High Viscosity Sodium Alginate and Gelatin Hydrogels - A study on the impact of Molecular weight of plasticizer on swelling behaviour

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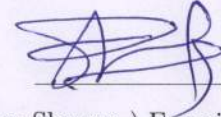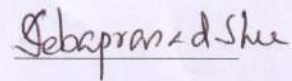
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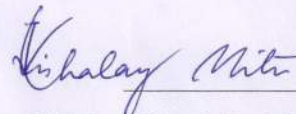
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Abstract

Biopolymers such as proteins and polysaccharides have a variety of applications in various fields including agriculture, biomedical and food industry. Blending of polymers is one of the simplest and cheapest way to enhance the properties of polymers. A polymeric blend of sodium alginate (SA) and gelatin display a unique pH responsive behavior which attracts them for their use in various fields. However, high water solubility and poor mechanical strength are the two main drawbacks of biomaterials prepared from them. Plasticizers are generally added to improve the mechanical properties of biomaterials by reducing the intermolecular forces. The objective of this study were to evaluate the effect of molecular weight (Mw) of plasticizer on the swelling behaviour of SA and gelatin hydrogels. Three different MW of Poly-ethylene Glycol (PEG) (2000, 4000 and 6000) were employed in SA/G hydrogels for this study.

The swelling experiments were carried out in Phosphate buffered saline (pH=7.4) and 0.1N HCl (pH=1.2) solution at $37\,^{\circ}$C. A high viscosity grade of SA was used in the blend to decrease the rate of disintegration in presence of solvent. An increase in content of high viscosity alginate increased the swelling ratio in PBS whereas in 0.1N HCl swelling ratio was found to decrease. Equilibrium swelling ratio (SR_{eq}) of SA/G hydrogels was found to decrease with the increase in Mw of PEG in PBS as well as in 0.1N HCl. Interestingly, it was found that Mw of PEG alters the intermolecular interactions between SA and Gelatin. FTIR spectra also reflects the variation in intermolecular interactions between SA and Gelatin chains due to the addition of different Mw of PEG. Scanning Electron microscopy (SEM) studies has shown that the miscibility of SA and Gelatin has decreased with the increase in Mw of PEG. Diffusion kinetics parameters were also evaluated and a considerable difference in nature of solvent diffusion was observed among SA/G hydrogels with PEG 2000, 4000 and 6000. Optimum viscosity level for the polysaccharide and lower Mw of PEG are found to be the most influencing properties to achieve controlled swelling of polysaccharide-protein blends with considerable mechanical stability.

Contents

Nomenclature

ν_{CH_2} Wavenumbers associated with $-CH_2$ peaks

ν_{OH} Wavenumbers associated with $-0H$ peaks

D Diffusion coefficient

DEA Diethanolamine

DEG Di-ethylene Glycol

EG Ethylene Glycol

F Fractional water uptake

GLY Glycerol

K_D Swelling constant

M_∞ Amount of solvent diffused into the hydrogel at infinite time

M_t Amount of solvent diffused into the hydrogel at time t

Mw Molecular Weight

n Swelling exponent

PE Plasticizing Efficiency

PEG Poly-ethylene Glycol

PG Propylene Glycol

r Radius of hydrogel

SA Sodium Alginate

SEM Scanning Electron Microscope

SR Swelling Ratio

SR_{eq} Equilibrium Swelling Ratio

TEA Triethanolamine

TEG Tri-ethylene glycol

W_d Initial dry weight hydrogel

W_s Weight of swollen hydrogel

W_{eq} Weight of equilibrium swollen hydrogel

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Chapter 1

Introduction

1.1 Motivation

In recent years, the research community has been focused on the materials prepared from biopolymers mainly because of their biodegradability. Biomaterials prepared from biodegradable polymers have been used extensively in various areas including agriculture, food and biomedical industry. The most important property of these polymers their ability to imbibe high amount of solvent due to high hydrophicity i.e. swelling capacity. Major limitation addressed by these polymers is their degradation in the presence of water. The degradation process involves diffusion of water into the hydrophilic polymers leading to the formation of a swollen system which ultimately dissolves on further uptake of water. Various efforts have been made to control their swelling and degradation in the presence of solvent. The additives like plasticizers and cross-linkers are generally used to improve their mechanical and swelling properties. Increasing the stability of biomaterials (e.g Hydrogels, films etc.) without using toxic chemical crosslinkers is a major area of research. An intrinsic property of polymers such as viscosity, Mw can be used to increase their stability in the presence of solvent. Additives like plasticizers are also added to improve mechanical properties via reducing inter molecular hydrogen bonding between polymer chains while increasing molecular spacing. This project mainly aims at presenting the impact of Mw of plasticizer on the swelling behaviour of hydrogel prepared from a blend of high viscosity SA and Gelatin.

1.2 General

Hydrogel is a three dimensional network of hydrophilic polymers that are physically or chemically cross-linked capable of retaining a large amount of water or biological fluids [1]. Hydrogels synthesized from non-toxic, biocompatible and biodegradable natural polymers have been extensively used for controlled release system. The benefits in the use of natural polymers are considerable, mainly because of their biocompatability, biodegradability and non-toxicity. A variety of natural (Alginate, Gelatin, Chitosan, cellulose, guar-gum, cyclo-dextran) and synthetic (poly-lactic acid, poly-ethylene glycol, poly-glycolic acid, poly-caprolactone) polymers have been used for the synthesis of hydrogels. However, the major drawback associated with hydrogels synthesized from natural polymers is their fast dissolution or degradation due their high hydrophilicity. Various approaches have been used to improve the various physical properties of hydrogels through: (i) Natural/Chemical crosslinkers (ii) Blending of polymers

Various crosslinkers are generally employed in the hydrogels to improve their physical properties such as crystallinity, thermal sensitivity, swelling ratio, and mechanical strength. To avoid the disintegration of hydrogels in solvent generally two types of crosslinking are done: physical, and chemical crosslinking. Chemical crosslinking of biomaterials is generally avoided for their application in biomedical and food industries due to their potential toxicity [2]. Examples of chemical cross-linkers for Gelatin includes Formaldehyde [3], Glutaraldehyde [4], and Calcium Chloride [5], carbodiimide [6] for SA. Physical crosslinking of biomaterials has advantage over chemical crosslinking. The main stabilizing force in physically cross-linked hydrogels are intermolecular forces such as hydrogen or ionic bonds, van der Waals interactions, crystal formation, physical entanglements or a combination of these [7]. Usually physically crosslinked hydrogels are not as strong as covalent cross-linked hydrogels by chemical cross-linker. Hence, the characteristic water insoluble behaviour of hydrogels is attributed to the presence of chemical or physical crosslinks, which provide a three-dimensional network structure and physical integrity to the system [8].

Blending two polymers is another efficient, simple, and cheaper way to improve various physical and chemical properties of biomaterials [9]. Polymeric blends are physical mixtures of structurally different polymers or copolymers which interact with secondary forces such as hydrogen bonding, dipoledipole forces and charge-transfer complexes. The gain in new properties depends upon the compatibility and miscibility between polymer [10]. The hydrogels prepared from polymeric blends without the use of crosslinker is known as physical hydrogel.

1.3 Problem Definition

A blend of proteins and polysaccharides display many advantageous features due to various types of non-covalent interactions like electrostatic interaction, steric exclusion, hydrophobic interaction and hydrogen bonding [11]. Biomaterials such as hydrogels and edible films prepared by blending of proteins and polysaccharides have been extensively studied for a variety of applications including biodegradable polymer based drug delivery systems [12] and food packaging [13]. The major limitation of these biomaterials is their low mechanical integrity which is mainly due to their hydrophilic nature. To improve mechanical properties and rate of disintegration of biomaterials in presence of solvent, these are cross-linked either physically or chemically. Chemical crosslinking due to its potential toxicity [2] is generally avoided in biomedical and food industries. Hence, Physical crosslinking is the desirable choice over chemical crosslinking. Physical crosslinking between two polymers basically depends on the compatability with each other. Protein and polysaccharides have complementary properties which makes them suitable candidates for physical crosslinking. However, the hydrogels prepared from proteins and polysaccharides are usually brittle and display poor mechanical properties which restricts their use in food and biomedical industry [14]. To overcome these inherent limitations, plasticizers are added, which improves the flexibility, workability and elasticity of polymers [15]. By changing three dimensional molecular organisation of polymers and disrupting the intermolecular interactions between polymer coils, plasticizers modify materials physical properties [16].

SA and Gelatin are two naturally occurring biopolymers with diverse applications [17, 18, 19, 20, 21, 22, 23]. SA and Gelatin are abundant, renewable, non-toxic, hydrophilic, bio-degradable and bio-compatible [24]. SA and Gelatin are polyelectrolyte in nature, which allows them to form a physically cross-linked hydrogel that depicts a unique pH dependent swelling and de-swelling behaviour [25]. High viscosity SA can be used in order to increase the amount of secondary bonds, which usually prevents the disintegration of hydrogels and results in the formation of strong networked structure. A blend of SA and Gelatin polymer has been studied for their performance in several applications like controlled drug delivery, wound dressing, tissue engineering, microencapsulation in the form of films, sponge, fibres, micro particles, hydrogels etc. mainly because of their compatibility with each other [26, 27, 28, 29].

Considering the advantage and unique property of a polymeric blend of protein and polysaccharide as mentioned previously, it is important to address the effect of plasticizer on their physical properties. Several studies have been performed on the effect of different plasticizers and their con-

centration on the physical properties of Gelatin based films [30, 31]. Compatibility of plasticizer with polymer is particularly important for effective plasticization which further depends on various factors like Mw, dielectric constant, polarity and solubility parameters [16, 32]. Polyols have been found to be particularly effective plasticizers for hydrophilic polymers [33, 34, 7, 35, 30, 36]. It was observed that the Mw of PEG was an important factor that controlled the plasticization, because of its polar chain with terminal hydroxyl groups [37]. However, only little information is available describing the effect of plasticizer on various properties of a polymeric blend of protein and polysaccharide. The effect of Mw of plasticizer on various properties of a polymeric blend of protein and polysaccharide were not taken into consideration. PEG was used as a plasticizer for this study mainly because of its hydrophilicity biocompatibility. The main drawback with lower Mw of PEG is their leaching out from the system [30]. To avoid this, we have selected PEG 2000, 4000 and 6000 for this study.

This project presents the impact of Mw of plasticizer on the swelling behaviour of hydrogel prepared from a blend of polysaccharide (i.e. SA) and protein (i.e. Gelatin). In this study, we analysed the various effects of Mw of PEG on the polymer blend starting from its effect on swelling to morphology and intermolecular interactions between high viscosity SA and Gelatin.

Chapter 2

Literature Review

Renewable and biodegradable polymers are considered as promising candidates for the preparation of biomaterials with relevant industrial applications in agriculture, packaging, pharmaceutical etc [12, 38, 39]. Their hydrophilicity and ability to control the release of active agents imparts an additional value to them. Hydrogels are the polymeric materials containing a large amount of hydrophilic groups capable of holding a large amounts of water [2]. They undergo a volume phase change with response to changes in the external environment such as pH, ionic strength, temperature, electric field, solvent and pressure [40]. The hydrophilicity of hydrogels is mainly due to the presence of hydrophilic functional groups hydroxyl, carboxyl, and amide group in their network. All these properties of the hydrogels enabled them as an important material in a variety of industrial applications as well as in consumer items.

Blending of natural polymers is an effective, simplest and cheaper way to improve their properties. To improve the performance of the individual natural polymeric biomaterials, many blend for example poly(vinyl alcohol)-chitosan [41, 42], cellulose-alginate [43], sodium alginate-silk fibroin [44], chitosan-gelatin [21], starch-chitosan [45] have been reported. Blending improves the property profiles of resulted biomaterials mainly due to the interaction between two polymers. An additional advantage of polymer blends is that the properties of the materials can be modified by combining component polymers and changing the blend composition [46]. The excellent mechanical properties, water solubility, body compatibility, and biodegradability are vital for their applications in food and medical industry. Generally, when the two polymers used for blending are compatible, biomaterials can form a homogeneous structure and exhibit better performance than the individual polymer. Biopolymers such as proteins and polysaccharides are generally used in food and medical

industry. Unique structure (contains 20 different amino acids) of proteins provides a wide range of functional properties and a high intermolecular binding potential. Polysaccharides are carbohydate molecules containing long linear chains of monosaccharide units linked together by glycosidic bonds. Net attraction between protein and polysaccharide mainly mediated through electrostatic interactions, complex coacervation or associative phase separation occurs, giving rise to the formation of protein-polysaccharide complexes [47]. The protein-polysacchaide complexes exhibit better functional properties (solubility, water-holding capacity, viscosity, gelation, coagulation, adhesion, emulsifying, and foaming properties) than that of the proteins and polysaccharides alone. Physicochemical factors such as pH, ionic strength, ratio of protein to polysaccharide, polysaccharide and protein charge, and Mw affect the formation and stability of such complexes. These properties of protein-polysaccharides complexes can be utilized to develop a novel biomaterial with desired mechanical, swelling and degradation property.

SA and Gelatin both are natural polymers with properties like biocompatibility, biodegradability, and hydrophilicity [24]. SA is a linear anionic polysaccharide extracted from sea weeds and consist of random or alternating units of β-D-mannuronic acid and α-L-guluronic acid in varying sequential arrangements and proportions **(Figure 3.1(a))** [48]. Gelatin, on the other hand, is obtained by a controlled hydrolysis of the fibrous insoluble collagen, which is a protein widely found in nature and is the major constituent of skin, bones and connective tissue [26]. Being a protein, Gelatin backbone contains a large number of amino acids like glycine (repeats almost 1 in 3 residues), proline and 4-hydroxyproline residues [20]. SA and Gelatin contain hydrophilic groups in their structure viz. hydroxyl, carboxyl, and amide groups respectively **(Figure 3.1(b))**. Therefore, these polymers are considered as better raw materials for making biomaterials. Gelatin is positively charged below its isoelectric point and can be able to form a polyelectrolyte complex with SA, which will have negative charges at lower pH [49]. During polyelectrolyte complexation, carboxyl groups in polysaccharides interact with amino groups in protein to form a complex that contains amide linkages. As a result, SA and Gelatin can be blended to form a physically cross-linked hydrogel matrix [50]. The pH-responsive behaviour of biomaterials prepared from blend of SA and Gelatin are likely to have potential application in food encapsulation, smart drug delivery, separation of biomolecules, enzyme immobilization, etc. [29]. Many reports have been published for their applications in medicine, pharmacy, agriculture and food industry [26, 51].

Due to high hydrophilicity of SA and Gelatin, biomaterials prepared from blending of SA an Gelatin display poor mechanical properties due to degradation in the presence of water. To increase their stability in the presence of solvent, several chemical crosslinking agents such as formaldehyde

[3, 52], glutaraldehyde [4, 53, 51], water-soluble carbodiimide [27, 6], diepoxy compounds [54], diiso-cynates [55], have been used. But the toxicity of a chemical crosslinking agent can not be ignored while developing a biomaterial that will be directly in contact with organisms. To overcome this problem, physical crosslinking between SA and Gelatin can be employed. It was reported that the coacervation is favoured by increasing the dextran with soy globulin [56]. In this study we have used a high viscosity grade of SA (i.e high Mw). It was thought that high viscosity SA will become more accessible for Gelatin chains in solution to form a more stable polyelectrolyte complex. Considering their pH-responsive behaviour [29], biodegradability and non-toxicity, a blend of SA and Gelatin can find application in food packaging [57], macroencapsulation [48], controlled drug delivery [26, 51], dehydration membranes [50], sponges for wound dressing [27], enzyme immobilization [58]. Generally, biomaterials prepared from protein or polysachharide exhibit poor mechanical properties which requires the addition of plasticizers. According to the definition of IUPAC(International Union of Pure and Applied Chemistry): "Plasticizer is a substance or material incorporated in a material (usually a plastic or elastomer) to increase its flexibility, workability, or distensibility" [15]. Several theories have been proposed in order to explain the mechanism of plasticizer. Among them, "Free Volume Theory" postulated by Fox and Flory [59] precisely explains the phenomenon of plasticization. According to Free Volume theory, the introduction of plasticizer molecules into the polymer involves the addition of more free volume and so, more flexibility, ease of movement to macromolecules through reduction of intermolecular forces between polymer chains [60].

Several reports have been published detailing the effect of type and concentration of polyols glycerol (GLY) , poly-ethylene glycol (PEG), ethylene glycol (EG), di-ethylene glycol (DEG),tri-ethylene glycol (TEG) and propylene glycol (PG), sorbitol as a plasticizer on polysaccharide based materials [61, 62, 63, 64, 45]. Several investigations have also been performed on the effect of different plasticizers and their concentration on the physical properties of protein based films [65, 31]. The thermal and functional properties of pig skin Gelatin-based films were improved by adding polyols such as GLY, PG, DEG, and EG as plasticizers [66]. Five different concentrations of plasticizer were used and their compatiblity with Gelatin was studied, producing flexible and easy handling films. In terms of functional properties, GLY presented higher plasticizing effect and efficiency. In order to modify mechanical and barrier properties of Gelatin based film other plasticizers such as sucrose, oleic acid, citric acid, tartaric acid, malic acid, PEG, sorbitol, mannitol, EG, DEG, TEG, EA, di-ethanolamine (DEA) and triethanolamine (TEA) were also studied citeCao2009. Polyols have been found to be particularly effective plasticizers for hydrophilic polymers such as proteins and polysaccharide [34, 65]. EG is a hydrophilic, bio-compatible and a non-toxic compound, frequently blended

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with biopolymers to improve the physical properties of biomaterials including mechanical strength [7], ductility [35], water barrier properties [30], and diffusion kinetics of drugs [36]. Compatibility of plasticizer with polymer is particularly important for effective plasticization which further depends on various factors like Mw, dielectric constant, polarity, and solubility parameters [16, 32]. The molecular structure of a plasticizer, chemical composition and Mw, plays an important role in polymer-polymer interactions and hence in the degree of plasticization [7]. In the case of PEG as plasticizer, Mw is an important factor that controls the plasticization, because of its polar chain with terminal hydroxyl groups [37]. Plasticizer has been used to modify not only the mechanical properties but also the thermal property, water absorption behavior, and adhesive property of polymeric films [67]. Plasticizer characteristics also effect the interactions between the plasticizers and the polymer, including its ability to attract water to the plasticized protein which further effect the performance and integrity of the final products [68]. Very little information is available on the effect of Mw of plasticizer on the swelling behaviour of hydrogels. In this study, the effect of Mw of plasticizer (PEG) on the swelling behaviour of SA and Gelatin hydrogels was evaluated.

2.1 Objectives of thesis

This research work was primary intended to gain greater insight into the effect of Mw of plasticizer (PEG) on the swelling behaviour of hydrogels prepared from a blend of polysaccharide (SA) and protein (Gelatin). The second point of interest was how to optimize properties such as strength and swelling of these biomaterials. This was carried out following the route of polymer blending of two complementary polymers for eg. SA and Gelatin. Blending involves the complextion between two polymers via secondary bonds such as hydrogen bonding, ionic bonding, Van der walls interactions which give will rise to the formation of insoluble complexes without using crosslinkers.

Chapter 3

Experimental

3.1 Materials

The following materials were used as received and all of them were of analytical grade: Gelatin A 175 bloom, Sodium salt of alginic acid High Viscosity (1000-1500 cps, 1% in water), Glycerol (99.8% ACS), Polyethylene glycol (PEG) Mw 2000, 4000 and 6000, Sodium chloride (NaCl) and Phosphate buffer saline-PBS (pH=7.4) were purchased form Alfa Aesar (A Jhonson Matthey Company). All the experiments were carried out three times to ensure the reproducibility.

Materials	60/40	70/30
Sodium Alginate (g)	2.4	2.8
Gelatin (g)	1.6	1.2
PEG (g) (Mw 2000/4000/6000)	2	2
Glycerol (ml)	2	2
NaCl (g)	0.2	0.2
Water (g)	20	20

Table 3.1: Composition of SA/G hydrogels

3.2 Equipments

A list of equipment used in the current project is as follows:

1- Weighing Balance (Sartorious)

2- Hot Plate with magnetic stirrer (IKA CMAG)

3- Hot Air Oven (Nabertherm)
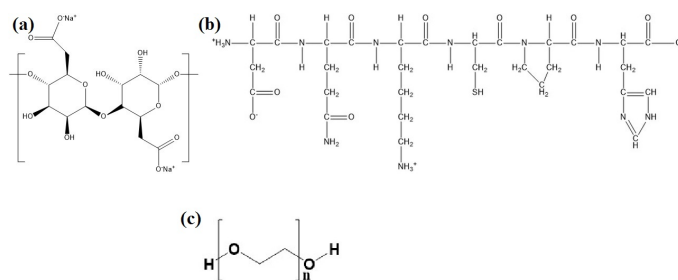
4- FTIR Apparatus (Bruker Alfa-P)

Figure 3.1: (a) Sodium Alginate (b) Gelatin polypeptide (c) Poly-ethylene Glycol

Figure 3.2: Representative image of SA/G hydrogel

5- Scanning Electron Microscope (Phenom World ProX)

3.3 Methods

3.3.1 Procedure for sample preparation

SA/G hydrogels with and without PEG was done by solvent evaporation method, according to method developed by Saarai.et.al [69]. Firstly, aqueous polymeric blend were prepared using the required weight of SA and Gelatin. Gelatin was added to deionized water at a temp of 60 °C and stirred at 400 rpm to prevent aggregation until a homogeneous solution was obtained then other components i.e SA, Glycerol, PEG (Mw 2000/4000/6000) were added to the mixture to form a viscous solution (**Table 3.1**). This solution is then poured on to the 60×15 mm petridishes and dried for 72 hours at 25 °C to form physically cross-linked SA/G hydrogels (**Figure 3.2**).

3.3.2 Swelling behaviour

Swelling behaviour of the physically cross-linked SA/G hydrogels was studied in PBS (pH=7.4) and 0.1N HCl (pH=1.2) at a temperature of 37 °C. Dried hydrogels were immersed in PBS and at different time intervals hydrogels were weighted after removing excess PBS from the hydrogel

surface using tissue paper. Using swelling experiment data of SA/G hydrogels, swelling ratio (SR) and equilibrium swelling ratio (SR_{eq}) were calculated using following equation:

$$SR(\%) = \frac{W_s - W_d}{W_d} \times 100 \tag{3.1}$$

$$SR_{eq}(\%) = \frac{W_{eq} - W_d}{W_d} \times 100 \tag{3.2}$$

where W_s, W_d, W_{eq} denotes the initial dry weight of hydrogel, weight of swollen hydrogel, and weight of equilibrium swollen hydrogel respectively.

3.3.3 Diffusion kinetics

Diffusion kinetics of SA/G physical hydrogels were evaluated in order to understand the change in the nature of diffusion of solvent over SA/G hydrogels due to addition of PEG (Mw 2000, 4000 & 6000. Diffusion characteristics of SA/G hydrogels were evaluated from fractional water uptake (F) using **Eq (3.3)** [70]:

$$F = \frac{M_t}{M_\infty} = K_D t^n \tag{3.3}$$

where M_t and M_∞ represents the amount of solvent diffused into the hydrogel at time t and at infinite time, K_D is swelling constant and n is swelling exponent respectively. K_D is related to the macromolecular structure of the hydrogel network, and the swelling exponent (n) is a numerical value to determine the type of diffusion followed by the solvent.

Diffusion coefficient (D) of the cylindrical SA/G hydrogels were calculated from **Eq (3.4)** using K_D and n obtained from Eq (3.3) [71, 18]:

$$D = \pi r^2 (\frac{K_D}{4})^{1/n} \tag{3.4}$$

3.3.4 Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The influence of PEG on the molecular interactions of SA/G hydrogels was evaluated using a Fourier Transform infrared (FTIR) Spectrometer (Model: Bruker Alfa-P). Scanning of SA/G hydrogel samples was done in ATR mode (500 to 4000 cm^{-1}) .

3.3.5 Scanning Electron Microscope (SEM)

The changes in morphology of SA/G hydrogels with PEG (2000,4000 and 6000) were analyzed by using table top SEM (Model Phenom World ProX). The SA/G hydrogels samples were frozen to $-15\,°C$ using a cooling stage to avoid moisture. The analysis of SA/G hydrogels microstructure were performed at an accelerating voltage of 15kV at the required magnification.

Chapter 4

Results and Discussion

Biomaterials prepared from a blend of biopolymers such as protein and polysaccharide have their applications in various fields. The most important property of these biomaterials is their ability to imbibe high amount of water i.e. swelling capacity. When a dehydrated hydrogel is placed in a solvent medium, solvent start diffusing inside the hydrogel to equilibrium over a period of time.The influence of PEGs Mw on the swelling behaviour of biopolymer hydrogels cast from aqueous blend of SA and Gelatin was investigated and results are summarized below.

4.1   Swelling Study

Firstly, SR of SA/G hydrogels of different weight ratio without (w/o) the addition of PEG was investigated in PBS (pH=7.4) **(Figure 4.1(a))** . SR_{eq} was plotted against time (t) as shown in **Figure 4.1(b)** to find the optimal from different weight ratios selected. It can be seen that swelling behaviour of SA/G hydrogels increased with the increase in alginate content up to 60/40 weight fraction due to increase in hydrophilicity of hydrogel matrix. Beyond 60/40, swelling ratio of SA/G hydrogel is decreased which is due to the enhanced viscous effect of SA [72]. Increased viscosity of SA has caused retardation of the movement of PBS inside hydrogel and resulted in lower ESR [5]. The effect of viscosity was found to be prominent between 60/40 and 70/30 w/o PEG SA/G hydrogels. For further studies, these two combinations were selected as they have higher ESRs with a visible impact of polymer viscosity.
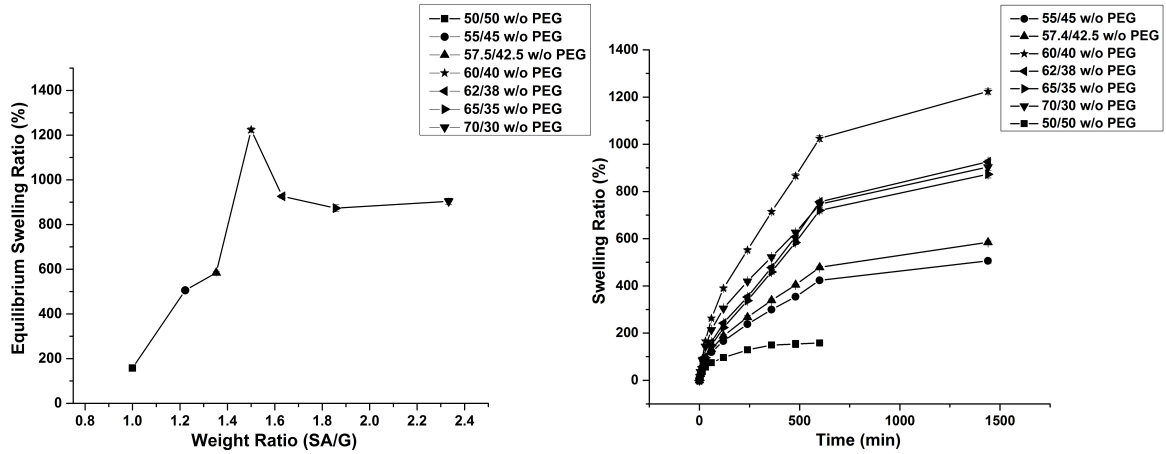
Figure 4.1: Swelling ratio of SA/G hydrogels (w/o PEG)

4.1.1 Swelling behavior in PBS

Swelling ratio of 60/40 and 70/30 SA/G hydrogels with respect to different Mw of PEG (2000, 4000 & 6000) are shown in **Figure 4.2(a)**. The SR_{eq} of hydrogels w/o PEG (60/40 and 70/30) was highest because molecular chains of SA and Gelatin are accessible to each other. As a result, at pH=7.4 a strong electrostatic repulsion was existing between negatively charged groups in SA and Gelatin which caused a rapid relaxation of polymeric chains and induced an enhanced PBS uptake. **Figure 4.2(a)** depicts the fact that with the increase in Mw of PEG, ESR of SA/G hydrogels decreased. The swelling behavior of SA/G hydrogels with respect to Mw of PEG was in the order of PEG 2000>4000>6000. The observed decrease in the SR_{eq} of hydrogel is mainly due to decreased plasticization efficiency (PE) of PEG with the increase in its Mw. Upon addition of PEG, it was distributed in the SA/G matrix, which has finally separated the molecular chains of SA and Gelatin. This in turn has reduced the direct interaction between them. Polar hydroxyl groups (-OH) on PEG backbone are believed to implicate polymer-plasticizer hydrogen bonds, replacing the polymer-polymer interactions between SA and Gelatin. Hence, a reduced repulsion between COO- groups of SA and Gelatin, might have resulted in reduction of ESR. PE decreases with the increase in Mw of PEG due to decrease in number of polar groups (OH) per mole [73]. PEG with lower Mw can thus form more hydrogen bonding with SA and Gelatin. This increases inter-chain spacing/ free volume as compared to PEG with higher Mw, further causing an increase in SR_{eq} with lower Mw of PEG. SR_{eq} of 70/30 SA/G hydrogels was found to be greater than 60/40 SA/G hydrogel with PEG, which may be attributed due to increase in electrostatic repulsion between negatively charged groups of SA (more in 70/30).
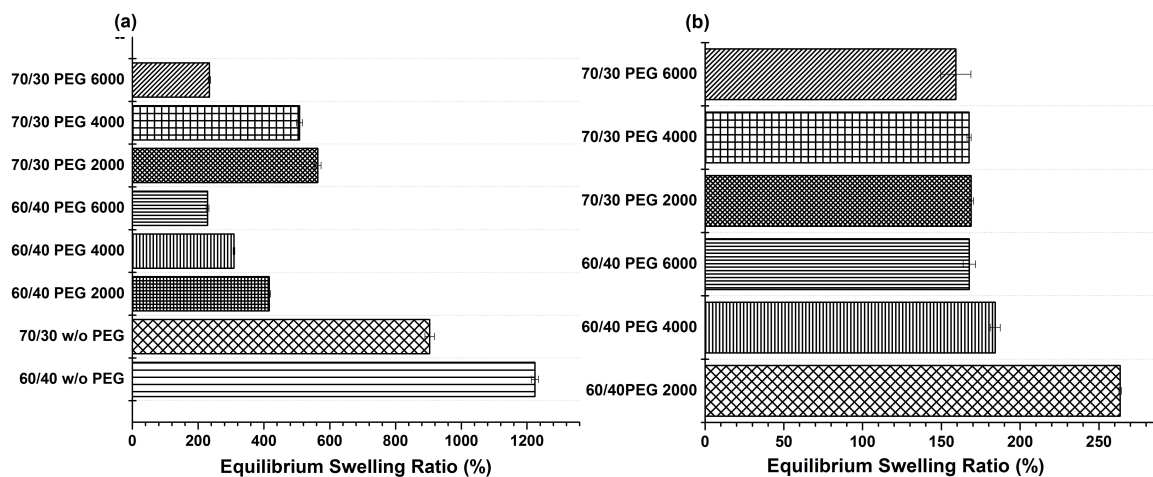
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Figure 4.2: Equilibrium Swelling Ratio of SA/G hydrogels at (a) pH=7.4 and (b) pH=1.2

4.1.2 Swelling behavior in 0.1 N HCL

SR_{eq} of 60/40 and 70/30 SA/G hydrogels in 0.1N HCl with PEG (2000, 4000 & 6000) is shown in **Figure 4.2(b)**. SR_{eq} of hydrogels decreased with the increase in Mw of PEG which might be due to decrease in interchain spacing or free volume with the increase in Mw of plasticizer [60]. However, the SR_{eq} of 70/30 SA/G hydrogel was lower as compared to 60/40 hydrogels which might be due to decrease in hydrodynamic volume of hydrogels. At pH=1.2, acid groups of SA and Gelatin remains protonated. As a result, a more rigid network is formed between SA and Gelatin with the increase in SA content due to ionic bonding between positively charged Gelatin and negatively charged SA which limits the movement of water inside hydrogels. Reduction in SR_{eq} of 70/30 SA/G hydrogels confirmed the reduction of hydrodynamic volume due to enhanced viscosity of SA.

A comparison of swelling behavior of SA/G hydrogels in PBS (pH=7.4) and 0.1 N HCl is shown in **Figure 4.3(a)& (b)**. Swelling behavior results of SA/G hydrogels clearly express the pH dependent swelling behavior. SA/G hydrogels swelled extensively in PBS (pH=7.4) as compared to 0.1N HCl (pH=1.2) which is due to difference in ionization behavior of amine groups of Gelatin and carboxylate groups of SA.

It can be concluded that the swelling behaviour of initially dried SA/G hydrogels was significantly affected by the Mw of PEG in both PBS and 0.1N HCl.

4.1.3 Diffusion kinetics

Diffusion kinetics study was performed to understand the influence of Mw of PEG in the diffusion behaviour of solvent inside SA/G hydrogel. The diffusion kinetic parameters (swelling exponent (n),
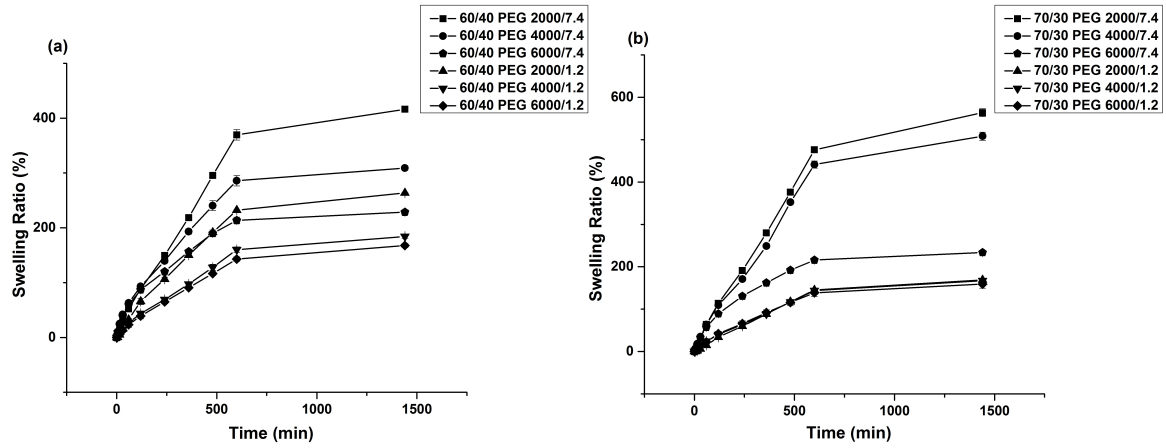
Figure 4.3: Swelling ratio comparison between pH=1.2 and pH=7.4 (a) 60/40 hydrogels and (b) 70/30 hydrogels

swelling constant (K_D), diffusion coefficient (D)) of SA/G hydrogels were evaluated from non-linear fitting of 60% (i.e. till 360 min) of the SR obtained experimentally using **Eq 3.3 and Eq 3.4** in MATLAB 2014 **(Figure 4.1)**. The numerical value of swelling exponent (n) delivers the information regarding mechanism of swelling of SA/G hydrogels. The plots of F versus t for hydrogel samples in pH=7.4 and pH=1.2 were illustrated in **Fig 4.5**. The swelling exponent (n) of SA/G hydrogels for PBS and 0.1N HCL are represented in **Table 4.1**. For cylindrical shapes, n=0.45 represents Fickian diffusion, which indicates solvent diffusion rate is much faster as compared to polymer relaxation ($R_{diff} > R_{relax}$). Whereas 0.45<n<1.00 corresponds to non-fickian diffusion, which represents the rate of solvent diffusion and polymer relaxation are comparable ($Rdiff \sim Rrelax$) [74]. From **Table 4.1**, swelling exponent (n) values of SA/G hydrogels in pH=7.4 indicates that the diffusion of PBS into SA/G hydrogels is fickian for PEG 2000 where diffusion of solvent dominates over polymeric chain relaxation. At pH=7.4, an increase in PEG Mw lead to the shift in the mode of diffusion from fickian to non-fickian in PEG 4000 and 6000 SA/G hydrogels due to decrease in free volume or inter-chain spacing with the increase in PEG Mw. It can also be noticed that the swelling exponent (n) of SA/G hydrogel in pH=1.2 were above 0.45 which represents that the diffusion of 0.1N HCl in SA/G hydrogels exhibited non-fickian behaviour due to the formation of a rigid network at lower pH (Kim et al., 2003). Hence, it can be concluded that the diffusion behaviour of solvent (0.1 N HCl/PBS) in SA/G hydrogels is mainly influenced by the change in molecular interactions between SA and Gelatin as a function of plasticization efficiency (PE) of PEG which further depends on its Mw.
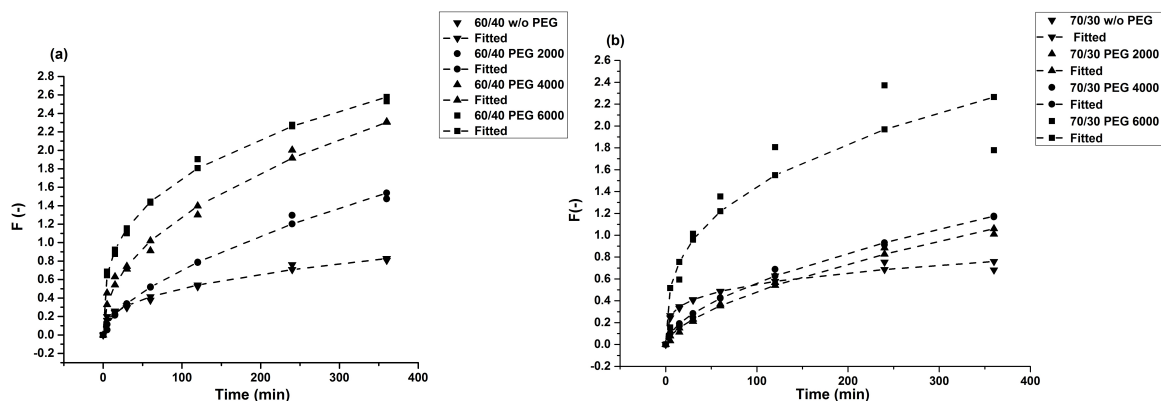
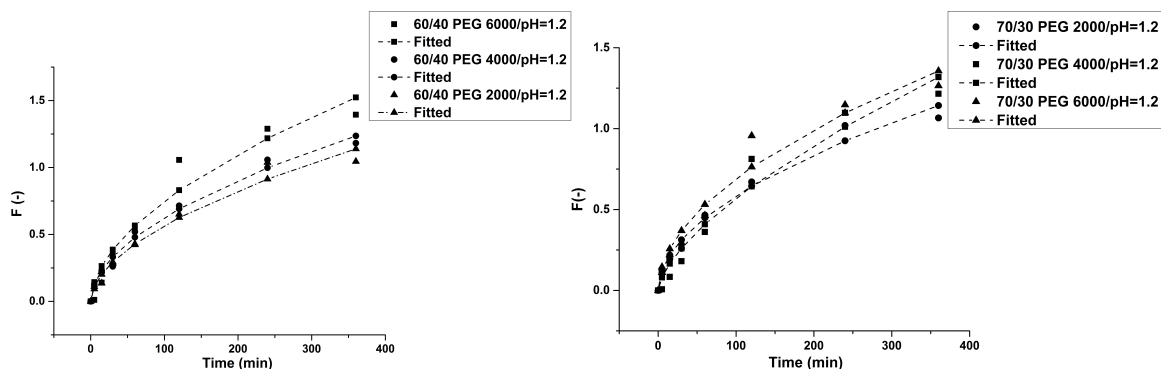Figure 4.4: Plot of F versus t of (a) 60/40 (b) 70/30 hydrogels in pH=7.4

Figure 4.5: Plot of F versus t of (a) 60/40 (b) 70/30 hydrogels in pH=1.2

4.2 Atteunated Total Reflectance -Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Further attempt of confirming the above mentioned interactions between plasticizer and polymer, was performed with the help of ATR-FTIR study The IR spectra of SA, Gelatin and SA/G hydrogels was recorded between 500 cm^{-1} to 4000 cm^{-1} **(Figure 4.6)**. Samples were prepared to investigate the wavenumber shifts of certain functional groups, which reflected the changes in intermolecular interactions in SA/G hydrogels associated with the addition of PEG (Mw 2000, 4000 & 6000).

ATR-FTIR spectrum of alginate (Figure 4.6(a)) showed characteristic absorption bands at 3214.42 cm^{-1}(-OH group), 2909.97 cm^{-1}(C-H stretch), 1591.63 cm^{-1}(COO- asymmetric stretch), 1404.71 cm^{-1}(COO- symmetric stretch) and 1023.80 cm^{-1}(C-O-C stretching) [50]. In FTIR spectrum of Gelatin (Figure 4.6(a), following characteristic absorption bands were observed: 3292.30 cm^{-1} (-NH stretching), 1641.19 cm^{-1} (amide I , C=O and C-N stretching), 1537.8 cm^{-1} (Amide II) and 1241.87 cm^{-1} (Amide III)[75]. The characteristic peaks confirming the presence of PEG in SA/G hydrogels **(Figure 4.6 (d), (e), (f) (h), (i) and (j))** are: 945-962 cm^{-1} (-CH out of plane

| Sample	pH= 7.4			pH=1.2		
	n	K_D	D ($\times 10^3$)	n	K_D	D ($\times 10^3$)
60/40 w/o PEG	0.38	0.08	0.33	–	–	–
60/40 PEG 2000	0.6	0.04	4.08	0.55	0.06	3.41
60/40 PEG 4000	0.45	0.16	5.84	0.53	0.5	2.13
60/40 PEG 6000	0.32	0.38	5.03	0.54	0.05	1.96
70/30 w/o PEG	0.24	0.17	0.023	–	–	–
70/30 PEG 2000	0.61	0.03	2.23	0.65	0.03	3.59
70/30 PEG 4000	0.57	0.04	2.29	0.52	0.05	1.77
70/30 PEG 6000	0.34	0.29	3.78	0.52	0.06	2.48

Table 4.1: Parameters for diffusion of solvent(PBS/0.1N HCl) into SA/G hydrogels
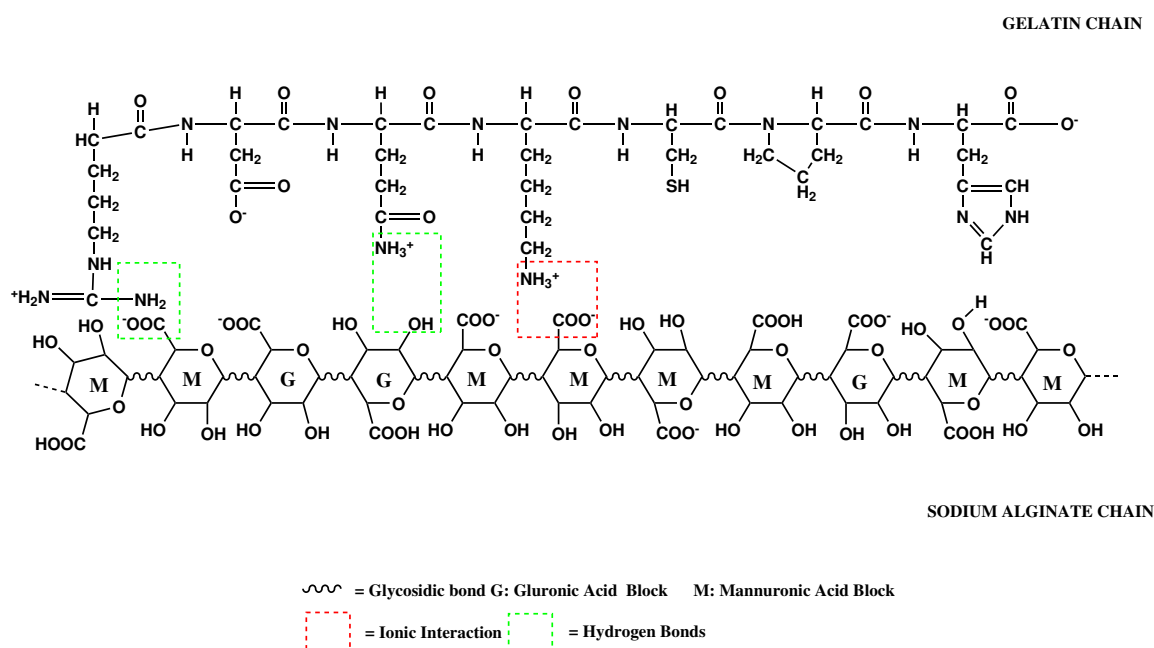
Scheme 4.1: Schematic diagram representing intermolecular interactions between SA and Gelatin polymer chains

bending vibration) 1105-1108 cm^{-1} (-C-O-C- ether stretch band), 2884 cm^{-1} ($-CH_2$ stretching vibration) [8]

The wavenumber of -OH and amide I sterching vibrations of SA/G hydrogels were summarized in **Table 4**. ATR-FTIR spectra of SA/G physical hydrogels w/o PEG **(Figure 3.5 (c) and (g))** revealed a significant peak for amide formation at 1563.31 cm^{-1} and 1547.33 cm^{-1} (C=O stretching of Amide) in 60/40 w/o PEG and 70/30 w/o PEG which confirmed formation of complex due to reaction between amino group ($-NH_3$) of Gelatin and carboxyl group ($-COO^-$) of SA [29]. Further, the absorption band around 3292.30 cm^{-1}, associated with the vibration of N-H group stretching in Gelatin shifted to a lower wave number at 3272.26 cm^{-1} and 3280.01 cm^{-1} in 60/40 w/o PEG and 70/30 w/o PEG, which reflects an increase in intermolecular hydrogen bonding between SA
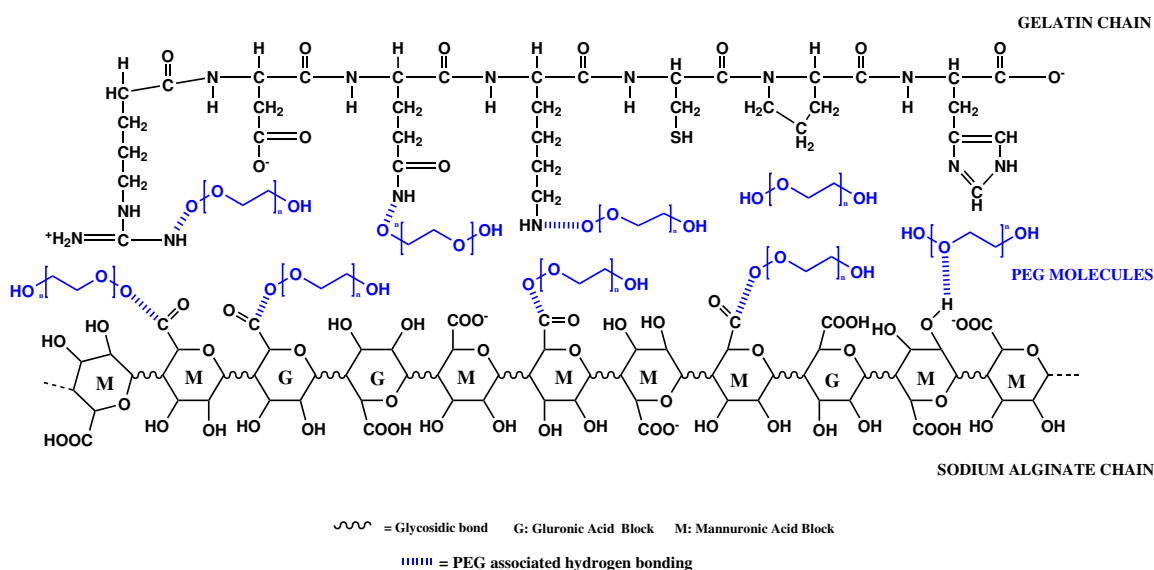
Scheme 4.2: Schematic diagram representing hydrogen bonding of PEG among SA and Gelatin polymer chains

and Gelatin[26]. Meanwhile, the peak at 1641.19 cm^{-1} corresponding to amide I in Gelatin showed significant changes, it shifted to lower wave-number 1625.53 cm^{-1} in 60/40 w/o PEG and 1627.06 cm^{-1} in 70/30 w/o PEG which also confirmed that the negatively pendant groups $(-COO^-)$ of SA might associate with positively charged pendant groups $(-NH_3)$ of Gelatin. In the absence of PEG, these changes provides enough evidence of inter-molecular interaction between SA and Gelatin. These results are in accordance with the swelling study of SA/G hydrogels witout PEG. At pH=7.4, a strong electrostatic repulsion between negatively charged moieties $(-COO^-)$ in the polymer backbone induced an enhanced uptake of solvent inside SA/G hydrogels w/o PEG.

The effect of PEG Mw (2000, 4000,and 6000) in SA/G hydrogels was analyzed by comparing the IR spectra of PEG containing SA/G hydrogels to SA/G hydrogels w/o PEG. The main peaks in SA/G hydrogels w/o PEG are 3272.26 cm^{-1}, 1625.53 cm^{-1} (**Figure 4.6(c)**) in 60/40 w/o PEG and 3280.01cm^{-1}, 1627.06 cm^{-1} (**Figure 3.5(g)**) in 70/30 w/o PEG corresponding to -OH and amide I stretching vibration. On comparing the IR spectra of SA/G hydrogel containing PEG 2000, 4000 and 6000 (**Figure 4.6(d), (e), (f), (h), (i) and (j)**),it can be seen that the wavenumbers associated with -OH and amide I stretching vibration in 60/40 w/o PEG and 70/30 w/o PEG shifted to a higher wave-number. This shift in peaks to a higher wavenumber clearly indicated the weakening of inter-molecular interaction between SA and Gelatin molecular chains. Due to polar nature of plasticizer (PEG), it can interact with both SA, Gelatin and decrease the inter-molecular interactions (hydrogen bonds, van der Waals or ionic forces, etc.) between them **Scheme**
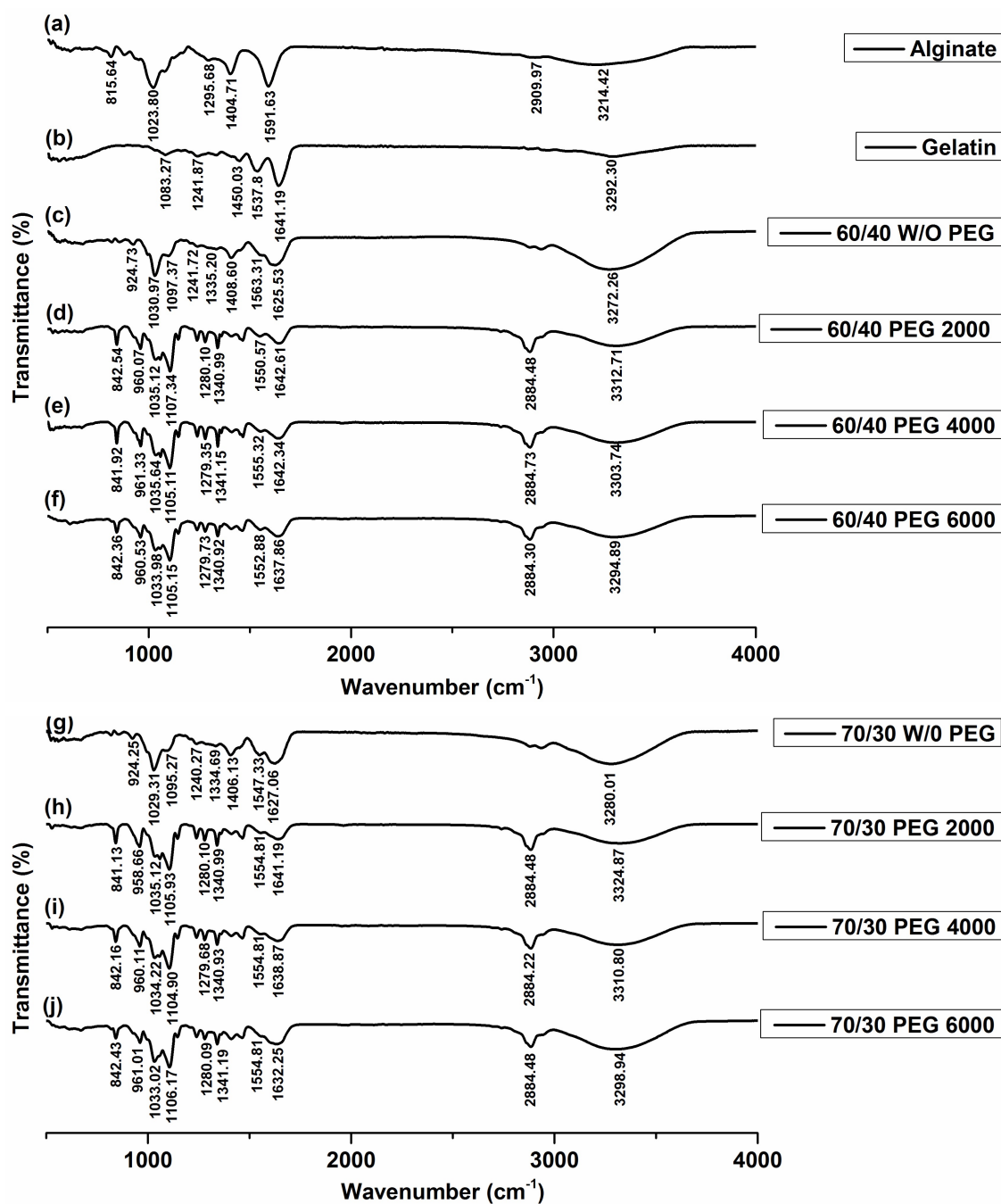
Figure 4.6: ATR-FTIR spectra of (a) SA (b) Gelatin (c) 60/40 w/o PEG (d) 60/40 PEG 2000 (e) 60/40 PEG 4000 (f) 60/40 PEG 6000

1. Accordingly, at pH=7.4, electrostatic repulsion between negatively charged moieties $(-COO^-)$ is intrupted/screened with the introduction of PEG which results in decreased SR of SA/G hydrogels.

It can be seen from **Table 4.2** and **Figure 4.6(d), (e), (f), (h), (i) and (j)** , the wavenumbers associated with -OH and Amide I stretching bands shifted in the order of PEG Mw 2000>4000>6000.

This suggest a decrease in the interaction of PEG with SA and Gelatin molecular chains due to significant reduction in number of hydroxyl groups per mole, solubility and polarity with the increase in Mw of PEG and hence its ability to interact with polymer chains decreases [73]. On comparing the FTIR spectra of 60/40 and 70/30 SA/G hydrogels with PEG, the peaks corresponding to -OH stretching shifted to a higher wavenumber with the increase in content of SA. These results depicts a reduction in the extent of intermolecular interactions between SA and Gelatin molecular chains. With the increase in SA content, more hydrophilic groups (-OH/-COOH) groups will be available for PEG-SA and/or PEG-Gelatin interactions rather than SA-Gealtin interactions. Therefore, from ATR-FTIR spectra it can be concluded that PEG (Mw 2000, 4000, and 6000) had a better PE in 70/30 SA/G hydrogels. Among PEG 2000, 4000 and 6000, PEG 2000 proved to be a better plasticizer due to its small size and higher number of hydroxyl groups per mole. Its relative impact on the SA/G interaction is the largest among the other cases.

Plasticization efficiency (PE) of PEG along with increase in Mw was also calculated according to Lim et al. [37], by monitoring band shift instead of change in relative intensity in the FTIR spectra of SA/G hydrogel. As, plasticizer usually works by intruppting the intermolecular interaction between the polymeric chains (Turhan et al., 2001). Plasticizing efficiency (PE) of PEG (Mw 2000, 4000 and 6000) in SA/G hydrogel was determined by examining the changes in intermolecular interaction between SA and Gelatin upon introduction of PEG. These relative changes in plasticizer-plasticizer and polymer-plasticizer intermolecuar interactions were related to the wavenumber shifts. Thus, the ratio of change in wavenumber (ν_{OH}) due to addition of plasticizer associated with -OH stretching band to the wavenumber of $-CH_2$ stretching band (ν_{CH2}) which is not affected due to intermolecular interaction between molecular chains of SA, Gelatin and PEG (**Eq 4.1**) gives the plasticizing efficiency (PE) [37]:

$$PE(\%) = \frac{\nu_{OH}}{\nu_{CH_2} + \nu_{OH}} \times 100 \tag{4.1}$$

Where, ν_{OH} and ν_{CH_2} represents the wavenumbers of -OH and CH2 peaks in SA/G hydrogels. From **Figure 4.7**, it can be seen that PE of PEG (2000, 4000 & 6000) was found to be greater in 70/30 SA/G hydrogels as compared to 60/40 SA/G hydrogels. PE has decreased as Mw of PEG is increased. ATR-FTIR analysis of SA/G hydrogel reflected a reduction in intermolecular interactions between SA and Gelatin molecular chains upon introduction of PEG Mw 2000, 4000 and 6000. These intermolecular interactions are also responsible for change in swelling behaviour of SA/G hydrogels. Thus, one can indeed take a control on the swelling behaviour of SA/G hydrogels by controlling the

Mw of PEG as plasticizer.

Sample	ν (-OH)	ν (-Amide I)
60/40 w/o PEG	3272.26	1625.53
70/30 w/o PEG	3280.01	1627.06
60/40 PEG 2000	3312.71	1642.61
60/40 PEG 4000	3303.74	1642.34
60/40 PEG 6000	3294.89	1637.86
70/30 PEG 2000	3324.87	1641.19
70/30 PEG 4000	3310.80	1638.87
70/30 PEG 6000	3298.94	1632.25

Table 4.2: Wavenumbers (-OH and Amide I stretching modes) of SA/G Hydrogels
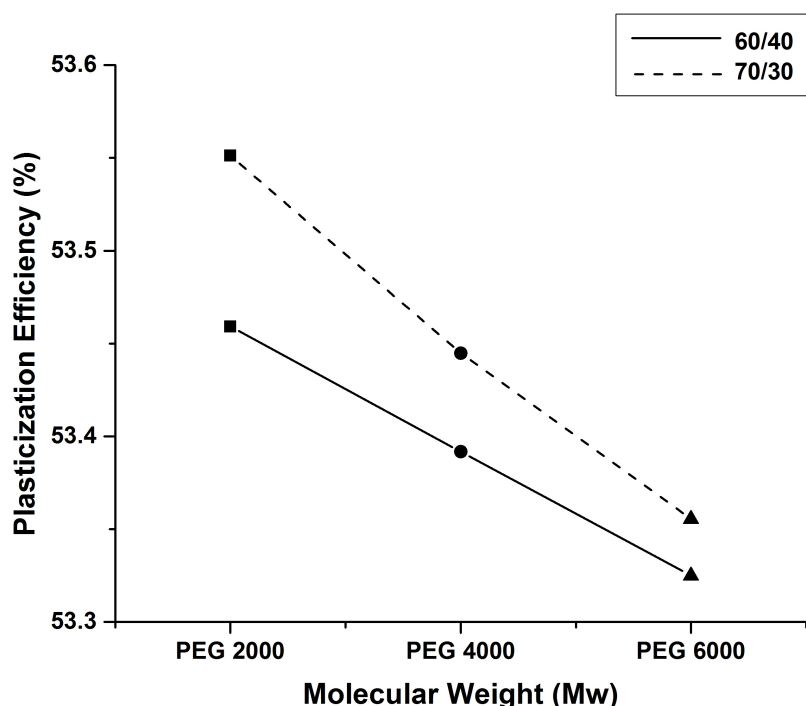

Figure 4.7: Plasticizing Efficiency of 70/30 and 60/40 SA/G hydrogel

4.3   Scanning Electron Microscope (SEM)

The change in surface morphology due to difference in the blending of SA/G hydrogels containing different PEG Mw (2000, 4000 & 6000) was characterized using SEM. **Figure 4.8(a), (b), (c), (d)** represents the SEM micrograph of 60/40 w/o PEG and 60/40 PEG 2000, 4000 & 6000 respectively. The SEM micrograph of 70/30 w/o PEG and 70/30 PEG 2000. 4000 & 6000 are represented in **Figure 4.8(e), (f), (g) and (h)**. **Figure 4.9(a), (b), (c)** represents the SEM micrograph of SA/G hydrogel samples swelled at 37 °C and dried again in order to examine the morphological changes
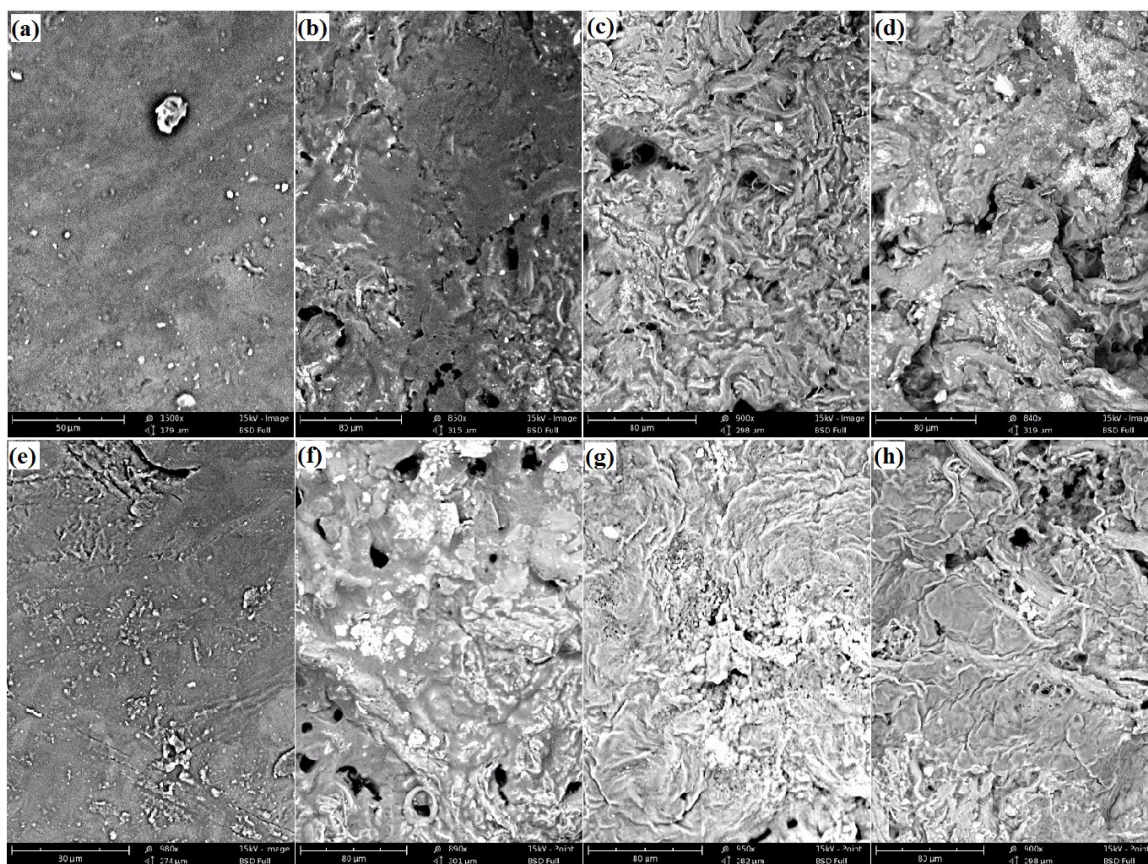
Figure 4.8: SEM micrograph of fabricated 60/40 PEG (a) w/o (b) 2000 (c) 4000 (d)6000 and 70/30 PEG (e) w/o (f) 2000 (g) 4000 (h) 6000
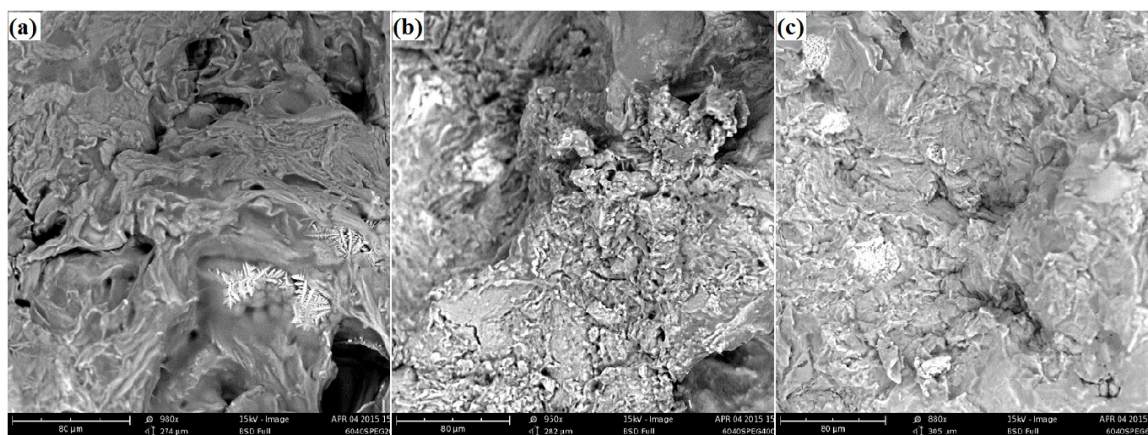
Figure 4.9: SEM micrograph of swelled (in PBS) and dried 60/40 PEG (a) 2000 (b) 4000 (c) 6000

incurred after swelling in different solvent medium (PBS and 0.1N HCl). FTIR-ATR studies of SA/G hydrogels without PEG suggests a good miscibility between SA and Gelatin due to intermolecular hydrogen bonding and ionic interactions between them. It is clear from SEM micrographs, that the surface morphology of SA/G hydrogels is dependent on the Mw of plasticizer. The SEM micrograph

of SA/G hydrogel without PEG **Figure 4.8(a)** shows a smooth and homogeneous surface without micro phase separation due to high miscibility between SA and G [9]. Upon introduction of PEG 2000 in SA/G blend **(Figure 4.8(b))**, the surface becomes rough and heterogeneous with some island type structure distributed over the surface which might be induced due to hydrogen bond formation of PEG 2000 with SA and Gelatin, which has effectively plasticized SA and Gelatin blend [76]. Whereas, SEM micrograph of SA/G hydrogel with PEG 4000 and 6000 (Figure 4.8(c) and (d)) show coarsening of morphology or micro phase separation of PEG. The observed micro phase separation in SA/G hydrogels with PEG 4000 and 6000 might be due to decrease in hydrogen bonding capacity of PEG as a result of the decrease in polar groups (-OH) with the increase in Mw. These results are also in agreement with the ATR-FTIR analysis of SA/G hydrogels with PEG 2000, 4000 & 6000.

The difference in morphological characteristics of swollen samples is consistent with the swelling behaviour represented by SA/G hydrogels. It can be seen from**Figure 4.9(a), (b) and (c)**, SA/G hydrogels with PEG 2000 facilitated bigger pores as compared to SA/G hydrogels with PEG 4000 and 6000 due to higher SR of SA/G hydrogels with PEG 2000.

4.4 Conclusion

In present study, the effect of increase in Mw of PEG and more SA content (thus higher polymer viscosity) on the swelling behaviour of the SA/G hydrogels was investigated. The high viscosity of alginate increased the stability of SA/G hydrogels through increasing the amount of secondary bonds between SA and Gelatin. The Mw was inversely related to the ESR of SA/G hydrogel. It can be concluded that Mw of PEG had an important role in restricting the interaction between SA and Gelatin molecular chains. Higher PE was exhibited by lower Mw weight PEG due to increased number of polar groups (-OH), which interrupt SA and Gelatin interaction resulting in more free volume. The higher PE of PEG (Mw=2000) was also confirmed by ATR-FTIR spectra. The SEM study also illustrated the change in morphology of SA/G hydrogels due to increase in Mw of PEG. SEM micrographs showed good miscibility with PEG (Mw=2000) and the results were also in agreement with the swelling and FTIR study of SA/G hydrogels. Hence, Mw of plasticizer can also play an important role in the swelling behaviour of SA and Gelatin hydrogels among other different properties which might affect the drug release characteristics of SA/G hydrogels. Consequently, a proper choice of plasticizers Mw is important for better optimization between swelling property and other physical (thermal and mechanical) properties of biopolymer based materials.

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