CHARACTERISATION AND APPLICATION OF NATIVE AND CROSS-LINKED CASHEW (*Anacardium occidentale* L.) GUM IN TABLETTING

BY

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A DISSERTATION SUBMITTED TO THE POST GRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICS

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DECLARATION

I declare that the work in this Dissertation entitled "Characterisation and Application of Native and Cross-linked Cashew (*Anacardium occidentale* L.) Gum in Tableting" has been carried out by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, under the supervision of Prof. A. R. Oyi (Mrs), Dr. A.B. Isah and Dr. I. Odidi. The information derived from literature has been duly acknowledged in the text and list of references provided. No part of this Dissertation was previously presented for another higher degree or diploma in any University.

_____ _____

Abdulrahman ABDULSAMAD Date

CERTIFICATION

This Dissertation entitled "Characterisation and Application of Native and Cross-linked Cashew (*Anacardium occidentale* L.) Gum in Tableting" by Abdulrahman ABDULSAMAD meets the regulations governing the award of the Doctor of Philosophy in Pharmaceutics of Ahmadu Bello University and is approved for its contributions to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents **Alhaji Abdulsamad** and **Hajia Hassu**, my wife **Bilkisu** and my children **Adam** and **Muhammad**.

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All praises are due to the almighty **Allah**, the all knower and the master provider, by whose grace this work was accomplished. Peace and blessings be upon His noble prophet **Muhammad** (SAW).

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ABSTRACT

The exudate from cashew tree, referred to as cashew gum (CG) had been investigated for use as binder in conventional tablet formulations. In this study, cross-linked derivatives of the gum were synthesized and investigated for their physicochemical and physicotechnical properties and applicability in modifying drug release in tablet formulations. The crude dried latexes of CG were purified by extraction using acetone. Two cross-linked derivatives of the gum were synthesized by heating (a), a 1:1 weight ratio of CG and citric acid (CTR) at 140 ^{o}C for 30 min and (b), a 1:1:1 weight ratio of CG, CTR and glycerol (GLY) at 170 ^{o}C for 30 min. In each case, monobasic sodium phosphate was used as catalyst at 2 %w/w concentration. The cross-linking was demonstrated qualitatively by Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) and ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) and quantitatively by polymer yield. Determinations of moisture content, moisture loss on drying, swelling ratio, water holding and water sorption capacities were used to characterize the derived polymers. Sub-acute toxicity studies were carried out to ascertain the safety or otherwise of the cross-linked derivative, CrosCCG. Wistar rats were fed with the polymer over a 28-day period and blood samples from the animals were assessed for changes in hematological and biochemical parameters. The purified CG and its derivatives were used to formulate tablets of Venlafaxine hydrochloride (Ven. HCl), a water soluble antidepressant, using the wet granulation method. Increasing concentrations of CG and its admixtures with MCC PH 102 were used as binder/matrix former in formulating the tablets. The synthesized derivative, CrosCCG, was assessed for drug release modification when used either alone or in admixtures with HPMC ER (K100). The drug release

enhancing property of the derivative was also investigated and compared with some known superdisintegrants, Croscamellose and Crospovidone at 3 %w/w disintegrant concentration.

Results obtained revealed that cross-linking of CG with CTR yielded an esterified derivative that was insoluble in all common solvents. The esterification reaction occurred between –OH of the sugar from the gum and –COOH of CTR. While being insoluble, on contact with water, however, the polymer sorped water and swelled. Cross-linking of CG with a combination of CTR and GLY yielded a polymer with improved yield and swelling potentials. Toxicity studies showed the polymer to have no apparent harmful effect on the animals and did not cause changes in the blood parameters tested. When used in tableting, CG was found to form good tablets of Ven. HCl with a crushing strength of 5.9 KN and friability of 0.03 %. Increasing concentration of CG or its admixture with MCC PH 102 offered no added advantage. CrosCCG could not modify the release of drug from the formulated tablets when used alone. Admixtures of CrosCCG at concentrations of 15 and 25 %w/w respectively with HPMC K100 at 7.5 %w/w showed a further delay in release of the drug. Concentrations lower than 10 %w/w of CrosCCG, however, showed a release enhancing ability. At 3 %w/w concentration, CrosCCG surpassed both Croscamellose and Crospovidone in enhancing drug release as observed from a 30 min *in vitro* dissolution data. It can thus be concluded that, this work has led to the discovery of a safe novel cross-linking process that uses safe and environmentally friendly cross-linking agents in derivatisation processes. Cross-linking CG using this method has led to the production of a powerful drug release enhancing agent.

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ACRONYMS AND ABBREVIATIONS

API	Active pharmaceutical ingredient
Ven. HCl	Venlafaxine hydrochloride
CG	Cashew gum
CrosCC	Cashew gum cross-linked with citric acid
CrosCCG	Cashew gum cross-linked with citric acid and glycerol
CrosCE	Cashew gum cross-linked with epichlorohydrin
EPC	Epichlorohydrin
PCA	Polycarboxylic acids
ADA	Adipic acid
SCA	Succinic acid
CTR	Citric acid
BTCA	Butanetetracarboxylic acid
MCC	Microcrystalline cellulose
HPMC	Hydroxypropyl methylcellulose
LV	Low viscosity
ER	Extended release
HCl	Hydrochloride
ODT	Orally dissolving tablet
MTD	Maximum tolerable dose
MEC	Minimum effective concentration
GIT	Gastro intestinal tract
R & D	Research and development
DSC	Differential scanning calorimetry

UV Vis	Ultraviolet visible region
FTIR	Fourier transform infrared
GC	Gas chromatography
KF	Karl Fisher
SS ^{13}C NMR	Solid state carbon 13 nuclear magnetic resonance
min	Minutes
s	Seconds
h	Hour
g	Gram
g/s	Gram per second
g/ml	Gram per milliliter
Kg	Kilogram
KgF	Kilogram force
rpm	Revolutions per minute
r^2	Correlation coefficient
$T_{80\%}$	Time of 80 % dissolution
t	Time
Vs	Versus
Ft	Feet
In	Inch
µm	Micrometer
ml	Millimeter
cm	Centimeter
m	Meter
h	Height

d	Diameter
r	Radius
G	Granule
T	Tablet
θ	Theta
L	Liter
^{o}C	Degrees Celsius
%	Percent
NaOH	Sodium hydroxide
NaH_2PO_4	Sodium dihydrogen Phosphate
KBr	Potassium bromide
w/w	Weight over weight
v/v	Volume over volume
USA	United States of America
USP	United States Pharmacopoeia
-OH	Hydroxyl
-COOH	Carboxyl
TiO_2	Titanium dioxide
DI	Deionised
PCV	Packed cell volume
TWBC	Total white blood cells
ALP	Alkaline phosphatase
AST	Aspertate transaminase
ALT	Alanine transaminase
SGOT	Serum glutamic oxaloacetate transaminase

SGPT	Serum glutamic pyruvate transaminase
etc	Etcetera
e.g.	Example
CO_2	Carbon dioxide
ppm	Parts per million
LOD	Loss on drying
± SD	Plus or minus standard deviation
et al	and others
TMS	Tetramethylsilane

CHAPTER ONE

INTRODUCTION

1.1 Introduction to Solid Dosage Forms

Perhaps after air, water and food in order of priority for survival in humans, is medicine. Medicines are agents used in preventing, treating/combating disease conditions or often used just to provide palliative measures. These agents, therefore, need to be readily available and relatively inexpensive to make life meaningful to people. Medicines come in different forms and are administered via varying routes. Several factors including physicochemical properties of the drug, disease condition and patient's response/compliance, individually or collectively, inform the choice of dosage form design in drug delivery. Medicines are thus formulated as solid, semi-solid or liquid dosage forms including injectables to ensure effectiveness, convenience and safety in use.

Solid dosage forms are a group of medicaments prepared and presented in their solid state. They range from loose powdered drugs to compressed products. Absence of moisture in these products affords them the one advantage of microbial stability, without the necessity of including a preservative. Microbial contamination is a factor that bedevils their liquid dosage form counterparts. Powdered drugs are rarely used these days but tablets and capsules remain popular. Tablets and capsules offer the patient the advantage of ease and convenience of administration when compared with, for example the injectable dosage forms, where administration mostly requires the attention of qualified personnel.

The oral solid dosage forms such as tablets and hard gelatin capsules had been in existence since the 19^{th} century and still remain popular, accounting for about half of all medicines

licensed (Davies, 2009) or 90% of all dosage forms used to provide systemic administration of therapeutic agents (Jones, 2008). Table 1.1 presents the commonly available solid dosage formulations.

Table 1.1: Types of Solid Dosage Forms

Dosage form	**Design of dosage form**	
Powders/granules	Divided powders/granules	
	Bulk powders/granules	Large dose oral powders
		Dusting powders
Tablets		
	Conventional compressed tablets	
	Multiple compressed tablets	
	Coated tablets	Sugar coated
		Film coated
		Enteric coated
	Modified-release tablets	Sustained-release
		Extended-release
		Targeted-release
	Effervescent tablets	
	Solution tablets	
	Dispersible tablets	
	Mouth dissolving tablets	
	Chewable tablets	
	Buccal and sublingual tablets	
	Vaginal tablets	
Capsules	Hard gelatin capsules	
	Soft gelatin capsules	
Others	Pastilles	

Culled from Allen et al. (2005); Ding et al. (2005); Alderborn (2007); Davies (2009)

1.2. Powders/Granules as Solid Dosage Forms

Powders/granules as dosage forms represent one of the oldest dosage forms in history which has now been replaced largely by tablets and capsules (O'Connor *et al.*, 2005). They are possibly one of the simplest and cheapest dosage forms to prepare as they do not require sophisticated machinery or expensive precautionary measures during formulation. Powders as dosage forms are medicated and intended to be used internally or externally depending on the powder type (Lund, 1994; Allen *et al.*, 2005). The powdered drug is commonly mixed with excipients, also in powdered form, to produce the final product. Those powders intended for use internally are normally mixed with water prior to use while those for use externally are dusted on the affected area from a sifter type container or applied from a powder aerosol (Allen *et al.*, 2005).

Granules as dosage forms are developed by aggregating the particles of the powder to form larger particles. Though the granule size is much bigger than the granules used in forming tablets, the method of manufacture is similar (Summers, 2007). The following had been presented as the advantages and possible drawbacks of use of powders/granules as dosage forms (Summers, 2007):

1.2.1 Advantages of Powders/Granules as Dosage Forms

- Solid preparations are more chemically and microbiologically stable than their liquid counterparts with shelve lives going into years.
- By circumventing the process of disintegration, orally administered powders and granules of soluble medicaments have a faster rate of dissolution than tablets and capsules.
- They provide a convenient and more acceptable way of administering drugs with a large dose e.g. compound magnesium trisilicate oral powder with a dose of 1-5 g is easier taken as a draught after dispersion in water.

1.2.2 Disadvantages of Powders/Granules as Dosage Forms

- It is difficult to mask unpleasant tastes in drugs presented as powders, though taste masking via effervescence has been employed in some products.
- Accuracy in dosing is a set back in using potent drugs in powdered form. Attempt to remedy this has been by presenting the medicament as divided preparations.
- Bulk powders or granules are far less convenient for the patient to carry than a small container or blister pack of tablets or capsules.
- Powders and granules are not suitable for the administration of drugs that cause irritations in the stomach, are inactivated in the gastric acid or by gastric enzymes and those liable to first pass effect.

1.3 Tablets as Solid Dosage Forms

Tablets are hard solid dosage forms obtained by compression of a powdered or granulated mixture of substances. They typically comprise of an active agent commonly referred to as active pharmaceutical ingredient (API) admixed with other agents referred to as excipients, which aid or facilitate the compression of the tablet into a compact uniform dosage unit. The process of compaction is carried out using a tablet press. The popularity of solid dosage forms is owed to tablets which remain the most popular of all dosage forms in contemporary pharmaceutical dosage form design. The advantages associated with tablets are not a product of serendipity but a result of some meticulous designs. Some drug powder mixes are compressed without further treatment while others require some tactful engineering. For a powder to be directly compressible it must possess an inherent binding ability or else a binder, as excipient, is incorporated in the tablet formula. Where the powder's flowability is poor, the added binder is used to impart granular properties to facilitate the requisite flowability. Granulation reduces the interparticulate attractions thereby enhancing the flow property of the powder. Uniformity in dosing is governed by uniformity in dosage units

which, in tablets, is dependent on the appropriate flow of the powder or granules into the die cavity (Summers and Aulton, 2007; Jones, 2008).

Tablets are formed by a series of processes that are normally preceded by a unit operation called granulation where granule sizes are optimized. Tablet size and shape is predetermined by the choice of die setting, otherwise referred to as tooling. The precise amount of powder or granule to fill the die is also predetermined by adjusting the depth the lower punch can go as it descends. The powder or granules as the case may be, is then made to flow via a hopper into the die cavity. The lower punch in the die setting descends to admit the powder into the die cavity before the upper punch in turn descends to compress the powder to make the tablet. Tablet presses are the machines used in compressing tablets. The single punch press produces few tablets per min, while multiple punch presses or rotary presses may have capacity of producing multiples of thousands of tablets an hour (Jani *et al.,* 2009). Jones (2008) presented the following as the reasons why tablets have an edge over other dosage forms as he equally listed some of the dosage form's drawbacks.

1.3.1 Advantages of Tablets as Dosage Forms

- Tablets are convenient to use and are an elegant dosage form.
- A wide range of tablet types are available, offering a range of drug release rates and durations of clinical effect.
- Tablets may be formulated to release the therapeutic agent at a particular site within the gastrointestinal tract to reduce side-effects, promote absorption at that site.
- Tablets may be formulated to contain more than one therapeutic agent (even if there is a physical or chemical incompatibility between each active agent).
- With the exception of proteins, all classes of therapeutic agents may be administered orally in the form of tablets.
- It is easier to mask the taste of bitter drugs using tablets than for other dosage forms e.g. liquids.
- Tablets are generally an inexpensive dosage form.
- Tablets may be easily manufactured to show product identification.

- The chemical, physical and microbiological stability of tablet dosage forms are superior to other dosage forms

1.3.2 Disadvantages of Tablets as Dosage Forms

- The manufacture of tablets requires a series of unit operations and therefore there is an increased level of product loss at each stage in the manufacturing process.
- The absorption of therapeutic agents from tablets is dependent on physiological factors e.g. gastric emptying rate and shows inter-patient variation.
- The compression properties of certain therapeutic agents are poor and may present problems in their subsequent formulation and manufacture as tablets.
- The administration of tablets to certain groups, e.g. children and the elderly may be problematic due to difficulties in swallowing. These problems, however, may be overcome by using effervescent, dispersible or solution tablets dosage forms.

1.3.3 Methods of Tableting

Several methods of tableting are employed in pharmacy (Table 1.2), which include direct compression, dry granulation or wet granulation. Choice of method is guided by the physical and chemical stability of the active agent, the nature of the excipient, available facilities and operational cost (Jones, 2008).

1.3.3.1 Granulation Methods

When a drug material or the diluent lacks the ability to flow well, the chances of non-uniform die fill is high, leading to possible gross and unacceptable intra and inter-production batch variations. This is commonly countered by a process called granulation where the particles are agglomerated to give larger free flowing agglomerates otherwise called granules. Granulation is commonly achieved by the use of a formulation aid called granulating agent or binder. Two types of granulation processes are in use, the dry

granulation and the wet granulation, depending on whether granulating liquid is involved in the process or not.

Table 1.2: Advantages and Disadvantages of Different Methods of Formulating Tablets

Method	Advantages	Disadvantages
Direct compression	Simple, cheap process. No heat or moisture, so good for unstable compounds. Prime particle dissolution.	Not suitable for all drugs, generally limited to low-dose compounds. Segregation potential. Expensive excipients.
Wet granulation (aqueous)	Robust process suitable for most compounds. Imparts flowability to a formulation. Can reduce elasticity problems. Coating surface with hydrophilic polymer can improve wettability. Binds drug with excipient, thus reducing segregation potential.	Expensive: time- and energy-consuming process. Specialized equipment required. Stability issues for moisture-sensitive and thermolabile drugs with aqueous granulation.
Wet granulation (nonaqueous)	Suitable for moisture-sensitive drugs. Vacuum-drying technique can remove/reduce need for heat.	Expensive equipment: explosion proof; solvent recovery.
Dry granulation (slugging)	Eliminates exposure to moisture and drying (heat)	Dusty procedure. Not suitable for all compounds. Slow process.
Dry granulation (roller compaction)	Eliminates exposure to moisture and drying (heat)	Slow process

Adapted from Davies (2009)

1.3.3.1.1 Dry Granulation

Tablet formulation that requires granulation but contains therapeutic agent(s) that is/are sensitive to moisture or heat is better granulated using the dry method. This is the granulation process that does not involve the use of granulating solvent; it rather involves the application of high stresses to the mixed powder. Two methods employed in dry granulation are slugging and roller compaction (Jones, 2008; Davies, 2009).

1. **Slugging**

This is a process whereby the powder to be made into tablet, comprising of the active agent, a binder, diluent and a disintegrant is dry mixed and compressed into a large tablet which is subsequently broken and milled. The stress applied brings the powder particles close together and upon milling the slugs, the particles remain as aggregates, with an acquired better flow property. The granules are thereafter lubricated and compressed into the desired tablet form.

2. **Roller Compaction**

In roller compaction, the powder mix is made to pass between two oppositely rotating rollers, a process that transforms it to a sheet like material. This sheet is then turned to granules by milling; the granules are then lubricated and compressed to tablets.

1.3.3.1.2 Wet Granulation

This is the most commonly used method for the manufacture of tablets (Jones, 2008). As the name implies, a liquid usually water or ethanol or a mixture of the two are employed as granulating liquid in the formation of granules. The ingredients excluding lubricant and glidant are dry mixed using an appropriate mixing device. The granulating liquid is either used to make mucilage of the binder before incorporation into the powder mix or it is added straight into the powder mix. The liquid is added and the mix sheared to form the granules using shear facilities at appropriate shear rates. The wet mass is dried and milled to a desired granule size as obtained in large scale operations or it is screened through a mesh of appropriate aperture and dried, a common practice in small scale laboratory productions (Davies, 2009).

1.3.3.1.3 Fluidized Bed Granulation

This is another form of wet granulation where the powder is fluidized and sprayed with binder liquid. The powder is admitted into fluid bed dryer and is fluidized by a stream of hot air. While in this state, the granulating liquid is sprayed onto it which imparts cohesive properties and causes agglomeration of the particles. The airborne state is maintained until the formed granules are dried. This process has the advantage of granulating and drying in the same chamber.

1.3.3.2 Evaluation of Granules

Some simple tests carried out on granules to assess their integrity and also to ascertain status of the imparted flow properties include granule size analysis, density (bulk and tapped) measurements, flow rate and angle of repose determinations.

1.3.3.2.1 **Granule Size Analysis**

Granule size distribution is determined by sieve analysis using a nest of sieves arranged on a shaker like is obtained with the Endecott sieve shaker. A stack of tarred sieves of different apertures are arranged on the shaker in ascending order with the one having the largest aperture size on top and the dust collecting pan at the bottom. A certain amount of the granules, usually 50 g, is placed onto the top most sieve and the shaker is set to vibrate for a preset time in minutes. The amount of granules retained on each sieve is collected and weighed. The quantity of granules retained on each sieve is determined using the differences in the weight of each sieve when empty and when containing granules.

1.3.3.2.2 **Flow Rate**

The angle of repose of the granules is determined using generally two types of approaches, the funnel method or the flow rate tester. The time taken for a 50 g sample of the material to pass through the orifice is determined and the rate in g/s is calculated. In the funnel method, the funnel is clamped on a retort stand, the opening of the funnel blocked and the material is introduced into the funnel. Removing the block from the opening of the funnel will allow the material fall from a height of commonly, 10 cm, and the time taken for the granule to completely flow through is noted.

1.3.3.2.3 Angle of Repose

The arrangement is similar to that of determining flow rate using the funnel method. The conical heap from the base formed by the material is now used to determine the angle of repose. The angle of repose is calculated from the geometry of the heap of the granules using equation 1:

$$\text{Angle of repose, } \tan\theta = h/r \qquad (1)$$

Where h is the height of the heap, r is the radius of the base of the heap.

Values of θ smaller than 25^{o} is an indication of good flow (Allen *et al.*, 2005; Staniforth and Aulton, 2007).

1.3.3.2.4 Bulk and Tapped Densities

The bulk and tapped densities of each batch of the powders or granules are determined using a 100 ml measuring cylinder. A 50 g quantity of the granules is placed in the measuring cylinder and the volume it occupied is used to calculate the bulk density. Thereafter, the cylinder is dropped fifty times from a height of 2 cm and the new volume after 3, 10, 25 and 50 taps is recorded and used to calculate the tapped density. Automated systems of determining densities are also available e.g. the USP density measurement machine, where cylinders of two capacities, 50 ml and 100 ml are used. A weighed amount of the material is introduced into either of the cylinders and the machine operated for preset droppings. The cylinder is dropped at a constant rate and from a constant height. After each round, volume occupied is noted and entered into the machine which in turn calculates and print out the results of the respective densities.

1.3.3.3 Direct Compression Method

This is the method adopted for compressing powders with inherent flow and compressibility properties. For a drug to be formulated into high dose tablet using the direct compression method, the API would have to be free flowing and compressible (Shiihii *et* a*l*., 2012). On the other hand, the bulking agent needs to possess appropriate flow and compressibility properties for it to be used in formulating low dose drugs (Muazu *et al.*, 2011). Powder particle size distribution is optimized prior to compression to ensure uniformity in dosage unit. Direct compression is becoming popular as it circumvents lots of unit operation processes associated with other tableting techniques especially the wet granulation method. This makes it the manufactures' preferred method as it offers the advantage of saving cost and time (Davies, 2009).

1.3.4 Components of Tablets

Tablet as a dosage form is hardly formulated containing only the active agent but almost always with other substances referred to as excipients or aids. In most cases, the excipients while expected to be inert, collectively account for the largest weight proportion in tablet formulation formula. Excipients give a tablet the structure it requires to serve as dosage unit. Diluents offer a tablet the bulk to make it of compressible size as binder affords it the requisite adhesive strength to hold together during formulation, transportation and handling by the patient. Disintegrants are included in the formulation to counter the effect of binder by breaking the tablet upon contact with a liquid medium. Lubricants and glidants are to give the tableting process the "smoothness" it requires (Rudnic and Schwartz, 2005; Alderborn, 2007).

1.3.4.1 Active Pharmaceutical Ingredient (API) or Active Agent

This is the tablet. Almost all tablets, except may be placebo tablets for experimental purposes, contain one or more active agents. This is the agent responsible for the desired or observed pharmacological effect. It is what makes the "dose" in tablet as a dosage unit. When the proportion of the API in the tablet formula is large, the tablet is termed "high dose" (Shiihii *et al.,* 2012) and when on the contrary, it is called "low dose" (Muazu *et al.,* 2011) tablet. The terms analgesic, antibiotic, tranquillizer etc. used to describe a tablet are basically defining the nature or type of the API. An example of API is Ven. HCl, the test drug in this study.

1.3.4.1.1 Venlafaxine Hydrochloride (Ven. HCl)

Ven. HCl with molecular structure $C_{17}H_{27}NO_2$.HCl (Figure 1.1), molecular name (R/S)-1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride and molecular mass 313.87 is a white to off-white crystalline solid with 501 mg/ml solubility in water at pH 7.09 (Wyeth, 2012). The high water solubility of this drug makes it a potential candidate for studies on tablet drug-release modification. It is an orally active serotonin and noradrenalin reuptake inhibitor used in the treatment of major depressive disorders (Doddayya *et al.*, 2011; Wyeth, 2012). It is a structurally novel phenylethylamine antidepressant which is chemically unrelated to tricyclic, tetracyclic or other available antidepressant agents. It was first introduced in 1994 for the treatment of major depression in its immediate release form. The extended-release forms of this medication are now greatly employed in the treatment of major depressive disorder, generalized anxiety disorder, panic disorder, and social anxiety disorder. Ven. HCl undergoes extensive

metabolism in the liver primarily to an equiactive and equipotent metabolite, O-desmethyl-venlafaxine (Martin and Jacobs, 2007).

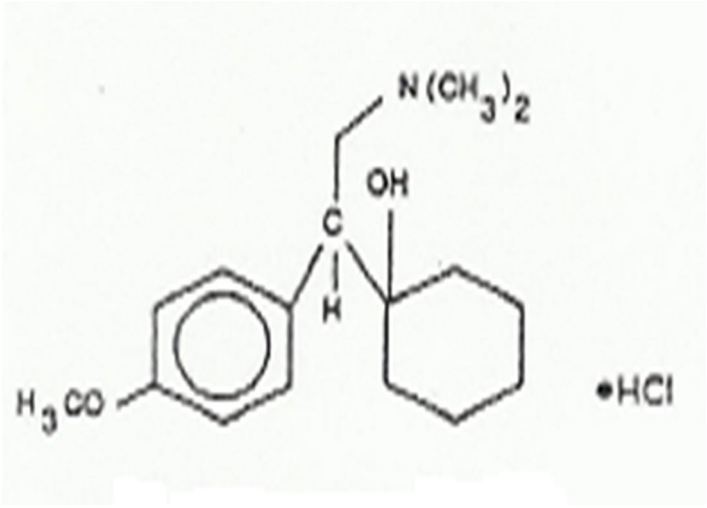


Figure 1.1: Venlafaxine hydrochloride (Rao *et al.,* 2009; Wyeth, 2012).

1.3.4.2 Diluents

Diluents are excipients commonly used in tablet formulations to bulk up the size of the drug material for ease of compression into tablets. Examples of commonly used diluent types are given in Table 1.3. Some drugs, owing to their potency, are needed in quantities so small that processing becomes a problem and diluents are used to bulk them up at least to a size of 50 mg (Rubinstein, 1988). These agents are equally used to promote the flow properties and compression characteristics of tablet mix (Allen *et al.*, 2005). Sometimes quantities of diluents large enough to completely define the properties of the powder mix could be used, and because of this, diluents are expected to have unique properties

1.3.4.2.1 Properties of Diluents

A diluent should be inert physiologically and pharmacologically

- It should not have any compatibility problems with either the drug substance or remaining excipients.
- It should be chemically and microbiologically stable.
- Its physical and chemical characteristics should be consistent.
- It should have an appealing color or better still, colorless.
- It should have optimum particle size or be capable of being size reduced to match the particle size distribution of the API.

Table 1.3: Some Diluents used in the Wet Granulation Method

Diluent	Comments
Dextrose	Hygroscopic
Dicalcium phosphate	Inexpensive, insoluble in water
Lactose BP	Inexpensive, relatively inert; the most frequently used diluents
Mannitol	Freely soluble; used particularly for chewable tablets
Microcrystalline cellulose	Excellent compression properties; has some disintegrating ability
Sodium chloride	Freely soluble; used for solution tablets
Sucrose	Sweet taste but hygroscopic; may not be diluted with lactose

Adapted from Rubinstein (1988)

1.3.4.3 Granulating or Binding Agents

Formulation of a good tablet is dependent on the flow properties of the powder particles to be compressed and also their ability to stick to one another after compression. While some powders may posses the inherent ability to flow well and gum together after compression, a sizeable number of materials need to have such qualities imparted on them. Binders are used

as excipients to form agglomerates, otherwise called granules, of these particles, which eventually change the physicochemical properties of the powdered material. Optimum concentration of binder could form granules that would flow better to ensure uniform die fill during compression and give the tablet the requisite hardness to withstand rigors of transportation and handling. Insufficient binder gives soft tablets while too much of it leads to the production of too hard tablets that may present with prolonged disintegration time and associated problems with bioavailability. Performance of a binder in tableting is also dependent on the type of binder used. Starches, celluloses and gums had been and are still in use as binders in tableting. These agents are often modified as in the pregelatinisation of starch or methylation of celluloses, a procedure that enhances the materials solubility and promotes its usability. Table 1.4 presents some of the binders and their concentrations in common use as pharmaceutical aids.

Table 1.4: Commonly used Granulating/Binding Agents

Granulating agent	Normal use concentration (%)	Comments
Starch	5-25	Was once the most commonly used binder. The starch has to be prepared as a paste, which is time consuming
Pregelatinised starch (PGS)	5-10	Cold water soluble. Easier to prepare than starch
Acacia	1-5	Requires preparation of paste prior to use. Can lead to prolonged disintegration time if used at too high a concentration
PVP	2-8	Available in range of molecular weights/viscosities. Can be added whether dry or in solution. Soluble in water or in ethanol
HPMC	2-8	Available in range of molecular weights/viscosities. Soluble in water and ethanol
MC	1-5	Low-viscosity grades most widely used

Abbreviations: PVP, polyvinylpyrollidone; HPMC, hydroxyprophyl methylcellulose; MC, methyl cellulose (Davies, 2009)

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1.3.4.4 Disintegrating Agents

Tablets after administration/ingestion and upon getting in contact with gastric fluid, are expected to deaggregate/disintegrate back to the former granular or particulate state, prior to their getting dissolved and finally absorbed to elicit the expected pharmacological activity. Disintegration of tablets to granules, facilitated by a disintegrant, presents more surface area for the action of the dissolution medium. A disintegrant, therefore, is that ingredient added in tablets and responsible for the breakup of tablets into granules/particles. Rate of disintegration of tablets is often dependent on type and concentration of disintegrant used. Different classes of polysaccharides produce disintegration in tablets at varying times and

through varying mechanisms as shown in Tables 1.5 and 1.6. Disintegrants are graded based on the speed with which they deaggregate the tablet into granules or particles. A special class of disintegrants which produces disintegration at low concentrations is referred to as superdisintegrants.

Table 1.5: Types of Disintegrating Agents

Disintegrant	**Normal Use Concentration (%)**	**Comments**
Starch	5-10	Probably work by wicking; swelling is minimal at body temperature
Microcrystalline cellulose		Strong wicking action. Loses disintegrant action when highly compressed
Insoluble ion exchange resins		Strong wicking tendencies with some swelling action
Sodium starch glycollate	2-8	Free flowing powder that swells rapidly on contact with water
Croscamellose sodium	1-5	Swells on contact with water,
Gums-agar, guar, xanthan	<5	Swells on contact with water; form viscous gels that can retard dissolution, thus limiting concentration that can be used
Alginic acid, sodium alginate	4-6	Swell like gums but form less viscous gels
Crospovidone	1-5	High wicking action

Adapted from Davies (2009)

Table 1.6: Mechanism of Disintegrant Action

Disintegrant action	Explanations
Water wicking	This describes the ability of a disintegrant to draw water into the porous network of a tablet thereby causing the breakup of the tablet. Water is drawn into tablets without necessarily being accompanied by volume increase.
Swelling	This is probably the most widely accepted mechanism of action for tablet disintegrants. A positive correlation is noted between the rate of swelling and the disintegrant action for some disintegrants. The swelling of some disintegrants is dependent on the pH of the media.
Deformation recovery	This implies that the shapes of the disintegrant particles are distorted during compression, and that the particles return to their precompression shape upon wetting, thereby causing the tablet to break apart. Crospovidone and starch are said to act by this mechanism
Repulsion theory	According to this theory, water penetrates into the tablet through hydrophilic pores and a continuous starch network which conveys water from one particle to the next, imparting a significant hydrostatic pressure. The water then penetrates between starch grains because of its affinity for starch surfaces, thereby breaking hydrogen bonds and other forces holding the tablet together. At present, this theory is not supported by adequate data.
Heat of wetting	During wetting, particles e.g. of starch were noted to exhibit slight exothermic properties, which was thought to cause localized stress resulting from the expansion of air retained in the tablet matrix aiding breakup of the tablet. Heat of wetting alone, however, is probably inadequate to explain disintegration.

Culled from Encyclopedia of Pharmaceutical Technology by Swarbrick and Boylan (2001)

1.3.4.4.1 Super Disintegrants

A sub class, in the classification of disintegrants, is the super disintegrants. This class of disintegrants produces rapid disintegration of tablets at disintegrant concentration range of 2-4 %w/w (Rudnic and Schwartz, 2005). The super disintegrants (Table 1.7) are characterized by their hydrophilicity and great ability of swelling and the mechanism they follow in causing disintegration of tablets. Disintegrants generally are widely believed to exert their action by swelling (Table 1.6). The polymer strands absorb water and this makes them to swell and consequently disrupt and break the tablet. Superdisintegrants are a product of derivatisation of polymeric materials. Even dispersible polymers like gums when appropriately cross-linked are rendered insoluble, absorb water and swell (Silva *et al.*, 2006).

Table 1.7: Super Disintegrants

Disintegrant	Normal Use Conc. (%)	Nature	Mechanism of disintegration
Sodium starch glycollate	2-8	Cross-linked starch; insoluble material	Free flowing powder that swells rapidly on contact with water
Croscamellose sodium	1-5	Cross-linked sodium carboxymethyl cellulose; insoluble material	Swells on contact with water
Crospovidone	1-5	Cross-linked polyvinylpyrollidon; insoluble material	High wicking action

Adapted from Swarbrick and Boylan (2001); Davies (2009); Deshmkh *et al.*, 2012)

1.3.4.5 Sweetening Agents

Some therapeutic agents are bitter and so is their tablet after formulation posing palatability problem especially in children. Bitter tastes in tablets are masked by a process called coating. Tablets are coated using three main methods: sugar coating, film coating and compression coating (Davies, 2009). Mannitol and sucrose are examples of materials commonly used to sugar coat tablets.

1.3.4.6 Coloring Agents

These are used principally for aesthetic reasons, so as to make the tablet more appealing to the patient. Allam and Kumar (2011) referred to colorants as cosmetics for the pharmaceutical dosage forms. Also color in tablet helps the manufacturer to control product during its preparation as well as serve as a means of identification to the user.

During tableting, colorants are usually incorporated with the binder solution prior to granulation. This is however associated with the problem of color migration during the process of drying. Solvent migration leads to transfer of colors giving rise to the production of tablets with varying color shades (mottled appearance). This can however be prevented when the granules are dried slowly with constant stirring. A similar colored lubricant can also be used to achieve proper color. Materials used as coloring agents include Red No. 3, Yellow No. 6 (Sunset yellow FCF), Blue No.2 etc (Otto *et* al., 2005; Allam and Kumar, 2011).

1.3.4.7 Lubricating Agents

Lubricants are agents used to reduce friction between moving parts. In tableting, they perform multiple functions like: (1) ensuring smooth ejection of the tablet from the die cavity; (2), facilitating frictionless movement between the punches and the die during compression and ejection of tablet; and (3), enhancing the flow properties of the granules (Rubinstein, 1988; Davies, 2009). They are added in small amounts commonly not greater than 1 % of the tablet weight. Insufficient quantity of lubricants may give rise to picking, capping, sticking, binding to the die cavity while too much of it may lead to decrease in tablet hardness, increase in tablet disintegration times and decrease in rate of dissolution of the tablet (Davies, 2009). Magnesium stearate, talc and stearic acid are examples of lubricants with magnesium stearate being the most popular.

1.3.4.8 Glidants

These are aids that enhance the flow characteristics of a powder mixture; they regulate the flow characteristics of the granules from the hopper into the die cavity. Glidants act by reducing the inter-particulate cohesive forces and consequently permit the granules' smooth and uniform flow. Uniform flow ensures uniform fill of die which eventually gives uniform tablet weights. Like lubricants, they are also added in small quantities. The glidant of choice is fumed silica (Lund, 1994), with others commonly used including starch and talc.

1.3.5 Types of Tablet Dosage Forms

Tablets as solid dosage forms are numerous and varied depending on the intended use. Tables 1.1 and 1.8 give an overview of the various types of tablets in common use. Some of these tablet types shall be described in relative details. Tablets are formulated containing different types or amounts of excipients which generally define where the tablet is intended to disintegrate and or dissolve and be absorbed to elicit the desired effect. Some tablets are intended to quickly dissolve in the mouth while some are desired to disintegrate and dissolve in the colon. This presents a wide range of environments that the drug gets exposed to and so to define where or when the tablet is to disintegrate and or dissolve requires some tactful approaches. Tablets can be broadly classified into: 1, those designed to instantly dissolve upon contact with a liquid medium like the saliva in the mouth e.g. the orally dissolving tablets (Shukla *et al.*, 2009; Rao *et al.*, 2010); 2, those expected to dissolve in the stomach after getting in contact with the gastric fluid and this class of tablets otherwise called conventional immediate release tablets are expected to dissolve within 30 min (DeVane, 2003; Pathak *et al.*, 2011; Govedarica *et al.*, 2011); and 3, those tablets whose rate of drug release has been modified and the drug is released usually over an elongated period of time running to several hours e.g. the extended release, enteric coated formulations (Heinig *et al.*, 1997; York, 2005; Ding *et al.*, 2005; Collate and Moretone, 2007).

Newer approaches in drug delivery are geared towards formulating drug delivery systems that will bring about the improvement in the overall bioavailability of the drug with minimal toxic effects. Such delivery systems' effectiveness is demonstrated by the eventual pharmacokinetic disposition of the drug in the body (Shargel *et al.*, 2005).

*T**able 1.8:** Types of Tablet Dosage Forms and Their Properties*

Formulation type	***Description***
Immediate release	*The dosage form is designed to release the drug substance immediately after ingestion*
Delayed release	*The drug substance is not released until a physical event has occurred, e.g. time elapsed, change in pH of intestinal fluids, change in gut flora*
Chewable tablets	*Strong, hard tablets to give good mouth feel*
Lozenges	*Strong slowly dissolving tablets for local delivery to mouth or throat. Often prepared by a candy molding process*
Buccal tablets	*Tablets designed to be placed in buccal cavity or mouth for rapid action*
Effervescent tablets	*Taken in water, the tablet forms an effervescent, often pleasant-tasting drink*
Dispersible tablets	*Tablets taken in water, the tablet forms a suspension for ease of swallowing*
Soluble tablets	*Tablets taken in water, the tablet forms a solution for ease of swallowing*
Pastilles	*Intended to dissolve in mouth slowly for the treatment of local infections. Usually composed of a base containing gelatin and glycerin*

Adapted from Davies (2009)

1.3.5.1 Mouth Dissolving Tablets

A drug delivery system that is fast growing and greatly gaining acceptance is the orally disintegrating tablet (ODT), also called intraoral dosage form or mouth dissolving tablet; where the tablet, without the necessity of using water, is designed to dissolve in the mouth (Goel *et al*., 2008). These tablets are designed to instantly disintegrate and dissolve once they get in contact with a liquid medium; thereby leading to rapid onset of action. The tablet is placed on the tongue, where it disperses and release the drug in the saliva which is subsequently absorbed into the systemic circulation. Agents that quickly facilitate drug release e.g. the superdisintegrants, are employed in designing this type of tablet dosage form. Super disintegrants had been used to develop fast disintegrating tablets of felodipine (Hariprasanna *et al.*, 2010), ketorolac tromethamine (Patil *et al.*, 2009), metoprolol tartrate (Namdev, 2010), zidovudine (Rao *et al.*, 2010a).

The following have been presented as the ideal properties of mouth dissolving tablets, and the advantages of same had also been highlighted (Fu *et al.,* 2004; Shulka *et al.*, 2009; Sahora *et al.*, 2010; Bala and Pawar, 2011; Konapure *et al.*, 2011; Ravichandiran *et al.*, 2011; Kashyap *et al.*, 2011).

1.3.5.1.1 Ideal Properties of Mouth Dissolving Tablets

- It should disperse in minimal amounts of liquid, use of water should not be necessary for the table to disintegrate and dissolve in the mouth within seconds of administration
- Have a pleasing mouth feel
- Leave minimal or no residue in the mouth after administration
- Be compatible with the required formulation excipients including taste masking agents
- Be adaptable and amenable to existing processing and packaging machinery

- Allow the manufacture of tablets using conventional processing and packaging equipment at low cost
- Allow high drug loading
- Have sufficient strength to withstand the rigors of the manufacturing process and post manufacture handling

1.3.5.1.2 Advantages of Mouth Dissolving Tablet as Dosage Forms

- Ease of administration to patients that experience difficulties in swallowing tablets e.g. pediatric, geriatric and psychiatric patients
- Convenience of administration has improved compliance
- Benefits of liquid medication in the form of solid preparation
- There is precision of dose unlike when the drug is given in liquid form
- Pregastric absorption may promote bioavailability by circumventing the first pass effect; this may allow use of reduced doses with associated reduction in side effect without compromising efficacy
- Rapid onset of drug action following absorptions in the mouth, pharynx and esophagus
- Increased bioavailability
- Does not require the use of water for dissolution
- Allows high drug loading

1.3.5.2 Conventional Immediate Release Tablets

These are the oldest known tablet types. A large proportion of tablets in use today are designed to immediately release the entire medicament upon contact with a solubilising liquid e.g. the gastrointestinal fluid. They are commonly compressed while containing an appropriate amount of binder that holds the tablet together and a disintegrant that will cause the rupture of the tablet on contact with a liquid medium. Releasing the medicament at once may not pose any problem with the less potent drugs or when the medicament is taken once a day or occasionally, but worries may arise with multi-dose therapies, especially of potent drugs. Drugs with short half-lives, prepared as rapid release products, often need to be given at frequent intervals to maintain blood concentrations within the

therapeutic window. Peaks and valleys commonly associated with these types of formulations and the possible patient non-compliance may affect the outcome of the therapy (Ding et al., 2005; Shargel et al., 2005; Varshosaz et al., 2006; Collett and Moreton, 2007). Lloyd (1999) reported that there is an inverse correlation between the frequency of dosing and patient compliance.

Ding, et al. (2005) highlighted the potential problems inherent in multiple dose therapy as:

- *If the dosing interval is not appropriate for the biological half-life of the drug, large peaks and valleys in the drug blood level may result. For example, drugs with short half-lives require frequent dosing to maintain constant therapeutic levels.*
- *The drug blood level may not be within the therapeutic range at sufficiently early time points, an important consideration for certain disease states.*
- *Patient non-compliance with the multiple-dosing regimen can result in failure of this approach.*

Following oral administration of a dose, the drug is absorbed into the systemic circulation at a rate and extent that is dependent on the pharmacokinetic parameters of the drug. In multiple dose therapies, the half life of the drug is used to guide the frequency of administration of doses such that the concentration of the drug in the plasma is within the therapeutic window [i.e. below the maximum tolerable concentration (MTD) but above the minimum effective concentration (MEC)] as shown in Figure 1.2. However, aptness or otherwise in administering the dose will define the therapeutic outcome. Doses taken too close to each other may predispose the patient to toxic concentrations when plasma drug levels of the drug go beyond the MTD, while delayed dosing may lead to risks of plasma concentrations dropping below the MEC. The former case is more pronounced when dealing with potent drugs that have narrow therapeutic windows, while the latter may lead to development of resistance as seen with sub-curative concentrations of antimicrobial agents

(Shargel *et al.*, 2005; Ding *et al.*, 2005; Chaubal, 2006).

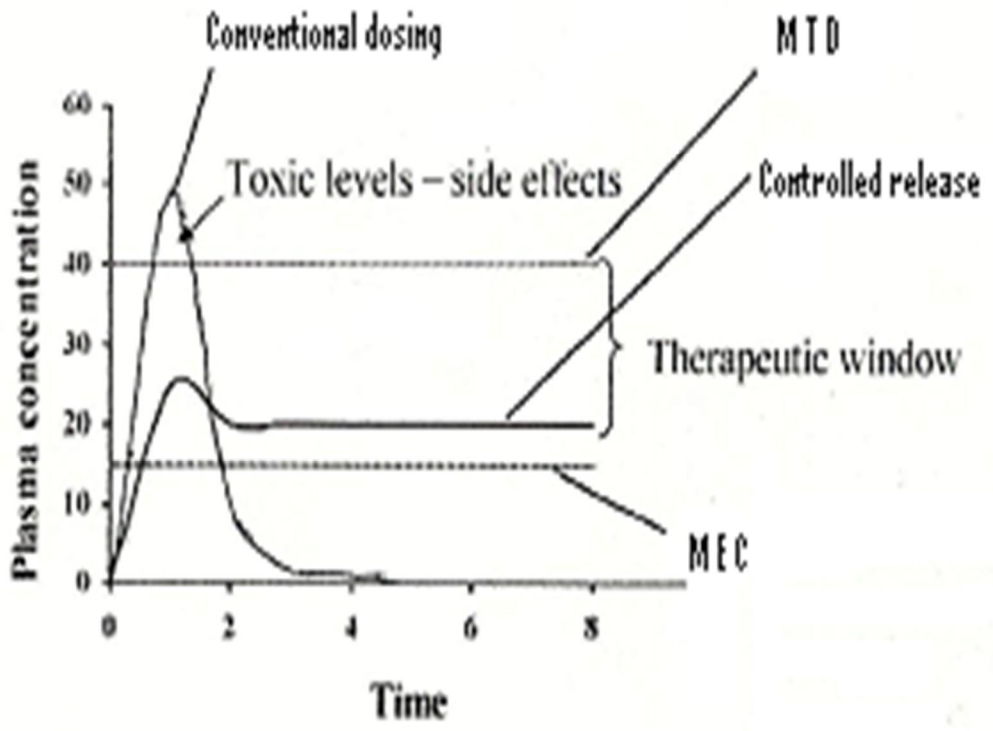


Figure 1.2: Comparison of typical pharmacokinetic profiles seen for conventional versus controlled release formulations. Key: MTD, minimum tolerable dose; MEC, minimum effective concentration (Chaubal, 2006)

1.3.5.3 Modified-Release Tablet Dosage Forms

Dosing interval factored by the half-life of the drug, associated with chances of patient non-compliance and the drug's toxicity had led to the emergence of modified-release dosage forms. Researches into ways of improving therapeutic outcomes had given rise to the modification of tablet release profiles culminating into the emergence of delayed release, extended release, receptor targeted release or site specific drug products through use of some specialized polymeric materials (Allen et al., 2005; Ding et at., 2005; Shargel et el., 2005). These products are designed to offer the potential benefits of sustained drug blood levels, site specific drug release, attenuation of adverse effects and promoting patients' compliance, eventually improving the therapeutic outcome. Delayed release, extended release, receptor targeted release are some terminologies used in modified-release dosage applications and are often mixed up and interchanged (Allen et al., 2005) and so, Ding et al. (2005), offered some definitions to these terminologies as shown in Table 1.9.

The modified-release oral dosage forms are becoming increasingly popular over the conventional immediate release dosage forms owing to some advantages they are associated with as shown in Table 1.10. Different methods are used to control the rate of release of drugs from delivery systems (Ding et al., 2005).

Table 1.9: *Definition of Some Terminologies used in Describing Modified-Release Formulations*

Terminology	***Definition***
Delayed-release systems	*These systems use repetitive intermittent dosing of a drug from one or more immediate release units incorporated into a single dosage form, or an enteric delayed release system*
Extended-release systems	*These include any dosage form that maintains therapeutic blood or tissue levels of the drug for a prolonged period. If the system can provide some actual therapeutic control, whether this is temporal or spatial or both, of drug release in the body, it is considered a controlled drug delivery system. This explains why extended-release is not equivalent to controlled release.*
Site-specific and receptor targeting	*This refers to targeting a drug directly to a certain biological location. In the case of site-specific release, the target is adjacent to or in the diseased organ or tissue; for receptor release, the target is the particular drug receptor within an organ or tissue. Both of these systems satisfy the spatial aspect of drug delivery requirement and are also considered controlled drug delivery systems.*

Adapted from Ding *et al.*, (2005**)**

Table 1.10: Advantages of Extended-Release Dosage Forms

Advantage	Explanation
Less fluctuation in drug blood levels	Controlling rate of release eliminates peaks and valleys of blood levels
Frequency reduction in dosing	Extended-release products frequently carry and deliver more than a single dose, hence may be taken less often than conventional forms
Enhanced convenience and compliance	With less frequency of dosing, a patient is less apt to neglect taking a dose, also greater convenience with day and night administration
Reduction in adverse side effects	Because of fewer blood level peaks outside therapeutic range and into toxic range, adverse side effects are less frequent
Reduction in overall health care costs	Although initial cost of extended-release dosage forms may be greater than for conventional forms, overall cost of treatment may be less because of enhanced therapeutic benefit, fewer side effects, reduced time for health care personnel to dispense and administer drugs and monitor patients

Adapted from Allen *et al*. (2005)

1.3.5.3.1 Some Disadvantages Associated with Extended-Release Dosage Forms (Shargel *et al.*, 2005) include:

- Higher cost
- Unpredictable or poor *in vitro in vivo* correlation
- Dose dumping
- Large dose drugs are not suitable for formulation in extended release forms
- Removal of drug in response to adverse drug reaction or intoxication is difficult
- Reduced potential for dose adjustment
- Poor systemic availability due to erratic or variable absorption from the GI tract

1.3.5.4 Rate-controlled delivery systems

These are systems designed to deliver drugs for systemic or local action at a predetermined rate for a specific time period. These systems are classified based on the mechanism used in optimizing drug release. Controlled drug release has been facilitated by designing drug-delivery that can be described by any or a combination of some of the following mechanisms, vis: diffusion, swelling, dissolution, osmotic or erosion controlled systems.

1.3.5.5 Diffusion systems

Diffusion systems are used to describe release of drugs via diffusion through an inert membrane barrier, usually an insoluble polymer. Drug release can be controlled by regulating the rate at which the drug dissolves or diffuses out of the tablet matrix. Matrices or reservoir devices are employed to control release of drug by controlling the rate at which water permeates into the device or the rate at which the drug leaches out after being dissolved from within the device. Drug release from such devices is described by the Higuchi equation (Ding *et al.*, 2005; *Shargel et al., 2005*) as described later in the text.

Monolithic matrix systems are very popular technologies used in controlled drug delivery because they are relatively inexpensive, easy to prepare and simple to use in formulations and are generally acceptable (Beneke *et al.*, 2009). Tablets are compressed by dispersing the drug in the matrix material prior to compression. The drug needs to be in small quantity compared to the matrix for it (the drug) to be properly protected and its release controlled (Shargel *et al.*, 2005). To ensure optimum delivery of doses, two types of granules are made with one containing the matrix former and the other part without the ingredient. Upon

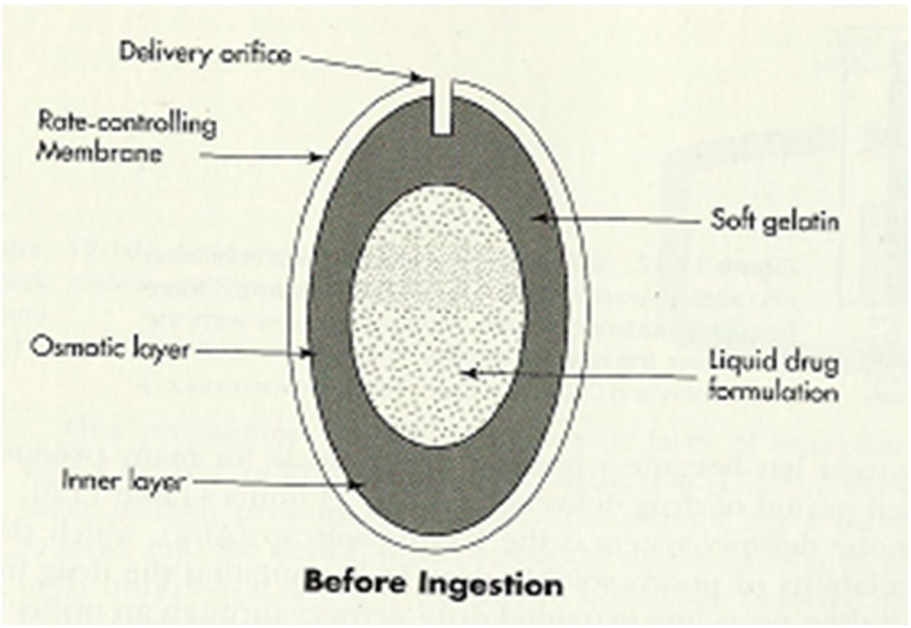
compression, the granules not containing the matrix former will provide the immediate effect and the drug-excipient granules provide extended action (Allen *et al.*, 2005). The hydrophilicity of the polymer used in forming the matrix allows for the gradual permeation of the device with water and the eventual dissolution of the drug, with the gelling property on the other hand, limiting the passage of the dissolved drug. Shargel *et al.* (2005) gave three examples of modified matrix-release mechanisms. The drug-matrix is coated with i, a soluble membrane; ii, an insoluble membrane and iii, an insoluble membrane with "windows" created by dissolving soluble parts of the membrane in water. Excipients including methylcellulose, gum tragacanth, Veegum® and alginic acid are said to form a viscous mass and provide a useful matrix for controlling drug dissolution. The gelling strength and concentration of the gum plays a very important role in such formulations (Shargel *et al.*, 2005).

1.3.5.6 Dissolution systems

A drug with a slow dissolution rate will yield an inherently sustained blood level. This principle has been used in extending drug release of highly water soluble drugs by reducing their solubility in water. Dissolution of highly water soluble drugs can be reduced by derivatizing or preparing an appropriate salt of the drug, by coating the drug with a slowly soluble material, or by incorporating it into a tablet with a slowly soluble carrier (Ding *et al.*, 2005).

1.3.5.7 Osmotic systems

This is a drug delivery system that relies on pressure buildup from within the tablet to cause release of the drug. A semipermeable membrane containing a laser-drilled hole, an osmotically active drug or osmotically inactive drug in admixture with an osmotically active salt are required in designing this type of dosage form. The semipermeable membrane allows passage of water into the device but prevents passage of drug except through the laser-drilled orifice. Upon contact with water, the osmotically active drug or agent generates osmotic pressure and the pressure difference created causes water to enter the device and the drug is forced out through the orifice (Ding *et al*., 2005; Shargel *et al.*, 2005) such device is shown in Figure 1.3. A newer device has been developed and is made to contain two compartments separated by a movable piston. In this case the drug and the osmotic agent are housed separately with the drug on the side of the orifice (Ding *et al*., 2005; Allen *et al*., 2005). Examples of products formulated as osmotic delivery device include Acutrim® and Procardia-XL® (Shargel *et al.*, 2005).



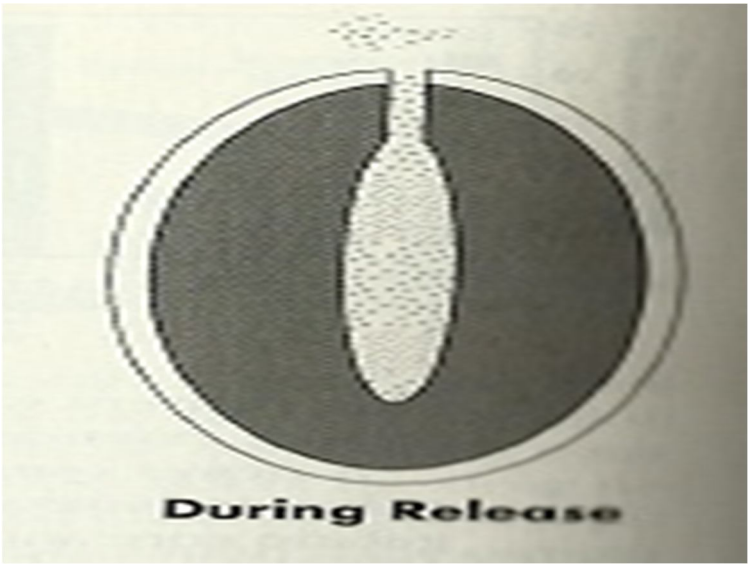


Figure 1.3: Osmotic delivery system (Shargel *et al*., 2005)

1.3.5.8 Swelling Systems

These systems, otherwise called gastroretentive drug delivery systems are designed to have prolonged resident time in the stomach to allow for the optimum release of the drug prior to its passage to the intestinal region of the GIT. Medicaments for treatment of gastric and duodenal ulcers and other gastrooesophagial ailments are good candidates for formulation as gastroretentive devices (Ray and Prusty, 2010; Krishnarajan *et al*., 2012). Dave *et al.* (2004) developed gastroretentive tablets comprising of guar gum, xanthan gum and hydroxypropyl methylcellulose as matrix formers and sodium bicarbonate was incorporated as a gas-generating agent to deliver ranitidine hydrochloride in the stomach. The authors observed that the sodium bicarbonate caused the generation of carbon dioxide (CO_2) in the presence of dissolution medium. The gas generated was trapped within the gel formed by the hydration of the polymer and this decreased the density of the tablet. The tablet became buoyant when the density became less than 1, and remained so for over 8 h (Singh and Kim, 2000). Tablets made with materials that can swell and expand without becoming buoyant can still be used to give spatial placement of a dosage form in the stomach. Gastric emptying is a size-dependent process and may prevent passage of swollen tablets into the duodenum thereby increasing the resident time of the drug in the stomach (Ding *et al.*, 2005). Arora *et al.* (2005) reported that for gastric contents to pass into the small intestine, their sizes will have to be in the range of 1 to 2 mm and that indigestible solids larger than the pyloric opening are propelled back thereby spending more time in the stomach (Figure 1.4). Valrelease® is an example of a product formulated as floating tablets of diazepam (Shargel *et al*., 2005).

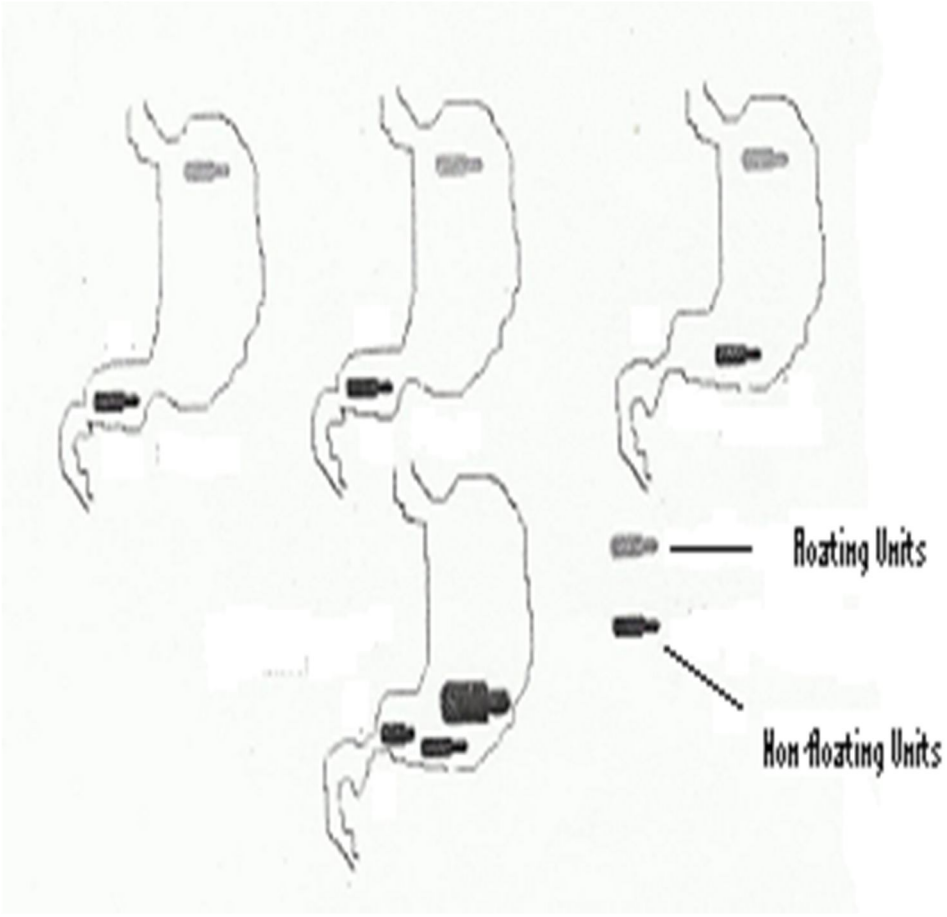


Figure 1.4: Intragastric residence positions of floating and nonfloating gastroretentive units (Arora *et al*., 2005**)**

1.3.5.9 Erosion Controlled Systems

These are systems that operate on the principle of the tablet eroding from the surface. Surface erosion of the polymer caused the controlled release of the drug. This is a relatively safe method of controlling drug release as possibility of dose dumping is eliminated. Drug release rate is proportional to polymer erosion and this can be controlled by changing polymer thickness or quantity of drug in the device (Ding *et al*., 2005). Constant-T® and Tenuate Dospan® containing theophylline and diethylpropion HCl respectively are examples of products whose drug release is controlled by erosion of the tablet (Shargel *et al.*, 2005).

1.3.5.10 Targeted Drug Delivery

These are a group of modified-release delivery systems designed to lodge the drug at therapeutic concentratios in specific sites with minimal access to nontarget sites (Ding *et al*., 2005). Colonic delivery is a form of targeted drug delivery intended for targeted drug release into the lower gastrointestinal (GI) tract. They are mostly used in the localized infections involving the colon such as irritable bowel syndrome, inflammatory bowel disease and colon cancer (Singh, 2007). Technologies developed in formulating the colonic delivery systems are broadly divided into (a) pH-dependent (or delayed-release) system designed to release the drug in response to change in pH, (b) time-dependent (timed-release) system designed to release the drug after predetermined time, (c) microbially-dependant (or microbially-controlled) system making use of the abundant enterobacteria in the colon, and (d) pressure-dependant system making use of luminal pressure in the colon (Singh, 2007).

Targeted delivery to pathological areas has also been of great benefit in the management of cancers (Torchilin, 2007). Desai and Kumar (2004) prepared and evaluated a novel buccal

adhesive system containing propranolol hydrochloride. In general, a targeted drug delivery system is preferred in the following situations (Ding *et al.*, 2005).

1. Pharmaceutical: drug instability, low solubility
2. Pharmacokinetic: short half-life, large volume of distribution, poor absorption
3. Pharmacodynamic: low specificity, low therapeutic index

1.4 Capsules as Solid Dosage Forms

Capsules are solid dosage forms in which the medicinal agent(s) is/are contained in a hard or soft shell made using gelatin. The medicinal agent could be in powdered or granular form and the shell is designed to dissolve upon contact with a liquid substance e.g. the gastric fluid. Hard gelatin capsules are used in most commercial medicated capsules. They come in different colors and sizes. Capsules are tasteless, easily administered and easily filled either extemporaneously or in large quantities. The shells are prepared separately to contain two sections. The drug is completely sealed by a slipping action of one part of the capsule over the other (Lund, 1994; Allen et al., 2005; Ding et al., 2005).

The soft gelatin capsules are also made of gelatin but to which glycerin or a polyhydric alcohol such as sorbitol has been added and commonly contain a preservative. They are designed to contain medicaments in liquid forms, as pastes or as solids (Allen et al., 2005).

1.5 Statement of Research Problem

Drug delivery is still a subject of great relevance in the horizon of scientific research. While the discovery or synthesis of novel therapeutic agents is taking the center stage in most pharmaceutical companies, excipients development is the next most important area worthy of being researched on. Excipients take the bulk of the floor in any research and development (R & D) unit of any pharmaceutical company, underpinning their relevance in drug delivery. An excellent therapeutic agent may be worthless if it cannot be formulated into an appropriate dosage unit, and this is commonly facilitated by optimally harnessing the varied functionalities of excipients or pharmaceutical aids required to make the dosage unit.

*Drugs/medicines are either imported into the underdeveloped/developing nations or the raw materials are brought in for local production of pharmaceuticals. The import of either case is that, it economically overburdens a large proportion of the populace who are predominantly poor. Most of the active agents or excipients needed for the production of such medicines, paradoxically, can be found relatively available in the local habitats of such nations and where they are absent, suitable substitutes abound. T*he underdeveloped world may be lacking in technological advancement but are blessed with abundance of natural resources. And, as *Chaubal (2006) opined, majority of polymers used in oral drug delivery are derived from natural sources.*

Gums and other polysaccharides have been found to be of great relevance in the production of pharmaceuticals and their derivatives are even of greater significance. Modification of drug release with its attendant beneficial effects has been made possible by derivatisation of polymeric materials. Explorations into the abundant natural resources may lead to valuable discoveries and derivatisations of the discovered polymers may pave way for the production

*of novel pharmaceutical actives or aids. Cashew is a tree that can thrive in a variety of environments and the gum it produces has so far been popular for use as varnishes. Its relevance in immediate release dosage formulation had been reported (*Okoye *et al.,* 2009; *Ofori-Kwakye et al., 2010; Gowthamarajan et al., 2011). Investigating the tableting potentials of cross-linked derivatives of this gum may open up another opportunity of getting yet another polymeric material for use in specialized drug delivery systems.*

In all, such discoveries may produce a multiplier effect in that the nation's foreign exchange may receive a boost if the product is of export quality, while the local people get employed for cultivation or harvest of the product. Health care delivery may as well become relatively cheaper when the actives and or excipients are produced locally.

1.6 Justification

Gums, starches and celluloses are greatly employed in pharmaceutical procedures to modify drug release profiles. Developing nations usually import these materials, a feat that is not economically reasonable in most nations endowed with locally available and easily renewable materials that can substitute the imported ones.

The cashew tree is well adapted to seasonally wet/dry tropical climates (Noel, 2000) and has become naturalised in many tropical countries, particularly in coastal areas (Pursglove, 1971). The tree is greatly abundant in some parts of Nigeria and if desired, can be grown in almost all parts of the country thereby multiplying the beneficial economic influences of the tree or its products on the common man and the nation in general.

The ability of CG to form matrices, the gum's abundance and the significance of the modified-release dosage forms in medicine, attract fuller investigation into applications of CG and its derivatives as matrices in the preparation of modified-release formulations.

Cashew gum can stand as prototype gum in this type of investigational procedure so that other gums and in general polysaccharides can be assessed thereby widening the researchable scope.

1.7 The Null Hypotheses

i. CG cannot be cross-linked using citric acid (CTR), a polycarboxylic acid.

i. Cross-linking CG with CTR does not confer tablet sustained-release properties to CG.

ii. Cross-linking CG with CTR does not confer tablet drug-release enhancing property to CG.

1.8 Aim of the Study

To study the tablet formulation properties of cross-linked cashew (*Anacardium occidentale* L.) gum.

The specific objectives of this work include:

i. *to collect and identify crude cashew exudates.*
ii. *to extract and produce the cashew gum powder from the crude cashew exudates.*
iii. *to cross-link the gum using epichlorohydrin.*
iv. *to cross-link the gum using polycarboxylic acids at varying ratios.*
v. *to cross-link the gum using citric acid and glycerol at varying ratios.*

vi. *to carry out differential scanning calorimetry (DSC), Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectral analysis of the gum and the cross-linked polymers.*

vii. *to determine the solubility profile of the cross-linked polymers.*

viii. *to study the physicochemical and physicotechnical properties including loss on drying, hydration, swelling, water content and water retention capacities of the developed polymers.*

ix. to determine drug-polymer compatibility between Ven. HCl (test drug) and CG, CrosCC or CrosCCG (excipient polymers).

x. to carry out formulation studies on tablets of Ven. HCl, a water soluble antidepressant, made with varying concentrations of CG or its admixtures with MCC PH 102 as binder or release retardant.

xi. to carry out formulation studies on tablets of Ven. HCl made with HPMC ER K100 as binder and varying concentrations of CrosCCG as release retardant.

xii. to carry out formulation studies on tablets of Ven. HCl made with HPMC ER K100 as binder and CrosCCG as drug-release facilitating gent.

xiii. to establish the mechanism and kinetics of Ven. HCl release from tablets containing HPMC ER K100 as binder and CrosCCG as release retardant by fitting release data into various drug-release models.

xiv. to statistically analyze and compare the results obtained from the studies as basis for the use of cashew gum in tablet formulations and also to substantiate any value added by its cross-linking.

xv. to establish the safety status of the cross-linked polymer (CrosCCG) using sub-acute toxicity studies on laboratory animals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Use of Natural Materials in Pharmaceutical Formulations

2.1.1 Polysaccharides

Carbohydrates, structurally made of carbon, hydrogen and oxygen, are the single most abundant class of organic compounds found in nature. They are a group of substances that include the sugars, starches, gums and celluloses. Carbohydrates are broadly classified into monosaccharides, disaccharides, oligosaccahrides and polysaccharides. Of these classes, the polysaccharides, owing to their long chains, prove to be of immense relevance in drug delivery in the pharmaceutical arena.

Polysaccharides are high molecular weight carbohydrates composed of multiples of repeating monomeric sugar units linked principally by β-1, 4- glycosidic linkages with a molecular weight range of 16,000 to 14,000,000 Daltons (Ali, 2002). They are the most abundant (by mass) of all organic substances in living organisms, comprising about two-thirds of the dry weight of the total biomass (BeMiller, 2009). Polysaccharides are termed homoglycan when the repeating sugar units are of the same type or heteroglycan when comprising of more than one sugar type. Polysaccharides serve as reserve nutrients e.g. in starches, skeletal materials as with celluloses or protective against disease conditions in gums (Balbaa, 1978; Agarwal, 2000).

*The polysaccharides, comprising of starches, gums and celluloses; and their derivatives, represent a class of polymeric compounds largely used in pharmaceutical formulations and in systems for controlled drug delivery (Coviello et al., 2005; Rajesh et al., 2009; Ogaji et al., 2012). Polysaccharides with varying physicochemical properties can be extracted from plants at relatively low cost and can be chemically modified to suit specific needs (*Beneke *et al.*, 2009). *They are thus indispensable components in the manufacture of medicines for human or animal use where they are employed to provide varied functions (Table 2.1) as dictated by the polymer's physicochemical properties*

Researches in pharmaceutical technology are aimed at improving the technology of production/derivatisation of pharmaceutical aids via explorations into the vast untapped natural resources. This is desirous especially in the developing nations, as it offers the opportunity of discovering raw materials with pharmaceutical relevance possessing higher or comparable performance that can possibly, often after modifications, present themselves as affordable substitutes.

The polysaccharides commonly used in pharmaceutical processing comprise of long linear or branched chains of monomeric sugar units that are held together principally by 1-4 but often with additional 1-6 glycocidic linkages. They have varied physicochemical properties that are commonly defined by the polymer's molecular weight, chain length and or branching. They thus range from soluble gums to insoluble starches and celluloses. These properties are what guide the employment of polysaccharides in pharmaceutical processing. Structural modifications have been used to effect some changes that eventually tailor the properties of polysaccharides to suite a particular interest. The polysaccharides currently in use include celluloses, starches and gums.

Table 2.1: *Classes of Polysaccharides Commonly Employed in Pharmaceutical Processing*

Class	***Ingredient***	***Use in pharmacy***
Celluloses	*Methyl cellulose (MC)*	*Suspending and or viscosity-increasing agent; tablet binder; coating agent*
	Ethyl cellulose (CMC)	*Tablet binder; coating agent*
	Sodium carboxymethyl cellulose (SCMC)	*Tablet binder and disintegrating agent, viscosifier*
	Hydroxypropyl methyl cellulose (HPMC)	*Suspending and or viscosity-increasing agent; tablet binder; coating agent; film former; emulsion stabilizer*
Starches	*Maize starch*	*Binder, filler for tablets and capsules; disintegrant in tablets; coating agent*
	Potato starch	*Tablet binder*
	Cassava starch	*Tablet binder, filler and disintegrant*
Gums	*Acacia gum*	*Emulsifier and suspending or viscosity increasing agent, tablet binder*
	Xanthan gum	*Thickening , stabilizing and suspending agent, controlled release agent*
	Guar gum	*Tablet binder and disintegrant*
	Tragacanth gum	*Viscosifier or suspending agent*

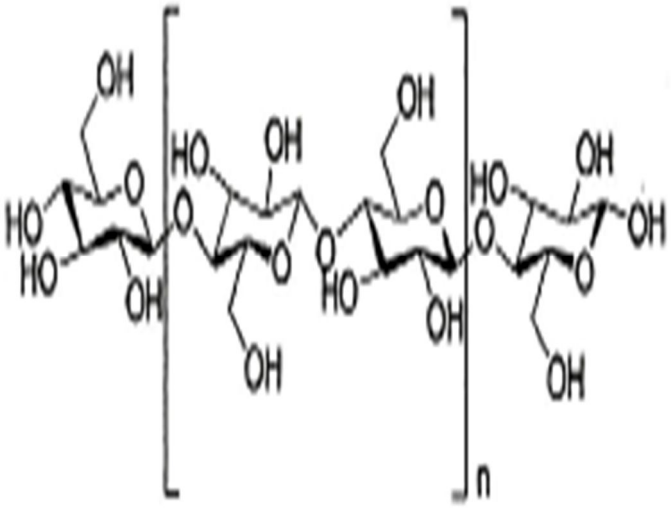
Adapted from (Jani et al., 2009; Rana et al., 2011)

2.1.1.1 Celluloses

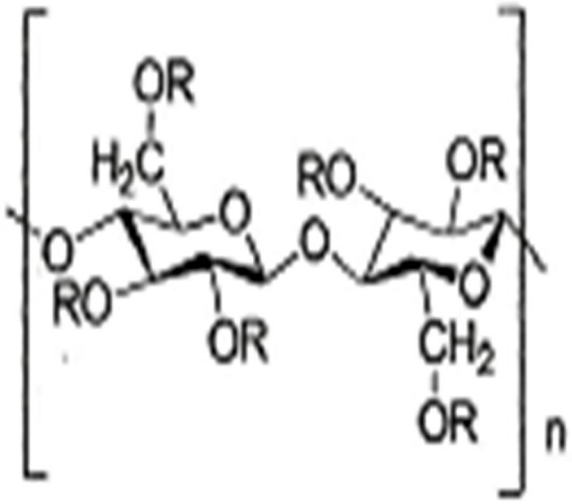
Cellulose is a colorless solid, insoluble in water and organic solvents but readily soluble in ammoniacal cupric hydroxide solution (Agarwal, 2000) consisting of β-1,4-linked D-glucose units (Figure 2.1). It is the most abundant, widely distributed and greatly used plant related material in pharmacy. Cellulose and its derivatives are greatly employed excipients in pharmaceutical preparations as binders, coating agent, suspending agent, viscosity enhancing agents etc. Cellulose, whose structure is shown in Figure 2.1a, is insoluble in

water and indigestible by the human body and this has reduced its applicability in formulations where solubility in water is desired. This has, however, been overcome by the development of cellulose derivatives with improved performances. Derivatisation of celluloses through cross-linking, etherification, esterification or graft copolymerization has yielded polymers like hydropropyl methyl cellulose, cellulose acetate, etc, which have been used in modification of drug release profiles (Beneke *et al.*, 2009; Ogaji, *et al.*, 2012). Cellulose derivatives had been used to make monolithic matrix systems or in film coating techniques to prepare enteric coated tablets or prepare semi-permeable membranes for osmotic pump delivery systems (Beneke *et al.*, 2009). Example of an etherified derivative of cellulose popularly used in controlled drug release is hydroxyprophyl methylcellulose (HPMC) (structure shown in Figure 2.1b). HPMC was used as reference binder/matrix former in assessing the drug release modification of the cross-linked derivatives of CG in this study.

HPMC has been described as the most important hydrophilic carrier material used for the preparation of oral controlled drug delivery systems. It functions by forming a gelatinous barrier layer around the tablet upon contact with water (Siepmann and Peppas, 2001; Levina and Rajabi-Siahboomi, 2004).



a



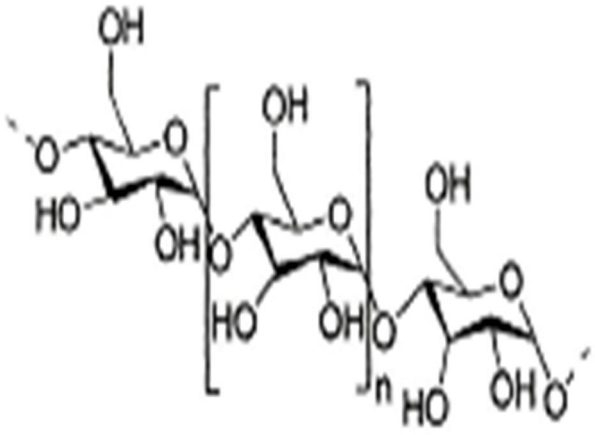
b

Figure 2.1: Chemical structure of a) cellulose and b) hydroxypropyl methylcellulose (HPMC). The substituent R represents either a $–CH_3$; a $–CH_2CH(CH_3)OH$, or a hydrogen atom (Siepmann and Peppas, 2001; Beneke, 2009)

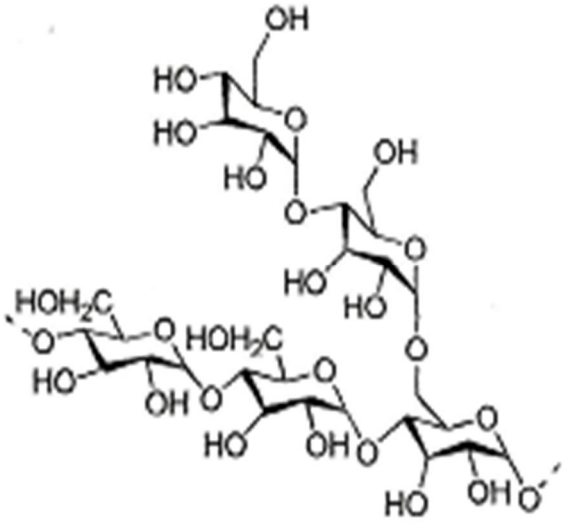
2.1.1.2 Starches

Starch is comprised of two polymers namely amylose and amylopectin. Amylose is a non-branching helical polymer consisting of α-1, 4 linked D-glucose monomers (Figure 2.2a), while amylopectin on the other hand is a highly branched polymer consisting of both α-1, 4 and α-1, 6 linked D-glucose monomers as shown in Figure 2.2b (Beneke *et al.*, 2009). Tables 1.4, 1.5, 1.7 and 2.1 reflect the worthiness of starches in pharmacy. Starches are used in tablet formulations as filler, binder or disintegrant, depending on the formulation design. Starches from maize, potato and cassava are very popular in the formulation arena.

Oyi *et al.* (2009) reported use of wheat and rice starches in tablet formulation, where they concluded that wheat starch made stronger tablets and could thus be suitable for the formulation of chewable tablets and lozenges. Garr and Bangudu (1991) compared sorghum starch with maize starch when used as tablet binder and disintegrants. Ibezim *et al.* 2008) reported the role of ginger starch as a binder in acetaminophen tablets. The results obtained from this work showed that at a binder concentration of 2.0-8.0 %w/w, ginger starch produced tablets that were as good as those produced using gelatin. Uhumwangho *et al.* (2006) while working on the influence of some starch binders on the brittle fracture tendency of paracetamol tablets compared the binder effects of cassava and cocoyam starch with that of maize starch. The authors found that cassava starch which was the most viscous, displayed the highest potential for ameliorating brittle fracture during manufacture of paracetamol tablets.



a



b

Figure 2.2: Chemical structure of starch, with a) amylose and b) amylopectin (Beneke *et al*., 2009)

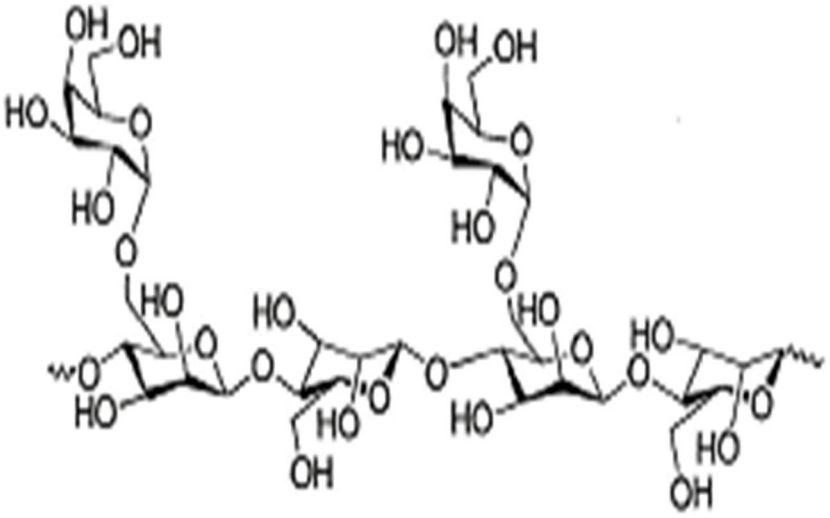
2.1.1.3 Gums

Gums are naturally occurring polymeric materials of plant or animal origin. They are components of plants, making both higher and lower plants serve as good sources of gum for pharmaceutical applications. Gums were formally known mostly in book binding but have now found use in foods (Nussinovitch, 2003) and also in pharmaceutical processing as raw materials for formulation of both conventional and novel dosage forms. Abundance, sustainability, biodegradability and biosafety had made the polysaccharide gums a subject of intensive research in industrial raw material development (Jani *et al.,* 2009; Amelia *et al*., 2011; Rana *et al*., 2011, Ogaji *et al*., 2012). Gums can be of exudative, extractive or seed source.

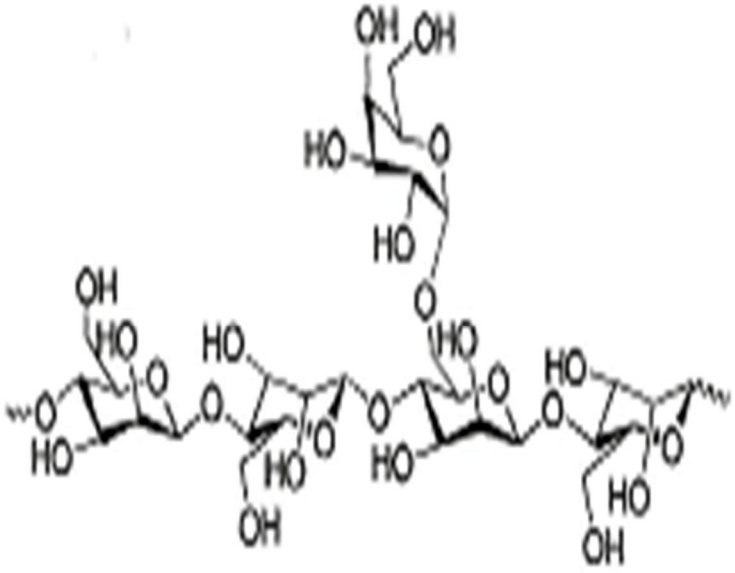
The plant tree gums are obtained as exudates on the bark of the tree trunks or branches following accidental or deliberate incision on the plant part. As a protective measure following incision on the plant part, a viscous fluid is secreted by the tree that flows over the affected area and even beyond in some instances and eventually dries to a brittle, translucent, glassy hard mass. Gums are thus said to be pathological products produced mainly as protective in consequence to an injury inflicted on the plant (Ogaji *et al.*, 2012).
These authors reported that the application of gums in pharmacy has been recognized since the 1800s with the United States Pharmacopoeia of 1820 containing gums like tragacanth, acacia, sterculia, while the National Formulary of 1947 reporting on sodium alginate and agar. And also that goggle browser checks have shown that gums and mucilages are the most highly investigated materials in recent time as potential pharmaceutical excipients.
Gums are now used as binders, drug release modifiers, disintegrants, emulsifiers, thickeners, viscosifiers etc. when employed in their natural forms or as derivatives after some structural

modifications (Anthony and Sanghavi, 1997; Hagerstrom, 2003; Singh *et al.*, 2010; Nep and Conway, 2010; Onunkwo, 2010; Singh *et al.*, 2010; Srivastava *et al.,* 2010; Avachat *et al.*, 2011; Chaudhari and Patil, 2011; Rana *et al*., 2011). Gums like gum Arabic, guar gum (structure shown in Figure 2.3a) and locust bean gum (structure shown in Figure 2.3b) have shown excellent potential as carrier materials in matrix type controlled release dosage forms such as microparticles, beads, tablets and cross-linked hydrogels (Beneke, 2009). Gums are therefore versatile in their use as pharmaceutical aids and hence need to be very readily available and also relatively inexpensive. Most local habitats abound with gum producing plants, exploration of which is thus, desirable for formulation and economic concerns. Pharmaceutical applications or uses of gums (Table 2.2) as culled from a larger table on pharmaceutical applications or uses of gums and mucilages (Jani *et al.*, 2009) have shown the versatility of gums in pharmacy.

Other gums investigated for pharmaceutical applications include mango (*Mangifera indica*) gum, which has been characterized and evaluated for use in formulations of paracetamol tablets (Singh *et al.*, 2010). The gum, extracted using acetone, was found to make good tablets when used as binder at a concentration of 5 %w/w. Locust bean gum derived from the seeds of the plant carob, *Ceratoniasiliqua* Linn has been combined with chitosan to modify the release of mesalazine (Ogaji *et al.*, 2012).



a



b

Figure 2.3: Chemical Structure of a) Guar Gum, b) Locust Bean Gum (Beneke, 2009)

Table 2.2: *Pharmaceutical Applications of Gums*

Common name	***Botanical name***	***Family***	***Pharmaceutical application***
Albizia gum	*Albizia zygia*	*Leguminoseae*	*Tablet binder*
Cashew gum	*Anacardium occidentale*	*Anacardiaceae*	*Suspending agent*
Guar gum	*Cyamompsis tetraganolobus*	*Leguminoseae*	*Binder, disintegrant, thickening agent, sustained release agent*
Gum acacia	*Acacia arabica*	*Leguminoseae*	*Binder, emulsifier, suspending agent*
Gum ghatti	*Anogeissus latifolia*	*Combretaceae*	*Binder, emulsifier, suspending agent*
Gum tragacanth	*Astragalus gummifer*	*Leguminoseae*	*Suspending and emulsifying agent, sustained-release agent*
Karaya gum	*Sterculia urens*	*Sterculieaceae*	*Suspending and emulsifying agent. Sustained-release agent*
Khaya gum	*Khaya grandifolia*	*Meliaceae*	*Binding agent*
Leucaena seed gum	*Leucaena leucocephata*	-	*Emulsifier, suspending agent, disintegrant, binder in tablets*
Xanthan gum	*Xanthomonas lempestris*	-	*Suspending agent, emulsifier, sustained-release agent*
Gellan gum	*Pseudomonas elodea*	-	*Disintegrant*

Adapted from Jani *et al.* (2009)

2.1.1.3.1 The Cashew Tree and its Medicinal and Economic Values

The cashew, *Anacardium occidentale* (Plate 2.1) is a resilient and fast growing evergreen tree that can grow to a height of 20 m (60 ft) (Davis, 1999). It is a member of the Anacardiceae family and has been described as a multipurpose tree with great economic importance principally because of the nutritious kernel used extensively in confectionary. The tree is well adapted to seasonal wet/dry tropical climates, and has the capacity to grow and yield satisfactorily on well drained, light textured soils with minimum inputs. Cashew tree has good adaptability to wide ecological differences (Grundon, 2000; Ezeagu, 2002; Hammed *et al.*, 2008). Nonetheless, a statistician has been reported describing cashew tree as the most variable agricultural plant he has worked with, when he noticed trees producing up to 40 kg of nuts in a year and by the following year the production got reduced to zero (Hilton, 1998).

Cashew tree is popular because of its edible nut. The nut or seed (Plate 2.2) is kidney-shaped and resembles a large bean (Ike and Chukwuji, 2005). It develops below the peduncle or apple and is usually separated from the apple during harvest by a twisting action (Ezeagu, 2002). The nut is roasted and consumed as salted nuts locally where processing is a problem (Hilton, 1998). Adequate processing, however, especially in nations with processing industries, has made cashew nut an international trade commodity used as popular dessert nut (Davis, 1999; Grundon, 2000; Mitchell, 2004).

Processing of cashew nut is associated with the emission of a poisonous substance called cashew nut shell liquid (CNSL). This substance, containing 90 % anacardic acid and 10 % cardol (Davis, 1999) has been used as brake fluids in automobiles and airplanes, antimicrobials, varnishes, insecticides and electrical insulations (Grundon, 2000; Ezeagu,

2002). The pseudo-fruit or apple of cashew (Plate 2.2) is the swollen red or yellow fleshy peduncle where the nut is attached. It is fibrous but juicy, sweet, pungent and rich in vitamins A and C (Davis, 1999) and has been used in making drinks, wines, pickles, candy and jam as well as in animal feed (Davis, 1999; Grundon, 2000).

Extracts of leaves from cashew tree have been shown to have antifungal, antibacterial and nematicidal activity (Grundon, 2000), while the bark is chewed for sore gums and toothache (Davis, 1999).

Cashew tree has been used as an ornamental and alley tree and greatly employed in afforestation and erosion control schemes in Nigeria (Ezeagu, 2002; Hammed *et al.*, 2008). The tree exudes a gum that can be used in varnishes or in place of gum Arabic (Davis, 1999, Lima *et al.*, 2002). This gum presents a great potential for industrialization (Miranda, 2009).



Plate 2.1: Cashew (*Anacardium occidentale* L.) Tree



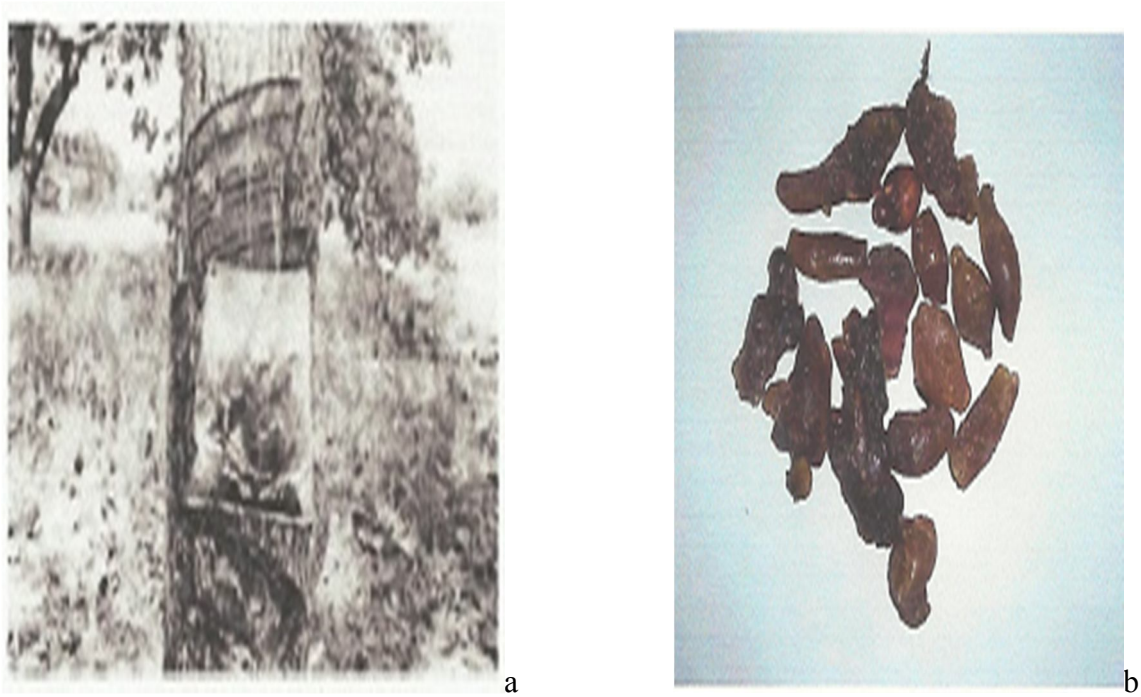
Plate 2.2: Cashew (*Anacardium occidentale* L.) nut and peduncle (false fruit)

2.1.1.3.2 Cashew Gum and Modes of Collection

A gum from the cashew tree, commonly called cashew gum is the subject of discussion in this work. Its physicochemical properties had been investigated (Marques and Xavier-Filho, 1991; de Paula *et al.,* 1998; Mothe and Rao, 1999; Lima *et al.,* 2002; Gyedu-Akoto *et al.,* 2007). A study of cashew gum from Brazil revealed the presence of arabinose, mannose, xylose, rhamnose, galactose and glucose sugars after hydrolysis (Paula and Rodrigues, 1995); while Abdulsamad *et al.* (2008) reported identifying glucose, xylose galactose, ribose and rhamnose sugars in a sample from Nigeria. The differences in composition may be associated with differences in climatic conditions (Lima *et al.*, 2002).

Collection of cashew gum is achieved through two principal methods. One method is by deliberately making incisions into the stem or branches of the tree and the exudates collected as shown in Plate 2.3a. The other method is collection via plucking of the dried gum

generally found attached to the stem or branches. This method relies on the accidental breakages of tree parts as a result of strong winds or often after illnesses of the tree. Such collections appear nodular in shape as shown in Plate 2.3b, and in most cases require some separation from stem bark and other extraneous matter (Miranda, 2009).



a b

Plate 2.3: Cashew tree exudates (a) Collection of exudates following deliberate incision, (b) Dried exudates collected from tree bark (Lima *et* al., 2002; Miranda, 2009)

2.1.1.3.3 Relevance of Cashew Gum in Pharmacy

CG has been investigated for use as binder in conventional immediate release tablet formulations. Abdulsamad *et al.* (2008) investigated the binding properties of CG in metronidazole tablet formulations in comparison to acacia gum. The authors reported that the gum at 5 %w/w binder concentration effectively made good tablets of metronidazole. In

a similar study, Ofori-Kwakye *et al.* (2010) used CG as binder at concentration range of 4 - 8 %w/w to prepare metronidazole tablets. Gowthamarajan (2011) reported that the gum could be used as binder at concentrations as low as 2.5 %w/w to make paracetamol tablets. In a separate study, Okoye *et al.* (2009) concluded that CG at binder concentration range of 1.0 – 3.0 %w/w when used to formulate paracetamol tablets achieved very good release profile and mechanical properties and should thus be exploited as alternative to povidone and gelatin. This, they argued, could reduce cost of tablet production. Okoye *et al*., (2010) submitted that CG belongs to the same "family" as acacia when both gums were used in establishing the relevance of brittle fracture index (BFI) as a tool in the classification, grouping and ranking of some binders used in tablet formulation. The gum has been combined with other polymers to form drug release matrices, example with reacetylated chitosan (Maciel *et al.,* 2006), HPMC and Carbopol (Garnesh *et al.,* 2010). *Suspending and emulsifying potentials of CG had been investigated and compared to acacia (Abdulsamad et al., 2006b) and xanthan gum (Asantewaa et al., 2011).*

From the preceding paragraphs, it can be seen that CG has been and is still receiving attention as a researchable polysaccharide for use in formulation of tablets. However, no report has yet been found on the use of modified CG in tablet formulation. Although the gum is a pathologic product, no report has yet been found on the influence of exudation and collection of such exudates on the yield of other commercial products of the tree, like the nut, fruit etc. and no attempt was made in this study to verify that.

2.2 *Use of Modified polymeric materials in Pharmaceutical Formulations*

Structural modifications on gums and other polysaccharides have given rise to derivatives with improved functionality (Hennink and Nostrum, 2002; Rana, 2011). Gums generally known to be soluble and commonly used as binders for immediate-release tablets have been modified and used as disintegrants and in some cases to produce controlled drug release as shown in Table 2.3. Development of novel drug delivery systems and materials for other biotechnological applications can be achieved and should be pursued through discoveries of newer materials as well as modification of the existing structures of polymeric materials (Jani *et al.,* 2009).

2.2.1 Derivatisation Processes

Rana *et al.* (2011) opined that gums in their putative forms are required in very high concentrations to successfully function as drug release modifiers in dosage forms. Polymers whose structures have not been modified are generally of limited use in drug delivery, especially when drug release modification is desired. But, the simplest and most widely used method of effecting changes in the drug release profile of polymers is said to be by formation of cross-linked hydrophilic networks (Brannon-Peppas, 1994; Mukesh *et al.,* 2000).

Derivatisation is the process of imparting changes in the structure of existing molecules usually achieved using methods like addition of functional groups as in carboxymethylation or via cross-linking strands of polymeric materials. The main essence of derivatisation is to enhance suitability or promote functionality of an existing polymeric material.

Table 2.3: *Pharmaceutical Applications of Modified Gums*

Gum	Modification technique	Application
Karaya gum	Heat treatment in hot air oven	Disintegrant
Agar and Guar gum	Heat treatment with co-grinding of both materials	Disintegrant
Tragacanth	Chemical modification using epichlorohydrin	Disintegrant
Acacia gum	Chemical modification using epichlorohydrin	Disintegrant
Guar gum	Chemical modification of guar gum	Disintegrating agent
Sesbania gum	Chemical modification using tartaric acid	Sustained-release formulation
	Chemical modification using acetone:chloroform mixture	Gelling agent
Guar gum	Chemical modification using glutaraldehyde	Colonic delivery
	Chemical modification using isopropanol	Film coating hydrogel

Adapted from Jani *et al.* (2009)

Derivatisation in gums, as in other polymeric materials, is gaining popularity in recent times targeting the modification of these materials to better their performances in advanced drug delivery (*Enel and Mcclure, 2004;* Rane and Kale, 2009; Viral *et al.,* 2010). Polymer modification is required to impart specific properties to the modified material, such as enhanced thermal stability, multiphase physical responses, compatibility, impact response, flexibility and rigidity (Bhattacharya and Ray, 2009).

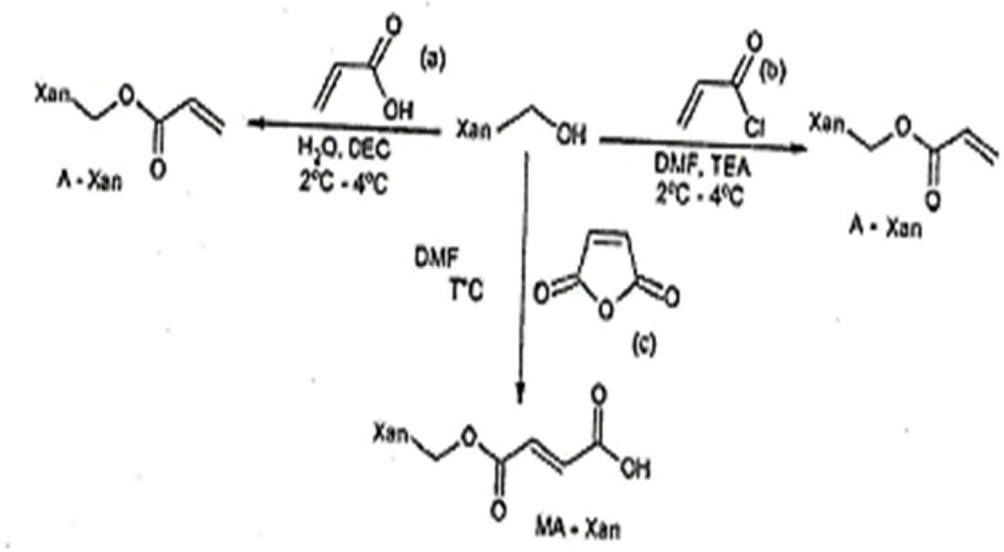
Derivatives or modified forms are semisythetic products obtained following some changes effected on the structure of an existing biomolecule. Such changes are introduced to address some limitations of the existing biomolecule or impart other desired physicochemical or biological properties. The resulting derivatized molecule will expectedly have superior properties over the original molecule. Bhattacharya and Ray (2009) reported that there are predominantly two methods of polymer modifications- grafting and cross-linking, with a less popular third being the reversible physiosorption.

2.2.1.1 Grafting

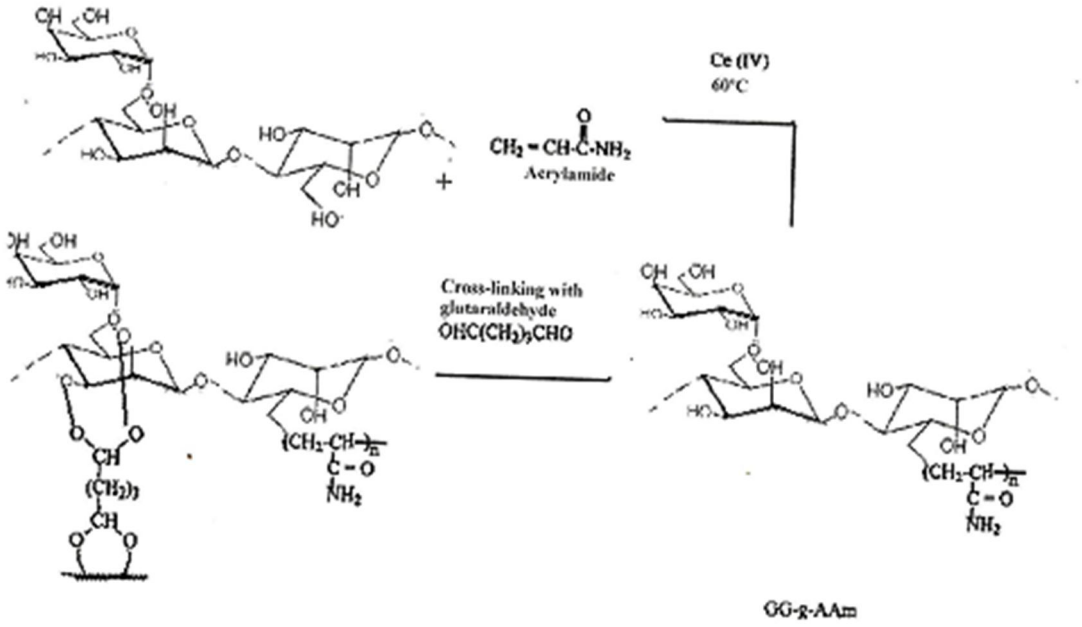
Grafting is an irreversible covalent attachment process where a functionalized monomer is made to react with the backbone polymer to form the grafted one (Bhattacharya and Ray, 2009). Grafting of polymers has greatly increased their physicochemical advantages and current demands in biomedical field, for instance cyclodextrins (CDs) had been grafted to make novel designs in many biomedical applications (Misra *et al*., 2009). Sand *et al*. (2010) reported graft copolymerization of 2-acrylamidoglycolic acid on to xanthan gum by free radical polymerization using bromated/thiourea redox pair in an inert atmosphere. The resulting graft copolymer showed better properties for swelling, metal ion sorption,

flocculation studies and was thermally more stable than pure xanthan gum. Mishra *et al.* (2008) developed a new green polymeric material of specialty application by grafting polyacrylamide with okra mucilage.

Fellows (2009) suggested that a method of following kinetics of grafting include monitoring of monomer consumption by FTIR, NMR, UV/Vis spectroscopy, calorimetry or use of a microbalance. The author equally proposed soxhlet extraction as way of verifying whether the "grafted" polymer is really grafted and not just entangled. Grafting has been used in the polymerization of xanthan gum as shown in Scheme 2.1 (Rana *et al.*, 2011). These authors also reported grafting of guar gum with polyacrylamide followed by cross-linking with glutaraldehyde (Scheme 2.2) and carboxymethylation of gums (Scheme 2.3). Graft copolymerization of dextran and polyacrilamide (Krishnamoorthi *et al.,* 2007) and 2-acrylamidoglycolic acid on to xanthan gum (Sand *et al*., 2010).



Scheme 2.1: Process of Grafting Xanthan Gum. Key: (a) acrylic acid; (b) Acryloyl chloride; (c) Maleic anhydride. A-Xan: Xanthan gum,TEA: Triethanolamine, DMF: Dimethylformamide, DEC: N'-[3-(dimethylamnopropyl)]-N-ethylcarbodiimide hydrochloride, MA; Maleic anhydride, ACT: Acetone (Rana *et al*., 2011)



Scheme 2.2: Grafting of Guar Gum with Polyacylamide (pAAm-g-GG) followed by Cross-linking with Glutaraldehyde (Rana *et al.*, 2011).

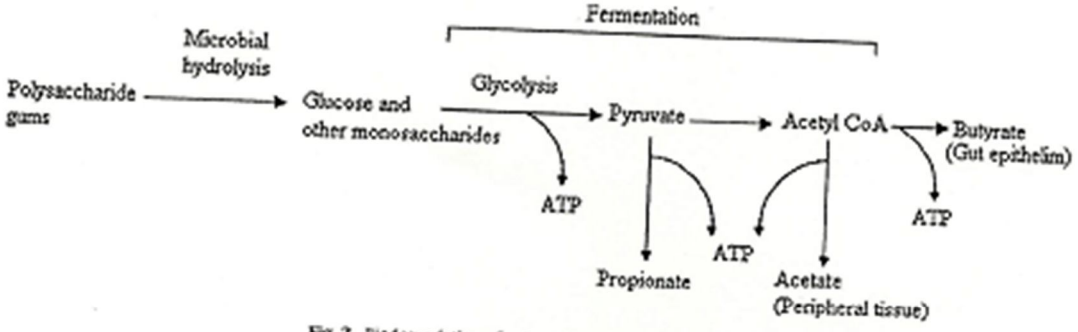
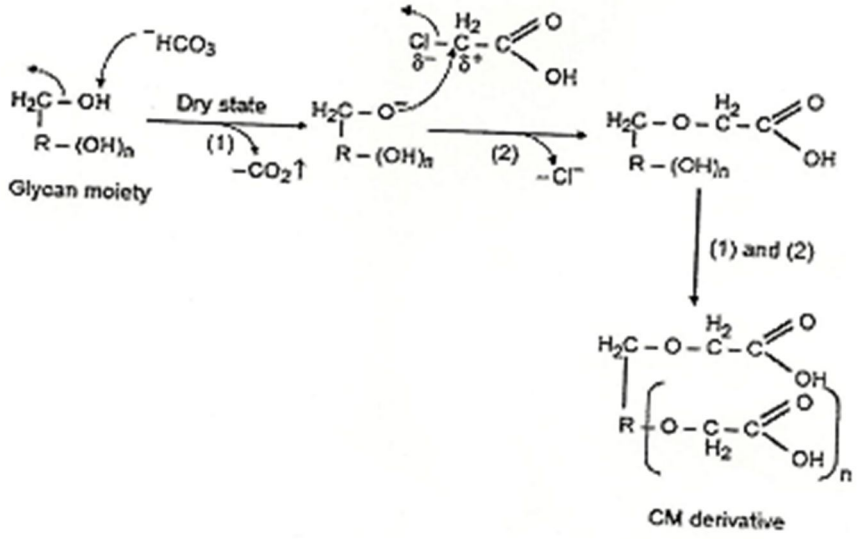


Fig. 2. Biodegradation of polysaccharides in the intestine.



Scheme 2.3: Carboxymethylation of Gums (Rana *et al.*, 2011)

2.2.1.2 Cross-linking

Cross-linking is used to describe the association of polymers through chemical or physical bond formations. The association could be intra- or intermolecular and in most cases is irreversible (Bhattacharya and Ray, 2009). Cross-linking has been used to make three-dimensional networks of water insoluble polymers, referred to as hydrogels that are of great pharmaceutical and biomedical relevance (Hoare and Kohane, 2008). Hydrogels are formed either by physical cross-linking, generally referred to as sol-gel transition, or by covalent bonding (chemical cross-linking) between polymer chains (Misra *et al*., 2009). Physically cross-linked hydrogels are obtained by processes like H-bonding, stereo-complex formation, hydrophobic and electrostatic interactions, protein interactions or by physically cross-linking amphiphilic block and graft copolymers (Hennink and Nostrum, 2002; Hoare and Kohane, 2008). Formation of hydrogels via chemical cross-linking is achievable through radical polymerization, chemical reaction of complimentary groups, by high energy irradiation or by enzyme action (Hennink and Nostrum, 2002). Hydrogels being products of cross-linking are insoluble in water but capable of imbibing large amounts of water, a process that make them swell (Peppas *et al.*, 2000; Hennink and Nostrum, 2002, Hoare and Kohane, 2008; Misra *et al*., 2009). While the kinetics of grafting is assessable, that of cross-linking is best monitored using gel fraction as rate of cross-link formation is rarely experimentally assessable (Fellows, 2009).

Cross-linked polymer networks are a group of polymeric materials that are synthesized or semi-synthesized by joining strands of the polymers through covalent or ionic bond formation, or by simple physical means. They have found relevance in pharmaceutical drug delivery courtesy of their ability to swell and had been principally categorized into two by

virtue of their uses i.e. as swellable devices or as disintegrants (Omidian and Park, 2008). These authors in describing agents and devices in oral drug delivery, grouped sodium carboxymethylcellulose, sodium starch glycolate, and cross-linked poly(vinyl pyrollidone) as swellable cross-linked products used as superdisintegrants, whereas acrylamide, acrylic acid, salts and esters of acrylic acid including sodium and sulfopropyl acrylates, 2-hydroxyethyl methacrylate were grouped as another class of cross-linked materials used as swellable devices in controlling drug release. In general, derivatisation of the natural polymers had availed the pharmaceutical industries of better performing or multifunctional biomaterials for use in drug delivery (Peppas, *et al.,* 2000; Hennink and Nostrum, 2002; Hoare and Kohane, 2008; Jani *et al.,* 2009; Rana *et al.,* 2011).

2.2.1.2.1 Chemical Cross-linking

Modification by chemical methods involves joining two or more polymer chains via formation of covalent or ionic bonds. Chemical c*ross-linking of polymer chains is achieved with the aid of a cross-linking agent that has at least two reactive ends capable of chemically attaching to specific functional groups on the cross-linking polymer strands. The cross-linking process eventually insolubilises a water soluble polymer giving a matrix that is highly hydrophilic and completely permeated by water. Aldehydes, ketones, carboxylic acids, sulfonic acids, glutaraldehyde, epichlorohydrin are examples of molecules used as cross-linking agents. Glutaraldehyde has been used to cross-link chitosan polymer strands as shown in Scheme 2.4.* Singh and Vashistha (2008) reported on the radiation-induced polymerization of sterculia gum.

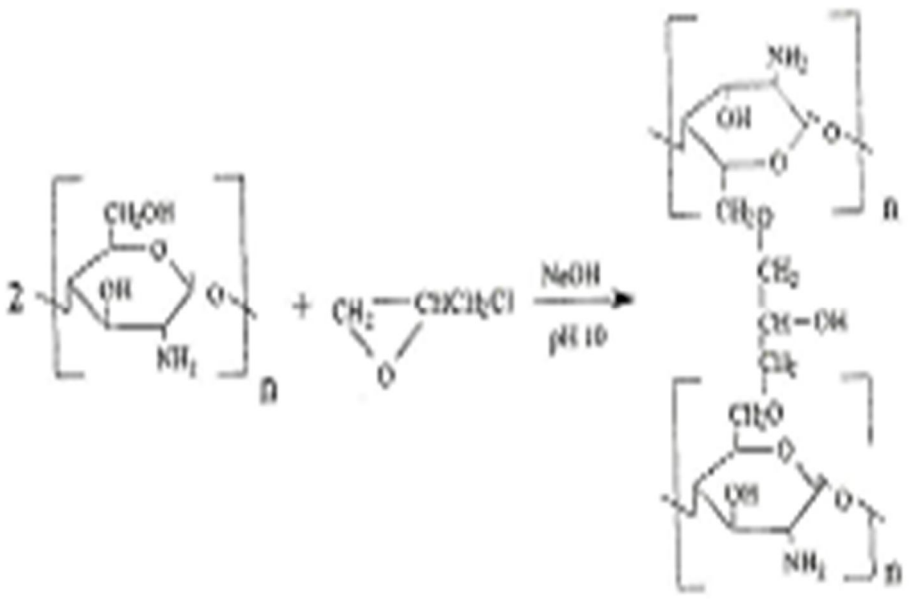


Scheme 2.4: Cross-linking Chitosan using Glutaraldehyde (Goncalves *et al.*, 2005)

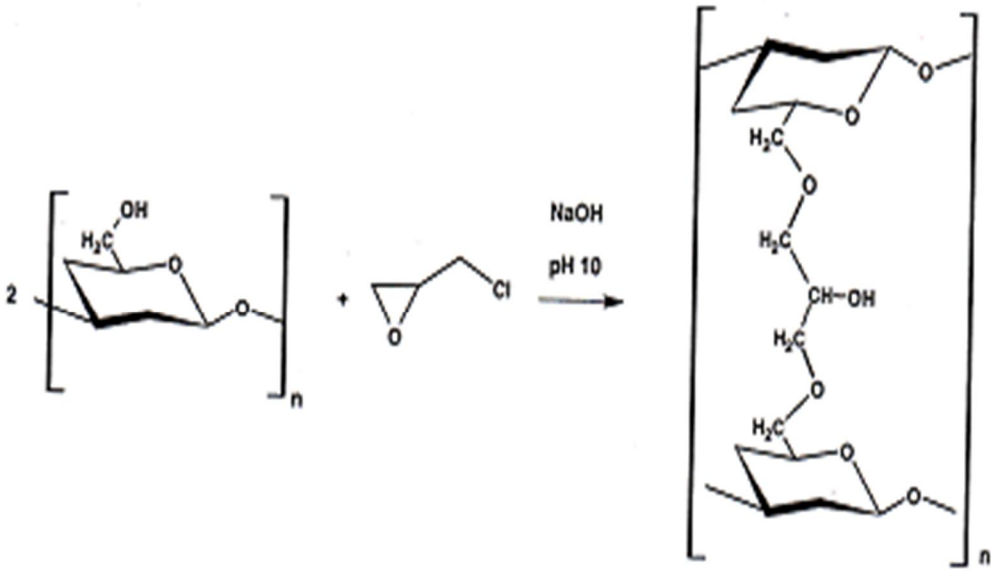
Epichlorohydrin is another powerful cross-liking agent and reports of its being used in polymerization of chitosan (Scheme 2.5) and cashew gum (Scheme 2.6) are in the literature (Goncalves *et al.*, 2005; *Silva et al., 2006)*. Cross-linking by irradiation was reported by Nagasawa *et al.* (2004) when they synthesized a new biodegradable starch derivative hydrogel by irradiation in highly concentrated solution. Chemical cross-linking of polymers based on poly(L-lactic acid-co-L-aspartic acid) has been reported by Elisseeff *et al*. (1997), while cross-linking sucrose polymer by thiol-ene photo-polymerization reported by Ortiz *et al*. (2010).

Employing the use of epichlorohydrin as cross-linking agent, Lima et al., (2002) had investigated the potential use of CG as chromatographic matrix and as a bioaffinity ligand for proteins (lectins). This etherified derivative of CG (Scheme 2.6) with its matrix forming ability has not been assessed for use in tablet formulations. Epichlorohydrin, though a powerful cross-linking agent that has found use in derivatisation processes involving starches, gums and celluloses (Loth and Philip, 1989; Hamdi et al., 2001; Paradossi et al., 2009), is nonetheless reported to be a potential toxic and hazardous substance (Solvay Chemicals Inc., 2003; Martel et al., 2005; Crini and Peindy, 2006; Solvay Chemicals, Inc., 2008).

Safety for use in pharmaceuticals and foods, handling concerns and environmental friendliness precludes the involvement of potential toxins during processing or manufacture of foods and medicines. Epichlorohydrin may thus be a poor candidate as cross-linking agent in processing of foods and pharmaceuticals, warranting the search for alternative safer materials for use as cross-linking agents.



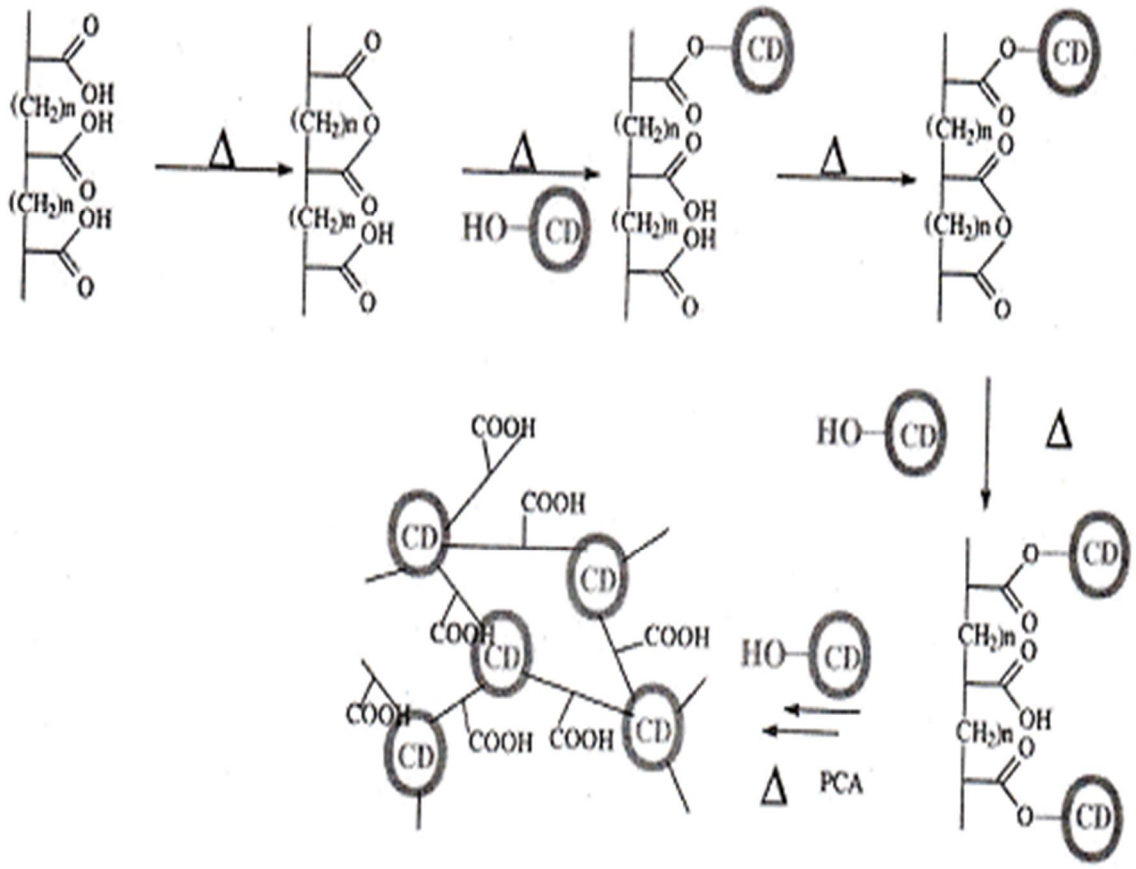
Scheme 2.5: Cross-linking chitosan using epichlorohydrin (Goncalves *et al.*, 2005)



***Scheme 2.6**: The structure of cashew gum cross-linked with epichlorohydrin (Silva et al., 2006)*

Polycarboxylic acids owing to the presence of –COOH groups in their structures have been reported to be used in cross-linking hydroxyl (-OH) containing starch (Reddy and Yang, 2010) and cyclodextrins (Welch, 1992; Dukoroy et al., 2007a, Martel et al., 2005,). In a process of esterification, the –COOH and –OH reacting groups are covalently joined to give an ester (Scheme 2.7). Gums with free alcoholic (-OH) groups seem to be good candidates for modification by cross-linking (Rana et al., 2011). Hydrogen bond formation between the –OH and –COOH molecules of the reacting substances aids cross-linking process (Brannon-Peppas, 1994, Hennink and Nostrum, 2002) while covalent bonds are principally responsible for holding the polymers together.

Copolymerization has generally been utilized to enhance the mechanical properties of hydrogels for use in pharmaceutical formulations (Peppas *et al.*, 2000). Copolymerisation of –COOH containing molecules with those carrying –OH may possibly enhance the polymerization yield and functionality of the polymer.



Scheme 2.7*: Scheme of cross-linking between citric acid and cyclodextrin (Ducoroy et al., 2007a) Key: CD - Cyclodextrin*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

- Epichlorohydrin, Succinic acid, Adipic acid, 1,2,3,4-Butanetetracarboxylic acid Dichlorodimethyl silane, Dimethylformamide, Dimethylsulphoxide, Ethyl acetate, Toluene, Cyclo hexane, Sulphuric acid, Magnesium Chloride hexahydrate, Magnesium nitrate hexahydrate, (Sigma- Aldrich, USA)
- Acetone UN 1090, Citric acid, Sodium chloride, Potassium chloride, Aqualine^{TM} Complete 5, (Fisher Scientific, USA)
- Sodium phosphate monobasic, Methanol UN 1230, (Caledon Laboratories Ltd, Canada)
- Ethanol 95%/2-Propanol 5% (Commercial alcohols, Canada)
- Venlafaxine HCl (Cadila Health Care Ltd., India)
- Hydroxypropyl methycellulose - HPMC LV, HPMC ER (K100) (Dow Chemicals, USA)
- Polyplasdone XL (Crospovidone NF) (ISP Technologies, USA)
- Croscamellose Sodium (FMC Biopolymer, USA)
- Lactose anhydrous (Kerry Bioscience, USA)
- Microcrystalline cellulose (MCC PH 102) (FMC Biopolymer, Ireland)
- Silicone Dioxide (Evonik Industries, USA)
- Magnesium stearate, (Tycol Healthcare, USA)
- Cashew gum was as purified in the laboratory (IntelliPharmaceutics Inc., Canada)
- CrosCE, CrosCC and CrosCCG were as synthesized in the laboratory (IntelliPharmaceutics Inc., Canada)

3.2 Methods

3.2.1 Collection and Identification

The dried exudate and leaf samples obtained from the cashew trees from where the gum was to be collected were identified in the Herbarium of the Biological Sciences, Ahmadu Bello University, Zaria; and assigned Voucher Number 184. The dried latexes from the back of cashew trees were plucked from the identified plantation in Likoro along Zaria - Kano road in Northern Nigeria.

3.2.2 Extraction/Purification of Cashew Gum (CG)

Manually, tree barks and other relatively big particles were picked out of the collected gum. Five hundred (500) grams of the gum was weighed and stirred in 1.5 L of deionised (DI) water contained in a 3 L beaker. The beaker was placed over a boiling water bath (Isotemp. 210, Fisher Scientific, USA) and occasionally stirred until the gum dissolved to give a viscous solution otherwise referred to as "mucilage". The mucilage was filtered using suction through a fine muslin cloth to remove all extraneous matter.

About 1.5 L of acetone was used to precipitate the gum from the aqueous solution of the mixture. One (1.0) L portion of the acetone was gradually poured into the beaker containing the mucilage while stirring to ensure effective distribution of the acetone in the mucilage. In a process of coacervation, the gum began to get separated from the water, becoming more solid on further additions of acetone. Stirring was continued even after the last part of the 1 L acetone has been added. The water- acetone solution was then decanted and the remaining 500 ml acetone was poured onto the precipitated gum to further the extraction and

purification of the gum. The gum was then separated from the acetone by a process of sedimentation and decantation, and the wet mass was spread on non-stick baking trays and dried in an oven (Memmert, Germany) at 40 ^{o}C for 5 h.

The extraction yield (%) was calculated using the relationship:

$$\% \text{ Yield} = \frac{(W2)100}{W1} \quad (2)$$

Where W_1 is weight of crude gum, W_2 is weight of purified gum

3.2.3 Cross-Linking of CG

3.2.3.1 Cross-linking of CG using Epichlorohydrin (EPC)

Epichlorohydrin was used as cross-linking agent in the cross-linking of CG to give a product coded CrosCE (Table 3.1) using a method described by Silva *et al.* (2006) with some modifications.

The gum (100 g) was mixed with 120 ml of 5 M NaOH solution and 70 ml of deionised (DI) water until a homogenous paste was formed. To this mixture was added 40 ml of epichlorohydrin and further kneaded for homogenization. The homogenized mixture was transferred into a culture dish and heated in an oven (Memmert, Germany) first at 40 ^{o}C for 24 h followed by a second heating time of 15 h at 70 ^{o}C. The oven was placed in a fume hood (Mott. Manufacturing Ltd., Canada). DI water was poured on the resulting gel that was clung to the dish, to soak it and get it separated from the dish. The gel was placed on a fine muslin cloth lining a mesh and washed with DI water. The gel was washed by passing DI water until the dark colour associated with the washings ceased to appear. The gel was then

soaked in DI water for 24 h and rinsed thoroughly with more DI water and thereafter dried in an oven (Memmert, Germany) at 40 ^{o}C for 5 h.

Cross-link yield (%) was calculated using the following relationship:

$$\% \text{ Yield} = \frac{\quad}{\quad} \qquad (3)$$

Where Mgel is mass of cross-linked polymer, Mgum is mass of gum used

Table 3.1: Formula for Cross-linking CG using EPC

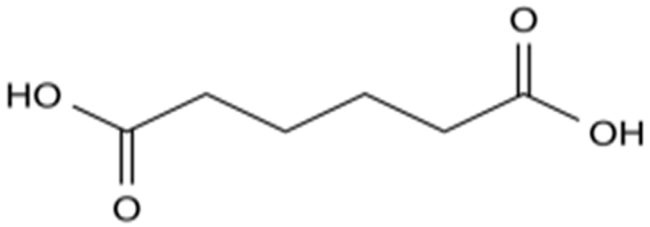
Ingredients	Quantity
CG	100 g
5 M NaOH	120 ml
De-ionised (DI) water	70 ml
Epichlorohydrin	40 ml

3.2.3.2 Cross-linking of CG using Polycarboxylic Acids (PCAs)

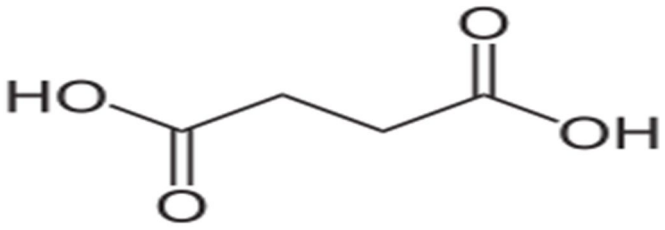
Types of PCAs used for the cross-linking

1. Di-Carboxylic acids, Adipic acid (ADA) and Succinic acid (SCA)

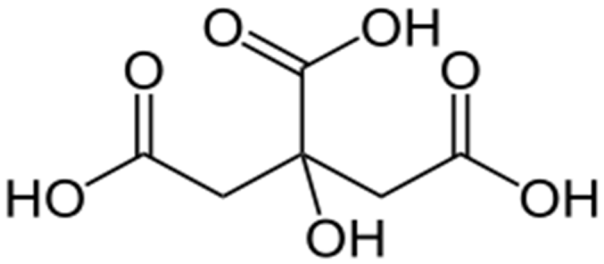
ADA -



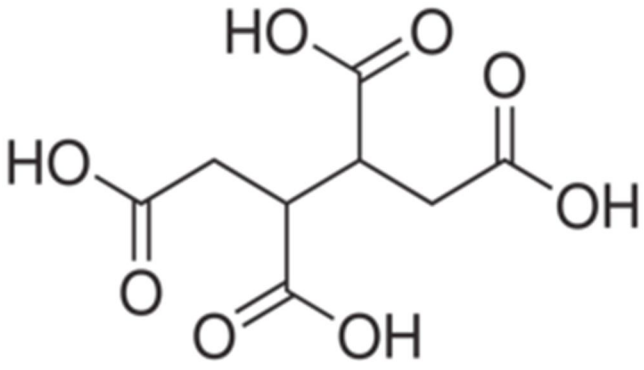
SCA -



2. Tri-Carboxylic acid, Citric acid (CTR)



3. Tetra-Carboxylic acid, 1,2,3,4-Butanetetracarboxylic acid (BTCA)



The methods described for polymerization of cyclodextrins by Martel *et al.,* (2005), Ducoroy *et al.* (2007a), Ducoroy *et al.,* (2007b), Zhao *et al.*, (2009a), Zhao *et al.*, (2009b) where varying proportions of cyclodextrins (a polysaccharide) and polycarboxylic acids (cross-linking agents) were employed, were used with some modifications to crosslink cashew gum, another polysaccharide.

An appropriate quantity of the gum to make 10 %w/v mixture (Ducoroy *et al.,* 2007a) in DI water was weighed into a beaker. Polycarboxylic acid (adipic acid, succinic acid, citric acid or 1,2,3,4-Butanetetracarboxylic acid) and sodium dihydrogen phosphate (serving as catalyst) were weighed and mixed with the gum as shown in Table 3.2. About 100 ml of DI water was added into the beaker, while being stirred and the total volume made to 200 ml with more DI water. The mixture was stirred over boiling water bath until homogenous.

Table 3.2: Formulae for Cross-linking CG using PCAs (ADA, SCA, CTR or BTCA)

	Batch 1	**Batch 2**	**Batch 3**
Ingredient	CG:PCA (1:1)	CG:PCA (2:1)	CG:PCA (1:2)
CG (g)	20	20	20
PCA (g)	20	10	40
NaH_2PO_4 (g)	2	2	2
DI water to (ml)	200	200	200

The homogenous mixture was transferred into a culture dish and concentrated by heating at 40 ^oC for 18 h. The resulting solid mass was then heated at 140 ^oC for 30 min for the cross-linking process to take place (Martel *et al.,* 2005). The material was thereafter allowed to cool and some quantity of DI water was poured on to the polymer, now clung to the plate,

which made the latter to swell and separate from the plate. The now fibrous looking material was transferred onto a mesh, lined with fine muslin cloth, and thoroughly percolated with DI water. The material was soaked in DI water for 24 h and further rinsed with more DI water before the water was drained off and the polymer spread on a non-stick baking tray and dried at 40 ^{o}C for 5 h. The polymer was milled using a coffee bean mill (Bodum$^{(R)}$, China) and packed in a moisture free container.

Cross-link yield was calculated using the following relationship:

$$\% \text{ Yield} = \left(\frac{W1-W2}{W1}\right) x\ 100 \qquad (4)$$

Where W_1 is the sum of the weights of the CG, PCA and sodium dihydrogen phosphate

W_2 is the weight of the cross-linked polymer

3.2.3.3 Cross-linking CG using CTR and/or glycerol (GLY)

Appropriate quantities of CG, CTR, GLY and NaH_2PO_4 as coded for convenience (Table 3.3) were weighed and mixed with some DI water contained in a beaker. The mixture was rendered homogenous by stirring over a boiling water bath and thereafter made to volume with more DI water. The homogenous mixture was transferred into a culture dish and concentrated by heating at 40 ^{o}C for 18 h. The solid mass was heated at 140 or 170 ^{o}C for 30 min for the cross-linking process to take place, and thereafter DI water was poured on to the polymer, now clung to the plate, which upon soaking with sufficient DI water, swelled and got separated from the plate. The now fibrous looking material was transferred onto a mesh, lined with fine muslin cloth, and percolated with DI water.

The material was soaked in DI water for 24 h and thereafter rinsed with more DI water before the DI water was strained off and the polymer spread on a non-stick baking tray and dried at 40 ^{o}C for 5 h. The polymer was milled using a coffee bean mill (Bodum$^{(R)}$, China) and packed in a moisture free container. The yield was determined using equation (4).

Table 3.3: Formulae for Cross-linking CG using CTR and or GLY

Ingredient	**CG:CTR (CrosCC)**	**CG:GLY**	**CG:CTR:GLY (CrosCCG)**		
Ratio (g)	1:1	1:1	1:1:1	1:1:2	2:2:1
CG (g)	20	20	20	20	20
CTR (g)	20	-	20	20	20
GLY (g)	-	20	20	40	10
NaH_2PO_4 (g)	2	2	2	2	2
DI water (ml) to	200	200	200	200	200

3.2.4 Residual Solvent Analysis by Gas Chromatography (GC)

CG was extracted using the following three extraction solvents: (1), acetone; (2), acetone followed by washing with DI water (to rid the gum of acetone); and (3), ethanol (95%) + 2-propanol (5%). Five hundred (500) mg samples of CG obtained by the respective methods were weighed and used in the determination of residual solvent using gas chromatograph (Agilent 6850 Series System, USA). Acetone and ethanol (95%)/2-propanol (5%) were used as reference solvents for the test.

3.2.5 Thermal Analysis using Differential Scanning Calorimetry (DSC)

Heat flux DSC (NETZSCH-Geratebau, Germany) was used to study the degradation temperatures of crude, processed and cross-linked CG. Crude CG is a sample of the gum as obtained from the plantation. Processed CG is the gum after extraction with acetone while the cross-linked CG is the polymer obtained after the cross-linking processes with the polycarboxylic acids. About 10 mg samples of the respective polymers were encapsulated in aluminium disposable pans. DSC scans were run to measure the energy changes associated with heating the samples to 500 ^{o}C at a scan rate of 10 ^{o}C/min using nitrogen as purge gas.

The thermogram obtained was used to ascertain whether the use of acetone for extraction affected the integrity of the gum. This was done by overlaying the two thermograms to compare the degradation temperatures of the crude and the processed CG as reflected on their respective thermograms. DSC was also used to obtain valuable information on the cross-link formation between CG and the PCAs. Thermograms of the cross-linked polymers were laid over those of the uncross-linked CG and observed differences were noted.

Compatibility status of CG or its cross-linked derivatives as excipients and Ven. HCl as active pharmaceutical ingredient (API) was assessed by the following procedure. About 10 mg samples of Ven. HCl and a thoroughly triturated 50/50 physical mixture of powders of CG or its cross-linked polymers on the one hand and Ven. HCl on the other, were respectively encapsulated and the DSC measurements taken as mentioned earlier. These thermograms were used to determine the compatibility or otherwise of Ven. HCl with the polymers as excipients.

In all the cases, appearance or disappearance of peak(s) in the overlaid thermograms would suggest the formation of new bonds/structures (Saunders, 2008).

3.2.6 Fourier Transform Infra Red Studies (FT-IR)

Fourier transform IR (FT-IR) spectra of CG and its cross-linked derivatives were obtained using JASCO IR spectrophotometer (model 4200, Jasco Inc. Japan). The powdered samples were mixed with potassium bromide (KBr), compressed into pellets and analysed between 400 and 4000 cm^{-1}. The spectrum of CG was compared with other spectra of CG reported in literature (Silva *et al.,* 2006; Chunha *et al.,* 2007) for identification purposes (Hsu, 1997).

The spectra of CG and that of the cross-linked CG (CrosCC and CrosCCG) were respectively overlaid to reveal the presence or absence of IR absorption bands representative of respective functional groups of interest. Appearance, disappearance or broadening of absorption band(s) on the spectra of the cross-linked polymers in comparison with the spectrum of CG was used to assess the cross-linking of CG (Hsu, 1997; Coates, 2000).

3.2.7 Determination of Solubility of the Polymers

The method of evaluating solubility as described by NICEATM (2003) was followed with some modifications. Samples of CG, CrosCC and CrosCCG weighing 200 mg each were respectively dispersed in 10 ml of each of the solvents contained in test tubes (Table 3.4). At ambient temperature the tubes were shaken using vortex mixer (Touch mixer model 231, Fisher Scientific, USA) for 2 min, sonicated for 5 min, using sonicator (Model FS220, Fisher scientific, Mexico), allowed to stand for 10 min and observed for the ability of the powders to go into solution.

Table 3.4: Solvents used for Solubility Determination

Class of solvent	Solvent
Non-polar	Diethyl ether, cyclohexane, toluene
Polar aprotic	Ethyl acetate, dimethylformamide (DMF), dimethyl sulfoxide (DMSO)
Polar protic	Isopropanol, ethanol, water

3.2.8 Solid State ^{13}C Nuclear Magnetic Resonance (SS ^{13}C NMR)

The solid state ^{13}C MAS NMR experiments on CrosCCG were performed on a Bruker AVANCE, 400 spectrometer, equipped with a magic angle-spinning (MAS) inclined at 57.4^{o}. The sample (about 5 mg) was spun at 8.0 kHz in a 4.0 mm outer diameter zirconia ceramic rotor spinner. The proton decoupling was applied. The recycle delay time was set to 5 ms. ^{13}C chemical shifts were referenced to tetramethylsilane (TMS) using solid adamantine as a secondary standard.

3.2.9 Moisture Loss on Drying

A weighing balance (AT 261 DeltaRange®, Mettler Toledo, Switzerland) was used to weigh 2 g samples (W_1) of powders of CG, CrosCC and CrosCCG respectively into three tarred evaporating dishes. The dishes with covers were earlier conditioned by heating at 105 ^{o}C for 30 min after which they were cooled in a desiccator for 30 min. The samples were heated at 105 ^{o}C for 2 h, cooled in a desiccator for 30 min each and weighed. The samples were heated again for two more periods of 30 min each, cooled and weighed (W_2). Three (3) h of

total drying time brought the sample to constant weight and the drying was thereby stopped. The moisture loss on drying (MLD) was determined using the following relationship

$$\% \text{ MLD} = \frac{(W1-W2)\ 100}{W1} \quad (5)$$

Where W_1 and W_2 are weights of powders before and after drying respectively

3.2.10 Water Content Determination Using Karl Fisher Titrimetry

Karl Fisher titrator has been described as an appropriate method of determining water contents of both solid and liquid samples (Przybylek, 2010; Shaikh *et al*., 2012; Tavcar *et al.*, 2012). Karl Fisher titrator (Mettler Toledo DL38, Switzerland) was used to determine the percent water content in CG, CrosCC and CrosCCG respectively, as described by Przybylek (2010). Two samples weighing 2 g each of CG and its cross-links were spread on tarred sample dishes and stored (1) over activated silica gel and (2) over water for a period of 7 days, respectively. A third 2 g sample was weighed out directly from the container of the powders kept at ambient temperature and humidity. Quantities weighing 150 mg of powders from the three samples were tested for percent water content. Karl Fisher volumetric reagent, Aqualine^{TM} Complete 5 and anhydrous methanol were used for the tests.

3.2.11 Residue on Ignition/Sulphated ash

The residue on ignition was determined as described in the USP (USP, 2011). Three porcelain crucibles and their covers, placed side by side, were conditioned by ignition at 600 ± 50 ^{o}C in a 48000 Barnstead/thermolyne furnace (Clarkson Laboratory & Supply Inc., USA) for 30 min. With the aid of tongs, the crucibles were covered, removed and placed

inside a desiccator containing activated silica gel for 30 min to cool. After cooling, the crucibles with the covers were weighed using a balance (AT 261 DeltaRange® Mettler Toledo, Switzerland). Using the same balance, 1 g quantities of CG were weighed and placed respectively into the crucibles and the weights of the crucibles with the material were again noted. From inside a fume hood, about 1 ml of concentrated sulphuric acid was used to wet the sample. The crucibles containing the samples were then placed inside the furnace, the covers removed and placed beside them and heated gently with the furnace lid slide open. The furnace temperature was gradually increased until the evolution of white fumes ceased. The sample was then ignited by closing the furnace lid and setting the furnace temperature to 600 ± 50 ^{o}C. The contents of the crucible were checked after every 30 min to observe whether the material is charred. After 2 h of ignition, the sample became totally charred and so the crucibles were covered, removed and allowed to cool in a desiccator for 30 min and then weighed. Concentrated sulphuric acid was used to wet the ash and the process of heating and igniting was repeated for two more times. This procedure was repeated to determine the residue on ignition for CrosCC and CrosCCG respectively.

3.2.12 Determination of Moisture Sorption/Desorption over Saturated Salt Solutions

Saturated salt solutions of magnesium chloride, magnesium nitrate, potassium chloride and sodium chloride were prepared as in Table 3.5 to provide controlled changes in relative humidity (Greenspan, 1976; Airaksinen, 2005). This was achieved by changing the type of salt solution after a 7 day exposure as shown in Figure 3.1. Three sample dishes containing 2 g weights of dried CG, CrosCC and CrosCCG respectively were prepared. An assembly of sample and saturated salt solution all contained in a desiccator was made such that, the salt

solution is placed at the bottom while the samples contained in tarred dishes were placed on top of the separator plate. The assembly was allowed to stand for 7 days during which the dishes containing the powders were brought out and weighed daily, to monitor the mass change as a function of time. Care was taken to avoid over exposure of the material to outside humidity. No attempt was made at controlling the temperature and the prevailing temperature was 22 ± 2^{o}C.

Table 3.5: Composition of Saturated Salt Solutions

Salt	Humidity (%) at 25 ^{o}C	Salt (g)	Water (mL)
Magnesium Chloride	33	200	25
Magnesium Nitrate	53	200	30
Sodium Chloride	75	200	60
Potassium Chloride	84	200	80

Culled from Jowitt and Wagstaffe (1989)

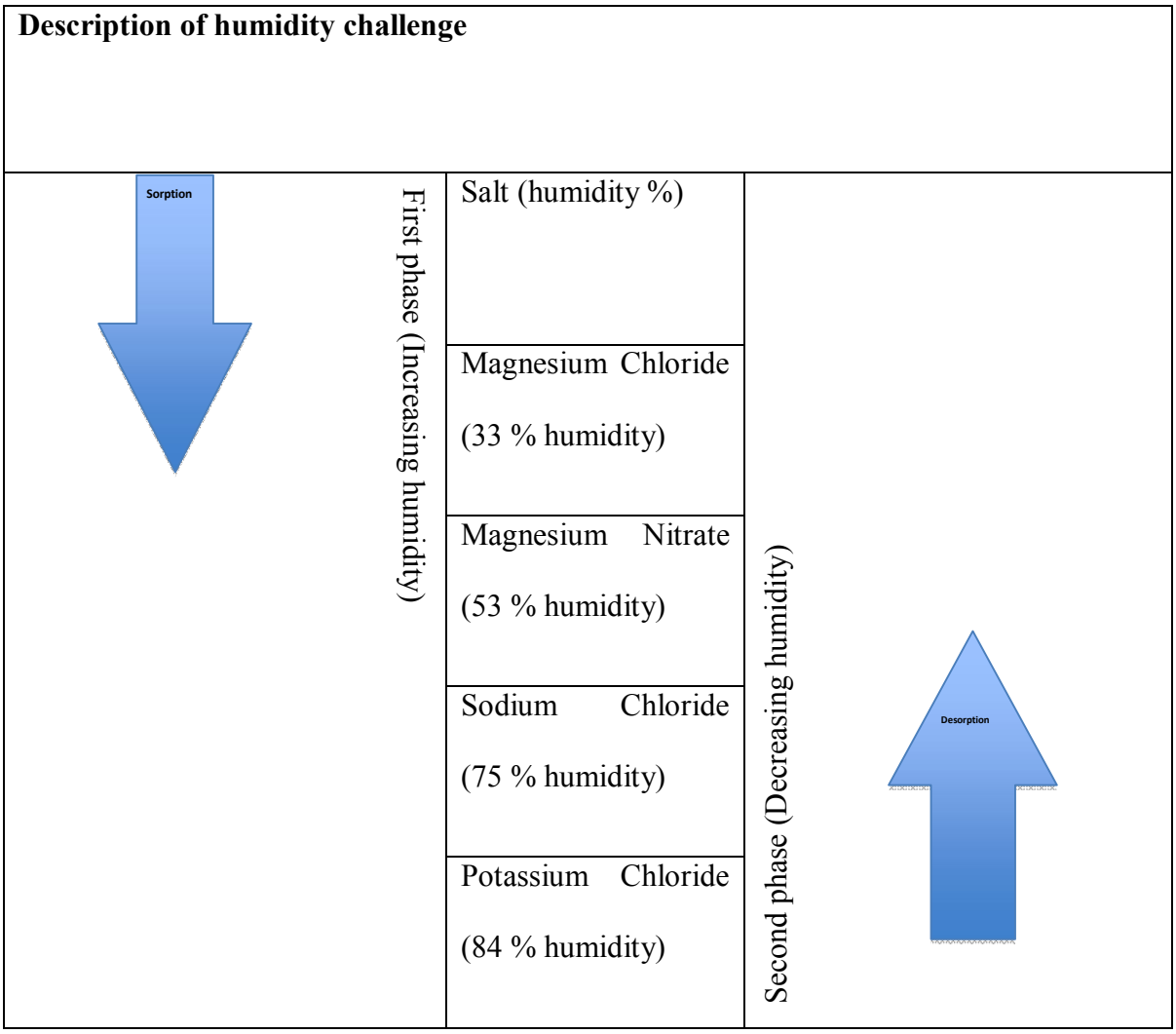


Figure 3.1: Moisture Sorption/Desorption Studies Over Some Selected Saturated Salt Solutions

3.2.13 Swelling Ratio Determination

The siliconised cylindrical tube model used for the estimation of contact angles in liquid penetration studies (Odidi and Newton, 1993) was employed in determining polymer swelling ratio with some modifications.

The swelling rate and extent of the polymers were determined using a simply assembled measuring device (Figure 3.2). A cylindrical tube, open on both ends, measuring 12 cm in length with 1.25 cm internal diameter was used for the determinations. A cheaply constructed stopper designed to retain solid particles but allow water to seep through, was used to block the lower end of the tube. The stopper was made of a perforated pen cover, with the narrower end inserted into the cylindrical tube to the point the pen cover is broad enough not to pass through. Into the hollow portion of the stopper is inserted a spongy material that is impermeable to solids but allow water to seep through. The tube was first siliconised to limit its adsorption of water by the following method:

3.2.13.1 Siliconisation of Cylindrical Tube

The procedure of Noteboom and Will (1982) was adopted with some modifications. Cylindrical glass tubes for swelling ratio determination were washed and siliconised as follows:

1. **Washing of the Glassware**

Formula for making 1 L of nochromix (cleaning) solution

Sodium peroxide (3 %v/v) solution	500 ml
Conc. sulphuric acid	500 ml

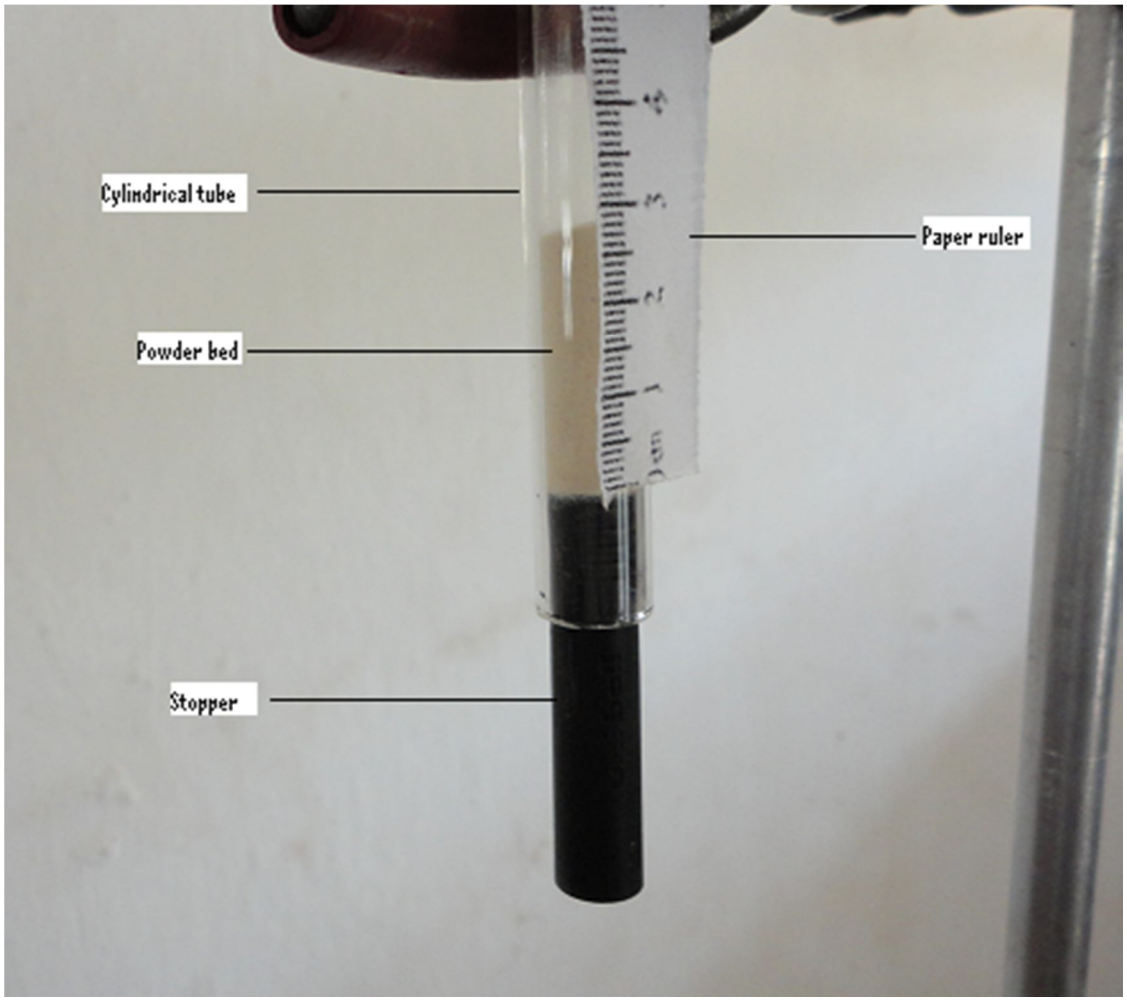


Plate 3.1: Siliconised Cylindrical Tube Assembly for Determination of Polymer Swelling Ratio

The cleaning solution was prepared by placing 500 ml of 3 %v/v hydrogen peroxide solution into a glass bottle placed in ice water contained in rubber jacket. This was followed by the slow addition while swirling of 500 ml of conc. sulphuric acid. The solution was allowed to cool completely while surrounded with the ice water.

The glass tubes were thoroughly washed with water, rinsed with DI water and then completely immersed in the prepared nochromix solution for about 30 min. The tubes

were removed and drained completely by tilting them at an angle of about 45^{o} in fume hood. They were then rinsed with DI water and thoroughly dried.

2. **Siliconisation of the Glassware**

Formula for making 100 ml of siliconising solution

Dichlorodimethylsilane		1 ml
Toluene	to	100 ml

The cleaned and dried tubes were immersed in the prepared 1 %v/v solution of dichlorodimethylsilane in toluene and their surfaces made to contact with the solution. The tubes were rinsed with a little quantity of toluene and allowed to air dry in a fume hood. The dried tubes were baked in an oven at 80 ^{o}C for 3 h.

3.2.13.2 Measurement of Swelling Ratio using the Cylindrical Tube

One (1) gram weight of the powdered sample was placed into the siliconised cylindrical tube with the lower end blocked with the designed stopper (Figure 3.2) and the tube tapped 100 times from a distance of about 2 cm (Odidi and Newton, 1993). The tube was thereafter clamped to a retort stand and lined with a paper ruler in millimeter units using which the powder bed height was determined. With the aid of a pipette, 40 ml of DI water was added into the tube with caution not to disturb the settled powder bed. The height attained by the powder bed upon swelling was noted using a timer. The swelling was assessed every 1 minute for the first 10 min and thereafter every 10 min for the next 90 min. The powder bed was made to remain below the water level by adding more DI water. The height attained by the swollen polymer bed (H_S) in relation to the original

height of the dry polymer bed (H_D) was used to determine the swelling ratio. The experiment was replicated five times and the average used to calculate the swelling ratio (SR) using the following relationship:

$$\% SR = \frac{HS-HD}{HD} x\ 100 \quad (6)$$

3.2.14 Water Holding Capacity (WHC)

The method described by Vittadini and Vodovotz (2003) was followed with some modifications. Two (2) g samples of CrosCC and CrosCCG were placed in tarred siliconised 50 ml centrifuge tubes and weighed (W_1). DI water (30 ml) was added into the tubes, shaken and allowed to stand for 1 h. The samples were centrifuged (Hanil Science Industrial Co. Ltd., Korea) at 4,500 rpm for 25 min but as the samples were not seen to settle satisfactorily, the centrifuge speed was changed to 5,000 rpm and allowed to run for 25 min. A syringe was used to suck out the liquid from the tube. The tubes were thereafter placed in a forced-draft air oven (Model 1370 F, VWR Scientific Products, USA) at an angle of 15–20^o, allowed to dry at 50 ^{o}C for 25 min and then cooled in a desiccator for 30 min. The tubes with the dried samples were weighed (W_2) using AT 261 DeltaRange$^{(R)}$ Balance (Mettler Toledo, Switzerland). The WHC was calculated using equation 7:

$$\% WHC = \left(\frac{W2-W1}{W2}\right) x\ 100 \quad (7)$$

Where W_1 and W_2 are weights of dry and hydrated samples, respectively.

3.2.15 Evaluation of Powder Flow Properties

3.2.15.1 Density Measurements

Densities of powders of CG, CrosCC and CrosCCG were measured using tap density tester USP (Electrolab, Model ETD-1020, Globepharma, U.S.A.), where the bulk and tapped densities as well as compressibility index and Hausner ratio were determined using the USP 1 method. A weighed quantity of the powder was poured into the cylinder, the powder bed noted and the cylinder made to drop at a rate of about 300 drops/min from a height of 14 mm ±2. Three rounds (1, 2, and 3) of taps were designed to subject the cylinder to 10, 500, and 1250 taps respectively, with each powder bed height noted and imputed in subsequent tap round. A variation of not more than 2 ml between powder bed heights readings marked the end point for the measurement. Densities were determined using the following relationships:

$$\text{Initial Density} = (W/Vo) \quad (8)$$

$$\text{Tap Density} = (W/Vf) \quad (9)$$

$$\text{Hausner Ratio} = (Vo/Vf) \quad (10)$$

$$\text{Compressibility Index} = [(Vo-Vf)/Vo] \times 100 \quad (11)$$

Where W is weight of sample, Vo is Initial Volume and Vf is the Final Volume

3.2.15.2 Powder Flow Rate

Glass funnel held by a retort stand with the tip 10 cm from the surface of the table top was used to measure the flow rate of powders of CG and its cross-linked derivatives. Fifty (50) g sample was poured into the funnel with the opening blocked with a gloved finger. Using a timer, the time taken for the material to pass through the opening upon removal of the block was determined. The average of three readings was used to calculate the flow rate.

3.2.15.3 Angle of Repose

The height (h) and the diameter (d) formed by the CG and its cross-linked derivatives powder heaps in earlier flow measurement were measured and used to calculate the angle of repose (θ) of the material using equation 1.

3.2.15.4 Determination of Powder Particle Size Distribution

Ro-Tap (Model RX-29, W.S. Tyler, USA) was used for the determination. Tarred sieves (W_1) with different sizes were arranged such that 600 µm was on top, followed by 425 µm, then 250 µm, 150 µm, 106 µm, 53 µm and finally 0 µm (pan) at the bottom. Powder weighing 50 g was placed on the topmost sieve and the shaker operated for 10 min. The sieves with the retained powder were weighed (W_2). The weight of powder retained on each sieve was calculated using the following relationship

$$\text{Weight of powder retained} = W_2 - W_1 \qquad (12)$$

3.2.16 Tablet Formulation Studies

3.2.16.1 Formation of Granules

The Ven. HCl, Lactose DT and the ingredients used as binder/release retardant/release enhancing agents (Table 3.6 to 3.9) were accurately weighed and dry mixed for 6 min using a low shear mixer (Kitchen aid, St. Joseph, Michigan USA) operated at moderate shear rate. Intermittently, the mixer was stopped and the powder laying on its surfaces scrapped back and further mixed with a spatula. DI water, as granulating liquid, was added in aliquots of 10 ml to a total quantity just enough to wet mass the powder mix. The damp mass was sheared using appropriate spindle run at speed 2 for 6 min to obtain optimum granulation. The damp granule mass was spread on paper laid on a tray and dried in a forced air oven (Model 1370 F, VWR Scientific Products, USA) for 3 h or when moisture content determined using moisture analyser (Model LJ 16, Mettler Toledo, Switzerland) was lower than 2%. The dried granules were milled by passing through a mill (Quadro Comil, Model no. 197 R, Quadro Engineering Inc., Ontario Canada) fitted with a metal screen affixed to the granulator.

Table 3.6: Ven. HCl Tablet Formulae Containing MCC PH 102 and/or CG as Binder

Ingredients	**Batch Number/Quantity (%)**					
	G1	G2	G3	G4	G5	G6
Venlafaxine HCl	30	30	30	30	30	30
Lactose DT	59	64	59	44	54	34
MCC PH 102	10	-	-	-	10	10
CG	-	5	10	25	5	25
Silicone dioxide	0.5	0.5	0.5	0.5	0.5	0.5
Magnesium stearate	0.5	0.5	0.5	0.5	0.5	0.5
TOTAL	100	100	100	100	100	100

Table 3.7: Ven. HCl Tablet Formulae Containing HPMC or CrosCCG as Binder

Ingredients	**Batch Number/Quantity (%)**			
	G7	G8	G9	G10
Venlafaxine HCl	30	30	30	30
Lactose DT	64	64	61.5	59
HPMC LV	5	-	-	-
HPMC ER	-	5	7.5	-
CrosCCG	-	-	-	25
Silicone dioxide	0.5	0.5	0.5	0.5
Magnesium stearate	0.5	0.5	0.5	0.5
TOTAL	100	100	100	100

Table 3.8: Ven. HCl Tablets Formulae Containing HPMC ER 7.5% as Binder and Some Polymers as Release Retardant

Ingredients	**Batch Number/Quantity (%)**		
	G11	G12	G13
Venlafaxine HCl	30	30	30
Lactose DT	51.5	46.5	36.5
HPMC ER	7.5	7.5	7.5
CrosCCG	10	15	25
Silicone dioxide	0.5	0.5	0.5
Magnesium stearate	0.5	0.5	0.5
TOTAL	100	100	100

Table 3.9: Ven. HCl Tablet Formulae Containing HPMC ER 7.5% as Binder and Some Polymers as Disintegrants at 3% w/w Concentration

Ingredients	**Batch Number/Quantity (%)**				
	G14	G15	G16	G17	G18
Venlafaxine HCl	30	30	30	30	30
Lactose DT	61.5	61.5	61.5	61.5	61.5
HPMC ER	7.5	7.5	7.5	7.5	7.5
CrosCCG	3	-	-	-	-
CrosCC	-	3	-	-	-
Croscarmellose	-	-	3	-	-
Crospovidone	-	-	-	3	-
CG:EPC	-	-	-	-	3
Silicone dioxide	0.5	0.5	0.5	0.5	0.5
Magnesium stearate	0.5	0.5	0.5	0.5	0.5
TOTAL	100	100	100	100	100

3.2.16.2 Tests on Granules

The granule density, size distribution and flow properties were determined using methods described earlier (see section 3.2.15).

3.2.16.3 Addition of Extragranular Excipients

Weighed quantities of silicone dioxide and magnesium stearate (Tables 3.6 to 3.9) were added to the granules and blended using the shear mixer and afterwards thoroughly shaken in cellophane bag. Granule flow rates were determined as earlier described (section 3.2.15.2).

3.2.16.4 Compression of Granules

Betapress Manesty Machine (No. 74182, Manesty Machines Ltd., England) was used to compress the granules (Tables 3.6 to 3.9) using 0.2756 in (7mm) punch and die setting and compression pressure of 4.5 metric tonnes to produce a batch of 1,000 tablets with a target weight of 167 mg.

3.2.16.5 Evaluation of Tablet Properties

3.2.16.5.1 Uniformity of Weight

Twenty (20) tablets were randomly selected and individually weighed using AT 261 DeltaRange balance (Mettler Toledo, Switzerland). The mean weight and deviations from the mean were calculated.

3.2.16.5.2 Tablet Crushing Strength

The crushing strength (KgF) of 10 tablets selected at random from each batch was determined using VK 200 Tablet Hardness Tester (Vankel, USA). The mean and standard deviations were calculated.

3.2.16.5.3 Tablet Friability Test

FAB-2 Friability tester (Logan Instruments Corp., USA) was used to carry out the friability test. Twenty (20) tablets from each batch were taken and weighed and then placed on the friabilator, which was then operated for four (4) min at 25 rpm (100 revolutions). The tablets were de-dusted reweighed and the difference in weight was determined using the following relationship:

$$\% \text{ Friability} = (W_1 - W_2 / W_1)\, 100 \qquad (13)$$

Where W_1 = original weight, W_2 = final weight.

3.2.16.5.4 Tablet Dissolution Test

Dissolution test was carried out using the paddle method on D 800 Dissolution tester (Logan Instruments Corp., USA) as described in the USP (USP 2011). The automated tester was set to analyze the amount of drug released after every 15 min of dissolution time. Paddle speed was 100 rpm as quantities amounting to 900 ml of DI water were used as dissolution medium, while the temperature was set at 37 ^{o}C ± 0.5 ^{o}C. Drug released was assessed at the Ven. HCl wavelength of 229 nm.

3.2.16.5.5 Drug Release Data Model Fitting

The drug release data was fitted into various drug release kinetic models. Zero order, first order, Hixon Crowell and Higuchi kinetic models (Table 3.10) were employed in describing the drug-release kinetics while Korsmeyer-Peppas equation provided valuable information on the drug-release mechanism. The model that best fits the data was taken as the model describing the drug-release mechanism for the preparation.

Table 3.10: Drug-release mechanism exponents

Release exponent (n)	**Diffusion mechanism**
0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous (non-Fickian) diffusion
0.89	Case II transport
n > 1	Super case II transport

Adapted from Talukdar *et al.* (1996); Shoaib *et al*. (2006); Dash *et al.* (2010)

3.2.16.5.6 The kinetic equations

1. **Zero Order Model**

Zero order model describes drug release rate that is independent of the concentration of the drug. It is represented by equation 10 (Kalam *et al*., 2007) and a plot of cumulative % drug release vs. time will be linear (Shoaib *et al.*, 2006)

$$A_0 - A_t = Kt \quad (14)$$

Where A_0 is the initial amount of drug in the dosage form; A_t is the amount of drug remaining after time "t" and K is the proportionality constant.

2. First Order Model

Drug release rate from dosage forms agreeing with first order model is concentration dependent and can be depicted by the equation 11 (Kalam *et al*., 2007) and a plot of log cumulative of percent drug remaining vs. time will be linear (Shoaib *et al.*, 2006).

$$\text{Log } A_t = \text{Log}A_0 + K_1 t/2.303 \quad (15)$$

Where A_t is the amount of drug released in time "t", A_0 is the initial amount of drug or the amount of drug at zero time, K is the first order rate constant.

3. Hixon-Crowell Model

Hixon-Crowell model is used to evaluate the drug release with changes in the surface area and the diameter of the particles/tablets (Kalam *et al.*, 2007) using the following equation:

$$A_0^{1/3} - A_t^{1/3} = K_s t \quad (16)$$

Where A_0 is the initial amount of drug in the dosage form, A_t is the remaining amount of drug in the dosage form at time "t" and K is the rate constant for Hixon-Crowell rate equation. A plot of cube root of percent drug remaining in matrix vs time will be linear.

4. Higuchi Model

This model is used to describe release of water soluble and low soluble drugs incoperated in semi-solid and solid matrices (Kalam *et al.*, 2007) and is given by the following equation. A plot of cumulative percent drug released vs square root of time will give a linear relationship.

$$A = Kt^{1/2} \quad (17)$$

Where A is the amount of drug released at time "t" and K is the Higuchi dissolution constant.

5. Korsmeyer-Peppas equation

The Korsmeyer-Peppas equation otherwise called the power law equation is used to determine the mechanism of diffusion in polymeric networks. The mechanism is described by the value of "n" obtained from equation 18, and used to characterize different release mechanisms as given in Table 3.10.

$$A_t/A_\infty = Kt^n \quad (18)$$

Where A_t/A_∞ is fraction of drug released at time t, K is the rate constant and n is the release exponent

3.2.16.5.7 Uniformity of dosage unit test

The USP method (USP 2011) was used to determine the content uniformity using Hewlett Packard diode array spectrophotometer. Ten (10) tablets were weighed individually and the

mean weight determined. The tablets were crushed and three samples of approximate weights to the mean tablet weight were assayed. The average of three values was taken as the drug content. Ten (10) tablets were weighed individually and the weights were used to determine the uniformity of the dosage units proportionately according to the USP method.

3.2.17 Sub-Acute Toxicity Studies

The method described by Tamta *et al.* (2009 was adopted with some modifications. The experiment was designed and the animals were treated in accordance with the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) 1986 Animal Guidelines. The animals (Wistar rats) were grouped into four with each group having 6 animals. Subgroups of 3 males and 3 females were made on each group. The animals were conditioned as per the environment, mode of feeding and water supply.

CrosCCG was not soluble and so could not be made into solutions for oral or intraperitonial (IP) administrations and this precludes the conduction of acute toxicity studies. Sub-acute toxicity studies were thus carried to ascertain the safety or otherwise of the polymer. Group 1 was made to serve as control while groups 2, 3 and 4 were fed 2.5, 5.0 and 10.0 grams of CrosCCG per 1 Kg feed over a 28 day period, respectively. The polymer was added in its solid state into the feed mix prior to being molded. The same mold formula as per respective groups was used to feed the animals throughout the 28 day period of treatment. Continuous water supply was ensured, *ad libitum*, using clean drinkers. Deaths, loss of weight, inability to eat or drink during the study period were recorded.

The animals were sacrificed on the 29 th day and blood samples were collected. Hematological parameters including packed cell volume (PCV), hemoglobin count,

neutrophils, lymphocytes, monocytes, eosinophils levels were also determined. Biochemical parameters including alkaline phosphatase (ALP), aspertate transaminase (AST, SGOT), alanine transaminase (ALT, SGPT) were tested.

3.2.18 Statistical Analysis

All the results obtained that require statistical analysis were analyzed using GraphPad Prism software package. Results were expressed as mean ± SD and differences between means were considered significant at $P < 0.05$ using the analysis of variance (ANOVA) or student's t test.

CHAPTER FOUR

RESULTS

4.1 Production Yield

4.1.1 Percentage Yield for the Extraction of CG

A yield of 78.0% was obtained following extraction of CG using acetone as extraction solvent.

4.1.2 Percentage Yield of Cross-linked CG with EPC

A yield of 18.81% was obtained following cross-linking of CG with EPC.

4.1.3 Percentage Yield of Cross-linking CG with PCAs

Cross-linking CG with PCAs at 140 ^{o}C led to varied yields dependent on the type and concentration or proportion of the reactants used. The dicarboxylic acids, ADA and SCA, gave a cross-link yield only when used at a CG:PCA weight ratio of 2:1 (Figure 4.1). At this ratio, the yields obtained for the cross-linked products CG:ADA and CG:SCA were 32.13 % and 34.81 %, respectively. At 1:1 and 1:2 weight ratios, neither ADA nor SCA was found to be effective in cross-linking CG, and so no yield was recorded. The tricarboxylic acid, CTR cross-linked CG at all the three ratios tested. The yields recorded for CG:CTR weight ratios of 2:1, 1:1 and 1:2 in order of increasing cross-linker concentration, were 51.11, 57.61 and 57.95 % respectively. The tetracarboxylic acid, BTC on the other hand, gave a yield of 54.84, 56.99 and 69.89 % for the CG: BTAC weight ratios of 2:1, 1:1 and 1:2 respectively.

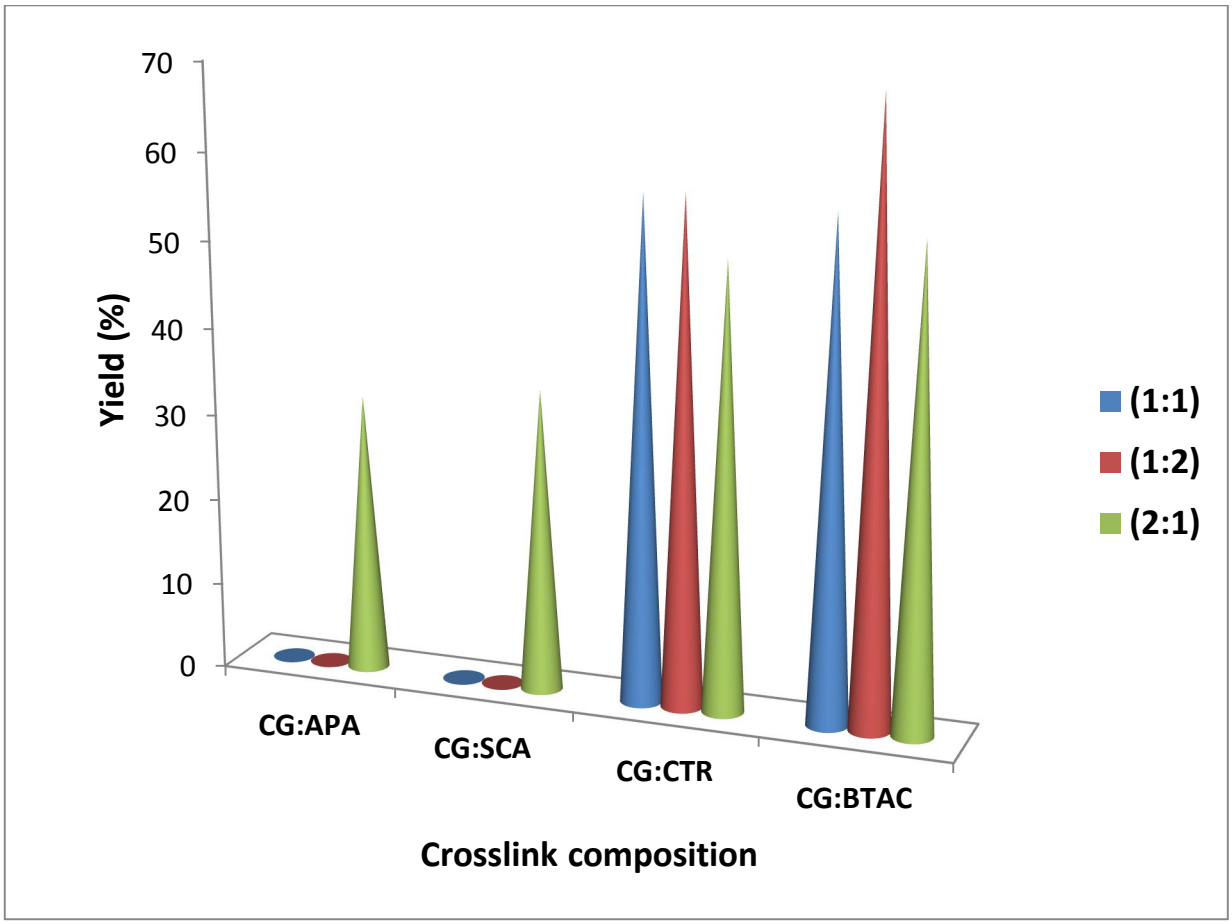


Figure 4.1: Effect of Composition on Polymer % Yield Obtained by Cross-linking CG with PCAs at 140 ^{o}C for 30 min

4.1.4 Percentage Yield of Cross-linking of CG with CTR and/or GLY

Figure 4.2 shows the cross-link % yield of cross-linking reactions between CG, CTR and or GLY conducted at 140 ^{o}C for 30 min. Heating the mixtures under this condition gave a cross-link % yield of 64.72 ± 1.13 and 26.22 ± 1.59 for cross-links of CG:CTR (1:1) and CG:CTR:GLY (1:1:1) in weight ratios, respectively. No cross-link yield was obtained when CG:GLY mixture was heated at the 140 ^{o}C for 30 min. A whitish solid mass that readily dissolved in water was obtained, instead.

At 170 ^{o}C (Figure 4.3), % yields of 54.73 ± 2.88 and 73.60 ± 3.61 were obtained for cross-linking weight ratios of CG:CTR (1:1) and CG:CTR:GLY (1:1:1), respectively. Here also, no yield was gotten by subjecting CG:GLY (1:1) to the same experimental conditions. A statistically significant ($P<0.005$) decrease in cross-link yield of CG:CTR (1:1) was observed when the cross-linking temperature was changed from 140 ^{o}C for 30 min to 170 ^{o}C for 30 min. In contrast, a statistically significant ($P<0.0001$) increase in cross-link yield of CG:CTR:GLY (1:1:1) was observed following similar change in temperature.

Figure 4.4 shows the cross-link yield obtained when weight ratios of CG and CTR were maintained but that of GLY varied and the samples heat treated at a temperature of 170 ^{o}C for 30 min. A slight increase in yield was observed as the concentration of GLY was increased. When CG:CTR:GLY mixture at (2:2:1) weight ratio was heat treated at the said condition, a yield of 74.89 ± 2.30 % was obtained. Increasing the GLY ratio concentration to 1:1:1 and 1:1:2 gave yields of 75.1 ± 2.80 % and 75.3 ± 3.80 %, respectively. Differences in the yield obtained was not found to be statistically significant ($P>0.05$).

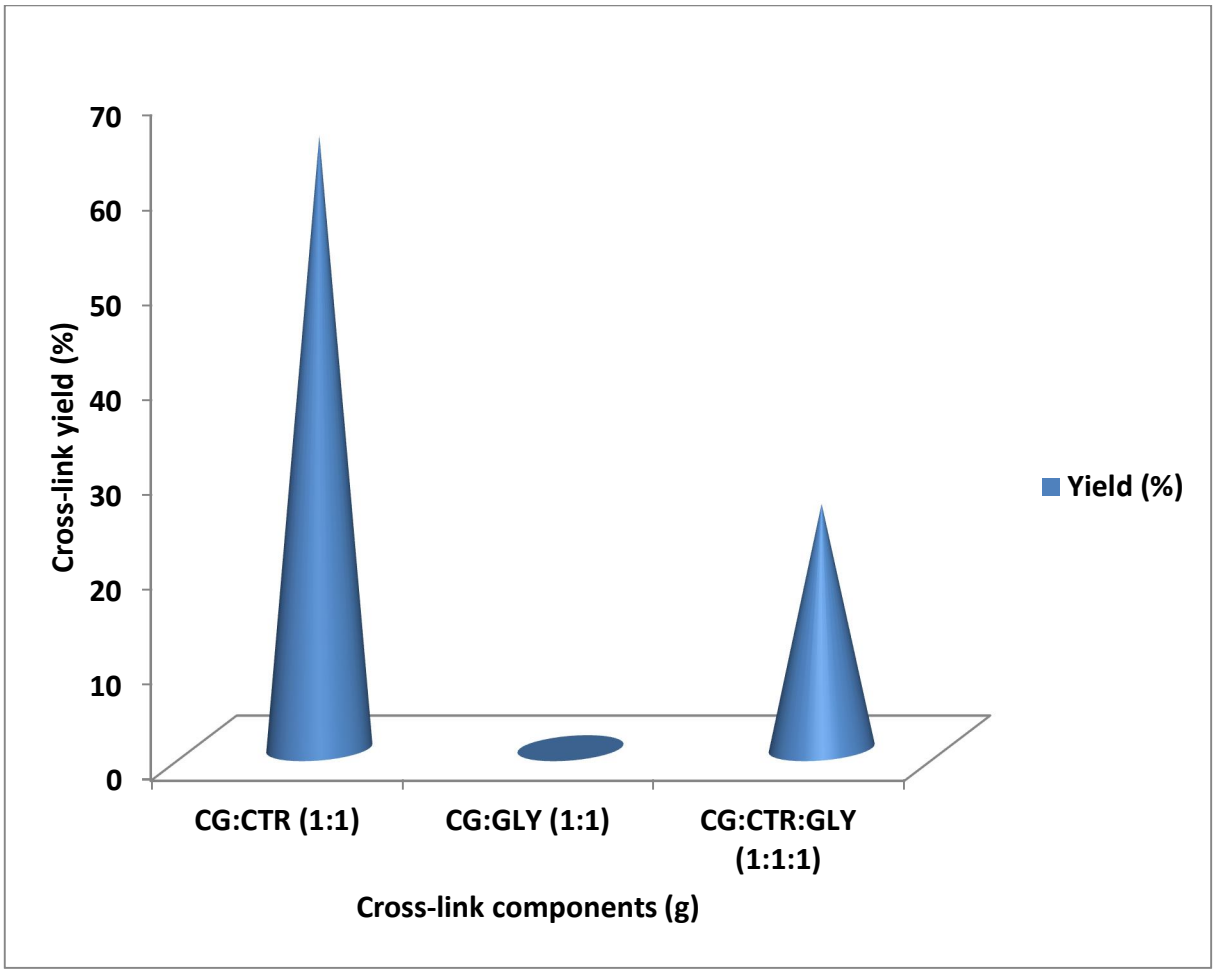


Figure 4.2: Effect of Composition on Polymer Yield (%) Obtained by Cross-linking CG with CTR and/or GLY at 140 ^{o}C for 30 min

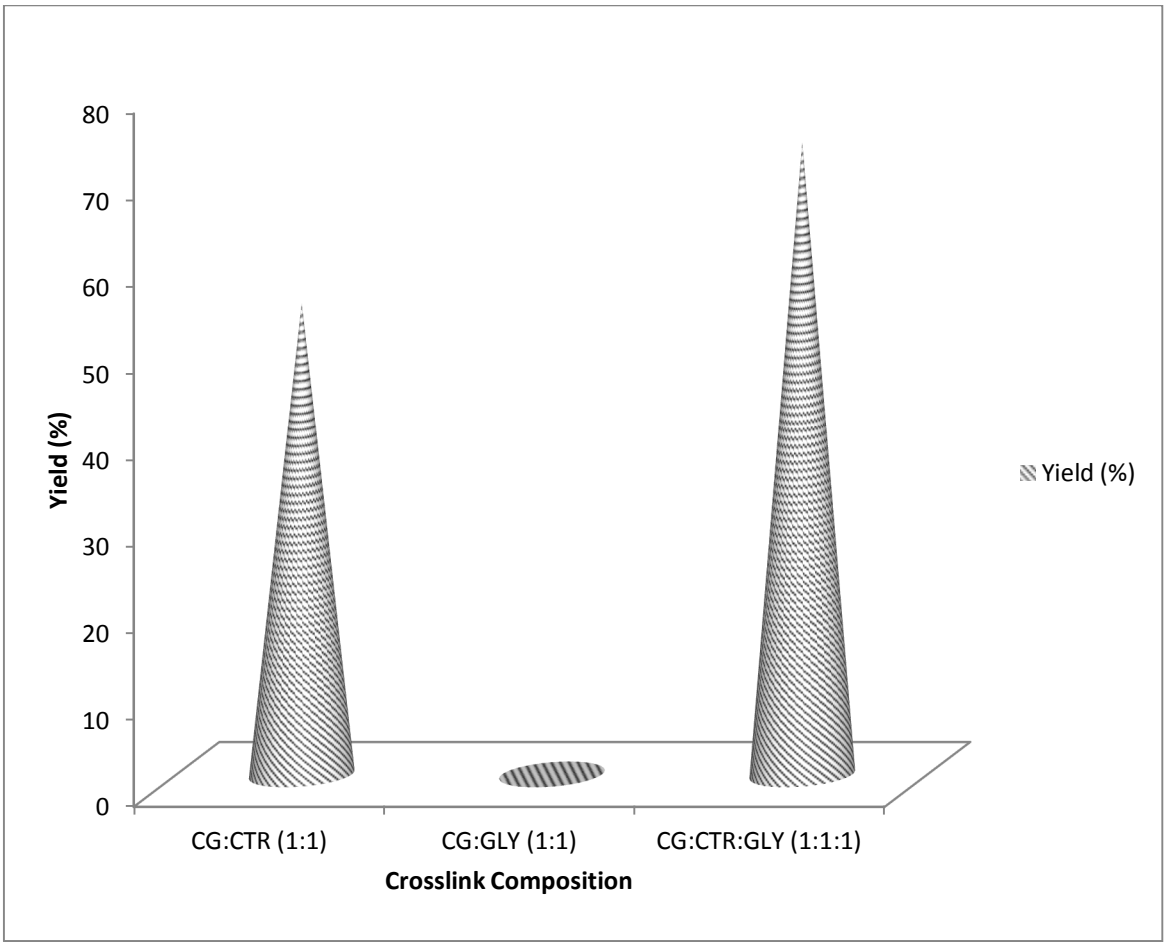


Figure 4.3: Effect of Composition on Polymer Yield (%) Obtained by Cross-linking CG with CTR and/or GLY at 170 ^{o}C for 30 min

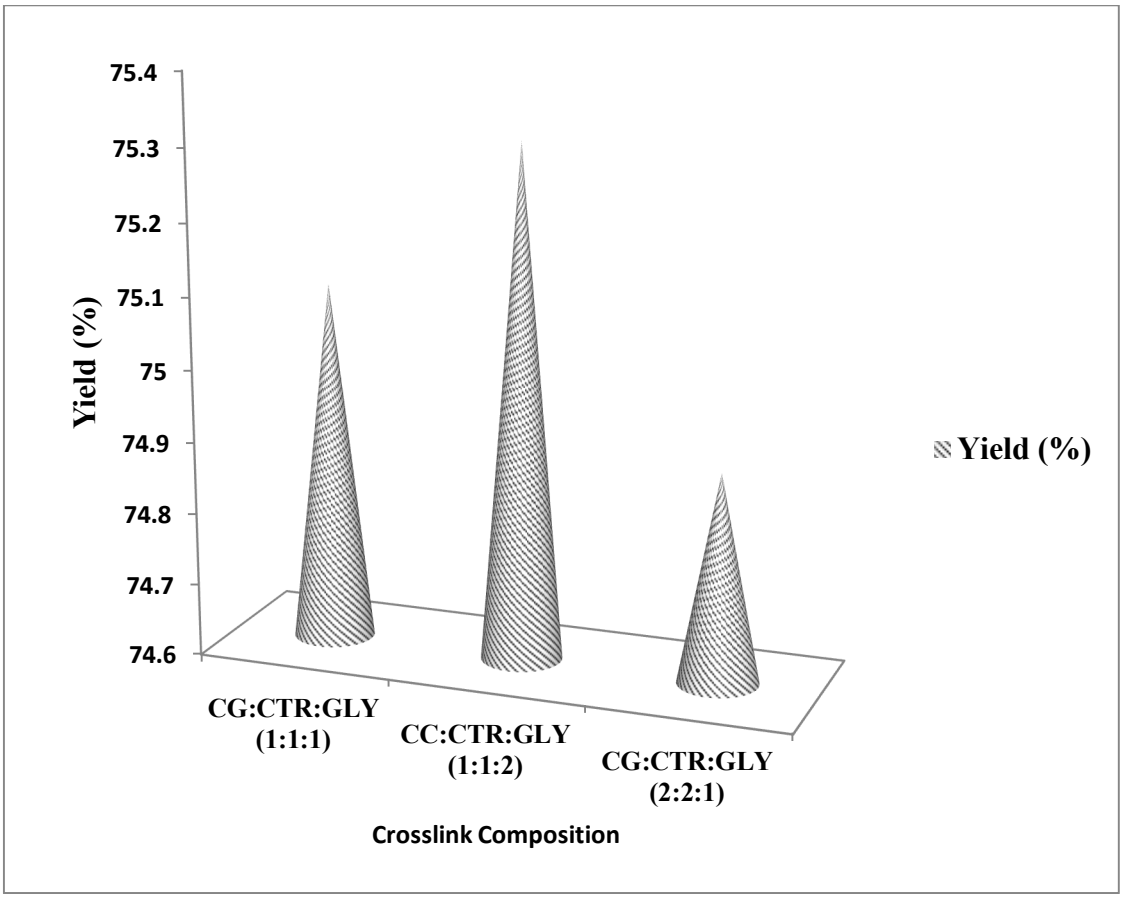


Figure 4.4: Effect of Composition on Polymer Yield (%) Obtained by Cross-linking CG with CTR and GLY at 170 ^{o}C for 30 min

4.2 Physical Appearances of the Cross-linked Polymers

Plates 4.1 to 4. 4 represent the physical appearances of the products obtained following cross-linking of CG with EPC or CTR. Plates 4.1 and 4.2 show the appearances of two cross-link products immediately after heat treatment and prior to washing with water. Plate 4.1 was the product of cross-linking CG with EPC, a product that displayed some hollow off-white swellings as against the brown to dark brown flaky or spongy appearance (Plate 4.2) of CG cross-linked with CTR. Washing the product in plate 4.1 gave an insoluble globular polymer, while washing the CG:CTR cross-link product with water yielded a brown spongy insoluble polymer as shown in Plate 4.3. Plate 4.4 was CG heat treated at the same temperature and time of 140 ^{o}C for 30 min used to cross-link CG with CTR, but without the cross-linking agent.



Plate 4.1: A picture of CG:EPC Cross-linked Polymer After Treatment with Heat and Prior to Washing

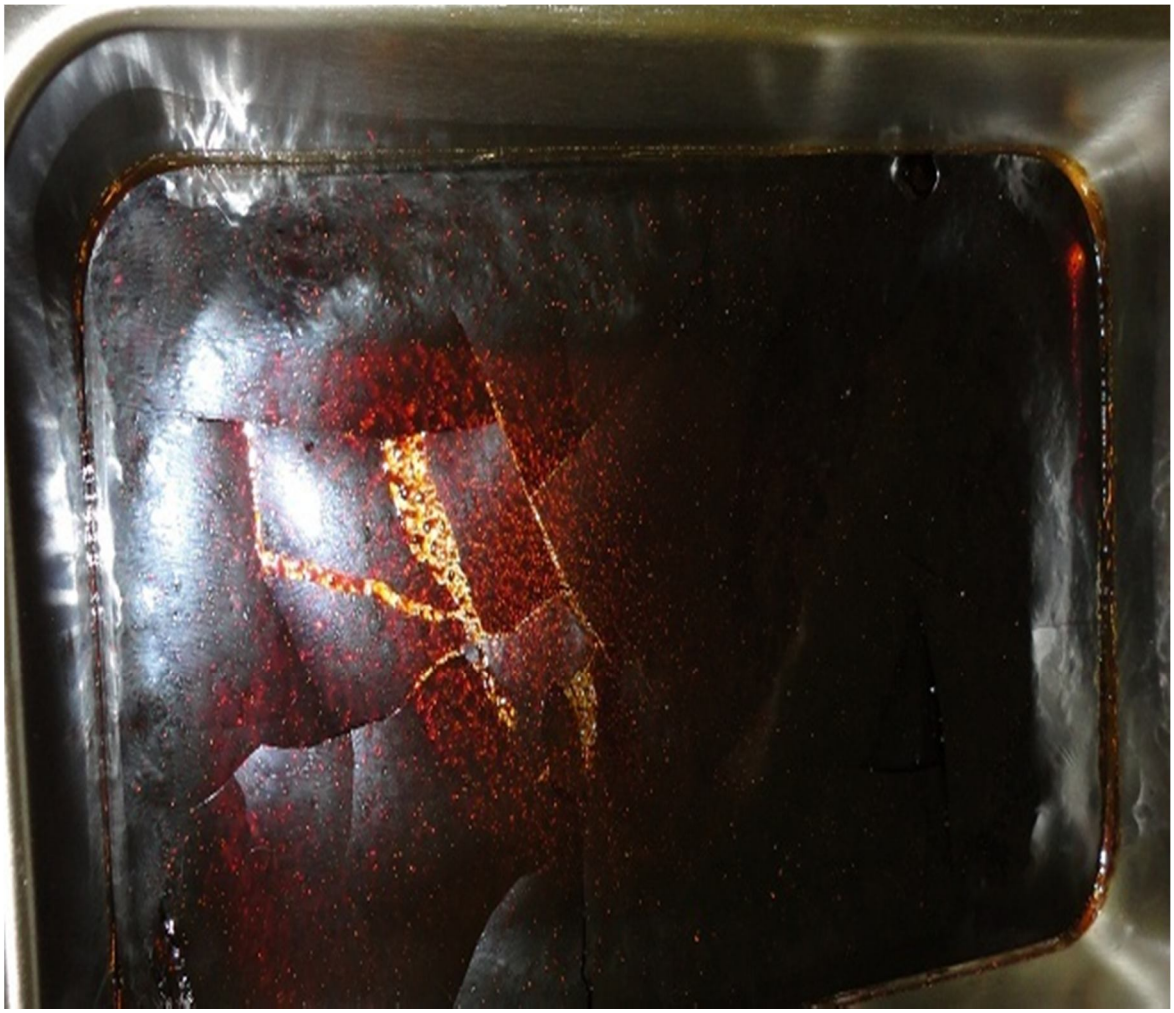


Plate 4.2: A Picture of CG:CTR Cross-linked Polymer Heat Treated at 140 ^{o}C for 30 min



Plate 4.3: A Picture of Washed and Dried CG:CTR Cross-linked Polymer Heat Treated at 140 ^{o}C for 30 min



Plate 4.4: A Picture of Plain (uncross-linked) CG, Heat Treated at 140 ^{o}C for 30 min

4.3 Residual Solvent Analysis by Gas Chromatography (GC) on CG

Both acetone and ethanol were present as residual solvents in concentrations above the permissible level of 5000 ppm (Figure 4.5) when tested using GC. The residual level of acetone in the tested gum sample was found to be 7446 ppm. Washing the precipitated gum with distilled water (Lima *et al.,* 2002) reduced the residual solvent quantity to a relatively lower value of 5730 ppm. Ethanol at 95 %v/v concentration when used to extract CG was found to be retained as residual solvent at a concentration of 6424 ppm, while 2-propanol that made up the ethanol solution used for the extraction was present at a concentration of 266 ppm.

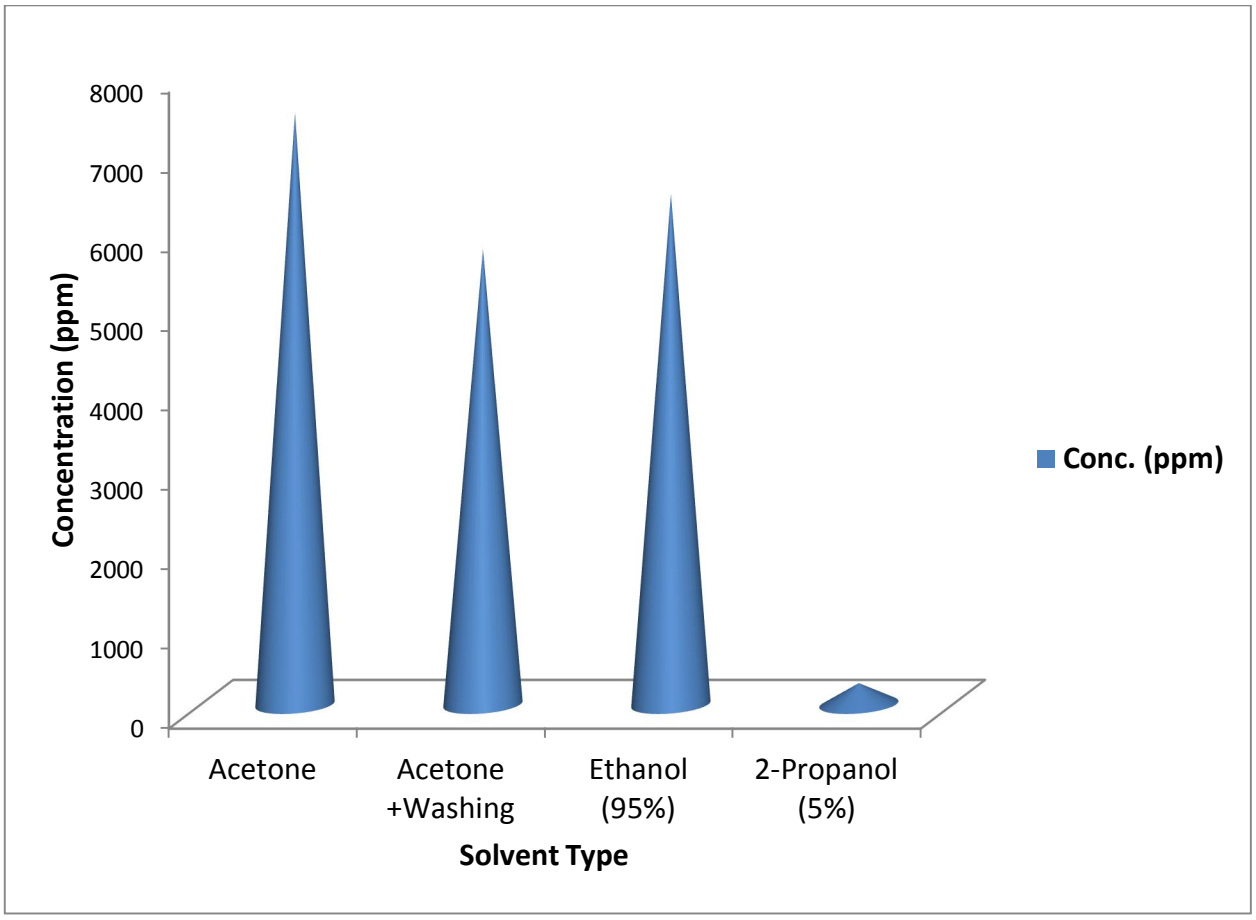


Figure 4.5: Residual Solvent Concentrations (ppm) of Acetone and Ethanol in CG

4.4 Thermal Analysis by DSC

Figure 4.6 is the DSC thermogram of crude CG powder showing the degradation temperatures of CG at 311.6 ^{o}C while the event at 25 -200 ^{o}C was due to evaporation of water (Silva *et al.*, 2006). Overlay of this thermogram upon that of CG processed using acetone (Figure 4.7) showed some level of variations in the curves. The figure showed processing the gum to have caused a slight increase in the degradation temperature peak at 313.6 ^{o}C.

Figure 4.8 is the overlay of DSC curves of CG and CrosCE synthesized polymer. The degradation temperature of the cross-linked polymer was found to be 324.4 ^{o}C, a figure much higher than the 311.6 ^{o}C of CG.

CTR was found to have a melting temperature peak of 156.3 ^{o}C (Figure 4.9). This peak was however noticed to be completely missing following the usage of CTR in cross-linking CG as shown in the DSC curve of CrosCC (Figure 4.10) and better demonstrated by the DSC overlay of curves of CrosCC and binary mixture of CG and CTR (Figure 4.11). Figure 4.12 is the DSC overlay of thermograms of CG, CrosCE and CrosCC showing varying degradation temperatures.

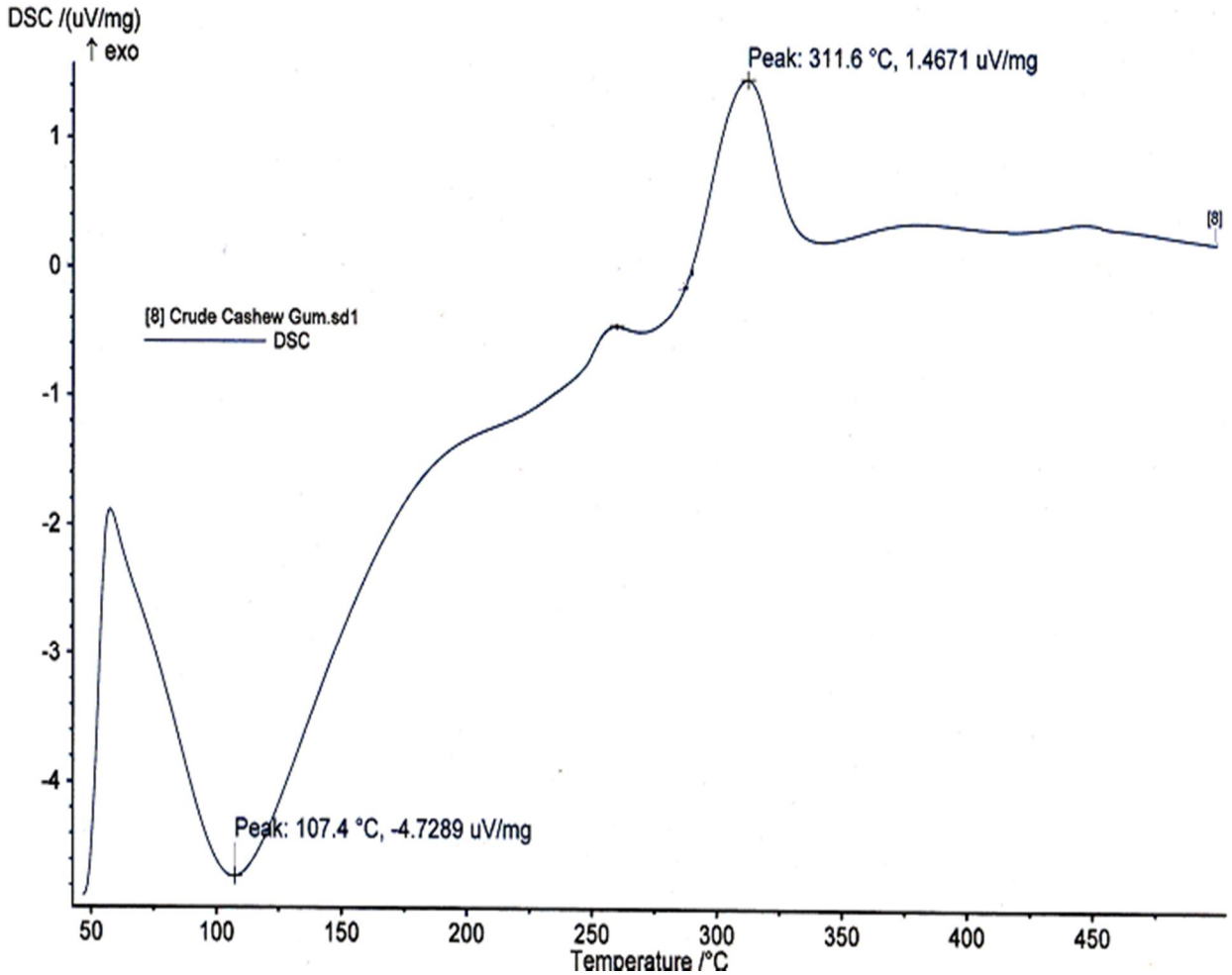


Figure 4.6: DSC Thermogram of Crude CG Powder

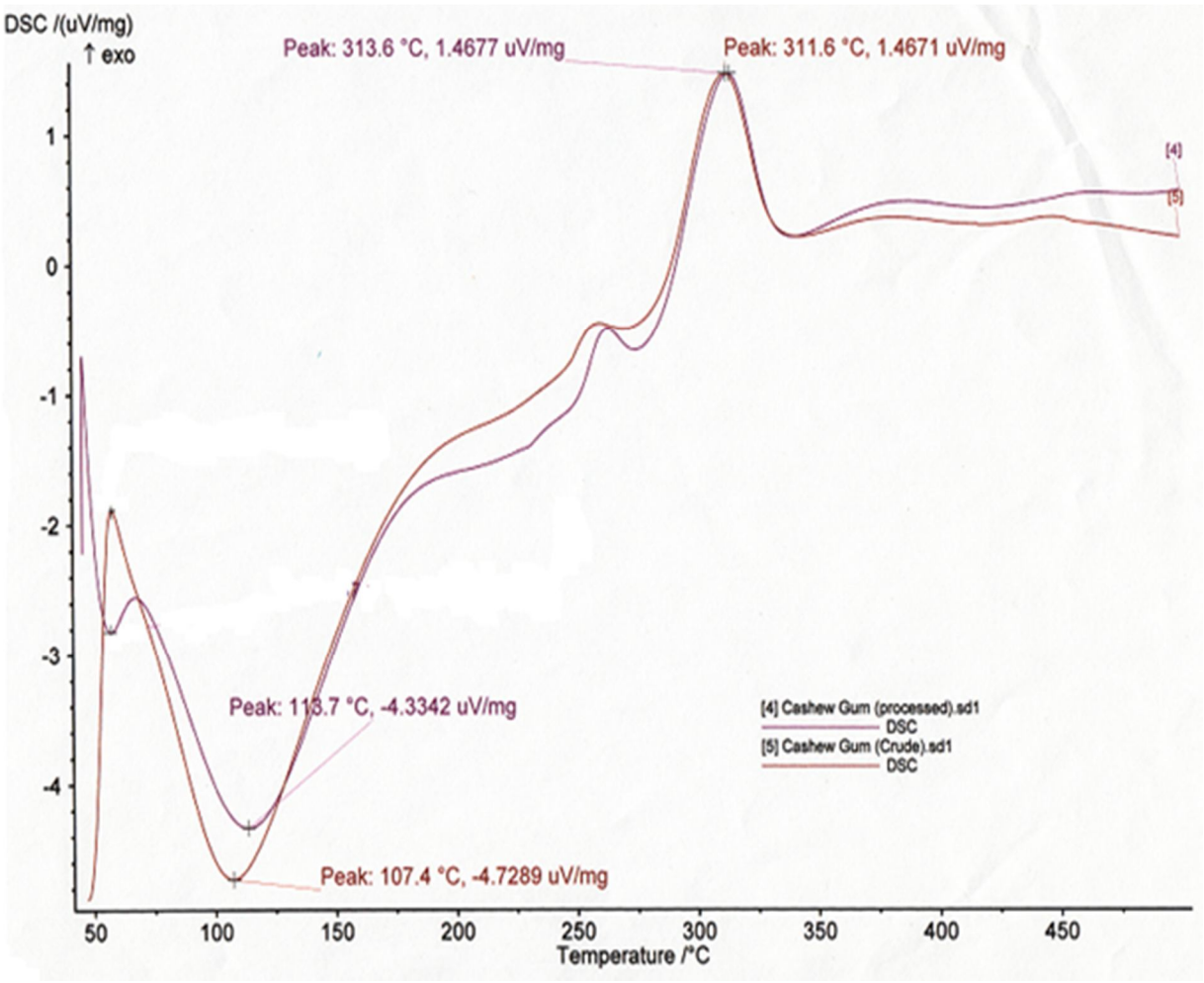


Figure 4.7: DSC Overlay of Crude and Processed CG Powders

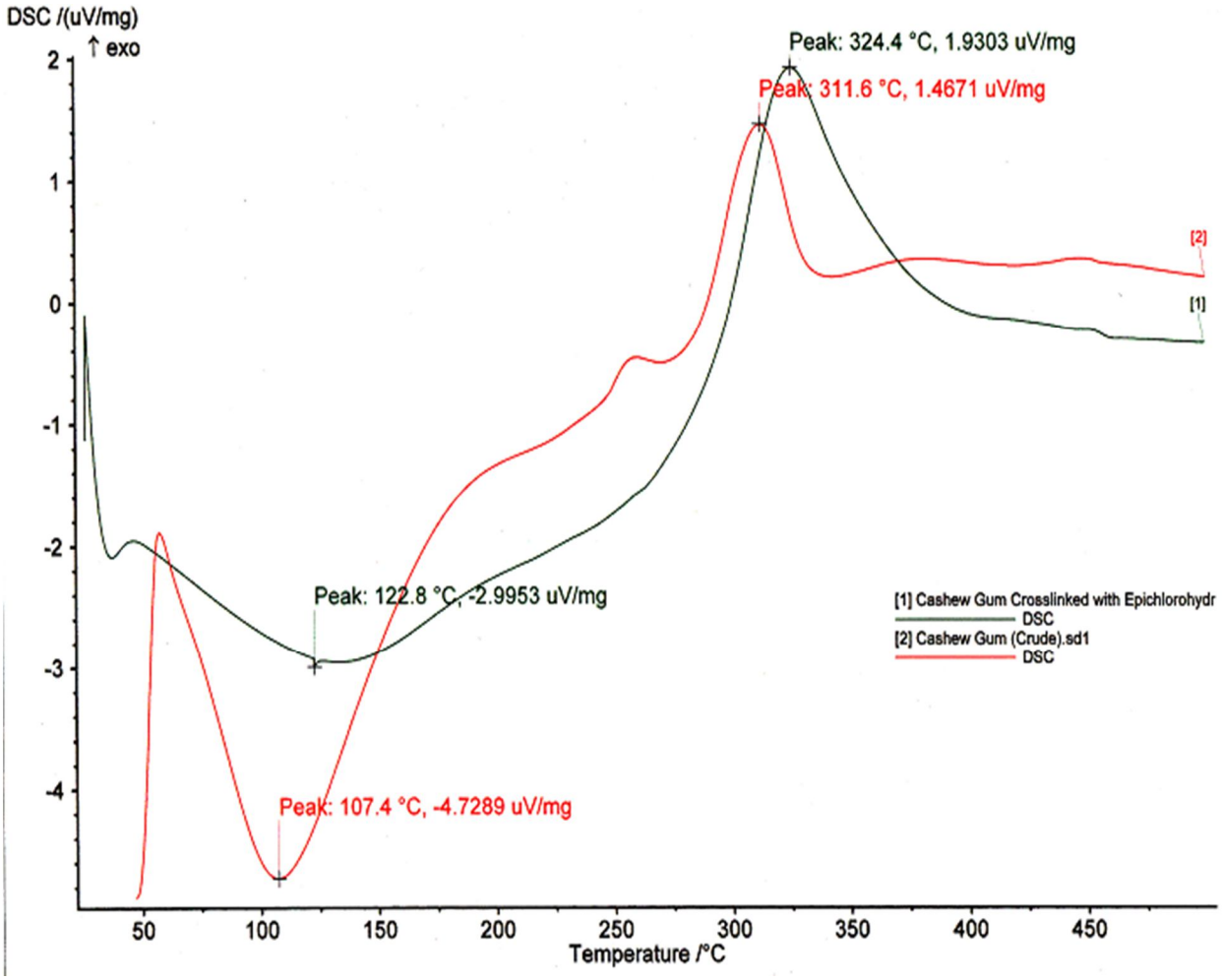


Figure 4.8: DSC Overlay of Powders of CG and CrosCE

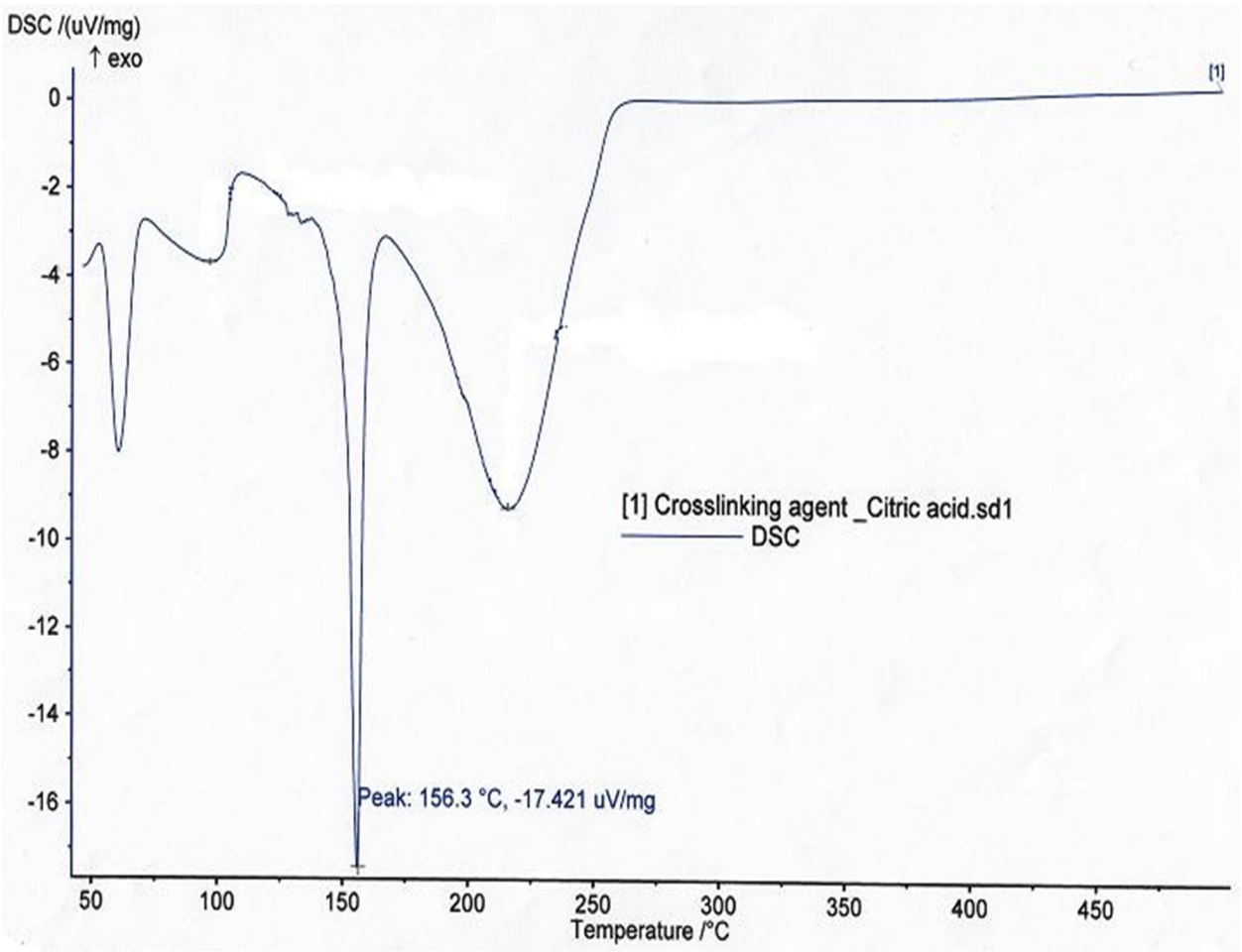


Figure 4.9: DSC of CTR (cross-linking agent)

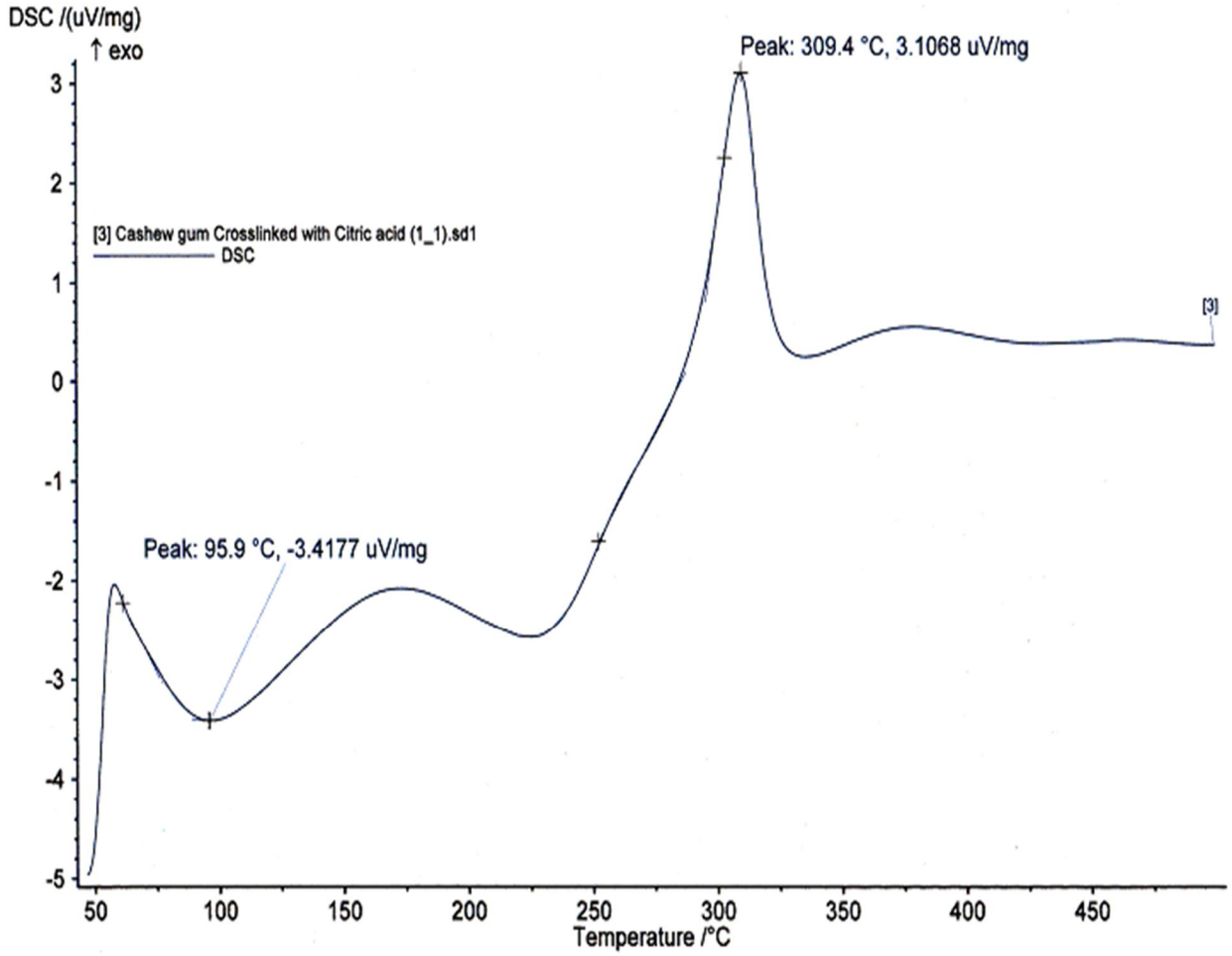


Figure 4.10: DSC of CrosCC Polymer

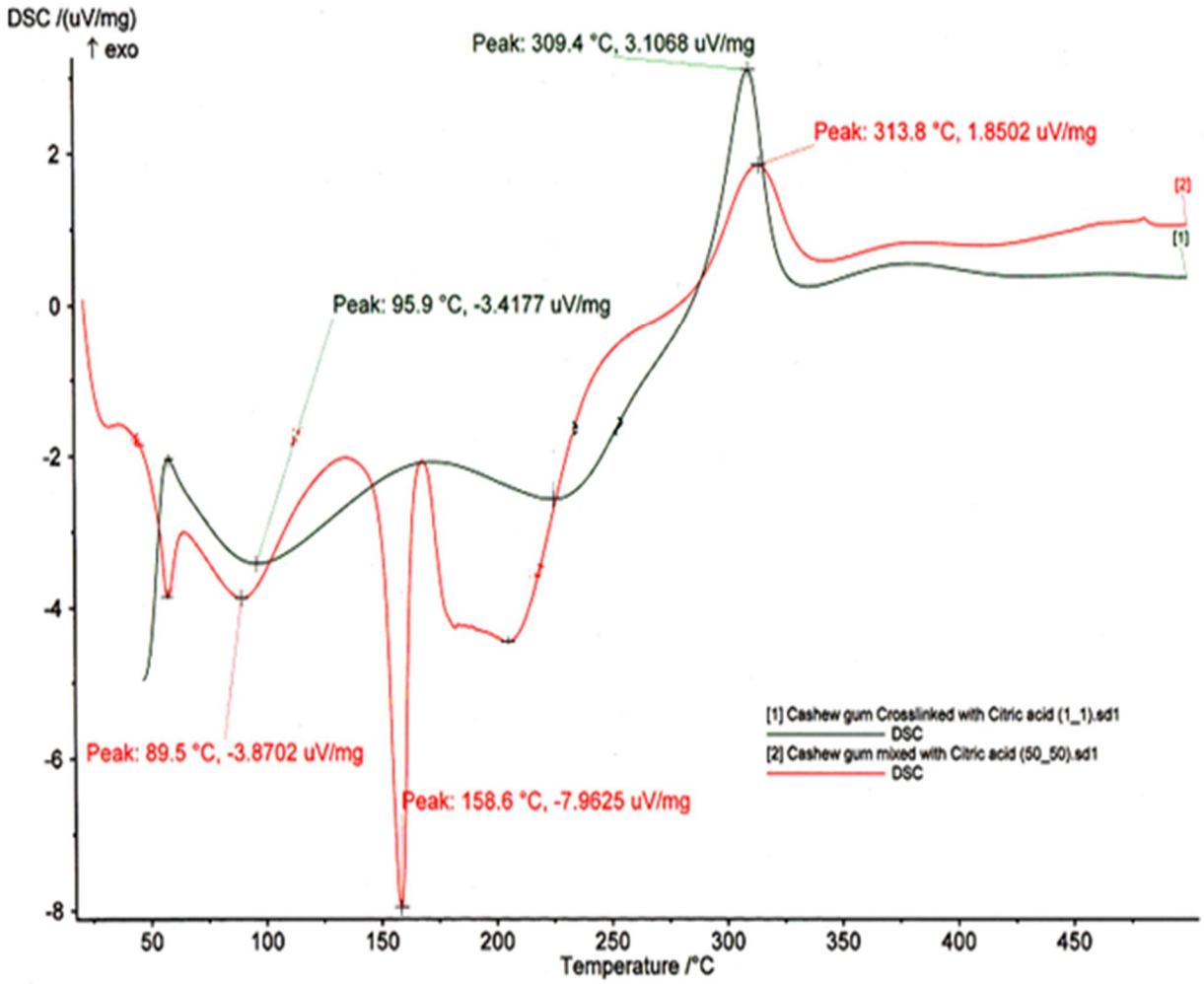


Figure 4.11: DSC Overlay of CrosCC and 50:50 Binary Mixture of CG and CTR

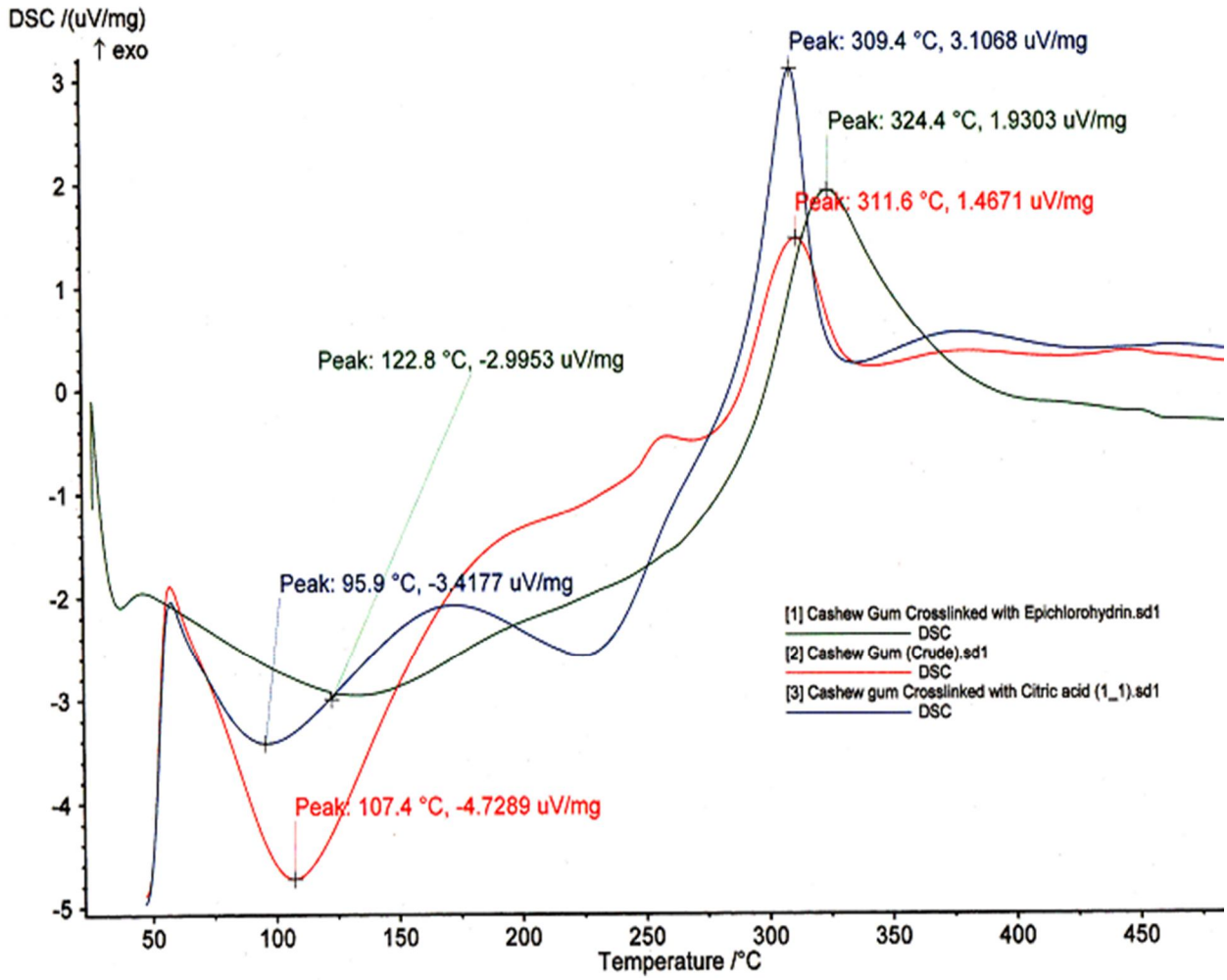


Figure 4.12: DSC Overlay of CG, CrosCE and CrosCC Powders

4.5 Fourier Transform Infrared (FT-IR) Spectral Studies

Figure 4.13 is the FT-IR spectrum of plain (uncross-linked) CG showing the characteristic absorption bands associated with aromatic sugars of carbohydrates. The band with peak at 3388.32 is H–bonded OH stretch band of aromatic compounds (Hsu, 1997). Figure 4.14 is the spectrum of CTR showing the characteristic stretch carbonyl frequency of carboxylic acids at 1726 cm^{-1} (USP, 2011).

Figure 4.15 is the FT-IR spectrum of 50/50 binary mixture of CG and CTR showing the absorption bands associated with the stretch carbonyl frequency of carboxylic acids appearing at 1726 and or 1713 cm^{-1}. Figure 4.16 is the spectrum of CrosCC, a cross-linked product of CG and CTR. Similar absorption bands can be seen as those found with the uncross-linked CG (Figure 4.13). The strong 1729 cm^{-1} carbonyl frequency band of CTR seemed to have disappeared and in its place appeared a strong and sharp band at 1735 cm^{-1} associated with the carbonyl frequency of ester group.

Figure 4.17 is the overlay of FT-IR spectra of CG and CrosCC highlighting the formation of the prominent peak at 1735 cm^{-1} assigned to the new ester molecule formed in consequence to the cross-linking process. Figure 4.18 shows the spectrum of CrosCCG which appeared very similar to that of CrosCC.

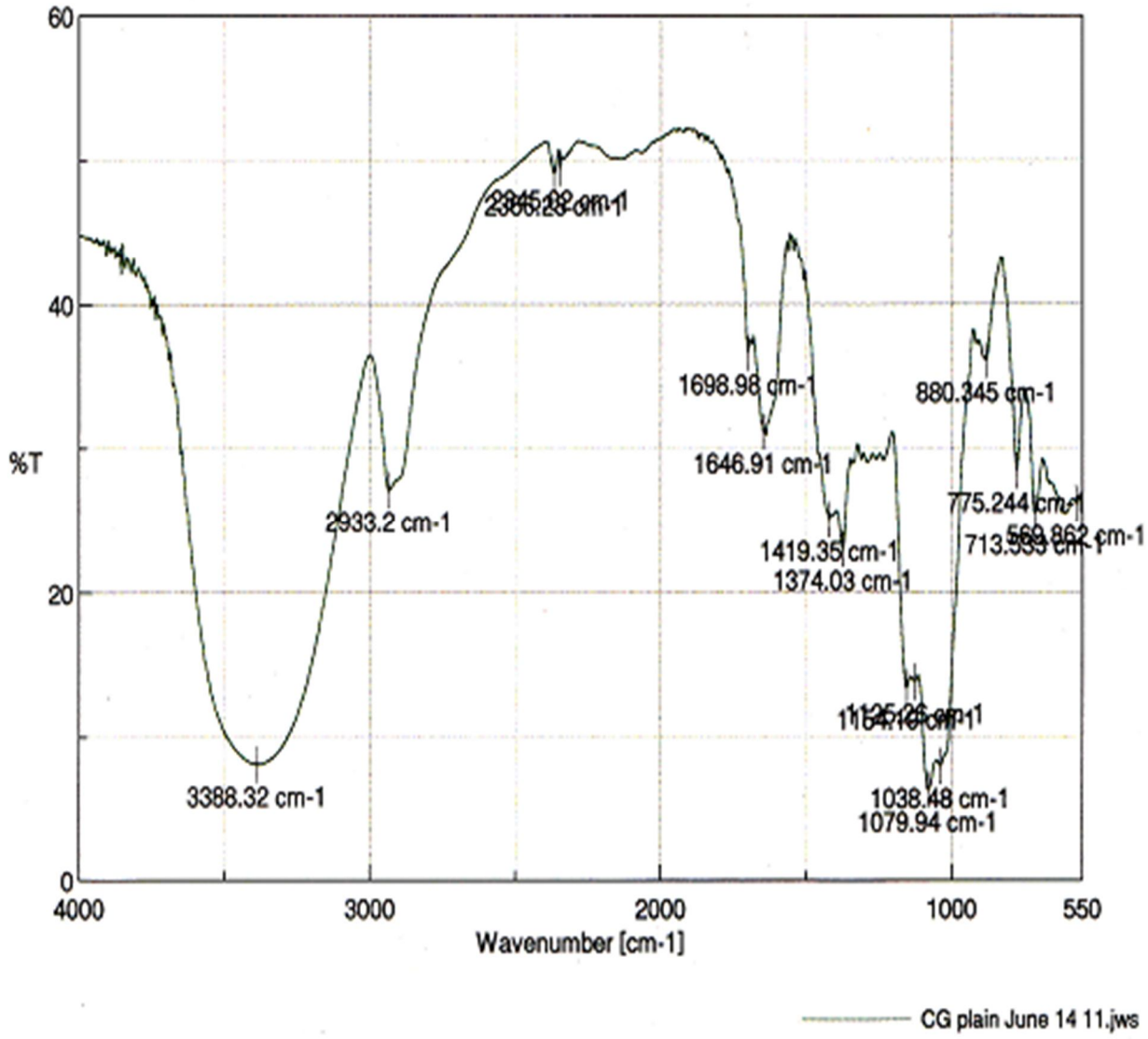


Figure 4.13: FT-IR Spectrum of Uncross-linked CG Powder

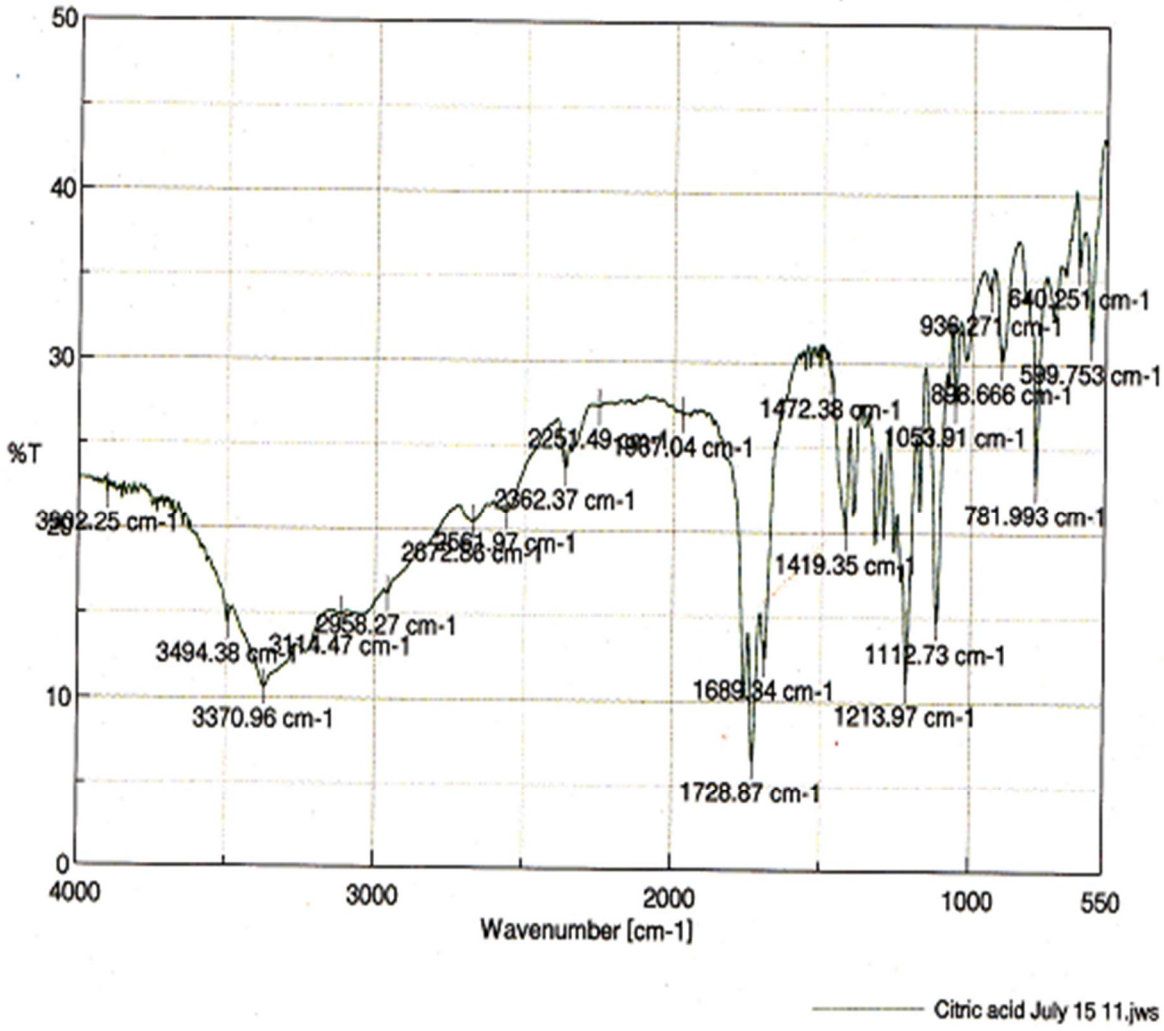


Figure 4.14: FT-IR Spectrum of CTR

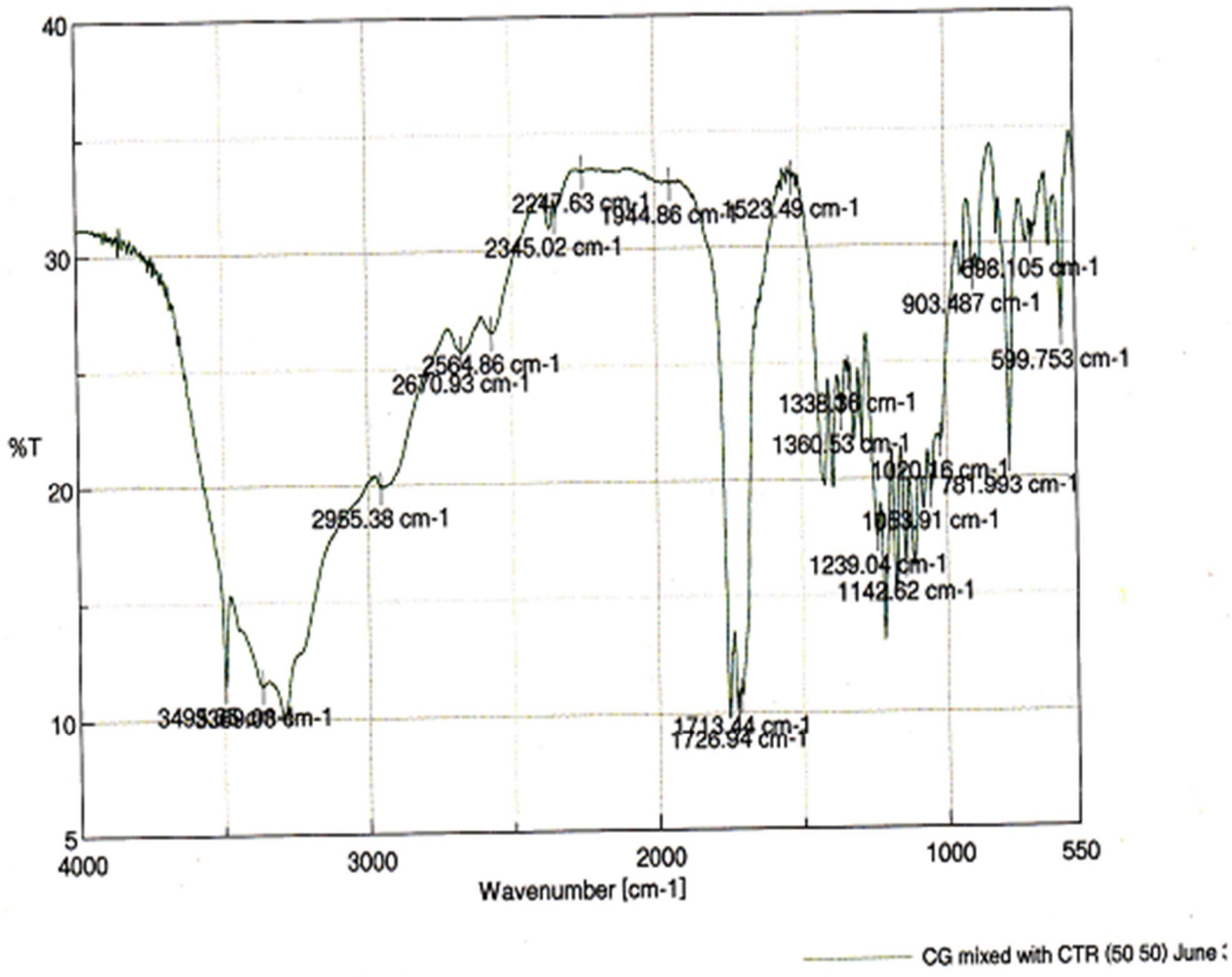


Figure 4.15: FT-IR Spectrum of Binary (50:50) Mixture of CG and CTR

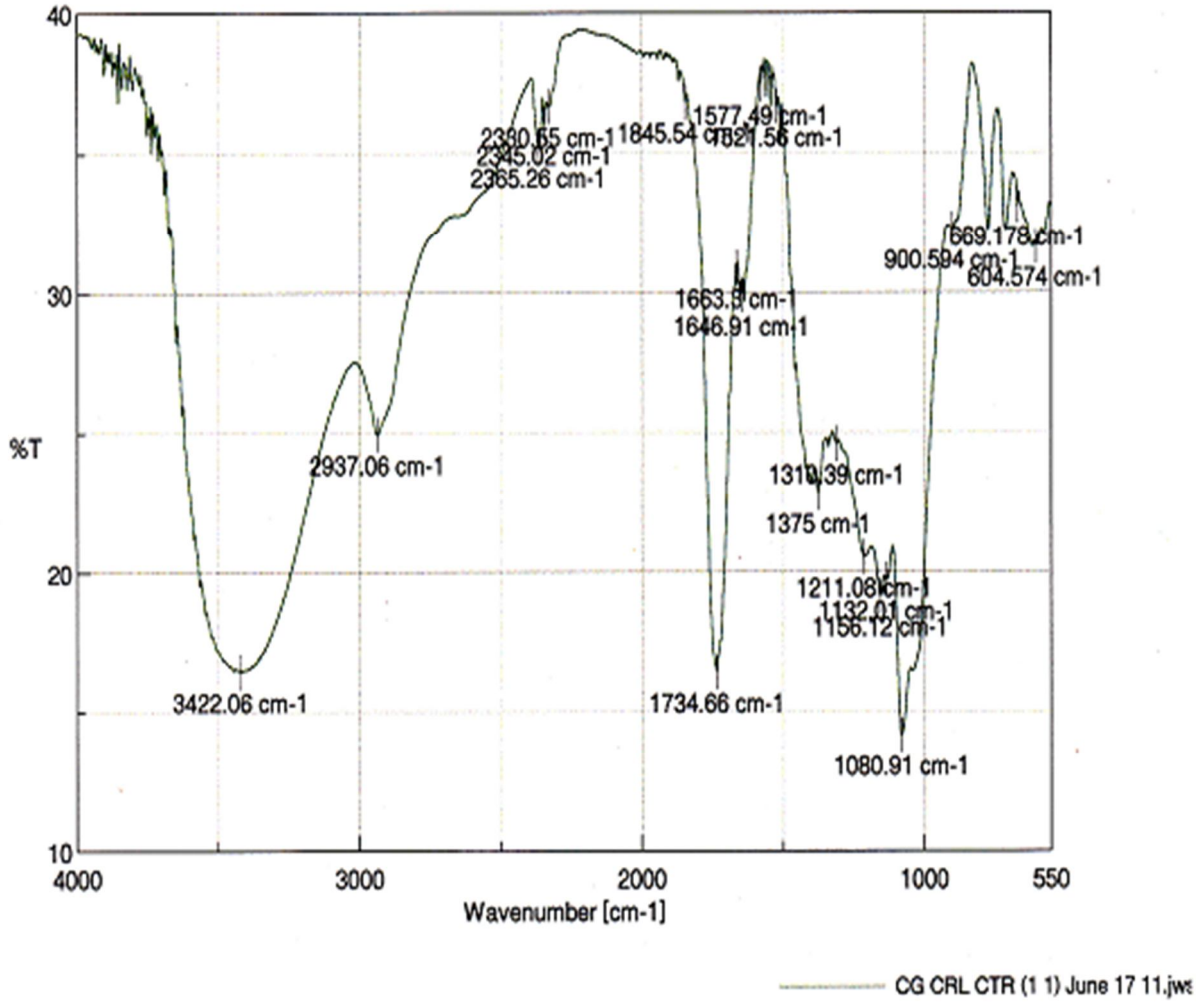


Figure 4.16: FT-IR Spectrum of CrosCC Polymer

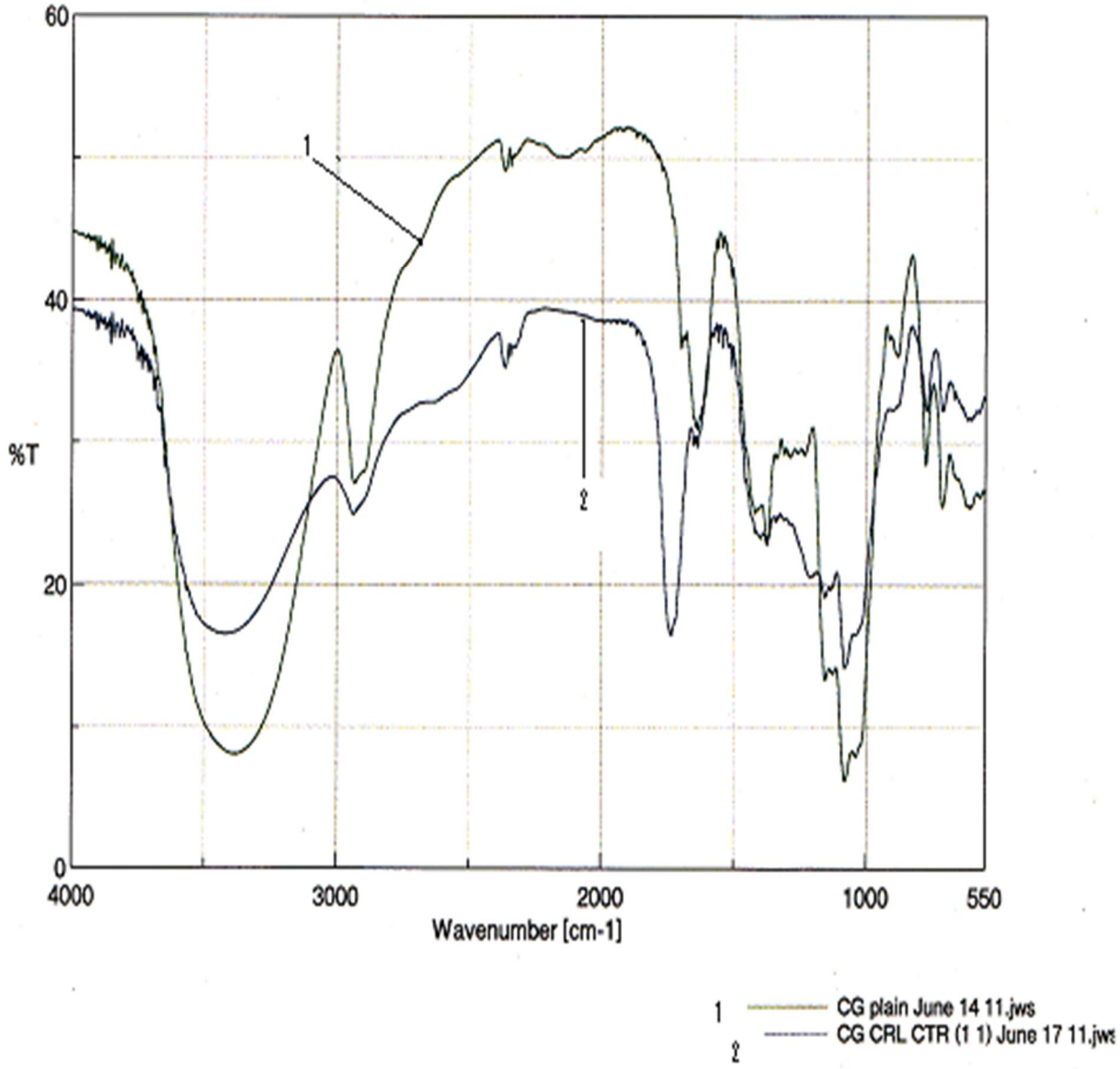


Figure 4.17: FT-IR Spectral Overlay of (1) CG and (2) CrosCC

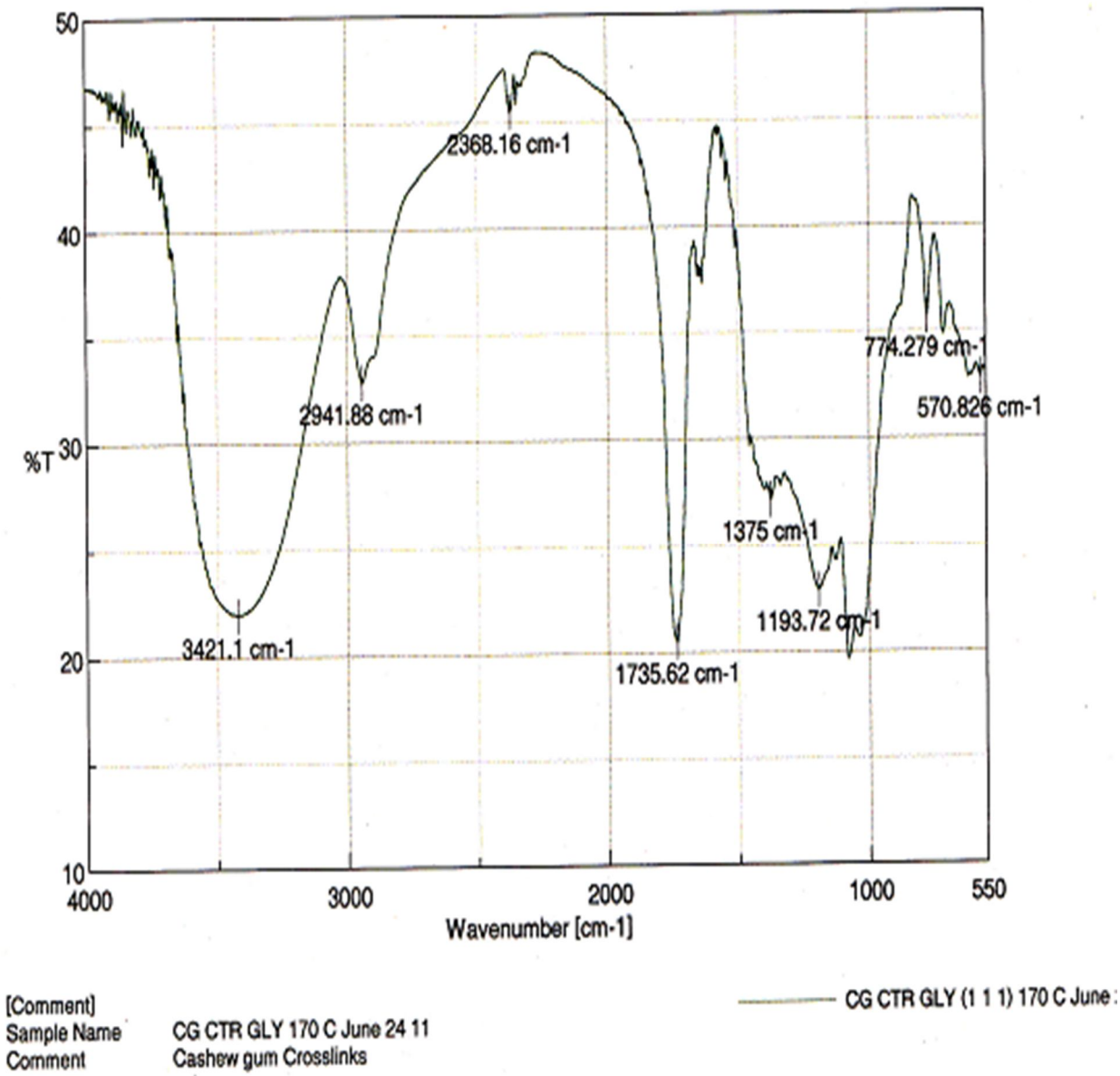


Figure 4.18: FT-IR Spectrum of CrosCCG

4.6 ^{13}C NMR Spectral Analysis of CrosCCG Polymer

Figure 4.19 is the solid state ^{13}C NMR spectrum of CrosCCG showing the various resonances associated with respective resonating carbons as interpreted in Table 4.1.

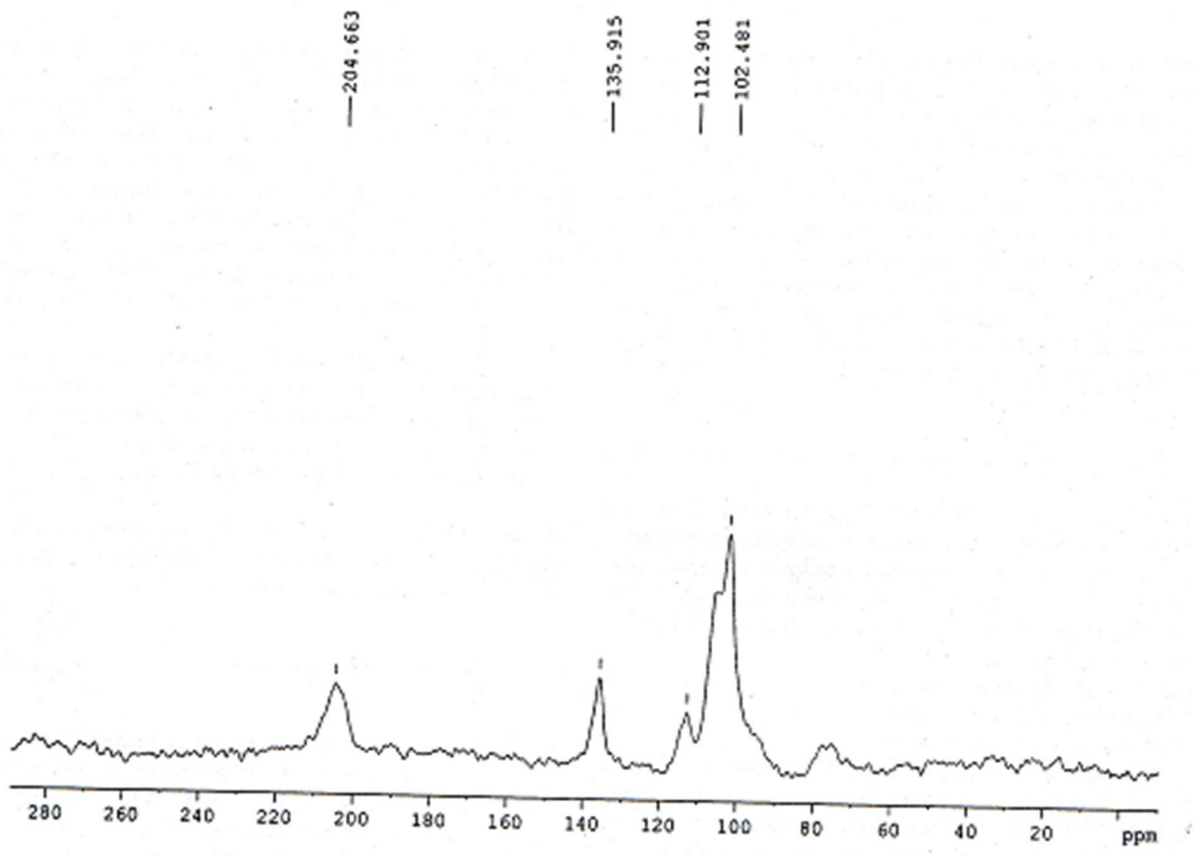


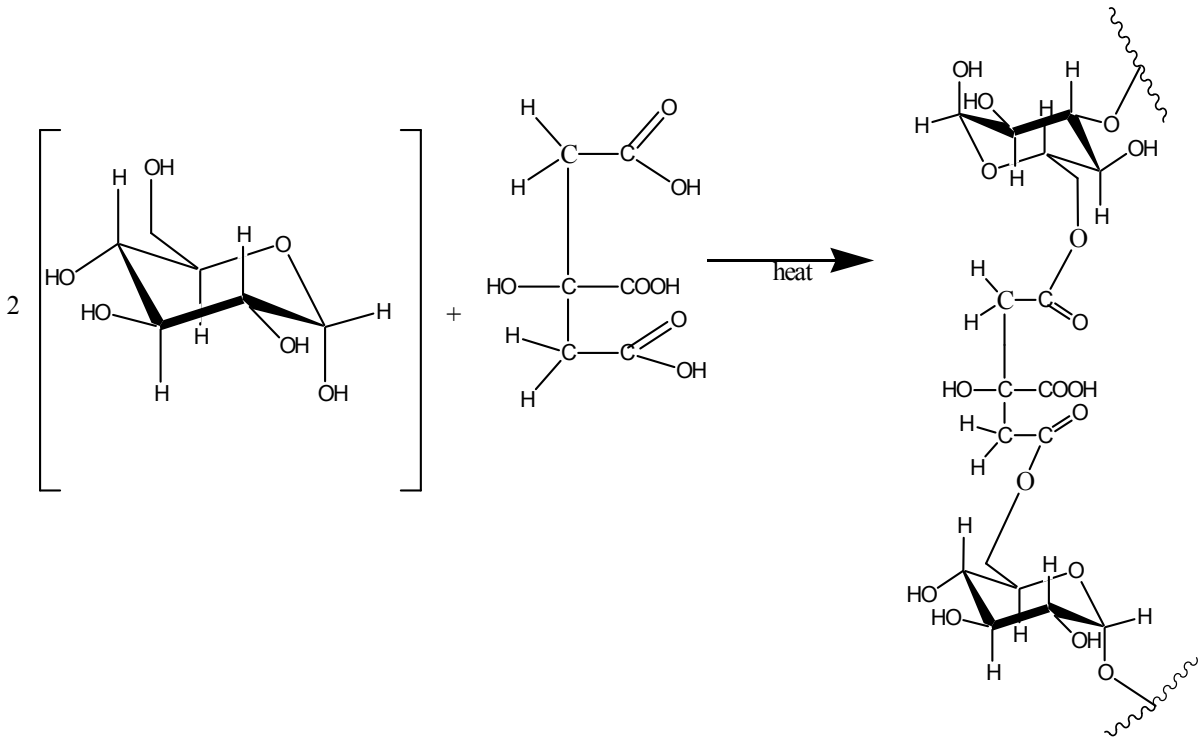
Figure 4.19: Solid State ^{13}C Nuclear Magnetic Resonance of CrosCCG

Table 4.1: Interpretation of the Solid State ^{13}C Nuclear Magnetic Resonance of CrosCCG

Resonance (ppm)	Responsible Carbon
18.0	Methyl group of Rhamnose
73.0	C-2, C-4, C-5
102.5	Anomeric carbon resonance of glucose, galactose and xylose sugars
112.9	Anomeric carbon resonance of β-D ribofuranose
204.0	Carbonyl frequency

4.7 Proposal on the CG and CTR Reaction Scheme

Scheme 4.1 shows the proposed mechanism of formation of CrosCC resulting from cross-linking CG with CTR. The scheme depicts a molar ratio reaction components involved in the synthesis of CrosCC. Two (2) moles of the CG reacted with 1 mole of CTR to give 1 mole of CrosCCG. The attachment of the sugar molecule was stepwise with one attached before the other and in each case 1 mole of water is eliminated. Steric hindrance may have prevented the attachment of the third sugar molecule onto the third and unreacted carboxyl group situated at the center of the structure.



Scheme 4.1: Proposed Structural Representation of CrosCC Synthesis

4.8 Properties of CG, CrosCC and CrosCCG Powders

4.8.1 Organoleptic/Physicochemical Properties

Table 4.2 shows the organoleptic and some physicochemical properties of CG, CrosCC and CrosCCG. CG was light brown to off white in color, tasteless and odorless. The Colour of CrosCC and CrosCCG was found to range between dark brown to light brown with CrosCCG polymers generally having lighter colorations. Hypochloric acid was found to bleach these polymers to white colour.

The various solvents used for determination of solubility of CG and the cross-linked polymers (Table 3.4) show that variation in polarity was used in selecting the solvents for use in these tests. CG was found to disperse only in water among all the solvents tried. The cross-linked polymers, CrosCC and CrosCCG failed to dissolve or disperse in any of the solvents used for the study.

Moisture loss on drying (LOD) was found to be somewhat similar for the three polymers. CrosCC had the lowest LOD value of 7.21% followed by CrosCCG with 7.39% and finally CG with 8.47 %.

CrosCCG was found to possess greater capacity of holding water in its macromolecular structure than CrosCC. CrosCCG with a water holding capacity of 244 % almost doubled the 143 % value for CrosCC. Water holding capacity could not be determined for CG because of the gum's solubility in water.

Table 4.2: Organoleptic and Some Physicochemical Properties of the Polymers

Parameter	**Polymer type**		
	CG	CrosCC	CrosCCG
Color	Light brown, Off white	Brown →White (bleached)	Brown →White (bleached)
Taste	Tasteless	Tasteless	Tasteless
Odor	Odorless	Odorless	Odorless
Solubility (Solvent)	Soluble (Water)	Insoluble	Insoluble
Sulphated ash (± SD)	1.39 ± 0.03	1.91 ± 0.04	2.70 ± 0.04
% Moisture loss on drying (± SD)	8.47 ± 0.65	7.21 ± 2.10	7.39 ± 3.81
Water holding capacity % (± SD)	ND	143.4 ± 19.57	244 ± 5.25

Key: ND = Not done; SD= Standard deviation

4.8.2 Water Content Determination Using Karl Fisher (KF) Titrimetry

The percentage water content determined for CG, CrosCC and CrosCCG using the Karl Fisher titration is reported in Figure 4.20. CG was found to contain a relatively higher percentage of water than did the derivatives. At ambient temperature and humidity, samples collected from sealed storage containers showed CG to have 8.53 % water content while CrosCC and CrosCCG had 7.12 and 7.04 %, respectively. Determinations on samples stored for one week over activated silica gel contained in a desiccator gave a 4.59, 3.15 and 2.96 % water contents for CG, CrosCC and CrosCCG, respectively.

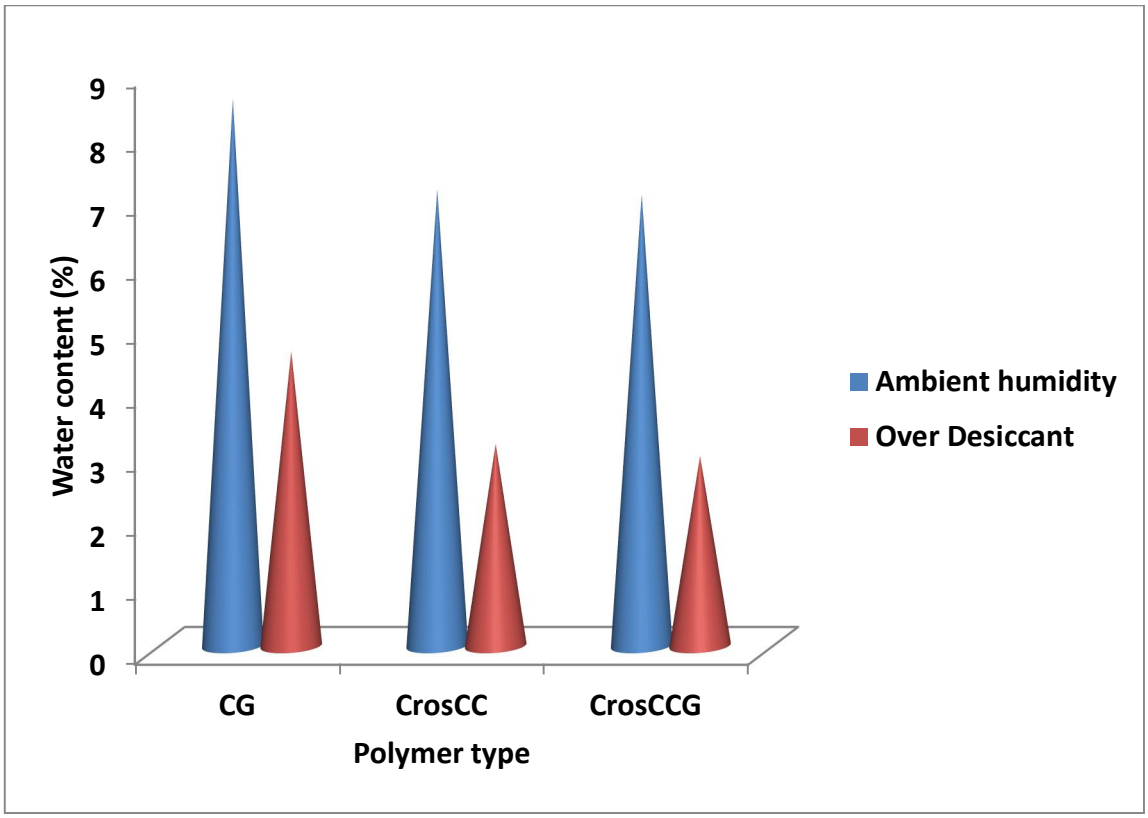


Figure 4.20: Water content (%) for CG, CrosCC and CrosCCG Determined by Karl Fisher Titration

4.8.3 Flow Properties of CG, CrosCC and CrosCCG Powders

Table 4.3 shows the parameters describing the flow of CG, CrosCC and CrosCCG. Among the powders, CG had the lowest flow with a rate of 10 g/s compared to CrosCC and CrosCCG where each showed a rate of 12.5 g/s. Angles of repose for CG, CrosCC and CrosCCG were found to be relatively uniform with values 30.96 ^{o}, 30.11 ^{o} and 29.24 ^{o}, respectively. CrosCCG, nonetheless, showed the lowest angle of repose. Density measurements showed CG to be more compressible than the cross-linked polymers with compressibility index and Hausner ratio of 17.30 % and 1.22, respectively. Compressibility of CrosCC was found to be slightly higher than that of CrosCCG as can be seen from the table. Particle size distribution (Figure 4.21) showed that all the powders had sizes that skewed to the larger size fraction. CG appears to have relatively more of the smaller sized particles than the derivatives.

Table 4.3: Flow Properties of CG, CrosCC and CrosCCG Powders

Parameter	**Powder type**		
	CG	CrosCC	CrosCCG
Flow rate (g/s)	10	12.5	12.5
Angle of repose (^{o})	30.96	30.11	29.24
Bulk density (g/ml)	0.732	0.556	0.549
Tap density (g/ml)	0.897	0.739	0.714
Compressibility index (%)	17.30	21.92	23.00
Hausner ratio	01.22	01.26	01.29

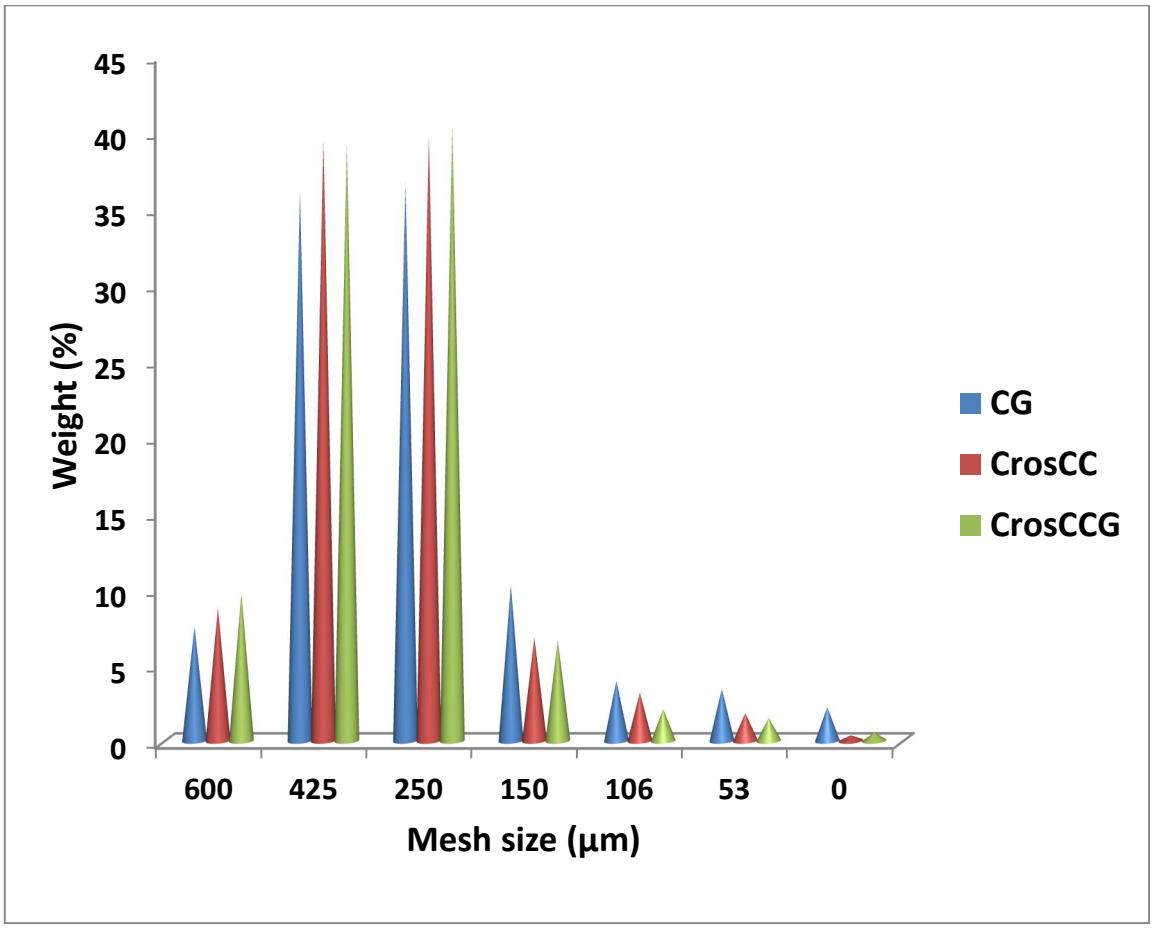


Figure 4.21: Particle Size Distribution of CG, CrosCC and CrosCCG Powders

4.8.4 Moisture Sorption/Desorption Determination

Figure 4.22 and Apendix C show the moisture sorption/desorption isotherm of 2 g samples each of CG, CrosCC and CrosCCG subjected to humidity changes (Table 3.5 and Figure 3.1). All the samples were found to sorp moisture to a maximum on the 4th week, corresponding to exposure over saturated salt solution of potassium chloride. The samples sorped least quantites of water at weeks 1 and 7 corresponding to sorption and desorption equivalents respectively following exposures over magnesium chloride. CrosCCG sorped a maximum of 0.59 g at week 4, corresponding to 0.13 g and 0.23 g of water sorped by CG and CrosCC respectively. Similarities were noted in quantities of moisture sorped and that desorped at each round of exposure.

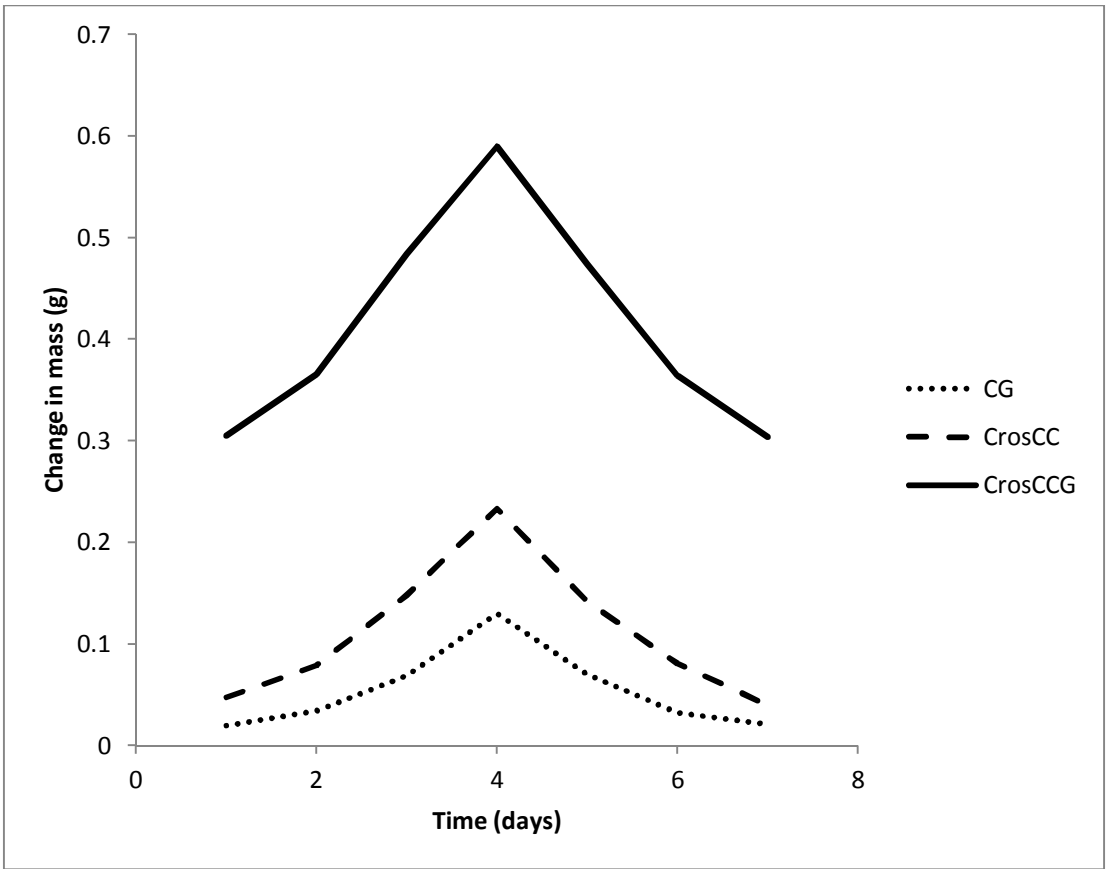


Figure 4.22: Moisture sorption/desorption isotherm of CG, CrosCC and CrosCCG after subjection to varying humidity challenges

4.8.5 Swelling Properties of CG, CrosCC, CrosCCG and CrosCE Powders

Figure 4.23 shows the effect of cross-linking on the swelling properties of CG over 10 min period of swelling. CG has been found to sorp some amounts of water (Figure 4.22), but this did not make it swell to an appreciable level. CrosCC, a cross-linked derivative of CG was found to have a swelling ratio 0.0 % at 0.0 min, 31.6 % at 1 min, 54.2 % at 2 min and 82.2 % after 10 min contact with water. In an apparent 1st order process, swelling of CrosCC was faster within the first 5 min and thereafter the rate dropped and the polymer almost stopped swelling.

Figure 4.24 and Appendix D show the effect of cross-linking of CG with CTR and GLY on the swelling property of CG. Cross-linking CG with a combination of CTR and GLY produced a polymer with a greater swelling capacity over that of CrosCC, a polymer obtained by cross-linking CG with CTR alone. The swelling ratio of CrosCCG was found to be 0.00% at 0.00 min, 87.5 % at 1 min, 120.3 % at 2 min and 195.3% after 10 min contact with water. Swelling in CrosCCG was found to be faster within the first 1 min and then it became steady to the end of the 10 min study.

Figure 4.25 shows the swelling ratio (%) of CrosCE and CrosCCG. CrosCE was found to possess greater swelling capacity than CrosCCG, with the former having a swelling ratio of 166.7% as against the latter's 87.5% within the first one minute. In both cases the rate of swelling was steady within the 10 min period of study. The difference in the rate was statistically significant ($P < 0.05$).

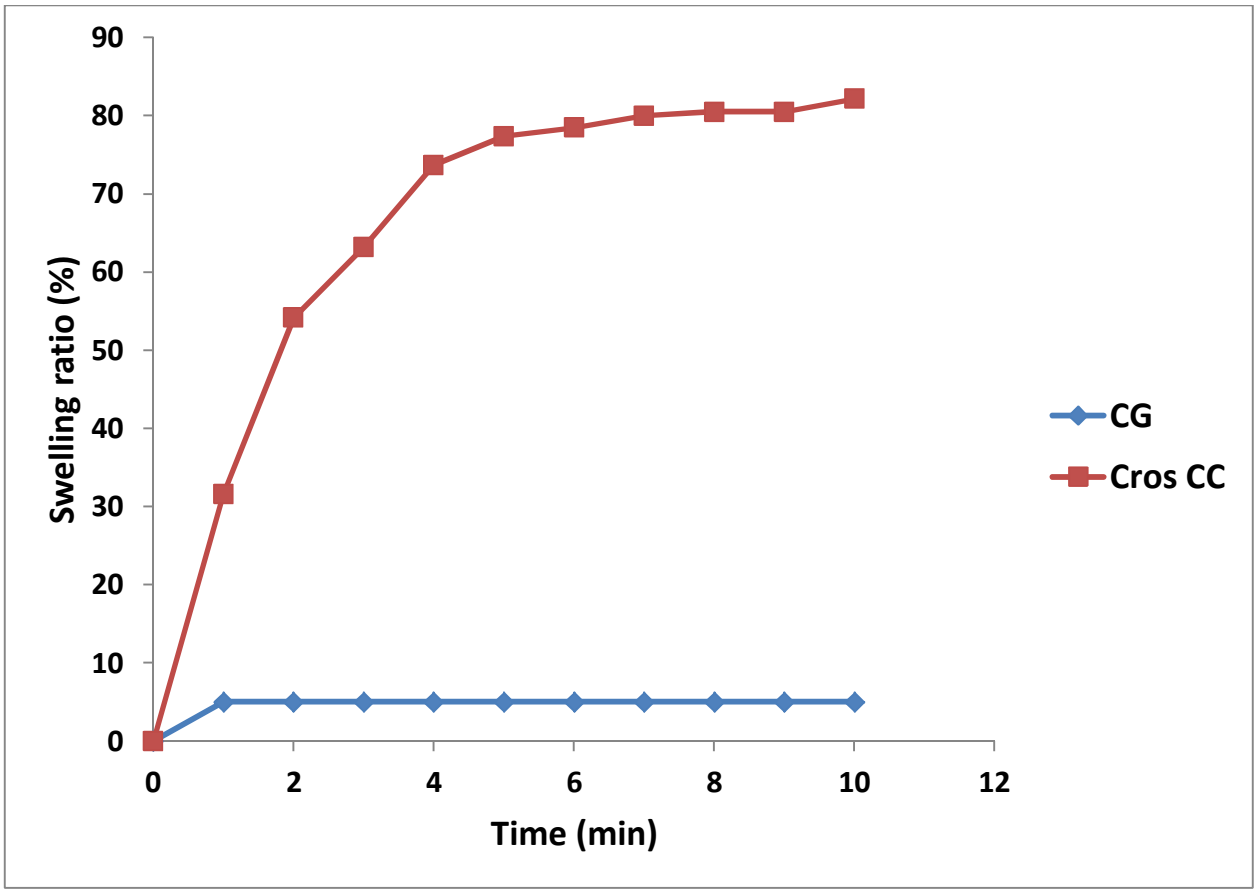


Figure 4.23: The Effect of Cross-linking on Percentage (%) Swelling Ratio of CG and CrosCC

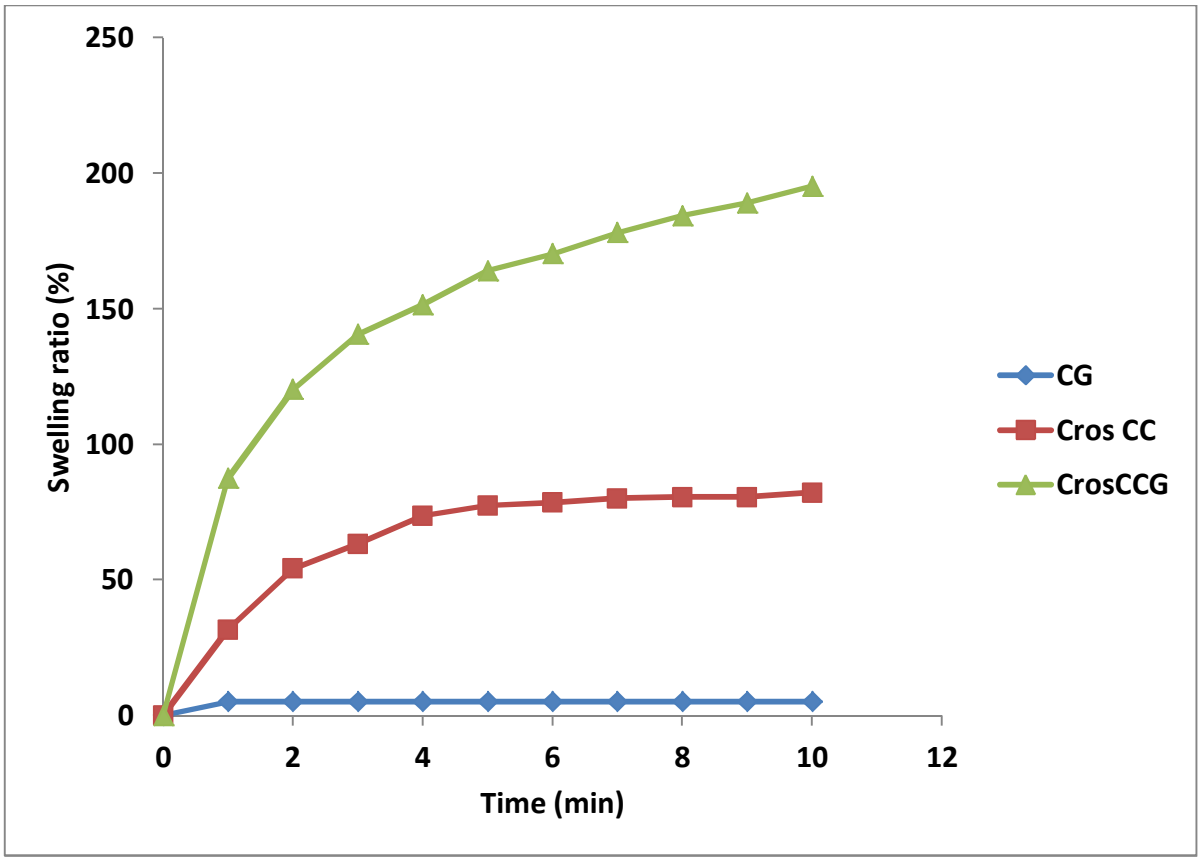


Figure 4.24: The Effect of cross-linking on Percentage **(%)** Swelling Ratio of CG

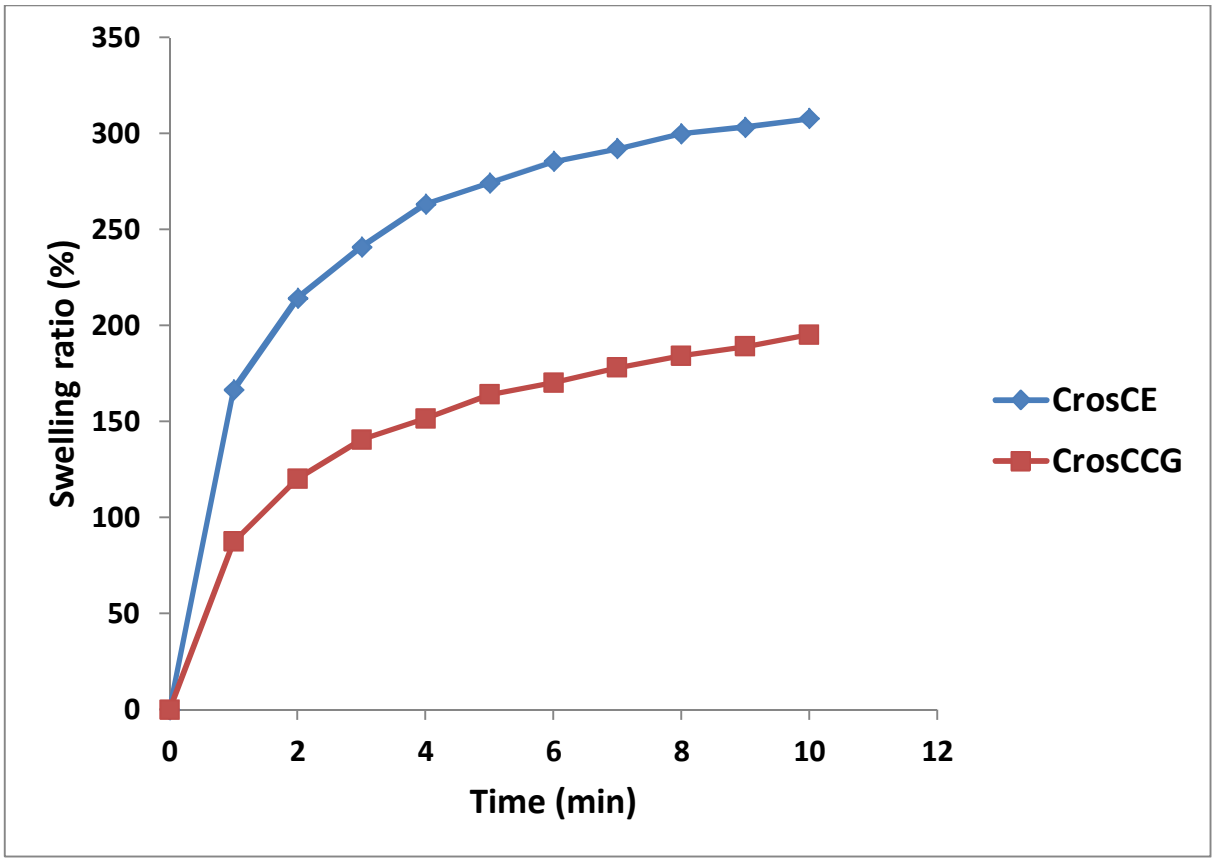


Figure 4.25: The Effect of Cross-linker type on Percentage (%) Swelling Ratio of CrosCE and CrosCCG

4.9 Formulation Studies

4.9.1 Drug Polymer Compatibility Studies using DSC

Figures 4.26 and 4.27 show the DSC measurement curves demonstrating the compatibility or otherwise of Ven. HCl (test drug) against CG and CrosCCG (test polymers), respectively. Figure 4.26 shows the DSC measurement curve of 50/50 binary mixture of Ven. HCl and CG overlaid on that of the pure drug. Figure 4.27 displays the DSC thermograms overlay of 50/50 binary mixture of Ven. HCl and CrosCCG on that of the pure drug. In both cases, the melting temperatures of the drug (Ven. HCl) in its pure form appeared sharper and more prominent than after its admixture with the polymer (CG or CrosCCG). The curve in the binary mixture was shorter, broader and of lower peak melting temperature.

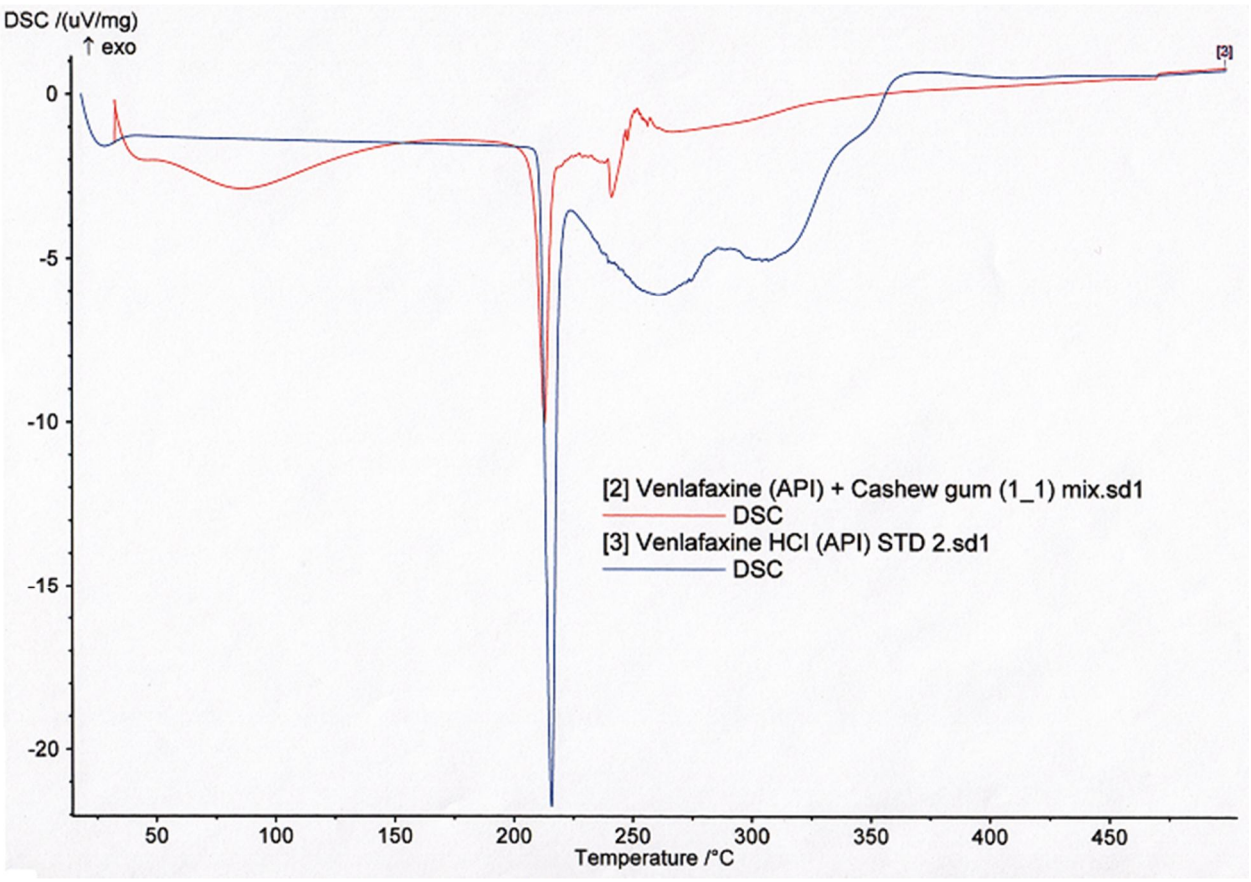


Figure 4.26: DSC Overlay of Ven. HCl (API) and its 50/50 mixture with CG (excipient)

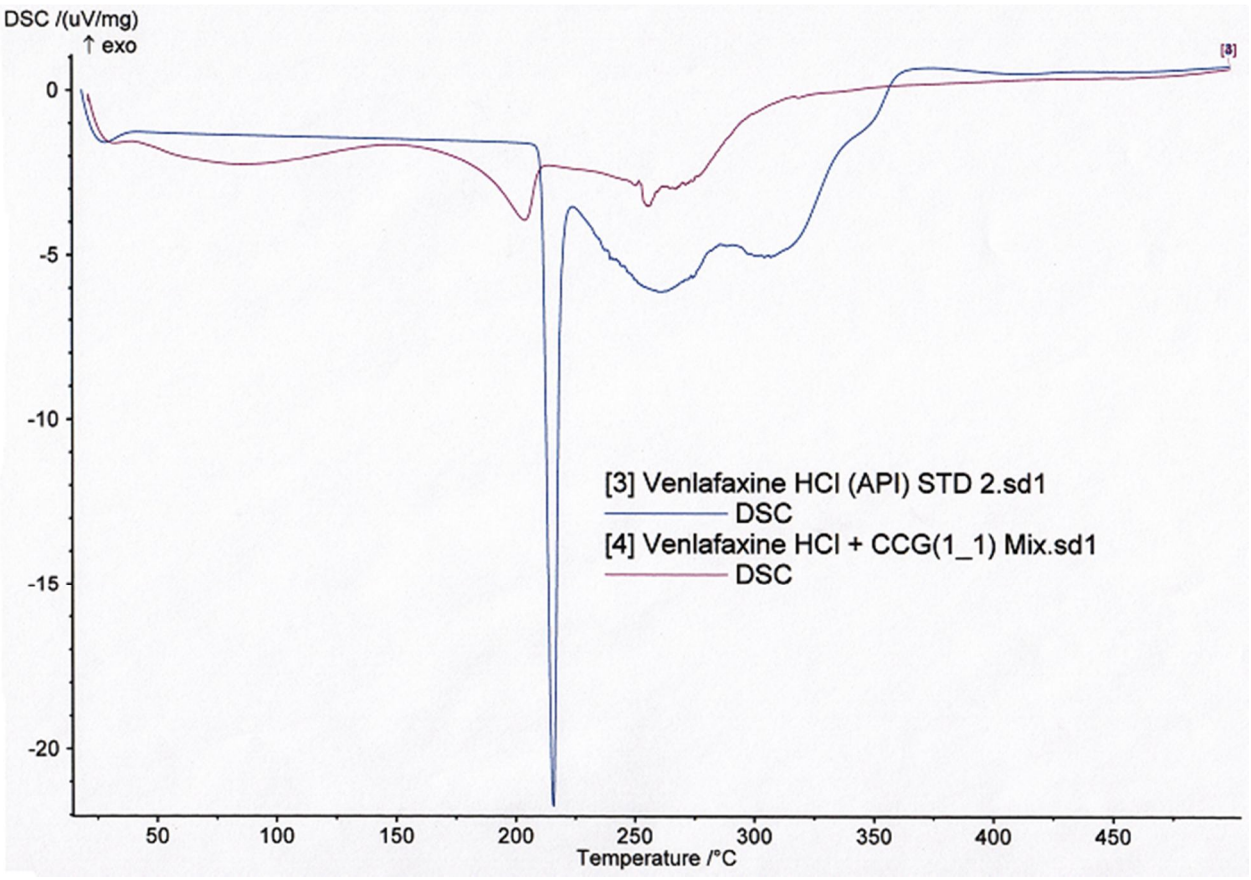


Figure 4.27: DSC Overlay of Ven. HCl (API) and its 50/50 mixture with CrosCCG (excipient)

4.9.2 Formulation Properties of Ven. HCl Tablets Containing MCC PH 102 and/or CG as Binder

4.9.2.1 Granule Properties

Table 4.4 shows the properties of granules of Ven. HCl made using MCC PH 102 or CG or admixtures of the two. Batch of granules containing MCC PH 102 as (G1) binder can be seen to present with a compressibility index of 25.20 % and Hausner ratio of 1.35, which were higher than those obtained for the other batches containing CG. All the other batches (G2 – G6) appear to share some similarities in values of compressibility index and Hausner ratio. Mean granule size was not widely different in all the batches. Flow rate showed some variations between the granule batches with G5 containing a mixture of CG and MCC PH 102 in 1:2 ratio having the highest rate. G1 and G6 had comparatively the poorest flow. All the batches had angle of repose values that were above 30^{o} with the highest being 33.51 for G6 and the lowest being 30.47 for G2.

Table 4.4: Properties of Ven. HCl Granules Containing Mcc and or CG as Binder

Parameter		Batches					
		G1	G2	G3	G4	G5	G6
Bulk density (g/ml)		0.566	0.657	0.511	0.680	0.657	0.680
Tapped density (g/ml)		0.767	0.839	0.663	0.877	0.839	0.877
Compressibility index (%)		25.20	20.60	21.80	21.40	20.6	21.40
Hausner ratio		1.35	1.29	1.29	1.28	1.27	1.28
Mean granule size (µm)		148.37	145.23	146.72	146.9	146.21	148.98
Flow rate (g/s)	**B/L**	2.49	2.73	3.0	3.13	4.28	2.5
	A/L	2.41	2.54	3.13	3.29	3.8	2.27
Angle of repose (^{o})	**B/L**	33.37	31.90	32.12	31.72	33.66	33.51
	A/L	32.29	30.47	31.49	31.03	30.85	33.51

Key: G=Granule,; G1=MCC PH 102 10%, G2=CG 5%, G3=CG 10%, G4=CG 25%, G5=MCC PH 102 10% +CG 5%, G6=MCC PH 102 10%+CG 25%; B/L and A/L=before and after lubrication, respectively.

4.9.2.2 Tablet Properties

Properties of Ven. HCl tablets compressed using either MCC PH 102 or CG or a combination of the two as binder is shown in Table 4.5 and Figures 4.28 - 4.29. The tablets showed some uniformity in tablet weights across the batches. MCC PH 102 at 10 %w/w (T1) produced the hardest tablets with crushing strength of 8.05 KN and friability of 0.00 %, while CG at 5 %w/w (T2) produced the softest tablets that failed at 5.90 KN with friability of 0.03 %.The other batches had crushing strength values lying in-between. All the batches released 80 % of their drug content within 15 min, except those batches made with 25 % CG

either alone or in admixture with MCC PH 102, which showed a $T_{80\%}$ of 30 min each. Contents of active ingredient for all the batches were between 95 and 105 %. Drug release profiles of the batches made with MCC PH 102 or increasing concentrations of CG are shown in Figure 4.28 and Appendix F while those of the admixtures of MCC PH 102 and CG are shown in Figure 4.29 and Appendix G.

Table 4.5: Properties of Ven. HCl Tablets Containing MCC PH 102 and/or CG as Binder

Parameter	**Batches**					
	T1	T2	T3	T4	T5	T6
Weight variation (mg ± SD)	165.55 ± 1.32	166.31 ± 3.1	164.93 ± 2.47	165.01 ± 1.20	161.85 ± 1.73	167.9 ± 2.69
Crushing Strength (KN ± SD)	8.05 ± 0.88	5.90 ± 0.46	6.79 ± 0.91	6.11 ± 0.87	6.18 ± 0.79	6.89 ± 0.57
Friability (%)	0.00	0.03	0.26	0.13	0.06	0.06
Content of active ingredient (%)	98.79	96.93	102.01	99.10	98.92	100.21
$T_{80\%}$ (min)	15	15	15	15	15	15
Diameter (mm ± SD)	7.040 ± 0.006	7.040 ± 0.006	7.040 ± 0.005	7.040 ± 0.005	7.040 ± 0.006	7.040 ± 0.006

Key: T=Tablet; T1=MCC PH 102 10%, T2=CG 5%, T3=CG 10%, T4=CG 25%, T5=MCC PH 102 10%+CG 5%, T6=MCC PH 102 10%+CG 25%

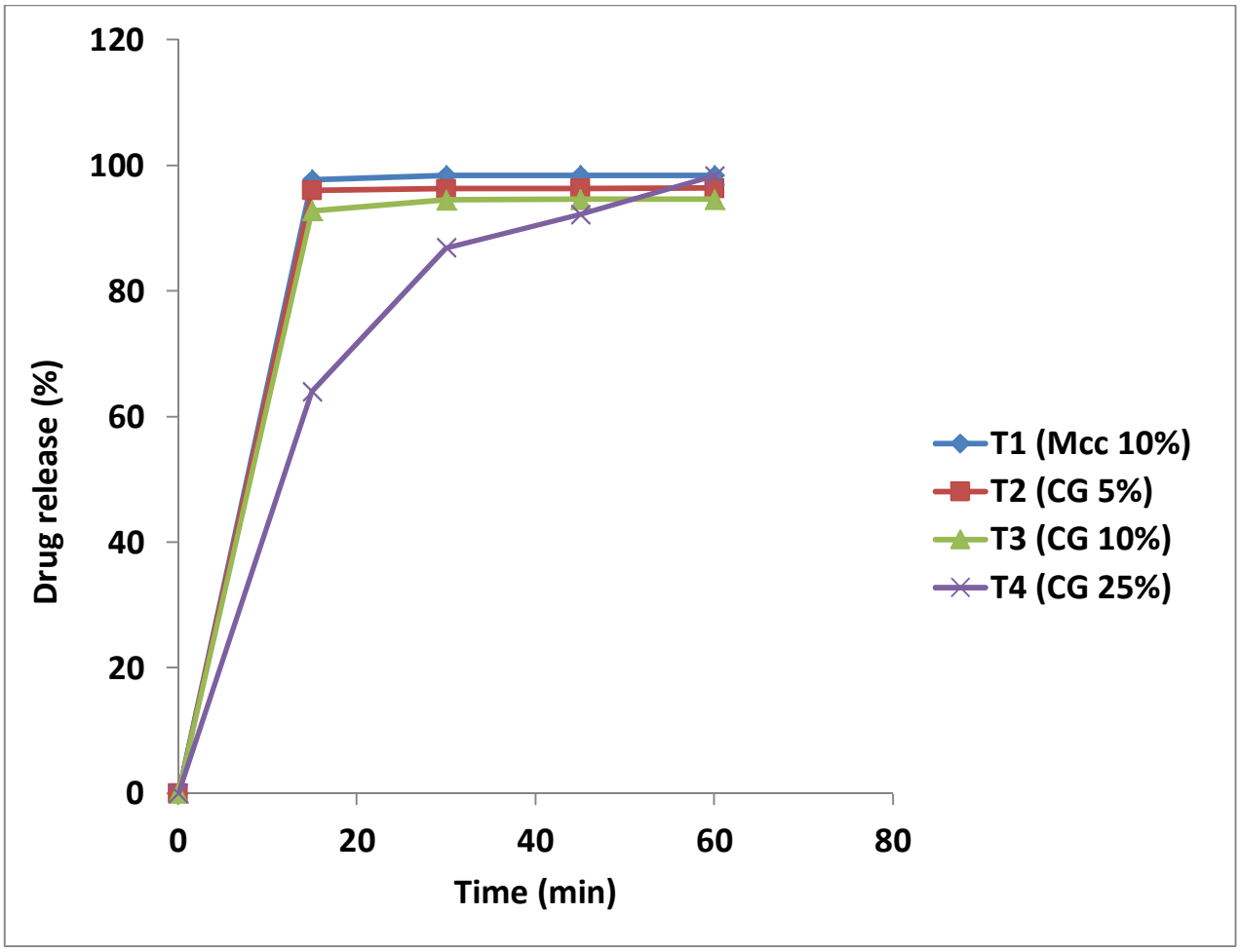


Figure 4.28: Drug Release Profile of Ven. HCl Tablets Containing MCC PH 102 or CG as Binder

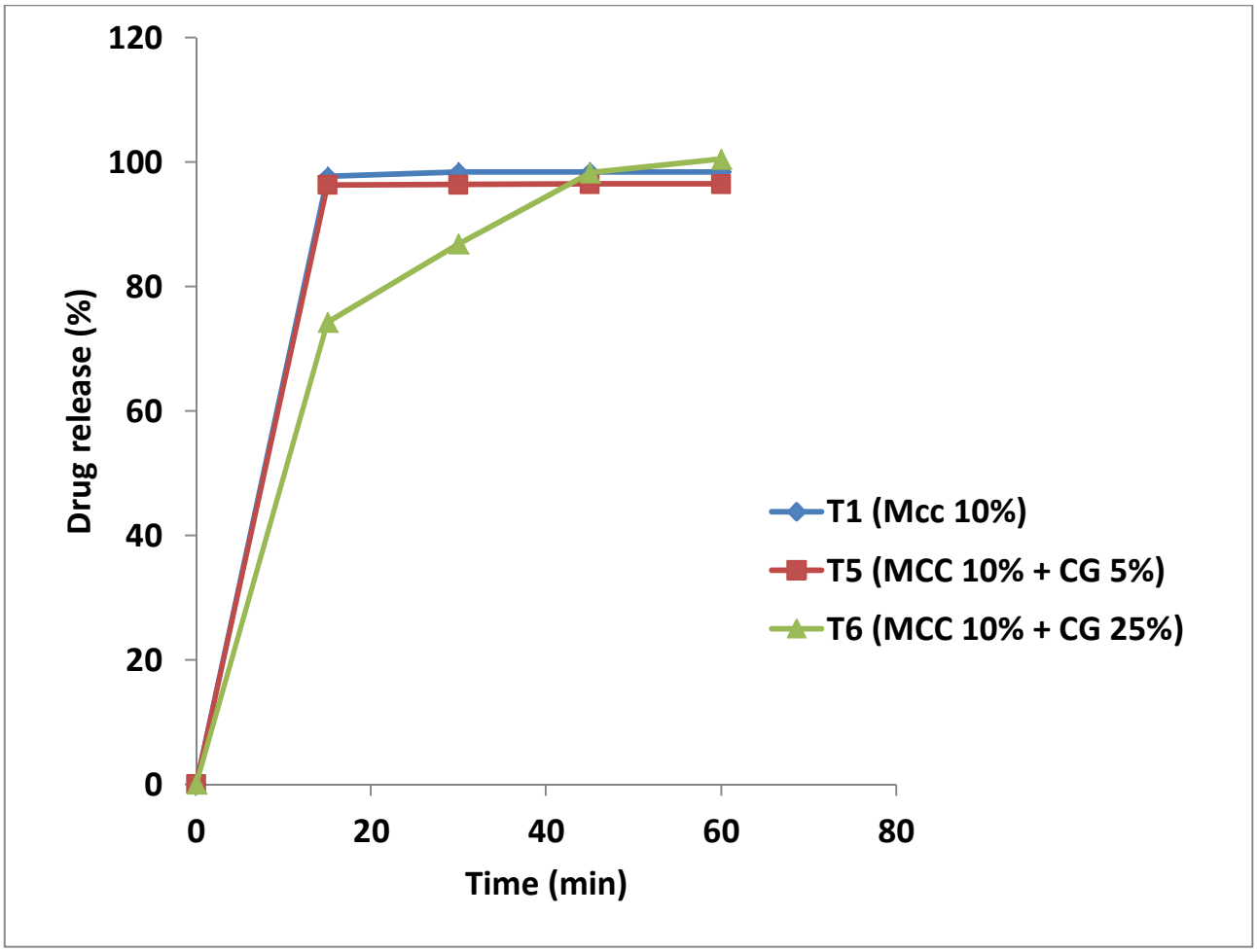


Figure 4.29: Drug Release Profile of Ven. HCl Tablets Containing MCC PH 102 in Admixture with CG as Binder

4.9.3 Formulation Properties of Ven. HCl Tablets Containing HPMC or CrosCCG as Binders

4.9.3.1 Granule Properties

The properties of granules formulated using HPMC LV at 5 %w/w, HPMC ER at 5 %w/w, and HPMC ER at 7.5 %w/w or CrosCCG at 25 %w/w are shown in Table 4.6. Granules formed using HPMC LV (G7) possessed the poorest flow properties by failing to flow through funnel and also having the highest compressibility index and Hausner ratio values of 39.00 % and 1.63, respectively compared with the other batches. Tablet batches containing HPMC ER (G8 and G9) also possessed relatively high values of compressibility index and Hausner ratio and failed to flow through funnel. G10 owing to the high content of CrosCCG possessed good flow together with lower values of compressibility index (17.70) and Hausner ratio (1.21).

Table 4.6: Properties of Ven. HCl Granules Containing HPMC (LV), HPMC (ER) or CrosCCG as Binder

Parameter		**Batches**			
		G7	G8	G9	G10
Bulk density (g/ml)		0.495	0.532	0613	0.556
Tap Density (g/ml)		0.812	0.791	0.846	0.676
Compressibility index (%)		39.00	31.50	26.50	17.70
Hausner ratio		01.63	01.48	01.38	01.21
Flow rate (g/s)	**B/L**	NF	NF	NF	4.17
	A/L	NF	NF	NF	3.85
Angle of repose (^{o})	**B/L**	-	-	-	30
	A/L	-	-	-	31.14

Key: G7=HPMC (LV) 5%; G8=HPMC (ER K100) 5%; G9=HPMC (ER K100) 7.5%; G10=CrosCCG 25%; NF= No Flow, - = No Reading

4.9.3.2: Tablet Properties

Table 4.7 and Figure 4.30 show the properties of batches of Ven. HCl tablets formulated using HPMC LV at 5 %w/w, HPMC ER at 5 %w/w, HPMC ER at 7.5 %w/w or CrosCCG at 25 %w/w as binders. The tablets compressed using CrosCCG (T10) as binder showed higher tablet weight values of 168.7 mg as against 164.9 mg for batch containing HPMC LV as binder. The low standard deviation values in all the groups showed that there was uniformity in the weights of the tablets. HPMC ER at 7.5 %w/w produced the hardest tablets amongst

the batches with a crushing strength of 5.6 KN. CrosCCG produced the softest and most friable tablets among all the batches with the tablets breaking at 4.0 KN and a friability of 0.58. HPMC LV and CrosCCG were found to release 80 % of their drug content within 15 min of starting the in vitro dissolution test (see Appendix H). HPMC ER at 5 %w/w had a $T_{80\%}$ of 30 min while HPMC ER at 7.5 %w/w displayed $T_{80\%}$ of 105 min. The tablets were of uniform diameter.

Table 4.7: Properties of Ven. HCl Tablets Containing HPMC (LV), HPMC (ER) or CrosCCG as Binder

Parameter	**Batches**			
	T7	T8	T9	T10
Weight variation (mg ± SD)	164.87 ± 2.90	167.20 ± 1.36	166.23 ± 1.97	168.71 ± 1.13
Crushing Strength (KN ± SD)	4.76 ± 1.10	5.48 ± 0.95	5.63 ± 1.31	3.95 ± 2.71
Friability (%)	0.37	0.41	0.12	0.58
Content of active ingredient (%)	96.20	91.87	101.56	97.69
$T_{80\%}$ (min)	15	30	105	15
Diameter (mm ± SD)	7.040 ± 0.006	7.040 ± 0.005	7.040 ± 0.007	7.040 ± 0.005

Key: T7=HPMC (LV) 5%, T8=HPMC (ER K100) 5%, T9=HPMC (ER K100) 7.5%, T10=CrosCCG 25%

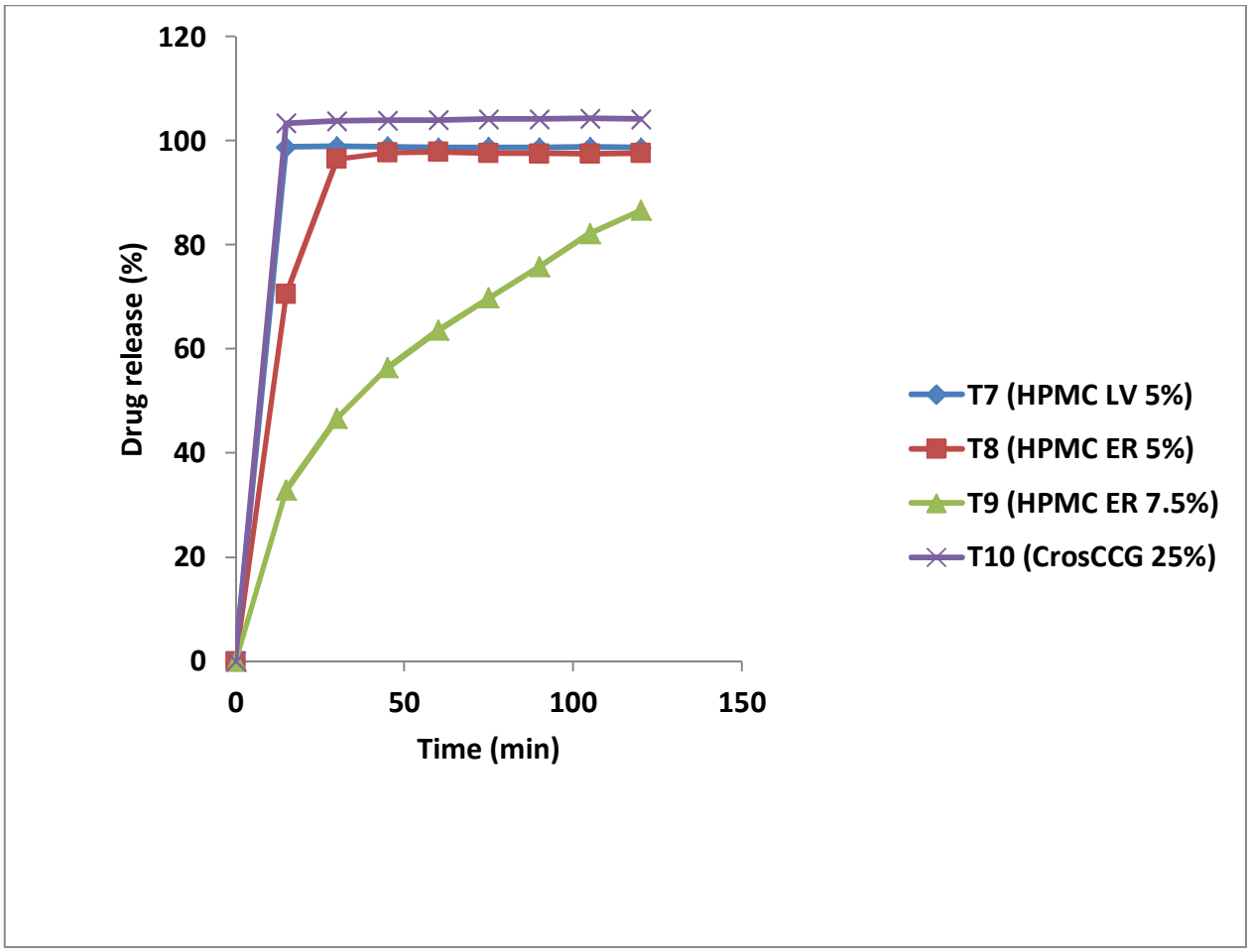


Figure 4.30: Drug Release (%) of Ven. HCl Tablets Containing HPMC or CrosCCG as Binders at Selected Concentrations

4.9.4 Formulation Properties of Ven. HCl Tablets Containing HPMC as Binder and CrosCCG Release Modifier

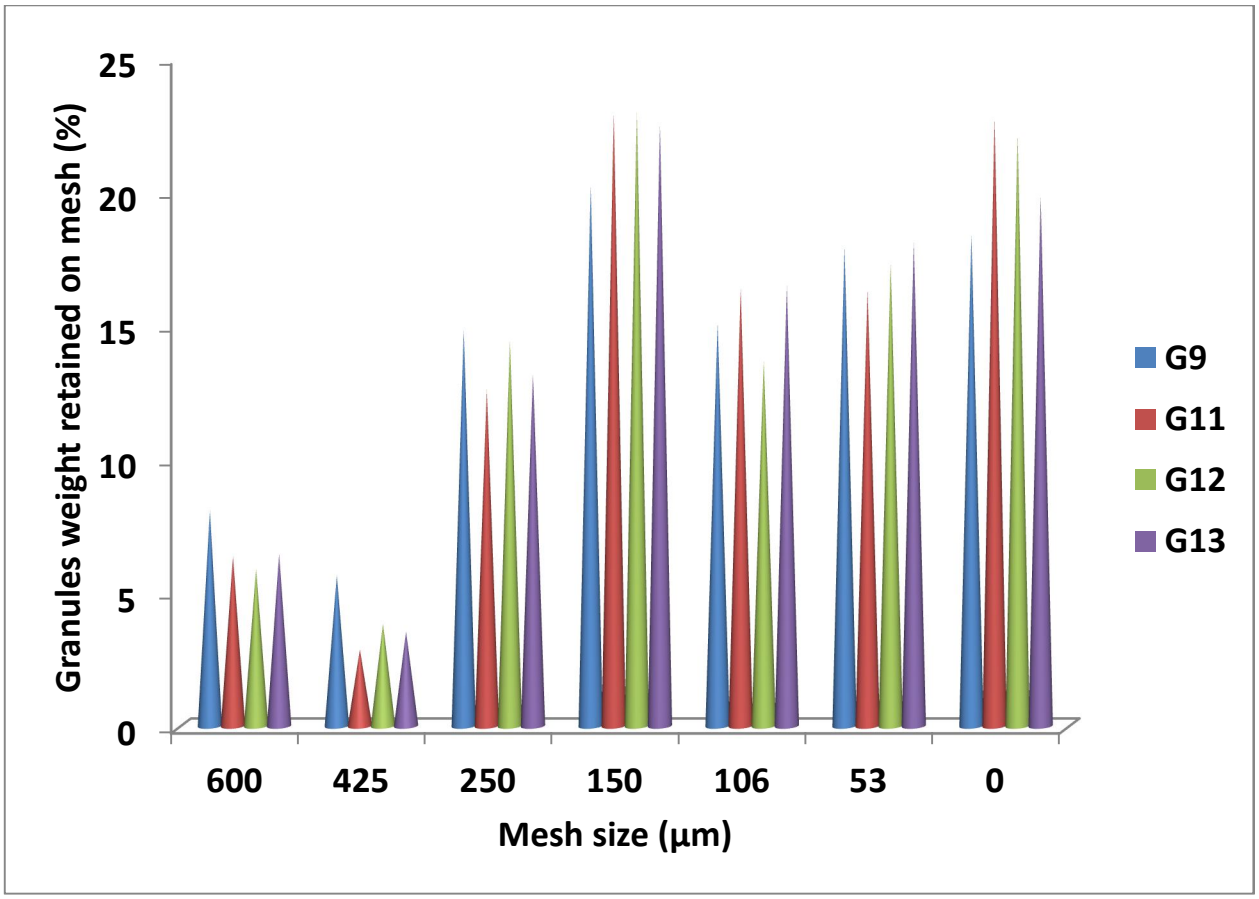
4.9.4.1 Granule Properties

The properties of granules formed using HPMC ER at 7.5 %w/w as binder and CrosCCG at varying concentrations as drug release modifier are shown in Table 4.8. The compressibility index and the Hausner ratio were found to decrease as the concentration of CrosCCG increased. At 10 %w/w concentration of CrosCCG, the granules were found to have a compressibility index and Hausner ratio of 24.20% and 1.34 respectively, while at 25 %w/w concentration the values were reduced to 20.60% and 1.27 respectively. As the concentration of CrosCCG increased, the flow rate increased while angle of repose was found to decrease. Inter-batch granule size distribution (Figure 4.31) was found to be somewhat uniform and skewed to the smaller size ranges. CrosCCG at 10 % (G9) produced relatively bigger granules with 28.45 % of the granule proportion being greater than 250 µm as against the 21.70, 24.05, and 23.00 % for G11, G12 and G13, respectively.

Table 4.8: Properties of Ven. HCl Granules Containing HPMC (ER) as Binder and CrosCCG as Release Modifier

Parameter		**Batches**		
		G11	G12	G13
Bulk density (g/ml)		0.619	0.645	0.682
Tap density (g/ml)		0.829	0.849	0.871
Compressibility index (%)		24.20	22.90	20.60
Hausner ratio		01.34	01.31	01.27
Flow rate (g/s)	**B/L**	1.87	2.12	2.42
	A/L	2.03	2.38	2.83
Angle of repose (^{o})	**B/L**	34.19	33.84	33.26
	A/L	33.51	33.11	32.40

Key: HPMC ER 7.5% + CrosCCG 10% (G11); CrosCCG 15% (G12); CrosCCG 25% (G13)



Key: HPMC ER K100 at 7.5%, alone (G9) plus CrosCCG 10% (G11); CrosCCG 15% (G12) and CrosCCG 25% (G13)

Figure 4.31: Size Distribution of Granules Made with HPMC ER K100 as Binder at 7.5% and Increasing Concentrations of CrosCCG as Release Retardant

4.9.4.2 Tablet Properties

Properties of tablets formulated using HPMC ER at 7.5 %w/w as binder and CrosCCG at varying concentrations as drug release retardants are shown in Table 4.9 and Figure 4.32. Determinations on weight variations of the tablets have shown that the intra-group weights were not so different, as shown by the values of the standard deviations obtained. Across the groups the tablet weights also seem somewhat similar. Intra and inter batch similarities in tablet crushing strength and friability were also observed as these parameters were studied on T11 – T13 tablet batches. Concentration of CrosCCG was found to affect drug release as T11 containing 10 %w/w concentration showed a $T_{80\%}$ of 90 min, while $T_{80\%}$ of 120 min was shown each for concentrations of 15 %w/w and 25 %w/w. Dissolution profile of the tablets (Figure 4.32) showed CrosCCG at 25 %w/w to have delayed the release of the drug most. At 15 min of commencing the *in vitro* drug dissolution test, the drug release from tablets containing CrosCCG at 10, 15 and 25 %w/w concentration was found to be 39.5 %, 31.56 % and 27.54 %, respectively. This trend was continued up to the 90^{th} min when release between concentrations of 15 and 25 %w/w became similar (see appendix I).

Table 4.9: Properties of Ven. HCl Tablets Containing HPMC (ER) as Binder and CrosCCG as Release Modifier

Parameter	**Batches**		
	T11	T12	T13
Weight variation (mg ± SD)	168. 10 ± 2.08	165.96 ± 1.74	167.03 ± 1.35
Crushing strength (KN ± SD)	5.12 ± 1.73	5.79 ± 2.21	5.44 ± 1.45
Friability (%)	0.43	0.39	0.57
Content of active ingredient (%)	100.81	94.23	99.08
$T_{80\%}$ (min)	90	120	120
Diameter (mm ± SD)	7.040 ± 0.005	7.040 ± 0.005	7.040 ± 0.006

Key: HPMC ER 7.5% + CrosCCG 10% (T11); CrosCCG 15% (T12); CrosCCG 25% (T13)

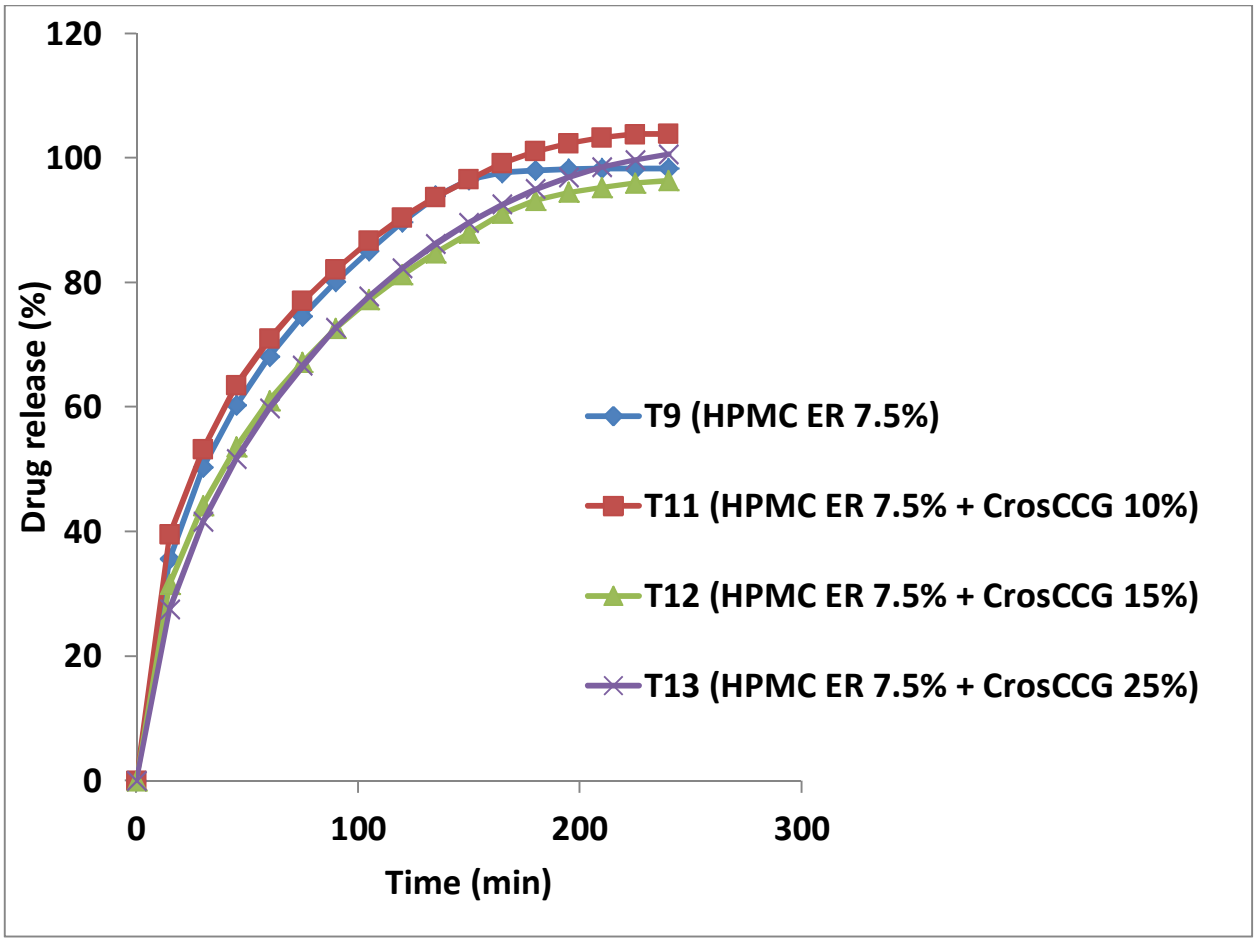


Figure 4.32: Effect of Concentration of CrosCCG on Drug Release of Ven. HCl Containing HPMC ER as Binder at 7.5 %w/w

4.9.5 Formulation Properties of Ven. HCl Tablets Containing HPMC as Binder and CrosCCG and Some Polymers as Release Enhancing Agents

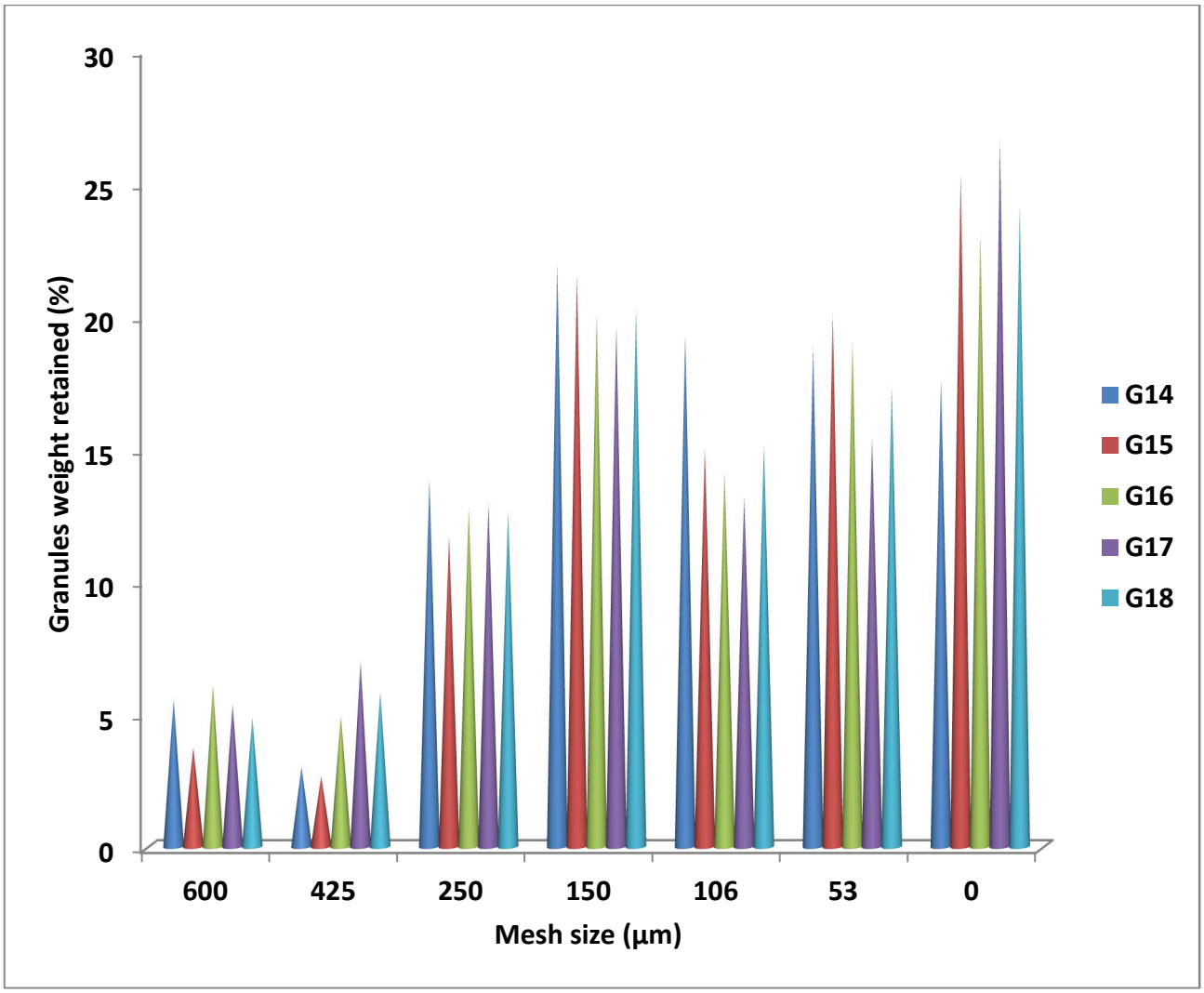
4.9.5.1 Granule Properties

Properties of granules made with 7.5 %w/w HPMC ER as binder and 3 %w/w concentrations of CrosCCG, CrosCC, CrosCE, Croscamellose and Crospovidone used as release enhancing agents are shown in Table 4.10. Granules from none of the batches were found to flow through the funnel. A near similar % compressibility and Hausner ratio were displayed by the granules from the respective batches. The compressibility index and Hausner ratio averaged 25 ± 0.707 % and 1.35 ± 0.01, respectively. Granule size distribution is shown in Figure 4.33, where the chart depicted granules with sizes which skewed to the small size fraction. The granules collected on pan accounted for an average of 20 % of each batch.

Table 4.10: Properties of Ven. HCl Granules Containing HPMC (ER) as Binder at 7.5 %w/w and Some Polymers as Disintegrants at 3%w/w

Parameter		**Batches**				
		G14	G15	G16	G17	G18
Bulk density (g/ml)		0.523	0.627	0.623	0.613	0.613
Tap density (g/ml)		0.708	0.843	0.843	0.841	0.838
Compressibility index (%)		25.00	24.40	25.00	26.00	25.70
Hausner ratio		1.35	1.34	1.35	1.37	1.36
Flow rate (g/s)	**B/L**	NF	NF	NF	NF	NF
	A/L	NF	NF	NF	NF	NF
Angle of repose (^{o})	**B/L**	-	-	-	-	-
	A/L	-	-	-	-	-

Key: HPMC ER 7.5% + CrosCCG 3% (G14); CrosCC 3% (G15); Croscamellose 3% (G16); Crospovidone 3% (G17); CrosCE 3% (G18)



Key: HPMC ER K100 plus CrosCCG 3% (G14); CrosCC 3% (G15); Croscamellose 3% (G16) Crospovidone 3% (G17) and CrosCE (G18)

Figure 4.33: Size Distribution of Granules Containing HPMC ER K100 as Binder at 7.5%w/w and Some Polymers as Release Facilitating Agents at 3 %w/w

4.9.5.2 Tablet Properties

Table 4.11 shows the properties of tablets formulated with HPMC ER as binder at 7.5 % concentration as binder and the CG cross-linked polymers: CrosCE, CrosCC and CrosCCG as well as two known superdisintegrants, Croscamellose and Crospovidone at 3 % concentrations as release enhancing agents. The tablets were found to have similar values in all the parameters tested except $T_{80\%}$. Batches T14 containing CrosCCG and T15 containing Croscamellose had similar $T_{80\%}$ values of 75 %, a figure relatively lower than 90 % for the remaining polymers tested. Figure 4.34 shows the dissolution profile of the tablets formulated with the various release enhancing agents. After 15 min and 30 min of the *in vitro* dissolution testing, the % drug release from tablets from batches T14 to T18 is shown in Figure 4.35. At 15 min, Crospovidone caused greatest drug release amounting to 49.96 % whereas CrosCE caused the least drug release with a value of 38.65 %. After 30 min of dissolution time, CrosCCG had the highest enhancement of drug release with a value of 64.37 %, while Crospovidone caused the least drug release with a value of 52.08 %. This trend continued until the 75th minute, when drug release caused by CrosCCG almost equaled that of Croscamellose. By the 90th minute however, Croscamellose achieved higher drug release with percentages of 87.07 for Croscamellose and 86.96 for CrosCCG (Figure 4.36 and Appendix J). Figure 4.36 shows the curve of % drug release against time for CrosCCG and Croscamellose when used as drug release enhancing agents at 3 %w/w concentrations. CrosCCG achieved greater drug release within the first 80 min after which Croscamellose caused higher drug to be released. Figure 4.37 shows the curve of % swelling ratio against time (min). CrosCCG swelled at a greater rate within the first four minutes after which the rate of swelling became slower. Croscamellose showed a slower rate of swelling

compared to CrosCCG within the first one hour but the rate increased thereafter thus achieving higher swelling than CrosCCG after about 80 min.

Table 4.11: Properties of Ven. HCl Tablets Containing HPMC (ER) as Binder and Some Polymers as Drug Release Facilitating Agents at 3 %w/w

Parameter	**Batches**				
	T14	T15	T16	T17	T18
Weight variation (mg ± SD)	166.67 ± 1.12	169.43 ± 3.10	165.91 ± 2.40	167.21 ± 0.75	166.45 ± 2.1
Crushing strength (KN ± SD)	5.63 ± 0.55	5.88 ± 0.52	5.50 ± 0.52	5.47 ± 0.79	5.38 ± 0.96
Friability (%)	0.19	0.20	0.03	0.17	0.13
Content of active ingredient (%)	98.39	98.08	99.21	96.87	103.54
$T_{80\%}$ (min)	75	90	75	90	90
Diameter (mm ± SD)	7.040 ± 0.006	7.040 ± 0.006	7.040 ± 0.006	7.040 ± 0.004	7.040 ± 0.006

Key: HPMC + CrosCCG 3% (T14); CrosCC 3% (T15); Croscamellose 3% (T16); Crospovidone 3%, (T17); CrosCE 3% (T18)

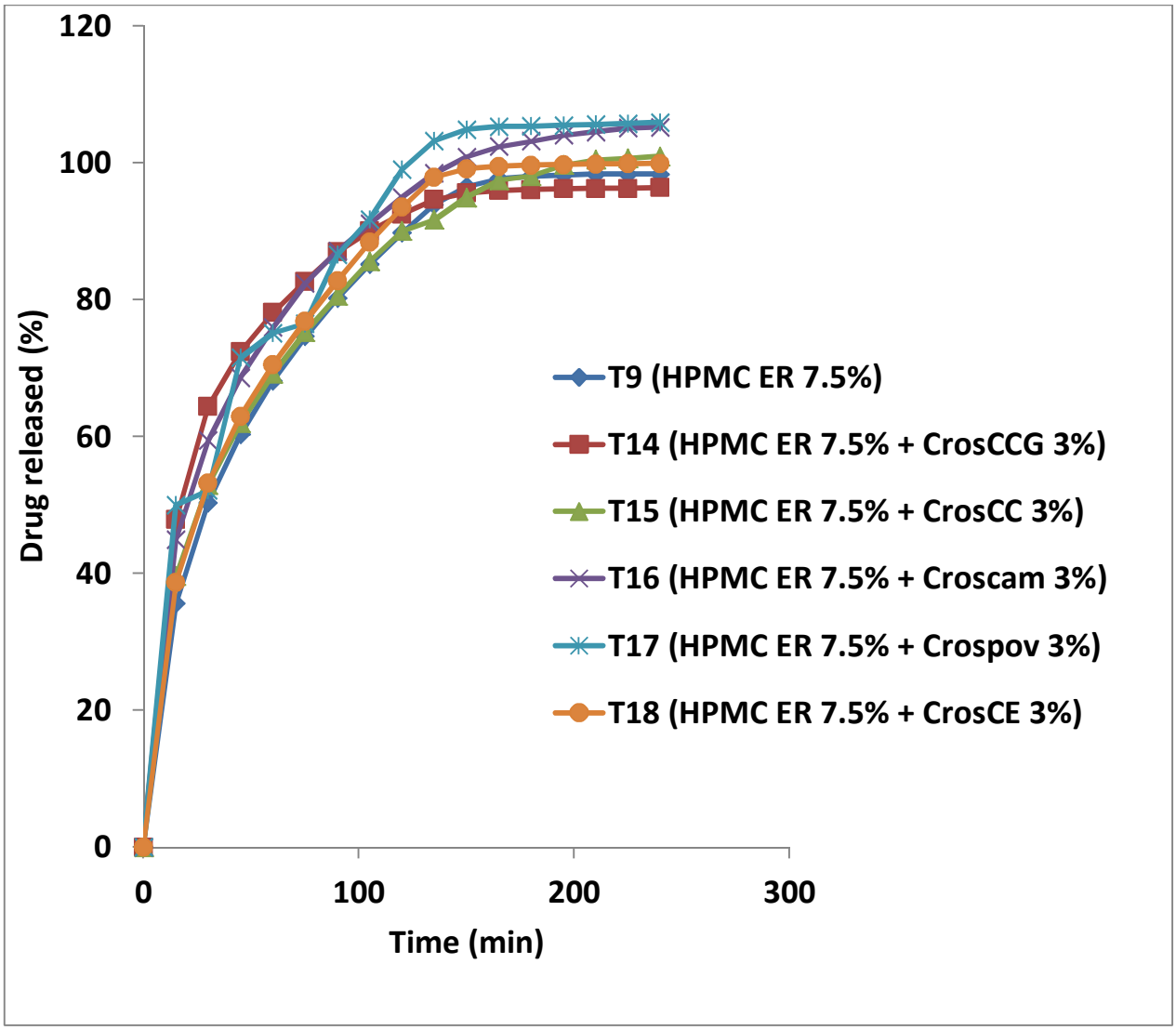
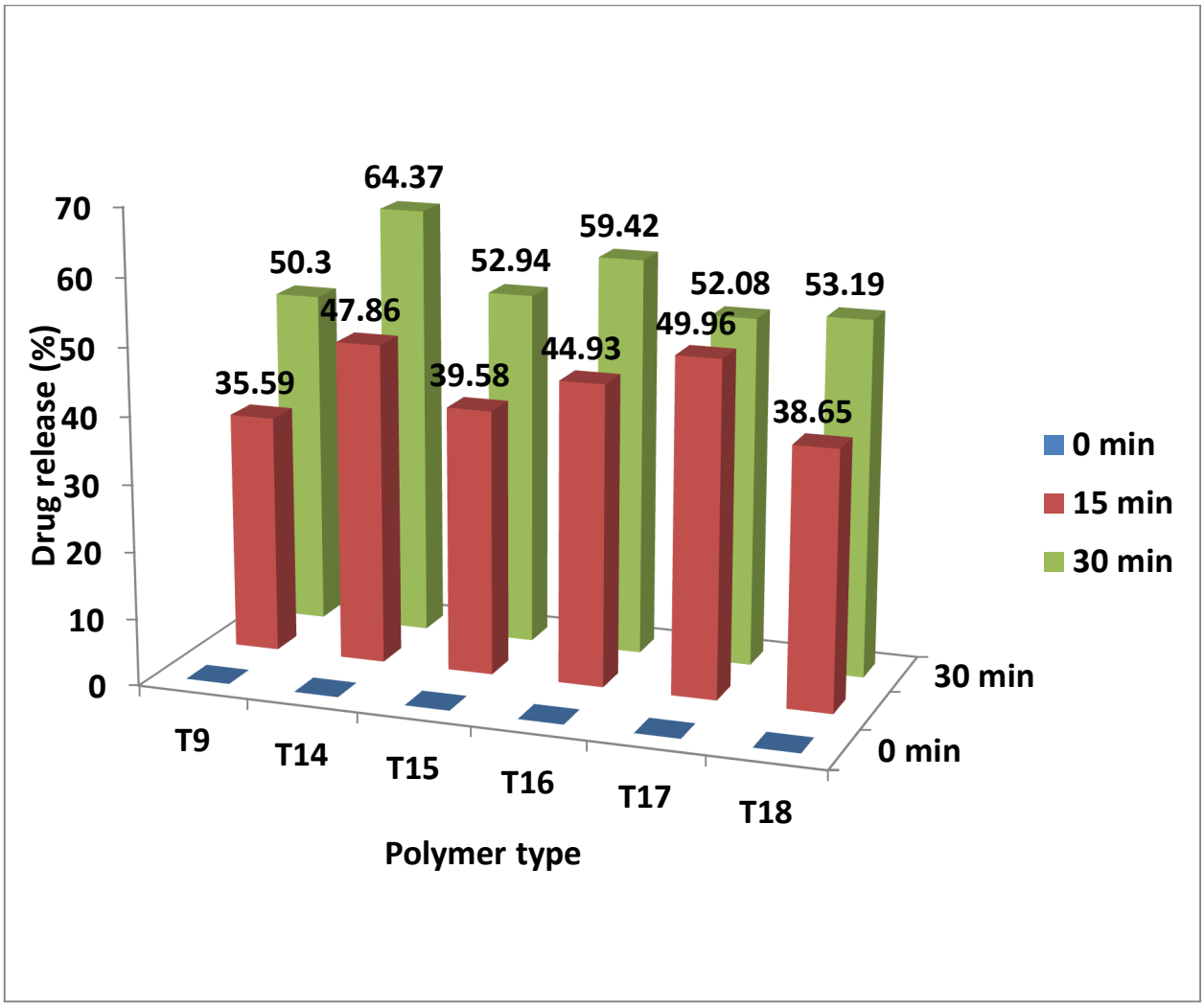


Figure 4.34: Effect of Various Polymers on Facilitating Drug Release from Ven. HCl Tablets Containing HPMC R 7.5% as Binder



Key: T9=HPMC ER 7.5%, HPMC ER 7.5% +CrosCCG 3% (T14), CrosCC 3% (T15), Croscamellose 3% (T16), Crospovidone 3% (T17) and CrosCE 3% (T18)

Figure 4.35: Effect of Various Polymers on Enhancing Drug Release of Ven. HCl from Tablets Containing HPMC ER 7.5% as Binder

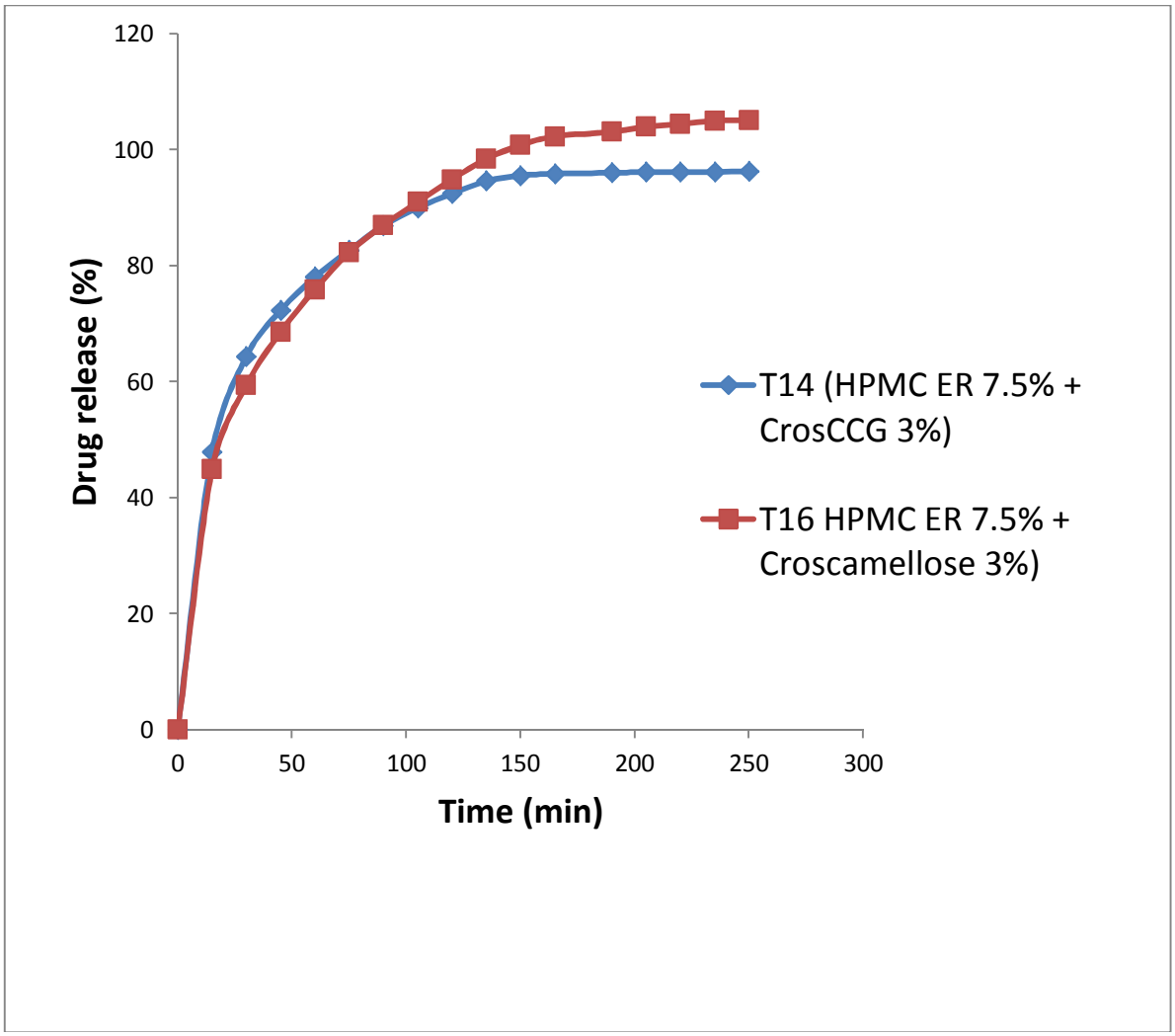


Figure 4.36: Effect of Time on Drug Release of Ven. HCl using CrosCCG or Croscamellose as Drug-Release Facilitators at 3 %w/w Concentration

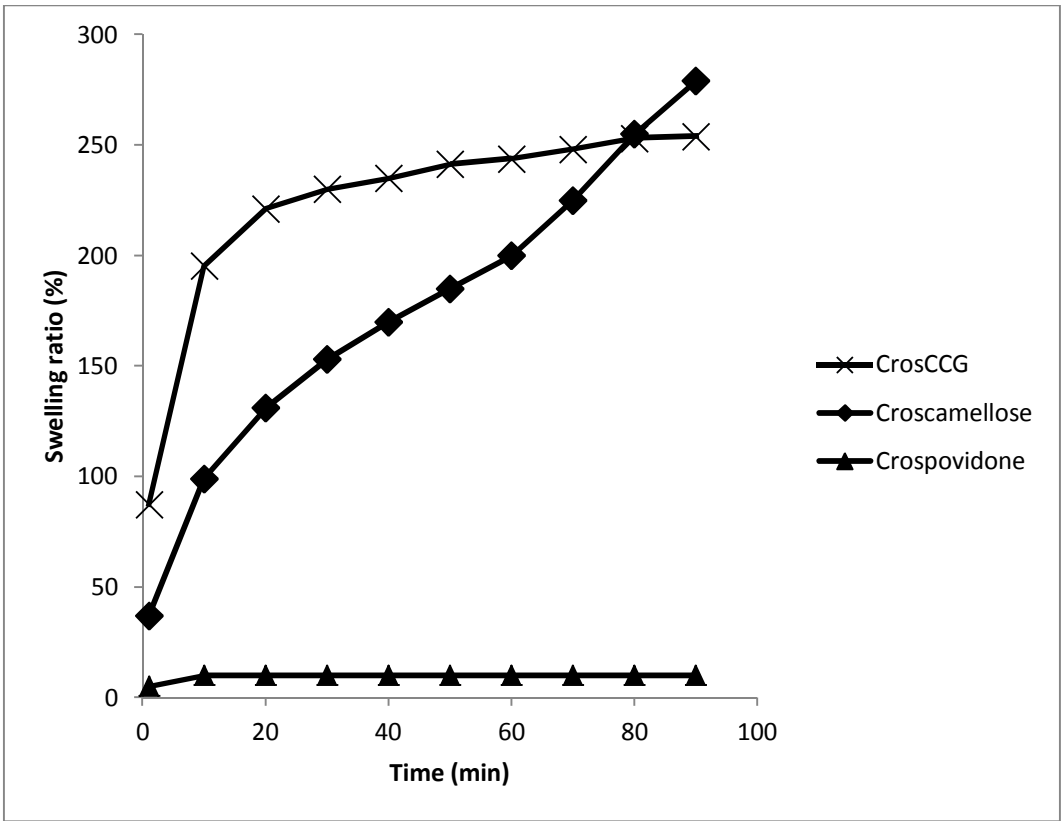


Figure 4.37: Effect of Time on the Swelling Ratio of CrosCCG and Croscamellose after 1.30 h Exposure

4.9.6 Drug Release Kinetics and Mechanism

Release of Ven. HCl from tablets formulated using HPMC ER at 7.5 %w/w as binder was found to follow the Higuchi model of drug release. The dissolution data fitted into the various release models was found to fit the Higuchi model with a regression coefficient (R^2)

of 0.996 (Table 4.12 and Appendix K). The value of n was found to be 0.45 indicating that the drug release was via Fickian diffusion (see Appendix L). Introduction of CrosCCG at 25 %w/w as drug release retardant was found to change the release mechanism from Fickian to anomalous (non-Fickian) diffusion with the value of n being 0.53 (see Appendix N).

Table 4.12: Mechanism and Kinetics of Drug-Release from Ven. HCl made with HPMC ER 7.5% as Binder and CrosCCG at 25 %w/w as Release Retardant

Mathematical model	**HPMC ER K100**		**HPMC 7.5% + CrosCCG 25%**	
	Regression coefficient (R^2)	n	Regression coefficient (R^2)	n
Zero Order	0.967		0.965	
First Order	0.908		0.882	
Hixson-Crowell	0.933		0.917	
Higuchi	0.996		0.997	
Korsmeyer-Peppas	0.997	0.45	0.997	0.53

4.10 Sub-Acute Toxicity Studies

Table 4.13 and Appendix O show the results of hematological studies carried out on Wistar rats fed with CrosCCG at the selected concentrations. Comparisons between the control group fed with feed not containing CrosCCG and test groups fed with increasing

concentrations of the material showed no significant differences (P>0.05). The biochemical test results (Table 4.14 and Appendix P) also showed no differences (P>0.05) between the control group and the test groups when tested for levels of the biochemical parameters.

Table 4.13: Hematological Parameters in Wistar Rats Fed with CrosCCG Over a 28 Day Period

Ex	de	BLOOD PARAMETER MEAN VALUE (SD)

	PCV (%)	Hemoglobin (g/dL)	Total protein (g/dL)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Band (%)	TWBC (x10^9/L)
AA1 (Cgrp)	44.0 (2.5)	14.6 (0.8)	6.6 (0.5)	15.8 (5.3)	81.0 (5.3)	1.8 (1.5)	-	-	1.3 (1.2)	11.2 (2.5)
AA2	44.2 (2.9)	14.6 (1.1)	6.7 (0.6)	16.2 (5.0)	79.5 (6.8)	1.7 (1.9)	0.3 (0.5)	1.0 (2.5)	0.8 (1.2)	8.1 (3.8)
AA3	44.5 (1.9)	14.6 (0.8)	6.6 (0.7)	16.0 (6.6)	79.5 (6.6)	2.0 (1.8)	1.7 (3.2)	-	0.5 (0.8)	9.8 (2.0)
AA4	46.0 (2.9)	15.3 (1.0)	6.7 (0.7)	20.8 (9.5)	74.7 (7.8)	2.7 (2.8)	0.3 (0.8)	0.3 (0.8)	1.2 (1.2)	8.2 (2.7)

Key: Cgrp = Control group; PCV = Packed cell volume; TWBC = Total white blood cells; Values in parenthesis represent SD

Table 4.14: Biochemical Parameters in Wistar Rats Fed with CrosCCG Over a 28 Day Period

Expt. Group	ALP	AST/SGOT	ALT/SGPT	Platelets	Urea
AA1 (Cgrp)	57.0 (23.7)	14.5 (10.1)	12.3 (16.1)	436.8 (120.3)	6.4 (2.9)
AA2	44.3 (21.7)	8.5 (6.7)	7.5 (2.9)	341.7 (151.5)	6.4 (0.7)
AA3	44.5 (17.4)	8.0 (1.5)	5.7 (2.0)	358.3 (152.1)	6.3 (1.9)
AA4	36.5 (14.3)	15.3 (6.9)	4.5 (2.3)	567.5 (171.0)	6.1 (2.6)

Key: ALP= Alkaline phosphatase; AST= Aspertate transaminase;
ALT= Alanine transaminase; Values in parenthesis represent SD

CHAPTER FIVE

DISCUSSION

5.1 Production Yield (%)

5.1.1 Extraction Yield of CG

A yield of 78.0 %, an average of a yield that ranged between 75 – 81% was obtained when batches of CG were extracted using acetone as solvent for extraction. This figure was much higher than the 55% yield reported for a separate extraction of CG using acetone (Kumar *et al.,* 2009) but similar to a 78.5% yield reported for extraction using ethanol (Ofori-Kwakwe *et al.,* 2010). In general, quality of the crude gum, purity of the extraction solvent and thoroughness of the extraction procedure may be responsible for variations in extraction yields of gums.

5.1.2 Yield of CrosCE (%)

EPC has been used in cross-linking various polymeric materials for varying application (Silva *et al.*, 2006; Xiao *et al.*, 2011). A relatively lower yield of 18.81 % was obtained when CG was cross-linked using EPC as compared to a yield of 33.5 % reported by Silva *et al.* (2006). The lower yield may possibly be due to the modification introduced in this study as per the method of purification.

5.1.2.1 Why EPC was dropped and the PCAs Selected for Cross-linking CG in this Study.

A variety of factors are considered in choosing a cross-linking agent needed for producing polymeric materials for use in foods and medicines. S*afety for use in pharmaceuticals and foods, handling concerns and environmental friendliness preclude the involvement of potential toxins/carcinogens during processing or manufacture.* EPC with CAS No. 106-89-8 was first listed in the fourth annual report on carcinogens. The International Agency for Research on Cancer (IARC) reported that there is sufficient evidence for the carcinogenicity of EPC in experimental animals, though same report says there is inadequate evidence for it to be considered a human carcinogen. While it was worthwhile, at least for comparison sake, to explore the potentials of CG cross-linked with EPC in formulation of dosage forms, concerns for safety of use and handling (IARC, 1976, 1982 and 1987; *Martel et al., 2005, Crini, 2005; Crini and Peindy, 2006)* limited its use in this study to some selected tests.

As EPC was considered somewhat unsafe for production of polymers for pharmaceutical processing based on reports of its being a potential carcinogen, safer alternative cross-linking agents were sought. The PCAs had been reported to be used in cross-linking cyclodextrins (Martel *et al.,* 2005; Ducoroy *et al.* 2007a; Ducoroy *et al.,* 2007b; Zhao *et al.*, 2009a; Zhao *et al.*, 2009b). The low cost and effectiveness (Welch, 1992), safety and environmental friendliness (Xu, 2001) made the PCAs ready alternatives in cross-linking CG. Results from preliminary studies supported by reports from the literature (Martel *et al.,* 2005; Ducoroy *et al.* 2007a; Ducoroy *et al.,* 2007b; Zhao *et al.*, 2009a; Zhao *et al.*, 2009b) suggested the choice of the two temperatures, 140 ^{o}C and 170 ^{o}C, for use in setting the reaction conditions of cross-linking CG with the PCAs in the present study.

5.1.3 Yield of Cross-linking of CG with PCAs (%)

The dicarboxylic acids, ADA and SCA showed dismal performances in cross-linking CG when used as cross-linking agents. An approximate yield of not more than 33.5 % was obtained when CG was cross-linked with either of the acids at a gum:acid weight ratio of 2:1 (Figure 4.1), a figure that was less than that obtained for PCAs with more than two carboxyl groups. At higher concentrations of the acid, formation of monolithic crystals (USP, 2011) was found to reduce the effective concentration of the acid for the cross-linking reaction. An unsuccessful use of some dicarboxylic acids in polycondensation reactions of cyclodextrins was also reported by Martel *et al.* (2005). Nonetheless, some uses of these agents in cross-linking processes have been reported. Adipic acid has been used in derivatisation of carboxymethylated polysaccharides (Bystricky *et al.*, 1999). Chen and Wang (2006) reported using succinic acid to cross-link cotton cellulose while Karimi *et al.* (2010) used it as cross-linking agent to attach titanium dioxide (TiO_2) to cotton. The dicarboxylic acids, based on poor yield, were thus ruled out as candidate cross-linking agents for use in this study.

It was observed that the more the number of carboxyl groups in the PCA the better the cross-link yield (Martel *et al.,* 2005) as shown in Figure 4.1. There was also increase in cross-link yield with increase in cross-linker concentration was observed. CG:CTR at 1:2 weight ratio produced the highest yield though not with a marked difference as was seen with CG:BTAC at the same ratio. CTR was selected over BTAC for use in formulation aspects of the study based on availability, while the 1:1 weight ratio of gum:acid was chosen based on convenience.

Cross-linking condition of 140 ^{o}C for 30 min was noticed to favour formation of CG:CTR with a yield of 64.5 % as against 54.5 % when the condition was 170 ^{o}C for 30 min. Cross-linking CG with CTR at 140 ^{o}C for 30 min was thus selected.

5.1.4 Yield of Cross-linking of CG with CTR and/or GLY (%)

Copolymerization processes have been used to synthesise hydrophilic matrices as was demonstrated by Guilherme *et al.* (2005) when they prepared novel super absorbent by copolymerization process using CG, glycidylmethacrylate and acrylamide. Peppas *et al.* (2000) in a review on hydrogels in pharmaceutical formulations reported that copolymerization has generally been utilized to enhance the mechanical properties of hydrogels. Cross-linking via the incorporation of safe and environmentally friendly dual cross-linking agents CTR and GLY, was considered to improve the cross-linking of CG. Gonzalez *et al.*, (1994) have used GLY to cross-link Carbopol in making hydrogels for buccal administration via ester formation between Carbopol carboxyl groups and glycerol hydroxyl groups. Hennink and van Nostrum (2002) reported that complexes formed between poly(acrylic acid) and poly(ethylene glycol) are held together by hydrogen bonding between the oxygen of the poly(ethylene glycol) and the carboxylic group of poly((meth)acrylic acid).

GLY alone as cross-linker containing –OH was not found to cross-link CG, similarly containing –OH functional group (Figures 4.2 and 4.3). The likely interaction to have occurred between these two compounds, and which may not have been strong enough to lead to cross-linking, was hydrogen bonding. GLY, however, in admixture with CTR influenced the net cross-linking process. Inclusion of GLY in the cross-linking mixture

offers two approaches to the overall CG cross-linking process. One is the ester formation with the unreacted (Ducoroy *et al.*, 2007a) carboxyl group of CG:CTR polymer and two, is the H- bond formation (Hennink and van Nostrum, 2002) with the carboxyl group or –OH of sugar from the gum. These claims could, however, not be verified in this study as the FT-IR spectral data could not be used to discern any increase in the ester or hydroxyl absorption peaks. What was obvious in this study was that inclusion of GLY imparted positively on the net cross-linking yield (Figure 4.3) and swelling (Figure 4.24) of the cross-linked CG polymer.

Temperature was found to influence the cross-linking of CG with the combination of CTR and GLY as cross-linker. Martel *et al.* (2005) and Ducoroy *et al.* (2007) reported on the influence of temperature on cross-linking β-CD with PCA, where water soluble or insoluble polymer fractions were obtained by varying the temperature. In this study, reaction temperature of 170 ^{o}C, a temperature beyond the flash point of GLY, was found to favor the cross-linking reaction when GLY was included in the reaction mixture (Figure 4.3). A significant ($P<0.0001$) increase in yield was noticed as the reaction temperature was changed from 140 ^{o}C to 170 ^{o}C in cross-linking CG with a combination of CTR and GLY. At a reaction temperature of 140 ^{o}C (Figure 4.2), a yield of not more than 30 % was obtained but when the temperature was set at 170 ^{o}C (Figure 4.3) the yield increased to about 70 %.

5.2 Physical Appearances of the Cross-linked Polymers

The cross-linking processes yielded polymers which were insoluble in water but swelled by absorbing some quantities of water. After the cross-linking reaction, the materials remained

clung to the culture dishes (Plate 4.2) such that would have been difficult to separate leading to likely product loss if separated in their dry state. The ability of the polymers to absorb water and swell facilitated their separation from the dishes. Upon addition of water, the material swelled giving a spongy appearance that was easily separated, washed and dried as shown in Plate 4.3. Heat treatment of CG alone at 140 ^{o}C or 170 ^{o}C for 30 min produced a white soft solid mass (Plate 4.4) that dispersed upon soaking in water. Absence of the formation of an insoluble polymer from this sample lends credence to the fact that the obtained polymers as in plates 4.1 and 4.2 were a result of cross-linking between CG and EPC, and CG and CTR in the presence of heat, respectively. While heat was necessary in the cross-linking processes, a cross-linking agent had to be present as heat alone was not found to cross-link CG.

5.3 Residual Solvent Analysis by Gas Chromatography on CG

Solvents are materials commonly used during the development of pharmaceutical raw materials or product manufacturing. Residual levels of these solvents often become unavoidable components of the drug product. But, as they are hardly of any benefit after the process and for safety reasons in some, the developed material needs to be, where possible, rendered free of the solvent. Washing (Lima *et al.,* 2002) and other maneuvers employed to rid the material of these solvents does not necessarily completely remove the solvent from the material. Quantitative evaluation of the residual levels of these solvents is often necessary as the ingestible amount permitted is a function of the toxicity of the solvent.

European Medicines Agency (2009) guideline grouped acetone and ethanol as belonging to the class of solvents with low toxic potential i.e. class 3 solvents. Available data indicate

that they are less toxic in acute or short-term studies and negative in genotoxicity studies. It is considered, according to the document, that residual amounts of these solvents up to 50 mg per day or concentration of 5000 ppm would be acceptable without justification; with higher amounts being acceptable provided they are realistic in manufacturing capability and good manufacturing practice (Niazi, 2009). Acetone and ethanol are commonly employed as solvents for the extraction of gums for pharmaceutical processing. Pharmaceutical application of gums containing these residual solvents as binders at low concentrations may not commonly pose threats of toxicity, as quantities used in doses, as in the present formulations, will not contain residual solvents in amounts over the acceptable 50 mg per day level.

Both acetone and ethanol were found to be present as residual solvents in concentrations above the permissible level of 5000 ppm (Figure 4.5) when tested using GC. Washing the precipitated gum with distilled water (Lima *et al.,* 2002) did not seem to reduce the residual solvent quantity to a value lower than the maximum allowed in the CG, but it inadvertently did dissolve some of the gum thereby causing a reduction in the overall gum yield.

5.4 Thermal Analysis by DSC

Figure 4.6 shows the DSC thermogram of crude CG powder showing an endothermic desolvation temperature peak of 107.4 ^{o}C and a degradation temperature of 311.6 ^{o}C while Figure 4.7 is the overlay of the DSC thermograms of crude and processed CG (extracted using acetone). The two samples showed similarities in degradation temperature with the crude having 311.6 ^{o}C while the processed had 313.6 ^{o}C. These figures fall within the maximum degradation temperature range of 270-320 determined for other polysaccharides

(Silva *et al.,* 2006). Extraction/purification, it appears, basically removes extraneous matter and water; and does not affect the structural integrity of the gum. This may lend credence to the worthiness of the purification process carried out. The extraction process has rid the gum of impurities commonly responsible for lowering melting temperatures of materials (Gabbott, 2008).

Figure 4.8 is the overlay of the DSC thermogram of powders of CG and CrosCE. The cross-linked polymer showed a higher degradation temperature of 324.4 ^{o}C when compared with that of plain CG. Modification of the gum structure consequent upon cross-linking must have led to the changes in the degradation temperature of the polymer. Silva *et al.* (2006) also observed that the CG cross-linked gels initiated their decomposition at higher temperatures than the uncross-linked gum. The authors recorded a decomposition temperature of 361 ^{o}C for CG cross-linked with EPC, which was higher than the 324.4 ^{o}C observed in this study.

DSC was used to demonstrate the cross-linking process between CG and CTR. Figures 4.9 to 4.11 were used to demonstrate the change in structure of CG through cross-linking with CTR. Figure 4.9 is the DSC of the cross-linking agent, CTR, showing a prominent characteristic endothermic peak at 156.3 ^{o}C caused by the melting of anhydrous citric acid (Chadha *et al.*, 2012). Thermogram of a 50/50 physical mixture of the CG and CTR (Figure 4.11) reflects that the characteristic melting temperature of CTR remained with the peak slightly shifted to 158.6 ^{o}C and the peak height shortened. Such slight changes may be caused by presence of impurities, with CG possibly being identified as the impurity in this case (Gabbott, 2008). After the cross-linking exercise the peak representing the melting temperature of CTR was completely consumed with the thermogram taking the shape of that

of CG as depicted in Figures 4.10 and 4.11. The cross-linking process apparently seemed to have "enveloped" the CTR between strands of the cross-linked polymer. and so, the absence of the melting temperature peak of CTR in the cross-linked polymer is suggestive of the formation of a new material with a structure that may not be that different from the parent material, CG (Ducoroy *et al.*, 2007). The thermogram, however, has shown that the CTR has either been completely altered or "enveloped". The constitution of this new material was not immediately apparent.

Appearance or disappearance of a peak(s) may be used to denote modification in structure of compounds normally because of addition or removal of some groups. Figure 4.12 shows thermogram of CG and that of its cross-linked polymers. The curves, though differing in the respective temperatures of events, are in the overall similar. This shows that, and as the name suggested, cross-linking did not tamper with the structural framework of the CG but simply linked strands of the polymer.

5.5 Fourier Transform Infrared (FT-IR) Spectral Studies

Qualitative analyses using FT-IR entails the timed calibration of the instrument including daily calibrations. The daily calibrations were thus carried out on each day the instrument was to be used for analysis. Two spectra were obtained to fulfill the calibration, one; air background scan (Appendix A) and two; Jasco polysterene scan (Appendix B). It is only when these spectra have met the stipulated correctness that the instrument was used.

The vibrational spectrum of a molecule is considered to be a unique physical property and is characteristic of the molecule (Coates, 2000). Figure 4.13 is the FT-IR absorption spectrum of plain CG, which showed bands similar to those noticed on other spectra of CG (Silva *et*

al., 2006). The strong broad band with a peak at 3388 is H-bonded -OH stretch band due to numerous OH groups in sugars. Hydrogen bonding has a significant influence on the peak shape and intensity, generally causing peak broadening (Hsu, 1997) as it ranged between 3600-3200 cm^{-1}. Presence of strong absorption bands in the region 910 to 650 cm^{-1} is representative of the out-of-plane bending of ring C-H bonds of aromatic and heteroaromatic compounds.

Figure 4.14 is the spectrum of CTR showing the characteristic carbonyl absorption band of carboxylic acid at 1728 cm^{-1}, a slight shift from the 1725-1700 cm^{-1} range for absorptions by carbonyl groups of acids (Coates, 2000). Other frequencies associated with the carboxylic acids are the C-O stretch that gives a strong band between 1210-1320 cm^{-1}, -OH stretch band at 2500-3300 cm^{-1} appearing strong and very broad. The spectra of a 50/50 binary mixture of CTR and CG showed peaks that can be likened to peaks in the spectra of CTR or CG. Of interest here, was the observation that the peak at 1729 cm^{-1} seen in spectrum of CTR (Figure 4.14) was also noticed in the spectrum of the CG and CTR binary mixture at 1727 cm^{-1} (Figure 4.15) but not in the spectra of the cross-linked polymers, where in its place appeared a band 1735 cm^{-1} (Figure 4.16 and 4.18).

A strong band around 1735 cm^{-1} (Figures 4.16 to 4.18) was indicative of the formation of an ester bond between the –OH from the sugar of the gum and –COOH of the cross-linking agent, CTR. This assertion was corroborated by the presence of a small band around 1193 cm^{-1} in the fingerprint region of the spectra (Figure 4.18), which was absent in the CG spectrum. Hsu (1997) reported that, esters not only show their carbonyl C=O stretch at 1750 to 1735 cm^{-1}, but also exhibit their characteristic absorption at 1300 to 1000 cm^{-1} from the coupling of C-O and C-C stretches. The strong 1735 cm^{-1} and the weak 1193 cm^{-1}

absorption bands were not present in the spectrum of uncross-linked CG (Figures 4.13, 4.15), while only the small band at 1193 cm^{-1} was present in the CTR spectrum (Figure 4.14). This finding confirms the formation of an ester bond between the –OH of sugar from the gum and –COOH of the acid leading to the formation of CrosCC.

Inclusion of glycerol in the cross-link formula led to the production of a polymer, CrosCCG, characterized by greater swelling properties and water sorption/holding capacity. FT-IR of this polymer (Figure 4.18) did not show any discernible changes or differences in the spectrum as compared with that of CrosCC. Glycerol with its –OH groups is a good candidate for formation of ester linkages with the –COOH of CTR (Garcia *et al.,* 2011). Addition of glycerol therefore must have served an additive or synergistic contribution to the overall esterification process. Another possible contribution of glycerol in increasing the water reactivity of CrosCCG is hydrogen bond formation with water. Dashnau *et al.* (2006) opined that glycerol interacts favorably with water to strengthen the H-bond network of the solvent. This claim, however, could also not be substantiated using FT-IR as there was no corresponding broadening of the O-H stretching frequency. Coates (2000) suggested that hydrogen bonding is important, but the effect tends to be weaker than for the hydroxyl group, and the overall effect on the spectrum is slightly less pronounced. A band at 1040 cm^{-1} used for quantitative determination of glycerol (Gorinstein *et al.,* 1980) was found present in the spectra of both uncross-linked CG and CrosCCG, though with higher intensity in the latter.

5.6 Solid-State ^{13}C NMR Spectral Analysis of CrosCCG Polymer

A report (Abdulsamad *et al.*, 2006a) on chromatographic analysis of the acid hydrolyzed sample of CG from Nigeria revealed the gum to contain in varying proportions glucose, galactose, xylose, rhamnose and ribose sugars. ^{13}C NMR spectra of CG have been reported (de Paula *et al.*, 1996; Guilherme *et al.*, 2005; Silva *et al.,* 2006). Failure of the CrosCCG to dissolve in water compelled the NMR spectral studies of the polymer in its solid state. Figure 4.19 shows the solid state ^{13}C NMR spectrum of CrosCCG, whereas Table 4.1 shows the various resonances of the C-atoms that made the structure of the polymer. As expected, the NMR spectrum showed peaks corresponding to resonances of the carbon atoms of sugars identified by Abdulsamad *et al.* (2006), but with an interesting additional carbonyl peak at 204.0 ppm. This peak was not seen in some reported ^{13}C NMR spectra of CG not subjected to cross-linking process (de Paula *et al.*, 1996; Guilherme *et al.*, 2005; Silva *et al.,* 2006). Presence of the carbonyl frequency in Figure 4.19, therefore, further supports the claim of the cross-linking of CG with CTR through esterification.

5.7 Proposal on the CG and CTR Reaction Mechanism

The results obtained upon carrying out DSC (Figures 4.6 to 4.11), FT-IR (Figures 4.13 to 4.18) and ^{13}C NMR (Figure 4.19) analyses led to the suggestion of a possible mechanism for the synthesis of CrosCC as shown in Scheme 4.1. Identification of ester group in FT-IR and the presence of an ester carbonyl absorption peak in the ^{13}C NMR were indicative of the nature of the bond formed during the cross-linking process. Esterification involving the –COOH of the CTR/GLY and the –OH of sugar from the gum led to the cross-linking of CG to form an insoluble but swellable CrosCCG. Similar mechanism was attributed to the cross-

linking of cyclodextrins with the PCAs containing at least three carboxylic groups (Martel *et al.*, 2005; Ducoroy *et al.*, 2007a; Ducoroy *et al.*, 2007b; Zhao *et al.*, 2009).

5.8 Properties of CG, CrosCC and CrosCCG Powders

5.8.1 Organoleptic/Physicochemical Properties

Table 4.2 presents the organoleptic and some physicochemical properties of CG and the polymers derived from it: CrosCC and CrosCCG. Both CG and the cross-linked polymers were found to be odorless and tasteless but while CG was off white in color, the cross-linked polymers were brown when first obtained. The intensity of the color was dependent on the amount of the cross-linker used; the more the proportion of the cross-linker, the darker the color. Colour, odour and taste of a material when not appealing, may pose problems to the use of such material in pharmaceutical formulations for human consumption. The cross-linked polymers were found to be bleached white with hypochlorite solution. The physicochemical, physicotechnical and formulation properties of the bleached polymers were not reevaluated after the bleaching process.

Solubility of CG and its cross-linked derivatives is shown in Table 4.2. CG when mixed with water forms a mucilaginous dispersion whose thickness is dependent on concentration (Zakaria *et* al., 1997; de Paula, 1998; Lima *et al.*, 2002; Abdulsamad *et al*., 2006). The procedure adopted for solubility determinations uses increasingly rigorous mechanical technique in an attempt to dissolve the material in any given solvent, with the determination of whether a chemical has dissolved based entirely on visual observation for the presence or absence of cloudiness or precipitate (NICEATM, 2003). While CG formed dispersions only in water, the cross-linked derivatives, CrosCE, CrosCC and CrosCCG, were found to be

insoluble and indispersable in all the solvents tested. Cross-linking reduces solubility (Koo, 2010) or even renders a hitherto soluble material insoluble, especially when established by covalent bond formation (Brannon-Peppas, 1994; Peppas *et al.,* 2000; Hoare and Kohane, 2008).

Sulphated ash values are also presented in Table 4.2 where some variations in the ash values obtained can be seen. The uncross-linked gum had the lowest ash value compared to the cross-linked derivatives. Ash values can serve as parameters for the determination of the purity of gums (Gundidza *et al.*, 2011b) and other polymeric materials. Gums like other plant based materials present with varied ash values necessitating the recommendation of limits for some commercially available polymeric materials e.g. acacia ≤ 5 %; tragacanth ≤ 5 %; starch ≤ 0.5 %; propylene glycol ≤ 0.01 %; cellulose acetate phthalate ≤ 0.1 % (Handbook of Pharmaceutical Excipients, 1993). *Agathis australis* gum has been reported to have sulphated ash value of 0.26 % (Singh, 2007). CG, CrosCC and CrosCCG with sulphated ash values 1.39, 1.91 and 2.7 %, respectively, can be categorized along with acacia and tragacanth powders; and can be said to have low sulphated ash values.

Loss on drying (LOD) is a determination used to quantify the amount of volatile matter of any kind that is driven off under any specified condition (Niazi, 2009). The method of drying 2 g ground sample at 105 ^{o}C for 2 h appears to be an optimum condition ensuring most of the water in the sample is given off (Ileleji *et al*., 2010) and is sensitive for determinations in materials with high or low moisture content (Gundidza *et al.,* 2011a). The moisture content determined in this study was found to be 8.47, 7.21 and 7.39 % for CG, CrosCC and CrosCCG, respectively. CG was found to have relatively more water content than the cross-linked polymers of the gum as was equally observed by Silver *et al.* (2006). A

slightly higher moisture content of 9.8 % was obtained for a different evaluation of cashew tree gum (Gyedu-Akoto *et al*., 2008). Official recommendations have specified values for some polymeric material evaluated for LOD e.g. acacia ≤ 15.0 %; starch ≤ 15 %; HPMC ≤ 10 (Handbook of Pharmaceutical Excipients, 1993). It can thus be seen that CG and its derivatives have moisture contents within the limits specified for similar polymers.

Determination of the water holding capacity (WHC) of a hydrogel made from carbohydrate polymers for use in foods and pharmaceutical is required to provide information on the storage and handling precautions to be adopted to avoid spoilage of the product (Mao *et al.*, 2000; Huda *et al.,* 2001; Bragadottir *et al.*, 2007). WHC as determined for CrosCC and CrosCCG showed the latter to have retained more ($P<0.0001$) water in its folds than did the former. This finding agreed with the result obtained when the moisture sorption/desorption isotherm of the materials (Figure 4.22) was determined. Cross-linking could be seen to increase the water sorption and swellability of CG. The additive cross-linking has aided the entrapment of water in the macromolecular structure of the cross-linked polymer (Brannon-Peppas, 1994; Peppas *et al.*, 2000; Hoare and Kohane, 2008).

5.8.2 Water Content Determination using Karl Fisher (KF) Titrimetry

Karl Fisher (KF) titration is a method in analytical chemistry that determines trace amounts of water in a sample using volumetric or coulometric titration (Koch *et al*., 2007). It is described as being accurate, less time consuming and is amenable to testing small sample sizes (Spahn *et al.,* 2006). KF has been used in determination of moisture in powder and lyophilized saffron where it was found to give the best result when compared with infrared balance technique (Naviglio *et al.*, 2010). Figure 4.20 presents the water content of CG,

CrosCC and CrosCCG as determined by KF titration. CG showed a higher value of 8.53 % while CrosCC and CrosCCG can be seen to have 7.12 and 7.04 %, respectively. Similarities in the values recorded for LOD, a test that quantifies the amount of volatile matter of any kind on one part and KF titration that specifically measures water content on the other, for CG, CrosCC and CrosCCG suggests that the loss in weight in these polymers was basically due to loss of water. Readings could not be obtained for samples stored over water as the CG powder sorped water and failed to disperse in the solvent. CG and the cross-linked polymers which were relatively hygroscopic for pharmaceutical concerns were found to lose water at lower humidity (Figure 4.22). Fears on the gum's spoilage due to its hygroscopic nature can therefore be allayed by including a pack of desiccant in its container.

5.8.3 Flow Properties of CG, CrosCC and CrosCCG Powders

The powders of processed CG and the cross-linked polymers, CrosCC and CrosCCG were all found to be free flowing. While processing of CG was found to yield a free flowing, moderately coarse powder, cross-linking of the gum produced a coarser and better flowing powder (Table 4.3). All the powders had a particle size distribution (Figure 4.21) that appeared skewed to the left signifying presence of larger particle size fraction with over 70 % of the particles having sizes greater than 250 µm, and particles with this size are free flowing (Staniforth and Aulton, 2007). CrosCC and CrosCCG were both found to have a flow rate of 12.5 g/s, whereas CG had a flow rate of 10 g/s. In a similar trend, the cross-linked polymers had relatively smaller angle of repose compared to CG. This may be attributed to the larger particle sizes in the cross-linked polymers as mentioned earlier. Poorer flow rate and larger angle of repose in CG may also possibly be connected with the

CG powder's higher water content (Figure 4.21), as the particle size distribution of the three powder types was similar. Sandler *et al.* (2010) in a study on the effect of moisture on powder flow properties of theophylline observed that moisture content can have a great effect on powder flowability. CG was found to have interestingly smaller values of both Hausner ratio and Carr's index compared to those of the cross-linked polymers (Table 4.3) in spite of having a relatively poorer flow rate. Good flow properties are a prerequisite for the successful manufacture of both tablets and powder-filled hard gelatin capsules (Davies, 2009).

5.8.4 Moisture Sorption/Desorption Determination

To monitor the interactions of water in solids, varying relative humidities are established by the respective use of saturated solutions of appropriate salts (Greenspan, 1977). The saturated salt solutions were prepared as shown in Table 3.5 (Jowitt and Wagstaffe, 1989) and used to vary the RH as depicted by Figure 3.1. The rate and extent of moisture sorption were found to be almost equivalent to the rate and extent of moiture desorption following sample exposure to varying RHs. This is demonstrated by the shape of the isotherm or hysteresis loop (Airaksinen, 2005), which is a measure of the interraction of CG, CrosCC and CrosCCG with water; and specifically highlighting the reversibility of the process (Figure 4.22). This figure also shows a relationship between the sample nature and the amount of moisture sorped. Cross-linking (Brannon-Peppas, 1994; Peppas *et al.*, 2000; Hoare and Kohane, 2008) was found to increase the water uptake ability of CG. The cross-linked polymers were found to sorp greater amounts of water than CG with CrosCCG demonstrating greatest water uptake. The extent of water uptake in CrosCCG could have

been improved as a result of additional esterification or hydrogen bonding (Brannon-Peppas, 1994; Hoare and Kohane, 2008) facilitated by incoporation of GLY in the cross-linking process (see Appendix C).

5.8.5 Swelling Properties of CG, CrosCC, CrosCCG and CrosCE Powders

Swelling plays a very important role in defining the performance of a polymeric material with regard to solute diffusion in drug delivery. Swelling can be described as the increase in weight, volume or length (Ganji and Vasheghani-Farahani, 2009). Knowledge of the swelling characteristics of polymers is therefore of great importance in pharmaceutical and biomedical applications (Brannon-Peppas, 1994). Hydrophylic cross-linked polymers owing to their swelling ability have found use as swellable matrices, superdisintegrants and swelling devices in drug delivery (Omidian and Park, 2008). Determination of swelling properties of polymers using the cylindrical tube method allows for the defining of the two very important parameters in swelling kinetics of polymeric materials i.e. rate and extent of swelling. The gravimetric method commonly used in determining swelling behavior in polymeric materials (Silva *et al.*, 2006; Martinez-Ruvalcaba *et al.*, 2009; Chavda and Patel, 2010) may only give the extent of swelling. Ganji *et al.* (2010) observed that one of the very important features of hydrogel is the rate of swelling. The cylindrical tubes were siliconised by treating with a 1 % solution of dichlorodimethylsilane in toluene so as to ensure that they do not interfere with the test by adsorbing any part of the water introduced. The water introduced into the tube would therefore be made available to the polymeric material for absorption and eventual swelling.

CG was not found to swell (Figure 4.23) even though it absorbed some amount of water as shown by the water sorption/desorption isotherm (Figure 4.22). Cross-linking CG with CTR caused the material to imbibe more water and most importantly, it imparted a remarkable swelling property on the polymer. This may be so because the macroporous structures of the cross-linked polymer make transfer of water molecules easier between the polymer matrix and the external aqueous phase (Caykara *et al.*, 2006). CrosCC, a cross-linked derivative of CG was thus found to swell to a greater height ($P<0.05$) compared to the uncross-linked variety, when made to contact water as shown in Figure 4.3. The swelling rate was more pronounced during the first 4 min after which the curve almost flattens out, signaling less and less swelling taking place. One of the very important features of hydrogel swelling is the rate of swelling or swelling kinetics (Ganji *et al.*, 2010).

Cross-linking of CG using a combination of CTR and GLY lead to the formation of a highly cross-linked polymer, CrosCCG, characterized by a faster swelling rate (Omidian and Park, 2008) compared to the uncross-linked CG or CrosCC (CG cross-linked with CTR alone) as depicted by Figure 4.24 and Appendix D. CrosCE showed the greatest ($P<0.05$) swelling ability as can be seen in Figure 4.25.

5.9 Formulation Studies

5.9.1 Drug-Polymer Compatibility Studies using DSC

It is important that the drug does not interact with any of the excipients in a way that is likely to reduce its efficacy; therefore, excipients compatibility is important when considering drug stability (Saunders, 2008). The objective is to quickly find those excipients

that should be avoided because of an obvious chemical incompatibility with a particular API (Moreton, 2006). DSC measurement curves (Figures 4.26 and 4.27) obtained from the present study showed decrease in melting temperatures of the samples, albeit slight. Ideally if no interaction occurs, then the mixture should show equivalent transitions to that of the individual components. If it does not, some interaction is indicated (Saunders, 2008). The author, however, observed that issues arise because physical interactions occur that have nothing to do with chemical interactions, which are the basic cause of concern. Presence of impurities in a sample could cause decreases in melting temperatures or shortening of peaks in a DSC curve commensurate to the level of impurity (Saunders, 2008). CG in this case could be identified as an "impurity" and so the decrease in both the intensity and measure of the melting temperature may not be an indication of interaction. DSC may not give an all conclusive indication of compatibility or otherwise of a given binary mixture and so HPLC, MS are often further used to guide on drawing rational inferences on a drug-polymer compatibility study. The insolubility of CrosCCG, in this study, precluded the conduct of these additional tests. Experienced analysts, however, have reported that useful information has been obtained from DSC interaction studies and it should be noted that data indicating non-interaction provide significant evidence that no interaction is occurring (Saunders, 2008). Drug release data should be used to substantiate this claim.

5.9.2 Formulation Properties of Ven. HCl Tablets Containing MCC PH 102 and or CG as Binder

5.9.2.1 Granule Properties

CG was found to produce good tablets when used as binder to formulate Ven. HCl tablets using the wet granulation method. The tablet formulae (Table 3.6) were designed to compare CG used as binder at increasing concentrations and also to explore whether there is any beneficial additive or synergistic effect when used as admixtures with MCC PH 102. MCC, in general, is an excipient greatly used in direct compression tablet formulations as diluents and also as disintegrant or binder in wet granulation (Jones, 2008; Davies, 2009). MCC, therefore, is the excipient to beat in tablet formulation.

The granules, upon assay, were found to be free flowing and with good compressibility. A 5% binder concentration of CG was used as the lowest amount, although concentrations as low as 1-3% had been suggested for immediate release tablet formulations (Okoye *et al.,* 2009). Increasing the concentration of CG was not found to significantly change the granule properties. An average Hausner ratio and compressibility index of 1.29 (± 0.004) and 21.27% (± 0.6) respectively, were recorded for granules made with CG as binder at 5 %, 10 % and 25 %w/w concentrations, while the angle of repose averaged 31^{o} (± 0.5).The low standard deviations attest to the similarity of the findings. Similar results were obtained when increasing concentrations of CG were used as binder in admixtures with MCC PH 102 at 10 %w/w. Granules made using MCC PH 102 alone as binder were found to be less compressible and not as free flowing as those made with CG alone or in admixture with MCC PH 102, which were attributed to some limitations of MCC when used in wet granulation (Sherwood and Becker, 1998).

5.9.2.2 Tablet Properties

CG was found to compare equally as binder with MCC PH 102 in the formulation of immediate release tablets of Ven. HCl. Both CG and MCC PH 102 produced tablets that had good crushing strength. MCC PH 102 when used alone, however, was found to produce the hardest tablets with a crushing strength of 8.05 KN, among the batches tested. MCC is known to produce hard tablets that disintegrate quickly (Galichet, 2006). All the batches showed good drug content that fell between 95% to 105% content of active ingredient (Table 4.5). Increase in concentration of the CG as binder or its admixtures with MCC was not found to significantly ($P > 0.05$) modify the drug release as all the batches released over 80 % of the drug in 30 min (Table 4.5; Figures 4.28 and 4.29 and Appendices F and G), an optimum time for immediate release tablet formulations of highly soluble drugs (Yu *et al.,* 2001). Low viscosity of CG dispersions (Lima *et al.,* 2002; Silva *et al.,* 2006) may account for its poor performance in modifying drug release as would have been expected when higher binder concentrations were employed (Rana *et al.,* 2011).

5.9.3 Formulation Properties of Ven. HCl Tablets Containing HPMC or CrosCCG as Binders

It was pertinent to decide on a reference material that possesses tablet binder properties but devoid of significant disintegrant action to assess binder and possible sustained release properties. Most of the excipients used as binder also possess disintegrant property. HPMC is used as a tablet binder at concentrations below 10 %w/w, while at higher values it is used to sustain drug release. Its performance is also a function of its viscosity i.e. low viscosity (LV) or high viscosity used for extended release (ER). Interestingly, HPMC does not posses

disintegrant property and tablet formulae (Table 3.7) were thus designed to aid selection of HPMC type and concentration to use for this study; as well as to evaluate the binding or sustained-release properties of CrosCCG.

In contact with water, HPMC hydrates rapidly and forms a gelatinous layer around the tablet. Type of polymer, drug, polymer drug ratio, particle size of drug and polymer, and the type and amount of filler used will influence rate of drug release from tablets made with HPMC (Levina and Rajabi-Siahboomi, 2004).

5.9.3.1 Granule Properties

Table 4.6 presents a report on the properties of granules formulated using HPMC LV, HPMC ER and CrosCCG. All the granule batches containing either HPMC ER or LV possessed poor flow characteristics, with the HPMC LV showing the poorest flow property. Powders with % compressibility of 35 – 38 are said to have very poor flow, while those with 28 – 35 % compressibility are said to have poor flow (Staniforth and Aulton, 2007). HPMC LV therefore, was found to possess the poorest flow property compared to the ER. The granules were not able to flow though the funnel. The good flow property of CrosCCG (Table 4.3) and a concentration of up to 25 % enhanced the flow property of granule batch G10.

5.9.3.2 Tablet Properties

Figure 4.30 shows the Ven. HCl release profiles from tablets formulated with the two varieties of HPMC i.e. low viscosity (LV) and the higher viscosity or extended release (ER); and CrosCCG at 25 %w/w binder concentration. HPMC ER (K100) at 7.5 %w/w binder

concentration showed a release of Ven. HCl that lasted beyond 2 h, time enough to monitor comparative drug release. CrosCCG at 25 %w/w binder concentration showed similar release profile as HPMC LV and HPMC ER at 5 %w/w binder concentrations, respectively. These materials at these concentrations displayed good binding ability but failed to demonstrate any extended release property. They all released 80 % of the drug within 30 min (see Appendix H).

HPMC ER at 7.5 %w/w was selected to study the influence of increasing concentrations of CrosCCG on Ven. HCl release from the tablets. CrosCCG did not sustain the release of Ven. HCl from the tablets when used alone. A combination, however, of CrosCCG and HPMC ER significantly ($P < 0.05$) delayed the drug release.

5.9.4 Formulation Properties of Ven. HCl Tablets Containing HPMC as Binder and CrosCCG as Release Modifier

5.9.4.1 Granule Properties

Table 4.8 and Figure 4.31 show the properties of granules formed with HPMC ER as binder at 7.5 %w/w and increasing concentrations of CrosCCG as release modifier. The amount of CrosCCG in the granules was found to influence the properties of the granules. The compressibility index as well as the Hausner ratio was found to decrease as the concentration of the polymer was increased. CrosCCG has been shown to possess good flow characteristics (Table 4.3) and the effect of such potential even after granulation was seen in batches of these granules. Generally, a decrease in compressibility index and Hausner ratio and angle of repose were observed as concentration of CrosCCG increased, while the flow rate on the other hand was found to increase.

5.9.4.2 Tablet Properties

Tablets of Ven. HCl were found to release 80% of the drug within 90 min when formulated using HPMC ER (K100) as binder at 7.5 %w/w (Figures 4.30 and 4.32). The drug-release data when fitted into various release models conformed to the Higuchi model of drug release (Table 4.12), with a regression coefficient (R^2) of 0.996 (see Appendix K). The mechanism of drug release was found to be by Fickian diffusion (Talukdar *et al.*, 1996; Harris Shoaib *et al.*, 2006) with a value of n=0.45 (see Appendix L). Drug release from HPMC hydrogel tablets has been reported to be diffusion-controlled (Lin and Metters, 2006). Inclusion of CrosCCG at 10 %w/w concentration increased ($P<0.05$) the amount of drug released even though $T_{80\%}$ still remained 90 min. At concentrations above 10 %w/w, CrosCCG admixed with HPMC ER at 7.5 %w/w was found to delay the drug release. Dissolution of a soluble drug in high concentration from an insoluble matrix follows the Higuchi square root equation wherein drug release is proportional to the square root of time (Fesssi *et al.* 2002; Gupta *et al.,* 2002). At 15 and 25 %w/w CrosCCG concentrations, $T_{80\%}$ became 120 min (Figure 4.32) and the difference in drug release compared to HPMC ER alone was statistically significant ($P<0.05$) and still follows the Higuchi square root equation (see Appendix M). Use of 15 or 25 %w/w CrosCCG, however was not found to be significantly different ($P>0.05$). At 15 %w/w, CrosCCG delayed the release, as 80 % of the drug was released in 120 min. At 25 %w/w concentration CrosCCG when admixed with HPMC ER 7.5 %w/w and used to formulate Ven. HCl tablets, caused the mechanism of drug release to change from Fickian to anomalous (non-Fickian) diffusion with $n > 5$ (Table 4.12 and Appendix N). Swelling properties of CrosCCG may be a factor responsible for the observed deviation. A swelling-controlled matrix used in drug delivery system must have satisfactory

swelling properties (Caykara *et al.*, 2003; Ganji *et al.*, 2010). Swelling in polymers plays an important role in causing deviations from Fickian diffusion usually as a consequence of the finite rates by which changes in polymer structure occur. The process of sorption-desorption in a polymeric material exposed to moisture leads to a flurry of events starting from swelling followed by stresses then structural changes and relaxations (Edwards, 1996; Kee *et al.*, 2005; Mazumder *et al.*, 2006).

5.9.5 Formulation Properties of Ven. HCl Tablets Containing HPMC as Binder and CrosCCG Release Facilitating Agents

5.9.5.1 Granule Properties

Granules formed for all the batches generally failed to flow through the funnel. This may be a result of higher proportion of smaller sized granules occasioned by the milling of the granules after drying and the small fraction of the insoluble polymers used. At 3 %w/w concentration, the polymers could not significantly influence the properties of the formed granules. They thus appear to have almost uniform flow characteristics (Figure 4.33) resembling those found with batch G9 that does not contain any release enhancing agents.

5.9.5.2 Tablet Properties

The type and amount of disintegrating agent employed in the formulation significantly control the overall rate of dissolution of dosage form (Kumar and Tewari, 2005). Tablet disintegration has received considerable attention as an essential step in obtaining fast drug release (Bele and Derle, 2008) especially in the rapidly disintegrating tablet formulations where superdisintegrants are the principal players (Nagar and Yadav, 2009; Rishabha, 2010;

Deshmkh *et al.*, 2012). The orally-disintegrating tablets also called rapidly dissolving, orodisperse, or fast dissolving tablets are gaining popularity because of an acquired convenience in use as they have increased compliance among pediatric and geriatric patients (Omidian and Park, 2008). In an analysis of patents on superdisintegrants, these authors reported that patent filing has been on the increase. Between the time ranges 1983 - 1988 only one patent application was recorded, while between 2001-2004 a total of 22 applications were recorded. Disintegrating agents that can otherwise be called drug-release enhancing agents are used to promote disintegration of tablets which in most cases is necessary for dissolution and eventual bioavailabity of an active agent. These agents are therefore worthy of being researched on. Due to some technical problems, determination of drug disintegration times were not carried out during this study but in its place, an equally revealing measurement, drug-release enhancing property, was determined using dissolution test data. As Banakar and Makoid (1996) opined, dissolution testing of pharmaceutical solid dosage forms has become the single most important test which will ensure the quality of the product.

Formulations in Table 3.9 were designed to evaluate the drug-release enhancing property of CrosCCG as compared to CrosCE and CrosCC as well as some known superdisintegrants, Croscamellose and Crospovidone. None of the polymers was able to cause rupturing of the tablet as it was held together by the powerful binding ability of HPMC ER at 7.5 %w/w. The rate of drug release was thus measured and the data used to evaluate the drug-release profiles of the respective polymers. CrosCCG at 10 %w/w concentration was found to facilitate drug release from Ven. HCl tablets containing HPMC ER 7.5 % as binder (Figure

4.34 and Appendix J). The increase in rate of drug release was significant ($P<0.05$) when compared to release from tablets containing HPMC ER at 7.5 %w/w alone.

Cross-linked polymers have been used to facilitate drug-release from tablets (Fu *et al.*, 2004; Zhao and Augsburger, 2005b). Figures 4.34 and 4.35 show the drug-release facilitating properties of CrosCCG and other polymers tested. All the polymers tested were found to facilitate drug release with statistically significant effect ($P<0.05$) when compared with drug-release from tablets containing HPMC ER alone or from one polymer to the other. Within the first 15 min of initiating the *in-vitro* dissolution process, the rate of drug release was found to be in the order Crospovidone > CrosCCG > Croscamellose > CrosCC > CrosCE > HPMC ER. Readings obtained after 30 min however changed the order to CrosCCG > Croscamellose > CrosCE > CrosCC > Crospovidone > HPMC ER.

Crospovidone, a cross-linked polymer, is a known superdisintegrant that causes tablet disintegration by wicking water up into the tablet thereby causing the tablet to rupture (Davies, 2009; Deshmkh *et al.*, 2012). In this study, it was found to cause greater amount of drug to be released within the first 15 min of dissolution time. Beyond 15 min of dissolution time, however, a change in events was noticed as Crospovidone caused the least release of Ven. HCl among the polymer tested. Of the polymers tested, Crospovidone was the only one that acts via capillary (wicking) action, a "first step" process that brings about the rapid penetration (Pahwa *et al.*, 2010; Bhowmik *et al.*, 2010; Desmkh, 2012) of the tablet surfaces by water causing instantaneous release especially of soluble drugs. This may possibly explain the greater release rate within the first 15 min noted for Crospovidone.

In trying to describe the rate of swelling of sodium starch glycollate and Croscamellose, Saharan *et al.* (2009) concluded that rate, force and extent of swelling have an important role in disintegrants that work by swelling. Swelling was found to be the main mechanism of enhancing drug release exhibited by the polymers synthesized in this study. A positive correlation exists between tablet disintegration time/cumulative percent of drug released and swelling (Zhao and Augsburger, 2005a) and also with water uptake (Zhao and Augsburger, 2005b). This relationship was classically demonstrated when drug-release facilitated by CrosCCG 3 %w/w and Croscamellose 3 %w/w from tablets containing HPMC ER at 7.5 %w/w were plotted against time (Figures 4.36 and Appendix J). CrosCCG caused Ven. HCl release at a faster rate within the first 80 min of *in-vitro* dissolution time, beyond which however, Croscamellose caused greater drug release. Interestingly, Figure 4.37 and Appendix E, showed how CrosCCG was superior to Croscamellose in swelling rate but not in extent of swelling. The figure showed how the two curves intersected around 80 min, after the first order swelling demonstrated by CrosCCG had seized and the curve had almost flattened out while the Croscamellose steadily swelled and eventually caused more drug release.

The superior swelling ratio (%) of CrosCE over CrosCCG (Figure 4.25) was not found to translate into superior drug-release facilitating property (Figures 4.34 and 4.35). Reason for this behavior was not immediately discernible, but cross-link bond type may possibly play a role.

Disintegrants by their hygroscopic nature could absorb moisture and pose threats to the stability of the formulated drug especially water soluble drug (Davies 2009; Bhowmik *et al.*, 2010). The ability of CrosCCG to desorb an almost equivalent amount of sorbed water

(Figure 4.22) demonstrates that fears of product spoilage due to hygroscopicity of the material can be allayed by inclusion of a sachet of an appropriate desiccant.

5.10 Sub-Acute Toxicity Studies

Acute toxicity studies conducted on CG has shown the gum to be non-toxic (Abdulsamad *et al.*, 2006b). However, acute toxicity studies could not be carried out on CrosCCG as the material was not soluble in any appropriate solvent. Sub-acute toxicity often referred to as sub-chronic toxicity assessment was therefore conducted on laboratory animals by administering the test material for 28 days and the animals sacrificed on the 29^{th} day (Aniagu *et al.*, 2005; Joshi *et al.*, 2007; Biswas *et al.*, 2012). Blood samples for the tests were collected via jugular vein puncture following administration of anesthesia. The material could only be administered in admixture with the animals' feed and this made it unrealistic to weigh the animals for the purpose of dosing, nonetheless, rats of similar age bracket were used for the test. The dosing was designed such that any dose dependent or sex related toxic effect of the material would easily be detected.

Physically, no apparent changes in the observable behaviours of the animals, associated with possible toxic effect of the material, were noticed over the 28 day study period. The monitored animals' feeding and drinking habits were not altered. None died, appeared dehydrated or emaciated either as a result of poor feeding, drinking or toxic effect of the test material. Both treated and concurrent control group displayed no clinical manifestations or signs of toxicity (Aniagu *et al.*, 2005; Tamta *et al.*, 2009).

Table 4.13 and Appendix O showed the mean values of hematological parameters tested. Blood parameters were used in assessing the effects of the material on the blood and

eventually on the body. The results obtained showed no differences (P>0.05) between the control group and the test groups. CrosCCG can be considered safe as it did not precipitate any untoward changes in the hematological parameters tested (Aniagu *et* al., 2005; Ramesh Babu *et al.*, 2010; Kengkoom *et al*., 2012)

The results of the effect of CrosCCG on the biochemical parameters tested are as shown in Table 4.14 and Appendix P. Intra- and inter- group statistical analysis of the alkaline phosphatase (ALP), serum glutamic pyruvate transaminase (SGPT, ALT), urea and platelets results using the one way Analysis of Variance (ANOVA) and treated with the Turkey's multiple comparison test showed no significant differences (P>0.05) between the groups. Results on serum glutamic oxaloacetate transaminase levels (SGOT, AST) showed some difference (P<0.05) when plasma enzyme levels in group AA1 females were compared with the group fed with the lowest dose, AA2 males, but other groups including AA4 that were fed with the highest dose showed no significant difference (P>0.05). This is taken to be an anomaly, possibly a result of physiological variations and not treatment related. Since increase in concentration of plasma enzymes (AST, ALT, LDH etc) is taken as characterizing liver cell damage (Aniagu *et al*., 2005), it can be concluded that CrosCCG has no deleterious effect on liver cells.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Tablets are still one of the most popular and promising dosage forms in terms of precision of dosing, chemical/microbial stability and convenience in use especially with the advent of modified release dosage formulations. Researches into discoveries or development of novel active agents or vehicles qualified for specialized delivery of the active agents is of paramount importance in promoting the health care delivery.

Polymeric materials including celluloses, starches and gums had been greatly employed in the formulation of medicines with the first two being of greater relevance, at least so far. Acacia gum, xanthan gum, guar gum, karaya gum are some of the few gums that had found use as gums of pharmaceutical relevance and had thus been listed as excipients in the official reference books. In recent years, cashew gum has been receiving attention through explorations into its potential relevance in drug delivery (Abdulsamad *et al.* 2008; Ofori-Kwakye *et al.* 2010; Okoye *et al*., 2010). Structural modifications on polymers had been used to advance performances of pharmaceutical polymers to provide specialized drug delivery. Structural modification has been tried on cashew gum and properties of such derivatives investigated.

In the present study, increasing concentrations of cashew gum or its admixtures with MCC PH 102 as binder have been used to formulate conventional release tablets of Ven. HCl. Tablet formulations containing, as binder/matrix, 5, 10, and 25 %w/w cashew gum were

made and compared with similar formulations made, but containing MCC PH 102 as binder at 10 %w/w concentrations. Binding properties of admixtures of CG at 5 and 25 %w/w with MCC PH 102 at 10 %w/w concentration were also compared with formulations containing MCC PH 102 at 10 %w/w as binder. At 5 %w/w concentration, CG produced good tablets characterized by conventional release. Increased concentration or admixtures with MCC PH 102 offered no added advantage.

CG has been cross-linked using epichlorohydrin as cross-linking agent but owing to the potential carcinogenicity of the cross-linking agent its use in the study was limited and safe alternative sought. Polycarboxylic acids reported to have been used in cross-linking cyclodextrins were considered safer alternatives and were thus used in cross-linking CG. The PCAs were found to cross-link CG via formation of ester bonds between the –COOH of the PCA and the –OH of the gum. Cross-linking of CG with a combination of CTR and GLY was found to produce a polymer, CrosCCG, with improved cross-link yield and greater swelling properties. Cross-linking CG with CTR had better yield when treated at 140 ^{o}C for 30 min, while cross-linking of the gum with a combination of CTR and GLY had better yield when carried out at 170 ^{o}C for 30 min. The cross-linking was demonstrated by DSC, FT-IR and solid state ^{13}C NMR spectral analyses.

Characterization of CG and the developed polymers, CrosCC and CrosCCG showed the polymers to be free flowing with particle size distribution that skewed to the bigger size fraction. The powder compressibility index was in the order CG > CrosCC > CrosCCG; while water sorption and swelling ratio were in the order CG < CrosCC < CrosCCG.

CG, CrosCC and CrosCCG were found to be compatible with the test drug, Ven. HCl, and this allowed their use in formulating the tablets. The release of Ven. HCl could not be modified by formulating tablets using CrosCCG alone as matrix former. Use of CrosCCG in concentrations above 10 %w/w in admixtures with HPMC ER at 7.5 %w/w was found to further delay drug release from tablets of Ven. HCl. Drug release from tablets containing either HPMC ER at 7.5% or its admixture with CrosCCG at 25 % concentration was found to show drug release kinetics that agreed with the Higuchi model. But while drug release mechanism from tablets formulated with HPMC ER at 7.5 % was found to be by Fickian diffusion, incorporation of CrosCCG at 25 % concentration changed the mechanism to anomalous (non-Fickian) diffusion. This deviation was attributed to the swelling ability of CrosCCG.

The drug release enhancing ability of the cross-linked polymers, CrosCE, CrosCC and CrosCCG, was also investigated and compared with the drug release by some known super disintegrants, Croscamellose and Crospovidone. The cross-linked polymers were all found to facilitate drug release at 3 %w/w from Ven. HCl tablet formulations containing HPMC ER at 7.5 %w/w as binder. The drug release enhancing ability as recorded within the first 15 min was in the order Crospovidone > CrosCCG > Croscamellose > CrosCC > CrosCE. This order, however, changed after 30 min of *in vitro* dissolution to CrosCCG>Croscamellose>CrosCE>CrosCC>Crospovidone. This finding highlights the potentials of the development of release enhancing or disintegrating agents by cross-linking gums with CTR and especially by cross-linking with a combination of CTR and GLY.

Sub acute toxicity studies on wistar rats showed CrosCCG to be safe. The polymer was not found to cause damage or changes in hematologic and biochemical parameters tested after feeding the animals with increasing quantities of the polymer over a period of 28 days.

6.2 Conclusion

CG in its unmodified state could find use as a binder in conventional immediate-release tablets of Ven. HCl and apparently in other water soluble drugs at concentrations not greater than 5 %w/w, with higher concentrations offering no additional advantage. CG can be cross-linked via etherification as was shown with polymerizations using EPC or esterification as depicted by cross-links with safer cross-linking agents, the PCAs. Cross-linking renders the material insoluble in almost all the common solvents. In water, while the cross-linked polymers do not go into solution, they absorb significant amounts of water which makes them swell appreciably. This property of swelling was exploited where the cross-linked polymer was used as binder/matrix former and also as drug release enhancing agent, in formulating tablets of Ven. HCl. Cross-linking of CG using a combination of CTR and GLY was found to give a polymer, CrosCCG, which possessed greater swelling abilities. CrosCCG when tested was found to be non-toxic on laboratory animals. Standing alone, CrosCCG could not sustain the release of Ven. HCl but when used in admixtures with HPMC ER, it facilitated drug-release at low concentrations; while at higher levels it displays a synergistic release retardant action.

6.3 Recommendations

Cross-linking studies using polycarboxylic acids with larger number of carboxyl groups; and also other cross-linking agents should be explored on cross-linking processes of cashew gum and other polysaccharides. Studies on formulations using varying particle/granule sizes should be undertaken to explore the possible influence of particle size on drug release. The effect of changes in pH on the formulation properties of the derivatized polymers should also be explored. Studies on formulation properties of cashew gum cross-linked polymers using water insoluble drug should be carried out.

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Tests on Hypotheses

i. CG cannot be cross-linked using citric acid (CTR), a polycarboxylic acid.

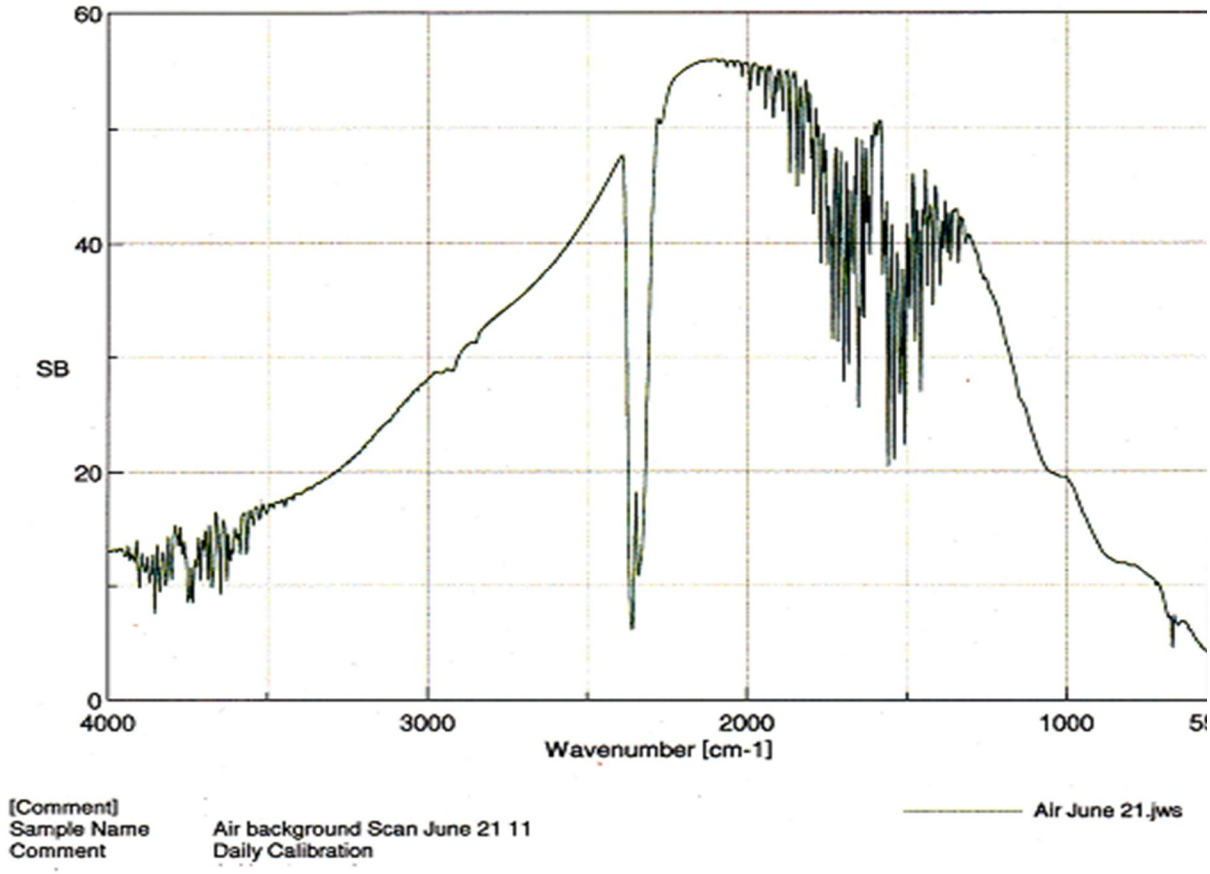
From the results of moisture sorption/desorption isotherm and swelling ratio studies, there is significant differences in the mean between uncross-linked CG and CG cross-linked using CTR. It is concluded that there is sufficient evidence to reject the null hypothesis (at the 0.05 level of significance). The results showed cross-linking to have imparted greater water reactivity on CG.

ii. Cross-linking CG with CTR does not confer tablet sustained-release properties to CG.

iii. Cross-linking CG with CTR does not confer tablet drug-release enhancing property to CG.

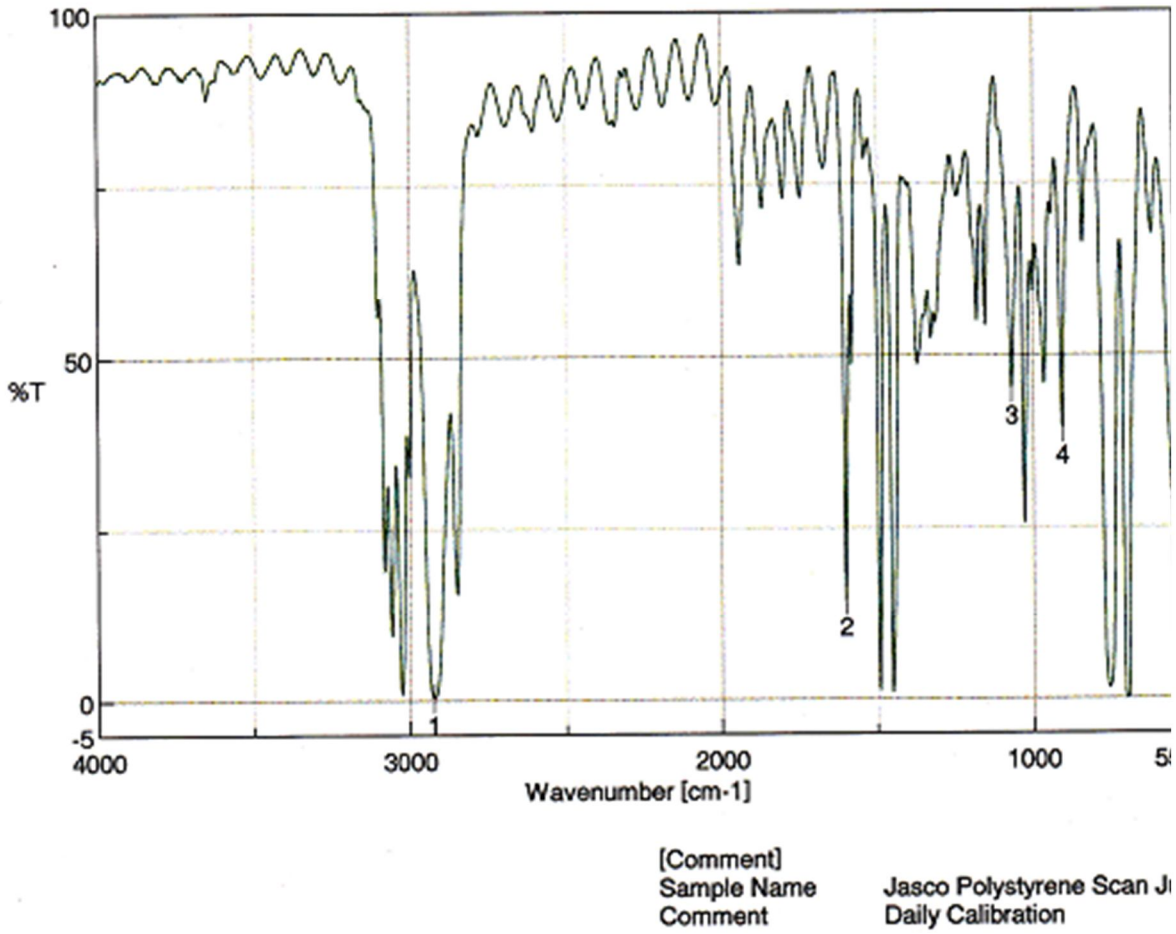
Results of drug-release enhancing property showed a significant difference in the mean between drug-release caused by the cross-linked polymer (CrosCC) and HPMC. It is concluded that there is sufficient evidence to reject null hypothesis (at the 0.05 level of significance). The results showed cross-linking to have enhanced greater drug-release.

Appendix A



FT-IR Air Background Scan (Daily Calibration)

Appendix B



FT-IR Jasco Polystyrene Scan (Daily Calibration)

Appendix C

Moisture sorption/desorption isotherm of CG, CrosCC and CrosCCG after subjection to varying humidity challenges

Polymer type	Weekly changes in mass (g ± SD)						
	1	2	3	4	5	6	7
CG	0.0197 ± 1.18	0.034 ± 0.94	0.0691 ± 0.99	0.1302 ± 0.55	0.0701 ± 0.76	0.032 ± 0.70	0.021 ± 0.89
CrosCC	0.0472 ± 1.74	0.079 ± 0.95	0.4839 ± 1.54	0.2334 ± 0.78	0.1422 ± 1.12	0.0806 ± 0.91	0.04 ± 1.32
CrosCCG	0.305 ± 2.92	0.3659 ± 1.01	0.4839 ± 0.59	0.5898 ± 0.87	0.4739 ± 0.98	0.3641 ± 1.10	0.3037 ± 0.77

Appendix D

Swelling ratio (%) of CG, CrosCC and CrosCCG

Time (min)	Polymer type		
	CG	CrosCC	CrosCCG
0	0	0	0
1	5	31.6	87.5
2	5	54.2	120.3
3	5	63.2	140.6
4	5	73.7	151.6
5	5	77.4	164.1
6	5	78.5	170.3
7	5	80	178.1
8	5	80.5	184.4
9	5	80.5	189.1
10	5	82.2	195.3

Appendix E

Effect of time on the swelling ratio (%) of CrosCCG, croscamellose and Crospovidone after 90 min exposure

Time (min)	Polymer type swelling (% ± SD)		
	CrosCCG	Croscamellose	Crospovidone
1	87.5 ± 2.45	37.0 ± 0.89	5.0 ± 0.50
10	195.3 ± 2.77	99.0 ± 1.09	10 ± 1.00
20	221.3 ± 3.65	131.0 ± 2.17	10 ± 1.00
30	230.0 ± 4.09	153.0 ± 2.80	10 ± 1.00
40	235.0 ± 4.16	170.0 ± 3.54	10 ± 1.00
50	241.30 ± 4.45	185.0 ± 3.67	10 ± 1.00
60	243.80 ± 4.80	200.0 ± 4.00	10 ± 1.00
70	248.10 ± 4.51	225.0 ± 4.20	10 ± 1.00
80	253.1 ± 4.76	255.0 ± 4.57	10 ± 1.00
90	254. 0 ± 4.72	279.0 ± 4.69	10 ± 1.00

Appendix F

Drug release profile of Ven. HCl tablets made with MCC PH 102 or increasing concentrations of CG

Time (min)	Drug released (% ± SD)			
	T1	T2	T3	T4
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
15	97.72 ± 2.54	96.03 ± 1.87	92.75 ± 2.01	64.00 ± 1.94
30	98.41 ± 1.98	96.27 ± 2.56	94.49 ± 2.49	86.89 ± 2.31
45	98.43 ± 2.14	96.31 ± 2.71	94.57 ± 1.99	92.23 ± 1.87
60	98.44 ± 2.20	96.38 ± 2.69	94.59 ± 2.46	98.37 ± 2.18

Key: T1 (MCC PH 102 10 %w/w); T2 (CG 5 %w/w); T3 (CG 10 %w/w); T4 (CG 25 %w/w)

Appendix G

Drug release profile of Ven. HCl tablets made with MCC PH 102 or its admixtures with CG

Time (min)	Drug released (% ± SD)		
	T1	T5	T6
0	0 ± 0.00	0 ± 0.00	0 ± 0.00
15	97.72 ± 1.02	96.38 ± 0.98	74.32 ± 1.21
30	98.41 ± 1.07	96.40 ± 1.10	86.89 ± 1.54
45	98.43 ± 0.98	96.51 ± 1.09	98.29 ± 1.61
60	98.44 ± 1.05	96.50 ± 1.12	100.52 ± 1.59

Key: T1 (MCC PH 102 10 %w/w); T5 (MCC PH 102 10 %w/w + CG 5 %w/w); T6 MCC PH 102 10 %w/w + CG 25 %w/w)

Appendix H

Dissolution profiles of HPMC LV (5 %w/w), HPMC ER (5 %w/w), HPMC ER (7.5 %w/w) and CrosCCG (25 %w/w) as binder/release retardant in Ven. HCl tablets

Time (min)	Drug dissolved (% ± SD)			
	T7	T8	T9	T10
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
15	98.81 ± 2.75	70.62 ± 8.62	35.59 ± 7.22	103.34 ± 2.37
30	98.9 ± 2.79	96.54 ± 2.58	50.30 ± 8.45	103.79 ± 2.47
45	98.84 ± 2.96	97.76 ± 1.10	60.27 ± 8.39	103.93 ± 2.43
60	98.69 ± 2.84	97.88 ± 0.51	68.12 ± 7.81	104.00 ± 2.43
75	98.72 ± 2.96	97.61 ± 1.06	74.61 ± 6.96	104.15 ± 2.41
90	98.69 ± 2.92	97.58 ± 1.02	80.18 ± 6.08	104.18 ± 2.42
105	98.81 ± 3.01	97.52 ± 1.02	85.11 ± 5.27	104.27 ± 2.38
120	98.69 ± 2.92	97.64 ± 1.10	89.75 ± 4.58	104.19 ± 2.42

Key: T7 (HPMC LV 5%); T8 (HPMC ER 5%); T9 (HPMC ER 7.5%); T10 (CrosCCG 25%)

Appendix I

Dissolution profiles of CrosCCG as release retardant at 10, 15 and 25 %w/w concentration in Ven. HCl tablets containing HPMC ER as binder at 7.5 %w/w

Time (min	Drug released (% ±SD)			
	T9	T11	T12	T13
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
15	35.59 ± 7.22	39.5 ± 6.97	31.56 ± 4.63	27.54 ± 4.37
30	50.30 ± 8.45	53.19 ± 8.05	44.19 ± 4.39	41.54 ± 4.55
45	60.27 ± 8.39	63.49 ± 7.98	53.58 ± 3.99	51.70 ± 4.43
60	68.12 ± 7.81	70.98 ± 7.69	61.04 ± 3.61	59.82 ± 4.30
75	74.61 ± 6.96	77.04 ± 7.20	67.19 ± 3.32	66.64 ± 4.14
90	80.18 ± 6.08	82.14 ± 6.66	72.68 ± 3.05	72.74 ± 3.89
105	85.11 ± 5.27	86.67 ± 6.21	77.23 ± 2.85	77.81 ± 3.59
120	89.75 ± 4.58	90.46 ± 5.65	81.27 ± 2.61	82.32 ± 3.31
135	93.88 ± 3.41	93.75 ± 5.22	84.79 ± 2.45	86.24 ± 3.04
150	96.53 ± 1.86	96.54 ± 4.39	87.95 ± 2.34	89.64 ± 2.80
165	97.63 ± 1.25	99.17 ± 3.48	91.10 ± 2.77	92.57 ± 2.50
180	98.01 ± 1.09	101.09 ± 2.81	93.22 ± 2.60	95.00 ± 2.27
195	98.19 ± 1.08	102.38 ± 2.24	94.49 ± 2.03	96.93 ± 1.94
210	98.3 ± 1.00	103.31 ± 1.97	95.30 ± 1.52	98.55 ± 1.71
225	98.28 ± 1.02	103.83 ± 1.65	96.00 ± 1.27	99.72 ± 1.42
240	98.32 ± 1.00	103.88 ± 1.47	96.33 ± 1.15	100.6 ± 1.26

Key: HPMC ER 7.5% (T9) + HPMC ER 7.5% +CrosCCG 10% (T11); +CrosCCG 15% (T12); +CrosCCG 25% (T13)

Appendix J

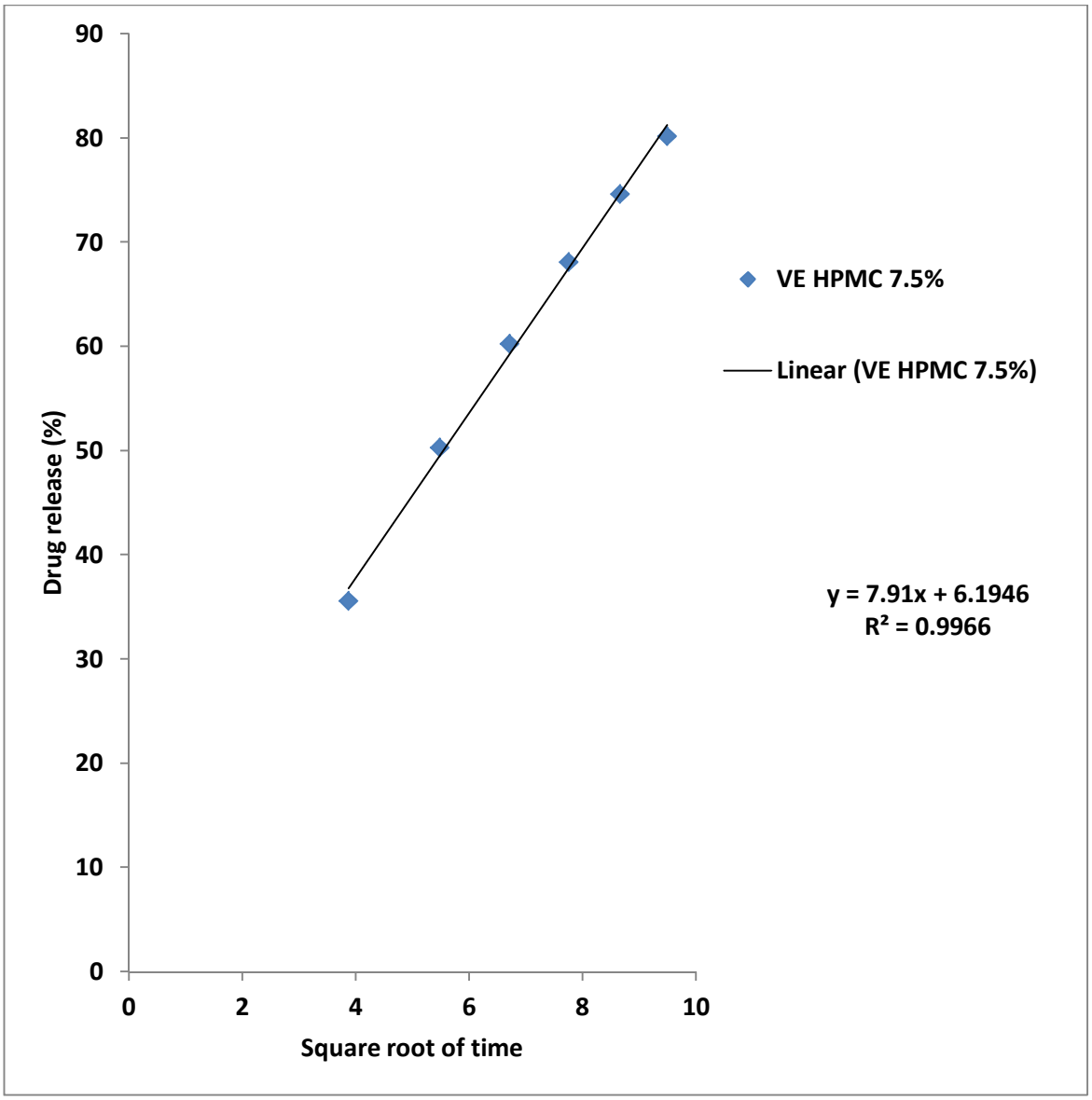
Dissolution profiles of CrosCCG, CrosCC, Croscam, Crospov and CrosCE at 3 %w/w

concentration in Ven. HCl tablets containing HPMC ER as binder at 7.5 %w/w

Time (min)	Drug released (% ± SD)				
	T14	T15	T16	T17	T18
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
15	47.86 ± 9.90	39.58 ± 6.31	44.93 ± 9.50	49.96 ± 11.71	38.65 ± 8.42
30	64.37 ± 13.68	52.94 ± 6.11	59.42 ± 10.77	52.08 ± 11.26	53.19 ± 8.07
45	72.34 ± 10.70	61.93 ± 5.72	68.58 ± 9.98	71.48 ± 12.20	62.9 ± 7.61
60	78.07 ± 8.36	69.13 ± 5.36	75.93 ± 9.23	75.09 ± 12.02	70.5 ± 7.21
75	82.65 ± 6.75	75.23 ± 4.89	82.33 ±9.69	76.50 ± 9.67	76.82 ± 6.80
90	86.96 ± 6.23	80.52 ± 4.59	87.07 ± 8.30	86.51 ± 7.75	82.71 ± 6.73
105	90.01 ± 5.18	85.57 ± 4.76	91.04 ± 6.79	91.71 ± 6.35	88.35 ± 6.87
120	92.47 ± 4.07	89.98 ± 5.31	94.91 ± 5.67	98.99 ± 4.04	93.46 ± 5.86
135	94.61 ± 4.01	91.67 ± 5.07	98.45 ± 4.96	103.17 ± 1.66	97.85 ± 2.78
150	95.57 ± 3.16	94.89 ± 3.77	100.81 ± 4.15	104.81 ± 1.13	99.09 ± 2.10
165	95.9 ± 3.36	97.45 ± 3.01	102.33 ± 3.18	105.27 ± 1.19	99.41 ± 2.05
180	96.05 ± 3.50	98.03 ± 2.96	103.12 ± 2.42	105.3 ± 1.34	99.61 ± 2.05
195	96.18 ± 3.55	99.64 ± 2.95	103.99 ± 1.89	105.43 ± 1.44	99.71 ± 2.12
210	96.22 ± 3.52	100.41 ± 3.00	104.47 ± 1.56	105.57 ± 1.46	99.77 ± 2.05
225	96.22 ± 3.58	100.6 ± 3.06	105.03 ± 1.37	105.7 ± 1.44	99.83 ± 2.07
240	96.33 ± 3.55	100.89 ± 3.09	105.16 ± 1.26	105.88 ± 1.45	99.88 ± 2.04

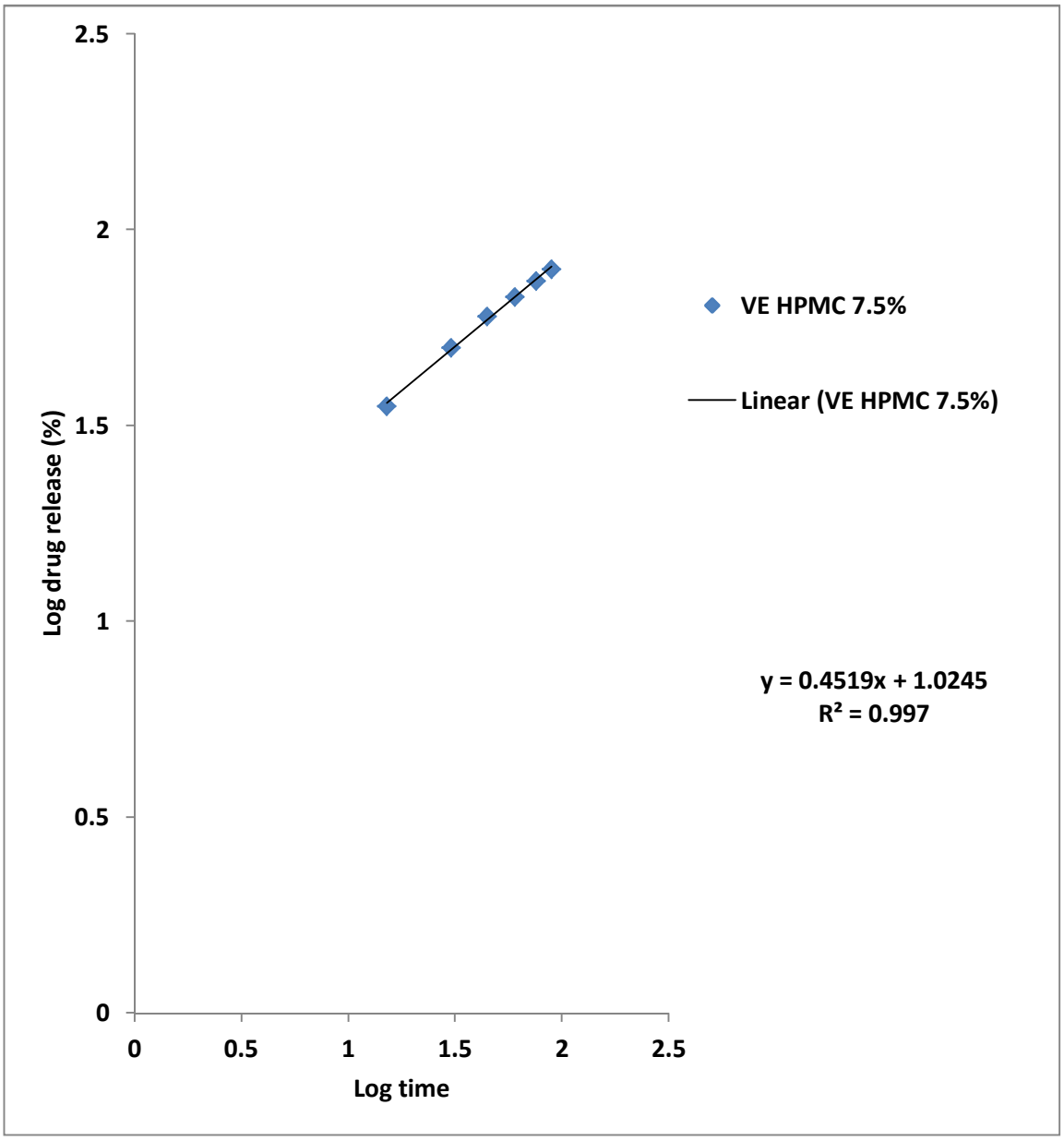
Key: T9=HPMC ER 7.5%, HPMC ER 7.5% +CrosCCG 3% (T14), CrosCC 3% (T15), Croscamellose 3% (T16), Crospovidone 3% (T17) and CrosCE 3% (T18)

Appendix K



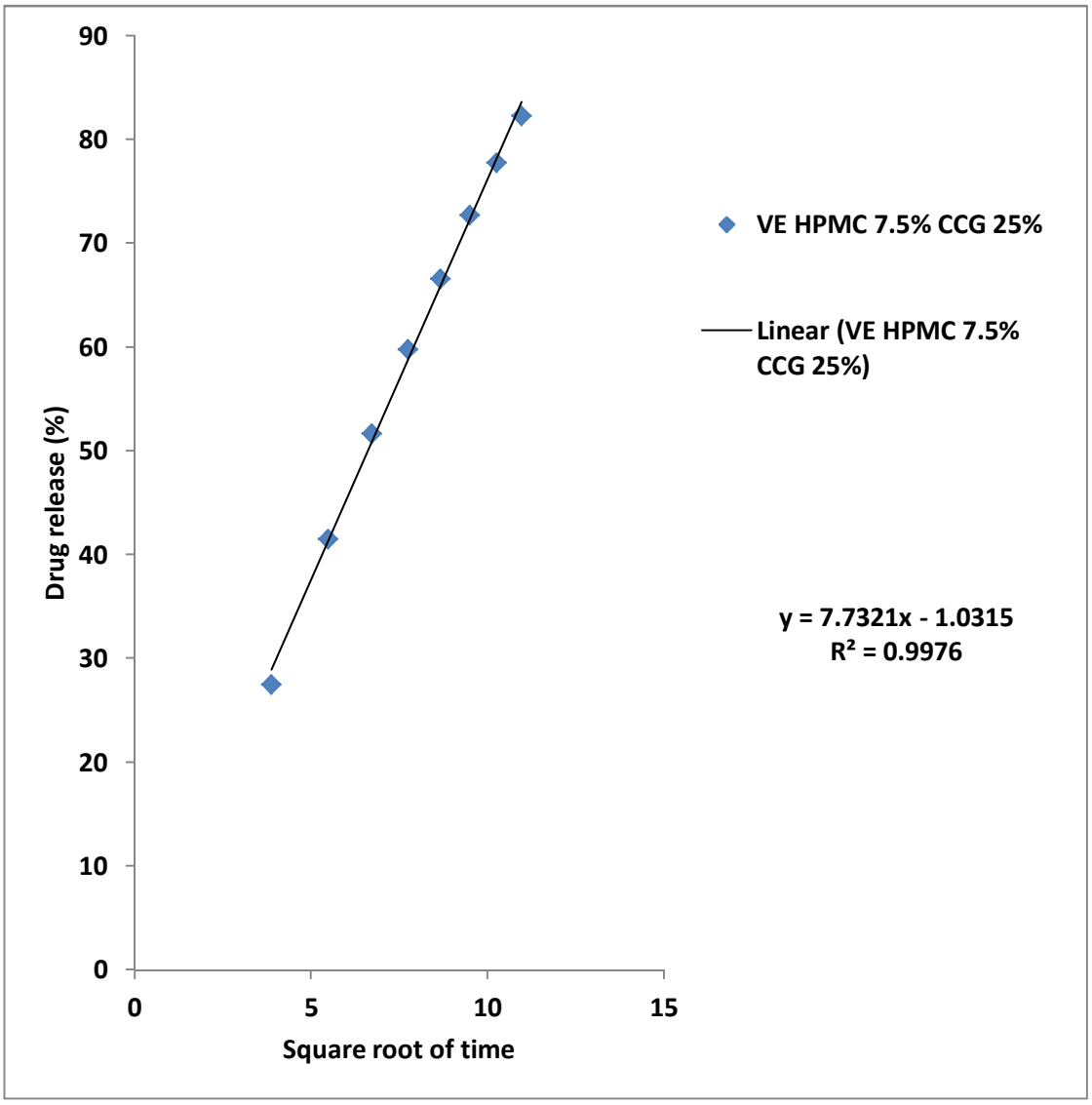
Drug release (%) Vs square root of time – Higuchi kinetic; Ven. HCl formulated with HPMC ER as binder at 7.5%

Appendix L



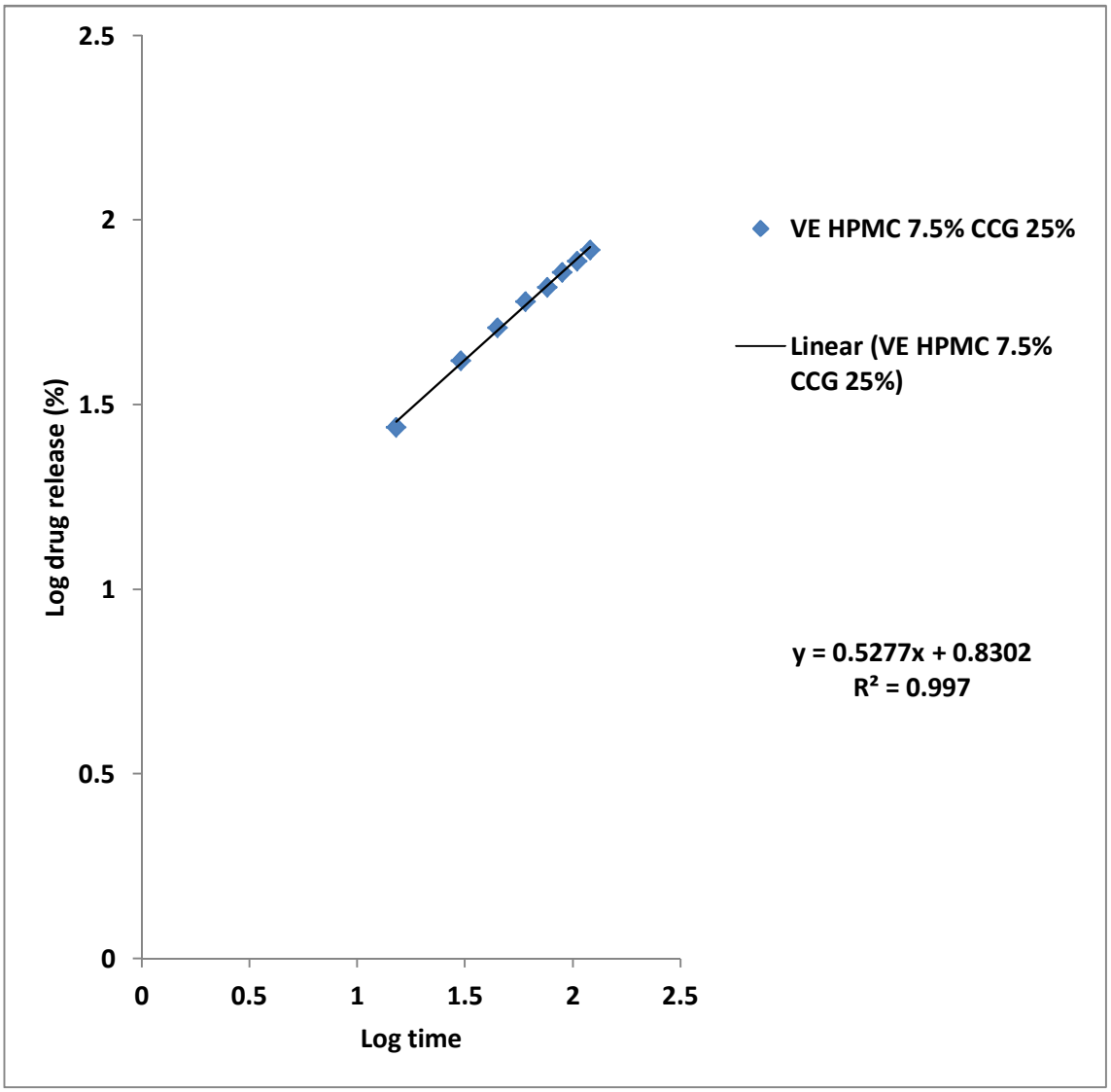
Log drug release (%) Vs Log time – Korsmeyer-Peppas kinetics; Ven. HCl formulated using HPMC ER as binder at 7.5%;

Appendix M



Drug release (%) Vs Square root of time – Higuchi kinetic; Ven HCl tablets formulated using HPMC ER as binder at 7.5 %w/w and CrosCCG as release retardant at 25 %w/w

Appendix N



Log drug release (%) Vs Log time – Korsmeyer-peppas model; Ven HCl tablets formulated using HPMC ER as binder at 7.5 %w/w and CrosCCG as release retardant at 25 %w/w

Appendix O

Blood Parameters of Wistar Rats Fed with CrosCCG over 28 Day Period

Clinic No.		Test type									
		Packed cell volume (PCV) (%)	Hemoglobin (g/dL)	Total protein (g/dL)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Band (%)	Total White Blood Cells (TWBC) ($x10^9$/L)
AA1	M1	42	14.0	6.1	12	84	3	-	-	1	10.8
	M2	41	13.6	5.9	13	83	1	-	-	3	10.8
	M3	48	16.0	6.8	13	85	-	-	-	2	9.5
	F1	44	14.6	6.6	21	77	2	-	-	-	12.4
	F2	45	15.0	7.2	24	72	4	-	-	-	8.2
	F3	44	14.6	7.2	12	85	1	-	-	2	15.5
AA2	M1	46	15.3	6.2	18	72	-	1	6	-	10.4
	M2	46	15.3	6.0	18	79	-	-	-	3	14.0
	M3	47	15.6	6.4	23	71	4	1	-	1	5.0
	F1	40	13.3	7.1	12	84	3	-	-	1	9.3
	F2	41	13.3	7.0	17	83	-	-	-	-	5.9
	F3	45	15.0	7.4	9	88	3	-	-	-	4.1
AA3	M1	41	13.6	6.0	18	80	2	-	-	-	7.0
	M2	48	16.0	6.2	12	84	2	-	-	2	11.0
	M3	43	14.3	6.0	28	71	-	-	-	1	9.8
	F1	44	14.6	7.0	15	83	-	2	-	-	12.7
	F2	43	14.3	7.0	9	87	4	-	-	-	8.6
	F3	45	15.0	7.6	14	72	4	8	-	-	9.5
AA4	M1	43	14.3	6.0	12	85	1	-	2	-	5.3
	M2	49	16.3	6.2	39	61	-	-	-	-	8.8
	M3	44	14.6	6.2	17	78	2	-	-	3	5.3
	F1	44	14.6	7.0	22	75	2	-	-	1	12.5
	F2	50	16.6	7.0	16	75	8	-	-	1	9.6
	F3	46	15.3	7.8	19	74	3	2	-	2	8.0

Key: AA1, AA2, AA3, AA4 are groups of animals fed with CrosCCG at 0.00, 2.50, 5.00 and 10.00 g/Kg feed, respectively

Appendix P

Biochemical Parameters of Wistar Rats Fed with CrosCCG over 28 Day Period

Clinic No.		Test type							
		Platelates	AST		ALT		Urea		ALP
AA1	M1	496.0	0.03	10	0.01	2	12	6.0	54
	M2	440.0	0.01	3	0.01	2	22	12.0	98
	M3	520.0	0.03	10	0.01	2	12	6.0	37
	F1	405.0	0.08	27	0.24	41	9	4.5	41
	F2	215.0	0.08	27	0.13	22	8	4.0	71
	F3	545.0	0.03	10	0.03	5	13	6.0	41
AA2	M1	130.0	0.01	3	0.01	2	15	6.5	37
	M2	445.0	0.01	3	0.05	8	13	6.5	20
	M3	225.0	0.01	3	0.04	7	14	7.0	64
	F1	410.0	0.06	19	0.06	10	13	6.5	57
	F2	300.0	0.04	13	0.06	10	10	5.0	20
	F3	540.0	0.03	10	0.05	8	14	7.0	68
AA3	M1	150.0	0.02	7	0.02	3	18	9.0	41
	M2	320.0	0.03	10	0.05	8	9	4.5	71
	M3	570.0	0.02	7	0.03	5	13		20
	F1	340.0	0.03	10	0.05	8	10	5.0	34
	F2	495.0	0.02	7	0.03	5	19	8.5	54
	F3	275.0	0.02	7	0.03	5	11	5.5	47
AA4	M1	680.0	0.04	13	0.05	8	7	3.5	20
	M2	390.0	0.02	7	0.01	2	18	9.0	34
	M3	735.0	0.06	19	0.03	5	11	5.5	54
	F1	340.0	0.04	13	0.01	2	7	3.5	47
	F2	715.0	0.04	13	0.03	5	13	5.5	44
	F3	545.0	0.08	27	0.03	5	19	9.5	20

Key: AA1, AA2, AA3, AA4 are groups of animals fed with 0.00, 2.50, 5.00 and 10.00 g/Kg;
ALP= Alkaline phosphatase; AST= Aspertate transaminase;
ALT= Alanine transaminase