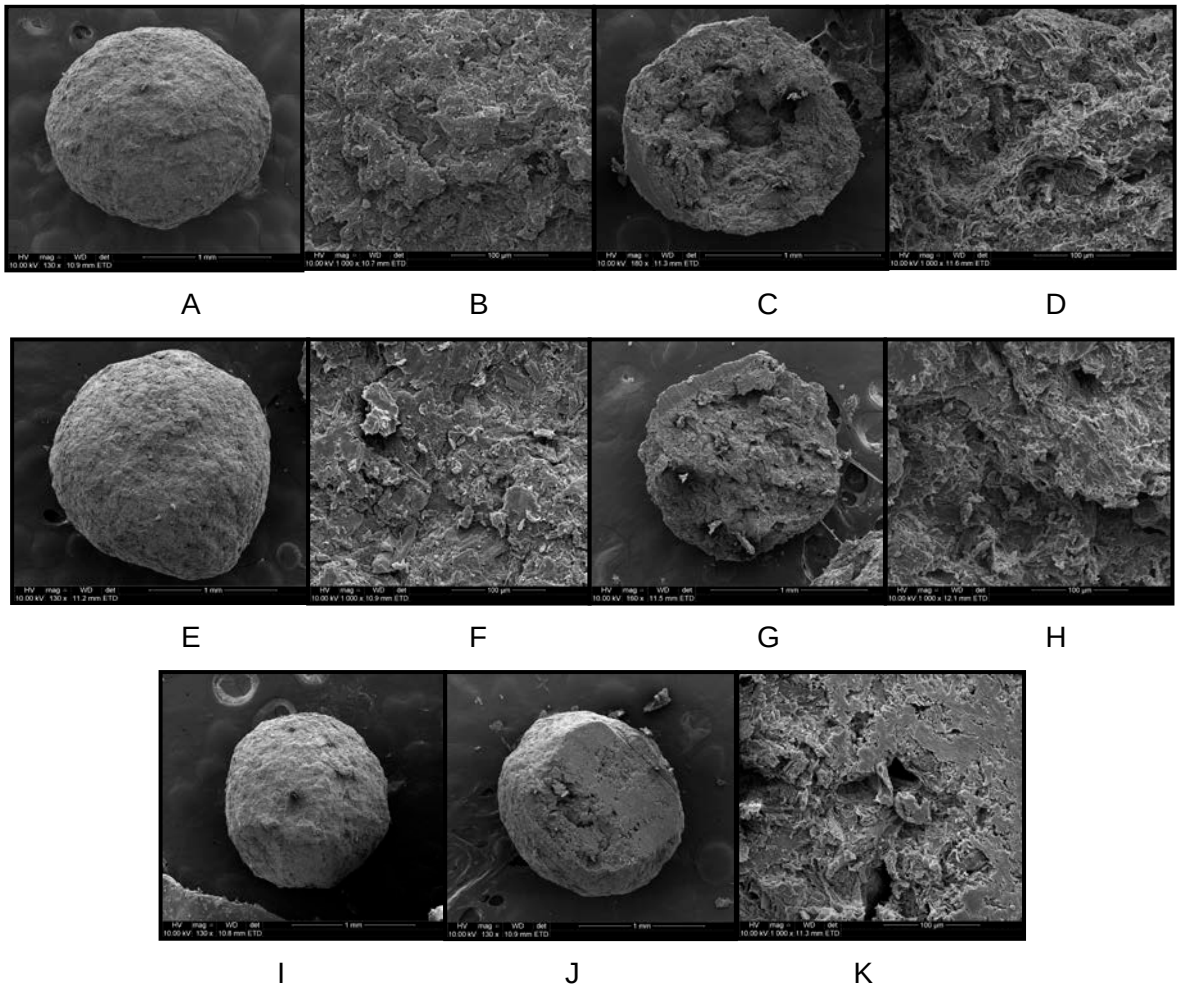


Figure 15: Scanning electron micrographs of bead formulations containing *Aloe marlothii* gel. A) External surface of a bead containing 3.75 % (w/w) AMG at a magnification of 130X, and B)1000X, C) its internal structure at 130X, D) and 1000X, E) external surface of a bead containing 7.5 % (w/w) AMG at 130X, F) and 1000X, G) its internal structure at 130X, H) and 1000X, I) external surface of a bead containing 10 % (w/w) AMG at 130X, J) its internal structure magnification 130X and K) 1000X

The surface morphology of the beads containing 3.75% (w/w) AMG was less smooth than that of the formulation containing MCC only, but it became smoother as the concentration of the AMG increased. Their internal structure appears to be more porous, specifically the formulation with the highest concentration AMG (Figure 15K), which indicates that incorporation of AMG will probably cause a faster drug release rate.

3.3 *Aloe vera* gel

The SEM micrographs of the beads containing *A. vera* gel are depicted in Figure 16A-K.

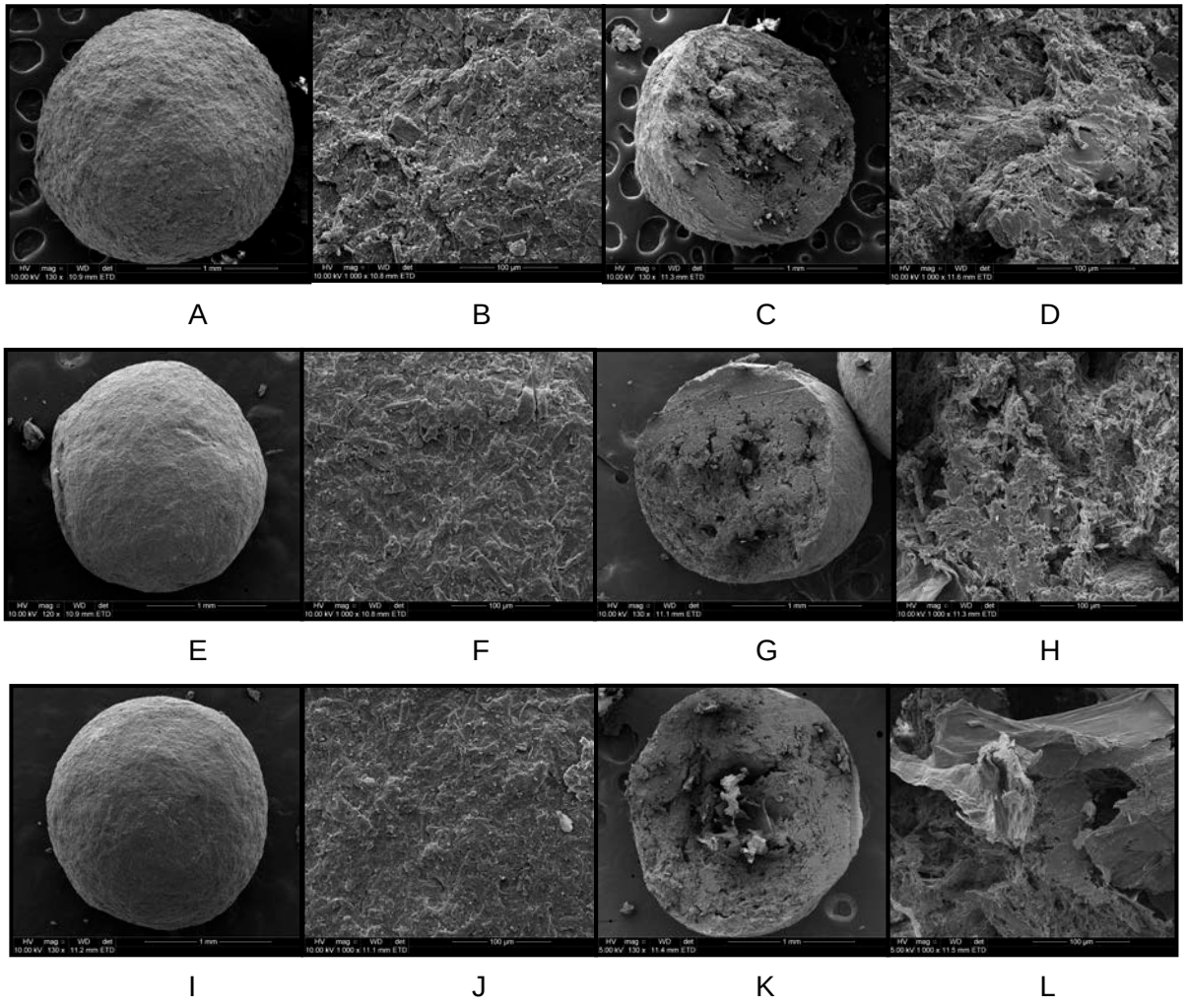


Figure 16: Scanning electron micrographs of beads containing *Aloe vera* gel. A) External surface of a bead containing 3.75 % (w/w) AVG at magnification of 130X, and B) 1000X, C) its internal structure at 130X, D) and 1000X, E) external surface of a bead containing 7.5 % (w/w) AVG at 130X, F) and 1000X, G) its internal structure at 130X and H) 1000X, I) external surface of a bead containing 10 % (w/w) AVG at 130X, J) and 1000X, and K) internal structure at 130X and L) 1000X

In general, the external surface morphology of the beads containing AVG was smoother than those containing MCC only (Figure 16A, E and I). The beads became more porous as the concentration of AVG increased to 10% (w/w) (Figure 16K). Porosity is an important aspect of beads in terms of drug release due to the entrance of water into the bead and the diffusion of the dissolved drug into the surrounding liquid (Gómez-Carracedo *et al*., 2009:302).

3.4 *Aloe marlothii* whole leaf

The micrographs of beads containing *A. marlothii* whole leaf are depicted in Figure 17A-L.

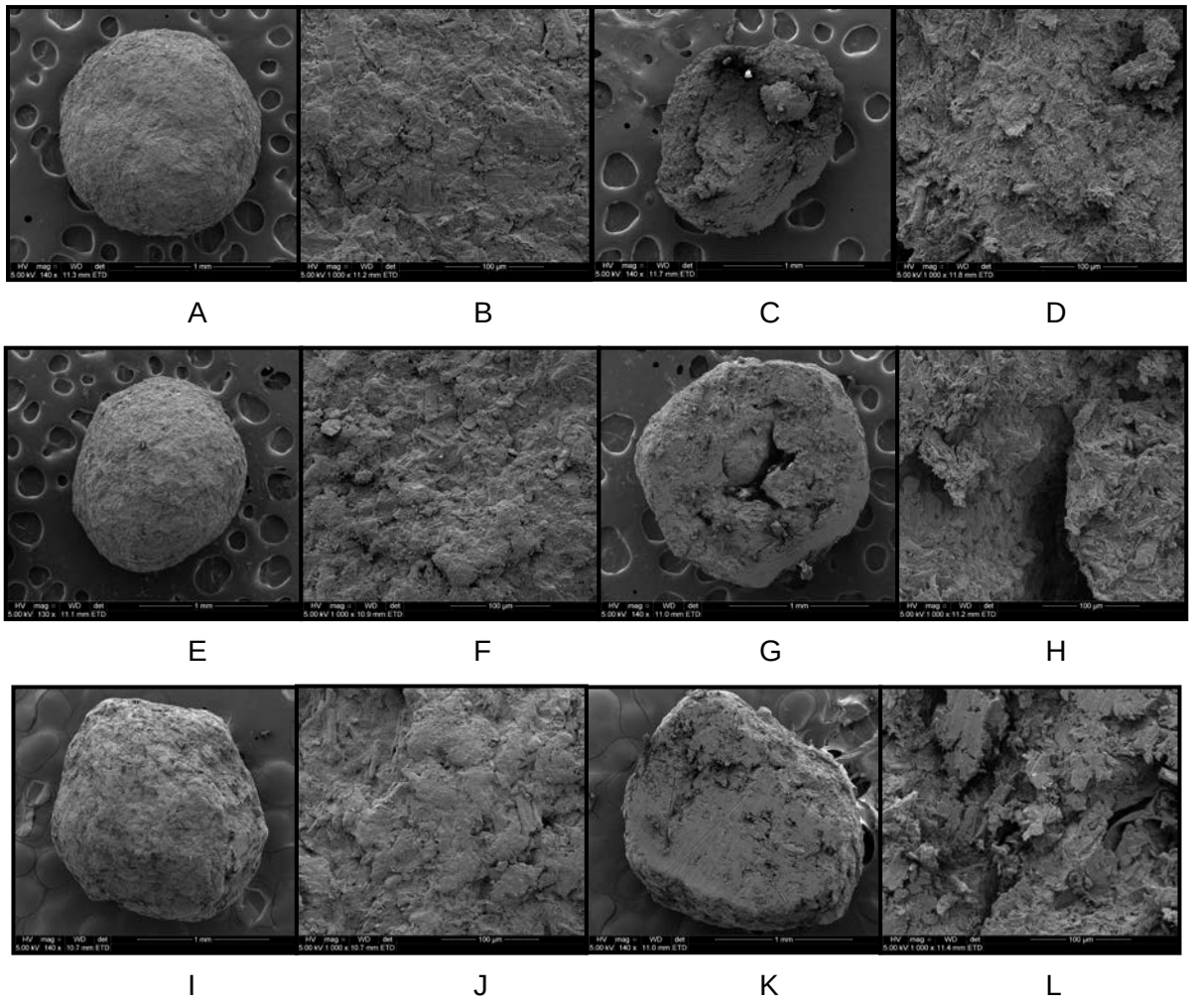


Figure 17: Scanning electron micrographs of beads containing *Aloe marlothii* whole leaf. A) External surface of a bead containing 3.75 % (w/w) AMWL at magnification of 130X, and B) 1000X, C) its internal structure at 130X, D) and 1000X, E) external surface of a bead containing 7.5 % (w/w) AMWL at 130X, F) and 1000X, G) its internal structure at 130X, H) and 1000X, I) external surface structure of a bead containing 10 % (w/w) AMWL at a magnification of 130X, J) and 1000X, K) the internal structure at 130X and L) 1000X

It is clear that the external surface morphology of the beads containing AMWL was rougher than that of the beads containing MCC only. The roughness of the bead surfaces seemed to become greater as the concentration of AMWL increased from 3.75 – 10% (w/w) (Figure 17B, F and J). Beads containing AMWL had internal structures that were more porous and exhibited crevices (Figure 17G) in the core of the beads. Inclusion of AMWL to the beads may therefore increase drug release rate from the beads due to the more porous structure.

3.5 *Aloe vera* whole leaf

The SEM photomicrographs of the bead formulations containing *A. vera* whole leaf (AVWL) are depicted in Figure 18A-H.

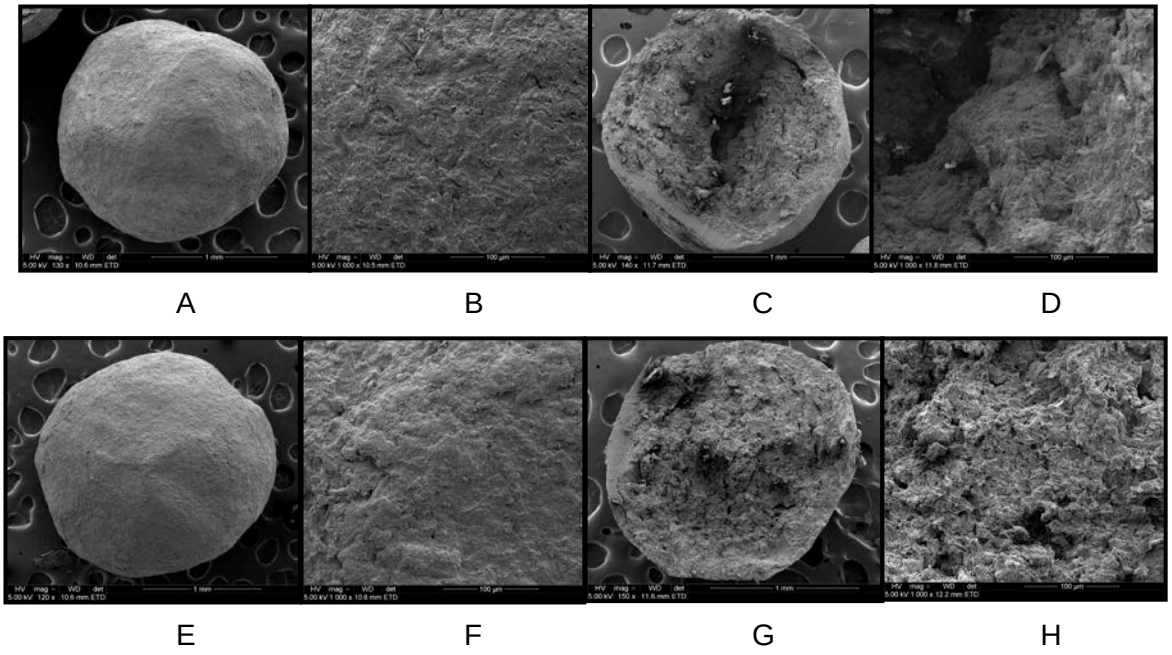


Figure 18: Scanning electron micrographs of bead formulations containing *Aloe vera* whole leaf. A) External surface of a bead containing 15 % (w/w) AVWL at a magnification of 130X, and B) 1000X, C) its internal structure at a magnification of 130X, D) and 1000X, E) external surface of a bead containing 30 % (w/w) AVWL at 130X, F) and 1000X, G) and its internal structure at 130X, H) and 1000X.

The external structure of the beads containing AVWL appeared smoother than that of the formulation containing MCC alone (Figure 18A, B, E and F). From the scanning electron micrographs of the internal structures of the beads containing AVWL, it is clear that the core of these beads was less compact with more pores, especially at higher concentration of AVWL (Figure 18G and H). It is therefore possible that the beads containing AVWL will release the drug at a faster rate than those containing MCC only.

4 Sphericity

The sphericity values of the bead formulations containing AMG are given in Table 2, while those containing AMWL are given in Table 3, AVG in Table 4 and AVWL in Table 5.

Table 2: Sphericity values of bead formulations containing *Aloe marlothii* gel

Concentration of MCC* (% w/w)	Concentration of PVP* (% w/w)	Concentration of AMG* (% w/w)	Average sphericity of 10 beads ± SD
90	0	10	1.02 ± 0.05
90	5	5	0.99 ± 0.05
95	0	5	0.97 ± 0.03
90	2.5	7.5	1.00 ± 0.04
93.75	2.5	3.75	0.98 ± 0.23
93.75	2.5	3.75	0.96 ± 0.03
93.75	2.5	3.75	1.027 ± 0.04
Average			**0.99 ± 0.02**

*MCC = microcrystaline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer, AMG = *Aloe marlothii* gel

The sphericity of the beads may influence aspects such as flow properties and uniformity in packing of the final product (Sinha *et al*., 2005:1). The bead formulation containing MCC only had a sphericity value of 1.17 ± 0.04. The sphericity values of the bead formulations containing aloe materials were relatively close to the desired value of 1.

The sphericity values of the beads exhibited an increase with generally improved concentrations of AMG. The beads containing 3.75% (w/w) AMG had a sphericity factor of 0.99 ± 0.01; those containing 5% (w/w) AMG had a sphericity factor of 0.98 ± 0.04; those containing 7.5% (w/w) had a sphericity factor of 1.00 ± 0.04 and those containing 10% (w/w) AMG had a sphericity factor of 1.02 ± 0.05. The average sphericity value of the beads containing AMG in different concentrations was 0.99 ± 0.02, which is very close to the desired sphericity value of 1.00. Inclusion of AMG up to 10 % (w/w) in beads prepared by extrusion spheronisation therefore provides acceptable sphericity.

Table 3: Sphericity values of bead formulations containing *Aloe marlothii* whole leaf

Concentration of MCC* (% w/w)	Concentration of PVP* (% w/w)	Concentration of AMWL* (% w/w)	Average sphericity of 10 beads ± SD
90	0	10	1.01 ± 0.06
90	5	5	1.03 ± 0.07
95	0	5	1.02 ± 0.05
90	2.5	7.5	1.01 ± 0.04
93.75	2.5	3.75	1.01 ± 0.05
93.75	2.5	3.75	1.09 ± 0.08
93.75	2.5	3.75	1.07 ± 0.08
Average			**1.03 ± 0.04**

*MCC = microcrystaline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer, AMWL = *Aloe marlothii* whole leaf

There was no correlation between the sphericity values and the concentration of the AMWL in the beads as the values stayed relatively constant. Inclusion of AMWL in beads made by extrusion spheronisation up to 10% (w/w) provided acceptable sphericity.

Table 4: Sphericity values of bead formulations containing *Aloe vera* gel

Concentration of MCC* (% w/w)	Concentration of PVP* (% w/w)	Concentration of AVG* (% w/w)	Average sphericity of 10 beads ± SD
90	0	10	1.04 ± 0.21
90	5	5	0.94 ± 0.06
95	0	5	1.03 ± 0.10
90	2.5	7.5	0.99 ± 0.04
93.75	2.5	3.75	1.00 ± 0.03
93.75	2.5	3.75	0.99 ± 0.03
93.75	2.5	3.75	1.01 ± 0.04
Average			**1.00 ± 0.03**

*MCC = microcrystaline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer, AVG = *Aloe vera* gel

There was no correlation between the sphericity values and the concentration of AVG in the beads. The sphericity values ranged between 0.94 ± 0.06 for the beads containing 5% (w/w) AMWL to 1.04 ± 0.21 for the beads containing 10% (w/w) AVG. Bead formulations containing 3.75% (w/w) AVG had a sphericity factor of 1.00 ± 0.01, those containing 7.5% w/w) had a value of 0.99 ± 0.04, while beads containing 5% (w/w) AVG had a sphericity of 1.03 ± 0.10. Inclusion of AVG up to 10% (w/w) in beads prepared by extrusion spheronisation provided acceptable sphericity. Inclusion of AVG in the formulations produced beads with sphericity values close to the target value of 1 and the sphericity was acceptable for all the concentrations of AVG included in the beads.

Table 5: Sphericity values of bead formulations containing *Aloe vera* whole leaf

Concentration of MCC* (% w/w)	Concentration of PVP* (% w/w)	Concentration of AVWL* (% w/w)	Average sphericity of 10 beads ± SD
70	0	30	1.13 ± 0.03
70	5	25	0.98 ± 0.10
85	0	15	0.97 ± 0.04
70	2.5	27.5	0.98 ± 0.03
83.75	2.5	13.75	1.18 ± 0.04
83.75	2.5	13.75	0.97 ± 0.02
83.75	2.5	13.75	1.11 ± 0.03
Average			**1.04 ± 0.09**

*MCC = microcrystaline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer, AVWL = *Aloe vera* whole leaf

The bead formulation containing 13.75% (w/w) AVWL had an average sphericity factor of 1.09 ± 0.11, while bead formulations containing 15%, 25% and 27.5% (w/w) AVWL had very similar sphericity values of 0.97 ± 0.04, 0.98 ± 0.10 and 0.98 ± 0.03, respectively. The bead formulation containing 30% (w/w) AVWL had a sphericity factor of 1.13 ± 0.03. The results show that the inclusion of AVWL up to 30% (w/w) provided beads of acceptable sphericity.

5 Mass variation

A fundamental quality attribute for all pharmaceutical preparations is the requirement for a constant dose of drug between individual dosing units in a batch. The uniformity of dose or dose variation can be measured using two tests namely uniformity of mass (or mass variation), which indirectly indicate dose variation and content assay (Aulton, 2002:417-418). The test for mass variation for capsules filled with beads was performed and the requirement was that the weight of individual capsules filled with beads from each formulation must not deviate from the average mass of the capsules filled with beads by more than ± 7.5% (BP, 1988:623).

The results obtained for mass variation of capsules filled with the different bead formulations are shown in Table 6 for beads containing AMG, Table 7 for those containing AMWL, Table 8 for beads containing AVG and in Table 9 for beads containing AVWL.

Capsules filled with beads containing AVWL had the highest weight of all the formulations containing aloe materials (648.76 ± 17.43 mg). This was followed by capsules filled with beads containing AMWL, which had an average weight of 610.97 ± 13.15 mg, then capsules filled with beads containing AMG (593.84 ± 13.14 mg) and the least weighing were capsules filled with beads containing AVG (555.57 ± 11.82 mg).

Table 6: Mass variation for hard gelatine capsules filled with beads containing *Aloe marlothii* gel

Formulation composition	Average mass (mg) of 20 capsules ± SD
3.75% AMG*	576.95 ± 8.99
3.75% AMG*	600.83 ± 5.81
3.75% AMG*	594.82 ± 11.31
5% AMG*	596.23 ± 8.39
5% AMG, 5% PVP*	615.51 ± 13.11
7.5% AMG*	593.80 ± 8.61
10% AMG*	578.79 ± 6.66
Average	**593.84 ± 13.14**

*PVP = Vinylpyrrolidone-vinylacetate-copolymer, AMG = *Aloe marlothii* gel.

There was no correlation between the concentration of AMG in the bead formulations and the weight of the capsules filled with beads. None of the capsules filled with beads containing AMG deviated from the average weight of 598.84 ± 13.47 mg by more than ± 7.5% indicating the uniformity of filling of the capsules with beads containing AMG.

Table 7: Mass variation for hard gelatine capsules filled with beads containing *Aloe marlothii* whole leaf

Formulation composition	Average mass (mg) of 20 capsules ± SD
3.75% AMWL*	598.41 ± 12.32
3.75% AMWL*	633.44 ± 9.95
3.75% AMWL*	621.29 ± 7.59
5% AMWL*	597.71 ± 12.69
5% AMWL, 5% PVP*	615.51 ± 13.11
7.5% AMWL*	606.70 ± 7.72
10% AMWL*	604.06 ± 8.15
Average	**610.97 ± 13.15**

*PVP = Vinylpyrrolidone-vinylacetate-copolymer, AMWL = *Aloe marlothii* whole leaf

There was no correlation between the weight of capsules filled with beads and the concentration of AMWL in the beads. None of the capsules filled with beads had a weight that deviated from the average weight of 610.97 ±13.15 by more than ± 7.5%.

Table 8: Mass variation for hard gelatine capsules filled with beads containing *Aloe vera* gel

Formulation composition	Average mass (mg) of 20 capsules ± SD
3.75% AVG*	557.46 ± 13.58
3.75% AVG*	542.00 ± 10.83
3.75% AVG*	552.76 ± 9.95
5% AVG*	552.60 ± 10.48
5% AVG, 5% PVP*	542.65 ± 9.93
7.5% AVG*	572.45 ± 13.82
10% AVG*	569.07 ± 10.51
Average	**555.57 ± 11.82**

* PVP = Vinylpyrrolidone-vinylacetate-copolymer, AVG = *Aloe vera* gel

Capsules filled with beads containing AVG weighed between 542.00 ±10.83 mgand 572.45 ± 13.82 mg. The weight of the capsules filled with beads increased as the concentration of the AVG in the capsules increased. None of the capsules filled with beads had a weight that deviated from the average weight of 555.57 ±11.82 mg by more than ± 7.5%.

There was uniform filling of the capsules as indicated by the low mass variation for the beads containing AVG, which can possibly be linked to the sphericity values of the beads containing AVG that were all very close to the target value of 1. As mentioned before, a spherical shape contributes to better flow and packing geometry of the beads leading to more uniform multiple-unit dosage form production.

Table 9: Mass variation for hard gelatine capsules filled with beads containing *Aloe vera* whole leaf

Formulation composition	Average mass (mg)of 20 capsules ± SD
13.75% AVWL*	664.07 ± 14.13
13.75% AVWL*	643.01 ± 15.40
13.75% AVWL*	626.88 ± 9.94
15% AVWL*	653.42 ± 11.47
25% AVWL*	625.48 ± 14.46
27.5% AVWL*	668.36 ± 10.79
30% AVWL*	660.15 ± 9.43
Average	**648.76 ± 17.43**

* PVP = Vinylpyrrolidone-vinylacetate-copolymer, AVWL = *Aloe vera* whole leaf

There was no direct correlation between the weight of capsules filled with beads containing AVWL and the concentration of AVWL in the beads. Capsules filled with beads containing AVWL had the highest weight compared to capsules filled with beads containing other aloe materials. None of the capsules filled with beads had a weight that deviated from the average weight of 648.79 ±17.43 mg by more than ± 7.5%. This confirms the uniform filling of capsules with beads containing AVWL.

6 Friability

The friability of solid oral dosage forms indicates their mechanical properties. The higher the friability, the more easily abrasion of the dosage form (e.g. beads) takes places. The beads must be tough enough to prevent crumbling during handling, but should be soft enough to release the drug upon contact with gastrointestinal fluids. The acceptable value for friability of solid oral dosage forms is less than or equal to 1% (USP 31, 2008:676).

Most of the bead formulations containing aloe materials had a friability value lower than that of the bead formulation containing MCC alone. The friability did not follow any specific relationship with the amount or type of aloe material included in the bead formulations. In general, bead formulations containing AMG had the lowest friability followed by the formulations containing AVWL, then formulations containing AVG. The bead formulations containing AMWL had the highest average friability and the formulation that contained 10%

w/w AMWL exhibited a friability value of 1.49% (Figure 19). Inclusion of AMG in the beads caused less brittleness, while AMWL increased the brittleness of the beads. This can possibly be explained by the fibrous materials present in the AMWL, while the AMG is a more dense powder.

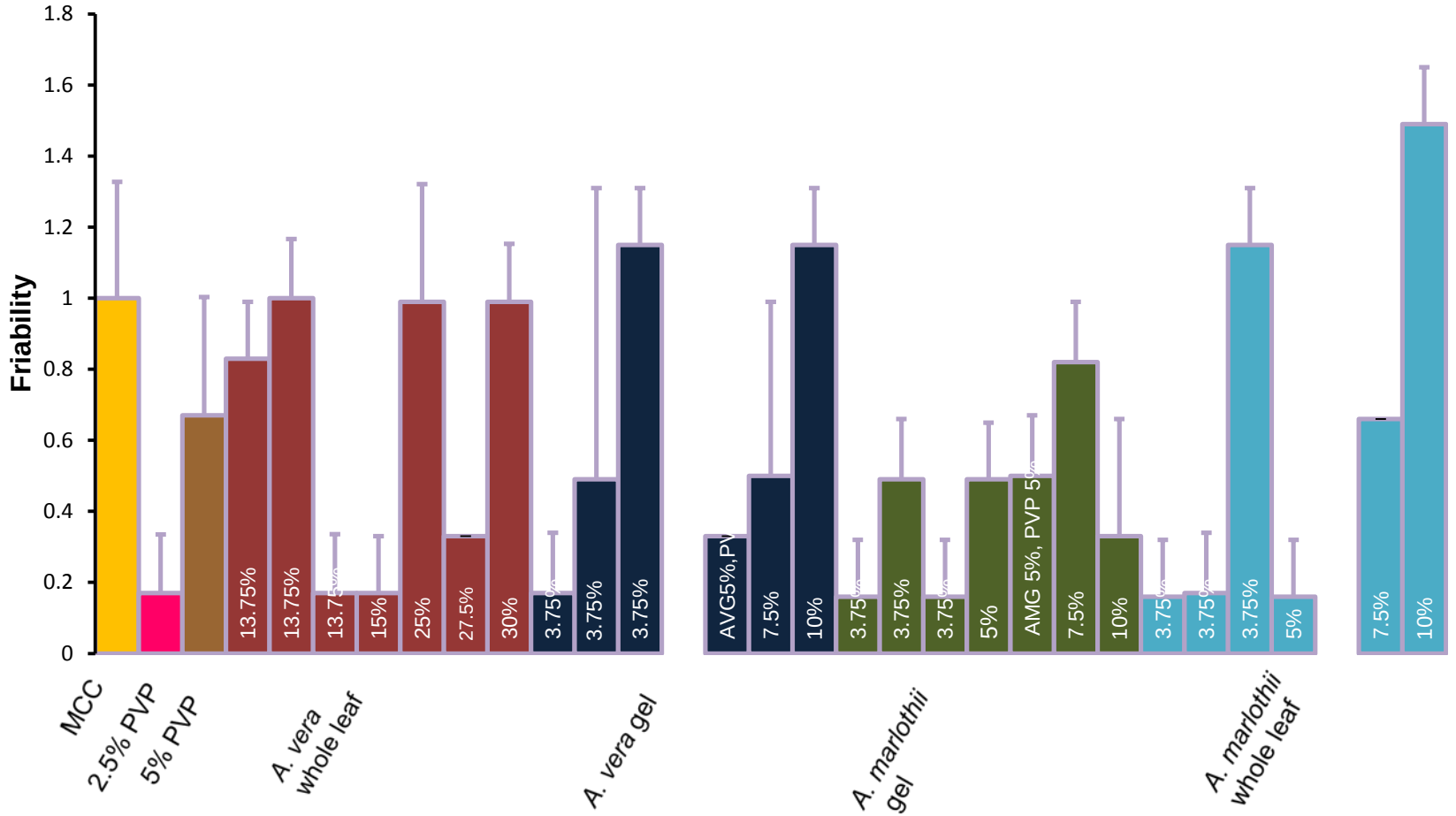


Figure 19: Friability for the different bead formulations

7 Analysis of samples by high performance liquid chromatography (HPLC)

7.1 Validation of HPLC method for ketoprofen

7.1.1 Linearity

The linear regression curve obtained for analysis of a series of ketoprofen solutions with HPLC is depicted in Figure 20.

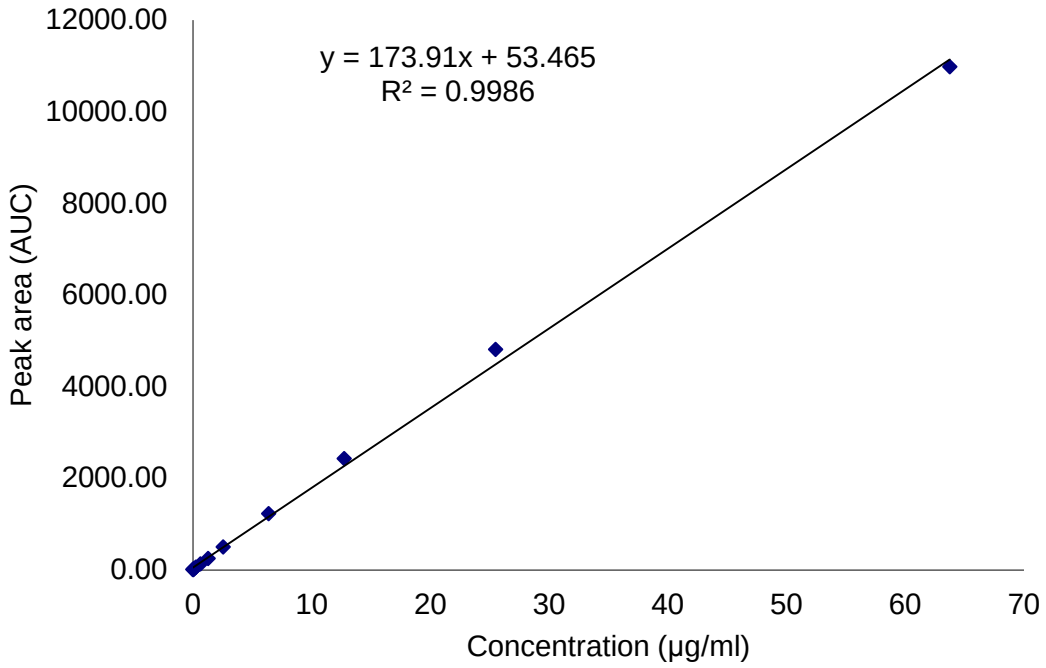


Figure 20: Linear regression curve for ketoprofen

A linear regression analysis should yield a regression coefficient (R^2) of ≥ 0.999 but for drug substances 0.998 is acceptable. Since an R^2 value of 0.9986 was obtained for the HPLC analysis method of ketoprofen used in this study, it complies with the requirement for linearity. The HPLC method was therefore able to give test results which are directly proportional to the concentration of ketoprofen in the samples.

7.1.2 Accuracy

The recovery values obtained from spiked samples of ketoprofen solutions are shown in Table 10.

Table 10: Recovery of ketoprofen from spiked samples

Concentration Spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
12.595	2386.6	2376.2	2381.4	12.25	97.29
12.595	2364.4	2371.1	2367.8	12.18	96.69
12.595	2379.0	2375.5	2377.2	12.23	97.11
25.19	4754.1	4775.0	4764.5	25.41	100.89
25.19	4764.8	4775.7	4770.3	25.45	101.01
25.19	4781.8	4767.7	4774.8	25.47	101.11
50.38	9171.7	9171.9	9171.8	49.75	98.75
50.38	9140.2	9140.2	9140.2	49.75	98.40
50.38	9161.2	9161.2	9161.2	49.69	98.63
				Mean	98.88
				SD*	1.74
				% RSD**	1.76

*SD refers to standard deviation

**% RSD refers to relative standard deviation

Recovery average of the analyte by means of HPLC must be between 98-102% in order for the method to be acceptable (Adamovics, 1997:10). The HPLC method used in this study to analyse ketoprofen exhibited an average recovery of 98.88% and therefore has an acceptable accuracy.

7.1.3 Ruggedness

The results showing the stability of ketoprofen over a 25 h period are depicted in Table 11.

Table 11: Results for ruggedness for ketoprofen analysis

Time (hours)	Peak Area	%
0	9033.4	100.0
1	9002.4	99.7
2	9036.5	100.0
3	9020.1	99.9
4	8962.9	99.2
5	8925.0.	98.8
6	8935.5	98.9
7	8896.7	98.5
8	8899.0	98.5
9	8926.4	98.8
10	8952.7	99.1
11	8932.3	98.9
12	8954.0	99.1
13	8923.5	98.8
14	8936.9	98.9
15	8966.6	99.3
16	8992.9	99.6
17	8968.0	99.3
18	9015.8	99.8
19	9001.7	99.6
20	9019.2	99.8
21	9031.8	100.0
22	9078.9	100.5

Table 11: Results for ruggedness for ketoprofen analysis (cont'd)

Time (hours)	Peak Area	%
23	9068.3	100.4
24	9069.7	100.4
25	9117.8	100.9
Mean	8987.20	99.5
SD*	58.27	0.65
% RSD**	0.65	0.65

SD* refers to standard deviation, RSD** refers to relative standard deviation

Samples should not be kept on the autosampler for a period longer than it takes the active ingredient to degrade by 2%. The model compound did not degrade by more than 2% for a period of 25 h.

7.1.4 Precision

The results showing the inter-day precision parameters for ketoprofen are listed in Table 12.

Table 12: Inter-day precision parameters for ketoprofen analysis

Concentration (µg/ml)	% Recovery		
	Day 1	Day 2	Day 3
25.19	100.89	100.91	98.86
25.05	101.01	100.77	98.86
25.09	101.11	100.09	98.86
Mean	101.00	100.59	98.86
SD	0.08	0.36	0.00
% RSD	0.08	0.36	0.00

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. In this study, days were

chosen as various samples containing ketoprofen were going to be analysed over a period of time on different days. The inter-day variation was less than 5% making the HPLC analysis method suitable for the analysis of ketoprofen in the samples under the same conditions on different days (ICH, 2005:10).

7.1.5 Repeatability (intra-day precision)

The results of the repeatability parameters for ketoprofen are shown in Table 13.

Table 13: Repeatability parameters for ketoprofen analysis

Injection number	Peak Area	Retention times (minutes)
1	4704.85	3.05
2	4665.81	3.04
3	4690.01	3.07
4	4642.26	3.06
5	4609.96	3.00
6	4604.30	2.98
Mean	4652.87	3.03
SD*	37.78	0.03
%RSD**	0.81	1.10

*SD refers to standard deviation, **RSD refers to relative standard deviation

The %RSD for the peak and retention time should not be greater than 2% and in this case it is 0.81 and 1.10, respectively. Therefore, the HPLC method expressed precision under conditions where there is the same analyst, the same equipment and identical reagents over a short time interval (Adamovics, 1997:9).

7.1.6 Specificity

Ketoprofen was analysed in the presence of salts and components used to prepare the dissolution media. The results obtained for the linearity of ketoprofen in media with pH values of 1.2, 4.5 and 6.8 are depicted in Figure 21 A-C.

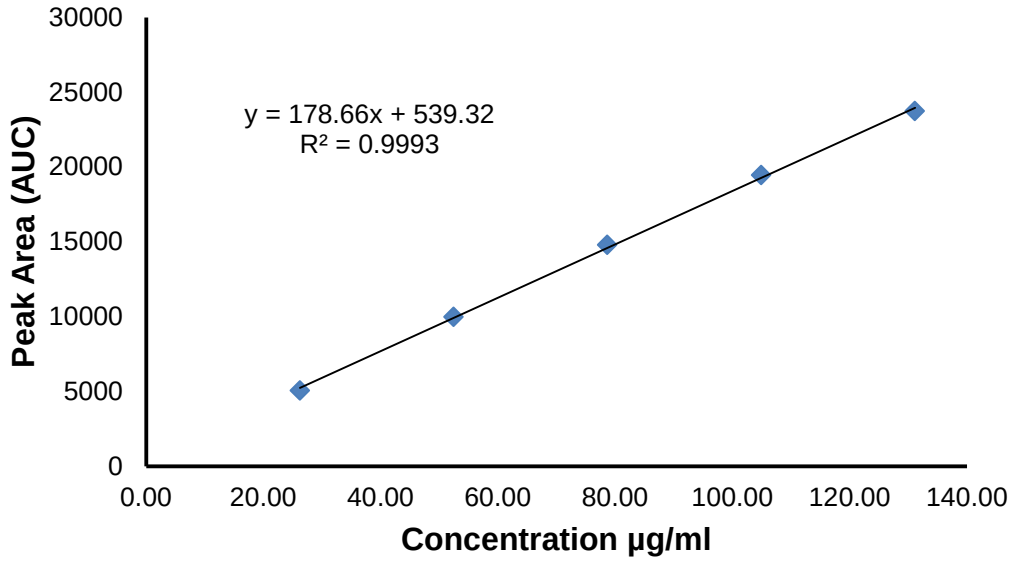


Figure 21A: Linear regression curve for ketoprofen in 0.1 N HCl (pH 1.2)

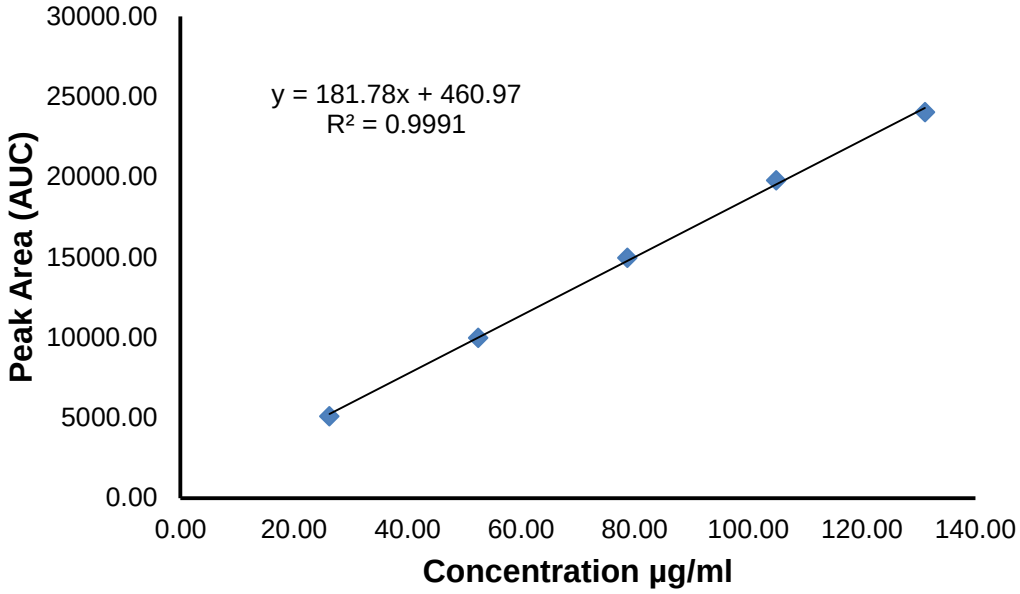


Figure 21B: Linear regression curve for ketoprofen in phosphate buffer (pH 4.5)

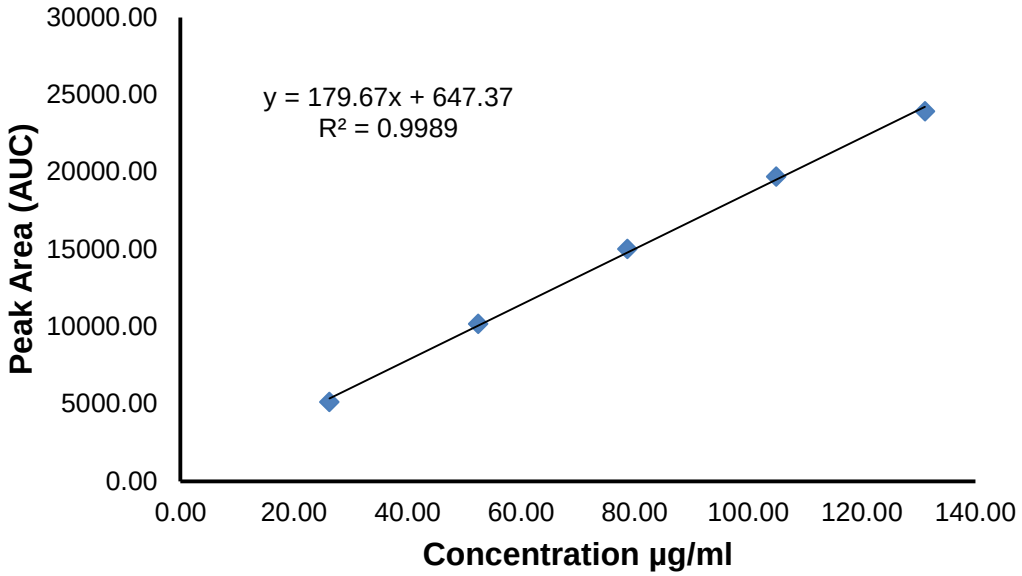


Figure 21C: Linear regression of ketoprofen in phosphate buffer (pH 6.8)

The regression coefficient (R^2) values of the analysis of a range of ketoprofen concentrations in buffers with different pH values were all above 0.99 (refer to Figure 10A-C). This showed that ketoprofen can be analysed with the HPLC method in media with pH values where dissolution of the beads are to be evaluated (i.e. pH of 1.2, 4.5 and 6.8).

A summary of the results for the validation of the HPLC analysis method of ketoprofen is shown in Table 13.

Table 14: Summary of results obtained for the validation of the analysis method for ketoprofen

Test	Result
Specificity	Complies
Range	100 µg/ml – 131 µg/ml
Linearity	R^2 = 0.9986
Accuracy	98.88%
Precision	RSD* = 0.81

*RSD = relative standard deviation.

8 Pre-optimisation drug release studies

Pre-optimisation dissolution studies were carried out for the various formulations at pH value 6.8 only. The dissolution profiles of the bead formulations containing the different aloe materials at pH 6.8 are displayed in Figure 22A-D.

The bead formulation containing MCC only as an excipient, which was used as a reference bead formulation in this study, displayed modified drug release properties due to the failure of these beads to disintegrate. The bead formulations containing MCC only as an excipient therefore released the drug slower than would be obtained with an immediate release dosage form with a burst release effect (Mallipedi*et al*., 2010:53). Incorporation of PVP in addition to the MCC without the aloe materials did not seem to have a pronounced effect on ketoprofen release and dissolution profiles similar with only a slight decrease to that of the formulation containing MCC only as an excipient were obtained (Figure 22B).

The bead formulations containing the different aloe materials (Figure 22A-D) exhibited dissolution profiles indicating faster release of ketoprofen compared to the formulation containing MCC only as the excipient.

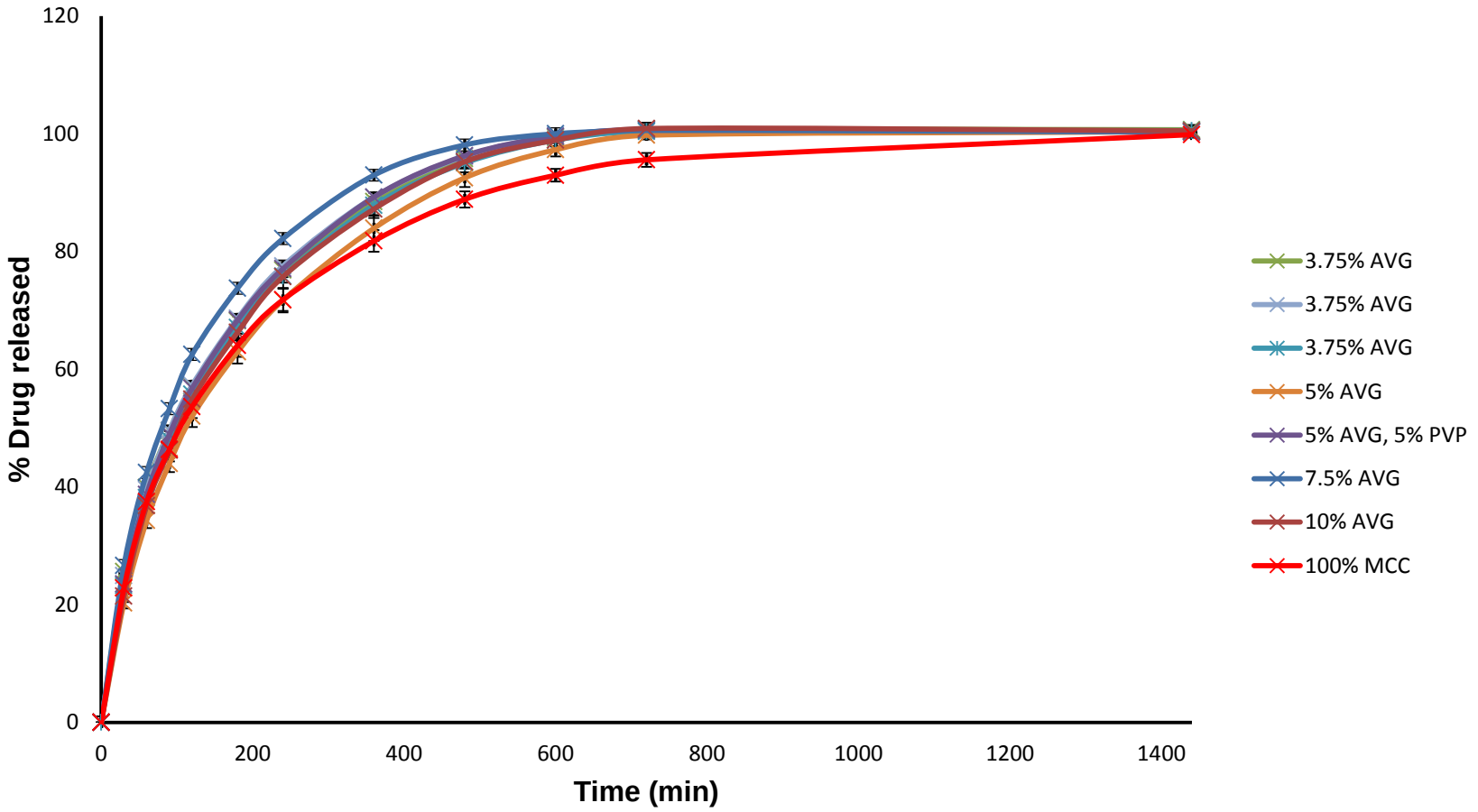


Figure 22A: Dissolution profiles of bead formulations containing different concentrations of *Aloe vera* gel (AVG) at pH 6.8

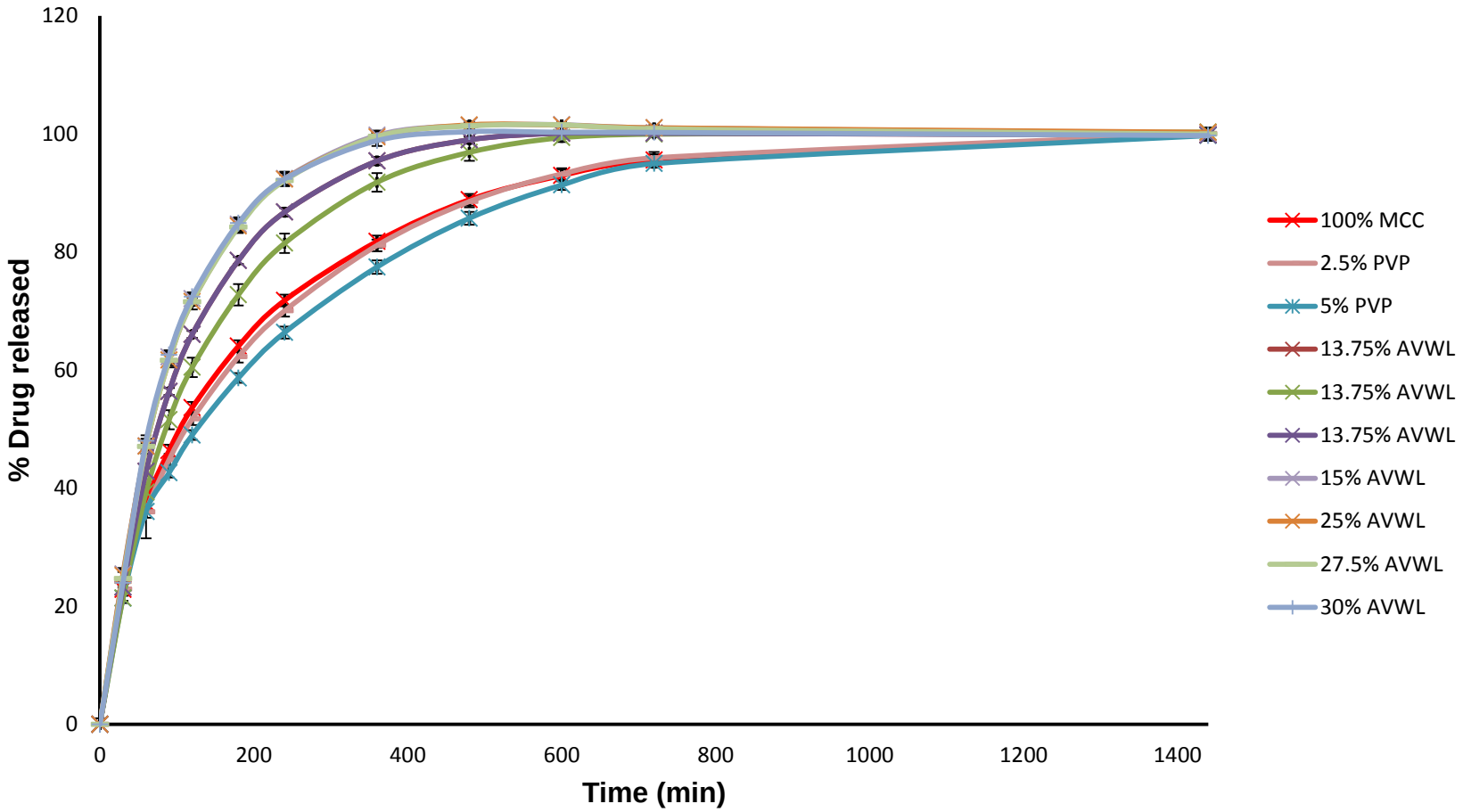


Figure 22B: Dissolution profiles of bead formulations containing different concentrations of *Aloe vera* whole leaf (AVWL) at pH 6.8

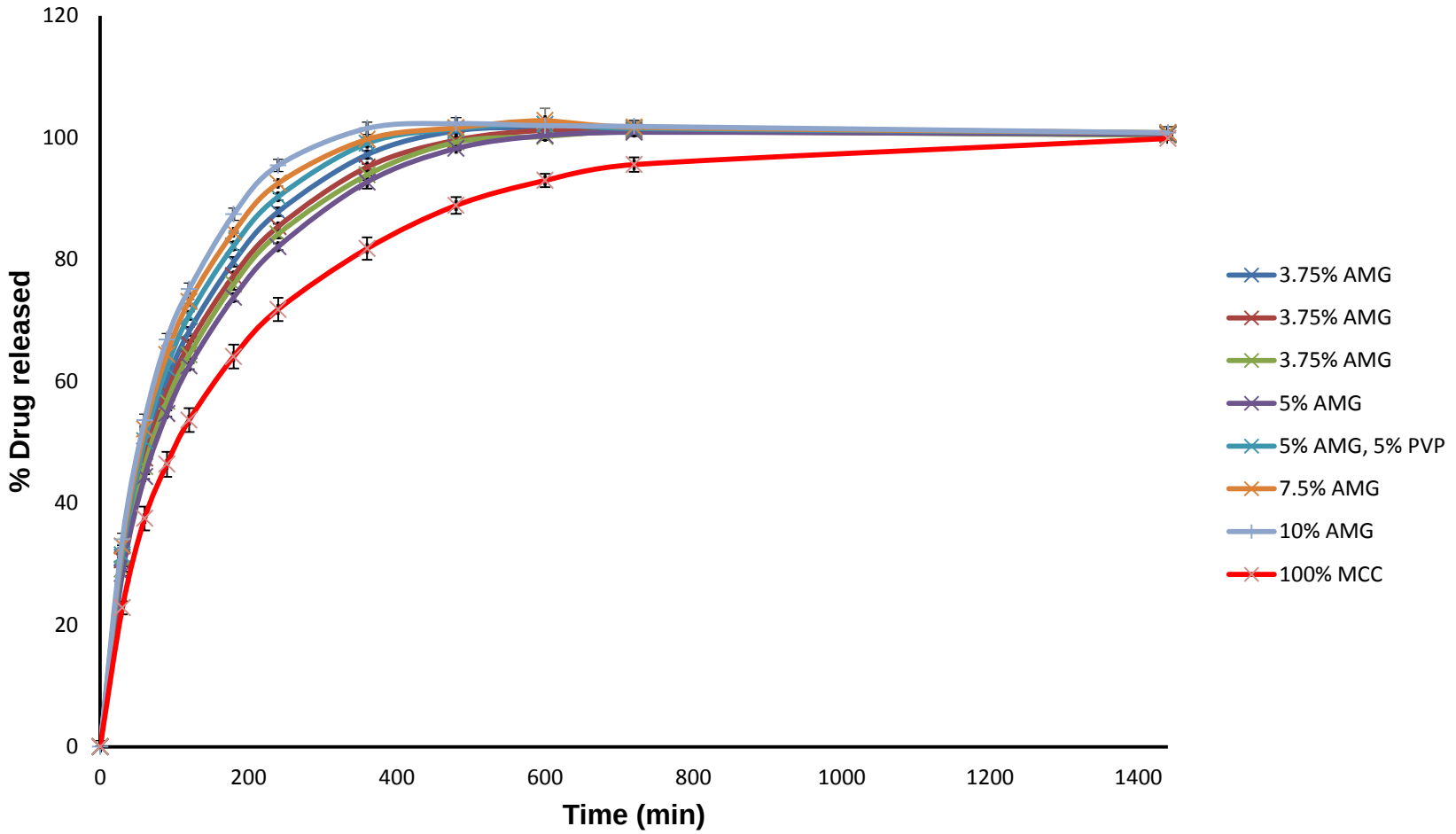


Figure 22C: Dissolution profiles of bead formulations containing different concentrations of *Aloe marlothii* gel (AMG) at pH 6.8

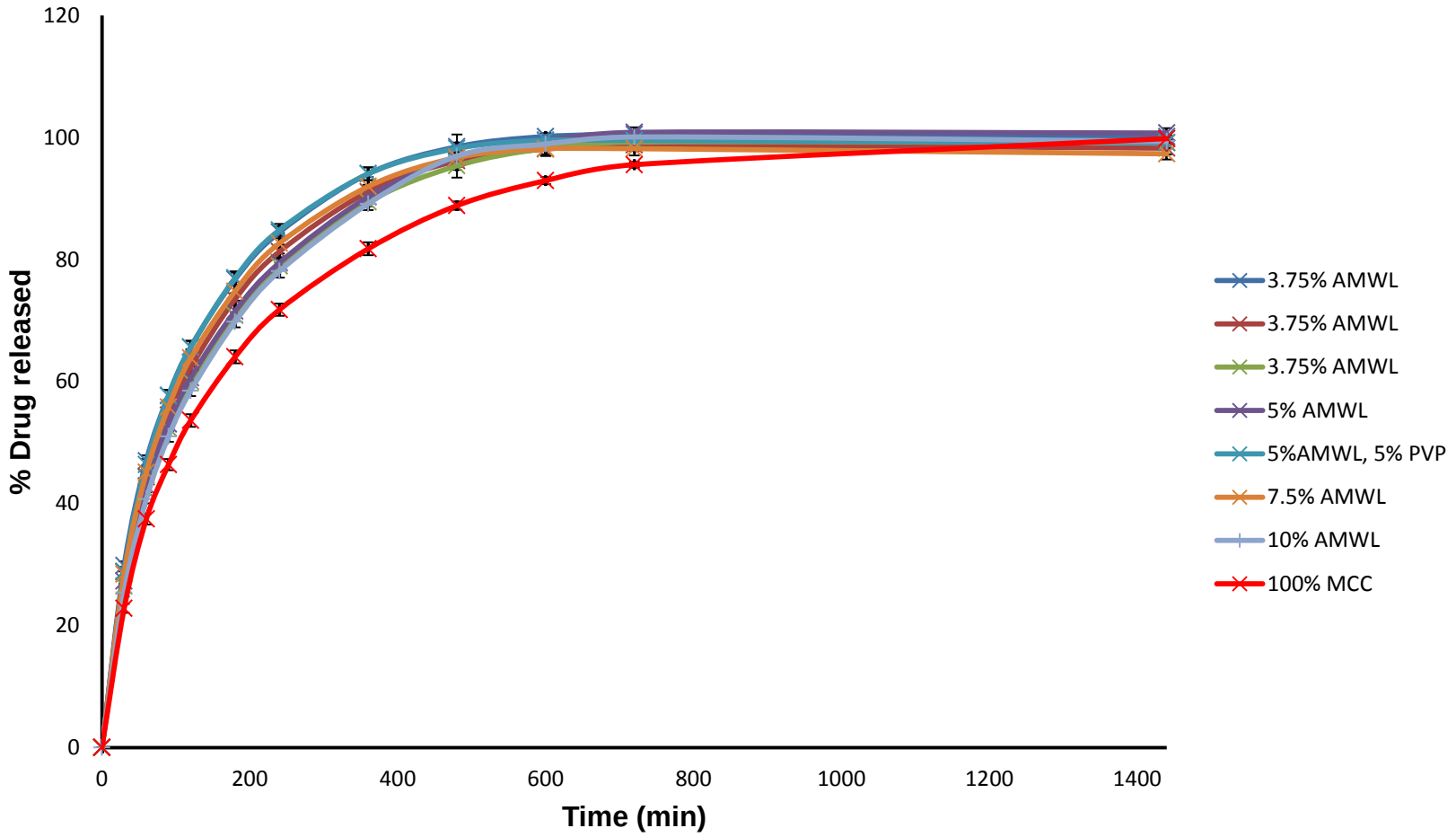


Figure 22D: Dissolution profiles of bead formulations containing different concentrations of *Aloe marlothii* whole leaf (AMWL) at pH 6.8

The faster release of ketoprofen in the formulations containing the aloe materials may be explained by the increased wettability of the drug by the polymers present in the aloe materials (Saharan *et al*., 2009:8). Furthermore, inclusion of the aloe materials indicated formation of micropores in the beads as elucidated by SEM, which may contribute to faster uptake of dissolution medium into the bead and thereby increasing drug release rate. The beads containing polysaccharides from the aloe materials may swell upon placement of the beads in the dissolution media and thereby increase the pore sizes and also drug release. However, the degree of swelling was not determined in this study and it is therefore not a conclusive statement.

8.1 Processing of dissolution profiles

From the data obtained with the dissolution of the different bead formulations, the mean dissolution time (MDT) and fit factors (f_1 and f_2) were calculated.

The MDT values for the bead formulations containing the different concentrations of aloe materials are shown in Figure 23. The formulation containing MCC only, used as the reference formulation in this study, had an MDT value of 43.24 ± 4.06 min. As mentioned before, the relatively slow drug release from the beads consisting of MCC only is due to the lack of disintegration of these beads, and therefore a long diffusion path of the drug through the intact matrix (Dukic-Ott *et al*., 2009:39).

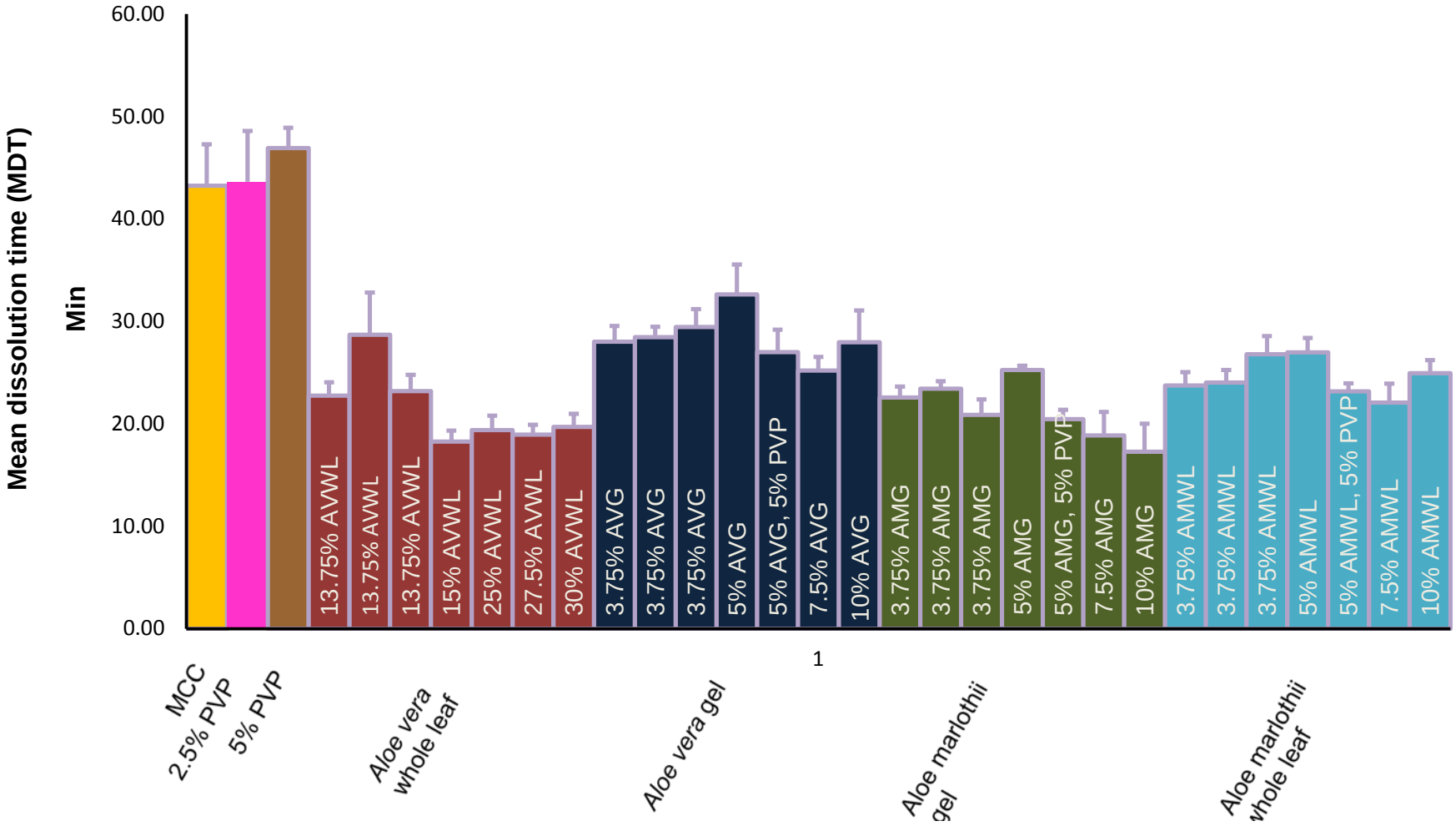


Figure 23: Mean dissolution time (MDT) values of the different bead formulations for each of the aloe materials at pH 6.8

The bead formulations containing PVP 2.5% and 5% (w/w) exhibited slightly higher MDT values of 43.65 ± 4.95 and 46.93 ± 1.97 min, respectively, compared to the beads containing MCC only. The MDT values were slightly increased (i.e. slower dissolution) with the addition of PVP, which can be explained by the binding properties of the PVP.

Addition of the AVWL to the bead formulations lowered the MDT (i.e. faster dissolution) compared to that of beads containing MCC only. Formulations with a lower concentration of AVWL 13.75% (w/w) had an average MDT value of 24.88 ± 3.31 min, which decreased with a higher concentration of AVWL 15% (w/w) to 18.28 ± 1.05 min. The MDT remained relatively constant in formulations containing 25, 27.5 and 30% (w/w), yielding MDT values of 19.39 ± 1.40, 18.96 ± 0.95 and 19.72 ± 1.28 min respectively. The decrease in MDT can possibly be attributed to the aloe material containing polymers, which at optimum concentrations produce a solubilisation effect and increase wettability and dispersibility, thereby enhancing dissolution (Saharan *et al*., 2009:112). Another possible explanation is the presence of weak acids in the aloe material such as iso-citric acid (Jambwa *et al.,* 2011:435). The weak acids dissociates at a pH value of 6.8 and thereby may lead to disentanglement of the polysaccharides inside the beads leading to faster movement of dissolution medium into the core with increased drug dissolution.

Formulations containing AVG had lower MDT values in comparison to the formulation containing MCC alone without a noticeable relationship between the MDT values and the amount of AVG in the formulations. The formulations containing 3.75% (w/w) of AVG had an average MDT of 28.65 ± 0.73 min, which increased in the formulation containing AVG 5% (w/w) to 32.63 ± 2.94 and slightly decreased to 27.00 ± 2.19 and 25.22 ± 1.33 in the formulation containing 5% (w/w) AVG as well as PVP and AVG 7.5% (w/w), respectively. The faster release of ketoprofen from the beads containing AVG compared to the bead formulation containing MCC only can also possibly be attributed to the beads being more porous and less compact as shown in the SEM micrographs. The MDT decreased in the formulation containing 10% (w/w) AVG to 27.97 ± 3.11 min, indicating slower ketoprofen release than from the beads containing other concentrations of AVG. In general, the beads containing AVG had slower ketoprofen release compared to the bead formulations containing the other aloe materials. The AVG may have provided a more viscous diffusion path for the drug and thereby decreasing the dissolution rate in comparison to the other aloe material containing bead formulations.

Bead formulations containing AMG had lower MDT values in comparison to the formulation containing MCC only as excipient. The MDT value was 22.30 ± 1.29 min for the formulation

containing 3.75% (w/w) AMG and 25.25 ± 0.42 min for the formulation containing 5% (w/w) AMG. The MDT value then decreased to the lowest MDT of all the bead formulations containing aloe materials (i.e. 17.28 ± 2.73 min) for the formulation containing 10% (w/w) AMG. Bead formulations containing AMG were more porous as indicated by SEM micrographs than the bead formulations containing MCC only, which promotes the entrance of the dissolution media into the beads thereby releasing the drug and enhancing dissolution.

Bead formulations containing AMWL showed a decrease in MDT values in comparison to the formulation containing MCC only and AVG, but higher MDT values when compared to the bead formulations containing the AVWL and AMG. The MDT values increased as the concentration of AMWL in the bead formulations increased up to the highest MDT value of 26.99 ± 1.39 min for the bead formulation containing 5% (w/w) AMWL. The MDT value slightly decreased in the formulation containing 5% (w/w) AMWL with PVP (23.18 ± 0.78 min) and the formulation containing 7.5% (w/w) AMWL (22.07± 1.86 min). The MDT value increased for the formulation containing 10% (w/w) of AMWL to 24.94 ± 1.28 min.

8.2 Fit factors

The fit factors, f_1 and f_2, are used respectively to compare the difference and similarity of dissolution profiles of test formulations with that of a reference formulation (in this study the formulation containing MCC only was used as the reference formulation). The fit factor f_1 is used to approximate the percent error between two curves where 0 represents identical curves and the value increases as the dissimilarity between two profiles increases. Values of f_1 must be ≤ 15 to indicate that the dissolution profiles are similar. The fit factor f_2 is a logarithmic transformation of the sum of squared error. It takes the average sums of squares of the difference between the test and reference dissolution profiles and fits the result between 0 and 100. The fit factor of f_2 is 100 when the test and reference are identical and approaches zero as the dissimilarity increases (Moore & Flanner, 1996:66). The value of f_2 must be ≥ 50 for the dissolution profiles to be relatively similar. From table 15 below the formulations containing 2.5% (f_1 = 2.46, f_2 = 89.24) and 5% PVP (f_1 = 5.50, f_2= 74.56) in addition to MCC have similar profiles to that of the formulation containing MCC only as shown by f_1 values below 15 and f_2 values above 50.

The fit factor (f_1 and f_2) values calculated from the dissolution profiles (pH6.8) of the bead formulations containing different aloe materials compared to that of the reference formulation containing MCC only are listed in Table 15.

Table 15: Fit factor (f_1 and f_2) values calculated from the dissolution profiles (pH 6.8) of the bead formulations containing different aloe materials compared to that of the reference formulation containing MCC only

Formulation	f_1	f_2
2.5% PVP, 97.5% MCC	2.46	89.24
5% PVP, 95% MCC	5.50	74.56
3.75% AMG	24.96	45.76
3.75% AMG	21.77	48.79
3.75% AMG	19.06	51.55
5% AMG	15.99	55.28
5% AMG, 5% PVP	27.65	43.48
7.5% AMG*	31.54	40.69
10% AMG*	27.65	43.48
3.75% AMWL*	20.55	50.12
3.75% AMWL*	17.81	53.09
3.75% AMWL*	12.13	61.06
5% AMWL*	12.45	60.67
5% AMWL, 5% PVP*	21.79	48.57
7.5% AMWL*	20.79	49.64
10% AMWL*	9.93	64.84
3.75% AVG*	7.62	69.38
3.75% AVG*	4.65	77.10
3.75% AVG*	7.32	70.85
5% AVG*	3.47	83.81
5% AVG, 5% PVP*	6.59	70.7
7.5% AVG*	14.81	56.29
10% AVG*	4.85	77.01

13.75% AVWL*	11.54	60.02
13.75% AVWL*	19.84	49.21
13.75% AVWL*	19.84	49.21
15% AVWL*	1.18	96.47
25% AVWL*	27.44	42.42
27.5% AVWL*	27.19	42.53
30% AVWL*	27.78	43.82

*AMG = *Aloe marlothii* gel, AMWL = *Aloe marlothii* whole leaf, AVG = *Aloe vera* gel, AVWL = *Aloe vera* whole leaf, MCC = Microcrystalline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer

According to the fit factors, most of the formulations containing AMG had dissolution profiles that were not similar to that of the formulation containing MCC, except for the formulation containing 5% (w/w) AMG. Formulations containing AMWL had more formulations with dissolution profiles similar to the formulation containing MCC only, namely the formulations containing 3.75% (w/w) (f_1 = 12.13 and f_2 = 61.06), 5% (w/w) (f_1 = 12.45 and f_2 = 60.67) and 10% (w/w) (f_1 = 9.93 and f_2 = 64.84) AMWL. All the bead formulations containing AVG had dissolutions profiles with f_1 values below 15 and f_2 values above 50, which displays their similarity to the dissolution profile of the formulation containing MCC only. Formulations containing AVWL had dissolution profiles dissimilar from the formulation containing MCC only, except for two formulations, the formulation containing 13.75% (w/w) AVWL (f_1 = 11.54 and f_2 = 60.02) and the formulation containing 15% (w/w) AVWL (f_1 =1.18 and f_2 = 96.47).

9 Optimised bead formulations

9.1 Response contour plots

After evaluation of the beads, the results in terms of sphericity and dissolution (pH 6.8) were entered as responses into MODDE® software. The optimised bead formulations for each of the four aloe materials were identified from the contour plots (Figure 24A-D).

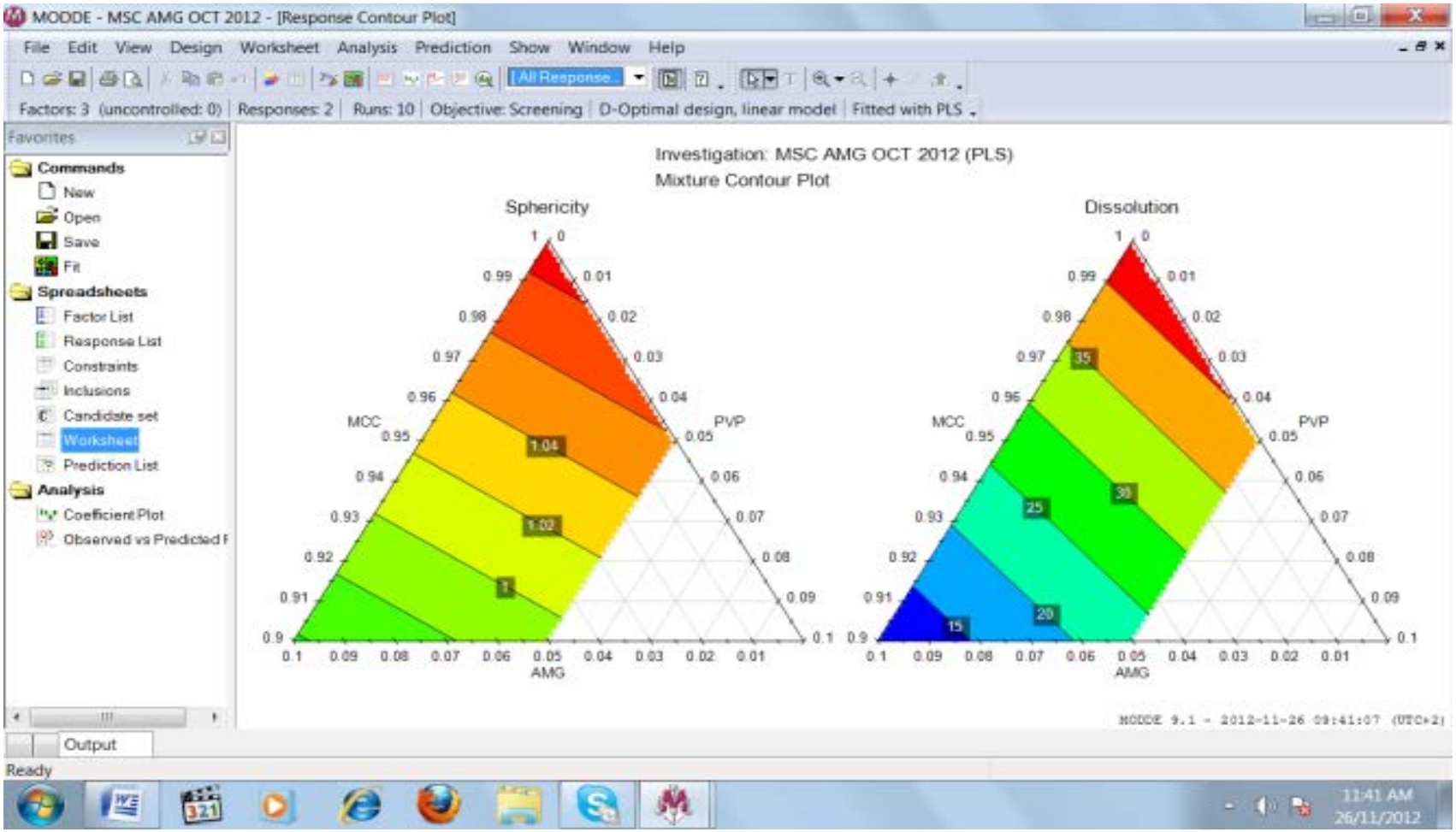


Figure 24A: The response contour plot for the bead formulation used to identify optimised formulations containing AMG

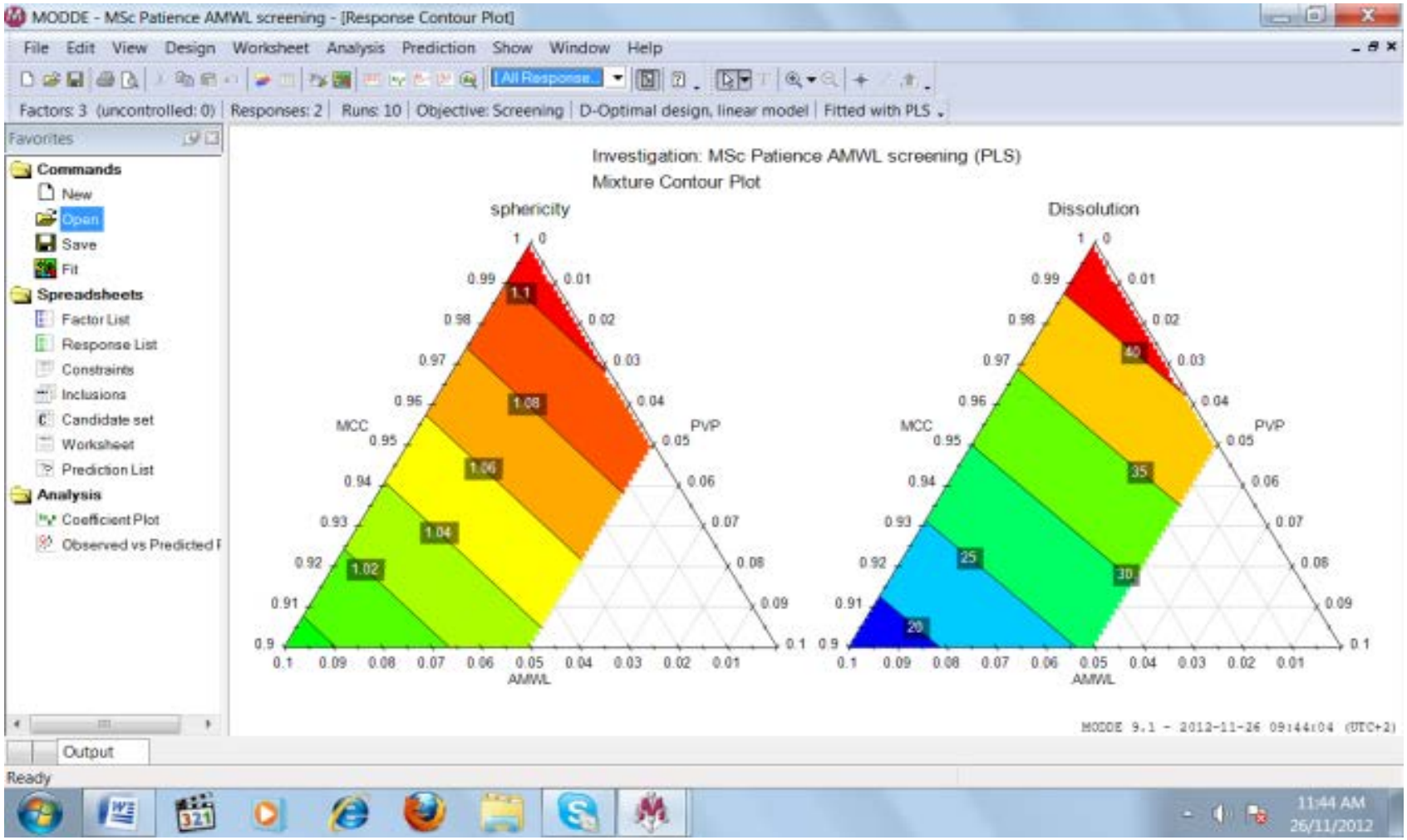


Figure 24B: The response contour plots for the bead formulation used to identify optimised formulations containing AMWL

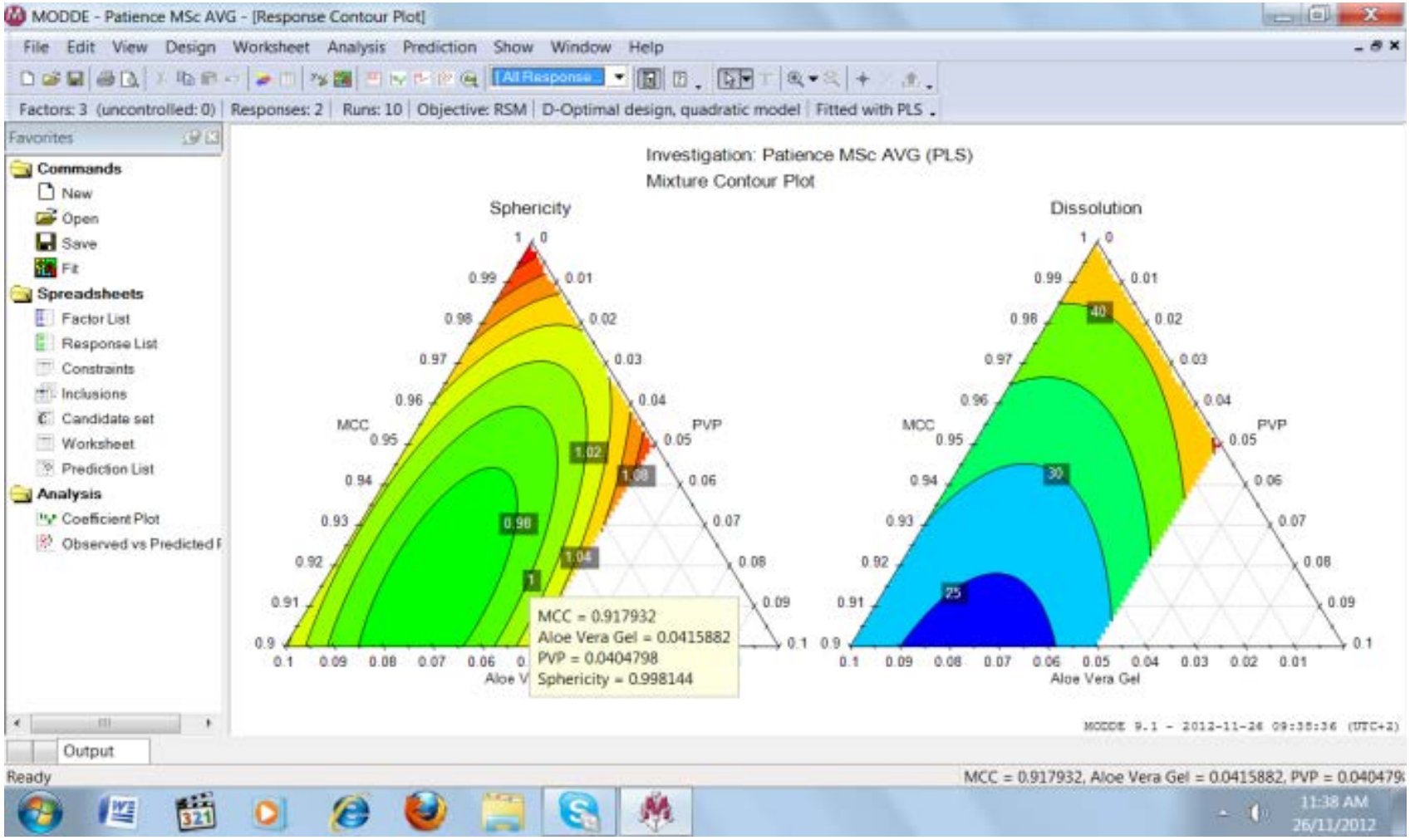


Figure 24C: The response contour plots for the bead formulation used to identify optimised formulations containing AVG

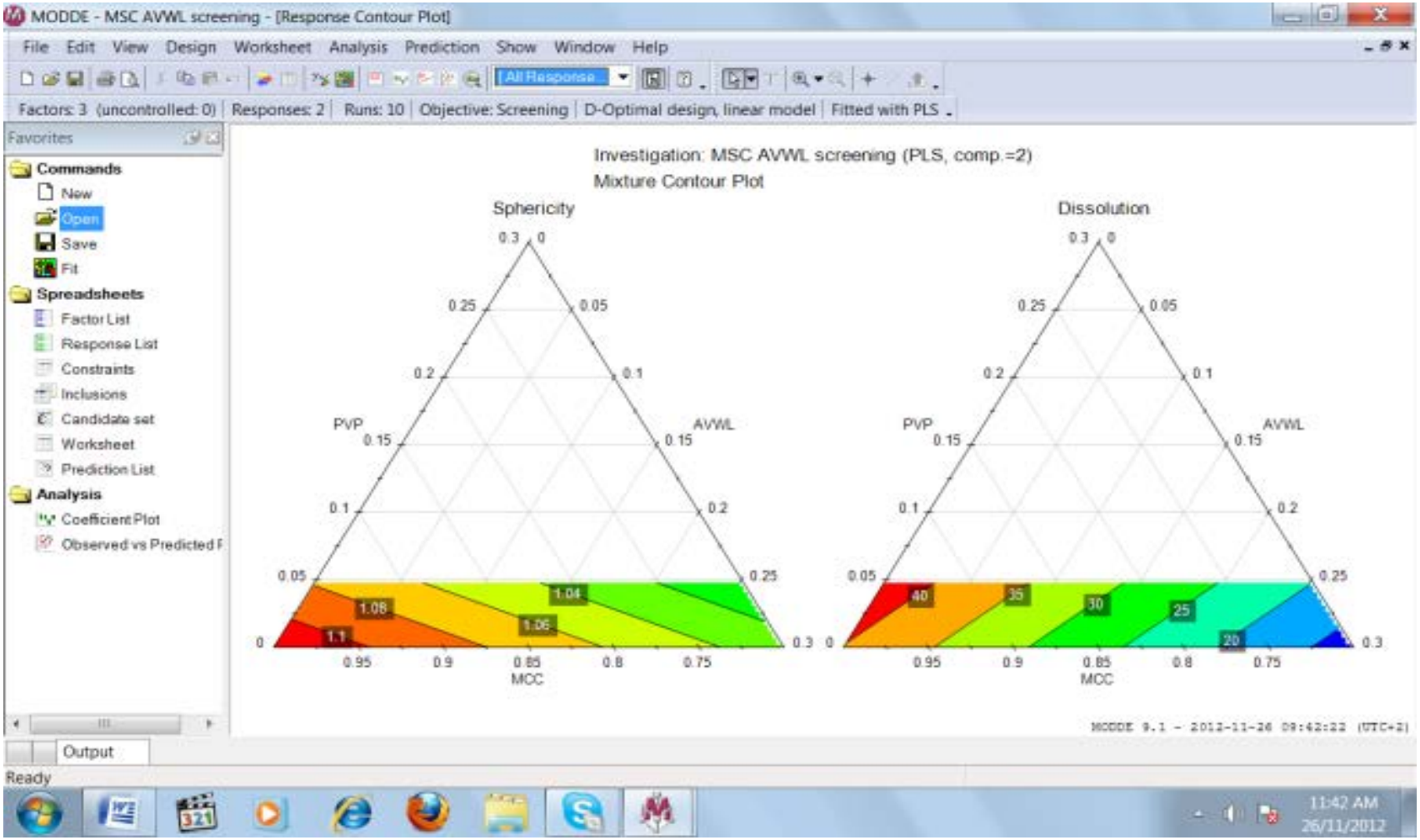


Figure 24D: The response contour plots for the bead formulation used to identify optimised formulations containing AVWL

The concentrations of MCC, PVP and different aloe materials for the optimised bead formulations is shown in Table 16.

Table 16: The composition of the optimised bead formulations as determined by (MODDE®)

AMG (% w/w)	MCC (% w/w)	PVP (% w/w)
3.5	93.7	2.8
AMWL		
5.7	93.1	1.2
AVG		
3.0	94.2	2.8
AVWL		
16.4	79.7	3.9

AMG = *Aloe marlothii* gel, AMWL = *Aloe marlothii* whole leaf, AVG = *Aloe vera* gel, AVWL = *Aloe vera* whole leaf, MCC = Microcrystalline cellulose, PVP = Vinylpyrrolidone-vinylacetate-copolymer

9.2 Evaluation of the optimised bead formulations

The optimised bead formulations for each of the four aloe materials were prepared by extrusion spheronisation according to the compositions as indicated by the contour plots and then evaluated in terms of sphericity, mass variation, friability and dissolution.

9.2.1 Sphericity

The sphericity values of the optimised bead formulations containing each of the four aloe materials are shown in Table 17.

Table 17: Sphericity values of the optimised bead formulations

Type of aloe in optimised formulation	Average sphericity ± SD
AMG*	1.19 ± 0.05
AMWL	1.14 ± 0.09
AVG	1.14 ± 0.14
AVWL	1.17 ± 0.08

AMG = *Aloe marlothii* gel, AMWL = *Aloe marlothii* whole leaf, AVG = *Aloe vera* gel, AVWL = *Aloe vera* whole leaf

Although the sphericity values of the optimised bead formulations were slightly above the desired sphericity value of one, they exhibited acceptable sphericity. There was not a large variation in the sphericity values of the four different optimised bead formulations indicating consistency of the models from the MODDE® software program to predict the optimised bead formulation for each of the aloe materials.

9.2.2 Mass variation

The mass variation results for the four optimised bead formulations filled in hard gelatine capsules are shown in Table 18.

Table 18: Mass of optimised bead formulations filled into hard gelatine capsules with the maximum % deviations from the average mass

Type of aloe in the optimised formulation	Mass ± SD	Maximum % deviation
AMG	610.47 ± 9.21	1.51
AMWL	582.06 ± 10.61	1.82
AVG	612.05 ± 8.83	1.44
AVWL	690.01 ± 9.93	1.44

AMG = *Aloe marlothii* gel, AMWL = *Aloe marlothii* whole leaf, AVG = *Aloe vera* gel, AVWL = *Aloe vera* whole leaf

Capsules filled with optimised beads containing AVWL had the highest average mass (690.01 ± 9.93 mg) followed by capsules filled with beads containing AVG (612.05 ± 8.83 mg) and AMG (610.47 ± 9.21 mg) and the lowest mass was obtained for capsules filled with

the bead formulation containing AMWL (582.06 ± 10.61 mg). All four the optimised formulations complied with the requirement of less than 7.5% deviation from the average mass. This indicates the ability of the optimised formulations to produce capsules with consistent doses.

9.2.3 Friability

The results obtained from the friability test for the optimised bead formulations are shown in Table 19.

Table 19: Friability results for the optimised bead formulations

Type of aloe in optimised formulation	Friability ± SD
AMG	1.66 ± 0.47
AMWL	0.67 ± 0.47
AVG	1.33 ± 0.00
AVWL	0.33 ± 0.00

AMG = *Aloe marlothii* gel, AMWL = *Aloe marlothii* whole leaf, AVG = *Aloe vera* gel, AVWL = *Aloe vera* whole leaf

Bead formulations containing AMG (friability = 1.66 ± 0.47%) and AVG (friability = 1.33 ± 0.00%) were more brittle than formulations containing AMWL (friability = 0.67 ± 0.47%) and AVWL (friability = 0.33 ± 0.00%).

9.2.4 Assay of ketoprofen content in optimised bead formulations.

Table 20: Content of ketoprofen in optimised bead formulations

Type of aloe in the optimised bead formulation	Area AUC (HPLC)	Amount of ketoprofen (mg)	% drug content
AMG	1022.00	47.26	94.5
AMWL	1054.25	48.82	97.6
AVG	964.46	45.98	91.8
AVWL	1016.34	46.90	93.8

AMG = *Aloe marlothii* gel, AMWL = *Aloe marlothii* whole leaf, AVG = *Aloe vera* gel, AVWL = *Aloe vera* whole leaf

The optimised bead formulations containing AMWL had the highest content of ketoprofen (97.6%), then those containing AMG (94.5%) and AVWL (93.8%). The optimised formulation containing AVG had the least ketoprofen content of 91.8%.

9.2.5 Dissolution

The dissolution profiles of the optimised formulations in comparison to the bead formulation containing MCC only in a dissolution medium with a pH of 1.2 are shown in Figure 25 on page 94.

In general, the dissolution of ketoprofen from the optimised bead formulations at pH 1.2 was relatively slow with the maximum amount of drug released being only about 45% after a period of 24 h from the formulation containing AVG. This can be explained by the fact that ketoprofen is mainly in the unionised state in an environment with a pH below its pKa value of 4.5. Formulations containing AMWL and AVG had a faster dissolution than that of the formulation containing AMG, which had a dissolution profile similar to that of the formulation containing MCC only. AVWL had the slowest drug release and only released 22.47% ketoprofen after a period of 24 h.

The dissolution profiles of the optimised bead formulations in comparison to the bead formulation containing MCC only at pH 4.5 are shown in Figure 26 on page 95.

The optimised bead formulations had a faster release rate at pH 4.5 than at pH 1.2 with the maximum drug release being 80% in the formulation containing AVWL. This is because more of the drug existed in the ionized form in a dissolution medium with a pH of 4.5 compared to a dissolution medium with a pH of 1.2. The dissolution profile of the bead formulation containing AVWL had the fastest drug release followed by the formulation containing AMWL, then the formulation containing AVG. These formulations had a faster dissolution than the one containing MCC only, which was similar to that of the formulation containing AMG.

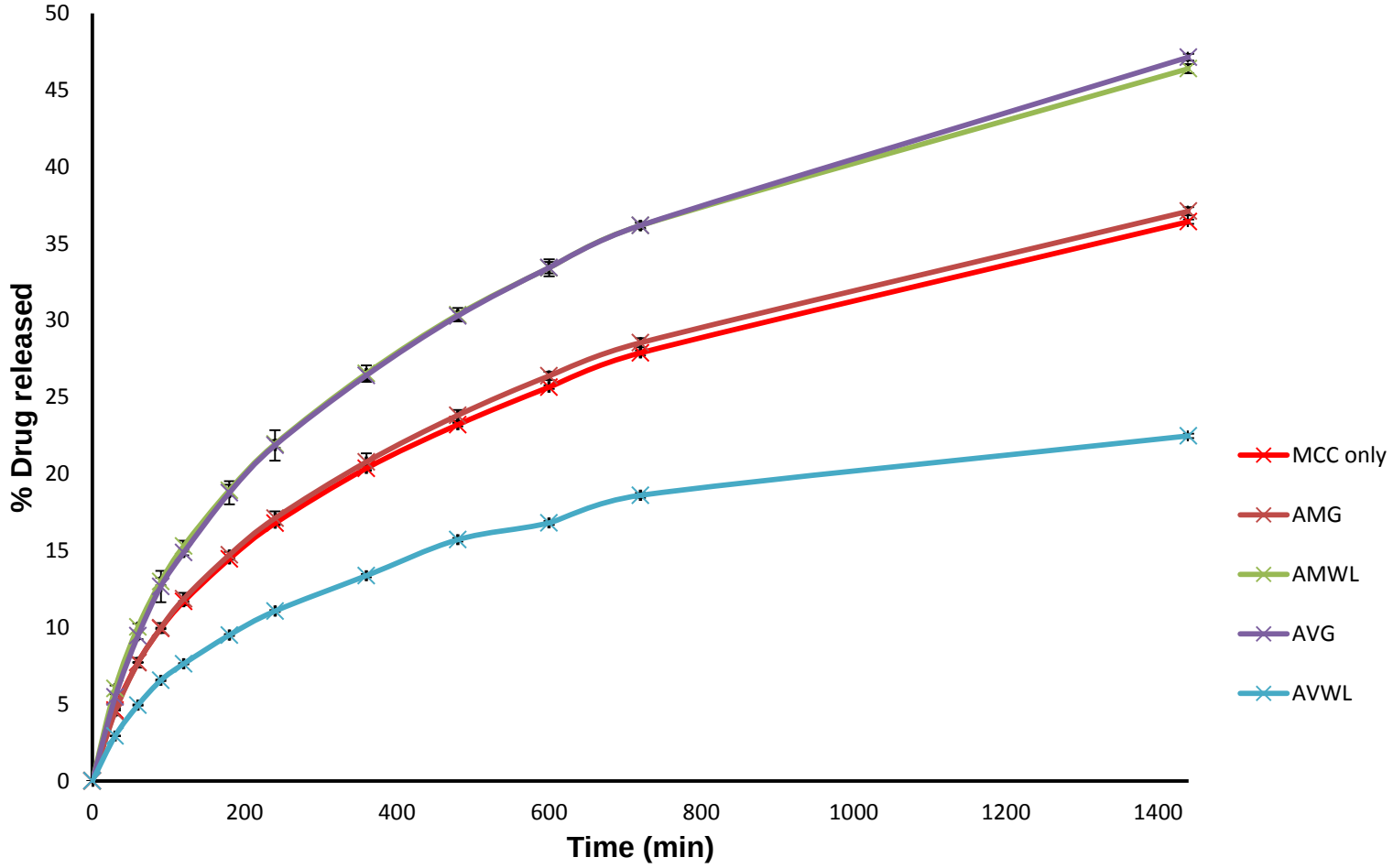


Figure 25: Dissolution profiles of optimised bead formulations at pH 1.2

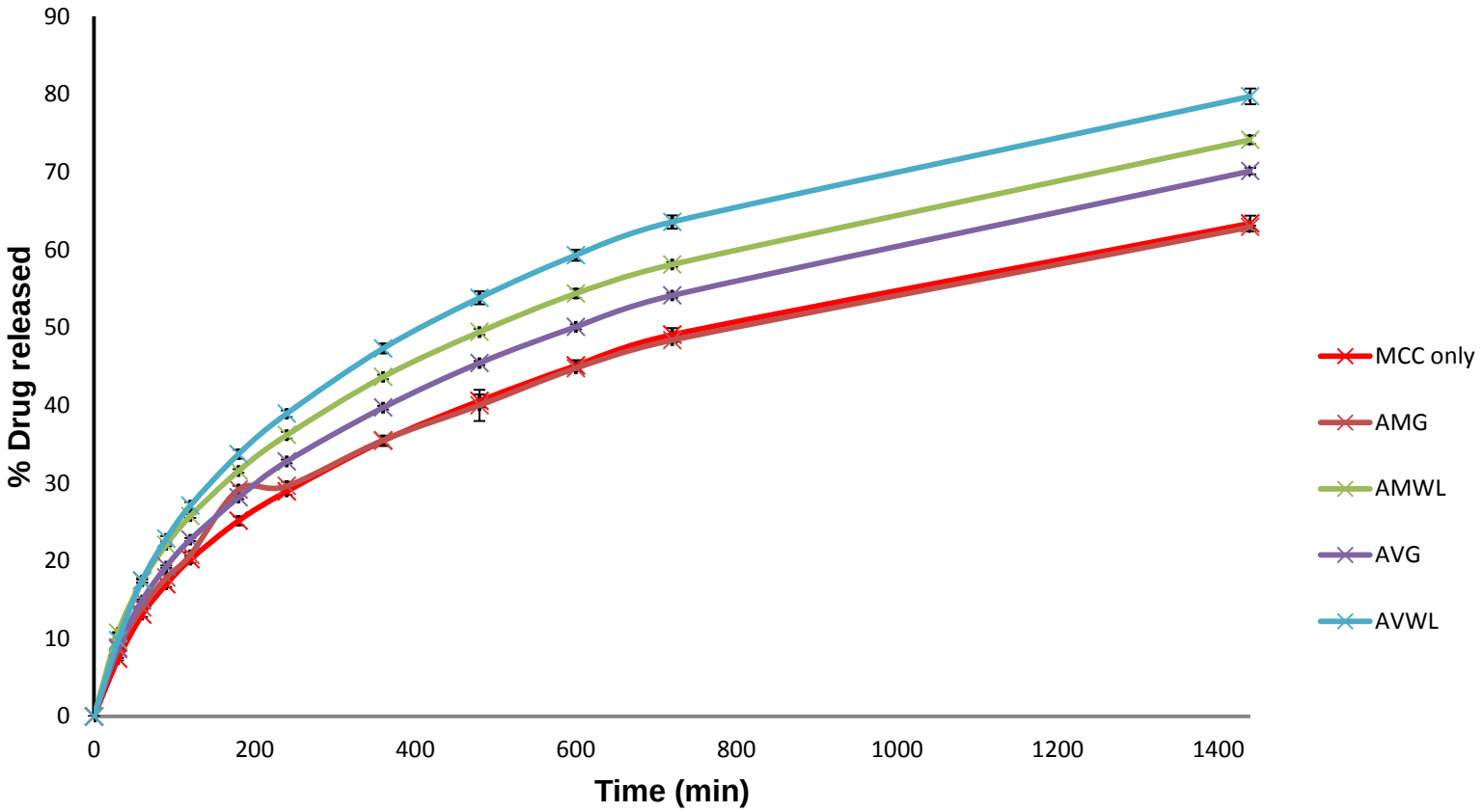


Figure 26: Drug release profiles of optimised formulations at pH 4.5

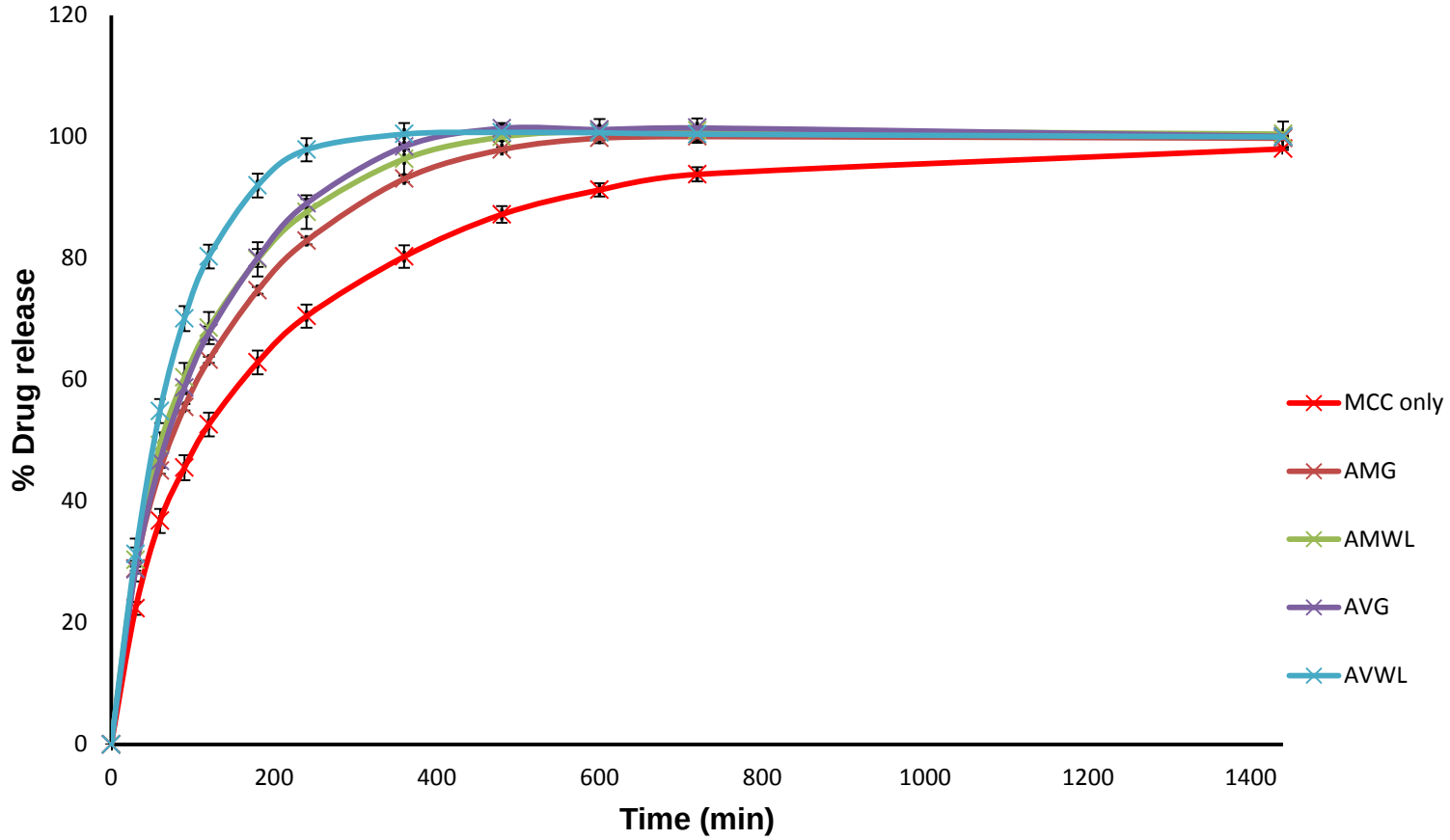


Figure 27: Drug release profiles of optimised formulations at pH 6.8

All four optimised bead formulations had dissolution curves that indicated faster ketoprofen release than that of the formulation containing MCC only at pH 6.8. The optimised bead formulation containing AVWL had the fastest ketoprofen release followed by bead formulations containing AVG and AMWL, which were relatively very close to the one for the formulation containing AMG.

Ketoprofen release from the optimised beads containing AVWL was the most affected by pH of the dissolution medium. In a dissolution medium with a low pH of 1.2, the ketoprofen release from the beads containing AVWL was pronouncedly lower than from the beads containing MCC only while an opposite effect was obtained in dissolution media with pH values of 4.5 and 6.8. This indicates that chemical components of AVWL material is most probably influenced by pH of the dissolution medium in such a way as to change the ketoprofen release when compared to beads containing MCC only.

9.2.6 Mean dissolution time

The mean dissolution time values for the optimised bead formulations are shown in Figure 28. At pH 4.5, the formulation containing AVG had the highest MDT value in comparison to the bead formulation containing MCC alone and the other optimised formulations. The bead formulation containing AVWL had the highest MDT value of 111.67 ± 1.70 min. As the pH increased to 6.8, the MDT values decreased as more of the drug was in its ionized form and therefore exhibited faster dissolution from the beads. The formulation containing MCC alone as an excipient had the highest MDT value (43.24 ± 4.06 min), followed by the formulation containing AMG (24.80 ± 1.36 min) then the formulations containing AVG and AMWL (18.98 ± 1.49 and 22.36 ± 3.31 min, respectively). The beads with the lowest MDT value were from the formulation containing AVWL (17.33 ± 1.61 min).

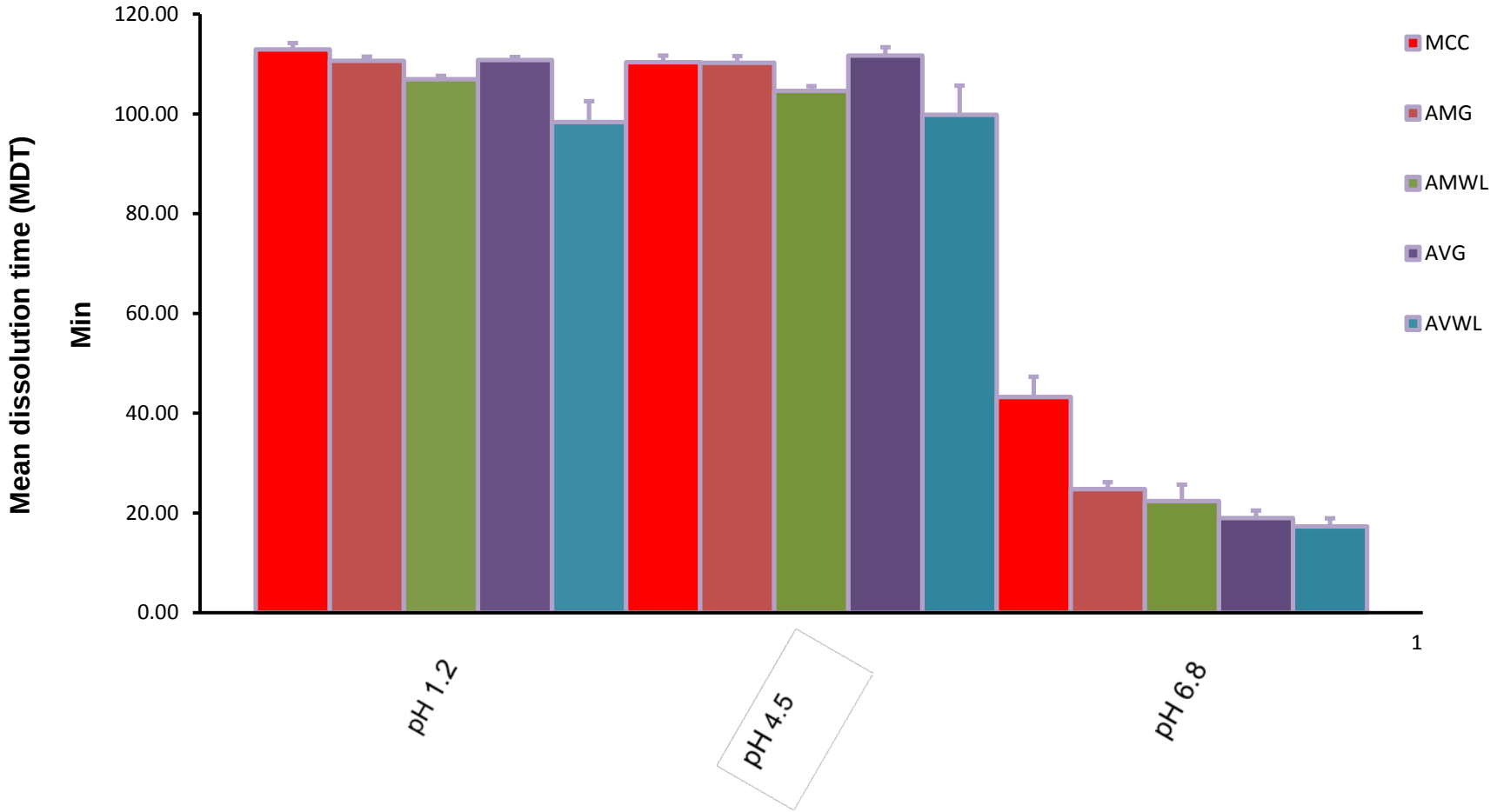


Figure 28: Mean dissolution time (MDT) values for the optimum formulations

9.2.7 Fit factors

The dissolution profiles of the optimised bead formulations were compared to the dissolution profile of the formulation containing MCC at the different pH values. The fit factor values for the optimised formulations are shown in Table 21.

The f_1 values between 1.00 (AMG) and 6.92 (AMWL) obtained at pH 1.2 indicate that the optimised profiles were similar to the dissolution profile of the formulation containing MCC only. This was confirmed by the f_2 values that are above 80% for all the formulations. At pH 4.5 the f_1 values between the optimised formulations and the reference formulation were lower than 15 with the highest f_1 value being 12.54 for the formulation containing AVWL. The f_2 values were above 50, which indicate that the optimised formulations had dissolution profiles that were relatively similar to that of the formulation containing MCC only. At pH 6.8, only the dissolution profile of the formulation containing AMG had a low f_1 value of 10.80 as it had a dissolution profile relatively similar to that of the formulation containing MCC alone (Figure 28). The rest of the aloe containing bead formulations had f_1 values above 15 and f_2 values below 50; hence, they were not similar to the dissolution profile of the formulation containing MCC alone as an excipient as is displayed in Figure 28.

Table 21: Fit factor values (f_1 and f_2) for dissolution profiles of the optimised formulations compared to the formulation containing microcrystalline cellulose only

Formulation	pH 1.2		pH 4.5		pH 6.8	
	f_1	f_2	f_1	f_2	f_1	f_2
AMG*	1.00	98.87	8.27	75.59	10.80	63.54
AMWL*	6.92	82.23	5.60	86.21	25.01	45.69
AVG*	1.62	97.14	2.23	95.68	24.18	45.59
AVWL*	6.71	80.45	12.54	71.10	39.43	35.34

*AMG = *Aloe marlothii* gel, AMWL = *Aloe marlothii* whole leaf, AVG = *Aloe vera* gel, AVWL = *Aloe vera* whole leaf

10 Conclusion

According to the ^{1}H-NMR spectra, the marker molecules for fresh *A. vera* materials were detected in AVG and AVWL used in this study, while AMG and AMWL did not contain aloverose as reported in previous studies. The composition of bead formulations for the different aloe materials was determined by a full factorial design and these were prepared by means of extrusion spheronisation. In general, the surface and internal micro-morphology of the bead formulations containing aloe material were more porous and less compact than that of the bead formulation containing MCC only.

The sphericity values for the different bead formulations were all close to the ideal value of 1. The beads exhibited good flow properties, shown by the uniform filling of hard gelatine as indicated by the compliance of all the formulations with the requirement that the average mass should not deviate by more than 7.5%.

Each of the bead formulations exhibited relatively low friability, which ranged between 0.00 and 1.49% with most beads meeting the < 1% friability limit. This makes the beads suitable for subsequent handling such as coating and filling into capsules. The friability may also influence the dissolution rate as the more brittle the beads are, the more easily they may disintegrate or erode in the dissolution medium yielding a faster dissolution rate.

The results showed that the inclusion of the selected aloe materials in the bead formulations increased the drug release rate. The effect of the aloe materials on the dissolution of ketoprofen was similar over the complete range of concentrations included in the beads. .

The optimised formulations exhibited acceptable sphericity, low friability, uniform mass variation and dissolution profiles resembling relatively faster release of the drug in comparison to beads containing MCC only. More than one factor seemed to influence the release of the drug from the bead formulations namely the ratio of MCC to aloe material in the bead formulations, the structure of the beads, friability and the pH of the dissolution media (based on data from optimised beads). Aloe materials could therefore be successfully included in bead formulations with MCC prepared by means of extrusion spheronisation, however, beads containing 100% of the aloe materials could not be produced by this technique.

Chapter 5

FINAL CONCLUSIONS AND FUTURE RECOMMENDATIONS

1 Final conclusions

Traditionally, excipients were included in drug formulations as inert vehicles that provided the necessary weight, consistency and volume for correct administration of the active ingredient, but in modern pharmaceutical technology, dosage forms are designed containing excipients that have multifunctional performing roles such as enhancing drug stability and bioavailability as well as controlling drug release kinetics according to the therapeutic needs. Excipients also function to enhance patient acceptability of the dosage form and performance of technological functions that ensure ease of manufacture (Beneke *et al*., 2009: 2603; Hamman & Tarirai, 2006:57; Hamman & Steenekamp, 2012). The main aim of this research project was to determine if *Aloe marlothii* and *Aloe vera* materials can be incorporated as excipients in the formulation of beads by extrusion spheronisation, which has been established. Unfortunately, beads could not be formed containing aloe material only as excipient. The concentration of aloe materials as excipients in beads prepared by extrusion spheronisation was restricted to 30% (w/w) for AVWL and to 10% (w/w) for AMG, AVG and AMWL.

The morphology of bead formulations containing aloe leaf materials indicated more porous internal structures than that of beads containing MCC only. An increase in the porosity of beads facilitates the entrance of water molecules into the beads thereby promoting faster dissolution of the drug. All the bead formulations had acceptable sphericity values that were close to the ideal value.

The sphericity of the beads resulted in the uniform filling of hard gelatine capsules as demonstrated by the positive results of mass variation tests. The low friability values of the beads displayed that the beads had acceptable mechanical strength and should be able to withstand handling as well as subsequent processing (e.g. coating). The HPLC method was valid for the analysis of ketoprofen in the samples. All the formulations containing aloe material had a faster drug release than that of the formulation containing MCC. This was contrary to the expected effect as aloe materials have been shown to slow down the release of drugs from mini-matrix type tablets (Jambwa e*t al*., 2011:439). However, the beads prepared in this study did not include a compression cycle as was the case with the mini-matrix type tablets. The aloe materials increased the drug release from the beads probably

by means of pore formation as indicated by the SEM micrographs as well as due to interaction with the dissolution medium and thereby may disentangle to increase the pore size even further.

Design of experiments (DoE) was used successfully to explore the effects of the aloe materials at different concentrations on the bead properties as well as to optimise the bead formulations. The optimised bead formulations had acceptable sphericity, friability, morphology and mass variations. The dissolution profiles of the optimised formulations exhibited immediate release of the drug. Therefore *Aloe marlothii* and *Aloe vera* leaf materials can be used successfully as excipients in the production of beads by extrusion spheronisation.

2 Future recommendations

It is recommended that further processing of the aloe leaf materials should be undertaken such as extraction of the polysaccharides. The separated polysaccharides can then be investigated as potential excipients in beads prepared by extrusion spheronisation with or without addition of MCC. Furthermore, other excipients than MCC and PVP could be incorporated into the beads with the aloe materials to optimise their properties. Excipients such as binders can be included to improve the friability of the beads and other polymers could be included to control the drug release rate.

The release of aloe leaf materials in addition to the release of the active pharmaceutical ingredient from the beads should be investigated. This is important for bead formulations where the aloe leaf materials are included as multifunctional excipients, e.g. to enhance drug absorption in addition to a faster release of the drug.

The drug delivery properties of beads containing aloe leaf materials should be investigated by means of *in vitro* transport techniques such as Caco-2 cell monolayers or excised animal tissue sheets. Once the bead formulations are optimised for enhanced drug delivery across the *in vitro* models, the performance of the beads should ultimately be investigated by means of *in vivo* bioavailability studies in animals and in humans.

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