Development of and characterization of gelatin-poly ethylene glycol composite hydrogels and gelatin-polysaccharide physical hydrogels

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This is to certify that the thesis entitled "**Development of and characterization of gelatin-poly ethylene glycol composite hydrogels and gelatin-polysaccharide physical hydrogels**" submitted by **Mr. Khade Shankar Mukundrao** in partial fulfillment of the requirements for the award of Master of Technology Degree in **"Biotechnology"** at the National Institute of Technology, Rourkela, Odisha is an authentic work carried out by him under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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ABBREVIATIONS

Abbreviation		Definitions
GS		Gelatin solution
u		Uncrosslinked
c		Crosslinked
PH		Physical hydrogels
PEG		Polyethylene Glycol
MZ		Metronidazole
CPDR		Cumulative percent drug release
DW		Distilled Water
w/w		Weight by Weight
w/v		Weight by Volume
T_m		Melting Point
SS		Stainless Steel
µm		Micrometer
R^2		Regression coefficients
SR		Swelling Ratio
OD		Optical Density
FTIR		Fourier Transform Infrared Spectroscopy
XRD		X-Ray diffraction Analysis
DSC		Differential scanning calorimetry
SC		Sodium Carboxymethyl cellulose
MD		Maltodextrin
DX		Dextran
GH		Gelatin blank
SR		Stress Relaxation
BE		Backward extrusion
Rb		Bulk resistance

REVIEW OF LITERATURE

Gels are the system which are highly elastic, capacity to absorb and retain large amount of solvent and also can form nanostructures [1]. Gels are widely used in the products of dairy, textured fruit, various processed meat products [2]. Emulsion filled gels have wide applications in cosmetic industries, food industries, and also in pharmaceutical industrial products [3].

Based on the drying methods the gel products are termed as: The product of super cooling drying of the gels is called 'aerogels'. While the product of liquid removal from gels are termed as 'xerogels'. And the product of freeze drying is called 'cryogels' [4].

Types of Gels:

There are 2 major types of gels i) Organogels and ii) Hydrogels [5]. The classification is based on nature of liquid, that the gel immobilizes.

i) **Organogels:**

The gels in which, the immobilized liquid is organic solvent are termed as organogels.

ii) **Hydrogels:**

Hydrogels contains high amount of water in there composition.

Hydrogels are the crosslinked network formed by hydrophilic polymers which have ability to absorb and retain large amount of water [6]. Because of the similarity with the soft tissues and their biocompatible nature, hydrogels are potentially used in the biomedical field [7]. Because of these properties hydrogels are used as a carrier for the controlled drug delivery [8].

The extent of crosslinking can be achieved with increase in the crosslinker concentration and decrease in the biopolymeric concentration.

Types of hydrogels:

Hydrogels are mainly divided into 1) physical hydrogels and 2) permanent hydrogels

1) **Physical hydrogels:**

These are the type of hydrogels which are formed by weak physical interactions between or within the polymers like wander vals force, non ionic interactions, hydrogen bonding etc [9]. In this study the physical hydrogels were prepared of following polymers:

- **Gelatin:**

Gelatin is a biopolymer which is obtained by denaturation of collagen [10]. Gelatin is a biopolymer obtained from fibrous protein collagen, which is the major component of animal skin, bone, and connective tissue. Gelatin contains a large number of glycine, proline and 4-hydroxyproline residues. A typical structure of gelatin is: Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.

In the food industries, gelatin is used for chewiness, texture, foam stabilization, creaminess, emulsification, gelling, water binding. In the pharmaceutical and medical engineering, gelatin is used as a matrix for implants, in injectable drug delivery. Gelatin also prominently used as a stabilizer in live attenuated vaccines for the immunization against measles, mumps, diphtheria, rabies and tetanus toxin.

- **Sodium salt of carboxymethyl cellulose:**

Sodium salt of carboxymethyl cellulose (SC) is a hydrophilic polymer of cellulose ether also referred as cellulose gum, sodium cellulose glycolate or simply carboxymethyl cellulose [11]. Due to the colloidal, binding, thickening, absorbing, stabilizing and film forming properties, it is widely used in the food, detergent, cosmetics, pharmaceutical industries.

- **Maltodextrin:**

  Maltodextrin (MD) is a non ionic dextrin polysaccharide product obtained enzymatically from starch [12]. Maltodextrins have wide applications in the food industry as a moisture conditioner, a food plasticizer, a crystallization inhibitor, a stabilizer, carrier and bulking agents. Maltodextrins are also used as binding agents for tablet formulations in wet granulation processes.

- **Dextran:**

Dextran (DX) a natural hydrophilic polysaccharide can be degraded easily. It is a polymer of glucose in which α-1,6- glucosidic linkages are predominant [13]. It is immensely used in food industries as a thickener, an emulsifier and a stabilizer.

2) **Permanent hydrogels:**

However these are the hydrogels which are formed by permanent interactions like covalent bonding after the use of crosslinker [14]. The crosslinking is mainly depend on the following three components:

- Crosslinker concentration
- Ionic content
- Hydrophilic content

These are mainly formed by using either natural crosslinker or synthetic crosslinker.

- **Natural crosslinker:**

These are the crosslinkers which are obtained from plants.

Ex.: Genipin:

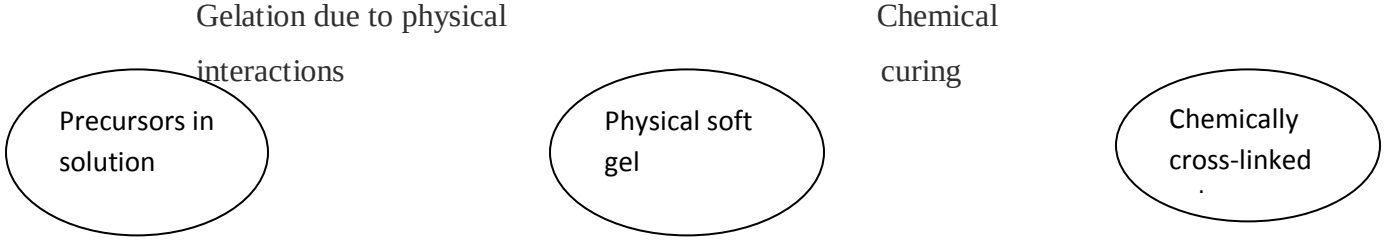
Genipin the natural crosslinker obtained from *Genipia Americana* [15]. It is less toxic crosslinker used for proteins, collagen, gelatin and chitosan.

- **Synthetic crosslinker:**

These are the crosslinkers which are synthesized chemically.

Ex.: Glutaraldehyde:

Glutaraldehyde is an excellent example of synthetic crosslinker. One of the advantage of using glutaraldehyde is that the presence of bifunctionality i.e. aldehydic and alcoholic group, due to which it forms crosslinks efficiently [16].



mechanical properties, irreversibility

Figure 5 : Formation of hydrogels

This study deals with the following biopolymers,

- **Gelatin**

As gelatin is described above.

- **Polyethylene Glycol (PEG):**

PEG is water soluble in nature and many organic solvents including toluene, methylene chloride, ethanol, and acetone. One of the most important properties is that PEG resists recognition from the immune system. It also resists protein and cell adsorption.

Characterization of Hydrogels

Following various methods have been used for the characterization of hydrogels:

Gel-Sol transition temperature (Tg)

Gel-sol transition temperature is the temperature below which gel doesn't show any distinct flow property. Gel loses its structural integrity at the temperatures above Tg. It is one of the important characteristic of hydrogel. It can be determined with either Differential Scanning Colorimeter (DSC), falling ball method, bubble motion, or by simple tube inversion method. Tg depends on physical and chemical properties of hydrogel samples, as well as their interactions (either physical or chemical). It increases with the rise in the gelator concentration.

Analytical methods

Analytical techniques like FT-IR, X-Ray diffraction analysis have been employed for the characterization of hydrogels. These methods provide important information regarding molecular

interactions among the polymers of hydrogels. FT-IR gives valuable information regarding Hydrogen bonding. XRD provides the nature of polymers in formed hydrogels either in dissolved or in crystallized form.

- **Biomedical/pharmaceutical applications of hydrogels**

1) **Hydrogels as tissue engineering matrices**

When parts or the whole of certain tissues or organs fail, there are several options for treatment, including repair, replacement with a synthetic or natural substitute, or regeneration.

2) **Drug delivery:**

The hydrogels are promisingly used for the drug delivery purposes. Due to the presence of network structures the loaded drug is released in a controlled manner. The drug release behavior is directly proportional to the swelling capacity of the hydrogels.
The delivery of drugs is facilitated in the following areas of

a) Oral drug delivery:

Oral drug delivery is most commonly used for the local treatment of mouth diseases such as periodontal disease, fungal and viral infections and cancers of oral cavity. Bioadhesive hydrogel plays an important role for the long term drug delivery against the salivary flow.

b) Gastrointestinal (GI) drug delivery:

GI tract is most popular area for the delivery of drugs by hydrogels due to the presence of large surface area for systemic absorption. For example in case of peptic ulcer diseases the stomach specific antibiotic can be delivered through hydrogels against *Helicobacter pylori* infection.

c) Rectal drug delivery:

For the local treatment of diseases associated with the rectum, such as hemorrhoids the rectal delivery is facilitated. The mucoadhesive polymers are used for rectal drug delivery.

d) Ocular drug delivery:

In ocular drug delivery, due to the presence of many physiological barriers such as excessive tear drainage, blinking, and low permeability through cornea, the conventional drug delivery is not effective. So due to this conventional eye drops possessing drug suspension are eliminated from eye rapidly.

To overcome this problem, scientists have developed hydrogels which are ocular drainage resistant in nature and prolonged retention property.

e) **Transdermal drug delivery:**

It has been known traditionally that the dermatological drugs are delivered to the skin to treat the skin diseases or for disinfections of skin. One of the benefit of transdermal drug delivery is that the drugs are delivered for the longer duration of time.

Recently iontophoresis and electroporation are the electrically assisted drug delivery methods for transdermal purposes.

f) Subcutaneous drug delivery:

Subcutaneously inserted external molecule may produce undesirable response causing inflammation, carcinogenicity and immunogenicity. Hence biocompatibility is most important in case of subcutaneous drug delivery. Hydrogels are generally considered as biocompatible in nature due to the presence of large amount of water.

3) Genetic engineering:

Now a day's hydrogels are prominently used as a carrier in the field of genetic engineering. The purpose of using hydrogels is to deliver the specific biomolecule such as DNA, RNA, or proteins at the target site.

UNIT 1

An insight on the properties of the gelatin and PEG based composite hydrogels

ABSTRACT

The present study describes properties of the gelatin/PEG based composite hydrogels. The hydrogels were prepared by varying the concentration of gelatin and PEG. The gels were prepared either with or without glutaraldehyde, a chemical crosslinker. The microstructures of both the hydrogels were studied under scanning electron microscope after converting them into xerogels. The hydrogels were further characterized by XRD, FTIR spectroscopy and DSC studies. The swelling, mucoadhesivity, pH, impedance and hemocompatibility properties of the hydrogels were also studied. The *in vitro* release behavior of metronidazole (MZ, a model drug) was studied. The results suggested that the properties of the hydrogels were dependant on the proportion of the gelatin and PEG. The pHs of the hydrogels were in the range of 4.00-6.00 and were hemocompatible in nature. The release of MZ from the hydrogels followed zero order kinetics.

Keywords: Hydrogel, crosslinking, swelling ratio, zero order kinetics.

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Hydrogels are crosslinked polymeric constructs having the ability to imbibe and hold water [1-2]. Hydrogels may be categorized either as permanent or physical hydrogels [3]. Permanent hydrogels (e.g. pMMA, pHEMA, glutaraldehyde crosslinked polymeric constructs) are formed due to the formation of covalent bonds whereas physical hydrogels (e.g. gelatin gel, agar agar gel) are formed due to the physical interactions [4-5]. Both physical and permanent hydrogels have been extensively studied as matrices for controlled drug or nutraceutical delivery [6-7].Gelatin is a protein-based biopolymer and have been used for the development of various products of biomedical importance (including drug delivery, cell immobilization, tissue engineering, wound dressing) due to its proven biocompatibility [8-9]. Polyethylene glycol (PEG) is a water soluble polymer of ethylene oxide [10-11]. PEG has been reported to be inherently biocompatible in nature and have been explored widely for making delivery vehicles. The present study has been designed to develop gelatin and PEG-4000 based composite physical and permanent hydrogels. Permanent hydrogels were prepared using glutaraldehyde as crosslinking agent. The physical properties of the hydrogels were studied thoroughly and were explored as vehicles for the delivery of metronidazole (MZ), model antimicrobial drug).

- The current study purposes the formulation and evaluation of gelatin/PEG 4000 based composite hydrogels for controlled delivery of metronidazole (MZ).
- To study the biocompatibility and characteristics of these hydrogels .

CHAPTER 2

MATERIALS AND METHODS

Gelatin was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. PEG-4000 and trisodium citrate were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Ethanol was procured from Honyon International Inc., Hong Yang Chemical Corpn., China. Glutaraldehyde (25%, for synthesis; GA) and hydrocholric acid (35% pure) were obtained from Merck Specialties Private Limited, Mumbai, India. MZ was obtained as a kind gift from Aarti drugs Limited, Thane, India. Distilled water (DW) was used throughout the study.

2.1 Preparation of the hydrogels

Hundred gram of 20% (w/w) gelatin solution (GS) was prepared by dissolving 20 g of gelatin in 70 g warm DW followed by making up the volume to 100 g using warm DW. In a similar manner, 10% (w/w) PEG solution (PS) was also prepared in DW. Physical and crosslinked hydrogels were prepared by homogenizing (800 rpm using an overhead stirrer) various proportions of GS and PS at 45 ºC for 15 min. The compositions of the developed hydrogels have been given in table 1. The homogenized solutions were poured into culture bottles and allowed to form gel under refrigeration (5 ± 1 ^{o}C). The physical gels, so formed, were kept under refrigeration and were regarded as uF gels. Permanent hydrogels (cFs) were prepared by adding 1.1 ml of crosslinking reagent (0.5 ml GA+ 0.5 ml ethanol + 0.1 ml HCl) to 20 ml of GS-PS mixture, maintained at 45 ^{o}C. The mixture was subsequently homogenized for 30 sec at 800 rpm and immediately poured into culture bottles. The culture bottles were stored at room-temperature (RT, 25 ± 1 ^{o}C) to promote formation of gels. Drug loaded gels were also prepared in the same manner. 1 % (w/w) MZ was dissolved in GS-PS mixture for the development of the hydrogels. The drug loaded physical and permanent hydrogels were regarded as uFD and cFD, respectively.

Table 1: Composition of hydrogels.

Sr. no	sample code	Vol. of 20% w/w GS (ml)	Vol. of 10% w/w PS (ml)	MZ (% w/w)
1	uF1	20	0	-
2	uF2	16	4	-
3	uF3	12	8	-
4	uF4	8	12	-
5	cF1	20	0	-
6	cF2	16	4	-
7	cF3	12	8	-

8	cF4	8	12	-
9	uF1D	20	0	1
10	uF2D	16	4	1
11	uF3D	12	8	1
12	uF4D	8	12	1
13	cF1D	20	0	1
14	cF2D	16	4	1
15	cF3D	12	8	1
16	cF4D	8	12	1

2.4 Thermal studies

The melting point of the uFs was carried out by falling ball method as per the previously reported literature [14]. In short, 2g of the uFs were filled in 10 ml of test tube and incubated at 5 ^{o}C for 2 h. A stainless steel ball (diameter: 1 mm; weight: 130 mg) was placed over the surface of the gels. The samples were then heated at 1 °C/min in a silicone oil bath. The temperature at which the ball starts moving into the gels was recorded as the melting point onset temperature (T1). The temperature at which the ball is completely submerged was recorded as temperature where the gel has been completely converted into sol (T2). The mid-point of T1 and T2 was regarded as the melting point. The permanent gels do not show any gel-to-sol transitions.

2.7 Mucoadhesive nature of the permanent hydrogels by wash-off method

The mucoadhesive property of the permanent hydrogels were analyzed by modified USP disintegration method as per the method reported earlier [9]. The experiment was carried out using freshly excised goat intestine, celaned and was used within 2 h of collection. The intestinal lumen was longitudinally cut open and was attached to glass slide (10 mm x 35 mm) using acrylate adhesives such that the mucosal surface was exposed. The rectangular pieces of hydrogels (10 mm × 10 mm) were put placed on the exposed mucosal surface and a weight of 5 g was kept over it for a period of 5 min. The glass slides were then transferred to USP Disintegration baskets containing 900 ml of phosphate buffer (PB; pH= 7.2), kept at 37 ± 1 ^{o}C

[18]. The study was conducted for 24 h and the disintegration of the hydrogels were regularly checked and noted down.

2.8 Swelling Behavior-

Rectangular pieces of permanent hydrogels (10 mm x 10 mm) were accurately weighted (W_o) and subsequently immersed in 100 ml of DW. At regular intervals of time (15min for first 1h and 30 min for next interval), the hydrogels were then taken out, wiped and weighed (W_t) accurately. The swelling behavior was studied until the equilibrium weight was achieved [20-21].. Swelling ratio (SR) of the hydrogels was then calculated from equation 4 [22-23].

$$SR = \frac{(W_t - W_0)}{W_0} . \qquad (4)$$

2.9 *In vitro* drug release

The drug release studies of the hydrogels were carried out in an in-house made modified Franz's diffusion cell. 1.5g of the molten physical hydrogels were poured into the donor of the diffusion cell. The donor compartment was lowered into the receptor, containing 50 ml of DW, such that donor and the receptor are in contact with each other and separated by a dialysis membrane. The receptor fluid was maintained at 37 ± 2 ^oC and stirred at 100 ± 5 rpm**.** At predetermined intervals of time, the receptor fluid was replaced with fresh DW. The experiment was carried out for 8 h.

The drug release studies of the permanent hydrogels were carried out in a 8- basket USP dissolution apparatus rate test apparatus for 8 h. The developed hydrogels were cut into rectangular pieces (5 cm x 5 cm) and were accurately weighed. The hydrogels were then put into the dissolution basket containing 900 ml of DW. The dissolution media was maintained at 37 ± 2 ^oC and stirred at 100 ± 5 rpm using a paddle. 5 ml of the dissolution fluid was withdrawn at predetermined regular intervals of time and was replaced with 5 ml of fresh DW. The experiment was conducted for a period of 8 h.

The collected samples (of both physical and permanent hydrogels) were analyzed in a uv-visible spectrophotometer (UV-3200 Double Beam Spectrophotometer, LAB INDIA, India) at 321 nm.

2.11 pH Measurement

The pH of hydrogels were measured by using a digital pH meter (Model 132E, EI products, Mumbai, India) [24]. The probe of the pH meter was dipped into the warm mixtures and the corresponding pH readings were noted [25].

2.12 Hemocompatibility studies

The hemocompatibility of the formulation was analyzed as per the method reported earlier [26]. Fresh citrated goat blood was used for the study. 100 ml of the citrated blood contained 3.8 g of trisodium citrate (TSC). TSC acts as an anticoagulant. 8 ml of the citrated blood was diluted with 10 ml of the normal saline. The leachants of the physical gels were used for the study whereas rectangular pieces of 10 mm x 10 mm of permanent hydrogels were used for the study.

1 g of the physical hydrogels were tied in a dialysis tubing and was dipped into 20 ml of PB and incubated at 37 ± 1 ^{o}C for a period of 1 h so as to allow the leaching of the harmful components (if any) to the external media (PB). 0.5 ml of the diluted blood was added to the 0.5 ml lechant. This mixture of the leachant and the diluted blood formed the test sample for the physical hydrogels. For the analysis of the permanent hydrogels, the pieces of the hydrogels were put in a mixture of 0.5 ml of diluted blood and 0.5 ml of saline. This formed the test sample for the permanent hydrogels. A mixture of 0.5 ml of the diluted blood and 0.5 ml 0.01 N HCl formed positive control whereas a mixture of 0.5 ml of the diluted blood and 0.5 ml of saline formed negative control. The entire test and the control samples were diluted to 10 ml with saline and incubated at 37^{o}C for 1 h. After 1 h of incubation, the samples were centrifuged at 3000 rpm for 15 min and the absorbance of the supernatant was taken at 545 nm using a uv-visible double beam spectrophotometer. % hemolysis was calculated from equation 5: [27].

$$\%\ Hemolysis = \frac{OD_{test} - OD_{Negative}}{OD_{positive} - OD_{Negative}} \times 100 \qquad (5)$$

The sample is said to be highly hemocompatible if the % hemolysis is <5 then, a value <10 indicates hemocompatible, while a value > 20 indicates non-hemocompatible [9].

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Preparation of Hydrogels-

The compositions of the developed hydrogels have been tabulated in table 3. The formations of the hydrogels were confirmed by the inverted tube method (figure S1). The transparency of the physical hydrogels (uFs) was increased with the increase in the proportion of the PS. The textures of the hydrogels were dependent on the concentration of the gelatin and PEG. Higher the proportion of gelatin was, firmer was the strength of the hydrogels. On the other hand, the hydrogels became softer with the increase in the proportions of PEG. Similar results were also obtained for the permanent hydrogels (cFs). The permanent hydrogels were pale brown in color. The saturation of the color was reduced with the reduction in the gelatin concentration.

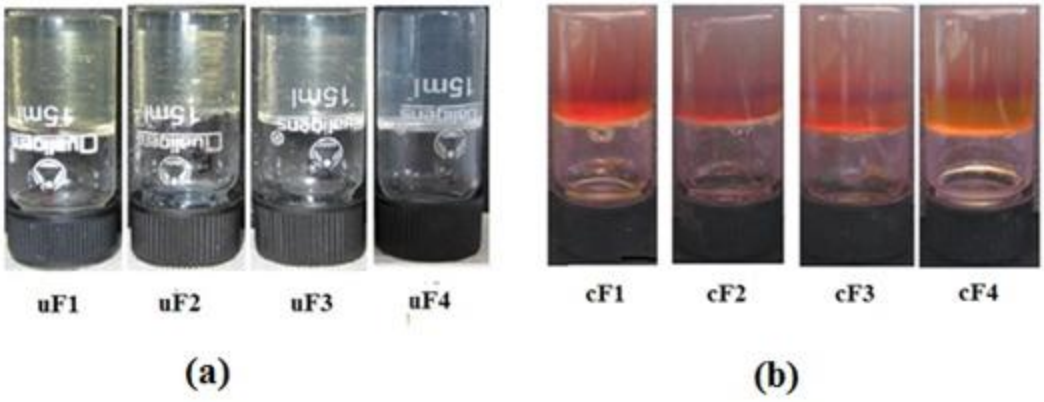


Figure S1: Inverted tube method to determine the gel formation of physical and permanent hydrogels

Table 3: Composition of Hydrogels

SR. NO	SAMPLE CODE	Vol. of 20% gelatin sol (ml)	Vol. of 10% PEG sol (ml)	GELATION PROPERTY	ODOUR
1	uF1	20	0	Gel formed	Pleasant
2	uF2	16	4	Gel formed	Pleasant
3	uF3	12	8	Gel formed	Pleasant
4	uF4	8	12	Gel formed	Pleasant

5	cF1	20	0	Gel formed	Pleasant
6	cF2	16	4	Gel formed	Pleasant
7	cF3	12	8	Gel formed	Pleasant
8	cF4	8	12	Gel formed	Pleasant

3.2 Melting point:

The melting points of the physical hydrogels were determined by the falling ball method and have been tabulated in table 4. The results suggested that the melting point was dependent on the concentration of gelatin and PEG in the samples. In general, there was a decrease in the melting temperature with the increase in the PEG concentration. This may be associated with the low melting point of the PEG used [33].

Table 4: Melting point studies of physical hydrogels

Sample code	Melting Temperature(°C)
PEG	55.7 ± 0.3
uF1	28.2 ± 0.9
uF2	25.66 ± 0.8
uF3	23.7 ± 1.1
uF4	20.63 ± 1.3

3.3 Mucoadhesive Property

Mucoadhesive properties of the permanent hydrogels were studied by *in vitro* wash-off method (modified USP disintegration test). The results showed that cF3 and cF4 hydrogels got detached at 1080 ± 20 min and 560 ± 25 min, respectively, from the mucosal surface. The cF1 and cF2 hydrogels did not detach during the experimental duration. The results suggested that the mucoadhesivity of the cF1 and cF2 hydrogels were much higher as compared to the cF3 and cF4.

3.4 Swelling Behavior-

The swelling properties of the permanent hydrogels were studied in-depth. The swelling profile of the hydrogels provides an insight about the water absorbing capacity of the hydrogels (figure 5). The swelling studies suggested that there was a decrease in the water up-take capacity of the hydrogels as the proportion of PEG was increased and were in the following order of cF1 > cF2 > cF3 > cF4. Swelling behavior of these hydrogels may be accounted to the hydration of gelatin polymeric chains by the capillary phenomenon [45]. It is known that the presence of protein in the hydrogel leads to a higher absorption of water due to the presence of polar groups. As the proportion of PEG was increased, there was a corresponding decrease in the proportion of GS which, in turn, resulted in the reduction of the polar groups per unit fraction of the hydrogel. This caused a reduction in the swelling of the hydrogels with higher proportions of PEG [46].

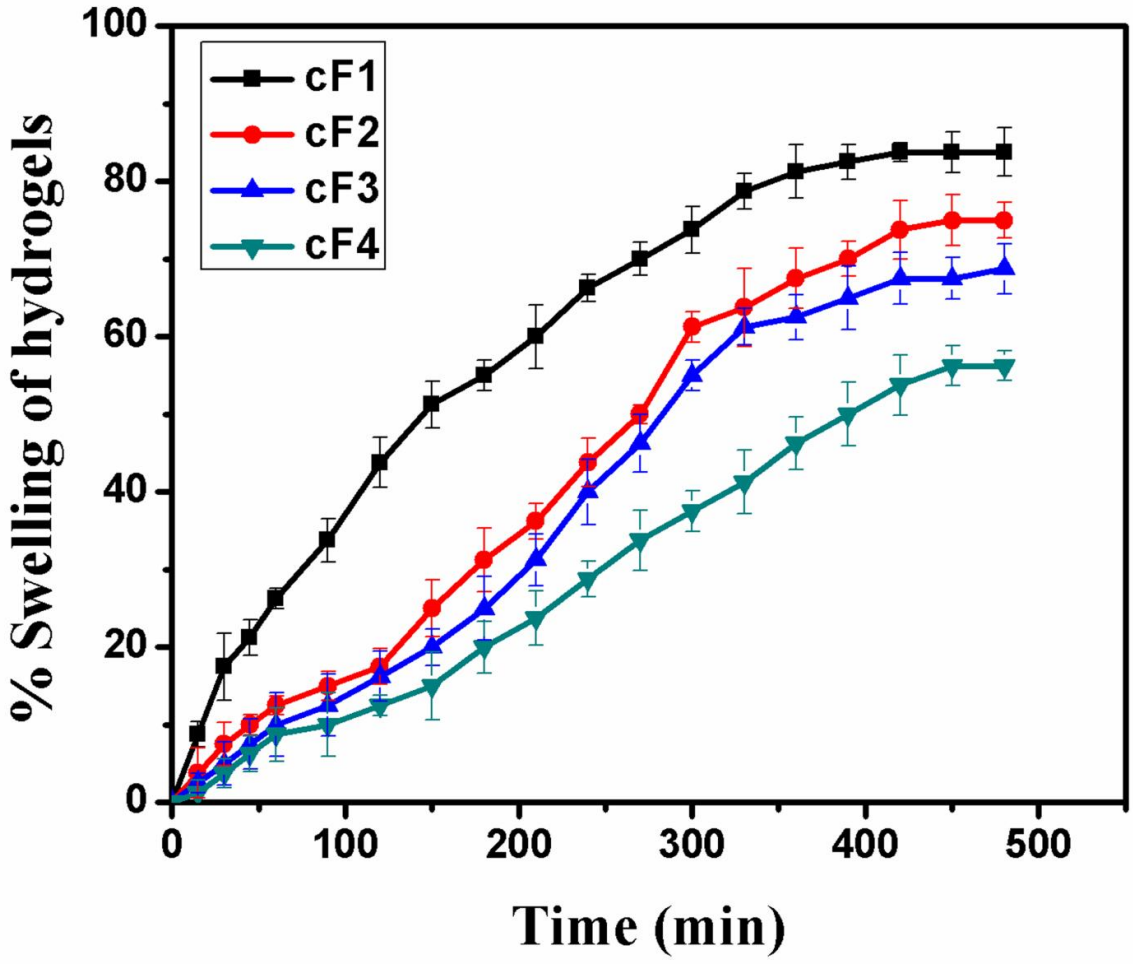


Figure 2: Swelling studies of permanent hydrogels

3.9 *In vitro* drug release

The release profiles of MZ from the physical hydrogels have been shown in figure 6. The drug release decreased in the following order uF1D>uF2D>uF3D>uF4D. Macromolecular relaxation of the polymer chains increase the mobility of the penetrating solvent and the drug [47]. Impedance studies suggested that the conductivity of the hydrogels with higher PEG was lower indicating the lower degree of polymer relaxation. Similar pattern was also observed in permanent hydrogels. This may be due to decrease in the hydrophilic groups in hydrogels with higher proportions of PEG, which lowered the migration of water into the hydrogels. The % release of the drug from the physical hydrogels was much higher as compared to permanent hydrogels. This may be accounted to the crosslinking of the matrices, which hampered the migration of the drugs within the matrices. The release of the drugs from the uF1D, uF2D and uF4D followed first-order release kinetics suggesting swelling independent release profile[48]. uF3D followed Higuchi release kinetics suggesting that the formulation was matrix type. The drug release kinetics from the permanent hydrogels suggested zero-order release kinetics (table 10).

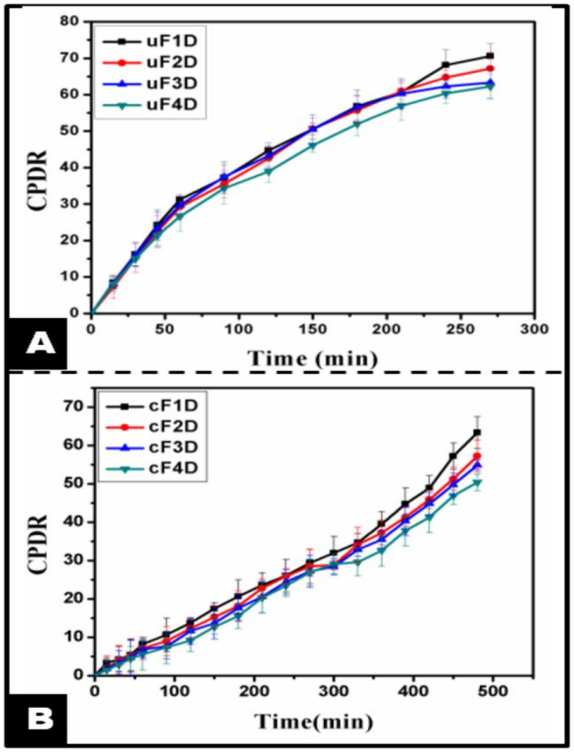


Figure 3: drug release studies of (A) physical and (B) permanent hydrogels

3.11 pH Measurement

The pH of the biomedical devices plays an important in deciding the applicability of the device for a particular application. If the pH of the device do not comply with the pH of the tissues, with which the device is to remain in contact, there might be cellular irritations in addition to serious immunological reactions [25]. The pHs of the physical hydrogels was ~5.75 whereas the pHs of the permanent hydrogels was ~4.7. The results suggested that the hydrogels may be used as matrices for drug delivery (table 12).

Table 12:pH of uFs and cFs hydrogels

Sample code	pH
uF1	5.72 ± 0.32
uF2	5.73±0.11
uF3	5.75 ± 0.24
uF4	5.74 ± 0.15
cF1	4.67 ± 0.14
cF2	4.69 ± 0.32
cF3	4.70 ± 0.23
cF4	4.77 ±0.17

3.12 Biocompatibility

The results of the hemocompatibility studies of the hydrogels have been tabulated in table 13. The results suggested that the developed hydrogels were highly hemocompatible as the % hemolysis was <5 %. The results suggested that the hydrogels may be biocompatible in nature.

Table 13: Biocompatibility study

Sample	% Hemolysis of crosslinked samples
uF1	2.42 ± 0.09
uF2	1.89 ± 0.06

uF3	1.52 ± 0.02
uF4	1.13 ± 0.06
cF1	4.4 ± 0.07
cF2	3.1 ± 0.08
cF3	2.9 ± 0.05
cF4	2.7 ± 0.10

CHAPTER 4

CONCLUSION

The study discusses the development of gelatine/PEG based composite hydrogels. The physical properties of the hydrogels were studied in-depth. The results suggested that the mechanical and the electrical properties of the composite hydrogels may be tailored by varying the composition of the hydrogels. Tailoring the composition of the hydrogels altered the rate of migration of solvent into the hydrogel matrices, which in turn, affected the release of the drug molecules from the hydrogel matrices. The hydrogels were found to be hemocompatible in nature. Based on the preliminary studies, the composite hydrogels were found to be suitable for controlled drug delivery.

CHAPTER 5

BIBLIOGRAPHY

1 Hoare, T. R. & Kohane, D. S. Hydrogels in drug delivery: Progress and challenges. *Polymer* **49**, 1993-2007 (2008).

2 Pal, K., Banthia, A. & Majumdar, D. Polymeric hydrogels: Characterization and biomedical applications. *Designed Monom and Polym* **12**, 197-220 (2009).

3 Aguzzi, A. & O'Connor, T. Protein aggregation diseases: pathogenicity and therapeutic perspectives. *Nat. Rev. Drug Disc.* **9**, 237-248 (2010).

4 Bordi, F., Paradossi, G., Rinaldi, C. & Ruzicka, B. Chemical and physical hydrogels: two casesystems studied by quasi elastic light scattering. *Phy. A: Stat. Mech. and its Appl.*, **304**, 119-128 (2002).

5 Hoffman, A. S. Hydrogels for biomedical applications. *Adv. drug delivery rev.*, **54**, 3-12 (2002).

6 Gupta, P., Vermani, K. & Garg, S. Hydrogels: from controlled release to pH-responsive drug delivery. *Drug Disc. Tod.*, **7**, 569-579 (2002).

7 Lozinsky, V. I. *et al.* Polymeric cryogels as promising materials of biotechnological interest. *Trends in Biotech.*, **21**, 445-451 (2003).

8 Taylor, S., Kabourek, J. & Hefle, S. Fish allergy: fish and products thereof. *J. of food sci.*, **69**, R175-R180 (2004).

9 Mallick, S. P. *Gelatin based emulsion hydrogels as a matrix for controlled delivery*, (2011).

10 Li, Y. *et al.* Nanostructured PEG-based hydrogels with tunable physical properties for gene delivery to human mesenchymal stem cells. *Biomat.*, **33**, 6533-6541 (2012).

11 Bayramoglu, G., Altintas, B., Yilmaz, M. & Arica, M. Y. Immobilization of chloroperoxidase onto highly hydrophilic polyethylene chains via bio-conjugation: Catalytic properties and stabilities. *Bioresource Tech.*, **102**, 475-482 (2011).

12 Pal, K., Banthia, A. & Majumdar, D. Polyvinyl alcohol—gelatin patches of salicylic acid: preparation, characterization and drug release studies. *J. of biomat. appl.*, **21**, 75-91 (2006).

13 Behera, B., Sagiri, S. S., Pal, K. & Srivastava, A. Modulating the physical properties of sunflower oil and sorbitan monopalmitate-based organogels. *J. of Appl. Polym. Sci.*, **127**, 4910-4917 (2013).

14 Raghavan, S. R. & Cipriano, B. H. in *Mol. Gels,* 241-252 (Springer, 2006).

15 Shibayama, M., Mizutani, S.-y. & Nomura, S. Thermal properties of copolymer gels containing N-isopropylacrylamide. *Macromol.,* **29**, 2019-2024 (1996).

16 Huysmans, M. C., Longbottom, C., Pitts, N. B., Los, P. & Bruce, P. G. Impedance spectroscopy of teeth with and without approximal caries lesions--an in vitro study. *J. Dent Res.,* **75**, 1871-1878 (1996).

17 Sadough, S., Rahmani, M. & Pouyafar, V. Rheological behavior, microstructure and hardness of A356 aluminum alloy in semisolid state using backward extrusion process. *Trans. of Nonfer. Met. Soc. of China*, **20**, s906-s910 (2010).

18 Tindall, J. A., Kunkel, J. R. & Anderson, D. E. *Unsat. zone hydrology for scientists and eng.*, (Prentice Hall Upper Saddle River, New Jersey, 1999).

19 Rajput, G. *et al.* Stomach specific mucoadhesive tablets as controlled drug delivery system: A review work. *Int J Pharm Biol Res.*, **1**, 30-41 (2010).

20 Basu, S. & Bandyopadhyay, A. K. Characterization of mucoadhesive nasal gels containing midazolam hydrochloride prepared from Linum usitatissimum L. mucilage. *Brazilian J.l of Pharma Sci.,* **47**, 817-823 (2011).

21 Balakrishnan, B., Mohanty, M., Umashankar, P. & Jayakrishnan, A. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomat.,* **26**, 6335-6342 (2005).

22 Thakur, G. *et al.* Crosslinking of gelatin-based drug carriers by genipin induces changes in drug kinetic profiles in vitro. *J. of Mat. Sci.: Mat. in Med.* **22**, 115-123 (2011).

23 Lee, W. F. & Chen, Y. J. Studies on preparation and swelling properties of the N-isopropylacrylamide/chitosan semi-IPN and IPN hydrogels. *J. of appl. polym. sci.,* **82**, 2487-2496 (2001).

24 Sutar, P. B., Mishra, R. K., Pal, K. & Banthia, A. K. Development of pH sensitive polyacrylamide grafted pectin hydrogel for controlled drug delivery system. *J. of Mat. Sci.: Mat. in Med.,* **19**, 2247-2253 (2008).

25 Shu, X., Zhu, K. & Song, W. Novel pH-sensitive citrate cross-linked chitosan film for drug controlled release. *Int. j. of pharma.,* **212**, 19-28 (2001).

26 Dong, L.-c. & Hoffman, A. S. A novel approach for preparation of pH-sensitive hydrogels for enteric drug delivery. *J. of Controlled. Release.,* **15**, 141-152 (1991).

27 Pal, K., Bag, S. & Pal, S. Development and coating of porous ultra high molecular weight polyethylene plates. *Trends Biomater. Artif. Org.,* **19**, 39-45 (2005).

28 Pal, K., Banthia, A. K. & Majumdar, D. K. Preparation and characterization of polyvinyl alcohol-gelatin hydrogel membranes for biomedical applications. *AAPS PharmSciTech.,* **8**, 142-146 (2007).

29 Firoozmand, H. & Rousseau, D. Microstructure and elastic modulus of phase-separated gelatin–starch hydrogels containing dispersed oil droplets. *Food Hydrocolloids* **30**, 333-342, doi:http://dx.doi.org/10.1016/j.foodhyd.2012.06.010 (2013).

30 Parmar, K. R., Shah, S. R. & Sheth, N. R. Studies in Dissolution Enhancement of Ezetimibe by Solid Dispersions in Combination with a Surface Adsorbent.

31 Kim, S., Nimni, M. E., Yang, Z. & Han, B. Chitosan/gelatin–based films crosslinked by proanthocyanidin. *J. of Biomed. Mat. Res. Part B: Applied Biomat.,* **75**, 442-450 (2005).

32 Purushothaman, M. & Vijaya, R. Formulation optimization and release kinetics of Metronidazole matrix, compression and spray coated tablets: Effect of organic acid on colon targeted drug delivery system. *Int J Res Pharm Sci.,* **1**, 551-562 (2010).

33 Joris, L., Schleyer, P. v. R. & Gleiter, R. Cyclopropane rings as proton-acceptor groups in hydrogen bonding. *J. of the American Chem. Soc.,* **90**, 327-336 (1968).

34 Perissutti, B., Newton, J. M., Podczeck, F. & Rubessa, F. Preparation of extruded carbamazepine and PEG 4000 as a potential rapid release dosage form. *Euro. j. of pharm. and biopharma.,* **53**, 125-132 (2002).

35 Leo, C., Subba Rao, G. & Chowdari, B. Studies on plasticized PEO–lithium triflate–ceramic filler composite electrolyte system. *Solid State Ionics* **148**, 159-171 (2002).

36 Pradhan, D. K., Samantaray, B., Choudhary, R. N. P. & Thakur, A. K. Effect of plasticizer on microstructure and electrical properties of a sodium ion conducting composite polymer electrolyte. *Ionics* **11**, 95-102 (2005).

37 Wieczorek, W., Such, K., Wyciślik, H. & Płocharski, J. Modifications of crystalline structure of PEO polymer electrolytes with ceramic additives. *Solid State Ionics* **36**, 255-257 (1989).

38 Smith, G., Duffy, A. P., Shen, J. & Olliff, C. J. Dielectric relaxation spectroscopy and some applications in the pharmaceutical sciences. *J. of pharm. sci.,* **84**, 1029-1044 (1995).

39 Murphy, E. & Morgan, S. The dielectric properties of insulating materials. *Bell Syst. Tech. J.,l* **16**, 493-512 (1937).

40 Chelkowski, A. (Elsevier, Amsterdam).

41 Pradhan, D. K., Choudhary, R. & Samantaray, B. Studies of structural, thermal and electrical behavior of polymer nanocomposite electrolytes. *Expr. Polym. Letters,* **2**, 630-638 (2008).

42 Alphonse, P., Bleta, R. & Soules, R. Effect of PEG on rheology and stability of nanocrystalline titania hydrosols. *J. of colloid and interface sci.,* **337**, 81-87 (2009).

43 Dhakar, R. C. *et al.* Rosiglitazone maleate microspheres for extending drug release: formulation and evaluation. *Int. J. pharma res. & dev.,* **2**, 56-65 (2010).

44 Nep, E. I. & Conway, B. R. Grewia Gum 2: Mucoadhesive Properties of Compacts and Gels. *Trop. J. of Pharm. Res.,* **10**, 393-401 (2011).

45 Lai, J.-Y. & Li, Y.-T. Functional assessment of cross-linked porous gelatin hydrogels for bioengineered cell sheet carriers. *Biomacromol.* **11**, 1387-1397 (2010).

46 Buhus, G., Peptu, C., Popa, M. & Desbrieres, J. Controlled release of water soluble antibiotics by carboxymethylcellulose-and gelatin-based hydrogels crosslinked with epichlorohydrin. *Cellulose Chem. & Tech.,* **43**, 141 (2009).

47 Mi, F.-L., Kuan, C.-Y., Shyu, S.-S., Lee, S.-T. & Chang, S.-F. The study of gelation kinetics and chain-relaxation properties of glutaraldehyde-cross-linked chitosan gel and their effects on microspheres preparation and drug release. *Carbohydrate Polym.* **41**, 389-396, doi:http://dx.doi.org/10.1016/S0144-8617(99)00104-6 (2000).

48 Zarzycki, R., MODRZEJEWSKA, Z. & NAWROTEK, K. Drug release from hydrogel matrices. *Ecolog. Chem. and Eng.,. S Selected full texts* **17**, 117-136 (2010).

UNIT 2

Modulating gelatin based physical hydrogels by various polysaccharides

ABSTRACT

The current study emphasizes on the properties of the gelatin and different polysaccharides based composite physical hydrogels (PHs). PH was prepared by varying the concentration of gelatin and polysaccharides in different ratios. The molecular interactions within the gel components were studied by FTIR spectroscopy. Mechanical properties were understood by using texture analyzer. The pH, impedance spectroscopy and hemocompatibility properties were also analyzed. The in vitro release profile of metronidazole (MZ) loaded PHs was studied for 8 h. The antibacterial efficiency of the MZ loaded PH was compared with that of the marketed MZ gel. FTIR spectroscopy suggested existence of intermolecular hydrogen bonding among the gel components. All the gels have shown good mechanical strength which varied according to the polysaccharides concentration. The impedance spectroscopy studies suggested capacitive dominant nature of the gels. The pH of the gels was in the range of 5.0-6.0 and the gels were highly hemocompatible in nature. The drug release profile of MZ followed zero order and Higuchian kinetic pattern. The PHs has shown comparable or better antimicrobial efficiency against gram positive and gram negative bacterium. Hence the developed gels may be used as a carrier for controlled drug delivery of bioactive agents.

Keywords: physical hydrogels, gelatin, polysaccharides, impedance spectroscopy

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Hydrogels are the cross-linked network composed of water soluble polymers which holds large amount of water or biological fluid [1-2]. Due to the presence of functional groups such as –OH, -CONH, -NH2, -COOH, hydrogels have greater ability to imbibe water in large extent [2-3]. Also formulations of hydrogels can be made into the different physical forms such as microparticles, nanoparticles and films. Hydrogels are the novel systems for the delivery of drugs which protect the drug from the enzymatic degradation and acidic environment. These hydrogels when put in excess water shows greater swelling capacity thereby retaining the water There are two types of hydrogels i.e. physical hydrogels and permanent hydrogels [4]. Physical hydrogels are those which are formed by the physical interactions in between the polymer(s) while permanent hydrogels are those which are covalently crosslinked with the help of crosslinking agent. Physical hydrogels can be defined as the network of polymers entangled with the molecular interactions like ionic, hydrogen bonding, and hydrophobic forces [5]. Besides pharmaceutical applicability hydrogels can also be used in genetic engineering for the delivery of biomolecules such as proteins, nucleic acid etc [6].

Gelatin is a protein obtained from the partial hydrolysis of collagen which has high levels of hydroxyproline, proline and glycine [7]. Due to its unique chemical and physical properties gelatin is commonly used in pharmaceutical, cosmetic and photographic purposes [8]. Gelatin finds its wider application in capsule preparation in pharmaceutical industry [9-10].

Sodium salt of carboxymethyl cellulose (SC) is a hydrophilic polymer of cellulose ether also referred as cellulose gum, sodium cellulose glycolate or simply carboxymethyl cellulose [11]. Due to the colloidal, binding, thickening, absorbing, stabilizing and film forming properties, it is widely used in the food, detergent, cosmetics, pharmaceutical industries [11].

Maltodextrin (MD) is a non ionic dextrin polysaccharide product obtained enzymatically from starch [7, 12].

Dextran (DX) a natural hydrophilic polysaccharide can be degraded easily [13]. It is a polymer of glucose in which α-1,6- glucosidic linkages are predominant [14]. It is immensely used in food industries as a thickener, an emulsifier and a stabilizers [15].

Taking inspiration from the aforementioned properties, it was decided to prepare physical hydrogels using gelatin and polysaccharides in different combinations. The study describes the preparation and characterization of physical hydrogels using gelatin and the polysaccharides in different compositions (gelatin (GH), gelatin- sodium carboxymethyl cellulose (GH-SC), gelatin-

maltodextrin (GH-MD), and gelatin-dextran (GH-DX). The gels were compared with the blank gelatin gels and their characterization was done to find its possible applicability in drug delivery system.

- To design gelatin and polysaccharide based physical hydrogel vehicles for controlled drug delivery.
- To study the biocompatibility and characteristics of these hydrogels.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Gelatin (GH), maltodextrin (MD) and dextran (DX) were purchased from Himedia, Mumbai, India. Sodium carboxy methyl cellulose (SC) was brought from RFCL, New Delhi. Trisodium citrate was procured from Loba Chemie Pvt. Ltd., Mumbai, India. Nutrient agar and dialysis tubing (MW cutoff: 60 kDa) were purchased from Himedia, Mumbai, India. Microbial cultures of *Bacillus subtilis* (NCIM 2699) and *Escheratia coli* (NCIM 2563) were obtained from NCIM, Pune, India. Metronidazole (MZ) was a gift sample from Arti drugs, India. Hydrochloric acid (HCl) was purchased from Merck specialist Pvt. Ltd. Mumbai, India. Ethanol was purchased from Honyon International Inc., Hong Yang chemical corporation, China. Goat blood was collected from the local butcher shop. Double Distilled water (DW) was used to carry out the experiment.

2.2 Methods

2.2.1 **Preparation of physical gel solutions**

Fresh 20% (w/w) stock solution of gelatin and 2% (w/w) stock solution of SC, DX and MD were prepared by stirring the mixture at ~50°C until a transparent solution was obtained. The physical hydrogel formulations were prepared by mixing the stock solution in different proportions as shown in table 1. The mixture was stirred for 10 mins at 500 rpm to confirm uniform mixing. The prepared gels were then kept in refrigerator for further analysis. 1% (w/w) MZ drug loaded samples were also prepared in the similar manner.

2.2.2 **Organoleptic evaluation**

The organoleptic behavior of the developed gels were evaluated physically for its colour, odour, texture, appearance and taste.

2.2.4 **pH measurement**

The pH of the formulations was measured using a digital pH meter (Model 132E, EI products, Mumbai, India) by dipping the probe into the freshly prepared hydrogels. The measurement was done in triplicate [19-20].

2.2.7 Thermal properties

The melting point of the developed hydrogels was determined by the falling ball method as per the reported literature [23]. The gel samples were melted and accurately weighed 2 g of the samples were transferred in 10 ml test tubes. The test tubes were further kept in freezer for 2h to form a solid gelled structure. A steel ball (110 mg, 1 mm) was then kept on the surface of the solid gels. Then samples were heated at the rate of 1^0C/min. The temperature at which the ball reached the bottom was regarded as melting point (T_m) of the gels.

2.2.9 Hemocompatibility studies

The hemocompatibility of the gel samples was calculated according to the earlier reported method [22]. In short, the citrated blood was diluted with the normal saline in the ratio of 8:10. The leachants of the gel samples were used for the study. The leachant preparation was done by putting ~ 1 g of sample in dialysis tubing which was further transferred in a conical flask containing 20 ml of phosphate buffer maintained at 37 ± 1°C for 1 h. After incubation 0.5 ml of leachant was mixed with 0.5 ml of diluted blood which is referred as test sample. The positive control was prepared by mixing 0.5 ml of diluted blood with 0.5 ml of 0.01 N HCl. However negative control was prepared by mixing 0.5 ml of diluted blood with 0.5 ml of normal saline. All the test samples including controls were diluted to 10 ml with normal saline and kept for incubation at 37°C for 1 h. After 1 h of incubation, the centrifugation of samples was carried out at 3000 rpm for 15 min and the absorption of the supernatant was carried out using UV-visible double beam spectrophotometer (UV-3200, LABINDIA, Mumbai, India) at 545 nm. The percentage hemolysis of the RBC's was calculated by using the formula [26].

$$\%\ Hemolysis = \frac{OD_{test} - OD_{Negative}}{OD_{positive} - OD_{Negative}} \times 100$$

Where,

OD_{test} - Absorbance of the test sample

$OD_{positive}$ - Absorbance of the positive control

$OD_{negative}$ - Absorbance of the negative control

2.2.10 *In vitro* drug release studies

The objective of this test was to study the release pattern of MZ from the drug loaded gel samples. In vitro drug delivery of the samples was carried out with the help of modified Franz diffusion cell. The release pattern was studied by weighing ~1.5 g MZ containing samples and was placed into the donor chamber of the diffusion cell. The chamber was separated with that of receptor chamber by dialysis membrane. 50 ml volume in the receptor chamber was kept maintained during the whole experiment. The study was carried out for 8 hours. During the experiment, at every 15 min sampling was performed for first hour and then subsequently at every 30 min for the next 7 hour. The sampling was done by replacing the whole receptor medium with that of the DW. Then finally the release of drug were studied by analyzing the presence of MZ at 321 nm using double beam UV-visible spectrophotometer (UV-3200, LABINDIA, Mumbai, India) [27].

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Preparation of physical hydrogels

The hydrogels were prepared by varying the types and proportion of polysaccharides with gelatin as shown in table 1. The formation of hydrogels was confirmed by inverted tube method. (figure 1). The samples were found to be transparent in nature. The transparency of the samples increased with the decrease in the concentration of gelatin.

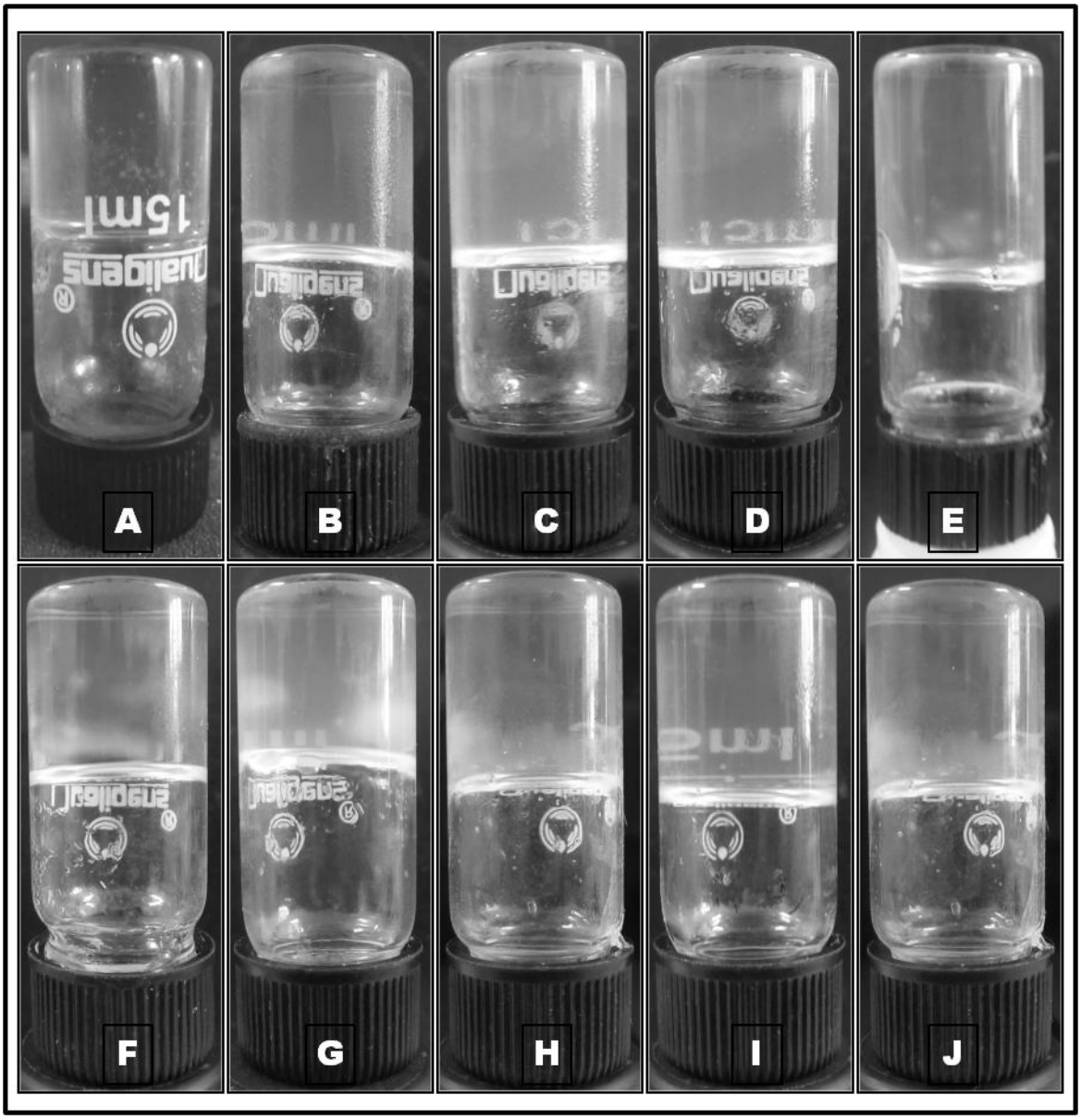


Figure 1: Confirmation of hydrogels by inverted tube method

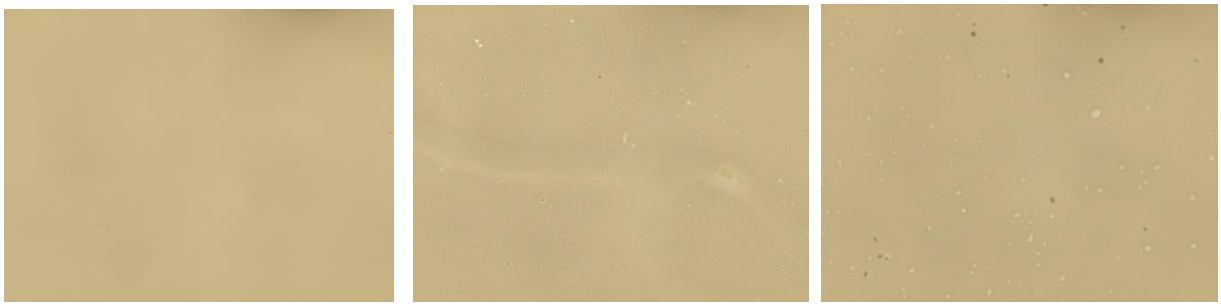
Table 1: Composition of physical hydrogels

S. No.	Sample code	Vol. of 20% (w/w) gelatin solution (ml)	Vol. of 2% (w/w) SC/MD/DX sol (ml)
1	GH	10	0
2	SC1	8	2
3	SC2	6	4
4	SC3	4	6
5	MD1	8	2
6	MD2	6	4
7	MD3	4	6
8	DX1	8	2
9	DX2	6	4
10	DX3	4	6

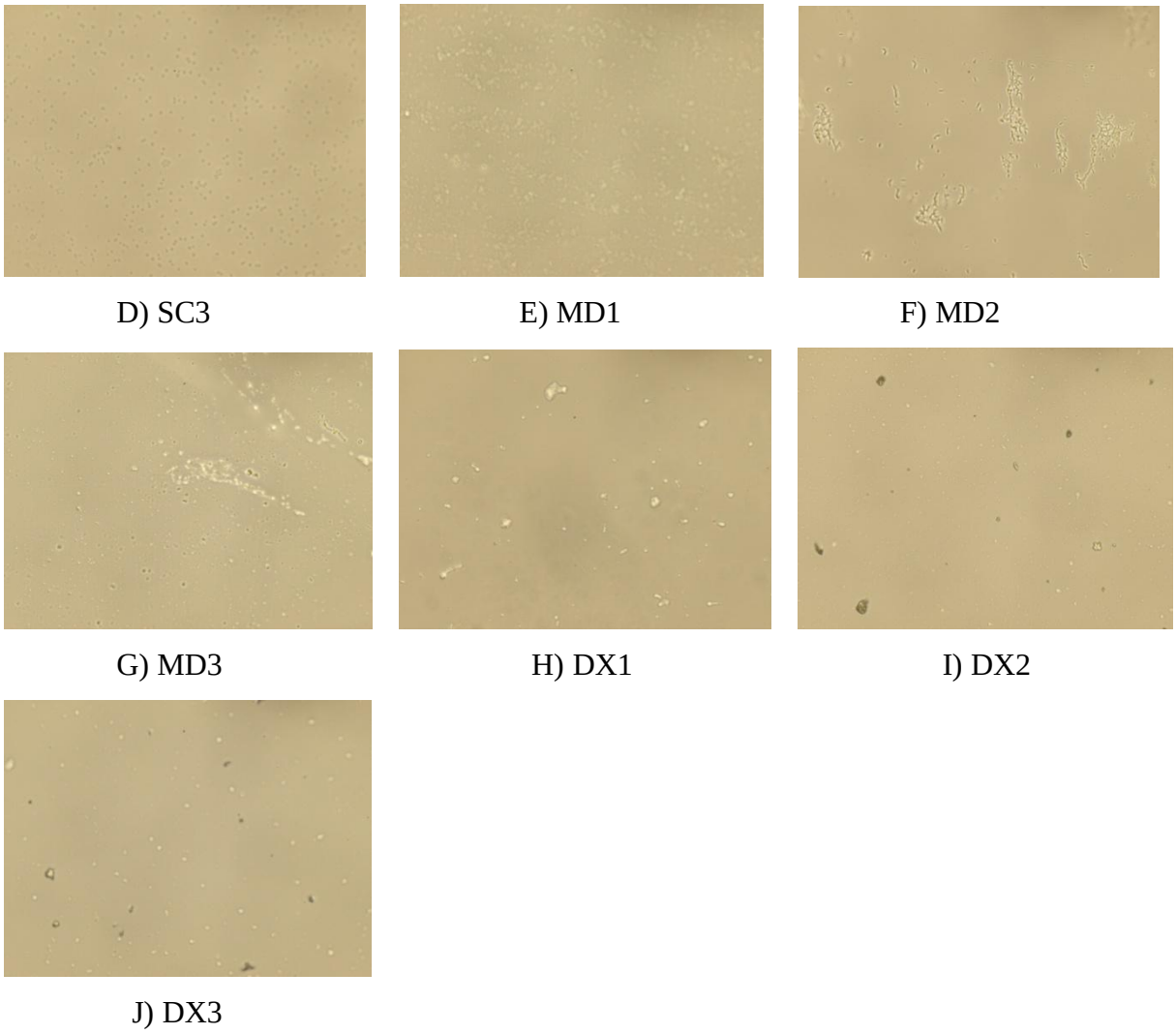
3.2 Organoleptic evaluation

The formulations were found to be homogenous and smooth in texture. The color of the formulations varied from light yellow to dark yellow. The intensity of the color increased with the increase in gelatin concentration. All the formulations were found to be odorless. They were sweet in taste. The sweetness increased with the increase in polysaccharide component.

3.3 Microscopic studies



A) GH B) SC1 C) SC2



D) SC3 E) MD1 F) MD2

G) MD3 H) DX1 I) DX2

J) DX3

Figure 2: phase contrast micrographs of the selected hydrogels

The microscopic evaluation of the hydrogels was done using phase contrast microscopy (PCM) and scanning electron microscopy (SEM). Bright field microscopy was unable to provide information about the structural organization of the gels. PCM images were taken at 40 x zoom. PCM micrographs have shown uniform type of arrangement throughout the matrix. No distinct demarcations were observed among the samples in the micrographs. Several spherical globules and elongated structures were observed distinctly which were dispersed non-uniformly though out the gel matrices.

pH Studies

The pH of hydrogel samples have been tabulated in the table 2. The pH of all the samples was found to be in the range of 5.0 to 6.0.

Table 2: pH determination of the physical hydrogels

Sample code	pH of physical hydrogels
GH	5.82 ± 0.02
SC1	5.72 ± 0.01
SC2	5.88 ± 0.04
SC3	5.95 ± 0.07
MD1	5.77 ± 0.06
MD2	5.80 ± 0.02
MD3	5.84 ± 0.03
DX1	5.86 ± 0.03
DX2	5.70 ± 0.04
DX3	5.64 ± 0.01

3.4 Thermal properties

The MPs of the selected hydrogels are shown in table 8. The MP of the samples was in the range of GH > SC > DX > MD. In general, the MP of the gels decreased with the decrease in gelatin concentration (DX and MD gels). The MP increased with decrease in gelatin concentration in SC gels. This indicated that increase in SC concentration resulted in strong molecular arrangement as compared to DX and MD where as gelatin gel has shown the strongest structural organization as compared to the SC/DX/MD gels.

Table 8: Melting point determination

Formulations	Melting Point (°C) ± SD
GH	29.4 ± 0.31
SC1	25.2 ± 0.54
SC2	27.5 ± 0.26
SC3	29.1 ± 0.23
MD1	19.7 ± 0.18
MD2	18.1 ± 0.22
MD3	16.9 ± 0.41
DX1	20.1 ± 0.27
DX2	19.4 ± 0.41
DX3	17.2 ± 0.51

3.9 Hemocompatibility studies

Table 11 shows the percent hemolysis of the selected hydrogels. The percent hemolysis < 5 % indicated that the developed gels may be regarded as highly hemocompatible.

Table 11: Hemocompatibilty studies

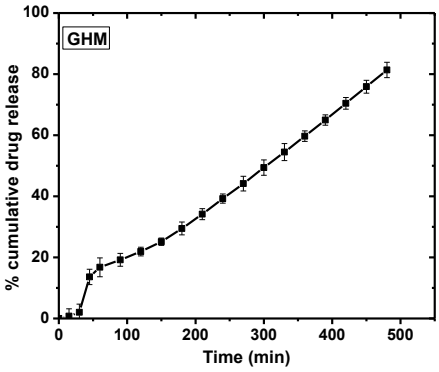
Formulations	% Hemolysis ± SD
GH1	3.21 ± 0.20
SC1	2.41 ± 0.11
SC2	1.81 ± 0.09
SC3	1.03 ± 0.14

MD1	1.84 ± 0.15
MD2	1.36 ± 0.07
MD3	0.69 ± 0.14
DX1	2.36 ± 0.16
DX2	1.64 ± 0.12
DX3	0.76 ± 0.28

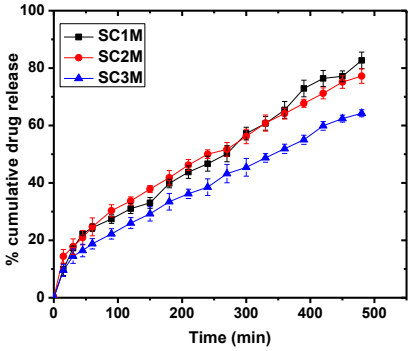
3.10 *In-vitro* drug release studies

The *in-vitro* release profile of MZ from different formulation matrices has been studied for 8 h and their cumulative percent drug release (CPDR) was calculated (Table 12). The drug release rate is highly dependent on the chemical composition of the formulation matrix. SCM samples have shown better CPDR as compared to MDM and DXM samples (SCM > MDM > DXM). It was also observed that the CPDR value decreased with the decrease in gelatin concentration and a corresponding increase in SC/MD/DX in all the samples.

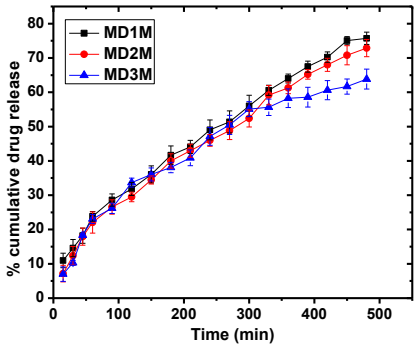
The drug release mechanism for MZ was predicted by fitting various kinetic model s.a. zero order, first order, Higuchian kinetics and Korsameyer- Peppas (KP) model. The drug release mechanism for the blank gelatin, the formulations containing higher concentration of gelatin (SC1M, MD1M and MD2M) and all the GH-DX formulations was found to follow zero order kinetics. This indicated that the release of MZ was constant rate process and was independent of concentration of the MZ incorporated into the gel matrix. SC2M, SC3M, MD gels (MD1M, MD2M, and MD3M) and DX1M has also followed Higuchian kinetic model indicating diffusion based mode of MZ-release. MD1M and MD2M has followed both zero order and Higuchian kinetic release pattern indicating concentration independent diffusion based drug release from the system. The n-value was determined from the KP model to understand the diffusion pattern of the MZ release from the gel matrix. The n-value > 0.45 indicated that the formulations followed non-Fickian diffusion [45-46].



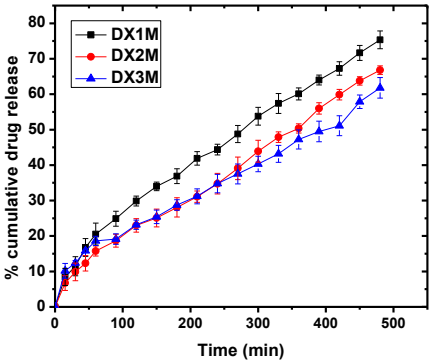
A) GELATIN



B) SCMC



B) MALTODEXTRIN



D) DEXTRA

Figure 16: *In vitro* drug release study

CHAPTER 4

CONCLUSION

The study reports the successful development of gelatin/polysaccharides based composite PHs. The physico-chemical properties of the hydrogels were studied in-depth. The results suggested that the mechanical and electrical properties of the developed hydrogels varied based on the variation of composition of gelatin/polysaccharide composition. Tailoring the composition affected the mechanism of drug release from the polymer composition. The developed hydrogels have shown zero order and Higuchian kinetics of drug release based on the gel composition suggesting concentration independent diffusion based release pattern. The diffusion kinetics was non-Fickian type. The gels were found to possess smooth texture and were highly hemocompatible. The preliminary results suggested that the gels may be used as a carrier for controlled drug delivery applications.

CHAPTER 5

ACKNOWLEDGEMENT

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CHAPTER 6

BIBLIOGRAPHY

1. Bardajee, G.R., et al., *UV-prepared salep-based nanoporous hydrogel for controlled release of tetracycline hydrochloride in colon.* Journal of Photochemistry and Photobiology B: Biology, 2011. **102**(3): p. 232-240.
2. Hamidi, M., A. Azadi, and P. Rafiei, *Hydrogel nanoparticles in drug delivery.* Advanced Drug Delivery Reviews, 2008. **60**(15): p. 1638-1649.
3. Vashist, A., Y.K. Gupta, and S. Ahmad, *Interpenetrating biopolymer network based hydrogels for an effective drug delivery system.* Carbohydrate Polymers, 2012. **87**(2): p. 1433-1439.
4. Aguzzi, A. and T. O'Connor, *Protein aggregation diseases: pathogenicity and therapeutic perspectives.* Nature Reviews Drug Discovery, 2010. **9**(3): p. 237-248.
5. Hoffman, A.S., *Hydrogels for biomedical applications.* Advanced Drug Delivery Reviews, 2012. **64, Supplement**(0): p. 18-23.
6. Haroun, A.A. and N.R. El-Halawany, *Encapsulation of bovine serum albumin within β-cyclodextrin/gelatin-based polymeric hydrogel for controlled protein drug release.* IRBM, 2010. **31**(4): p. 234-241.
7. Nickerson, M.T., et al., *Some physical properties of crosslinked gelatin–maltodextrin hydrogels.* Food Hydrocolloids, 2006. **20**(7): p. 1072-1079.
8. Balti, R., et al., *Extraction and functional properties of gelatin from the skin of cuttlefish (Sepia officinalis) using smooth hound crude acid protease-aided process.* Food Hydrocolloids, 2011. **25**(5): p. 943-950.
9. Bussemer, T. and R. Bodmeier, *Formulation parameters affecting the performance of coated gelatin capsules with pulsatile release profiles.* International Journal of Pharmaceutics, 2003. **267**(1–2): p. 59-68.
10. Zhang, N., et al., *Developing gelatin–starch blends for use as capsule materials.* Carbohydrate Polymers, 2013. **92**(1): p. 455-461.
11. Bhandari, P.N., D.D. Jones, and M.A. Hanna, *Carboxymethylation of cellulose using reactive extrusion.* Carbohydrate Polymers, 2012. **87**(3): p. 2246-2254.
12. Nickerson, M.T., et al., *Some physical and microstructural properties of genipin-crosslinked gelatin–maltodextrin hydrogels.* International Journal of Biological Macromolecules, 2006. **38**(1): p. 40-44.
13. Liu, Y. and M.B. Chan-Park, *Hydrogel based on interpenetrating polymer networks of dextran and gelatin for vascular tissue engineering.* Biomaterials, 2009. **30**(2): p. 196-207.
14. Wang, T., J. Nie, and D. Yang, *Dextran and gelatin based photocrosslinkable tissue adhesive.* Carbohydrate Polymers, 2012. **90**(4): p. 1428-1436.
15. Can, H.K., et al., *Effect of functional crosslinking agents on preparation and swelling properties of dextran hydrogels.* Carbohydrate Polymers, 2005. **59**(1): p. 51-56.
16. Song, J. and R.I. Hollingsworth, *Synthesis, conformational analysis, and phase characterization of a versatile self-assembling monoglucosyl diacylglycerol analog.* Journal of the American Chemical Society, 1999. **121**(9): p. 1851-1861.
17. Novales, B., et al., *Self-assembly of fatty acids and hydroxyl derivative salts.* Langmuir, 2008. **24**(1): p. 62-68.
18. Teodorescu, M., A. Lungu, and P.O. Stanescu, *Preparation and properties of novel slow-release NPK agrochemical formulations based on poly (acrylic acid) hydrogels and liquid fertilizers.* Industrial & Engineering Chemistry Research, 2009. **48**(14): p. 6527-6534.
19. Shu, X., K. Zhu, and W. Song, *Novel pH-sensitive citrate cross-linked chitosan film for drug controlled release.* International journal of pharmaceutics, 2001. **212**(1): p. 19-28.

20. Dong, L.-c. and A.S. Hoffman, *A novel approach for preparation of pH-sensitive hydrogels for enteric drug delivery.* Journal of Controlled Release, 1991. **15**(2): p. 141-152.
21. Vongsvivut, J., et al., *Quantitative determination of fatty acid compositions in micro-encapsulated fish-oil supplements using Fourier transform infrared (FTIR) spectroscopy.* Food Chemistry, 2012. **135**(2): p. 603-609.
22. Singh, V.K., et al., *Castor oil and sorbitan monopalmitate based organogel as a probable matrix for controlled drug delivery.* Journal of Applied Polymer Science, 2013.
23. Gaides, G.E. and A.J. McHugh, *Gelation in an amorphous polymer: a discussion of its relation to membrane formation.* Polymer, 1989. **30**(11): p. 2118-2123.
24. Satapathy, D., et al., *Sunflower-oil-based lecithin organogels as matrices for controlled drug delivery.* Journal of Applied Polymer Science, 2012.
25. Lee, K.H., et al., *Electrical Impedance of Spin-Coatable Ion Gel Films.* The Journal of Physical Chemistry B, 2011. **115**(13): p. 3315-3321.
26. Pal, K., A.K. Banthia, and D.K. Majumdar, *Preparation and characterization of polyvinyl alcohol-gelatin hydrogel membranes for biomedical applications.* AAPS PharmSciTech, 2007. **8**(1): p. 142-146.
27. Shah, D.K., et al., *Development of olive oil based organogels using sorbitan monopalmitate and sorbitan monostearate: A comparative study.* Journal of Applied Polymer Science, 2012.
28. Sagiri, S.S., et al., *Effect of Composition on the Properties of Tween-80–Span-80-Based Organogels.* Designed Monomers and Polymers, 2012. **15**(3): p. 253-273.
29. Saravanan, M. and K.P. Rao, *Pectin–gelatin and alginate–gelatin complex coacervation for controlled drug delivery: Influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules.* Carbohydrate Polymers, 2010. **80**(3): p. 808-816.
30. Muyonga, J., C. Cole, and K. Duodu, *Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (< i> Lates niloticus</i>).* Food Chemistry, 2004. **86**(3): p. 325-332.
31. Kim, S., et al., *Chitosan/gelatin–based films crosslinked by proanthocyanidin.* Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2005. **75**(2): p. 442-450.
32. Wang, X.-S., et al., *Homogenous polyelectrolyte complex membranes incorporated with strong ion-pairs with high pervaporation performance for dehydration of ethanol.* Journal of Membrane Science, 2013.
33. Chiu, H.-C., T. Hsiue, and W.-Y. Chen, *FTIR-ATR measurements of the ionization extent of acrylic acid within copolymerized methacrylated dextran/acrylic acid networks and its relation with pH/salt concentration-induced equilibrium swelling.* Polymer, 2004. **45**(5): p. 1627-1636.
34. Deng, Y., Q. Zhou, and S. Sun, *Analysis and discrimination of infant powdered milk via FTIR spectroscopy].* Guang pu xue yu guang pu fen xi= Guang pu, 2006. **26**(4): p. 636.
35. Purushothaman, M. and R. Vijaya, *Formulation optimization and release kinetics of Metronidazole matrix, compression and spray coated tablets: Effect of organic acid on colon targeted drug delivery system.* Int J Res Pharm Sci, 2010. **1**: p. 551-562.
36. Herculano, R.D., et al., *On the release of metronidazole from natural rubber latex membranes.* Materials Science and Engineering: C, 2011. **31**(2): p. 272-275.
37. Liu, Y., et al., *Thermal behavior and mechanical properties of physically crosslinked PVA/Gelatin hydrogels.* Journal of the Mechanical Behavior of Biomedical Materials, 2010. **3**(2): p. 203-209.
38. Durán, E., et al., *Low sugar bakery jams with gellan gum—guar gum mixtures. Influence of composition on texture.* Food Hydrocolloids, 1994. **8**(3–4): p. 373-381.
39. Winter, H.H. and F. Chambon, *Analysis of linear viscoelasticity of a crosslinking polymer at the gel point.* Journal of Rheology, 1986. **30**(2): p. 367-382.

40. Bot, A., et al., *Effect of Sterol Type on Structure of Tubules in Sterol + γ-Oryzanol-Based Organogels.* Food Biophysics, 2009. **4**(4): p. 266-272.
41. Zhang, Q., et al., *Physicochemical properties of nitrile-functionalized ionic liquids.* The Journal of Physical Chemistry B, 2007. **111**(11): p. 2864-2872.
42. Othman, L., K. Chew, and Z. Osman, *Impedance spectroscopy studies of poly (methyl methacrylate)-lithium salts polymer electrolyte systems.* Ionics, 2007. **13**(5): p. 337-342.
43. Shukla, A., et al., *Structural, microstructural and electrical studies of La and Cu doped BaTiO< sub> 3</sub> ceramics.* Physica B: Condensed Matter, 2010. **405**(1): p. 99-106.
44. Sinclair, D. and A. West, *Electrical properties of a LiTaO_ {3} single crystal.* Physical Review B, 1989. **39**(18): p. 13486.
45. Philip, A., M. Srivastava, and K. Pathak, *Buccoadhesive gels of glibenclamide: A means for achieving enhanced bioavailability.* Drug Delivery, 2009. **16**(7): p. 405-415.
46. Sawant, P.D., et al., *Drug release from hydroethanolic gels. Effect of drug's lipophilicity (log P), polymer–drug interactions and solvent lipophilicity.* International Journal of Pharmaceutics, 2010. **396**(1–2): p. 45-52.