# Design and Biological Evaluation of Acrylated Polyethylene Glycol Gel Containing Acarbose

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Thesis accepted in fulfilment of the requirements for the degree *Doctor of Philosophy in Biology* at the North-West University

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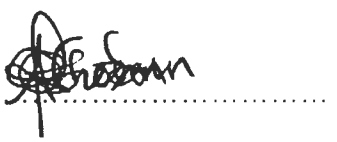
## DECLARATION

I, **Shesan John Owonubi** declare that this dissertation is my own work unless where acknowledged. It has not been submitted to any institution for the purpose of obtaining a qualification.



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## DEDICATION

This study is dedicated to my loving parents, Captain (rtd) and Mrs. J.J Owonubi, my siblings; Kemi, Seyi, Taiye and Kehinde, and finally my grandma Mrs. Felicia Durowaiye for their unconditional phenomenal love, prayers and support.

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# ABSTRACT

Diabetes is certain to be among the most challenging health problems in the 21st century. According to prevalence estimates of the International Diabetes Federation, 366 million people had diabetes in 2011; by 2030 it is estimated that this will have risen to 552 million. South Africa has about 2.3 million diagnosed people living with diabetes, Democratic Republic of Congo have about 2 million, Ethiopia having almost 1.5 million and Nigeria with barely over 1.5 million, as indicated by the IDF in 2015. The number of people with type 2 diabetes, which affects about 90% of all diabetic patients, is increasing in every country. The two most common forms of diabetes are: type 1 diabetes (diminished production of insulin) and type 2 diabetes (impaired response to insulin and β-cell dysfunction). Both lead to hyperglycemia, excessive urine production, compensatory thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in the energy metabolism.

Till date, researchers have struggled with a cure for this metabolic disorder and have only come up with means to manage the condition. Of the numerous means to manage type 2 diabetes, the use of an α-glucosidase inhibitor, acarbose is the focus of this study. Acarbose belongs to the group of noninsulinotropic oral antidiabetic agents. Simply put, it works by slowing the action of certain chemicals that break down food to release glucose (sugar) into your blood. Thus, it slows down food digestion and helps keep blood glucose from rising very high after meals. Because of its unique mode of action, acarbose not only plays an essential and direct role in carbohydrate uptake from food into the blood, but also has an indirect role in the optimization of glucose metabolism over the whole day.

The main objective of this study was to design Polyethylene Glycol (PEG) acrylated covalently bound acarbose drug delivery carrier. To achieve this we had two groups of PEG-drug attachments; PEG acrylated acarbose hydrogel (PEG-A), which was designed from acrylated acarbose (Ac-Ac) which we synthesized and then chemically attached it to polyethylene glycol (PEG) thiol arm and PEG acarbose hydrogel, which was synthesized by covalent crosslinking of pure acarbose dispersed within functionalized PEG thiol and acrylate derivates (PEGA).

For the confirmation of successful acrylation of acarbose, Nuclear magnetic resonance (NMR) was used and for the confirmation of successful integration of acarbose into the PEG polymer, also Fourier transform infrared spectroscopy (FTIR) employed to confirm successful integration of acarbose into the PEG carriers.

Swelling analysis were performed at varied pH and *in vitro* drug release behaviour and release kinetics suggested the pH sensitive capacity of the carrier system and it revealed that the PEG-A hydrogels exhibited a superior pH-induced drug release profile, which can trigger releasing the drug at physiological pH values at much better control levels than the PEGA hydrogels or free acarbose. Other characterization techniques were used to compare and explain the behaviour of the polymer-drug delivery carriers in comparison to free acarbose; Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform Infra-red Spectroscopy (FTIR), Xray Diffraction (XRD) Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC). The MTT toxicity study which was performed on the c2c12 cell line and comparison of the PEG carriers to free acarbose showed no significant toxicity to the cells line.

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| Ac | Acarbose |
| AFM | Atomic force microscopy |
| BMI | Body mass index |
| BSA | Bovine serum albumin |
| $D_20$ | Distilled water |
| DDS | Drug delivery system |
| DM | Diabetes Mellitus |
| DMEM | Dulbecco's modified eagle's medium |
| DMSO | Dimethyl sulfoxide |
| DPP-IV | dipeptidyl peptidase IV inhibitors |
| DSC | Differential scanning calorimetry |
| EDTA | Ethylenediaminetetraacetic acid |
| FCS | Foetal calf serum |
| FDA | United States Food and Drug Administration |

| | |
|---|---|
| FPG | fasting plasma glucose |
| FTIR | Fourier transform infrared |
| GI | Gastrointestinal |
| HbA1c | glycosylated hemoglobin |
| IDF | International diabetes federation |
| KDa | Kilo dalton |
| MTT | 3-(4, 5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide |
| NADH | nicotinamide adenine dinucleotide |
| NADP | nicotinamide adenine dinucleotide phosphate |
| NMR | Nuclear magnetic resonance |
| OGTT | oral glucose tolerance test |
| PBS | Phosphate buffered saline |
| PEG | Polyethylene Glycol |
| PEG-A /_A | Polyethylene Glycol acrylated to acarbose hydrogel |
| PEGA | Polyethylene Glycol with dispersed acarbose hydrogel |

| | |
|---|---|
| PEG-Ac | Polyethylene glycol acylate arm |
| PEG-SH | Polyethylene glycol thiol arm |
| PLA | Poly lactic acid |
| PVA | polyvinyl alcohol |
| RPM | Revolutions per minute |
| SDF | Simulated duodenum fluid |
| SEM | Scanning electron microscopy |
| SGF | Simulated gastric fluid |
| SIF | Simulated intestinal fluid |
| SPIF | Simulated proximal ileum fluid |
| SR | Swelling ratio |
| T1DM | Type I diabetes mellitus |
| T2DM | Type II diabetes mellitus |
| TEM | Transmission electron microscopy |
| TGA | Thermogravimetric analysis |

| | |
|---|---|
| UKPDS | United Kingdom Prospective Diabetes Study |
| UV | Ultraviolet |
| WHO | World health organization |
| XRD | X-ray diffraction |

## DEFINITION OF CONCEPTS

**Adipose:** This describes body tissue used for the storage of fat.

**Acrylation:** a chemical reaction involving the addition of an acrylate group.

**C2C12:** A mouse embryonic myoblast cell line.

**Diabetes mellitus:** A group of metabolic diseases with symptoms that result from a deficiency in insulin production or utilization, characterized by a failure in glucose transport from the blood into cells at normal glucose concentration.

**Hyperglycaemia:** An excess of glucose in the bloodstream, often associated with diabetes mellitus.

**Acarbose:** A drug belonging to the noninsulinotropic oral antidiabetic agents used to treat diabetes mellitus type 2.

**Myoblast:** Any of the cells derived from the mesoderm in the vertebrate embryo that develops into muscle tissue.

## PUBLICATIONS EMANATING FROM THIS RESEARCH

- **S.J. Owonubi, S.C Agwuncha, E. Mukwevho, B.A. Aderibigbe, E.R. Sadiku, O.F. Biotidara and K. Varaprasad**, Application of Hydrogel Biocomposites for Multiple Drug Delivery. Vijay Kumar Thakur, Manju Kumari Thakur and Michael R. Kessler (eds.), Handbook of Composites from Renewable Materials, Volume 6, (139–166) © 2017, Wiley-Scrivener Publishing LLC.

# CHAPTER 1

# INTRODUCTION AND STUDY BACKGROUND

## 1.1 BACKGROUND

Novel systems for drug delivery is the future of targeted drug delivery control and overtime the potentials of such systems, being essential steps towards a controlled drug targeted administration in the body, have led to the discovery of polymer hydrogels. The ability to deliver pharmaceutical agents into the body at a controlled rate, with minimal toxicity and also avoiding immunogenic response is the highpoint of any drug delivery system, but having the ability to be completely eradicated by the hepatic system makes polyethylene glycol (PEG) hydrogel macromolecules ideal as a potential delivery route of therapeutics. PEG has the potential to improve the delivery of anti-diabetic drug-acarbose for the management of diabetes, with its capacity to enhance the absorption of acarbose and rid the challenge of medications needed to be remembered to be administered.

This research focuses on the utilization of PEG hydrogels for the delivery of acarbose in order to improve its absorption and efficacy, by controlling the discharge of acarbose from the polymer network. Subsequent toxicological tests performed *in vitro* on mature c2c12 cells were to confirm its non-toxic nature for future application to diabetic rats.

Advances in drug delivery have overtime become the vital goal of scientists involved in pharmacology in order to improve the actual dosage release-to-time ratio of drugs in patients. This has led to the development and utilization of highly improved biomaterials

that have superior properties: biocompatibility, biodegradability, possible better tensile strength and responsiveness to external stimuli (pH, temperature, etc.)

## 1.2 PROBLEM STATEMENT

Diabetes mellitus is a metabolic syndrome and today it affects about 415 million people worldwide (International Diabetes Federation, 2016). Although it is known to be as a result of complications associated with plasma blood glucose levels, till date there is no cure for this disease. Several pharmaceutical agents are available to manage the disease, but with notable levels of side effects. There exists two main varieties of the metabolic disease, viz; type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). The focus of this research is on T2DM.

Obesity and T2DM are strongly linked with physical inactivity (Adams and Murphy, 2000). Hence, physical activity has been advocated as a means to alleviate the disease. Weight loss through exercise is found to ameliorate insulin resistance which is characteristic of obesity and the metabolic syndrome T2DM (Tock *et al.*, 2006). However, the amount of physical activity required for substantial weight loss is well beyond what is feasible in recent days (Popkin *et al.*, 2012).

Contemporary pharmacological agents such as metformin, acarbose and other drugs are employed as front line pharmaceutical agents to treat T2DM (Chiasson *et al.*, 1994). However, they are associated with detrimental side effects. In addition, the issue of patient non-compliance due to inconvenient drug taking has added to the advancement of this disease condition (Olokoba *et al.*, 2012). It has therefore become imperative to attempt to improve the delivery of the acarbose, in order to rid patient of having to be

bothered about taking multiple doses of drugs and potential reduction of the existing side effects. Also, the mechanism of action of acarbose overtime has shown that it demonstrates low rates of absorption, since it is widely indicated that on treatment with acarbose, only about 2 % of the drug is absorbed in its original form (Hanefeld, 2007). The drug is known to be potentially affected by some metabolic enzymes which are undesirable (Honda *et al.*, 2013); hence the improvement to bioavailability of acarbose is a necessity.

## 1.3 AIM

This research aims are to design an effective polymer-drug delivery mechanism for the successful delivery of acarbose, hoping to provide a solution to the issue of patience compliance to taking drugs periodically and to achieve an improvement in the challenge of low absorption of the drug.

## 1.4 OBJECTIVES

1. Design a PEG acrylated covalently bound acarbose drug carriers.

2 Perform the characterization of the PEG gel in order to confirm dispersion and integration of acarbose in the polymer network by using techniques such as: Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform Infra-red Spectroscopy (FTIR), Xray Diffraction (XRD) Thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC) and Nuclear Magnetic Resonance (NMR).

3. pH swelling analysis of the PEG acarbose bound gel.

4. To perform *in vitro* drug release studies in various buffers depicting the drug pathway in the biological system for the establishment of suggestive *in vivo* release kinetics.

5. *In vitro* toxicological evaluation of plain PEG gel in order to confirm the non -toxic nature of polymer on c2c12 cells.

## 1.5 SIGNIFICANCE OF THE STUDY

This study is intended to design PEG acrylated covalent bound acarbose gel which would show the capacity to assist in improving the potential bioavailability of hypaeglycaemic drug –acarbose. By performing various characterization techniques, we confirm the level of dispersion and integration of the drug in the polymer network and show the ability of the gel to biomimic the body tissues.

It is hypothesized that this derivatized PEG-acarbose form increases bioavailability of the drug and that findings achieved *in vitro* can be related directly to potential *in vivo* models.

# CHAPTER 2

# REVIEW OF LITERATURE

*** Part of this literature review has been published: S.J. Owonubi*, S.C Agwuncha, E. Mukwevho, B.A. Aderibigbe, E.R. Sadiku, O.F. Biotidara and K. Varaprasad, Application of Hydrogel Biocomposites for Multiple Drug Delivery. Vijay Kumar Thakur, Manju Kumari Thakur and Michael R. Kessler (eds.), Handbook of Composites from Renewable Materials, Volume 6, (139–166) © 2017, Wiley-Scrivener Publishing LLC.**



## 2.1 INTRODUCTION

This chapter brings to light the metabolic syndrome – diabetes, statistical information about diabetes worldwide, types of diabetes and means to manage the disease as no cure exists till date, drug delivery devices, challenges and benefits of varied drug delivery systems, polyethylene glycol (PEG) a polymer as a plausible drug delivery device, characterization techniques used for such polymer drug delivery devices and the use of the 3-(4, 5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide (MTT) assay for toxicity tests on polymer drug delivery devices is also highlighted.

## 2.2 DIABETES MELLITUS

Healthy individuals have normal concentration of glucose in plasma within a range of between (4.0 - 6.0 mmol/l) irrespective of the nutritional intake, exercise and other (physical or psychological) influences, and this can vary slightly between laboratories (Engelgau *et al.*, 2000). Insulin, a blood-glucose lowering hormone is responsible for the

stability of plasma glucose levels in any individual (Sprague and Arbeláez, 2011). The secretion of insulin occurs as short-lived bursts when there is a response to stimuli and continuous basal rates in normal situations. These basal normal secretions usually come about during the fasting state wherein it is required to prevent hepatic glycogenolysis, ketogenesis and gluconeogenesis which account for ~ 40 % of the daily total production of insulin. Increase in insulin secretion occurs in cases when the plasma glucose amount surpasses the range of between 4.4 - 5.6 mmol / l. This can only be restored by causing reactive glucose absorption and possible storage, a state of euglycaemia. By the stimulation of the peripheral glucose and storage by insulin, the glucose level only spikes up in a time of between 30-60 minutes after eating and returns to basal concentrations somewhere between 2-3 hours. This basal insulin secretion in addition to the reaction to various states within the body are key factors in ensuring the balance of glucose in healthy individuals, thus permitting stability and reproducibility of blood glucose (Service and Nelson, 1980; Guerci and Sauvanet, 2005).

Diabetes mellitus is a disease which is also denotes a collection of metabolic conditions that share the phenotypic condition of hyperglycaemia, a condition depicted by plasma glucose levels which unusually, are high levels. The disease condition occurs as a result of failings in the secretion of insulin, the action of insulin and in some cases both states fail. Distinct types of the condition - diabetes mellitus, caused by multifaceted interactions between factors of genetic nature and also environmental nature, but individuals majorly exhibit diabetes mellitus of distinct types: T1DM and T2DM.

These two distinct disorders account for between 5 % – 10 % and between 90 % - 95% of all identified diagnosis respectively (American Diabetes Association, 2015). T2DM is

characterized by a state of high plasma glucose, sometimes also with insulin resistance occurring, caused as a result of damage to the secretion of insulin (American Diabetes Association, 2009). Minor cases of T2DM result from issues with the secretion of insulin alone without insulin resistance, but in the case of T1DM, there is an absolute impairment of insulin secretion occurs.

The focus of this thesis is on T2DM and the management of the disease condition through the improvement of the delivery techniques of medication that are readily available. The dominance of T2DM, a lingering glucose metabolic disease is on the rise (Stumvoll *et al.*, 2008; Whiting *et al.*, 2011; Forouhi and Wareham, 2010). Two percent of the deaths worldwide were associated with diabetes according to the findings by The World Health Organization (WHO) in 2005. Predictions indicate that the prevalence of the disease is going to grow exponentially from 285 million in 2010 to 592 million in 2035 with Southern Africa having the largest increase, with South Africa being the forerunner (Guariguata *et al.*, 2014; Shaw *et al.*, 2010). An estimated 14.2 million adults aged between 20-79 years of age, have diabetes in sub-Saharan Africa as shown in Figure 1, demonstrating a prevalence of between 2.1 % - 6.7% in the region. The awareness within the region is very low; since the region still has the highest proportion of undiagnosed diabetes; with over two thirds (66.7%) undiagnosed cases. The majority (58.8%) of people with diabetes live in cities, even though the population in the sub-Saharan Africa region is predominantly (61.3%) rural as indicated by the International Diabetes Federation in 2015.

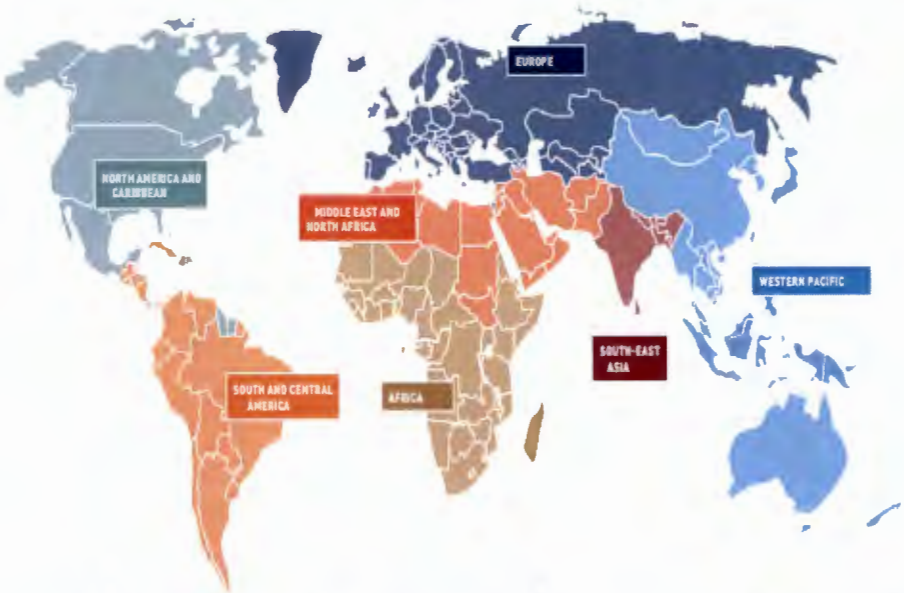
Some of Africa's most populous countries account for the most numbers of people with this disease; South Africa has about 2.3 million, Democratic Republic of Congo with

about 2 million, Ethiopia having almost 1.5 million and Nigeria with barely over Ethiopia's figure indicated by the IDF in 2015, with about half of all the adults in these four countries accounting for the statistical population of those with the disease in the region.

This evident escalation in the rate of people with diabetes has been caused majorly by the economic development and the way of life modifications which has resulted in the upsurge in obesity (Peer *et al.*, 2014; Kengne *et al.*, 2013). In South Africa the frequency of the disease in adults has almost doubled between the period 2000 and 2009 (Bradshaw *et al.*, 2007; Bertram *et al.*, 2013).

A research conducted by researchers at the Medical Research Council in 2015 indicated that South Africa is fast becoming a fat nation (Manyema *et al.*, 2015) and failure to act on the ever increasing rate of consumption of fizzy drinks, which might possibly result to a further increase to 1.2 million obese South Africans by the year 2017. Another research article titled "Cost of Inaction on Sugar-Sweetened Beverage Consumption: Implications for Obesity in South Africa", made calculations showing that if the intake of these fizzy drinks is increased by just over 2 %, the equivalent proportion that the trade of these fizzy beverages will bring could lead to a drastic increase in obesity and diabetes (Tugendhaft *et al.*, 2015). The most southern nation in Africa is already statistically the one with premier levels of the resultant condition of citizens with excessive weights in sub-Saharan Africa, according to their findings. The levels have been shown to spike to about 11 % in males and about 39 % in females between the period, 2003 and 2012 (Tugendhaft *et al.*, 2015). According to their findings, R7-billion a year is an initial approximation of the resultant of a 20 % tax levied on fizzy beverages

which the present South African government has put forward and this could be accepted into law if the state settles for such taxation policy. In addition to this extra revenue to the government is the potential positive reduction in the upsurge of citizens with obesity and associated medical disorders, which are exceedingly expensive to manage.




| | 2015 | | 2040 | | |
|---|---|---|---|---|---|
| IDF REGION | DIABETIC POPULATION | Total Population | DIABETIC POPULATION | Total Population | % Increase |
| Africa | 14.2 million | 441 million | 34.2 million | 926 million | 140.9 |
| Europe | 59.6 million | 660 million | 71.1 million | 663 million | 18.9 |
| Middle East and North Africa | 35.4 million | 387 million | 72.1 million | 635 million | 103.7 |
| North America and Carribean | 44.3 million | 344 million | 60.5 million | 413 million | 36.6 |
| South and Central America | 29.6 million | 315 million | 44.8 million | 411 million | 51.4 |
| South East Asia | 78 million | 926 million | 140 million | 1.31 billion | 79.5 |
| Western Pacific | 153 million | 1.6 billion | 215 million | 1.8 billion | 40.5 |

*Figure 2.1: 2015 statistics showing estimated age- adjusted prevalence of diabetes in adults (20 - 79) in 2015 and projected 2040 estimates (adapted from IDF atlas 2015).*

## 2.3 MANAGEMENT OF TYPE II DIABETES.

Currently, to manage T2DM, several classes of oral pharmacological agents are used (Figure 2). They include: metformin (a biguanide) (Bailey and Day, 1989; Cusi and DeFronzo, 1998; Zhou *et al.*, 2001), sulfonylureas (Lebovitz and Pasmantier, 1990; Matschinsky, 1996), dipeptidyl peptidase IV inhibitors (DPP-IV) (Green *et al.*, 2006; Green, 2012; Crepaldi *et al.*, 2007; Dicker, 2011), meglitinides (Weyer *et al.*, 1999; Dunning *et al.*, 2005), incretin based therapies (Nauck *et al.*, 2009; Cernea and Raz, 2011; Drucker *et al.*, 2010) , thiazolidinediones (Mudaliar and Henry, 2001; Sohda *et al.*, 1995; Miyazaki *et al.*, 2002; Kawamori *et al.*, 1998; Sohda *et al.*, 1982; Goldstein, 1999; Greene, 1999; Miyazaki *et al.*, 2001), and α-glucosidase inhibitors (Lebovitz, 1998; Puls, 1996). These medications can be employed individually, or in combination with one another, taking advantage of the fact that each class has its own generic mechanism of action.

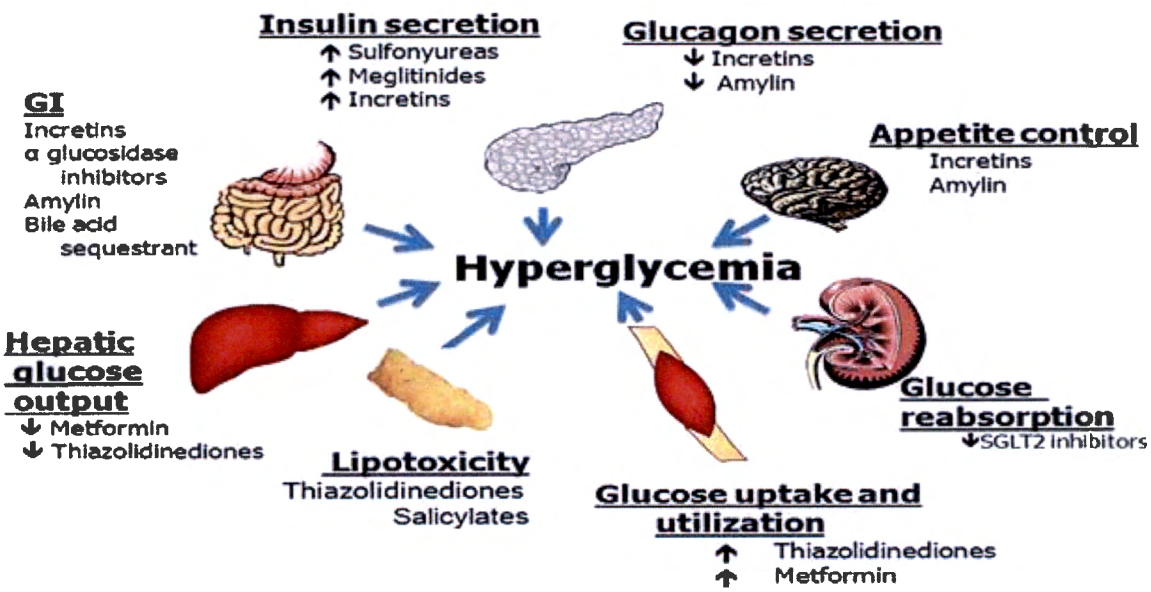


*Figure 2.2: Management of T2DM state of hyperglycemia by several classes of oral pharmacological agents (adapted from (Evans et al., 2013)*

Briefly, the mechanism of action of meglitinides and sulfonylureas encompasses the enablement of secretion of the hormone - insulin in different ways; thiazolidinediones on the other hand focuses on the bordering tissue insulin resistance, while metformin suppresses gluconeogenesis and α-glucosidase inhibitors act by inhibiting the breakdown of complex carbohydrates in the gut.

Researchers have shown that individuals with diabetes that is poorly-controlled tend to witness pronounced microvascular and increased macrovascular crisis (United Kingdom Prospective Diabetes Study (UKPDS) Group, 1998; Bonora *et al.*, 2002). However, the focus of this thesis is, therefore on α-glucosidase inhibitors – acarbose, an oral pharmaceutical compound.

## 2.4 α-GLUCOSIDASE INHIBITORS

This group of oral pharmaceutical compounds for the management of T2DM, is a class that is unique in their effectiveness to block the ability of enzymes to breakdown the complex carbohydrates molecules, in the small intestine, from food consumed (Lebovitz, 1998; Magner and Amatruda, 2000). Acarbose and miglitol are popular drugs associated with this group of oral anti-hyperglycemic compounds and they are inclined towards causing a reduction in the rise of glucose levels after eating and improvement of the glucose balance without aggregate threat for increase in weight or eventual development to hypoglycemia. By the late1990s, the United States Food and Drug

Administration (FDA) endorsed the use of these two drugs. Both result in a substantial reduction in post-prandial glucose, glycosylated hemoglobin (HbA1c) and fasting plasma glucose (DeFronzo, 1999; Lebovitz, 1998; Magner and Amatruda, 2000). In addition, acarbose has been approved for use as a form of combined therapy with metformin, insulin or sulfonylurea. Miglitol, on the other hand, is only permitted to be used together with a sulfonylurea. There is an additive effect of these compounds on the glycemic control when they are used together and this can be presumed to be as a result of the differences existing in their mechanism of action. α-glucosidase inhibitors such as acarbose are notably useful to people that have minor hyperglycemia or individuals that are disposed to hypoglycemia.

- **MECHANISM OF ACTION**

These glucose inhibitors have quite competitive characteristics; acting as inhibitors of pancreatic α-amylase and hydrolase enzymes which are bound to the intestinal membrane. The earliest α-glucosidase inhibitor to be discovered was acarbose. It is a pseudotetrasaccharide comprising of nitrogen, whereas miglitol is a chemical derivative of 1-deoxynojirimycin (DJN), commonly found in mulberry leaves. The mechanism of action of both acarbose and miglitol are comparable although they have quite some variations. The inhibitors tend to bind vigorously to the sites of oligosaccharide of the α-glucosidase enzymes and this results invariably to the prevention of hydrolysis by enzymatic action. The affinity of acarbose to bind to α-glucosidase enzymes is that glycoamylase binds better than sucrose and sucrose binds better than maltase, which binds better than dextranase (Lebovitz, 1998; Puls, 1996). Unlike miglitol, acarbose has very little attraction for isomaltase, but none towards lactase, a β-glucosidase enzyme

(Lebovitz, 1998). For sucrose and maltase, miglitol is a rather preferred inhibitor pharmaceutic than acarbose, but having no effect on α-amylase, however very effective to inhibit intestinal isomaltose (Lebovitz, 1998).

- **EFFECTIVENESS**

α-glucosidase inhibitors have been used as oral medication and overtime several clinical trials have been conducted establishing the fact that antihyperglycemic effectiveness results to about 50% less than that of either sulfonylureas or metformin. Acarbose is commonly used as a sole drug, predominantly affecting the expected spike in glucose levels (post-prandial), which as a result lowers the glucose levels spike after meal (DeFronzo, 1999; Scheen, 1998; Lebovitz, 1998; Magner & Amatruda, 2000; Martin & Montgomery; Coniff, 1997). Many researchers have confirmed that these inhibitors do not have substantial effects on insulin sensitivity in patients that have T2DM. Although substantive findings have shown that acarbose and voglibose, an analog of miglitol, are used in experimental trials in Japan and vogibose was observed to have caused post-prandial hyperinsulinemia in people having fasting blood glucose levels higher than 6 mmol / l (Chiasson et al, 1996; Shinozaki et al, 1996). α-glucosidase inhibitors are readily utilized effectively for combined therapy, since their mechanism of action differs from other oral agents, having a rather more additive effect in combination with others that have dissimilar means of action. HbA1c emanates when a protein, haemoglobin found in the red blood cells and carries oxygen through the human body, links with glucose in the blood, it makes the haemoglobin become glycated. It was observed by researchers that by using combined therapy of acarbose and sulfonylurea, the HbA1c decreases by 0.85%; whereas with combination of

acarbose and metformin, a form of combined therapy, the glycated haemoglobin decreases by 0.73%; also, when it is used together with insulin for therapy it decreases HbA1c by 0.54% (Lebovitz, 1998). The use of α-glucosidase inhibitors to manage T2DM seems to result in a lesser degree of secondary disorders associated with the drug when compared to sulfonylurea and metformin therapy and it offers the best therapy with regards improvement on post-prandial hyperglycemia.

- **SIDE EFFECTS**

Gastrointestinal disturbances are the foremost side effects of the α-glucosidase, occurring in 25 – 30 % of diabetic patients with discomforts relating to flatulence, diarrhea, bloating, and abdominal pains (Nakhaee and Sanjari, 2013; Playford *et al.*, 2013; Sweetser, 2012; Hollander, 2012; Evenepoel *et al.*, 2006). By regulating the dosage, these side effects can be minimized and some other times they tend to lessen over time. Acarbose is not advised for individuals with bowel diseases, high plasma creatinine of above 177 µmol / l or improper liver function. The use of acarbose as monotherapy rarely leads to hypoglycemia, but this rare condition can occur while the patient is on medication, which is combined to sulfonylurea, a meglitinide or insulin. The generally endorsed response to such a case of hypoglycemia by the patient is the administration of pure glucose, dextrose or milk orally.

As a result of the low absorption of acarbose by the body (Hanefeld, 2007; Bischoff, 1995), there is a need to deliver acarbose via an alternate delivery technique in order to compare the level of absorption and invariably improving the efficiency of acarbose.

## 2.5 DIAGNOSIS OF DIABETES MELLITUS

Overtime, hyperglycaemia has been accepted as an indication of DM (World Health Organization, 1999), with patients having blood glucose levels of up to 200mg/dL or higher. Thus measurements of fasting plasma glucose (FPG), which reveal a possible state of hyperglycaemia have been used to diagnose diabetes (McCance *et al.*, 1997).

An oral glucose tolerance test (OGTT) measures the blood glucose after a person fasts for at least 8 hours and 2 hours after the person drinks a glucose-containing beverage. This test can also be used to diagnose diabetes. The FPG test is most reliable when performed in the mornings, and it is the preferred test for diagnosing diabetes because of its convenience and low cost. However, it may sometimes not detect the conditions that are detectable by the OGTT. Researchers have overtime, shown that the OGTT is more sensitive than the FPG test for diagnosing pre-diabetes, but it is acceptably less convenient to administer. A random plasma glucose test, also called a casual plasma glucose test, measures blood glucose without regard to when the person being tested last ate. This test, along with an assessment of symptoms, is used to diagnose diabetes but not pre-diabetes. Test results indicating that a person has diabetes should be confirmed with a second test on a different day (Merz *et al.*, 2002).

HbA1c is the percentage of adult haemoglobin (Hb) that is glycated (Nathan *et al.*, 2007). The glycation process involves the binding of carbohydrates non-enzymatically to proteins such as Hb (Kilpatrick, 2000). HbA1 refers to charge separated haemoglobins of normal adult HbA0 (Kilpatrick 2000). Glycated Hb is a generic term for irreversibly Hb-bounded, in ketoamine form, to glucose (Brown and Bowes, 1985). This

includes HbA1, HbA1c and total glycated haemoglobin. HbA1, in addition may be further sub-classified as HbA1a1, HbA1a2, HbA1b and HbA1c (Kilpatrick, 2000).Total glycated Hb also includes the glycated Hb variants. HbA1c is the major sub-fraction of the glycated normal Hb (Brown and Bowes, 1985). Carbohydrate molecules, of which glucose is the major fraction, bind to Hb to form HbA1c. HbA1c was first identified as a minor fraction of normal adult Hb by ion exchange chromatography nearly 4 decades ago (Kilpatrick, 2000).

Some experts believed that HbA1c can be used for the diagnosis of diabetes, however there were concerns due to the limitations of the test (Mayfield, 1998): HbA1c is a dynamic test that is affected by: age, race, pregnancy, different laboratory standards and co-morbid diseases such as haemoglobinopathies and renal failure (Nielsen *et al.*, 2004).



HbA1c is a well-established monitoring tool for diabetes mellitus (DM) (Mayfield, 1998). HbA1c has been found useful in the identification of patients at risk of retinopathy and nephropathy (McCance *et al.*, 1994). In 2009, the American Diabetes Association started using HbA1c as a diagnostic tool for DM. Recently, the International Expert Committee extensively reviewed all the epidemiological evidence associated with HbA1c and found that it provides a reliable measurement of chronic glycaemia and suggested it may be a better means of diagnosing DM (McCance *et al.*, 1994).

HbA1c has its strengths. As mentioned, unlike the OGTT and FPG, no prior preparation or fasting is required (American Diabetes Association 2010). While collecting a blood sample for HbA1c, other biochemistry analysis can be concurrently performed. HbA1c is

collected and stored in tubes with ethylenediaminetetraacetic acid (EDTA), which acts as an anticoagulant. In summary, the HbA1c test is a clinically convenient test with less pre-analytical instability.

## 2.6 DRUG DELIVERY SYSTEMS.

Drug delivery systems (DDS) are defined as drug dosage formulations or devices that enable the introduction of a specific substance(s) into the body with improvement to its efficacy and safety, by having influence over the degree, frequency and final release destination of the substance. They involve the management of this substance(s) in question, the discharge of the components that are biologically active from the substance as well as the consequent movement of the active components across the internal body system to the target destination, with the least toxicity to the patient are the constituents of the entirety of the process (Kewal, 2008).

DDS have generally been known by researchers as the connecting mechanisms linking the drug formulation to the patient. Over decades, DDS have been the focus of quite a number of researchers to improve the remedial effect of drugs (Fundueanu *et al.*, 2008; Huynh *et al.*, 2008; Wang *et al.*, 2008b; Nakayama *et al.*, 2006). Today, drugs can be administered by various anatomical means; the choice of administration is dependent on the disease, the drug formulation and the pharmacokinetics of the drugs (Figure 3).

Given that drugs can be directed to the diseased tissue or alternatively directed via other techniques to target the diseased locale (Kewal, 2008), several routes for drug delivery exist and have been researched. However the oral mode till date is the desired approach for the conventional drug deliveries. This preference is due to: the ease

delivery, extensive approval by patients, and superior flexibility in the design of the dosage forms and also due to belief from patients that oral ingestion along with meals will be easier to get into the body (Pandey *et al.*, 2003).

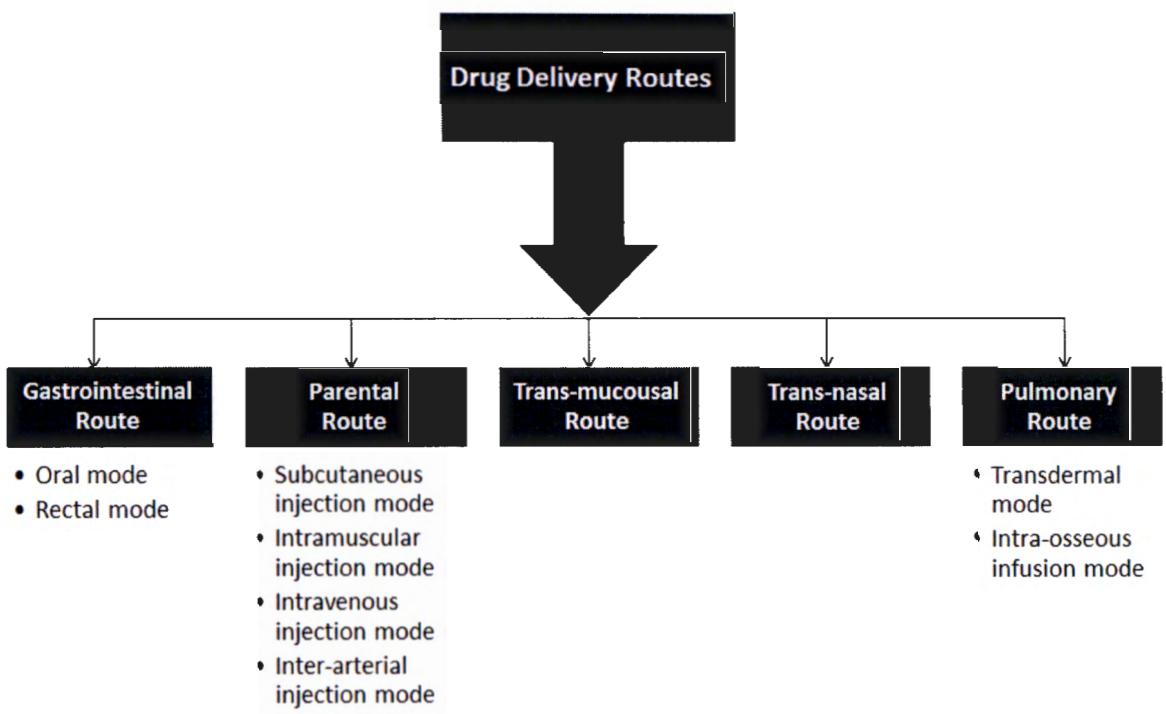



*Figure 2.3: An illustration of drug delivery routes.*

An oral drug delivery device design needs to take into consideration a number of properties. This is due to the fact that the dosage form interacts with various properties relating to the interior body system and if not formulated properly, the delivery system or device will be perceived as a foreign body by the internal physiology of the body and it is likely to be rejected, thus defeating the purpose of the drug efficiency as a delivery formulation. Therefore, the gastrointestinal physiology, pharmacokinetics, pharmacodynamics and the drug delivery device design are very crucial for an efficient

and effective drug delivery formulation (Ho-Wah, 1987; Chien *et al.*, 1982; Tingstad, 1976).

The more sophisticated a drug delivery formulation is, the better the required understanding of the body physiological make-up, which is essential to avoid failure in the development of an efficient delivery mechanism. Hence, the main aspects to consider for a successful drug delivery formulation consist of:

- The physiochemical, kinetics of the drug and the physiological effects of the drug on the body.
- The knowledge of the anatomy and the physiological make-up of the gastrointestinal tract.
- The physiochemical behavior of the drug delivery formulation.

Although widely spread and used, major limitations still exist in utilizing oral route of drug administration. These limitations are the driving forces behind researchers for improved oral means to administer drugs. Overtime, the limitations faced by researchers relating to oral drug delivery systems include, but by no means limited to:

i. Drugs taken orally possess inconsistent rates of absorption, hence there is the difficulty in ascertaining exactly how the drugs will be absorbed in the body. As a result of this variation in absorption rates, sustained and controlled-release systems have been developed.

ii. With degradable formulated products, in some cases they tend to be degraded before their intended site of absorption as a result of the acidic nature of the intestinal body system and the interaction with the digestive

enzymes. This is a crucial challenge for proteins constituents that are ingested; however, stimuli responsive drugs have been developed to overcome this challenge.

iii. Numerous macromolecules and polar complexes cannot successfully pass through the membranous cells of intestinal en route the blood stream, thus limiting their effects to the gastrointestinal tract.

iv. Several drugs tend to possess a limiting capacity of becoming insoluble at acidic pH levels faced in the digestive tract. This modification to being insoluble considerably decreases the bioavailability of the drug.

v. Some drugs can be neutralized within the liver en route the systemic circulation. For example, glyceryl trinitrates are incapacitated by certain hepatic enzymes during a phase of metabolism.

vi. Certain drugs irritate the gastrointestinal tract, an evidence of being rejected by the body physiology, but this irritation can be partially neutralized by coating the drug.

vii. The oral mode to drug delivery might be unsuitable for certain targeted drugs directed to certain organs, except when the path of the drug delivery is pre-studied and then the drug is specifically designed to overcome the physiological factors it will encounter before it gets to the target organ.

Despite the various limitations, the oral mode to deliver drugs is still the desired route because of constant evolution and research results that have been achieved using this

route. Some advancements in the formulation of drugs for improved effects have been researched upon, these involve alterations to the widely used methods and development of new variances to the oral route of DDS, with such modifications aiming to proffer solutions to the limitations previously highlighted.

Several variances to the oral route to drug delivery over time have been fashioned out to provide improved solutions to most of the limitations encountered to improve the overall qualities of the oral route of drug delivery that are widely used.

## 2.7 SUSTAINED DRUG RELEASE SYSTEMS

This is a DDS that is aimed at achieving the gradual discharge of the active ingredient from the drug formulation over an undefined time period, but being able to sufficiently provide the required therapy over time. Sustained drug release is that which is known to be as long acting, occasionally when being compared to conventional drug release systems. This description at times is also mistaken with controlled drug release which in its case defines a refined control mechanism of the discharge and not restricted to the discharge over a specific time. Sustained delivery of drugs may not be defined by the steady rate of delivery, but rather it being confined to a time span (Kewal, 2008; Pandey *et al.*, 2003).

Sustained drug release systems provide immediate doses for normal therapeutic responses as there is a gradual discharge of the drug in quantities adequate to ensure the required therapeutic responses, over a prolonged time.

The motives to design systems with sustained release profiles are:

- To prolong the period of drug mechanism in the body;
- To decrease the frequency to take drug doses;
- To reduce gastrointestinal irritation and irregular volumes of plasma levels;
- Development of enhanced drug use;
- Provide uniformity of the drug released; and
- Provide less adverse effects, i.e. toxicity as a result of too much drug within the body at a particular period.

## 2.8 CONTROLLED RELEASE SYSTEMS

Controlled release systems are also called sustained release system and they describe the management of the delivery of a drug by a DDS. Controlled DDS aim to transport the specific drug at a known frequency for a fixed duration, being dependent on the physiology of the body system. The release system does not only release the drug at a defined rate, but it is dependent on the design of the systems. For instance, drug 'X' could be released only when desired, whereas drug 'Y' programmed to circumvent the abdominal sectors, could yet be released at the small intestine by specific triggers. By such specific trigger release technique, large molecules can be genetically engineered and delivered at suitable amounts.

The drug distribution usually takes much longer with control release systems than in the case of sustained release which varies from days to years. Most times, this is used in place of sustained drug release systems, but restricted to being dependent on one or more factors in the gastrointestinal tracts which include, pH, enzyme concentration or specific enzymes and gastric motility among others (Gudsoorkar and Rambhau, 1993; Connal *et al.*, 2008).

Controlled release system has the mechanism of prolonged action within the drug formulation and is totally dependent on the method of release into the body. Controlled release can have methods incorporated that encourage easy release of drug at the required site, and are referred to as "site-specific and targeted" delivery systems. Some other factors influence the design of any DDS (Popli and Sharma, 1989; Joseph and Gwen, 2002; Jantzen and Robinson, 1996), in addition to the interactions within the gastrointestinal tract.

- **Half-life of the drug formulation.**

The half-life for any material is the time taken for half the amount of that material to be degraded. Drug formulations with half-life that are short are perfect for drug release systems that are sustained, because they enable the reduction in dosing frequency, thus leading to lower toxicity. This is not true for drug molecules with very long half-life as excessive amounts of the drugs will be required per dosage to manage the therapeutic levels, and this may result sometimes in ill effects to the concentration of the plasma levels, invariably causing toxicity to the patient.

- **Absorption**

The absorption of the drug formulation by the patient's body is the ultimate objective of any delivery system, hence its absorption degree into the body is a determining factor of the concentration in the blood stream. Absorption frequency constant is an apparent rate constant which depicts the release proportion of the dosage formulation. It is a well-established fact that the demonstration of low absorption rate constant for drug formulations are indications of them being poor candidates for sustained drug release systems.

- **Metabolism**

Drugs that are orally administered and considerably metabolized prior to absorption into the tissues of the body, release the drug slowly. However, this usually results in decreased bioavailability of the drug due to enzymatic degradation, thereby causing low active drug ingredients that are then released. Considerable care has to be taken to formulate drug dosage forms that are not affected by such enzymatic actions. Delivery systems need to be capable of withstanding levels of degradation by the enzymes, in order to have enough bioactive drugs in the formulation for efficient and sustained release.

- **Dosage size**

According to Popli and Sharma (1989) and Jantzen and Robinson (1996), a single dose of between 500-1000 mg drug formulations is considered the ideal range dosage form, even for sustained drug delivery systems. In addition, there is some form of preference to administering drug formulations made of large molecules with narrow therapeutic ranges.

- **pH stability and aqueous stability of the drug formulation**

The ionization potential (pKa) of a drug formulation influences the efficiency of the drug. Most drugs are known to be either weakly acidic or weakly basic. Overtime, it has become essential to observe the route which drug formulations take in the body, in order to predict the internal physiology it will encounter and know what properties would be beneficial to the drug for ease of absorption in its unaffected form.

The drug usually permeates across the lipid membranes, by diffusion or dissolution and will require solubility in aqueous medium in order to be able to achieve absorption. The lower boundary designed for absorption of a drug for formulation for a release system has been reported by researchers to be 0.1mg/mL.

- **Barrier co-efficient**

Drug formulations having relatively high barrier co-efficient are soluble in lipids and are not advisable. They possess quite low aqueous solubility and the compounds typically end up persisting in the organs for a lengthy period. This is a not beneficial because they can concentrate in the membranes of body cells, leading to toxicity in the cells.

- **Stability**

Formulation of bioactive compounds taken orally may be exposed to acidic or basic hydrolysis in some cases, in some others enzymatic breakdown. Some drugs are altered in the stomach by enzymes, thus these drugs require a means to remain intact for the duration in the gastrointestinal locale (GI) tract and thus such systems suitable for prolonged release mechanisms are designed. Drug formulation complexes that are unstable in the intestine can sometimes exhibit diminished bioavailability when distributed from a sustained prescribed form.

The distribution of drugs orally to date still remains the utmost desired means for drug administration as a result of the flexibility in drug design and broad range of patient acceptability. However, with regards to drug formulation and the delivery, there are various pH that the drug will interact with within the pathway through the body; mouth-saliva juices, stomach, gastro intestine, enzymes; therefore their influence on the drug

formulation should be carefully noted. Most orally sustained release systems rely on drug formulations being dissolved, diffused or at times a combination of both means, in order to allow for regulated slow drug constituent release to the gastrointestinal locale or location of interest (Rane *et al.*, 2010; Sampath Kumar *et al.*, 2010).

Drug delivery concepts have overtime experienced advancements. It has been noted that certain properties have to exist before certain devices can be classified as model macromolecular drug distribution or biomedical system (Kewal, 2008). These properties include:

- Ability of the device to possess influence over the entire structure of the drug molecule.
- Biocompatibility, possessing non-toxic functionality.
- Possessing clear-cut support abilities.
- Possessing high imaging-agent structures.
- Having a well-defined surface changeable functionality for targeting moieties.
- Not having properties that provoke immune responses in the body of a human or animals.
- Possessing suitable adhesion properties at cellular level, transportation of molecules within the cell and control to ensure beneficial drug distribution in the cytoplasm.
- Satisfactory biological elimination from the body at the end of its active lifespan.
- Being a trigger-able (being able to be stimuli activated) drug release.
- Having the drug molecules possessing inaccessibility and shielded against inactivation in the pathway to the specific release locale.

- Possessing low binding properties which are non-specific at the cellular level.
- Having easily methods of synthesis which are easily reproducible.

These existing delivery techniques have limitations principally associated with the management of illnesses confined to locales. These have brought about the development of substitute techniques to deliver drugs in order to increase their ability to be specific in action via targeting. An approach of interest is the use of bio-eliminable polymeric carriers as drug delivery devices, which have the ability to convey to and release the drugs to the site of interest for prolonged periods with slow discharge of the drug constituent over time. Since this polymeric substrate is degradable, non-toxic and biocompatible, it is hence eliminable from the body without the requirement of surgery after the drug constituent has been offloaded. Drug delivery systems utilizing such polymeric carrier to accomplish drug delivery via targeting have been referred to as smart drug delivery systems (Okuda and Kidoaki, 2012). The polymers employed as such carriers could be:

- Non-degradable polymers. These are polymers which are quite stable within the biological environ; thus they are readily adaptable as constituents of implantable drug carrier devices,
- Conjugated Drug polymers: These are polymers wherein the drug to be delivered is incorporated onto polymer carrier which are easily soluble by an attachment linkage. They tend to be difficult to access by healthy tissues. These conjugates are potential candidates for targeting of drugs by way of systemic distribution. Instances of such polymers are: polyacrylamide, albumin and dextran.

- Bio-eliminable polymers: These polymeric carriers are eliminated overtime by degradation of the constituents under normal natural circumstances to non-toxic by products.

## 2.9 POLYMERIC DRUG DELIVERY DEVICES

Polymers are large molecules that are made up of repeating subunits connected to one another by chemical bonds. They have unique properties and can be tailored depending on their intended purpose; hence, the design of polymers and their behavior depends on how their atoms and molecules are connected, as well as on which atoms and molecules begin the repeating subunits.

Much recently, the extent of studies utilizing smart stimuli responsive hydrogels has been steadily increasing, most importantly with drug distribution and biological sciences (Lee *et al.*, 2013). These intelligent hydrogels are cross-linked, water loving polymer complexes undergoing a physicochemical alteration in reaction to a variation in exterior stimuli, being in forms of pH concentration change, light and temperature, among others. (Hoffman, 1991; Qiu and Park, 2001; Caldorera-Moore and Peppas, 2009). These responses are often expresses in several forms which include change in hydrophobicity ability, change in ability to magnetize, bond breakings, or a combination of these (Hoffman, 1991; Peppas, 1997). The need for biocomposite materials having varying usefulness and ability of specific form of action is increasingly sought after. Today researchers have designed smart polymers, which are those polymers with sophisticated response to more than one stimuli in the environment with better mimic biological processes (Koetting *et al.*, 2015). They undergo intended changeable behaviours as a result of external variations in the environment; they show possible

uses for biomedical science researchers, being utilized as means of distribution of therapeutic agents, tissue engineering scaffolds, bio-separation techniques and actuators.

Hydrogels are typical examples of smart polymer which are characterized by hydrophilic networks that are cross-linked into an insoluble, but highly hydrophilic structure. They are a broad class of materials that can be prepared in many different ways and can exhibit significantly different behaviors. For instance, the crosslinks that form the hydrogel network may take on many forms such as covalent chemical links, ionic bonds, weak physical entanglements, hydrogen bonds, or other dipolar interactions (Huglin, 1989). They are increasingly becoming attractive because they can hold drugs in networks and control drugs release by means of diffusion, swelling or degradation and response to certain external stimuli (Lin and Metters, 2006). When stimuli-sensitive, they can be made to act as "smart" DDS as depicted by figure 2 (Kuckling *et al.*, 2002; Wu *et al.*, 2006). The interactions between repeating units of the polymers and the solvent that dissolves the polymer show sudden changes with change in several external stimuli.

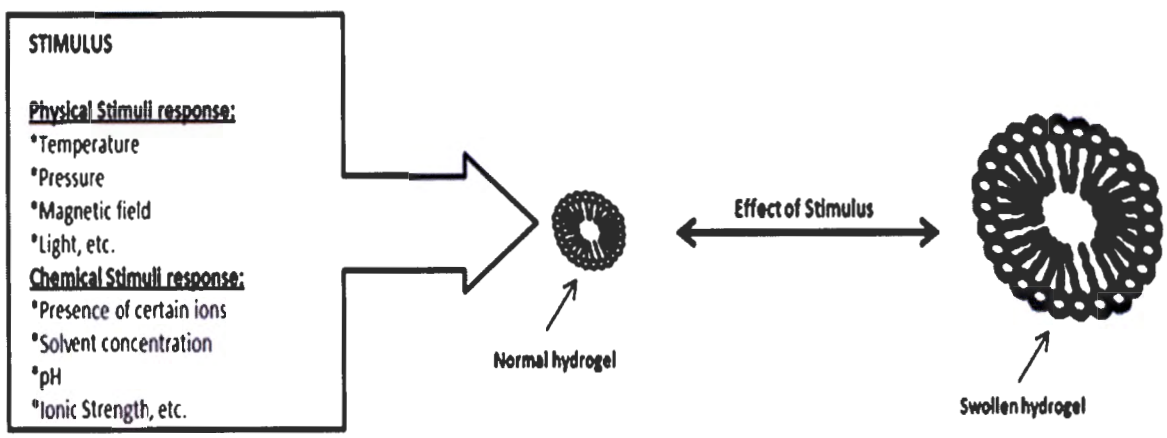


*Figure 2.4: Effect of stimuli on hydrogel (revised from (Wu et al., 2006; Kuckling et al., 2002).*

## 2.10 HYDROGELS – POLYMERIC DRUG DELIVERY DEVICES

The term hydrogels refer to insoluble polymers having three-dimensional, hydrophilic networks with the ability to swell in abundant volumes of water or biological fluids (Zisch *et al.*, 2003; Huglin, 1989; Brannon-Peppas, 1990).The swelling ability in the presence of biological fluids occurs with the gels retaining a significant fraction of water within their structure (Flory and Rehner, 1943; Flory, 1953). A diverse range of hydrogels that are made from natural and / or synthetic polymer materials do exist. Their tendency to take in biological fluids is as a result of the water loving side groups, such as: Hydroxyl (OH), Carboxylic acid amines (CONH), Amides ($CONH_2$), (Carboxylic Acid) COOH and Sulfonic acid ($SO_3H$) (Flory, 1953).

The unique characteristics possessed by hydrogels that make their application in drug delivery an important phenomenon, is their ability to imbibe large amounts of water (>90%, w/v). Hydrogels possess therapeutic discharge mechanisms which are quite dissimilar to hydrophobic polymers, since hydrogels display enlargement behavior on swelling in a manner that is determined by the variations with the environment. Responsive hydrogels exist and these are made of polymer materials which can be broken or swollen due to alterations to the physiological environment of the gels (Peppas, 1991). Unlike other classes of synthetic biomaterials, hydrogels resemble the body's natural living tissue and this is evident in their extraordinary capacity to take in biological fluids and their soft uniformity which is similar to that exhibited by natural

tissue (Ratner and Hoffman, 1976). This characteristic makes them ideal to mimic the body's internal tissues, which enables their application for contact lenses, biosensors, as constituents in artificial skin, coatings designed for synthetic hearts and as therapeutic delivery devices (Mühlebach *et al.*, 1997; Huglin, 1989; Martens *et al.*, 2002; Peppas and Langer, 1994; Peppas, 1997).

Hydrogels have good biocompatibility (Hu *et al.*, 2011b; Hu *et al.*, 2011a; Hu *et al.*, 2009), thus when introduced into the body system, they are not perceived as foreign objects and rejected by the body.

Hydrogels have the ability to take neutral or ionic forms based on their prospective application. Their ability to swell has been comprehensively investigated and it has been identified that the drive for a hydrogel to swell is as a result of the thermodynamic energy that exists between water and the polymer as well as the involvement of the elastic character of the polymer (Kudela and Jacqueline, 1976). It has been confirmed that ionic hydrogels possess interactions between their excited networks and their unrestricted ions, which perform well in contributing to their swellability (Katchalsky *et al.*, 1951).When these polymeric gels are effected by external stimuli, they tend to be able to be manipulated for certain intended use and are referred to as "smart" drug delivery systems (Kuckling *et al.*, 2002; Feil *et al.*, 1992; Wu *et al.*, 2006).

A smart polymeric gel or hydrogel is that variation which possesses the ability to react to specific changes in its physiological locale. Examples of such specific variations or changes are usually related to temperature, light, pH potential, enzyme presence,

strength of ions present and the magnetic field variation (Kato *et al.*, 1997; Zhao and Stoddart, 2009; Gu *et al.*, 2009; Straley and Heilshorn, 2009).

Hydrogels are increasingly becoming attractive because they can hold therapeutics in the mesh of polymeric network and they exercise control over the discharge of such therapeutics within the body through diffusion, swelling or degradation (Lin and Metters, 2006), thereby avoiding the need for surgery in order to eliminate the carrier vehicle (hydrogel) from the body. Overtime, simple and complex simulations have been developed to forecast the drug discharge from polymeric gels, having the time span of discharge for comparison. These simulations are centered on the rate related factors associated with controlled release (Susana *et al.*, 2012).

Over the years, the hydrogels prepared as controlled drug discharge transporters have employed the use of polymeric substances that are dissoluble in water, bioeliminable, natural or synthetic (Gudeman and Peppas, 1995; Park *et al.*, 1998; Wang *et al.*, 2004; Wen and Stevenson, 1993).

Hydrogels are designed when an appropriate amount of crosslinks forms among polymeric subunits; the characteristics are usually influenced by the properties of the individual monomers (natural or synthetic), functionalization and geometry of monomeric units and also the kind of linkages formed between these polymer subunits. These factors should all be taken into consideration when attempts are made to develop hydrogels that are appropriate for localized and controlled drug delivery.

## 2.11 NATURAL AND SYNTHETIC POLYMERS.

These are the building subunits of polymer networks. They are the subunits, which influence the overall properties such as possible magnetic properties, pH potential or/and other properties of the polymers (Lee and Mooney, 2001). Natural polymers can be obtained from animals; for example chitin, alginates, carrageenans, or plants; with cellulose, hemicellulose, glucomannan, agar, starch and pectin as examples. They are biocompatible and as a result of this biocompatibility characteristic, they have benefits in the biomedical field viz; scaffolds for cell growth, delivery of drugs and proteins, coating for immuno-suppression for implants etc. As a result of their useful applications, they are usually beneficial to be degradable, compatible with biological tissues and hence, they are not perceived as foreign (Kulkarni *et al.*, 2012). Synthetic polymers are also called plastics and they tend to enable greater alterations to the properties of the gels with reproducibility. They can also be laden with materials that are beneficial to the properties of interest. They have favorable mechanical properties and they are also thermally stable, in comparison to the natural polymers. Several limitations for natural polymers relating to their performance exist when compared to their synthetic forms. The natural forms cannot be altered to take numerous shapes and there is limitation to their availability. Extreme temperatures involved in polymer processing tend to have a negative effect on natural polymers; with the destruction of the structure, however, this is where the benefit of tenability of synthetic polymers lies. More recently, fusion of natural and synthetic forms 'blends' have been researched upon. These blends possess combined properties which make them biocompatible, but still possessing the individual properties of the subunits; withstanding high temperatures and favorable mechanical

properties which can be beneficial in biomedical applications (Risbud *et al.*, 2000; Li *et al.*, 2010; Sionkowska, 2011).

More recently, delivery devices have evolved in the utilization of synthetic polymers to distribute proteins, deoxyribonucleic acid (D NA), and drugs of interest. Some of the synthetic polymers of interest include; polyethylene glycol (PEG), polyacrylic acid, poly-N-isopropyl acrylamide, polylactic acid (PLA), poly-Nisopropylacrylamide (PIPAM), poly-N-vinyl-2-pyrrolidone and polyvinyl alcohol (PVA) (Lee *et al.*, 2000; Yamaguchi and Kiick, 2005; Miller *et al.*, 2005; Hoffman, 2002). With regards to the focus of this project, Polyethylene glycol hydrogels will be dwelt with.

## 2.12 POLYETHYLENE GLYCOL (PEG) HYDROGEL DESIGN

For several years, polyethylene glycol (PEG) gels have been widely researched upon for use as suitable carriers for controlled delivery of cells, proteins and therapeutics for relieve from diseased conditions, promoting cell growth and tissue regeneration (Drury and Mooney, 2003; Peppas *et al.*, 2000; Mellott *et al.*, 2001; Peppas *et al.*, 1999).

PEG gels are made of polymer materials that are dissolvable in water, non-toxic, biocompatible, biodegradable and lack of protein fouling (Fan *et al.*, 2015; Csucs *et al.*, 2003; Scott and Murad, 1998). PEG polymer chains are known to have weights lower than 20,000KDa, while polymer chains with considerably higher molecular weights are referred to as poly (ethylene oxide) (PEO). PEG is accepted widely and approved for use in biomedicine since it is easily and quickly discarded from the body (Peppas *et al.*, 2006). PEG is known to have the capacity to influence the properties of other biomaterials by covalently binding to other rather toxic molecules. By this binding, the

blend now becomes non-toxic, or molecules that are otherwise non-soluble become soluble (Harris, 1992). This understanding of the changeability of polyethylene glycol (Nuttelman *et al.*, 2008), in addition to its biocompatibility, has elicited the advancement of the various smart hydrogel systems for biomedical applications. PEG is non-reactive and has numerous unattached hydroxyl side-groups on the polymer chains, which are easily prone to substitution with other side groups that are susceptible to attachments (Zustiak and Leach, 2010).In the design of PEG hydrogels for controlled delivery; the actual tailoring of the PEG hydrogels plays a very key role. This influences the extent to which drug molecules will be tightly or loosely bounded to the polymer, thereby affecting the release of the therapeutic to the cells on treatment and also invariably affecting the cells functioning that is essential for cell existence and multiplication.

Overall, tailoring of PEG –drug attachment has an influence on the discharge of the drug particles and also an invaluable influence over the possible negative immunogenic responses from the cell and hence enabling the drug to be delivered and get to its point of action unaltered (Lin and Anseth, 2009). However, in the design of PEG-drug hydrogels, special care has to be taken. These include the careful consideration of the: physiological surroundings of the target tissues; gelation time of the PEG gel; pore sizes; drug loading and drug release kinetics; the properties of the drug for distribution; and the possible interactions with biological fluids.



The attachment of therapeutics to macromolecular carriers such as PEG has become a proven strategy for improving the pharmacological kinetics of the drug release. The attachment of therapeutics is achieved as a result of these tunable polymeric networks which can result from covalent of non-covalent crosslinks between polymers.

Therapeutics can be covalently attached to a PEG through cross linkages that on detection of certain stimuli, release the therapeutic (Greenwald *et al.*, 2003; Filpula and Zhao, 2008). PEG can form star shaped polymer structures, which are 3D hyper branched structures wherein linear arms of similar or dissimilar weights emanate from the central core. Commonly known are 4-arm and 8-arm star shaped PEG. They provide functional groups with high weight per volume and can be used as carriers for drug molecules (Merrill, 1993).

## 2.13 POLYETHYLENE GLYCOL CROSSLINKS

The methods by which the polymer subunits are cross linked/gelled together usually define the type of gels that will result; i.e. physical or chemical gels, while the incorporation of the therapeutic into the cross linked network can also be of either a physical or chemical nature.

Covalent crosslinking results in considerably stable structures within the PEG gel, with the individual cross links being tunable to varying properties such as; degradability, solubility in solvents, ability to withstand high impact, etc. (Peppas *et al.*, 2000; Lin and Metters, 2006). These PEG gels are fabricated by covalently linking of functionalized polymers together (Hennink and van Nostrum, 2002). On the other hand, physical crosslinks can as well be as a result of non-covalent interfaces existing among polymers or due to entanglements existing amongst spontaneous macromolecules. Physical crosslinking tends to occur when there exists cross links between functionalized polymers that have charged groups and species of the alternate charge (Appel *et al.*, 2012). The kinds of connections formed are usually easily cleavable, electrostatic,

induced by solvents and they are readily reversible (Haraguchi, 2007; Guvendiren *et al.*, 2012).

Attachment or dispersion of therapeutics within polymer macromolecular networks and their eventual release is ultimately controlled by the design of the intricate physical parameters of the linkages. Parameters such as: the crosslinking density, gel mesh size, the pore sizes, the swelling degree and rate of degradation have enormous influence over the attachment and release of the therapeutic from the polymer network (Hoare and Kohane, 2008; Weber *et al.*, 2009). Drugs chemically incorporated into the polymer network have a release mechanism that is reliant on the covalent bonds strength that exists between the drug and the gel (which can be cleaved by water addition, addition of enzymes, or in disulfide bonds breakages). This bond breakage yields a theoretically better controlled release mechanism than with drugs that are physically incorporated (Ahmed, 2015).

Several techniques exist for the formation of chemical covalent PEG crosslinked polymer networks that enable them possess the capacity of controlled drug delivery. These techniques include: free radical polymerization method, Michael-type addition method, reactions that are enzyme-catalysed, click chemistry reactions, thiolene chemistry reactions and amide coupling reactions (Şen and Güven, 2001; Zhang *et al.*, 2014; Liu *et al.*, 2015; Hoyle and Bowman, 2010; Srinivasan *et al.*, 2010). Michael-addition reaction technique as a method of chemical covalent formation of PEG crosslinked polymer network is the focus of this thesis.

## 2.14 DEGRADABILITY OR NON-DEGRADABILITY OF PEG CROSSLINKS

The need for degradability or non-degradability of cross-links is at the discretion of the researcher and it depends on the proposed application of the gel that is being prepared. The tendency of the cross links existing to be degradable or non-degradable depends on the nature of the cross links. The crosslinks made between functionalized subunits that are used to form the polymer gel can be made of degradable or non-degradable materials. Also, the bonds that enable attachment of the protein, drug, or cell to the polymer can also be degradable or non-degradable. Therefore, all these intricate bonds relate to the overall degradability or non-degradability of the PEG gel. Degradable gels are mostly of preference for *in vivo* experiments. This is because the chance of inflammation is significantly higher when the gel is exposed to the tissue or cells for considerably longer periods. In some experiments, where PEG gels are used as short-term 3D support for the growth of cells or proteins (Zisch *et al.*, 2003), the gels tend to be rather degradable, so that the by-products after degradation can be eradicated by the renal and hepatic pathways (Veronese and Pasut, 2005). Even though possible long-term inflammation can be decreased by the use of degradable gels, some researchers have experienced inflammation occurring by their use (Hahn *et al.*, 2007; Dobner *et al.*, 2009; Bezuidenhout *et al.*, 2013).

PEG polymer gels are prepared to discharge drugs over a prolonged time-span and still have relevant influence on their targets, but without immunogenic response are rather essential. Hubbel and co-workers have prepared degradable and non-degradable gels that are suitable for drug delivery without necessarily causing inflammation (Lutolf *et al.*, 2001; Lutolf and Hubbell, 2003; Metters and Hubbell, 2005; Schoenmakers *et al.*, 2004;

Jo *et al.*, 2009; Fittkau *et al.*, 2005; Elbert *et al.*, 2001). This has enabled other researchers to adopt their procedures and improve the approach to device new techniques for varied applications (Zustiak and Leach, 2010; DuBose *et al.*, 2005; Bezuidenhout *et al.*, 2013; Mather *et al.*, 2006).

## 2.15 MICHAEL-TYPE ADDITION REACTION

This chemical reaction is named after Arthur Michael and lately, it has been identified to be very useful for the synthesis of crosslinked polymers such as hydrogels (Rizzi and Hubbell, 2005). It is a straight forward chemical process occurring amongst nucleophiles and reactive olefins and alkynes. The reaction occurs and the nucleophile is added across possible carbon–carbon multiple bonds (Kaya, 2011). The reaction is favored by mild reaction environments, tolerance of the reactive groups and monomer units which are easily polymerized, very reactive precursors and advantageous reaction rates (Vernon *et al.*, 2003).

Simply, Michael-type addition reaction is a step growth crosslinking reaction with gelation occurring when there is a reaction between a minimum of two monomers that are multifunctional, possessing common reactive side groups and being chemically reacted with one another, but wherein the functionality of the average monomer is greater than two (Odian, 2004). The reactions are also referred to as the 1, 4-nucleophilic addition reaction and they can occur rapidly involving α, β-unsaturated carbonyl compounds, which are called the acceptors and the nucleophiles are also known as the donors (Mather *et al.*, 2006).This kind of conjugation reaction can be

performed devoid of commonly used free-radical initiators, by producing less defects in the eventual polymer structural formation (Malkoch *et al.*, 2006; Mather *et al.*, 2006), thus allowing for more concise management of the crosslinking density of the gel and the attendant and consequent properties of the end product. This concise management is reasonably significant in the distribution of therapeutics, in that it allows for the accurate scientific extrapolations subsequent to the distribution that is extremely valuable in defining suitable therapeutic drug dosing formulations and its potential discharge kinetics.

Over the years, the scope of the Michael-type addition chemistry has advanced to comprise wide-ranging acceptors and the possibility of having additions of non-carbon donors (Mather *et al.*, 2006). Several researchers have developed degradable hydrogels which were designed by a step-growth form of Michael-type addition reaction; wherein the chemistry occurs amongst polyethylene glycol polymer which were acrylated and reacted with thiol groups (Elbert *et al.*, 2001; Rizzi *et al.*, 2006; Rizzi and Hubbell, 2005; van de Wetering *et al.*, 2005). Evidently, it has been quite successful with the delivery of proteins. It is observed that the by-products of these gels after degradation do not release as by-products chains that possess high molecular weight that may exceed the glomerular filtration limit and in some instances result in defensive host immune response or rejection.

However, the focus of this project relates to the Michael-type addition reaction wherein there is a spontaneous reaction between Michael acceptors and Michael donors. On conjugation, the double bonds present in the system cause the terminal carbon to seek electrons and thus, make it likely to be attached by species that are rather nucleophic

(LoPachin *et al.*, 2008). These nucleophiles then get reacted with the electrophiles and then the protonation of the solvent is observed (shown in the Fig. 2.5)

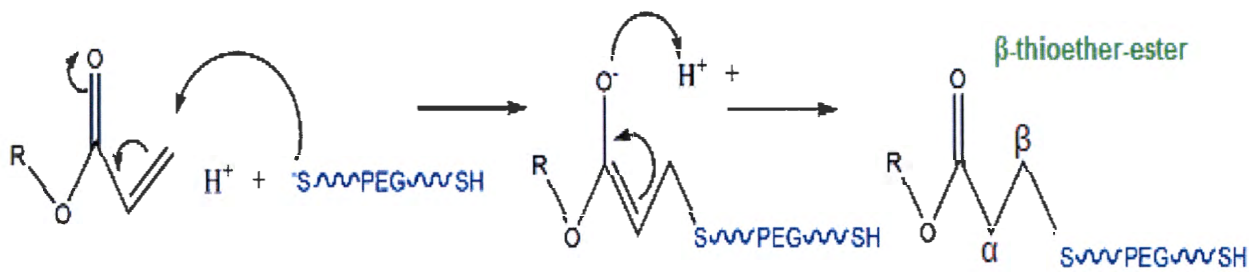



*Fig 2.5: Michael-type (1, 4, nucleophilic) addition reaction.*

Overtime, numerous researchers have employed this reaction for the formation of gels because it occurs at a pH of 7.4 and at ambient temperature and does not require any form of initiator, irradiation, thus avoiding unwanted by-products and hence, producing products which remain potentially non-toxic (Benoit *et al.*, 2007; Hahn *et al.*, 2006; Koehler *et al.*, 2013; Nehls *et al.*, 2016; Cai *et al.*, 2005; Lutolf *et al.*, 2001; Tae *et al.*, 2006). As reviewed, protein molecules and therapeutic molecules in theform of drugs and cells have been shown to be successfully encapsulated within PEG gels for the potential release to target location (Tae *et al.*, 2007; Smeds *et al.*, 2001; Garcia, 2014; Knop *et al.*, 2010) with their functionality easily influenced by varying their molecular weight and cross linker type and also the possibility exists for release from the gels to result in either degradation or non-degradation (Guvendiren *et al.*, 2012).

## 2.16 PEG MODIFICATION FOR ACARBOSE DRUG ATTACHMENT

Coupled with the issue of patient compliance, acarbose as an oral drug has a challenge of low absorption by the body after it has been taken (Ganesan *et al.*, 2011; Sönmez *et al.*, 2005; Hanefeld, 2007; Bischoff, 1995), thus the covalent attachment of the drug to a PEG polymeric macromolecule can possibly, enhance its absorption on a cleavage of the covalent attachment within the body as revealed by other researchers (Goto *et al.*, 2006). In order to achieve this modification to the PEG polymers, acrylate arms are attached to provide the feasibility of a Michael-type addition reaction to covalently attach the drug molecules to the polymer. These PEG polymers can now have acrylate arms or thiol arms to allow for covalent attachment of the drug molecules.

## 2.17 DRUG KINETICS AND RELEASE MECHANISM

The release of attached drugs from polymer macromolecule, which acts as drug delivery mechanism is the endpoint of the drug distribution process. It is that process wherein the drug solutes that are attached to the PEG polymer are discharged to the target location (Langer, 1990). The detachment of these drug molecules are as a result of the polymer materials properties and the nature of the attachment between the drug and the polymer, nonetheless it is also dependent on the physicochemical properties of the drug. The solubility of the drug, stability of the drug molecules, charges existing within the bonds, the chemical interface with the environment and the amount of the drug attached, are parameters to be considered. Also to be considered are the particular drug delivery system targeted, the technique employed for the preparation of the polymer drug attachment, the mesh size and pore sizes, the swelling and cleavage

of bonds attaching the drug to the polymer, the environment wherein the drug will be discharged, the pH state of the locale, the temperature of the targeted location and possible enzymes that will be encountered *en-route* the targeted location and the potential connections between these elements (Siepmann and Siepmann, 2008; Fu and Kao, 2010). Furthermore, these properties can influence the potential drug release from the PEG polymers depending on the form of attachment (Siepmann and Siepmann, 2008):

- Moistening of the polymers surface with fluid.
- Potential fluid infiltration into the polymers via the pores.
- Change in the transition state of carrier between different phase states (e.g glassy to rubbery).
- The ability of the drug to integrate fully into the polymer network.
- Low solubility of the drug and polymer carrier caused by varying solubility profiles at different conditions.
- Degradation of drug and polymer attachments.
- Solubility of the degradation by-products.
- Formation of pores which majorly constitute water.
- Reaction of the polymer mesh and the pore sizes to swelling.
- In Carbopol or other coated dosage forms, substantially balanced pressure can be formed in the delivery system.
- As a result of the pressure, drug release can result from delivery devices.
- Formations of fissures inside the PEG gels affect the discharge frequency.

- Formation of pH dependent micro-environments in the discharge carriers created by degradation products.
- Micro-environment pH is subject to changes, which changes the rate of drug and / or excipients degradation rate.
- Physical interface amongst the drug and the excipients, i.e., ion interaction and possible forces existing therein. These interactions can differ as the time and site changes as a resulting from the variations in the micro-environmental conditions, such as ionic strength, counter ions and changes in pH.
- Alteration to the micro-environmental conditions can directly affect the variations in drug and/or excipient.
- Distribution of drugs from the formulations with dependence to time and location diffusion coefficients.
- Distribution of drugs throughout the carrier device.
- Infiltration from the release medium into the drug delivery system of acids bases or salts.
- Limitation to additional drug discharge as a result of substantial drug concentrations in the release medium (non-sink conditions).
- Chemical reaction occurring between the drugs, excipients and/or water.
- Changes in the drug carrier's dimensions caused by shear forces.

Diffusion is the central element responsible for drug discharge, while the potential swelling of the polymer in solute, possible cleavage of bonds to cause wearing away of bonds also exists (Arifin *et al.*, 2006). The drug discharge mechanism from polymer

macromolecular attachments can be of three forms, though reliant on the physical nature of the polymers and the chemistry involved (Arifin *et al.*, 2006). The forms are:

- Controlled systems by diffusion–polymer carriers which are not biodegradable are reliant on the possible distribution of the solute from their polymer network.
- Controlled systems by swelling - The swelling ability of the polymer in this case, enhances the rate by which the drug diffuses from within the polymer network.
- Controlled systems by erosion - drug discharge as a result of carrier degradation and wearing away.

Breakages to the existing polymer bonds of the biodegradable polymer causes some form of wearing away which causes drug release. Although this breakdown is not rapid, the discharge from the drug-polymer network is still quite obvious. The release of the attached drug from non-biodegradable polymers is rather as a result of the difference in concentration gradient by diffusion mechanism or swellability of the polymer network (Arifin *et al.*, 2006). There are several mathematical empirical models that have been employed to describe the study of drug discharge mechanisms from polymer hydrogels.

## 2.18 DRUG KINETIC STUDIES

- **ZERO ORDER MODEL**

This kinetic release model is utilized for modeling drug dissolution in pharmaceutic forms which do not disaggregate ordinarily and discharge the therapeutic gradually. This is in assumption that the zone is constant and conditions of equilibrium are not reached. These assumptions have been depicted by the equation (i); (Costa and Sousa Lobo, 2001; Varelas *et al.*, 1995).

$w_o - w_t = kt$ .....equation (i)

$w_0$ = initial quantity of drug

$w_t$ = drug present at time t

$k$ = constant of proportionality

Equation (i) above can be further simplified this;

$f_t = k_o t$ .....equation (ii)

$f_t = 1 - \left( \frac{w_t}{w_o} \right)$ denotes a portion of drug dissolvable in time $t$.

$k_o$ = zero order constant.

This zero order model can also be of relevance in describing the dissolution of the drug which occurs via numerous discharge pharmaceutical dosage means including transdermal, matrix tablets with lowly dissolvable drugs, coated forms, osmotic system and many more (Varelas *et al.*, 1995; Costa and Sousa Lobo, 2001). Dosage forms which suit this model discharge the same quantity of the drug per unit time. This manner of release affords pharmacological prolonged action (Dash *et al.*, 2010).

The equation can be further simplified as equation 3:

$Q_t = Q_o - k_o t$ .....equation (iii)

$Q_t$ = the quantity of drug dissolvable in time t

$Q_0$ = the original quantity of drug in solution (the value of $Q_0$ is mostly = 0)

$k_0$ = zero order release constant.

For zero order, *in vitro* data is represented on a graph by increasing percent of discharge from drug against time changes (Dash *et al.*, 2010).

- **FIRST ORDER MODEL**

This model was first proposed in the late 1960s by two scientists (Gibaldi and Feldman, 1967), afterwards further knowledge was established and highlighted by Wagner (1969) for research in drug dissolution. By the early 1980s, more insight was discovered by (Gibaldi and Perrier, 1982) who employed the model to explain investigations in the absorption and/or elimination of some drugs. Although this model is hard to theorize, it has been accepted to be expressed by the equation (iv) below;

$$\frac{dc}{dt} = -kc \quad \text{.....equation (iv)}$$

The equation (iv) can be rewritten:

$$\log C = \log C_o - \frac{kt}{2.303} \quad \text{.....equation (v)}$$

$C_o$ = initial concentration of the drug

$k$ = first order rate constant

t = time.

The plot of the log of the cumulative percent of unreleased drug against time yields a linear graph with a slope of: $-\frac{kt}{2.303}$. First order model of drug release is applicable for

the dosage forms encapsulating drugs which are soluble within porous matrices (Dash *et al.*, 2010).

- **HIGUCHI MODEL**

Higuchi designed numerous models in order to explain, theoretically the study of the discharge of lowly soluble therapeutics and water soluble types, integrated in polymer matrixes. Arithmetic terminologies were acquired from disseminated discharges from a constant matrix acting as the distribution medium. In order to investigate the dissolution from a planar system with a consistent matrix, theoretical relationship was achieved. This relationship is shown by equation (vi): (Higuchi, 1961; Higuchi, 1963; Higuchi, 1962).

$$f_t = Q = \sqrt{D}(2C - C_s) \bullet C_s t \quad \ldots\ldots\ldots\ldots \text{equation (vi)}$$

$Q$ = the quantity of therapeutic discharged

t = time per unit area

$C$ = original drug concentration

$Cs$ = therapeutic dissolvability in the matrix medium.

$D$ = diffusion constant within matrix substance.

When Higuchi first developed this relationship, solubility of therapeutics in suspension form ointments and bases were his target. However, it is also applicable to other drug

dosage forms (Higuchi, 1961; Higuchi, 1963; Higuchi, 1962). The following hypotheses are the basis of the Higuchi (Higuchi, 1961; Higuchi, 1963; Higuchi, 1962).

- The drug solubility is much lower than the original quantity of the therapeutic in the matrix.
- Edge effect is negligible because therapeutic diffusion does not occur in three dimensions.
- The therapeutic particles are considerable slower when compared with the systems thickness.
- The solubility and the swellability of the matrix are neglected.
- Therapeutic potential to diffuse is constant.
- Seamless sink circumstances are constantly attained in the discharge location.

Equation (vi) holds for almost all situations, apart from instances wherein the total exhaustion of the therapeutic in the delivery system is reached, hence, the development of other models based on different shapes and degrees of dispersion of polymer was the focus of Higuchi. Equation (vii) below is one of such, which is applied to spherical homogenous matrix and planar/spherical granular heterogeneous matrix; with lower therapeutic concentration in the matrix than its solubility; thus discharge happens via the pores in the permeable matrix (Higuchi, 1961; Higuchi, 1963; Higuchi, 1962).

$$f_t = Q = \sqrt{\frac{\mathcal{D}\varepsilon}{\tau}}(2C - \varepsilon C_s)\, C_s t \quad \text{.....equation (vii).}$$

$Q$ =the quantity of therapeutic discharged in time $t$ by surface unity

$C$ = the original concentration of the therapeutic

$\varepsilon$ = the matrix permeability

$\tau$ = the tortuosity factor of the passageway

$Cs$ = the therapeutic dissolvability in the matrix

$D$ = the diffusion constant of therapeutic in the liquid

These Higuchi representations hypothesize that the system lacks surface coating and that significant changes are altered in the existence of water.

Furthermore, Higuchi recommended equation (viii), employed in a case whereby the therapeutic is dissolved from a saturated solution ($C_0$ - solution concentration) and then disseminated in a permeable matrix (Higuchi, 1962).

$$f_t = Q = \sqrt{2C_o\varepsilon\frac{D_t}{\tau\pi}} \quad \text{.....equation (viii)}$$

A polynomial equation for the matrix tablet shown in equation (ix) was proposed by some researchers in the 1970s (Cobby *et al.*, 1974a; Cobby *et al.*, 1974b).

$$f_t = Q = G_1k_rt^{0.5} - G_2\left(k_rt^{0.5}\right)^2 + G_3\left(k_rt^{0.5}\right)^3 \quad \text{.....equation (ix)}$$

$Q$ = the discharged quantity of therapeutic in time $t$

$kr$ = the dissolution constant

(1,2, 3) = shape factors

In order to facilitate the ease of using the Higuchi model it is simplified as follows:

$f_t = k_H t^{0.5}$ .....equation (x)

$kH$ = the Higuchi dissolution constant.

This constant has been employed in different manners by different authors and theories (Higuchi, 1961; Higuchi, 1963; Higuchi, 1962).

The information obtained from the use of this equation (x) is usually plotted as cumulative percentage therapeutic discharge against the square root of time (Dash *et al.*, 2010).

- **KORSMEYER-PEPPAS MODEL**

This model is a simple, semi-empirical design that was developed in 1983 (Korsmeyer *et al.*, 1983). It relates exponentially the therapeutic discharge to the time taken ($t$). The relation is shown in the equation (xi) and $a$ represents the constant incorporating the structure and geometric properties of the therapeutic forms, whereas, $n$ is a discharge exponent, indicating the mechanism of the therapeutic discharge and function of $t$ is $Mt$ / $M\infty$ (fractional discharge of the therapeutic).

$f_t = at^n$ .....equation (xi).

a = the constant incorporating structure and geometric characteristics of the dosage form.

n = the discharge exponent.

The relationship representing the diffusion of drugs from a controlled released polymeric plane sheet system was addressed by Korsmeyer *et al.* (1983) and presenting by equation (xii).

$$\frac{\partial c}{\partial t} = \frac{D\left(\partial^2 c\right)}{\partial x^2} \quad \text{.....equation (xii).}$$

D = therapeutic diffusion co-efficient (not concentration dependent) i.e. if therapeutic discharge occurs under perfect sink circumstances, below are the presumed original and borderline conditions;

| | | |
|---|---|---|
| $t$ =0 | $-d/2<x<d/2$ | $c = c_0$ |
| $t>0$ | $x = \pm d/2$ | $c = c_1$ |

$c_0$ = the original therapeutic concentration in the delivery device.

$c_1$= the concentration of the therapeutic at the polymer-water boundary.

In 1995, Crake proposed a solution equation under these conditions, i.e., equation (xiii).

$$\frac{M_t}{M_\infty} = 2\left(\frac{D_t}{\delta^2}\right)^{0.5}\left[\pi - 0.5 + \sum_{n=1}^{\infty}(-1)^n ierfc\frac{n\delta}{\sqrt[2]{D_t}}\right] \quad \text{.....equation (xiii).}$$

When values of $t$ are small, equation (xiii) is simplified into equation (xiv) and the second term of equation (xiii) disappears (Korsmeyer *et al.*, 1983).

$$\frac{M_t}{M_\infty} = 2\left(\frac{D_t}{\delta^2}\right)^{0.5} = at^{0.5} \quad \text{.....equation (xiv)}$$

If diffusion is the foremost discharge technique and a straight line is resonated from plotting the therapeutic quantity discharged against the square root of time and then, influenced by some investigational conditions, the discharge technique strays from the Fick's equation and follows non-Fickian, anomalous behaviour. For such experiments, equation (xv) is more relevant (Korsmeyer *et al.*, 1983; Costa and Sousa Lobo, 2001).

$$\frac{M_t}{M_\infty} = at^n \quad \text{.....equation (xv)}$$

The $n$ value was used (Peppas, 1985) to characterize different release mechanisms for different shapes.

## 2.19 CHARACTERIZATION TECHNIQUES OF THE PEG HYDROGELS

### 2.19.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

This technique is an absorption spectroscopy procedure that exploits the infrared part of the electromagnetic band in order to study samples chemical arrangement. It is based on the vibrational excitation of the bonds within the sample molecules by the way they absorb the infrared light energy (Taha *et al.*, 2013). The spectrophotometer produces a beam of infrared light that is split into two (Smith, 2011). The two beams are: one at a fixed length (mirror A) and the other at a variable length (mirror B) (Silverstein and Bassler, 1962).The varying distances between the two path lengths result in destructive and constructive interferences. Thus, there is variation in the intensities of the interferogram. The interferograms are converted by the fourier transformation to a single spectral position on the more conversant form of the frequency field (Silverstein and Bassler, 1962). The Fourier transform manages to result into a complete infra-red

spectrum from the variation of the length between mirrors A (at a fixed length) and mirror B (variable length). An analysis is performed by placing a sample in the path of the generated beam and determining at what frequencies the sample absorbs the light; this is used for identification purposes. The Perkin Elmer spectrum 100 spectrometer was utilized in the process of characterization.

### 2.19.2 UV-VISIBLE SPECTROPHOTOMETER

UV-visible spectrophotometry is a technique established on the basis that there are differences that exist on the way that material absorb visible light beam (Tissue, 2002). This difference in absorption may be represented as transmittance or comparative absorbance of the beam by the material. A light beam from an ultra violet source is split to its elemental wavelengths by a diffraction grating. Every distinct wavelength beam is divided into two identical strength beams by a device which is said to be half-mirrored. Then, the beam for the samples goes through a clear cuvette holding the sample material that is dissolved in a clear solvent. Another beam, a reference beam, goes through a similar cuvette, but holding the clear solvent alone. In order to analyze the sample, the comparisons of the strengths of the individual light beams are identified by electronic detectors.

### 2.19.3 SCANNING ELECTRON MICROSCOPY (SEM)

This technique employs the use of the scanning electron microscope which enables the production of micro-view images of the materials being viewed; reaching nano levels that the light microscope cannot observe, as a result of the nano sizer size. It is sometimes viewed as the scanning of merely the surface of the sample material, but at

a nano level (Ludwig, 1998). The SEM equipment consists of three major working parts namely: electron-optical column, vacuum system and the electron and display system (Lawes, 1987). The process is initiated by the generation of an electron beam which lights up the image and then more of such beams are produced at very high voltage by an electron gun. This voltage passes through an electron-optical column that is held under high vacuum. Several deflection coils navigate the electron beam over the surface area of the sample material and varying signals are produced. These signals are collected, compiled and interpreted by a detector to give an image (Yamamoto *et al.*, 1975; Chapman, 1986).

### 2.19.4 TRANSMISSION ELECTRON MICROSCOPY (TEM)

This is another procedure to produce images of materials at nano level and operates like a slide projector. The TEM equipment creates electrons that are focused on a single pinpoint spot on the sample. TEM is different from SEM because it uses a quite thin sample material and a higher accelerating voltage. The beam from the equipment produces electrons that interact with the specimen, traveling through it, rather than surface scanning of the sample, as does the SEM. Thus, these electrons travelling through it projects the image whilst enroute through it onto a screen, producing an image. The samples for this thesis were photographed with a dual camera from Gatan on a JEOL model JEM-1230 at a voltage of 80v. Darker parts of the image produced are as a result of some electrons failing to pass through the sample (Boon, 2000).

### 2.19.5 X-RAY DIFFRACTION (XRD)

X-ray microscopy is a great device used to obtain characteristic information on polymer materials which aids in determining the structure of a polymeric material (Alexander, 1969; Willard *et al.*, 1974; Allen *et al.*, 1989; Settle, 1997). The interatomic distances within crystals are widely accepted to be comparable to the wavelength of X-rays. The primary data obtained from the diffraction pattern is the arrangement of the crystals in the polymers with interatomic distances varying between 1 – 50 Å.

The sample is usually placed in the path of a monochromatized X-ray beam of low divergence. The disseminated X-rays from the regularly placed atoms hamper one another, giving evident diffraction patterns in particular directions. These directions of the diffracted beams are interrelated to the slope and dimensions of the unit cell of the crystalline lattice, and the diffraction intensity depends on the disposition of the atoms within the unit cell (Allen *et al.*, 1989). The intensity of the X-ray beams usually depends on the number of electrons in the atoms.

### 2.19.6 THEROMOGRAVIMETRIC ANALSIS (TGA)

Thermogravimetric analysis (TGA) is a thermal characterization technique which looks into the properties of certain materials in relation to change(s) in temperature. It monitors and measures the changes in the mass of the material in question as a function of temperature, time or both, as the material is exposed to defined temperature range within a confined space. The TGA equipment is operated at a temperature ranging from room temperature to 1000 °C (Settle, 1997). The analyzer - TGA Q500 obtained from TA instrument was used to perform the analysis.

The apparent weight loss of the material can easily be identified from the plot of the derivative weight loss curve versus the change in temperature. It provides the necessary information relating to the material characteristics like thermal stability, sample composition changes and possible kinetic parameters. This evident change in mass during this analysis gives rise to a number of possible different phenomena, viz:

- Physical changes –Gas adsorption, gas desorption, phase transitions, vaporization and sublimation.
- Chemical–Decomposition, break down reactions, gaseous reactions, chemisorption (adsorption by means of chemical instead of physical forces).

TGA is used to identify; thermal stability, material purity, determination of humidity and for corrosion studies (e.g. oxidation or reactions with reactive gases), gasification processes and kinetic processes.

### 2.19.7 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Differential scanning calorimetry (DSC) is a temperature based analytical technique that measures the dissimilarity in the heat necessary to raise the temperature of a sample and that of an inert reference at the same rate as a function of temperature (O'neill Michael and Watson Emmett, 1966). The following is the fundamental key to the technique: as the sample is heated, it goes through a physical change instigating chemical reactions, hence giving rise to a different amount of heat required to keep temperature of the sample increasing at the same rate as the reference and these changes are represented as peaks on a DSC thermogram. The machine is usually programmed to scan a temperature range by increasing linearly at a predetermined

rate. The apparatus can also be used to measure the total heat capacity, emission of heat and the purity of solid samples. In addition, it can be used to obtain phase diagram information and to provide kinetic data (McNaughton and Mortimer, 1975).

The resulting thermograms can be accessed by computer software to identify the phase change in the temperature ranges, enthalpy for heats of fusion and crystallization (cooling) processes and information about the material's glass transition temperature ($T_g$), amongst other parameters. Overtime, the DSC has also been used to characterize the energetics and mechanisms of temperature-induced conformational changes of biological macromolecules and pharmaceutical agents in general (Kurganov *et al.*, 1997; Chiu and Prenner, 2011; Gill *et al.*, 2010).

A few advantages of DSC over other analytical methods were summarized by (Androsits, 1999): (1) this technique is able to accommodate samples in different physical forms such as solid, liquid or gel by using a variety of sample vessels or attachments; (2) the study of the sample can occur over a wide temperature range while using various cooling or heating rates; (3) very minimal quantity of sample is required (0.1 µg-10 mg) (4); a DSC run can be completed in a very short time, depended on the sample run(heating rate) (5) the immediate vicinity of the sample can be controlled and standardized e.g having $N_2$ or Argon gas pumped into the chamber for a particular effect; (6) several sample runs can be programmed on one sample in series or different samples (if an auto-sampler is attached); and (7) data analysis software are informative and elaborate.

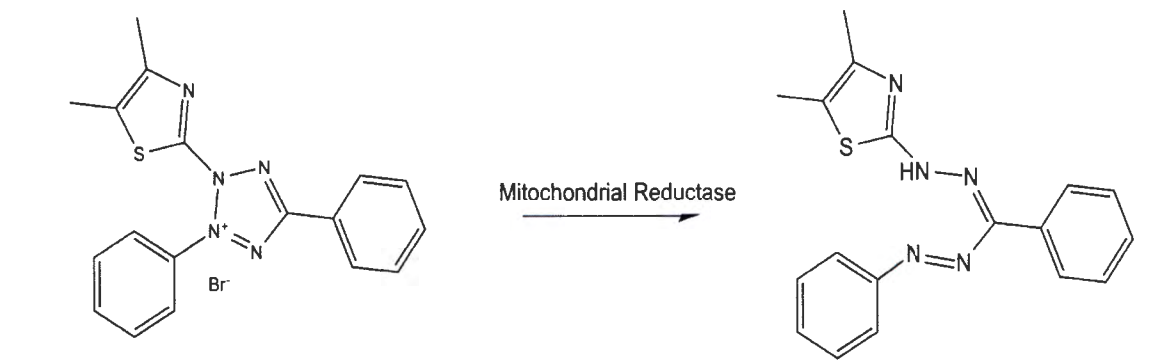
### 2.19.8 NUCLEAR MAGNETIC RESONANCE (NMR)

NMR is an established research technique which was first discovered in the early 1950s by two research groups of prominent scientists towards the latter end of the World War II (Purcell *et al.*, 1946; Bloch *et al.*, 1946).The technique constitutes a non-invasive, non-destructive atom/isotope discerning method which delivers information on a microscopic scale. NMR has been, overtime, relevant to various fields of research i.e. material science, chemistry, biochemistry, biology, physics and medicine. The electronic and magnetic properties of samples are the highlight of the NMR technique, providing vast information about the elemental spin state. The spectrum range can only be detected for nuclei that have a net spin. NMR spectroscopy is mainly concerned with the nuclei showing magnetic dipoles as a result of the electrical charge and mechanical spinning together with contrasting effect (Darbeau, 2006). Specifically, in relation to drug discovery (Pellecchia *et al.*, 2008) or possible drug attachment, the chemical shift mapping is identified by the NMR spectrum, hence aiding in the confirmation of intended drug molecule to the drug delivery vehicle.

## 2.20 MTT ASSAY

In order to understand the cells reactions to external factors introduced during *in vitro* experiments, researchers have focused on the cell growth and viability parameters. Cell's successful growth and viability *in vitro* can now be quantified and currently, the chemical reduction of tetrazolium salts is extensively recognized for being a dependable approach to study these parameters. The yellow colored tetrazolium MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide] has been established to undergo

a reduction reaction as a result of the presence of active cells that metabolize (Figure 6), and as a result and partly by the reaction of dehydrogenase enzymes produce nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) (Mosmann, 1983). By using UV spectrophotometer, absorbance can then be developed to quantify the purple colored soluble formazan that is obtained from the reaction. The cell growth rate and the eventual cell lysis by numerous means or the decrease in population can be identified by the MTT Cell Proliferation Assay. A straightforward relationship amongst the cell population and certain indicative signals has been proven, permitting for the precise quantification of the variations in the degree of cell proliferation (Ferrari et al., 1990). The procedure is centered on the reduction of the tetrazolium salt by active cells which are metabolized, thereby giving rise to the purple colored soluble formazan which is then quantified by a spectrophotometer at a wavelength of 650 nm.




3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
**(MTT)**

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan
**(Formazan)**

*Figure 2.6: Reduction of MTT to purple colored formazan.*

# CHAPTER 3



## 3.0 MATERIALS AND METHODS

### 3.1 INTRODUCTION

This section summaries and explains the experimental methodologies employed to achieve the set out objectives outlined in chapter 1. The methods include: preparation of the covalently bound PEG-acarbose gel; preparation of samples for characterization in order to confirm integration of acarbose into the PEG gel; pH swelling analysis; drug loading and release processes; cell culture and methodology of toxicological studies by using the MTT assay kit, The results from these processes are discussed in chapter 4 under "Results and Discussions".

### 3.2 CHEMICALS AND REAGENTS

All chemicals used in this project were analytical grade and were obtained from Sigma-Aldrich (Pty) Ltd. (Johannesburg, RSA) unless otherwise specified. Acarbose was purchased from Sigma Aldrich, Germany, 8-armed acrylated polyethylene glycol (PEG), having molecular weight of 20 kDa (20PEG8Ac) and 4 armed PEG thiol (SH) having molecular weight of 10 kDa (10PEG4SH) were obtained from Nektar Therapeutics, Huntsville, USA and Creative PEGworks, Winston Salem,USA, respectively.

## 3.3 SAMPLES PREPARATIONS AND CHARACTERISATION

### 3.3.1 INSTRUMENTATION

Perkin-Elmer Spectrum 100 spectrometer Fourier Transform Infrared (FTIR) spectroscopy, Thermofisher Multiskan GO microplate spectrophotometer, Jeol Jsm-5600 Scanning Electron Microscope (SEM), Thermogravimetric analysis (TGA), Tecnai G2 spirit Transmission Electron Microscope (TEM), Bruker DRX-400 NMR spectrometer and Cary 100 UV-visible spectrophotometer were employed to characterize the gels designed.

### 3.3.2 SAMPLE PREPARATIONS

#### 3.3.2.1 FOURIER TRANSFORM INFRARED (FTIR)

About 0.05 g of the hydrogel samples was grounded by using a pestle and mortar. The sample to be analyzed was put on the diamond sample surface of the machine. The number of scans used was 16 with a resolution of 4 $cm^{-1}$. A scan of the background was taken before the sample was analyzed in order to act as a control.

#### 3.3.2.2 UV-VISIBLE SPECTROPHOTOMETER

A Cary 100 UV-Visible instrument was employed to identify the quantity of the therapeutic released from the hydrogels. The spectrophotometer was switched on 20 mins before the analysis in order to allow sufficient heating up time. The wavelength used for the analysis varied according to the drug loaded onto the hydrogel. Auto zero and standards were run before the absorbances of the samples were recorded.

### 3.3.2.3 SCANNING ELECTRON MICROSCOPE

The SEM machine was filled with liquid nitrogen to enable analysis. A carbon tape was fixed on the stub of the sample holder and the sample was mounted onto the carbon tape. The sample was gold coated, and then the coated sample was loaded into a Jeol Jsm-5600 SEM. Proper focusing was done resulting in the formation of images.

### 3.3.2.4 TRANSMISSION ELECTRON MICROSCOPE

The samples were grounded into powdery forms and put in vials before sonication in distilled water for 5 minutes. A tweezer was used to hold the grid and used to pick up some samples form the vials. The grid was then put inside the tip of the sample holder. The tip was introduced into the TEM and appropriate adjustment and focusing were done in order to enable the production of an acceptable image.

### 3.3.2.5 XRAY DIFFRACTION (XRD)

Wide andgle X-ray diffraction (WAXS) was utilized to ascertain the level of interaction of the drugs within the polymer hydrogels. These measurements were carried out on dried and finely grounded samples on a P Analytical X'Pert PRO diffractometer ($CuK_{\alpha}$ radiation, with a wavelength, $\lambda$ = 0.1546nm) running at 45 kV and 40 mA. The sample was then packed into a hole of between 1–2 mm in diameter, in a piece of metal of ~ 1.5 mm thick. The metal piece was then mounted on a sample holder so that the X-ray beam passes through.

The XRD technique helped to resolve the phase structure of the sample so as to understand the change in the morphological structure and the change in the crystalline state.

### 3.3.2.6 THERMOGRAVIMETRIC ANALYSIS (TGA)

TA TGA Q500 was used for this thermal characterization technique. Between 5 mg–10 mg of the samples were measured and loaded into an analyzer and then an inert gas was used to purge the samples in order to avoid decomposition. Avoidance of moisture on the plates is necessary, so that the results are not affected by moisture. The instrument was set at a heating rate of 10 $^{o}$C / min, under air and ramped up to 900 $^{o}$C from 40 $^{o}$C. The plot of the weight loss against temperature was achieved from this analysis and this data can be further analyzed to get the points of inflection.

### 3.3.2.7 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The melting and crystallisation kinetics were studied by differential scanning calorimetry (DSC) measurements with a TA Q2000 instrument. Approximately 4 mg of each sample was loaded in an aluminium pan. The analyses were conducted from - 65 to 300 °C at a heating rate of 20 °C/min with a nitrogen flow rate of 25 mL / min.

### 3.3.2.8 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

All samples were dissolved 4 mg/mL in deuterated chloroform ($CDCl_3$) and distilled water ($D_2O$) and filtered (0.45 µm) prior analysis. MestreNova 10.0 software was used for phase correction, baseline subtraction and integration.

## 3.4 EXPERIMENTAL PROCEDURE

The entire experimental procedure entails the following subheadings:

### 3.4.1 HYDROGEL FORMATION BY MICHAEL-TYPE ADDITION REACTION

Hydrogels were produced by crosslinking 4 armed 10PEG4SH (PEG-SH) and 8 armed 20PEG8Ac (PEG-Ac) in a 1:1 stoichiometric ratio as shown in the Figure 3.1 below.

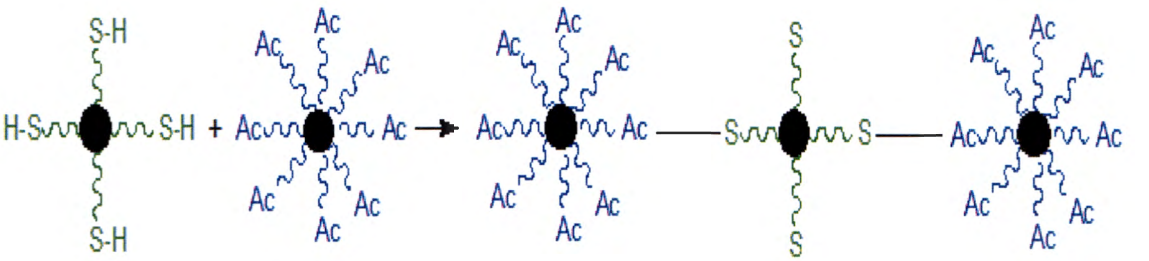



*Figure 3.1: Michael-type addition reaction between PEG-SH and PEG-Ac*

10 mg PEG-SH was dissolved in 50 µl IsoPBS solution, 10 mg PEG-Ac was also dissolved in 50 µl IsoPBS. After dissolution of both, they were reacted together and then by heating it in the oven for ~ 2mins, the polymerization occurred.

### 3.4.2 HYDROGEL SWELLING STUDIES

The pH swellability of a hydrogel is that property of a smart drug delivery system which enables it to hold therapeutics in the mesh of its polymeric network and control the release of such therapeutic to the body by means of diffusion, swelling or degradation (Lin and Metters, 2006). After the preparation of the PEG hydrogels, the pH swelling studies was performed. The equilibrium swelling behaviour of the hydrogels was measured by gravimetric method (Varaprasad *et al.*, 2010). As depicted in Figure 3.2,

individual hydrogels were swollen by subjecting them to varying pH solutions in order to determine their swelling capacities at different pHs.

The initial weights of the gels ($W_0$) of 50 mg were measured, and then the gels were put in beakers with the buffer solutions (1.2 and 7.4) of ~ 20 mL at room temperature. The absorbed water into the gel was determined by weighing every sample after blotting with filter paper, at various time intervals in order to obtain varied masses ($Wt_1$, $Wt_2$, $Wt_3$, $Wt_4$, $Wt_5$, $Wt_6$, $Wt_7$, $Wt_8$, $Wt_9$, $Wt_{10}$, and at equilibrium $Wt_{11}$). The gels that have swollen were weighed by an electric weighing scale. The readings were taken at intervals of 30 mins for 7 h and finally the equilibrium weight was determined at 24 h.

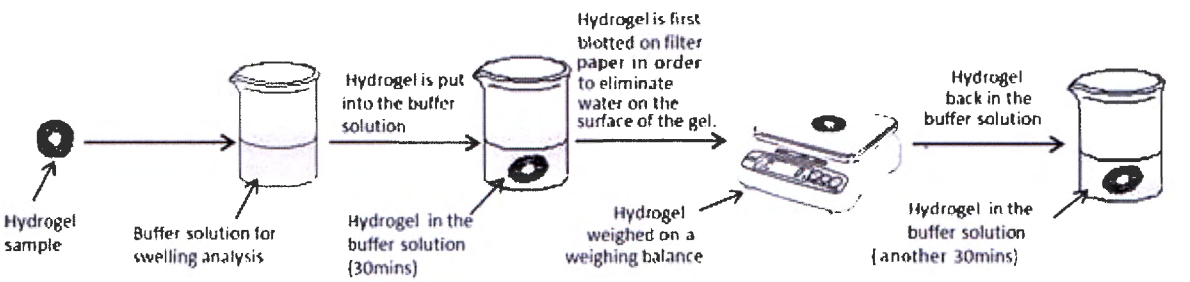



*Figure 3.2: pH swelling analysis of hydrogels.*

### 3.4.3 PROCEDURE FOR DETERMINATION OF SWELLING CAPACITY OF THE HYDROGELS.

Swelling of the gels was performed in the pH solutions at ambient temperature until the swelling equilibrium was attained over a period of 24 h. They were performed according to literature (Varaprasad *et al.*, 2010).

The swollen gels were removed and blotted with filter paper in order to get rid of excess water on the exterior of the gel and weighed. The hydrogels swelling ratio and

equilibrium swelling ratio in the buffer solution were estimated by gravimetric technique from the relation;

Equilibrium swelling ratio:

$$S_r = \frac{(w_t - w_o)}{w_o} \quad \text{..... equation 3.1}$$

$$S_{eq} = \frac{(w_s - w_t)}{w_s} \quad \text{..... equation 3.2}$$

$S_r$ = swelling ratio

$W_t$ = weight at time t

$W_o$ = initial hydrogel weight

$W_s$ = swollen hydrogel weight

$S_{eq}$ = swelling ratio equilibrium

Gels swellings were examined every 30 mins and the calculation of the solvent diffusion and polymer matrix easing influence were evaluated by probing the exponent 'n' from the equation below;

$$\frac{w_t}{w_\infty} = Kt^n \quad \text{..... Equation 3.3}$$

where:

Wt = weight of hydrogel at time, t

$W_\infty$ = weight of hydrogel at equilibrium

K = diffusion constant

n = diffusion exponent.

n = 0.5, specifies case I (Perfect Fickian diffusion). This means that the rate of the polymer matrix easing is quicker than the rate at which diffusion occurs.

n = 1.0, specifies a non-Fickian diffusion. This relates to cases wherein water transport is controlled and the degree of diffusion is quicker than the polymer matrix easing.

$0.5 < n < 1.0$, specifies that the degree of penetration, flexibility and segmental easing are compatible (Ritger and Peppas, 1987a; Ritger and Peppas, 1987b).

The value of 'n' was determined from the gradient of the graph of ln ($M_t/M_\infty$) against ln t for swelling ratio.

The coefficient of diffusion (D) was estimated by using:

$$S = 4\left[\frac{D}{\pi r^2}\right]^{0.5} \bullet t^{0.5} \quad \text{..... equation 3.4}$$

where;

D = coefficient of diffusion of the hydrogel

r = the hydrogel radius

S = fractional swelling

t = time

Diffusion coefficients were assessed from the plot of the fractional swelling (S) against $t^{1/2}$ and these were obtained from the slopes.

### 3.4.4 DRUG ATTACHMENT TO PEG HYDROGEL.

Two techniques were employed to attaching the drug to the PEG gel:

- Chemically attaching the acrylated acarbose to the reaction mix in the preparation of the PEG gel.
- Dispersing the powdered acarbose during the polymerization reaction mix between the PEG-Ac and PEG-SH.

### 3.4.5 CHEMICAL ATTACHMENT (PEG-A)

This occurred in two stages: Acarbose was firstly acrylated in order to enable the ease by which it is attached to the SH-bonds in the PEG hydrogel prepared. Then the acrylated acarbose was involved in the Michael-type addition reaction between PEG-Ac and PEG-SH. In order to achieve this acrylation, the following procedure was adapted.

For instances where close to total acrylation occurs, Figure 3.3 below to depicts this.

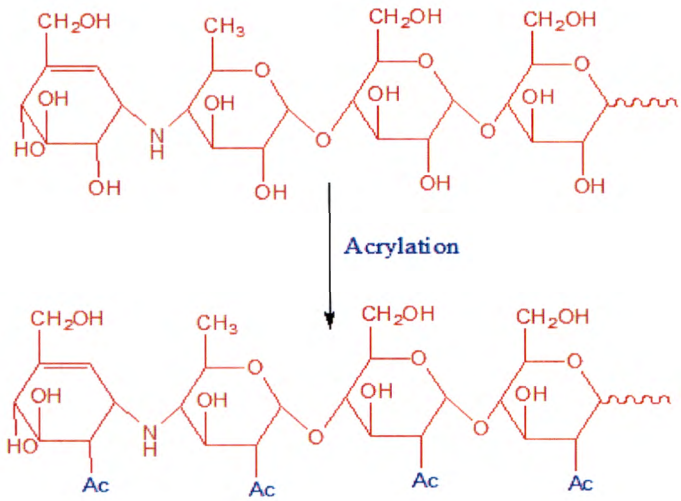



*Figure 3.3: Total acrylation of Acarbose for attachment to PEG hydrogel*

By reaction of the acrylated acarbose with the reaction mix to prepare the PEG hydrogel, the acylation of acarbose enables the drug molecule to be attached to the PEG polymer arms while polymerization of the PEG hydrogel occurred. This has been shown in the Figure 3.4 below:

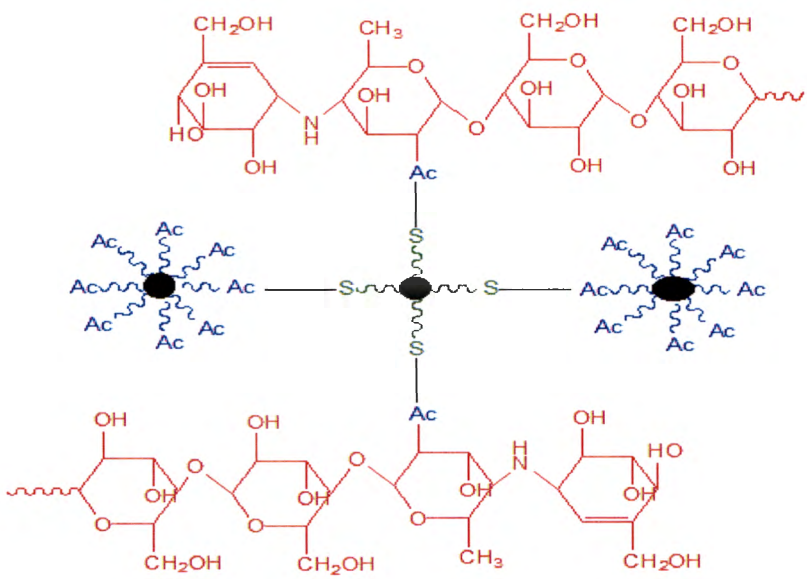


*Figure 3.4: PEG-acarbose attachment after acrylation of acarbose.*

### 3.4.6 DISPERSING THE POWDERED ACARBOSE (PEGA)

This is a more commonly used technique to load therapeutics onto polymer hydrogels. It was done by the dispersion of known amount of the powdered therapeutic into the preparation reaction mix of the PEG hydrogel. Thus, on solidification of the polymer gel, the therapeutic will be encompassed within the polymer network.

5 mg of acarbose was dispersed into the mixture of PEG-Ac and PEG-SH in order to have the therapeutic encompassed within the PEG polymer matrix. The loading was confirmed by various characterization techniques.

*Table 3.1: Loading of Acarbose onto PEG gel.*

| Hydrogel | Acarbose (mg) | Means of Loading Acarbose |
|---|---|---|
| PEG | - | - |
| PEGA | 5 | Dispersion |
| PEG-A | 5 | Chemical attachment |

### 3.4.7 DRUG RELEASE STUDIES

Drug release study was performed and the drug release profiles of the acarbose loaded hydrogel; as a result of a specific environmental changes (e.g. stimuli-pH), was evaluated.

Figure 3.5 shows the standing experiment; a buffer solution was prepared and placed in a water bath shaker-BS-06 Lab Companion (set at 37 $^{o}$C) overnight to equilibrate. On equilibration (100 RPM, 37 $^{o}$C), the hydrogel was placed in the buffer and at 30 mins

intervals, 4 mL aliquots of the buffer solution in the water bath, was pipetted and replaced with several 4 mLs buffer solution.

Each 4 mL aliquot pipetted was stored in the fridge and after 8hours of sampling, the aliquots were analyzed by using the UV spectrophotometer in order to ascertain the discharge concentrations of the drug released at the varying times.

Two buffers were used: 7.4 buffers were used to imitate the environment of the human blood, while 1.2 buffers were used to mimic the interaction with the gastric juice in the stomach.

The quantity of the drug released was measured by using ultraviolet visible spectrophotometer in both buffers; the absorption of the solution of acarbose was at 610 nm. Although it was not identified directly, it was identified by utilizing sodium hydroxide (NaOH) and potassium permanganate ($KMnO_4$) as an oxidizing agent. Before taking readings of the aliquot under the UV Spectrophotometer, 1 ml of 0.5 M NaOH was added, followed by 1 ml of 0.01 M $KMnO_4$ to the aliquot, only then could the absorbance of acarbose be identified accordingly.

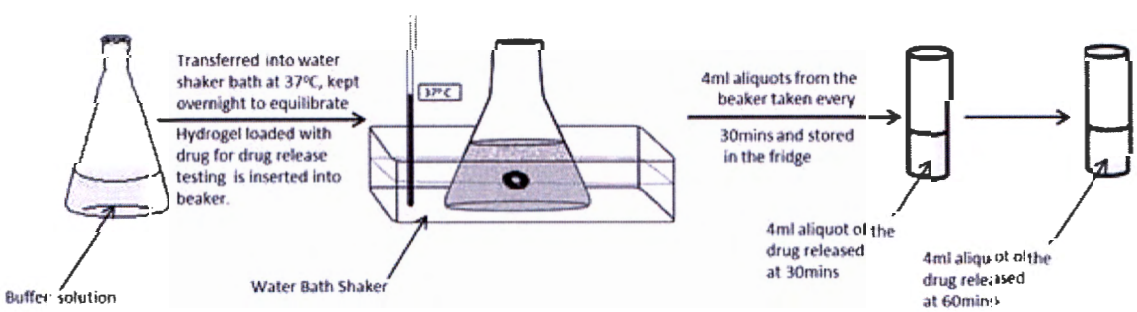



*Figure 3.5: Acarbose release from PEG gels*

## 3.5 GASTROINTESTINAL (GI) ROUTE.

In order to closely mimic the gastrointestinal route, researchers have tried to go a step further and by taking into consideration the various pH conditions that drug formulations would encounter in their journey to treatment. According to Bajpai *et al.* (2015), these varied pH conditions have been identified for the changes in the gastrointestinal routes simulating the complete GI environment and these have been categorized according to table 3.2.

*Table 3.2: Gastro intestinal locale categories described.*

| **Fluid name** | **Abbreviation** | **pH condition** |
|---|---|---|
| Simulated gastric fluid | SGF | 1.2 |
| Simulated duodenum fluid | SDF | 6.0 |
| Simulated proximal ileum fluid | SPIF | 7.0 |
| Simulated intestinal fluid | SIF | 7.4 |

The drug formulation was put in the SGF for 2 h, then moved to the SDF for 2 h, onto the SPIF for yet another 2 h and finally to the SIF for 2 h. This procedure should give a more closely mimicking of the gastrointestinal route.

## 3.6 CELL CULTURE

Cytotoxicity study was performed with c2c12 myoblast cell line and was composed of: positive control cells, cells treated with plain PEG gels and this experiment was executed in order to reveal the non-toxic properties of the plain gels to the c2c12 cells.

### 3.6.1 CELL GROWTH

The frozen cells were removed from -80 $^{o}$C, thawed at 37 $^{o}$C and grown for 1 week in 10 mL culturing media added to each plate made up of Dulbecco's Modified Eagle's medium (DMEM) (Sigma Life Science, USA) enhanced with 10% FSC (Biowest, South America) and 1% streptomycin (Gibco Life Technology, USA) at 37 $^{o}$C in 95% Humidity with 5% $CO_2$ (Media was replaced every second day).

Thereafter, cells were split, with the following procedure:

Growth media, PBS and Trypsin were pre-heated at 37 $^{o}$C. Media were decanted and plates rinsed with phosphate-buffered saline. Then, 1 ml of trypsin was added to each plate(s) and incubated for 5 minutes at 37 $^{o}$C. Next, 5 ml of media was then added to the plate(s) and transferred cells to centrifuge tubes. Cells were then centrifuged at 2000 rpm for 3 mins. Media were decanted and appropriate amount of media was added depending on number of plates and pellet was re-suspended. 1 mL of cells was transferred to each plate together with 9 mL of the medium for growth and incubated at 37$^{o}$C at 95% humidity with 5% $CO_2$. (Splitting is determined by the number of plates necessary for the study).

Once cell growing was completed, cells were allowed to differentiate at 97% humidity in Dulbecco's Modified Eagle's medium (DMEM), 1% streptomycin and 2% horse serum (Biowest, South America) for 1 week.

The growth of the cells was viewed under a light microscope, as shown in Figure 3.6 for: (a) 3 days cell growth. Lipid droplets forming in the early days of cell growth of the pre-adipocytes were evident and became more widespread as the number of cells increased.

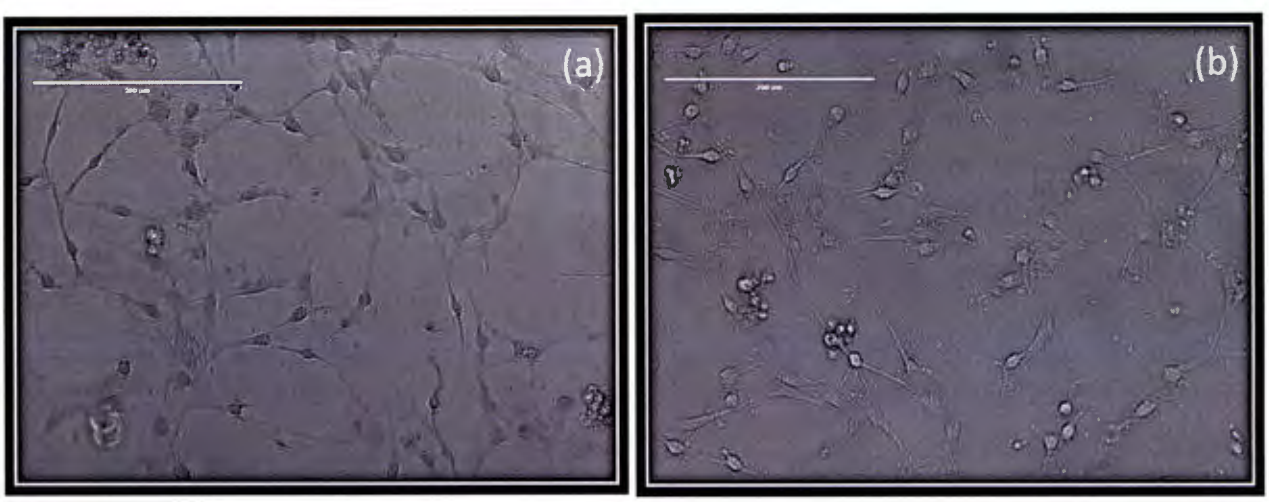



*Figure. 3.6: c2c12 cell growth viewed under the light microscope after 5 days.*

Cell differentiation was observed under the light microscope after 5 days of cell growth, as shown in Figure 3.7. The well-defined network of cells can be appreciated as the cells were differentiated.

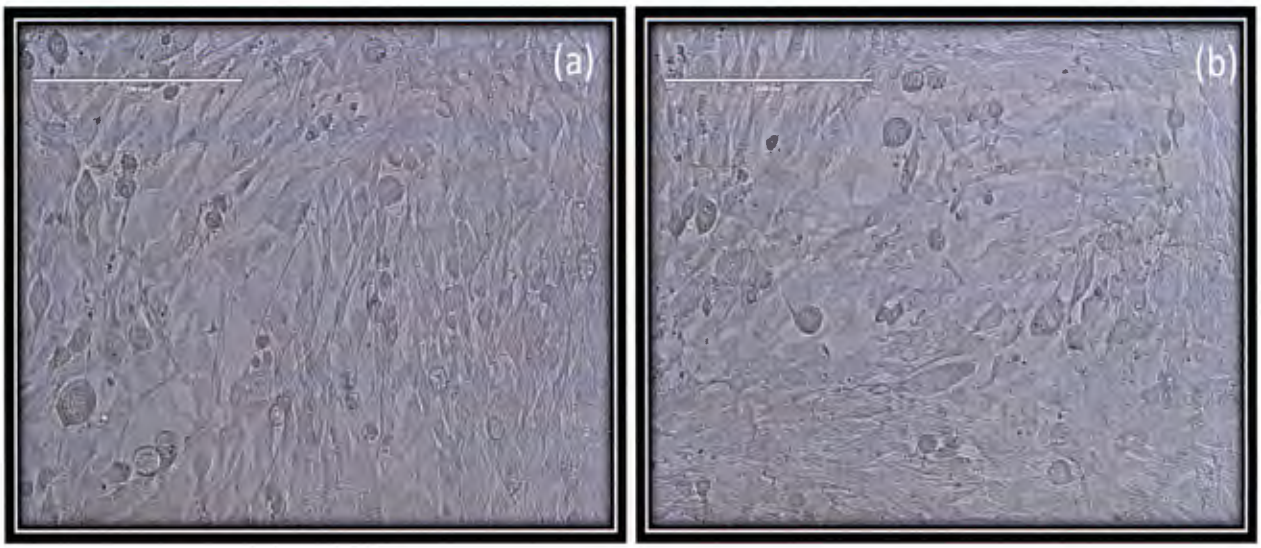



*Figure.3.7: c2c12 cell differentiation viewed under the light microscope after 3 days of differentiation.*

For backup cultures, some of the cells obtained were frozen. This was performed by the addition of freezing media consisting of 70% DMEM, 20% FCS and 10% DMSO to a set of cells after centrifugation. Cells were then stored at -80 $^{o}$C.

### 3.6.2 TREATMENT OF CELLS

After the cells were differentiated to allow for the expression of the required phenotypic characteristics, the cells were treated by using plain PEG gels in order to confirm the non-toxicity of the plain PEG gels. Three treatments with the plain PEG were performed and done in triplicates. The treatments were done with approximately the same sized gels (0.75mg) and the study was performed over a 72h time period.

### 3.6.3 TOXICITY TEST.

The differentiated mature c2c12 cells were treated with the plain PEG gels in order to investigate the effect of toxicity on the cells on treatment. This was done by using the MTT Assay kit and the procedure of utilization of the kit is as follows:

The viability of the cells was quantified by colorimetric assay which showed the MTT reduction by viable cells, as evident in published literatures (Mosmann, 1983; Denizot and Lang, 1986) by using a Thermofisher Multiskan GO microplate spectrophotometer. According to the manufacturer's procedure, 5 mg / ml MTT was prepared as a stock by dissolving 5 mg / ml MTT into PBS. The solution was then sieved, aliquoted and the leftover stored was at -20°C in eppendorf tubes. To each treated plates, 10 µl of the prepared stock was added and incubated for 4 h. 50 µl Dimethyl sulfoxide (DMSO) was then added to the plates for the dissolution of possible remaining formazan crystals. Further incubation for 10 min was followed at 37 °C and then measurement of the absorbance was done at 540nm.

# CHAPTER 4

# RESULTS AND DISCUSSIONS

## 4.1 INTRODUCTION

This chapter discusses, analyzes and highlights the relevance of the study and the results obtained from the previous chapter: morphological characterization, swelling and drug loading analysis, drug release and toxicity tests that were performed on the gels.

## 4.2 RESULTS AND DISCUSSIONS OF DATA OBTAINED FROM STUDIES.

### 4.2.1 HYDROGEL SWELLING ANALYSIS AND DRUG RELEASE

Swelling analysis of the PEG gels was performed in order to shed some light on the possible release mechanism of the loaded drugs from the polymer substrates. Acarbose was either dispersed (PEGA) or acrylated (PEG-A) to the PEG gels (substrates) and drug release analysis was performed.

Polymer swelling in varying buffer conditions has become very relevant in biomedical application since apart from biomimicking the internal biological environment of the patient, it suggests the actual mechanism of drug release that will occur from the polymer gel (Colombo *et al.*, 1996). Although hydrogels capacity to swell depends on a number of factors, the swelling is a significant element that can influence the rate of release of drug from the polymer matrix because it correlates with the rate of diffusion of drug in and out of the hydrogel matrix (Peppas *et al.*, 2000).

The swelling study was performed at ambient temperature over a period of 24 h in selected buffer solutions (SGF -pH 1.2, in order to imitate the stomach and interaction

with the acidic gastric juice and SIF -pH 7.4, in order to imitate the intestinal fluid-blood) according to (Bajpai *et al.*, 2015).

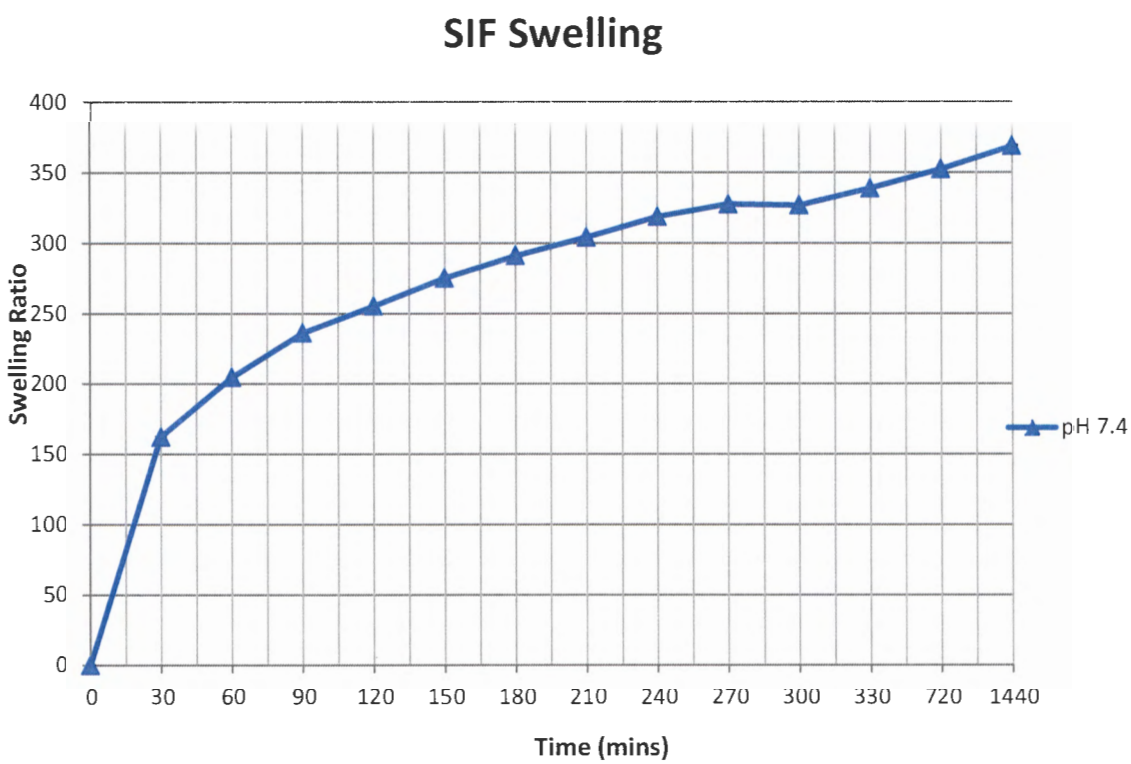



*Figure 4.1: PEG polymer swelling in SIF at ambient temperature.*

The swelling of PEG polymer gels were performed at ambient temperature and in triplicates. By 30 mins, the gel has swollen to about 1.5 times its original weight, and finally at 24 h about 3.5 times its original weight. Although between 270 – 300 mins, we can observer that there was almost no swelling recognized in the SIF swelling study. We can observe that the resulting PEG polymer gels contain hydrophilic groups which contribute to an increase in the overall density and water sorption ability, hence increase in the swelling rates. This initial swelling rate in the first 30 mins could indicate burst release of any drug loaded and this has been observed by several other

researchers using PEG and other polymers (Bartil *et al.*, 2007). The potential for burst release is evident with the initial swelling at the SIF as reviewed by researchers, but this burst release is affected by the drug loaded on the polymer to counter the negative effects possible (Huang and Brazel, 2001).

But in the SGF (Figure 4.2), the swelling rate is rather reduced with the gel swollen to only about 0.5times its original weight in 30 mins. After 24 h, the total swelling ratio was about 1.5 times the original weight. This information reveals a potential reduction in diffusion rates of potential loaded drugs within the polymer network. Thus a swelling rate observed within 30 mins of swelling in the SIF is only achieved on 24 h swelling in the SGF. Similar finding was observed by (Bartil *et al.*, 2007), indicating the amount of water absorbed by the polymer network in SIF was larger than SIF at the same time.

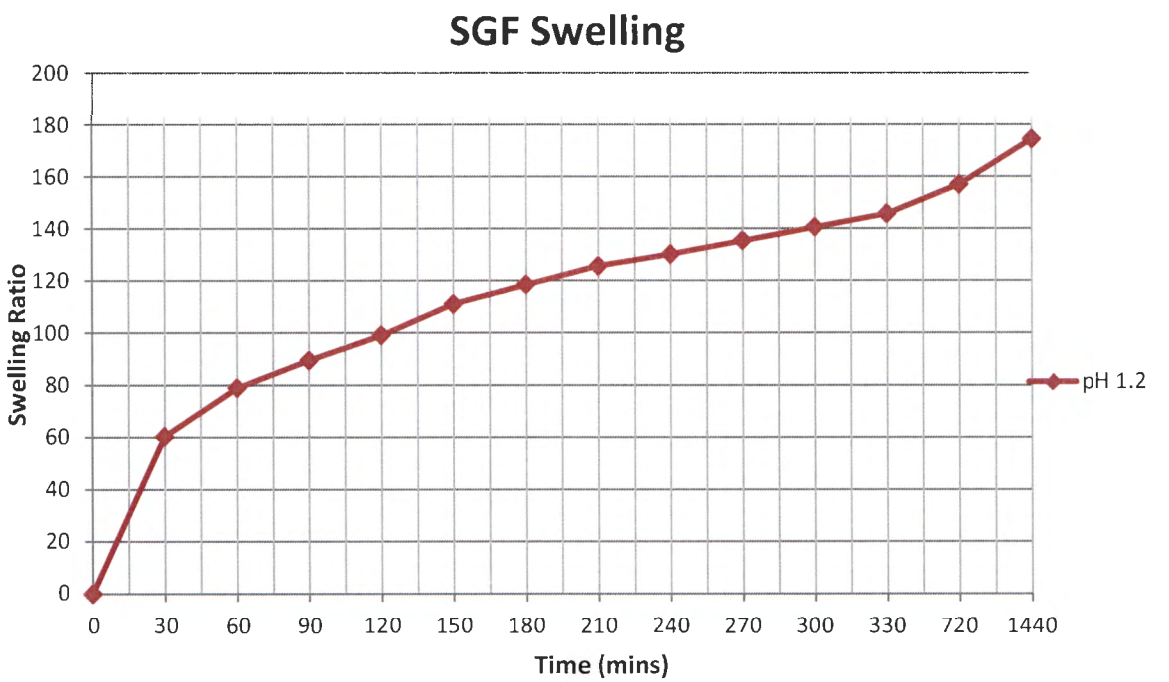



*Figure 4.2: PEG polymer swelling in SGF at ambient temperature.*

Hydrogel swelling studies give insight into the balance between the media penetration rate and the drug diffusion (Kortesuo *et al.*, 2001).With polymers in general, during swelling it is noteworthy that the presence of these buffers sufficiently changes the chemical potentials due to the elastic forces, and this is solely responsible for the change of volume fraction density of the polymer chains (Peppas and Merrill, 1977; Chai *et al.*, 2017). Kim and Peppas (Kim and Peppas, 2002) have performed studies which showed similar pH responsive swelling that has been observed in this study with PEG composite polymers, and it is widely known that the effect of polymer swelling relates directly to the discharge of the loaded drugs and the values obtained from the analysis indicate that these hydrogels have the potential to be used as pH responsive targeted delivery systems in biomedical applications.

Table 4.1 shows the analysis of the swelling ratio of the PEG gels in both buffer conditions. The determination of the swelling exponent (n) was calculated by the slope of graph of $\ln\left(\frac{M_t}{M_\infty}\right)$ versus $\ln t$

where: Mt = Mass of polymer gel at time (t)

M∞ = Mass after swelling to 24 h

t = Time

The swelling exponent (n) was 0.32 in SIF and 0.39 SGF, having coefficient of determination, $R^2$ between 0.97 - 0.99, in both buffers and showing good linearity.

The diffusion coefficient evaluated from the slope of the swelling ratio (SR) versus the square root of time ($t^{1/2}$) in both buffer conditions were calculated and shown in Table 4.1. According to Table 4.1, in the SIF, the slope (n) depicting the diffusion coefficient in this case was 0.14, while in the SGF, n was 0.07, with $R^2$ values ranging between 0.94 - 0.98, showing fair enough linearity.

These 'n' values obtained meant that the PEG gels had water penetration rate below the polymer chain relaxation rate, thereby classifying the diffusion rate as Less Fickian diffusion behavior (Wang *et al.*, 2008a).

*Table 4.1: Analysis of swelling ratio data obtained*

| Code | pH | $R^2$ (graph of *Ln* SR vs *Ln* time) | *n* | $R^2$ (graph of SR vs $t^{1/2}$) | n |
|---|---|---|---|---|---|
| **PEG gel** | 7.4 | 0.99 | 0.32 | 0.98 | 0.14 |
| **PEG gel** | 1.2 | 0.97 | 0.39 | 0.94 | 0.07 |

*In vitro* cumulative drug release of acarbose from both methods of attachment to the PEG polymer was performed in both the SGF and SIF conditions and the reading recorded with 30 mins interval for 6 h and then readings taken at 24 h, 48 h and 72 h in order to biomimmic the internal biological system of the body.

In the SIF, acarbose which was acrylated and attached to the PEG polymer was discharged fasted from the polymer capsule within the first 30 mins, but by 1 h, they both had discharged approximately the same amount of acarbose from the polymer carrier (Figure. 4.3a). As shown in Figure 4.3a, by 72 h ~ 61% of acarbose has been

released from the PEGA gel when compared to ~ 54.9% in the case of PEG-A. This confirms that acrylating acarbose to PEG provides some extra binding of the drug molecules to the polymer, which enables it to remain bound to the polymer a lot tighter than the dispersion technique.

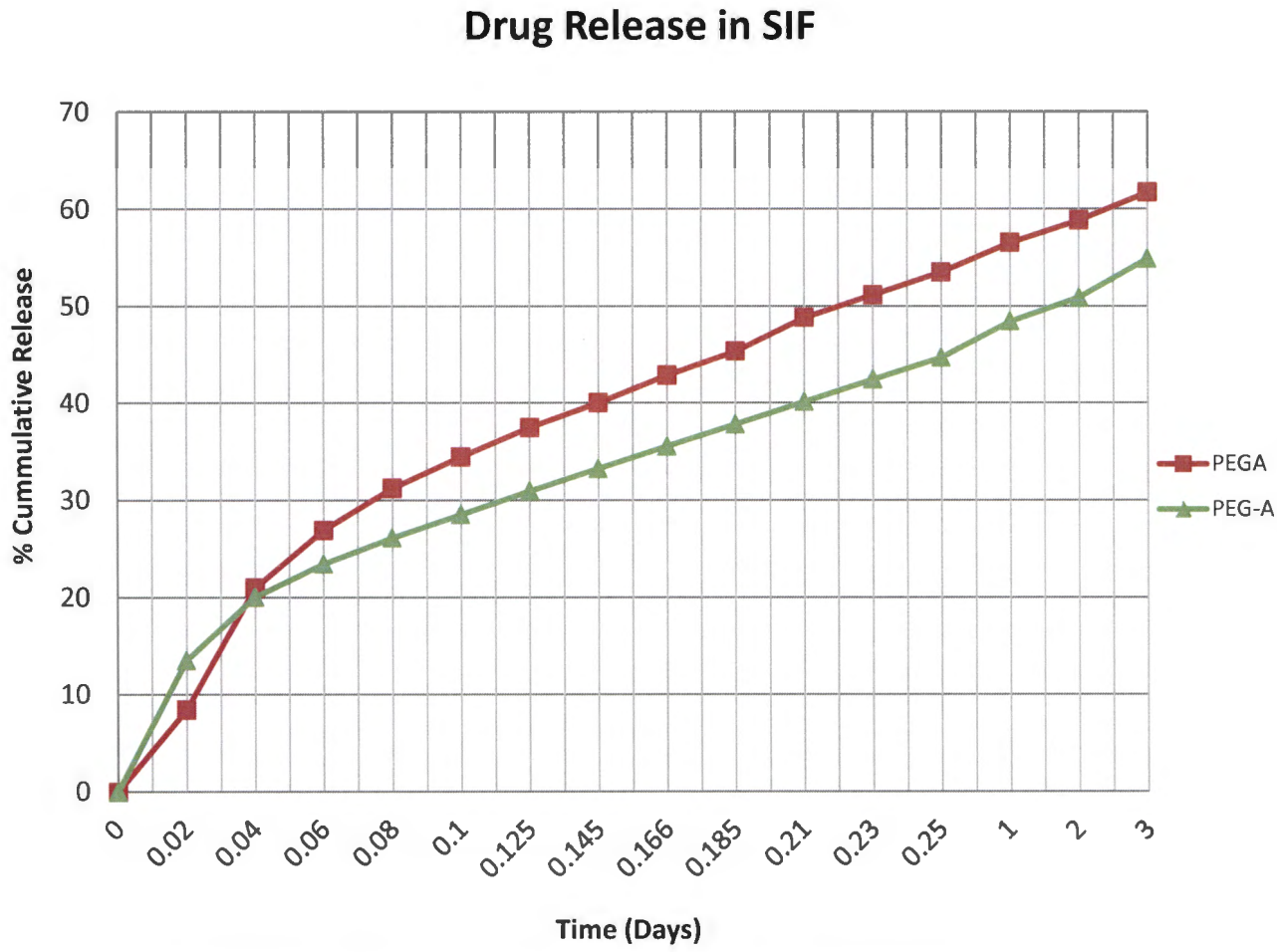



*Figure 4.3a: Cumulative release of acarbose in SIF at ambient temperature.*

This release in the SIF conditions is a steady state release and with evidence that by 72 h about 60% of the drug was released from the gel matrix. In the SGF, the rate of discharge of the drug molecule from the polymer matrix was considerably fas5t and this

is known for most hydrogels. As shown in Figure. 4.3b, the discharge of acarbose from both PEGA and PEG-A gels were approximately the same for 30 mins, but thereafter, the dispersed drug discharges a lot faster at a constant relative rate until ~72 h. At the 72 h timestamp, the PEGA had discharged ~ 72.7% of the dispersed acarbose drug, as opposed to 61.9% of the acrylated drug.

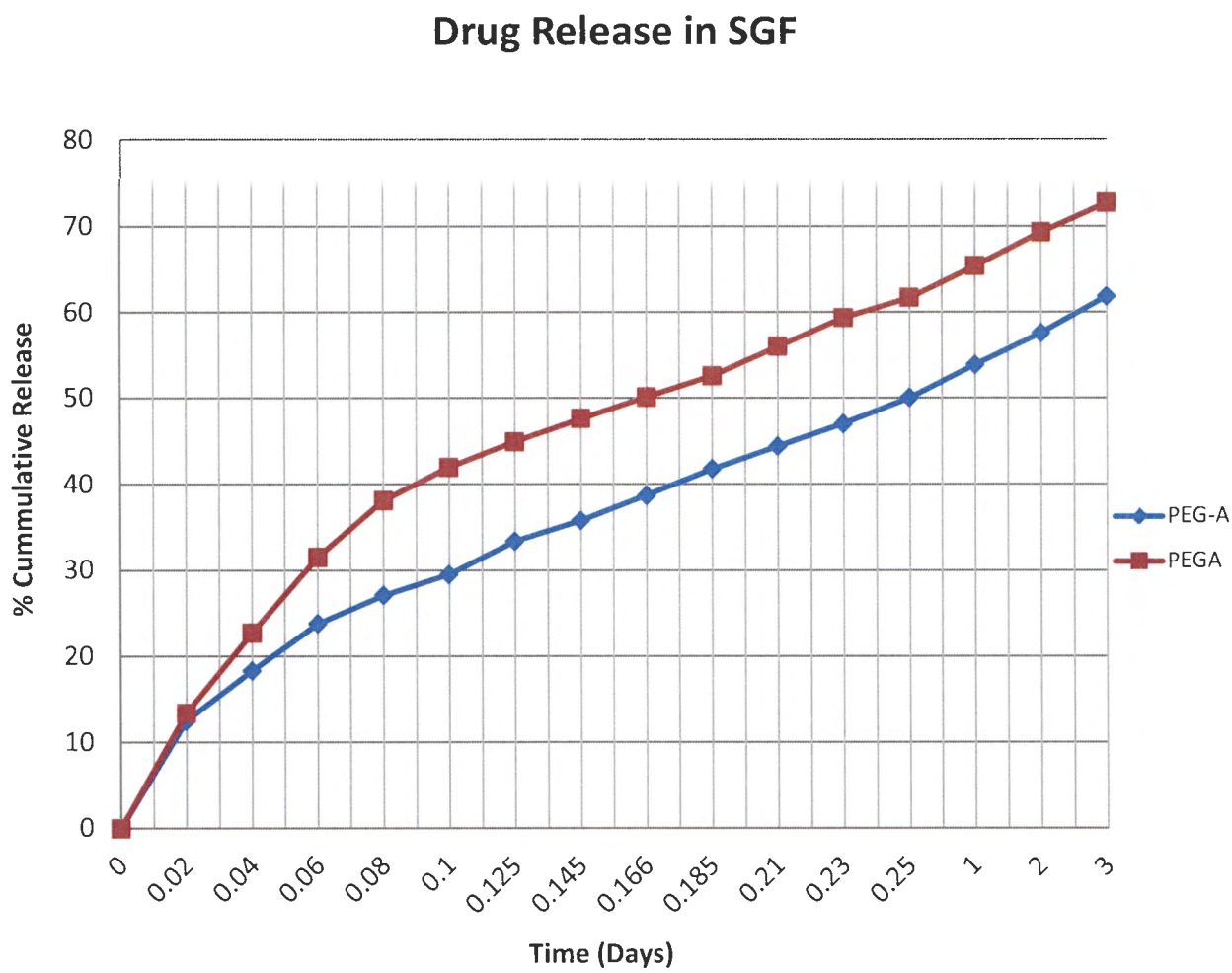



*Figure 4.3b: Cumulative release of acarbose in SGF at ambient temperature.*

This once again confirms that it is more difficult for the bonds of the acrylated acarbose to be broken than that of the dispersed acarbose from the polymer matrix.

Several models have been exploited in the past in order to analyze the mechanism of drug release. For this release mechanism, the Korsmeyer–Peppas model was used for the analysis of the fraction of drug released. This model was preferred because it is that theoretical model that studies the release of water-soluble and low-solubility drugs incorporated in semisolid and/or solid matrices (Higuchi, 1961; Siepmann and Peppas, 2011).

The Korsmeyer–Peppas model relates to the equation below:

$$\frac{M_t}{M_\infty} = Kt^n \quad \text{..... equation 4.1}$$

where: $\frac{M_t}{M_\infty}$ = fractional drug released.

t = time of release

K = constant incorporating the structural geometric characteristics of the hydrogel.

n = release exponent.

The diffusion exponent was estimated from the linear regression of log Mt/M∞ versus log t for only the 60 % of drug released (Wang *et al.*, 2008a; Korsmeyer *et al.*, 1983; Serra *et al.*, 2006). When n = 0.5, it indicates a Fickian diffusion, when 0.5<n<1, it indicates an anomalous or non-Fickian diffusion, which refers to a combination of diffusion and erosion-controlled rare release. When n = 1, it indicates case II transport phenomenon and when n>1, it indicates super case II transport phenomenon (Costa and Sousa Lobo 2001; Ritger and Peppas, 1987a).

The linear regression of log $M_t/M_\infty$ against log t was plotted and the release exponent values (n) and the correlation coefficient ($R^2$) of both gels in the individual release medium are shown in Table 4.2 below:

*Table 4.2: Release exponents, n and correlation coefficient of hydrogels*

| | pH = 1.2 | | pH = 7.4 | |
|---|---|---|---|---|
| | n | $R^2$ | n | $R^2$ |
| **PEGA** | **0.58** | **0.98** | **0.65** | **0.98** |
| **PEG-A** | **0.55** | **0.99** | **0.47** | **0.99** |

At both buffer solutions, the value of n ranged between n=0.5 to 0.5<n<1, which indicates Fickian to non-Fickian diffusion. The correlation coefficient values ranged between 0.98 – 0.99, showing good linearity and confirming that the Korsmeyer–Peppas model is a good fit for this hydrogel drug delivery system. The rapid release of the drug from the hydrogel networks in the SIF when compared to the SGF is in agreement with the swelling behavior of the PEG hydrogels observed earlier and the enhanced diffusion of the drug from the hydrogel networks and also reported by other researchers (Bajpai *et al.*, 2015).The slow release of the drug at acidic pH suggests that the hydrogels are potential good delivery devices for sustained release mechanism to the gastrointestinal region. There are similar findings reported by other researchers when using PEG based gels (Ayhan and Özkan, 2007; DiRamio *et al.*, 2005) and others when using acarbose for release studies (Bajpai *et al.*, 2015).The non-Fickian diffusion rate is desirable, as it indicates that the media penetration rate is in the same range as drug diffusion (Kortesuo *et al.*, 2001). Through the observations made in this study, the

use of PEG hydrogels represents a potentially effective pH sensitive sustained substrate for delivery device for long term delivery of drugs. The graph of the Korsmeyer- Peppas plots is shown in Figure 4.4 (a-d).

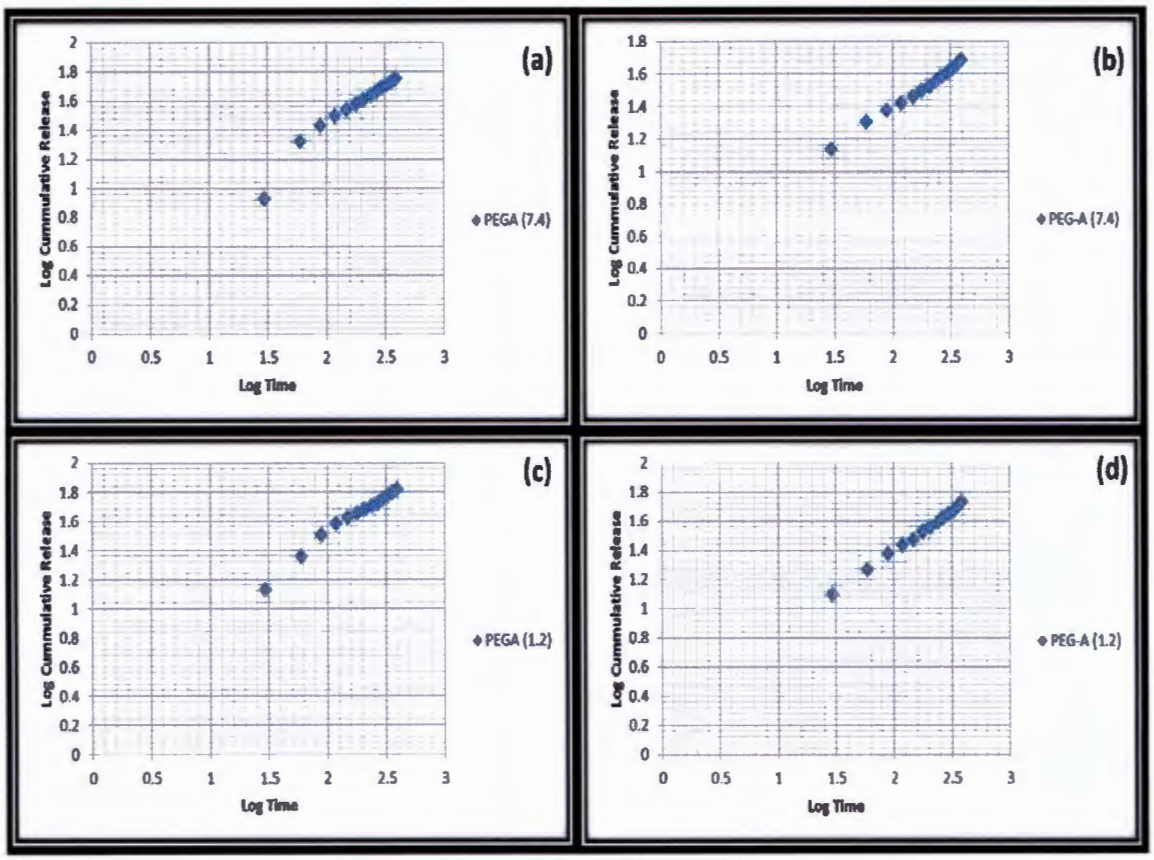



*Figure 4.4: Korsmeyer-Peppas mathematical kinetics model plots of PEGA and PEG-A gels dynamic release in SGF and SIF.*

### 4.2.2 DRUG RELEASE IN GASTROINTESTINAL LOCALE.

In addition to the standard drug release from a drug formulation, the polymer drug formulation was subjected to release studying in varying pH conditions as this better represents the gastrointestinal locale which is encountered by the drug formulation.

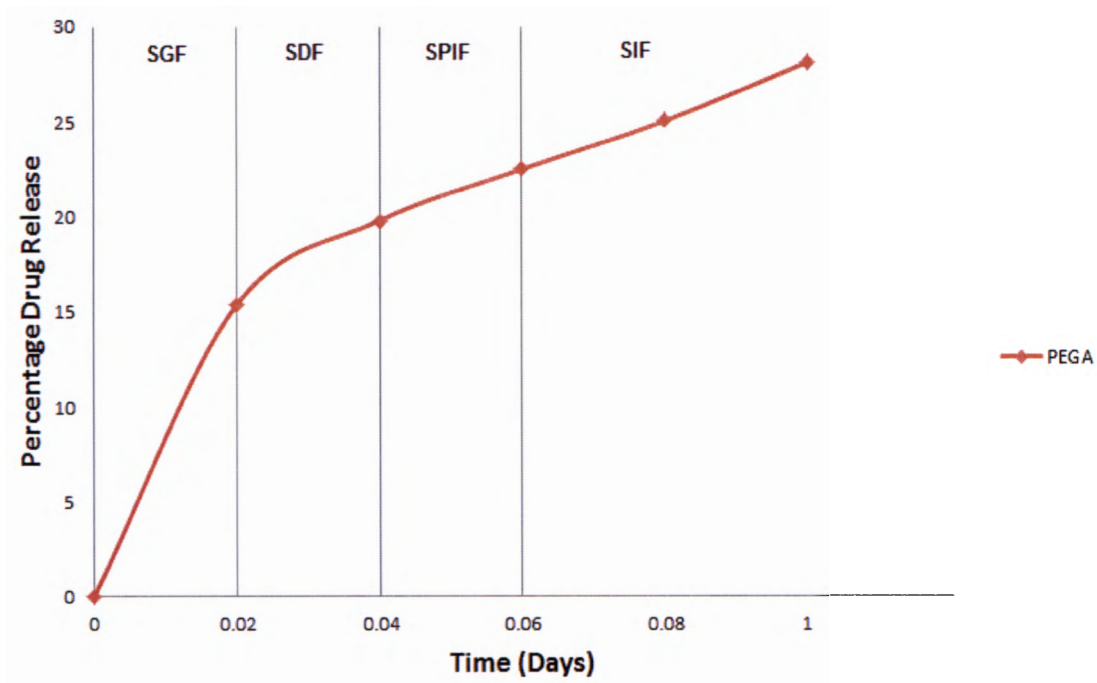



*Figure 4.5a: Dynamic release of acarbose from PEGA sample in the medium of varying pH.*

Simulation in the GIT was performed on both PEGA and PEG-A (Figure 4.5(a) and 4.5(b)). Firstly, the PEG-A released 7 % of the drug in the SGF during the first 2 h, while the PEGA released 15.3 % within the same time frame. This can be described as a burst release, which is not so beneficial in some instances, but with the observation of the PEG gel swelling analysis in the SGF, this is not unexpected. In the SDF, the release of acarbose increased up to 10.9% for the PEG-A, while the PEGA was at

19.8%. On transfer to the SPIF, a cumulative release of 13.2% was achieved from the PEG-A drug formulation, while from the PEGA drug formulation, 22.5% cumulative release of acarbose was observed. On further transfer to the SIF, the cumulative release of acarbose from the PEG-A polymer drug formulation had become 15.7%, while from the PEGA polymer drug formulation, 25.1 % acarbose was released. Finally, on leaving the polymer drug formulations for 24 h in the SIF, the cumulative drug release from the PEG-A drug formulation was 18.7 %, while that released from the PEGA drug formulation was 28.1%. This drug release experiment in the GIT further confirms that the level of control of the discharge of acarbose from the acrylated form of chemical attachment is better than that of the drug only.

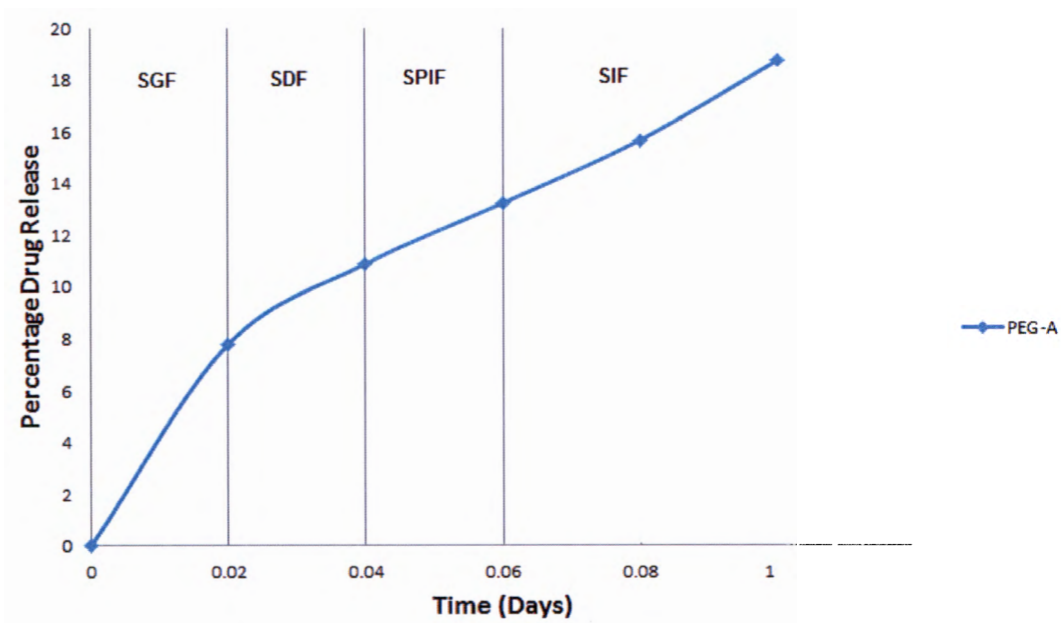



*Figure 4.5b: Dynamic release of acarbose from PEG-A sample in the medium of varying pH.*

### 4.2.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR).

The Fourier transform infrared spectroscopy spectra of the hydrogels were illustrated and the significant peaks highlighted and explained. According to Kumar and Sinha (2012), acarbose possess characteristic broad peaks at around 3371cm$^{-1}$ due to stretching of O-H, at around 1652cm$^{-1}$ showing the zone of C=C, at around 1418cm$^{-1}$ as a result of O-H bending and around 1152cm$^{-1}$ as a result of C-O stretching (Figure 4.6 (c)).

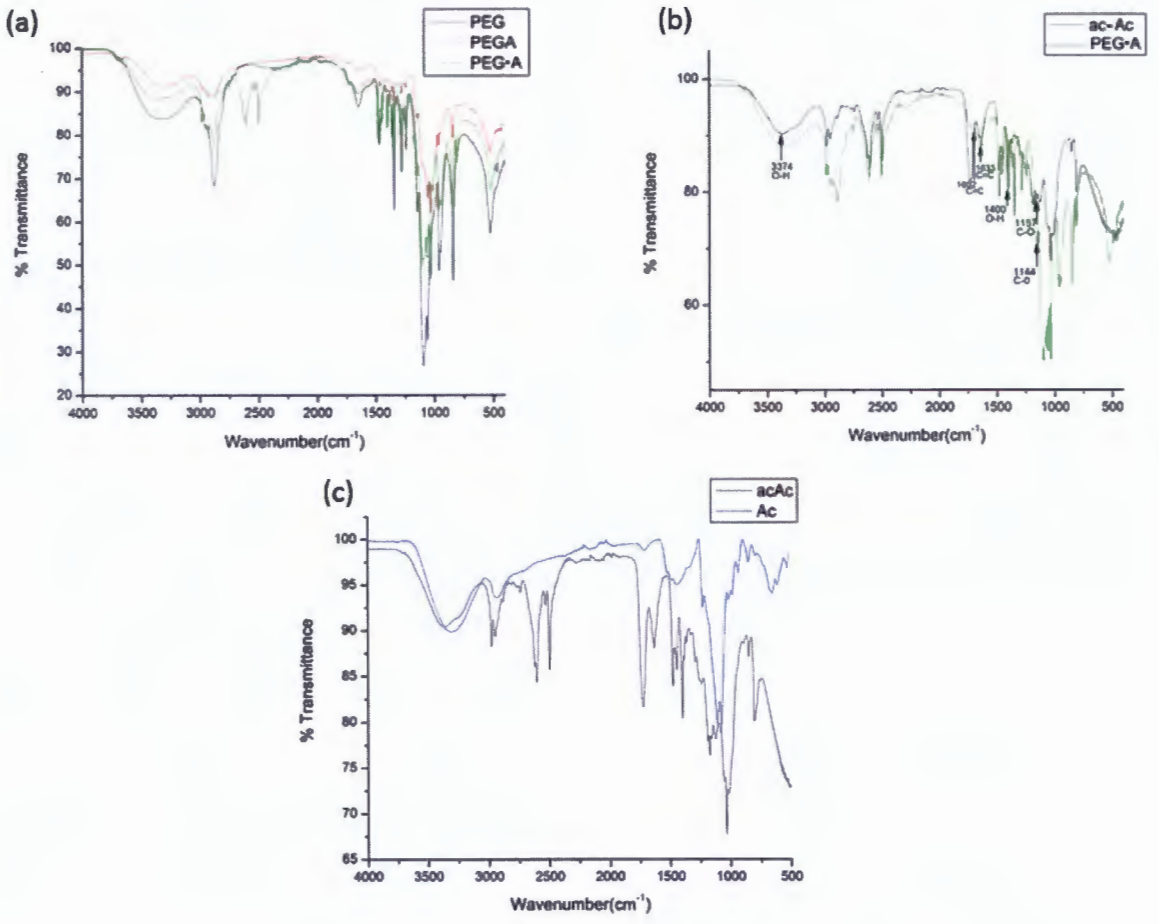


*Figure.4.6: FTIR Spectra: (a) PEG, PEGA and PEG-A gels (b)ac-Ac and PEG-A gel (c) ac-Ac and PEG-A gels*

As highlighted in Figure 4.6(b), similar peaks were observed by (Kumar and Sinha, 2012) depicting acarbose, which have been highlighted in both acrylated acarbose and the PEG-A gel and confirming encapsulation of acarbose into the PEG polymer and successful acrylation of acarbose. In Ac-ac, the broad peak was observed at 3374$cm^{-1}$ due to stretching of O-H, the zone of C=C was identified at 1633 $cm^{-1}$, while the O-H bending at around 1400$cm^{-1}$ and 1157$cm^{-1}$ as a result of the C-O stretching. On the other hand, the PEG-A gel shows corresponding broad peaks observed at 3374$cm^{-1}$ depicting the stretching of O-H and the zone of C=C was identified at 1652$cm^{-1}$, while the O-H bending at around 1400$cm^{-1}$ and 1144cm-1 as a result of C-O stretching.

Figure 4.6(a) shows the similarity of peaks observed between the PEG, PEGA and PEG-A gels, showing more closely related peaks between the PEGA and PEG-A gels as a result of acarbose present in both of them as opposed to the plain PEG polymer. When the plain PEG polymer alone was observed, similar peaks were reported by other researchers (Pramanik *et al.*, 2015; Reddy Polu and Kumar, 2011), with the main characteristic peaks of C-O-C stretching between 1050 -1150 $cm^{-1}$, C-O stretch between 1000 – 1260 $cm^{-1}$, C-H stretching between 2850 -2960 $cm^{-1}$, CH bending between 1300 -1450 $cm^{-1}$ and O-H stretching between 3200 -3600 $cm^{-1}$ (Shameli *et al.*, 2012; Tunc and Duman, 2008).

### 4.2.4 NMR SPECTROSCOPY

In addition to FTIR, Figure. 4.7 shows the nuclear magnetic resonance spectroscopy of both the initial simulated acarbose molecule and the acrylated acarbose molecule.

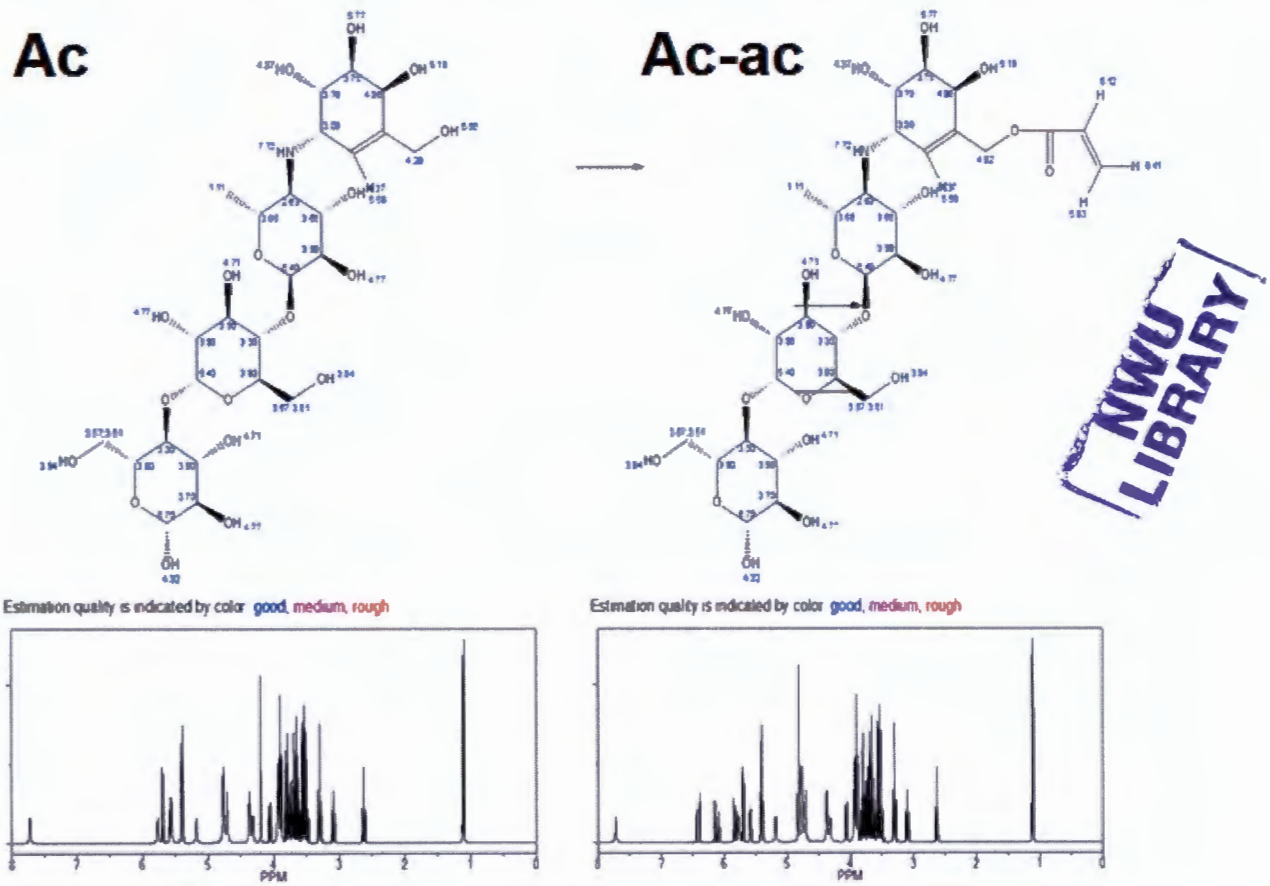





Figure. 4.7: $^{1}H$ NMR spectra estimation quality of Ac and Ac-ac with major signals.

Figure 4.8 on the other hand shows the NMR spectroscopy plots of the actual acarbose molecule and the acrylated acarbose molecules, which informed the level of acrylation that was present.

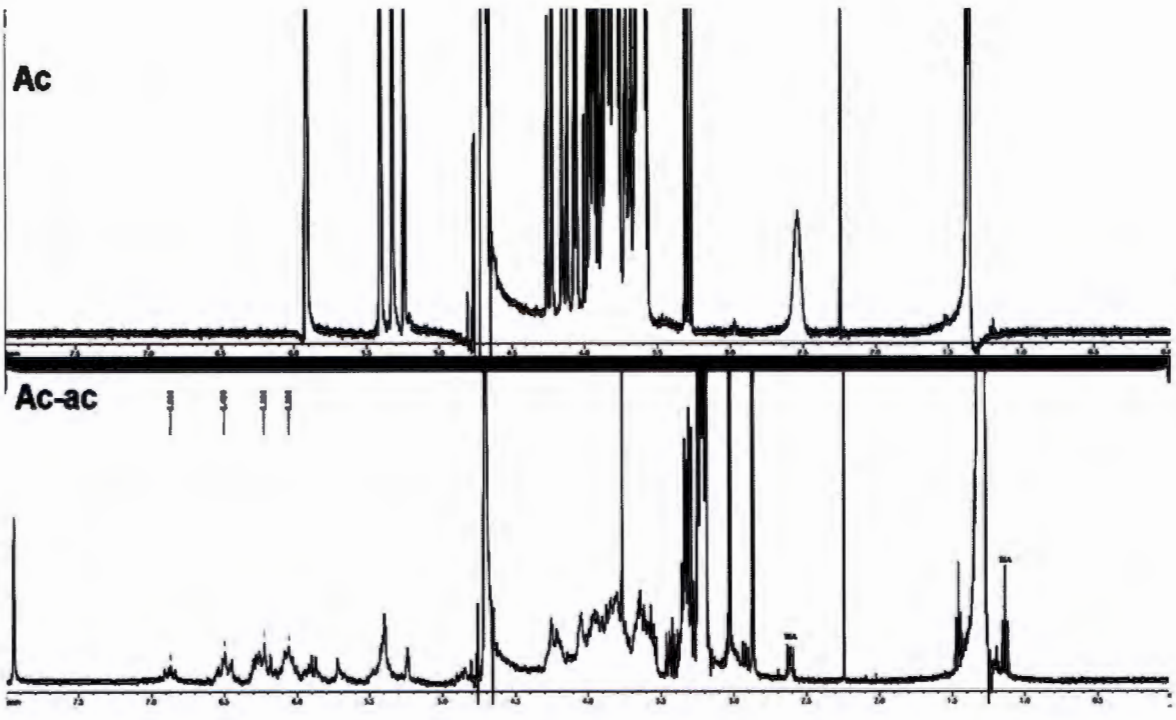



Figure. 4.8: $^{1}H$ NMR (400MHz, $D_2O$) spectra of acarbose (Ac) and acrylated acarbose (Ac-ac) with major signals.

The incorporation of the drug on the polymer was also confirmed with the results obtained from the NMR analysis, presented in Figure 4.8 (Ac and Ac-ac). The NMR spectrogram peaks for the neat polymer in Figure 4.8 (Ac) showed proton shifts at 1.11, 2.63, 3.30, 3.90 up to 7.72 for various hydrogen atoms present. Figure 4.8 (Ac-ac), which is the NMR result for the polymer-drug, showed similar proton shifts. However, there was an increase in the intensity of proton shift observed, from 4.20 to 4.82, with the hydrogen atom on the carbon bearing the OH group used for reaction with the drug molecule. This is indicative of a change in chemical environment of the hydrogen molecule. The presence of the drug molecule has induced a shift in the proton behavior of the mentioned hydrogen atom due to the presence of more electronegative atoms (i.e

a carbonyl oxygen molecule). This is a confirmation of a successful reaction and incorporation of the drug with the polymer molecule.

This information provided the basis to know the specific level of acrylation needed for specific quantity of PEG-Ac so as to have specific quantity of acarbose present for drug delivery. For it is known that PEG-Ac to PEG-SH are combined in ratio 1:1, but on acrylation and information form the NMR data, it is revealed that variation of the ratio exists, informing us on new ratios of combination to utilize for the study.

### 4.2.5 SCANNING ELECTRON MICROSCOPY.

Scanning electron microscopy micrographs of the hydrogels showing the surface morphology of the samples (with and without drugs) were viewed at an accelerating voltage of between 2 kV – 15 kV. Figure 4.9 (a-d) shows the micrographs of the plain PEG hydrogel without the drug attached. The micrographs were taken at different magnifications in order to reflect the surface morphology of the plain PEG gels before drug attachment.

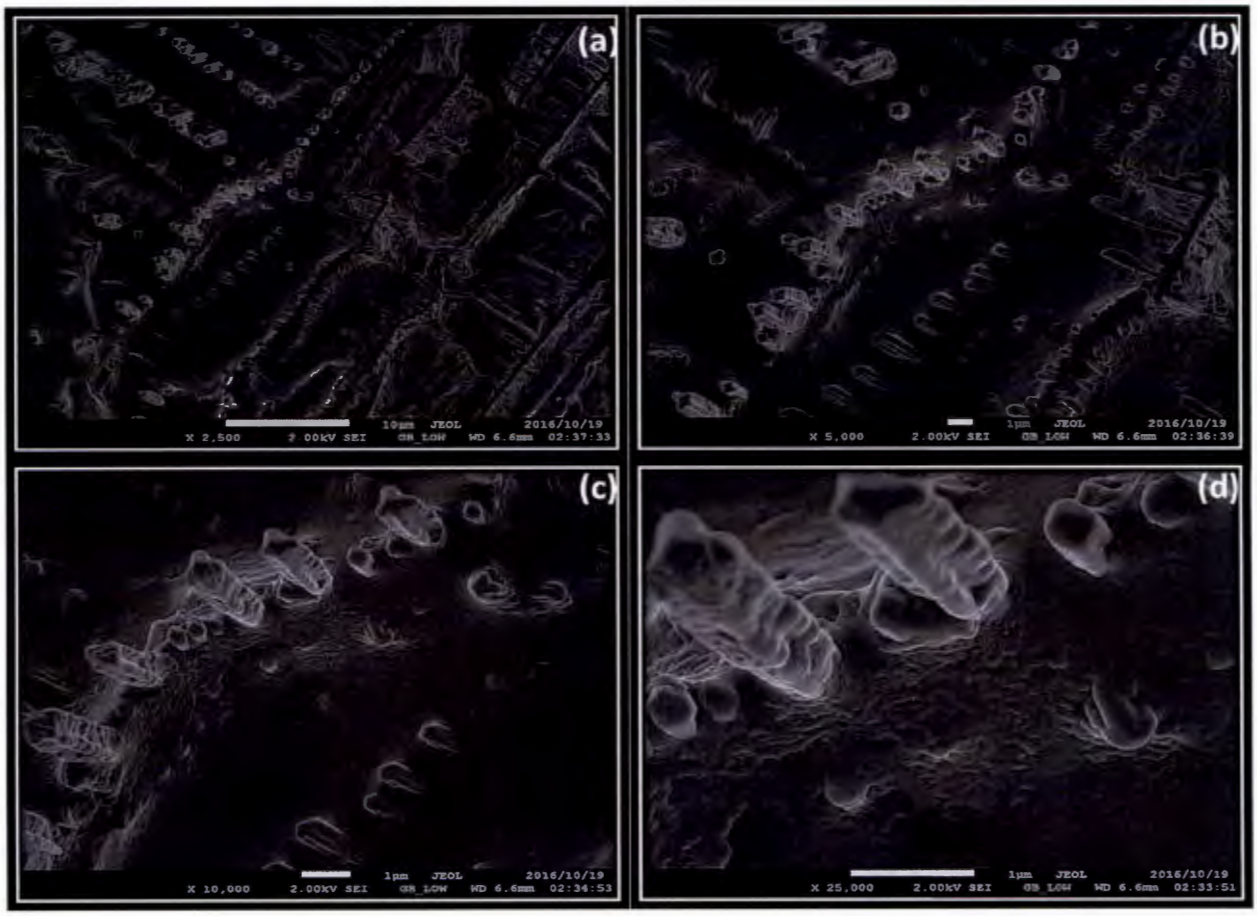



*Figure 4.9: SEM micrographs of plain PEG hydrogels.*

Figure 4.10 (a-d) shows SEM micrographs of acarbose, showing the surface morphology of the drug molecule. Irregularly spaced coarse patterns can be observed at varying magnifications. These types of images have been similarly observed by other researchers (Joshi *et al.*, 2014).

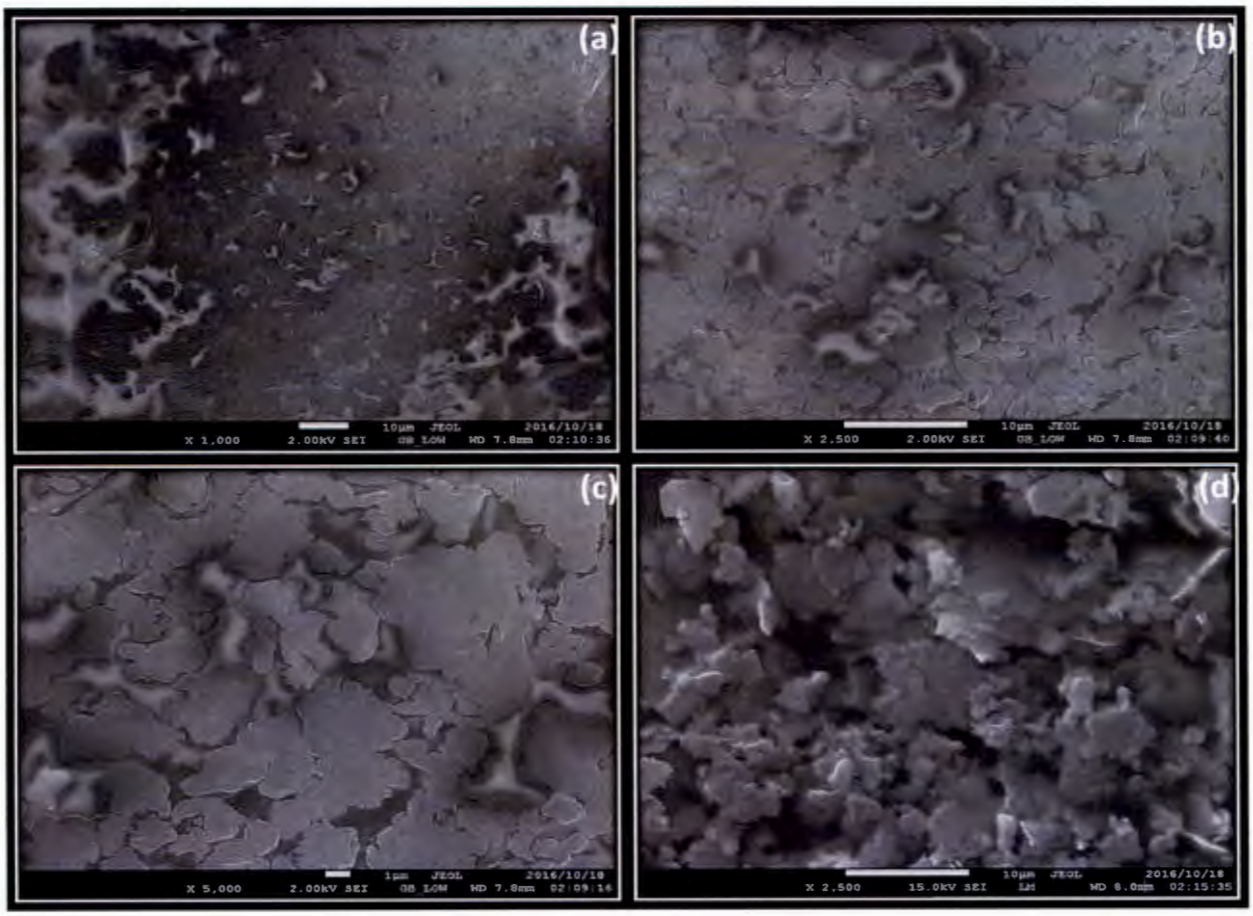



*Figure 4.10: SEM micrographs of acarbose.*

Figure 4.11(a-d) on the other hand shows the SEM micrographs of the acrylated acarbose molecule. A complete change in pattern of the micrographs is observed on acrylation. Evidence that some form of attachment has taken place is visible, with glass-like linkages between molecules. The acrylated acarbose form is hypothesized to enhance the drug release control over the acarbose molecule.

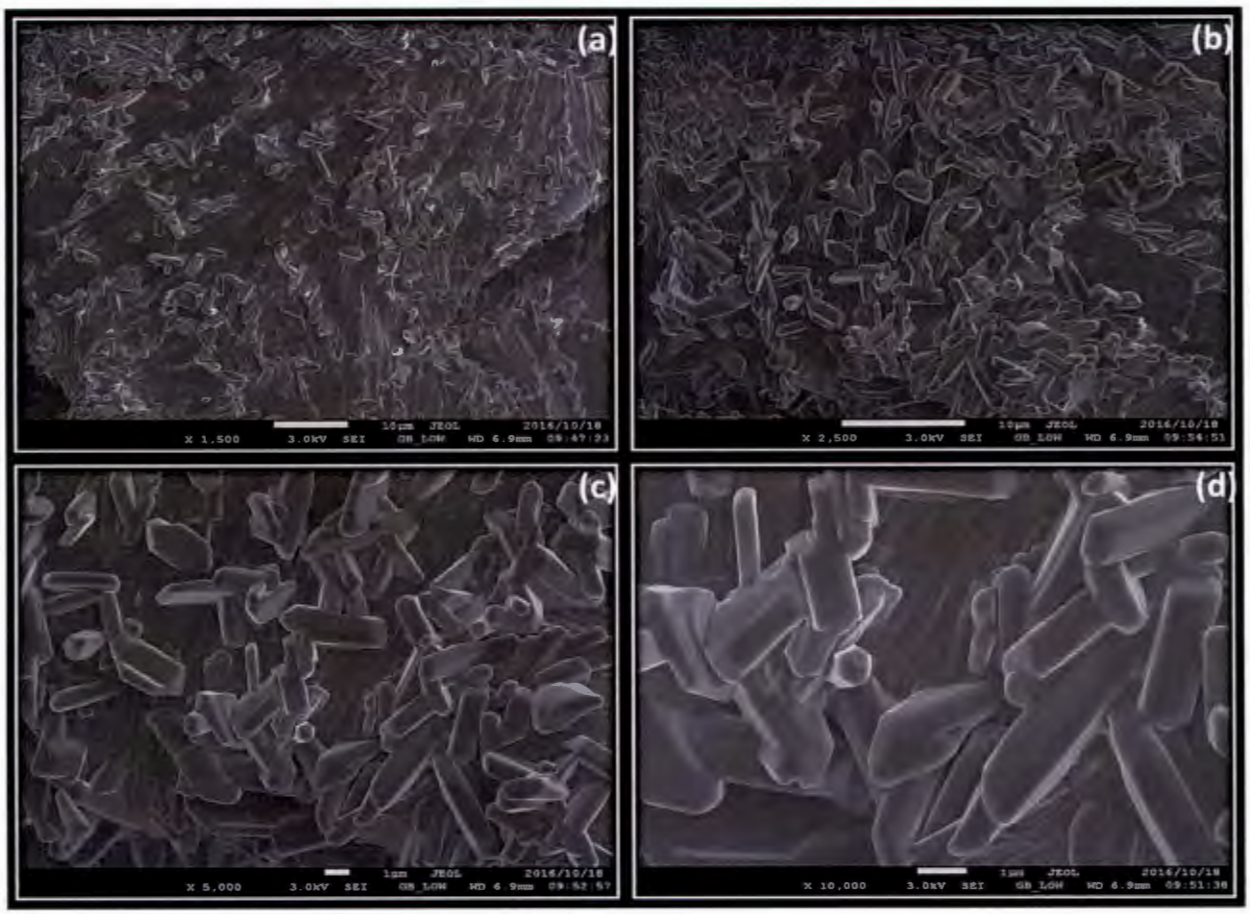



*Figure 4.11: SEM micrographs of acrylated acarbose.*

Figure 4.12 (a-d) shows the micrographs of PEGA gels after drug loading viewed at varying magnifications indicated. The cross sectional view of the PEGA gels obtained from the scanning electron microscope, reveal very irregular coarse surface patterns, but some smooth parts also exist at a lower magnification of X 1500. But on further magnification into the irregular coarse surface, it seems likely to be the technique by which the PEG and the drug bound to themselves.

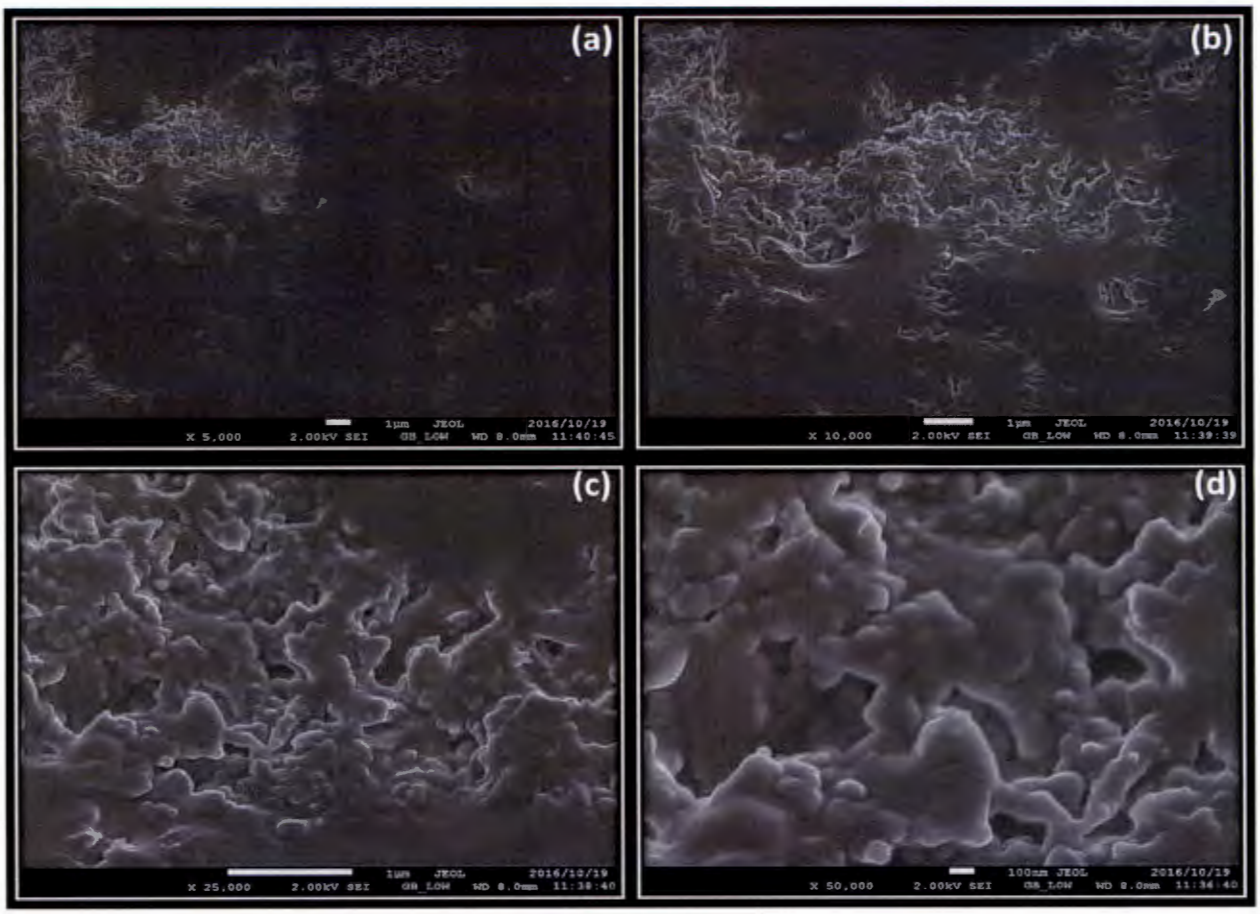



*Figure 4.12: SEM micrographs of PEGA hydrogels.*

Figure 4.13 (a-d) depicts the scanning electron microscopy images of the PEG polymer acrylated to acarbose, i.e. the PEG-A gels. There is evidently, a difference between the micrographs of the PEGA and PEG-A gels, with the molecules of the PEG-A gels shown to have images that are more tightly packed together. This is expected as the acrylated form of acarbose is expected to bind tighter to the PEG molecule as theory of swelling explains.

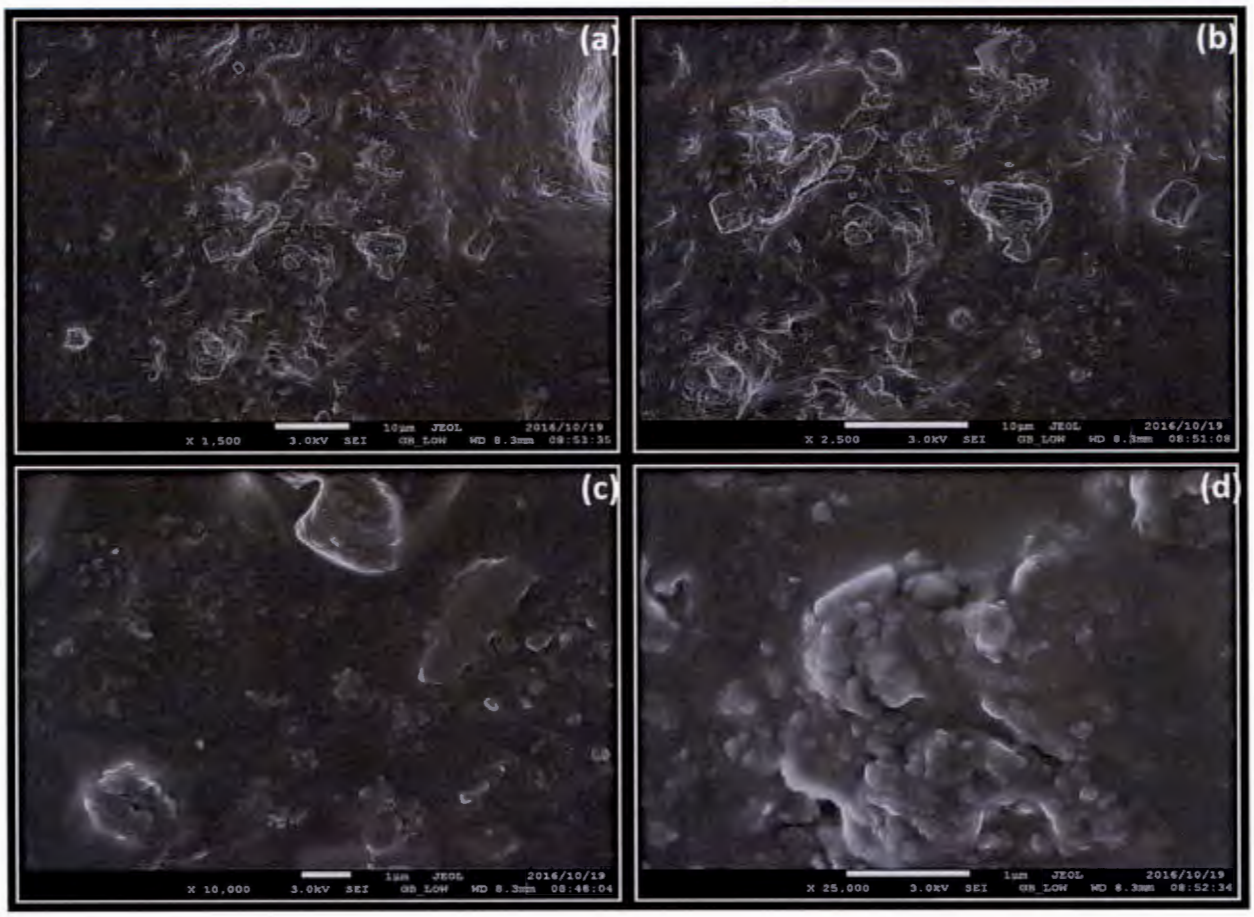



*Figure 4.13: SEM micrographs of PEG-A hydrogels.*

After performing the drug release *in vitro* in SGF and SIF conditions, the SEM micrographs of the PEGA and PEG-A gels were observed and they showed different surface morphological changes observed.

Figure 4.14 (a-d) shows PEGA after drug release in the SIF. It is clear that this polymer with acarbose dispersed, displayed some form of degradation, although minimal, when compared to Figure 4.12 shows degradation and is attributed to the SIF.

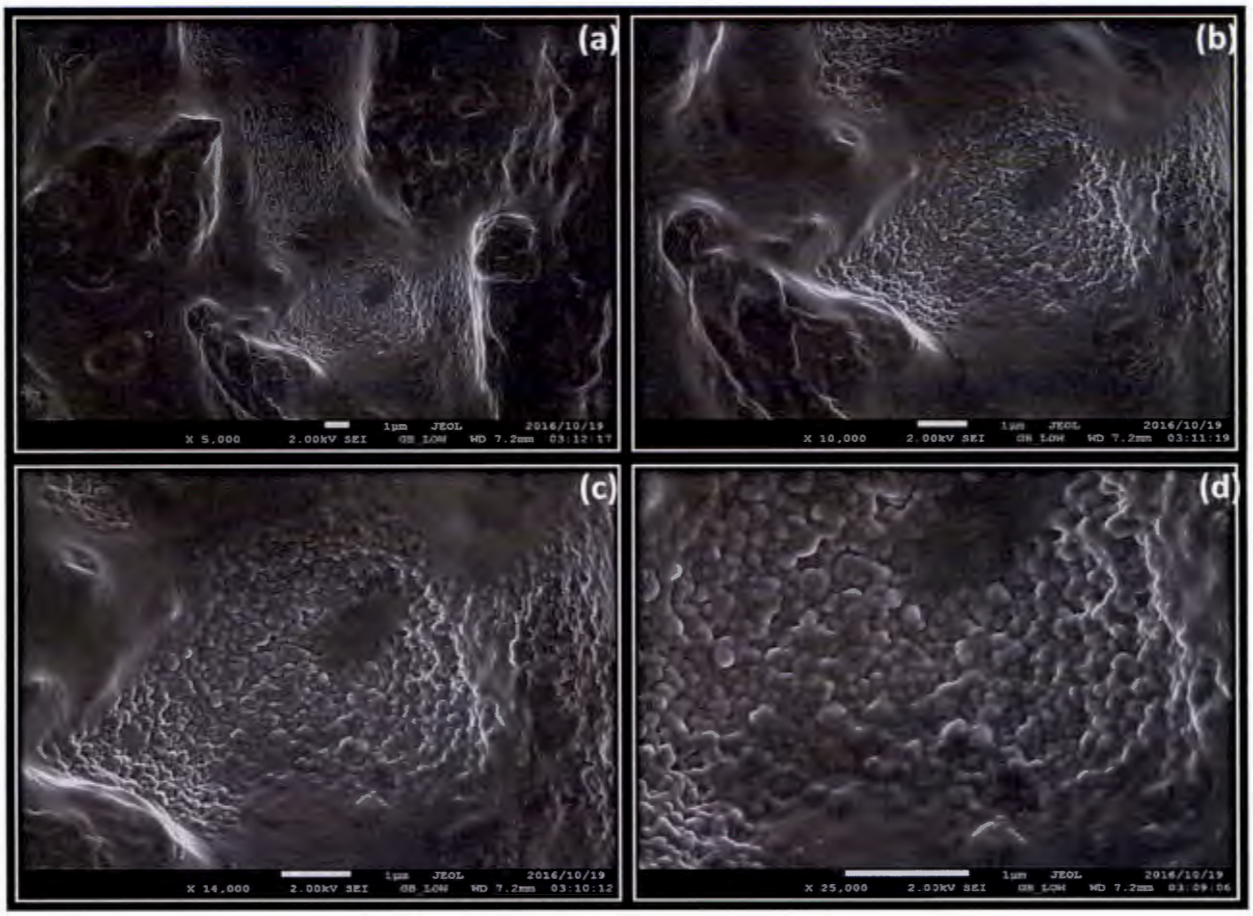



*Figure 4.14: SEM micrographs of PEGA after 48 h drug release in SIF*

Figure 4.15 (a-d) shows SEM micrographs of PEGA gels after drug release in SGF. It is evident that the level of degradation from the loosening of the existing mesh is significantly more in this buffer condition in comparison to the SIF condition. This is expected in relation to theory and swelling studies. At X25000 magnification, the straightening out of the polymer linkages can be appreciated.

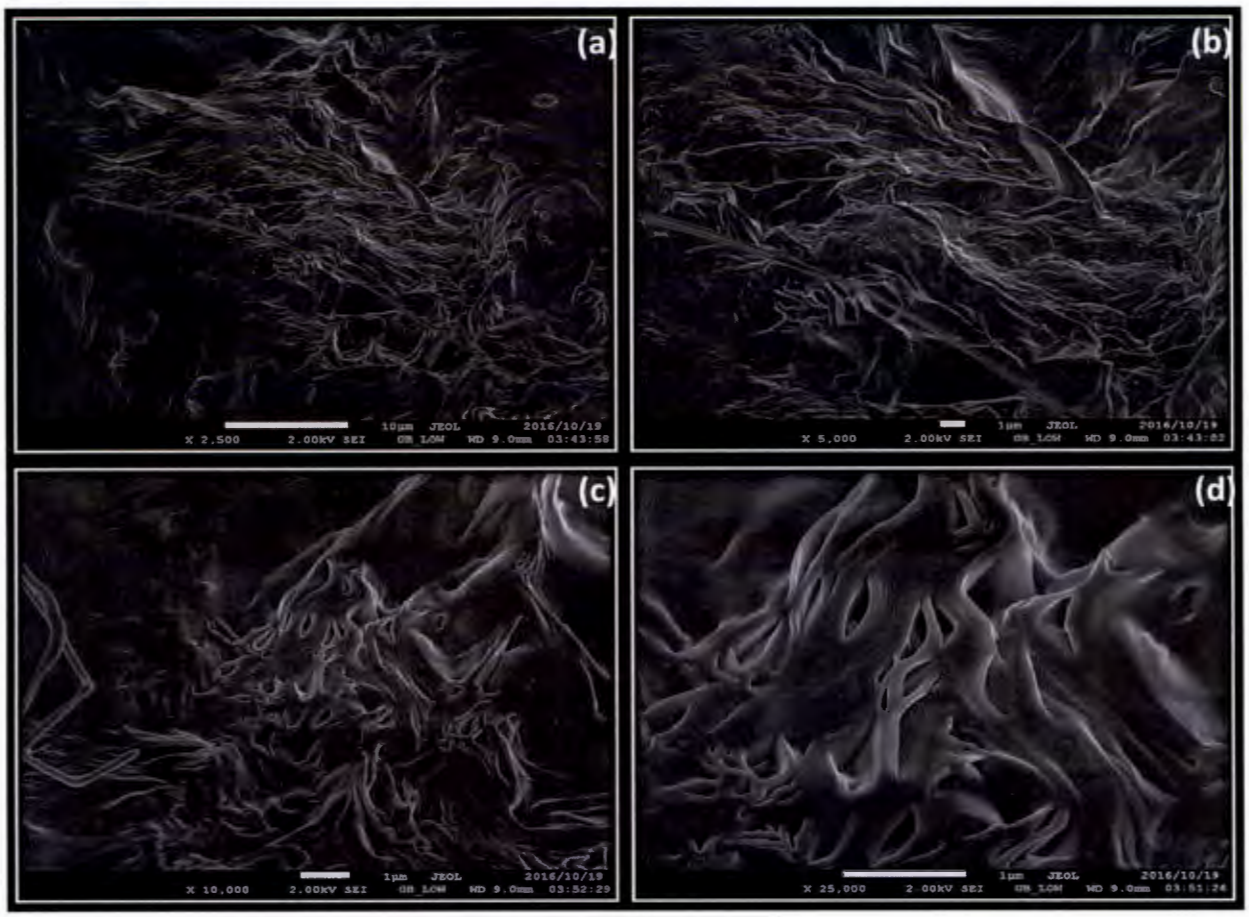



*Figure 4.15: SEM micrographs of PEGA after 48 h drug release in SGF.*

Figure 4.16 (a-d) depicts the SEM images of PEG-A gels after in vitro drug release in SIF. The image shows variation to the previous image of the PEG-A gels in Figure. 4.13, evidently as a result of the exposure to SIF. When compared to the disparity between the PEGA gels to its original SEM micrographs. It can be confirmed that the effect of the buffer on the polymer drug carrier is less dramatic in the acrylated form.

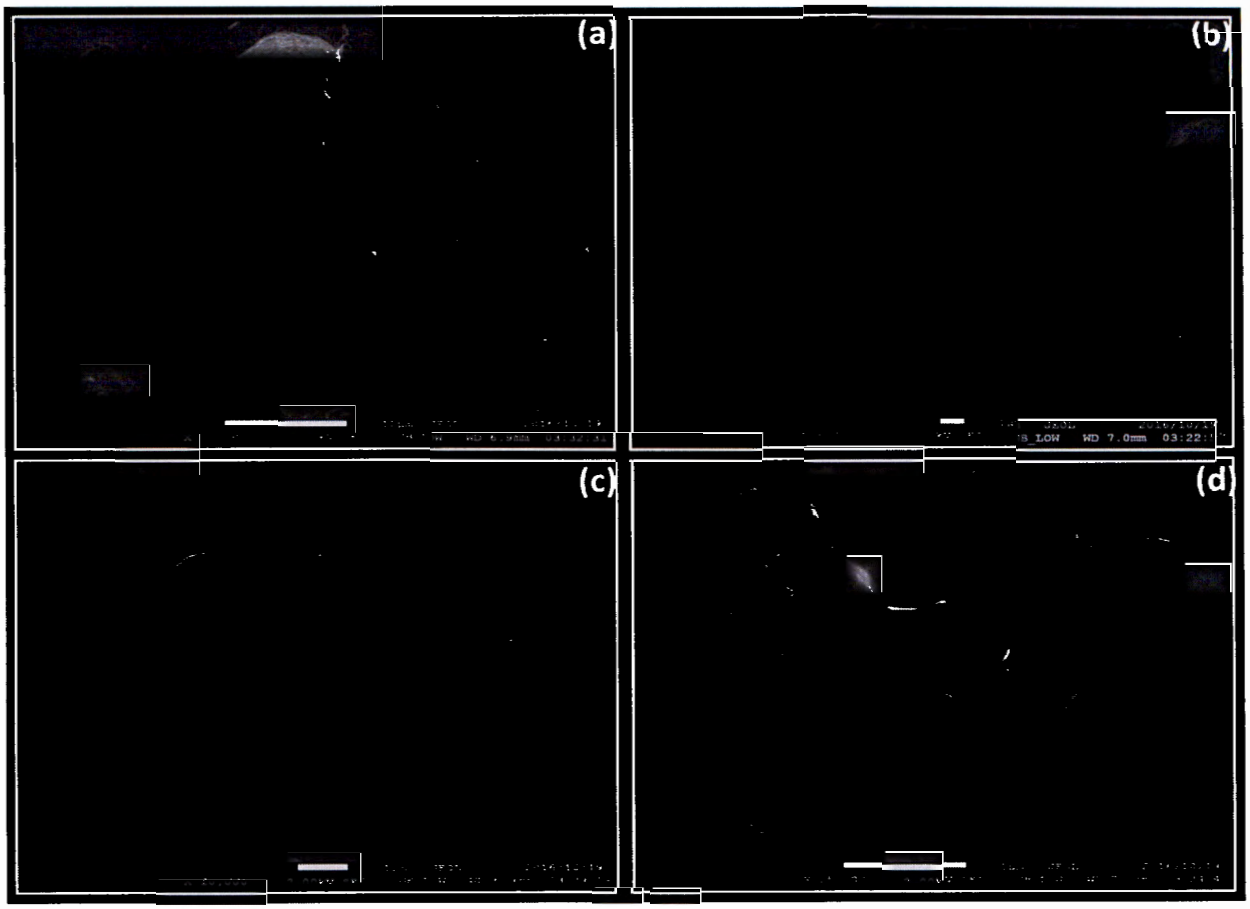



*Figure 4.16: SEM micrographs of PEG-A after 48 h drug release in SIF.*

Figure 4.17 (a-c) shows SEM micrographs of PEG-A after the *in vitro* drug release in SGF. The images reveal the degradation by loosening of the mesh within the structure of the polymer drug carrier at varying magnifications, confirming the theory and preliminary swelling and drug release data obtained in previous sections. But in comparison to the PEGA gels, the molecules of the acrylated form appeared to be better bonded together than the PEGA gels.

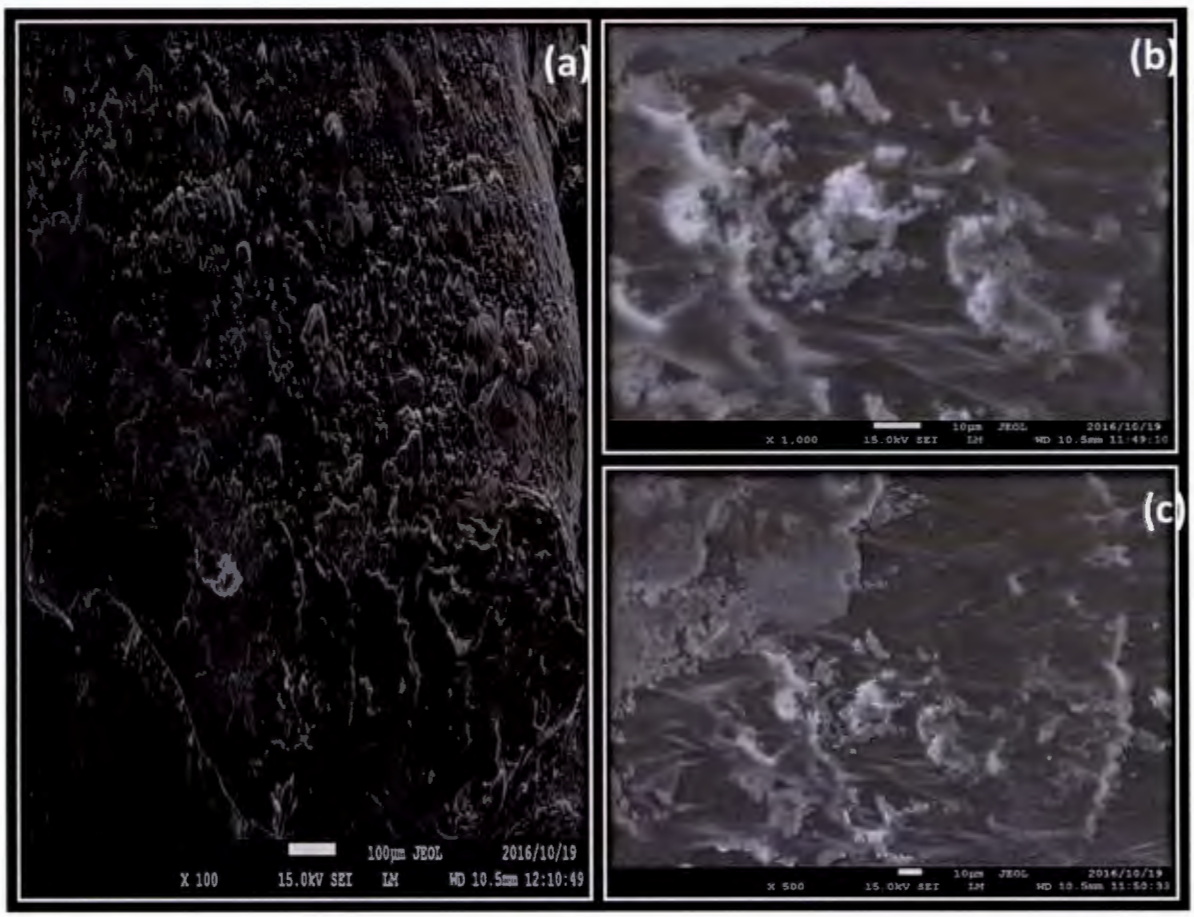



*Figure 4.17: SEM micrographs of PEG-A after 48 h drug release in SGF.*

### 4.2.6 X-RAY DIFFRACTION

XRD was used to study the change(s) in the morphological structure and the change(s) in the crystalline form, i.e. the diffraction pattern of the hydrogels pre and post drug loading into the polymeric matrix. It will be necessary to point out the broad diffraction pattern (Bragg's angle) of the gels in general; this is as result of the amorphous nature.

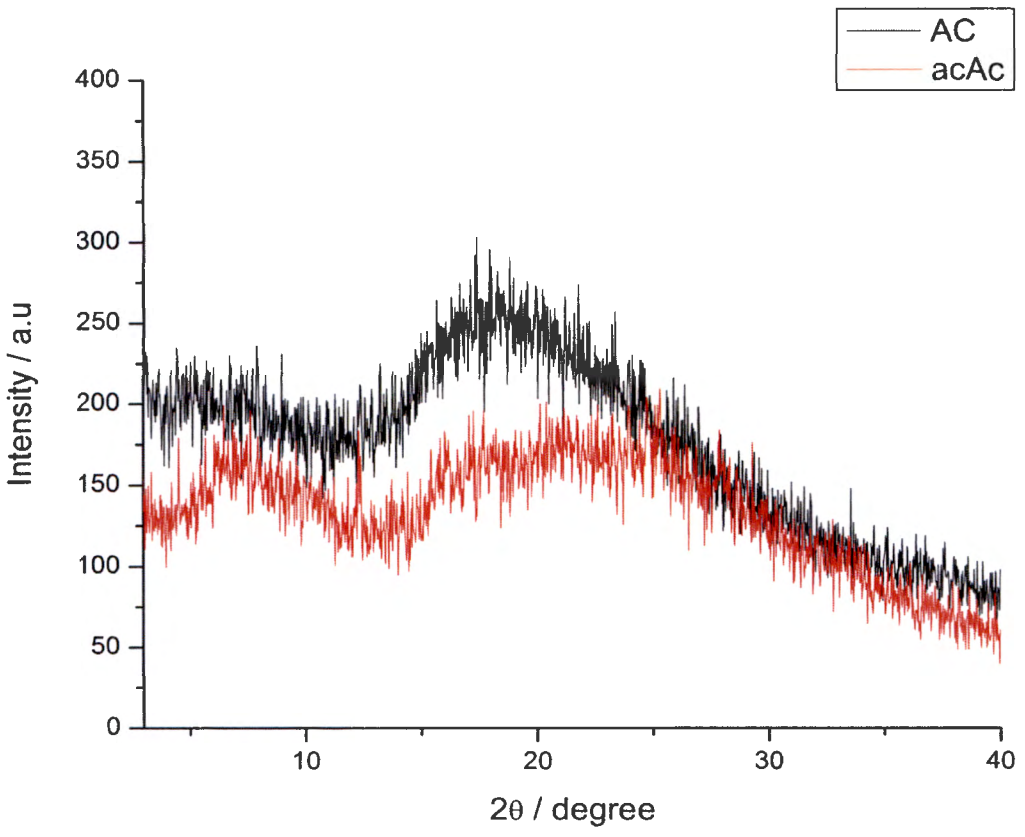



*Figure 4.18: XRD patterns of Acarbose (AC) and acrylated Acarbose (acAc).*

Figure 4.18 shows the XRD diffraction patterns of acarbose and acrylated acarbose. It can be observed that the major reflections for acarbose (AC) corresponding to the Bragg's angle 2θ were at about 6° and 18°, while for the acrylated acarbose (acAc), at about 7°, and 21°. They both show broad and diffuse peaks overall, with intensities ranging between 50 – 300.

Figure 4.19 depicts the diffraction patterns of the polymer gels prepared. The PEG polymer gel shows a broad and very diffuse peak with reflections corresponding to the Bragg's angle 2θ at 24°. The same reflection was observed in both PEG-A and PEGA gels, but was weakened.

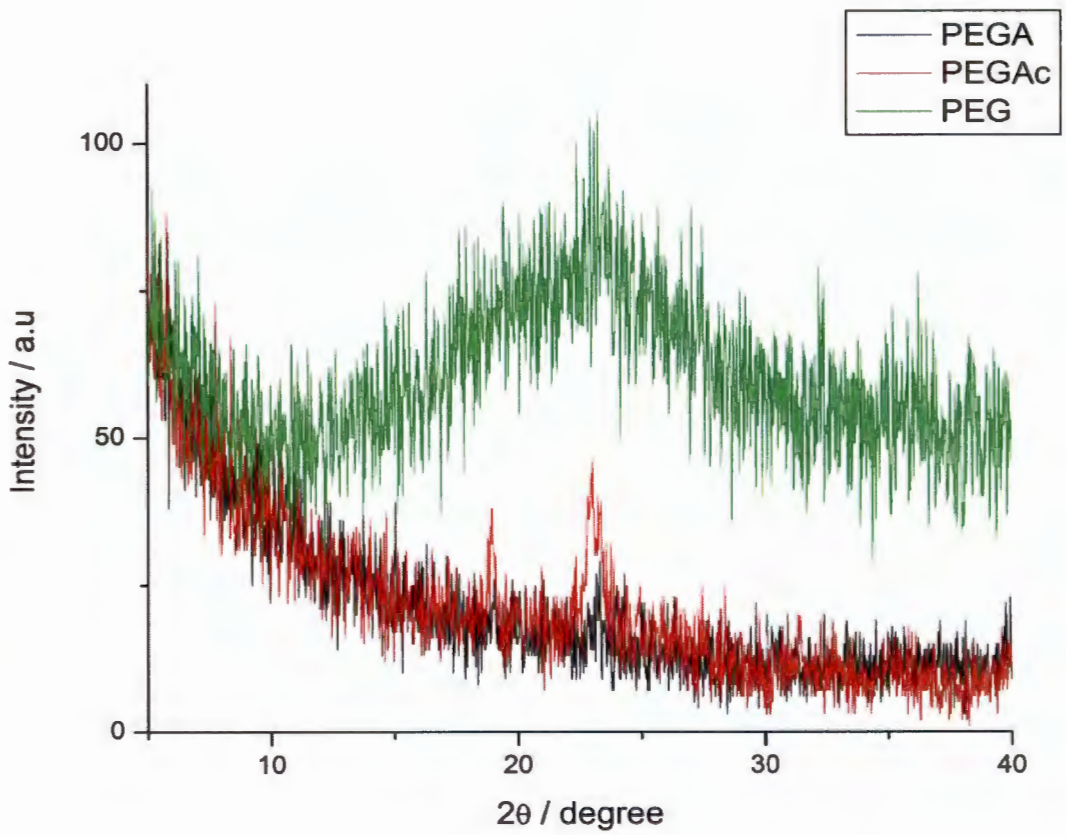





*Figure 4.19: XRD patterns of PEG, PEG-A and PEGA gels.*

The overall intensity of the reflections were influenced by the drug loading as the intensity was considerably lower between 0 – 100.

It is well known according to research findings that the intensity of the reflections is an indication of the crystallinity of a substance (Qian *et al.*, 2001). Thus it is expected that by entrapment of a lightly crystalline acarbose molecule or acrylated acarbose molecule onto PEG polymer carrier, the crystallinity would be compromised, as shown in the XRD patterns. This decrease in intensity is attributed to the interaction between the polymer molecule and that of the drug molecule(s) and it can be said that there is a relative

decrease in the molecular interactions, resulting in the reduction in crystallinity. As shown by Aderibigbe and Mhlwatika (2016), the increase in drug molecule loading increases the broadness of the peak and invariably, leading to reduction in crystallinity. This clearly shows that the drug molecules have successfully been dispersed in the case of PEGA gels and bound by acrylate links in the PEG-A gels. The lower crystalline gels show that there is evidently lower structural stability within the structure, which is desired for easy degradability of the polymer carrier molecule after drug release. Similar plots confirming the crystallinity of the acarbose molecule were observed by Joshi *et al.* (2014).

### 4.2.7. TRANSMISSION ELECTRON MICROSCOPY

Figure 4.20 (a-c) depicts the TEM images of PEGA gels. It shows that acarbose drug molecules agglomerated within the polymer, on close magnification. Thus, this suggests that the dispersion of acarbose in the polymer network was not uniform.

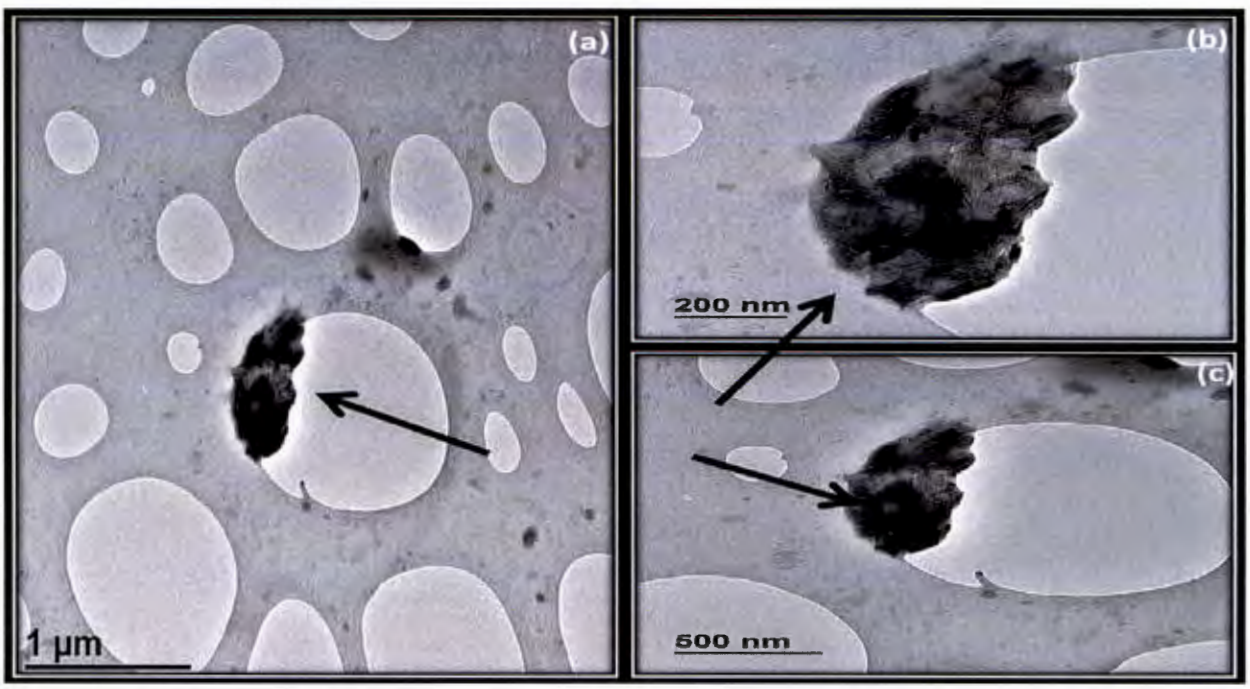



*Figure 4.20: TEM images of PEGA gels.*

The black clogged mass indicated in the micrographs, is believed to be the acarbose and it is evident that it is not fully dispersed. On the other hand, Figure. 4.21 (a-d) shows the TEM images of PEG-A gels. This obviously confirms a more uniform attachment and a predominantly uniform incorporation of acarbose within the polymer matrix.

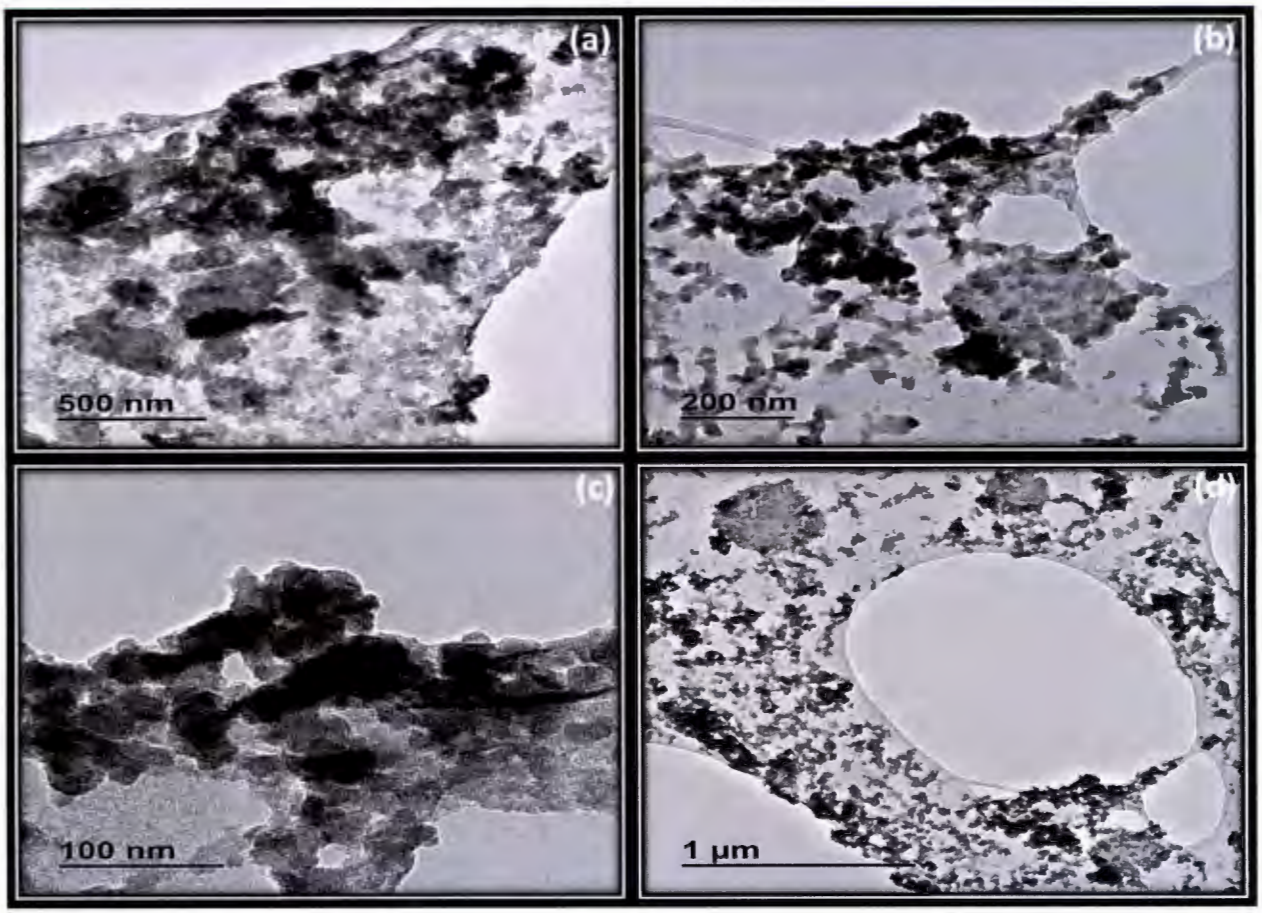



*Figure 4.21: TEM images of PEG-A gels.*

This further confirms that the dispersion of the drug alone did not suffice for uniform polymer drug integration.

### 4.2.8. THERMOGRAVIMETRIC ANALYSIS

The Thermogravimetric analysis (TGA) and the derivative TGA (DTGA) results are presented in Figure 4.22(a-b). From the results, the TGA analysis for Ac and Ac-Ac show three stages of degradations with the first stage, starting at 119$^{o}$C and 50$^{o}$C for Ac and Ac-Ac respectively. At this stage, it can be concluded that the occluded water molecules present inside Ac and Ac-Ac were removed. From the results, it was observed that the weight loss is about 5%. However, if it is the drug molecules that are degraded at the first stage as observed, it can be said that the thermal stability of the drug is very low. The DGTA result for Ac shows a small peak at 120$^{o}$C indicative of the decomposition of a small molecule from the drug material. The result for sample PEGA and PEG_A show improved thermal stability of the drugs when loaded onto the polymer as seen in Figure 4.22 (a-b).

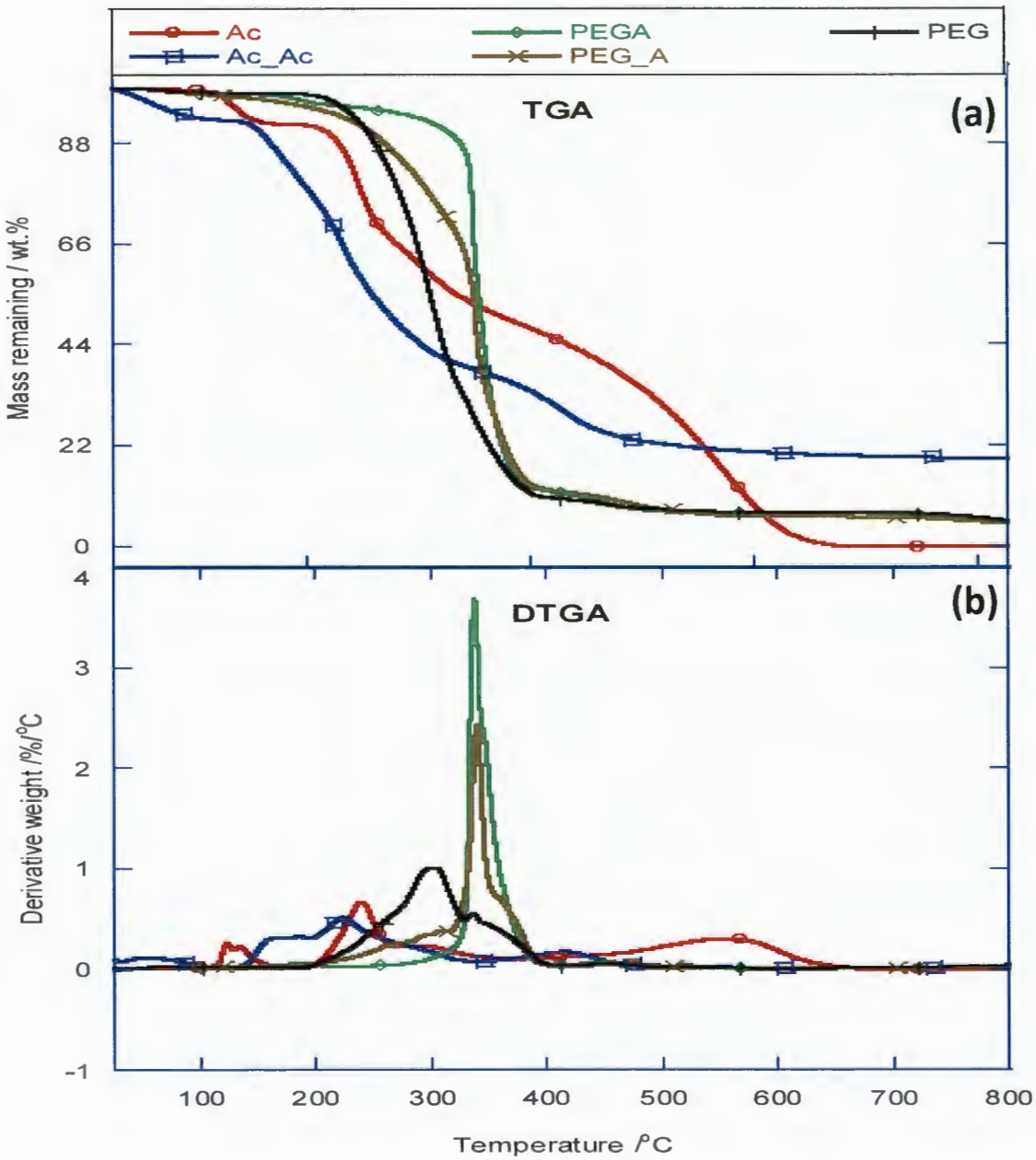



*Figure 4.22: (a) TGA and (b) Derivative TGA thermographs of Ac, Ac-Ac, PEGA, PEG-A and PEG hydrogels.*

This must have been as a result of the better thermal stability of the substrate, the plain PEG polymer over the pure Ac drug and Ac-Ac. The thermal stability of the polymer

loaded with Ac and Ac-Ac increased by approximately 100% from 119 to 330°C for Ac. This shows that the PEG polymer protected the drug sample thermal degradation at elevated temperature, however at 350°C, the polymer loaded drug showed maximum degradation (Figure 4.22 b). Therefore it can be concluded that the PEG polymer is a good platform for the loading Ac drug because of the improved thermal stability of the drug.

### 4.2.9. DIFFERENTIAL SCANNING CALORIMETRY

The DSC results for the samples are presented in Figure 4.23. Figure 4.23(a) shows the behavior of the materials during heating while Figure 4.23(b) shows the behavior during cooling. From the endothermic process (heating), it was observed that the pure drugs were generally amorphous in nature. The glass transition temperatures, $T_g$s were observed at 80.0 °C and 79.8 °C for Ac and Ac-Ac respectively. The pure polymer was observed to be highly crystalline with sharp and narrow peak indicative of a small range melting temperature. However, the polymers loaded with the drugs showed peak broadening due to the presence of the drugs.

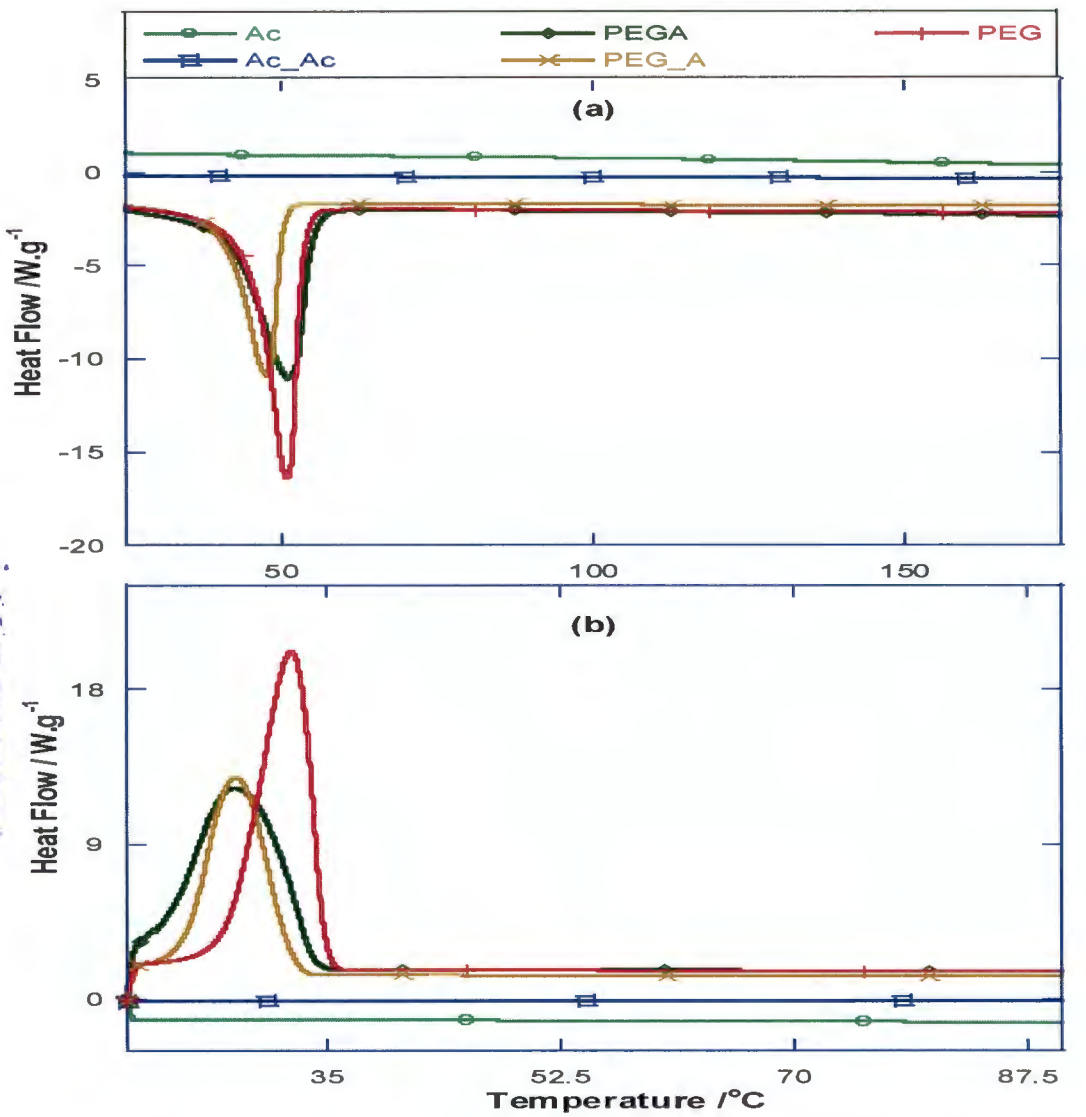





*Figure 4.23: DSC thermographs of Ac, Ac-Ac, PEGA, PEG-A and PEG hydrogels*

*(a) Behavior during heating (melting)*

*(b) Behavior during cooling (crystallization)*

The polymer loaded with the pure drug was observed to have led to about 8 % reduction in the initial melting temperature, i.e., from 45.3°C for plain PEG to 41.8 °C PEGA while the polymer loaded with the acrylated drug led to ~ 10% reduction in the

initial melt temperature, i.e., from 45.3 $^{o}$C to 41.0 $^{o}$C. This drop in the melting temperature starting-point is believed to have resulted from the presence of the amorphous drug which may have slowed down the formation of crystals and also inhibited to growth of the crystals formed leading to the presence of small crystals which started to melt at lower temperature. The melt enthalpy was observed to have conformed to this trend. The melt enthalpy for the plain polymer was found to be 81.8 J/g and 79.4 J/g for the crystallization enthalpy. These values were observed to have decreased due to the incorporation of the drug in PEGA and the acrylated drug (PEG-A). The melting enthalpy was observed to have decreased by ~ 10.5 % and ~ 23.1 % for PEGA and PEG-A respectively. This is an indication of the amount of reduction in the sizes of the crystals formed when the drugs were loaded. From the first DSC run as seen in Figure. 4.23 (a), the melting peaks of the PEG polymers were observed to be present as well as the amorphous reflection for the pure drug and that of the acrylated drugs at ~ 80 $^{o}$C. This was an indication that there was little or no chemical interaction between the polymer and the loaded drugs. However, the acrylated drug led to a decrease in the % crystallinity however greater than that observed with the pure drug as shown in Table 4. This may be a direct result of the molecular structure of the acrylated drug rather than any chemical interaction with the polymer molecules. Furthermore the increase in the % amorphous content may strongly affect the rate of release of the drug, as will be discussed later.

*Table 4.3: Glass transition temperature, Melt and Crystallization temperature and enthalpy for the samples.*

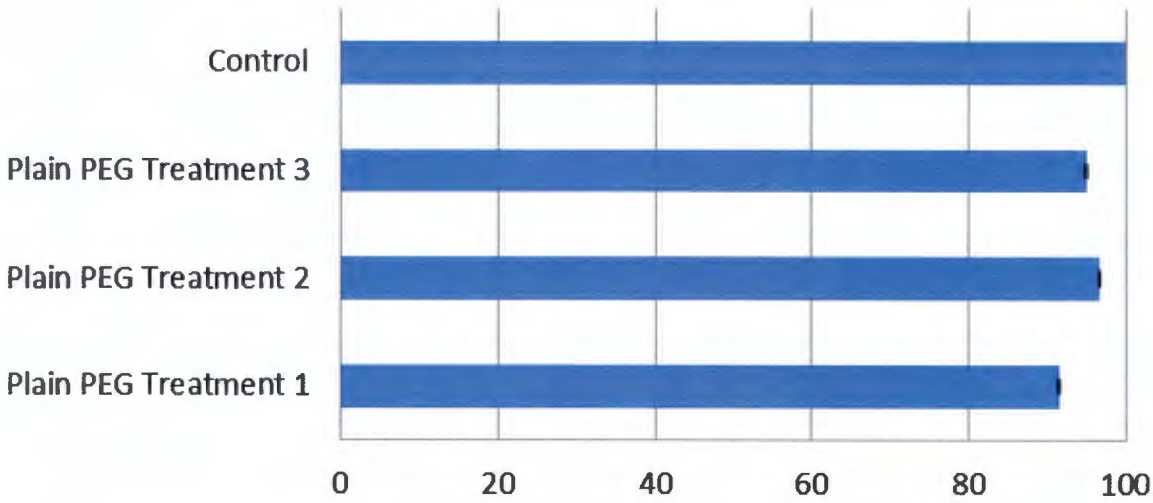
| Sample | | Melting temperature | | | Crystallization temperature | | |
|---|---|---|---|---|---|---|---|
| | $T_g$ (°C) | $T_i$ (°C) | $T_m$ °C) | $H_m$ (°C) | $T_{ci}$ (°C) | $T_c$ (°C) | $H_c$ (°C) |
| **Ac** | 81.0 | - | - | - | - | - | - |
| **Ac_Ac** | 79.8 | - | - | - | - | - | - |
| **PEG** | - | 45.3 | 50.7 | 81.7 | 34.8 | 32.3 | 79.4 |
| **PEGA** | - | 41.8 | 50.9 | 73.1 | 33.8 | 28.3 | 60.0 |
| **PEG-A** | - | 41.0 | 47.6 | 62.8 | 32.3 | 28.2 | 58.4 |

### 4.2.10 TOXICITY – MTT ASSAY

The MTT assay as earlier described provides a versatile method by which cells could be evaluated upon various treatments (Ahmadian *et al.*, 2009).

The c2c12 cells were cultured and after being differentiated, they are exposed to the MTT assay protocol described in chapter 3. The percentage cell viability (% cell viability) of the c2c12 cells after treatment with plain PEG gels, in order to confirm non toxicity to the c2c12 cells are shown in Figure 4.24.

In comparison to the positive control, > 90 % viability of the cells were observed, when they are exposed to the plain PEG polymer for the same period of time. This is to confirm that the plain PEG polymer in itself is non-toxic to the overall viability of the c2c12 cells. Similar non –toxic results have been reported by other researchers who employed PEG-based materials for cytotoxicity capacity in similar cell line (Hong *et al.*, 2009) and other cell lines (Liu *et al.*, 2014).




| | Plain PEG Treatment 1 | Plain PEG Treatment 2 | Plain PEG Treatment 3 | Control |
|---|---|---|---|---|
| % Cell Viability | 91.45932285 | 96.64978574 | 94.97022544 | 100 |

*Figure 4.24: MTT-assay toxicity test on c2c12 cells.*

## 4.3 DISCUSSION.

The pH swelling analysis, *in vitro* drug release behavior and release kinetics suggested the pH sensitive capacity of the carrier system and it revealed that the PEG-A hydrogels exhibited a superior pH-induced drug release profile, which can trigger releasing the drug at physiological pH values at much better control levels than the PEGA hydrogels or free acarbose. Further characterization of the PEG-drug carriers revealed an added advantage of acrylation of the drug to the polymer and thermal stability of the acrylated form over the other groups is worthy to note. The surface and transverse morphologies also clearly show the advantage of acrylation over dispersion.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 CONCLUSION

The research topic read 'design and biological evaluation of polyethylene glycol gel containing acarbose' and this was broken down into objectives which were achieved individually and explained in previous chapters. The aim of the research was firstly to potentially establish that acrylation of acarbose to specific functionalized polyethylene glycol hydrogels would improve the bioavailability of acarbose *in vitro; b*ioavailability in the sense of superior drug release profile and kinetics, which can then suggest for application of these findings in the future to *in vivo* hypaeglycaemic models to possibly witness potential sustained reduction in blood glucose levels.

The results section showed the potential advantage of acrylation of acarbose to functionalized polyethylene glycol acrylated and thiolated polymer hydrogels, when compared to the PEG acarbose dispersed counterpart. Confirmation of the presence of acarbose in the acrylated form was confirmed by several characterization techniques and the swelling ability of the hydrogel was determined. This swelling ability is known to give more insight into the drug release capacity of the hydrogel and it is evident from the results that by tunable acrylation, the release of the drug from the polymer can be controlled, as opposed to the dispersed drug form, which cannot be controlled. The burst release which is very common to the regular dispersion method can be avoided and potentially controlled. The thermal stability of the acrylated form of acarbose is shown to be more superior that the dispersed form and the morphology (surface and

transverse) of both hydrogels were compared by both TEM and SEM. Finally, MTT assay test was performed to investigate the potential toxicity of the polymer hydrogels to c2c12 myoblast muscle cells and there was no significant reduction in cell viability with the treatment with the PEG hydrogels as shown in the results section.

## 5.2 RECOMMENDATION

Considering the research objectives were fully met, the findings have revealed the potential benefit of acrylation of drugs to polymers, and this could be applicable to numerous other drug forms which can be acrylated. Already this is evident from other researchers acrylating heparin and utilizing the tunability of polymer chemistry to influence further its application. A further study is recommended to optimize the acrylation of acarbose to the functionalized PEG polymers and also potentially *in vivo* studies will be recommended for the confirmation of longer periods of sustained action of acarbose within the gut of the model animals.

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