Targeted delivery of insulin by *Psyllium* plant (*Plantago spaghula*) based hydrogels/membranes

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ABSTRACT. Developments in therapeutics are the need of hour for the benefit of mankind and to increase the quality of life. Diabetes mellitus is a non-curable disease affecting a great amount of world's population. This genetic disorder gets worse with time and ultimately leads to various complications like renal failure, lower limb amputation, heart diseases and blindness. Hydrogels for quite recent time are being used for the drug delivery. The present article is related to the fabrication of *Psyllium* based dermal patches insulin drug delivery system that could release insulin in a sustained and controlled manner. *Psyllium*, a natural polysaccharide, is a medicinally important dietary fiber and drug delivery system has developed a novel insulin loaded *Psyllium* based hydrogels for glucose lowering. They can be used for the transdermal delivery of insulin as hypodermic administration of insulin is painful, complicated, can also results in allergic reactions and infections. For the sustained and steady release of insulin from fabricated hydrogels were also modified with the addition of graphene oxide nanoparticles. To study the structural aspects of these various polymeric networks thus formed were characterized with FTIR

(Fourier-transform infrared spectroscopy) and SEM (Scanning electron microscopy) which identifies the interaction/bonding among different functional groups and also indicating the layer of graphene oxide nanoparticles on the surface of hydrogels stage and release related experiments such as degradability, swelling kinetics, swelling ratio and drug kinetics were also performed. Because of its functionalization these unique 3D drug loaded polymeric hydrogels will have the double prospective of curing diabetes. Different types of *Psyllium* based hydrogels were prepared, and the effect of ph on the release dynamics of insulin from drug loaded hydrogels has been studied to evaluate the drug release mechanism *in-vitro* and *in-vivo*. For *in-vivo* study avian skin was used a model. Fickian diffusion mechanism has been observed for the release of insulin at different ph both in-intro and *in-vivo* and therefore these drug loaded hydrogels can be potentially used to develop pH-sensitive systems. Keeping in view the results of different analysis it can be concluded that these hydrogels can prove to be very useful for the treatment of diabetes mellitus

Keywords: *Psyllium*; Insulin; Hydrogel; Drug Release; Transdermal Patches

INTRODUCTION

Diabetes Mellitus (DM), commonly known as diabetes, is a genetic and multifactorial group of metabolic disorders which is characterized by a high blood glucose level over a prolonged period of time. It is a common problem now-a-days which is caused by decreased production (IDDM) or by decreased ability to use insulin (NIDDM). Diabetics are unable to metabolize blood sugar in their body. Subcutaneous administration of insulin is the only mean of traditional treatment which is throbbing, complicated and can also cause infections. Two to four times in a day, the diabetic patients usually have to inject insulin hypodermically so that insulin helps them to control their blood glucose from getting too high (hyperglycemia) or too low (hypoglycemia). In order to avoid this sore treatment, various attempts have been made to develop an efficient method of insulin delivery (Babu et al. 2004; Yadav et al. 2009). So, to get rid of this injection method of administration insulin, vital interest is developed to introduce the development of dermal delivery system for insulin that could release the drug in a constant level for longer periods. Hydrogels have been shown to be promising candidates for such a system (Kim et al. 2003; Peppas et al. 2004). “Hydrogels are three-dimensional network of hydrophilic polymers that get swollen by a solvent i.e. water called hydrogels (Campoccia et al. 1998).” Hydrogels are usually chemically stable but they ultimately dissolve and break down. There are two types of hydrogels including “physical gels” if the network within them is held together by hydrophobic interactions and other interactions mainly hydrogen bonding. They are not homogenous in nature (Campoccia et al. 1998). The other type is known as “Chemical gels” if there are covalent interactions within the network. These hydrogels are formed either by the crosslinking between the polymers which are water soluble or by converting hydrophobic polymers to hydrophilic polymers and then crosslinking them (Wichterle and Lim 1960). Hydrogels have a wide variety of applications such as in cell culture, tissue engineering, directed drug delivery, biosensors, breast implants, and contact lens (Yetisen et al. 2014). Hydrogels can be architected into 3D shapes known as scaffolds and one of the major applications of this scaffold is tissue engineering of the damaged heart valves. Now-a-days, hydrogels are also being used to deliver drugs transdermal or orally which were mostly protein in nature and were previously intravenously administered. As those drugs which cannot pass through the harsh conditions of stomach (pH2) because they can easily be denatured (Sharpe et al. 2014). One of the most important agents for the oral/dermal delivery is insulin. Insulin is the major cause of worry for the world and it is usually administered subcutaneously. This procedure is very painful as at every administration patient’s skin is punctured. Usually protein is coated with such material which can easily pass

through the highly acidic conditions of stomach and can deliver the protein of interest to small intestine where its absorption takes place (Sharpe et al. 2014).

Hydrogels can be prepared in such a way that they show sensitivity to different pH values. The pH sensitivity of hydrogels is usually due to two factors: (1) Ionizable groups in response to change in pH (2) Bonds whose cleavage is pH sensitive. The pH sensitive hydrogels are generally divided into two types; cationic hydrogels and anionic hydrogels. In case of anionic hydrogels they are swollen at a pH greater than pKa of the polymeric network. These gels protect the drug from the stomach degradation and are ideal for the drug delivery in upper portion of small intestine and colon (Ronald et al. 1988). Cationic hydrogels are swollen at pH below the pKa of the polymeric network and these gels are mostly used for the delivery of drug in stomach while protecting it from the pH environment of the oral cavity (pH 5.8-7.4). These gels mostly serve as masking agents for the taste of drugs and are used for coating purposes (Douroumis et al. 2011). Alternatively, the passive transdermal delivery of drug which has been practiced for many years ago such as topical drugs applied on the skin for remedies. This form is now being practiced in advance form by the help of hydrogels which are acting as potential candidate for transdermal drug delivery because of their high water retention, swelling properties, strength and transparency indicating its biocompatibility with the native extracellular fluid. This way of drug incorporation looks attractive and alternative to both oral and skin injection (Mark et al. 2008). Hydrogels mimics with the natural conditions for drug delivery because of their moisture, thermo-sensitivity, biocompatibility and swelling kinetics. Wang et al. (2016) have reported the development of thermo-sensitive Poloxamer 407/Carboxymethyl cellulose sodium (P407/CMCs) composite hydrogel formulation with double functions of moisture and drug supply for acute dermatitis treatment. Hydrogels based iontotherapeutic devices are also being investigated for insulin, calcitonin and vasopressin through transdermal delivery (Soonkap, 1993; Valenta and Auner 2004). In addition to this, polysaccharide based diet is also a meant for controlling or causing delay in the absorption of glucose and hence inhibiting the hyperglycemic effect said by American Diabetes Association (Chandalia et al. 2000). This effect of dietary fibers based food lies in their viscous nature which can either trap the glucose or reduces its absorption in intestine and thus controls the rise of sugar in the blood (Jenkins et al. 1978; Vuksan et al. 2000). *Psyllium* is a fibrous form of Plantago plant husk which is medicinally proven helpful in gel forming and glucose lowering. *Psyllium* which represents several members of genus *Plantago* whose seeds when mechanically processed by milling/grinding to get the mucilage which accounts for 25% to the total seeds yield. The fibrous mucilage is water absorbing approximately tenfold or more making a clear, colorless mucilaginous gel. The gel nature and composition of the polysaccharides extracted from the seeds of the Plantagoovata has been reported in literature. These mucilaginous gels have several medicinal aspects in lowering blood glucose level, treating diarrhea, lowering cholesterol level and obesity (Singh 2007). In view of the pharmacological importance of *Psyllium* to reduce glucose absorption and drug delivery devices based on hydrogels, *Psyllium*, if suitably designed to prepare the hydrogels, can act as the double potential applicant to develop novel drug delivery vehicle. Therefore, the present study is an attempt to synthesize *Psyllium* based hydrogels by various cross-linked combinations of *Psyllium*, polyvinyl alcohol, polyacrylamide, casein hydrolysate, polyethylene glycol, graphene oxide in the presence of glutaraldehyde and N, N-methylenebisacrylamide as a cross-linker and ammonium persulfate (APS) as initiator and thereafter use as drug delivery devices. It also discusses the *in-vitro* and *in-vivo* release dynamics of insulin in a different release medium, for the evaluation of release mechanism and diffusion coefficients.

MATERIALS AND METHODS

Materials

Plantago *psyllium* husk was obtained from Qarshi industries (Lahore, Pak). Acrylamide was obtained from Merch Chemicals, Polyvinyl alcohol and acetone was purchased from MERCK Chemicals. Casein hydrolysate, pepsin, Bradford reagent and glycine were purchased from Sigma-Aldrich. Glutaraldehyde was purchased from Bio basic Inc. Bromophenol blue dye was purchased from Fisher Scientific. Bovine serum albumin purchased from Bio-World. Graphene Oxide (GO) was purchased from Sigma and dispersed in water for ultra-sonication (13 kHz frequency). Sodium bicarbonate and iso-butanol purchased from Riedel-de-Haën. Muller- Hinton agar purchased from TM media and formalin purchased from ACROS Organics.

Fabrication of Hydrogels/Membranes

*Psyllium*i spaghol husk (1g) was mixed with 200 ml of d.H_2O for overnight to swell homogenously. Then ispaghol (1%) and 2% polyvinyl alcohol were added in 250 ml measuring cylinder and placed on magnetic stirrer for 2h to which 1% casein hydrolysate and 0.016% GO was poured. After which 20% glutaraldehyde was added as a cross-linker. To this point, both A and B hydrogels have same composition but varies according to different components, following the further division on the basis of percentages used.

Hydrogels A and B (ISP-PVA-CH-PAA hydrogel)

In hydrogel A, 15% polyacrylamide, 10% APS (0.4 ml) and TEMED (24µl) were added. Hydrogel B had 20% PAA to which 10% APS (0.4 ml) and TEMED (24µl) were added whereas incubated at 45°C for overnight.

Characterization

These formulated hydrogels where characterized which have been discussed somewhere else (Amtul et al. 2017).

FTIR

Fourier Transformed Infrared spectroscopy of parent molecules and formulated hydrogel was done on Shimadzu IR prestige-21, Kyoto Prefecture Japan. The spectral resolution was 4 cm^{-1} at a range of 500-4000 cm^{-1} wavenumber.

Scanning Electron Microscopy (SEM)

SEM was used to understand and confirm the loading of drug on the hydrogels thus formed. The surface topology, to see the pores/channels of hydrogels the scanning electron microscope S-3700N Hitachi attached with EDX was used both for drug loaded and without drug that confirms the presence of the drug on hydrogels.

Swelling properties

Swelling ratio and kinetics: Buffers solution of pH 5.4 and pH 7.5 (0.1 M HCl-KCl buffer pH 5.4 and 0.1 M phosphate buffer pH 7.5) were prepared. 20 ml of each buffer was taken in separate flasks. Each hydrogel was weighed and immersed in 20 ml of buffers of different pH. The weight of the gels was recorded after every ten min till equilibrium was attained. All experiments were run in triplicates. The swelling degree of gels in each buffer was calculated using the formula:

$$Q = \frac{Ws - Wd}{Wd}$$

Where Ws is the weight of swelled hydrogel and Wd is the weight of dry hydrogel and Q is the extent of swelling (Rithe et al. 2014). To determine the swelling kinetics of drug (insulin) loaded ispaghol based hydrogels at various pH conditions recorded mass of hydrogels were used, and data were fitted to the Korsmeyer-Peppas Equation (Peppas and Franson 1983).

$$F(\%) = \frac{M^t}{M^\infty} = kt^n$$

Where F is the fraction uptake of swelling, Mt is the weight of the sample at time 'T' and M∞ is the weight of the sample at equilibrium, K is a constant and n is the diffusional exponent which ultimately determined the transport mechanism of hydrogel (Peppas and Franson 1983).

Folding endurance: To determine folding endurance of hydrogels (2.5 cm diameter) they were repeatedly folded at the same place, and the number of folds was counted until hydrogel was broken. Folding endurance was calculated using the formula:

$$Fd=\log 10d$$

Here, Fd indicates folding endurance; d is the number of double folds

Biocompatibility assays

Anti-microbial activity: The anti-microbial activity of insulin loaded ispaghol based hydrogel was analyzed by disk diffusion method. The freshly grown culture of E. coli (O.D600=0.6, 50ul) was spread on the LB agar plates (Korsmeyer et al. 1983). The sterilized hydrogels were then placed on the plate. All the plates were incubated at 37°C overnight the gels were removed next morning after incubation, and the contact inhibition was observed on the agar plate.

Fabrication of drug loaded hydrogels

Estimation of Insulin: For the preparation of drug (insulin) loaded hydrogel, the amount of insulin is measured by taking absorbance of number of standard solutions (BSA) by using UV visible spectrophotometer and a standardized graph is constructed and concentration of insulin is measured from this standard graph as the concentration with the corresponding absorbance of the solution (Havlinova et al. 2009).

Fabrication of drug (Insulin) loaded hydrogels: Insulin is loaded onto the hydrogels by following the swelling equilibrium method. Hydrogels were soaked in the drug solution of known concentration where insulin diffuses into the hydrogels due to slackening of the polymeric chain. Swelling of hydrogels occurs due to absorption of insulin. This process is carried out at 37°C for overnight. The hydrogels were kept at same temperature unless it dries to get the release device.

Drug release from hydrogels

Two different methods of drug release were used for determining the drug release from hydrogels.

***In-vitro* drug release:** Drug release was determined using extraction method in pH 5.4 and pH 7.5 buffers. In a conical flask 20 ml of 0.1 M Tris-Cl buffer was taken that acts as release medium. Hydrogel loaded with insulin was immersed in buffer solution at 37°C the drug release was monitored by taking absorbance at 280 nm after every thirty mins for 6 h.

Drug release through chicken skin model: The chicken skin model was prepared to examine the drug release through skin model. The chicken skin was obtained from a local market, excessive fats were removed, and skin was washed thoroughly with distilled water. It was then cut into the 3 × 3 cm sections. A donor compartment was made using a polypropylene tube. On one end of the vessel, the skin patch along with drug loaded hydrogel was mounted, the other end was capped. A receiving chamber was prepared using a petri dish containing Tris-Cl buffer pH 7.5 and other with pH buffer 2. The donor compartment with the skin and hydrogel patch was dipped in the buffer of receiving compartment. The receiving chamber with buffer was placed on the magnetic stirrer at 50 rpm. The buffer from the receiving chamber was taken after every 10 min and absorbance was taken at 280 nm. The concentration of the drug released was measured from the standard graph of insulin release. To study the release kinetics of the drug from the skin model the following equation is used:

$$Q_0 - Q_t = K_0$$

Where Qt is the cumulative amount of drug released at time‘t,’ Q0 is the initial amount of drug in the solution mostly zero, and K0 is the constant of zero order release. Data obtained from *in-vitro* drug release was plotted as cumulative% of drug release versus time t (Narasimhan et al. 1999).

Biological effects of the released drug

Albumin denaturation inhibition: Percentage inhibition of albumin denaturation was determined by following equation (Narasimhan et al. 1999).

$$Percentage\ inhibition = \frac{(Abs\ Control - Abs\ Sample) \times 100}{Abs\ Control}$$

Anti-proteinase activity: Anti-proteinase activity of drug released form hydrogel was evaluated (Narasimhan et al. 1999).

RESULTS

Physiochemical characteristics of formulated insulin drug loaded hydrogels were analyzed to govern their application as confined drug delivery system for transdermal patches. Glutaraldehyde cross-links the *Psyllium* and monomers, on which active sites were generated by APS as free radical mechanism that lead to formulation of polymeric network of hydrogels. This 3D polymeric hydrogel was used to study the drug release both *in-vitro* and *in-vivo* (Peppas et al. 2004).

Characterization of hydrogels A and B

Hydrogel A and B: Hydrogel 'A' is transparent and has intact structure with folding capacity (flexibility) due to presence of *Psyllium*, Polyvinyl Alcohol and 15% Polyacrylamide cross-linkages having strong hydrogen bonding while Hydrogel 'B' has more intactness with folding capacity (flexibility) due to presence of *Psyllium*, polyvinyl alcohol, Glycerol and 20% Polyacrylamide cross-linkages having strong hydrogen bonding and stretching ability (Figures 1 and 2).

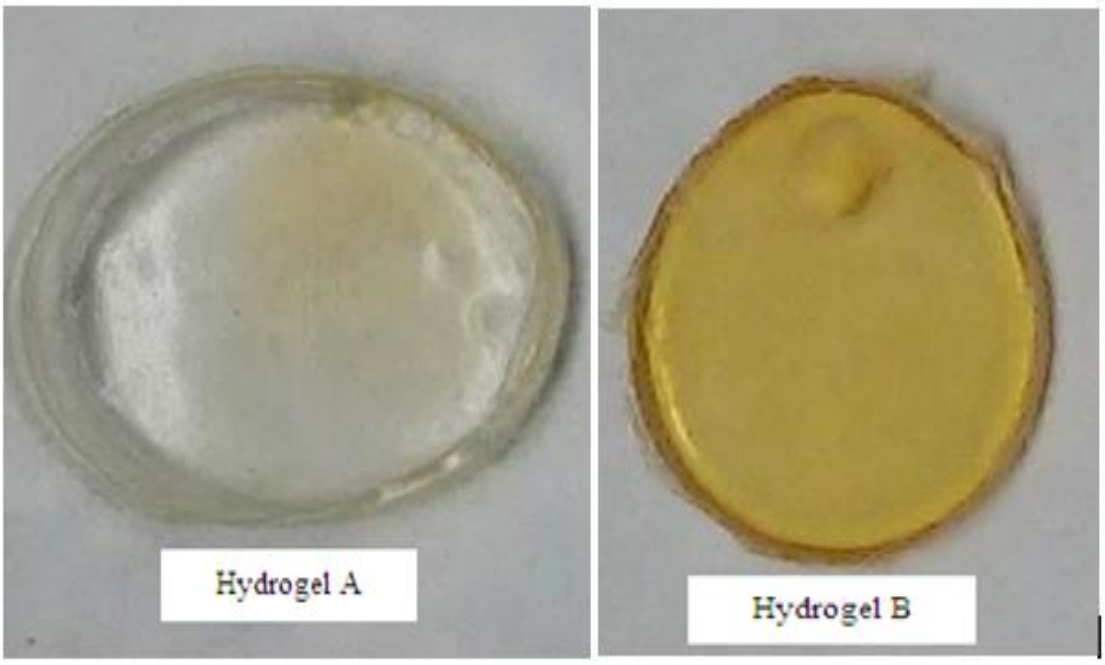

Figure 1. Hydrogel A (*Psyllium*-PVA-Casein hydrolysate-PAA) with 15% polyacrylamide, Hydrogel B (*Psyllium*-PVA-Casein hydrolysate-PAA) with 20% polyacrylamide.

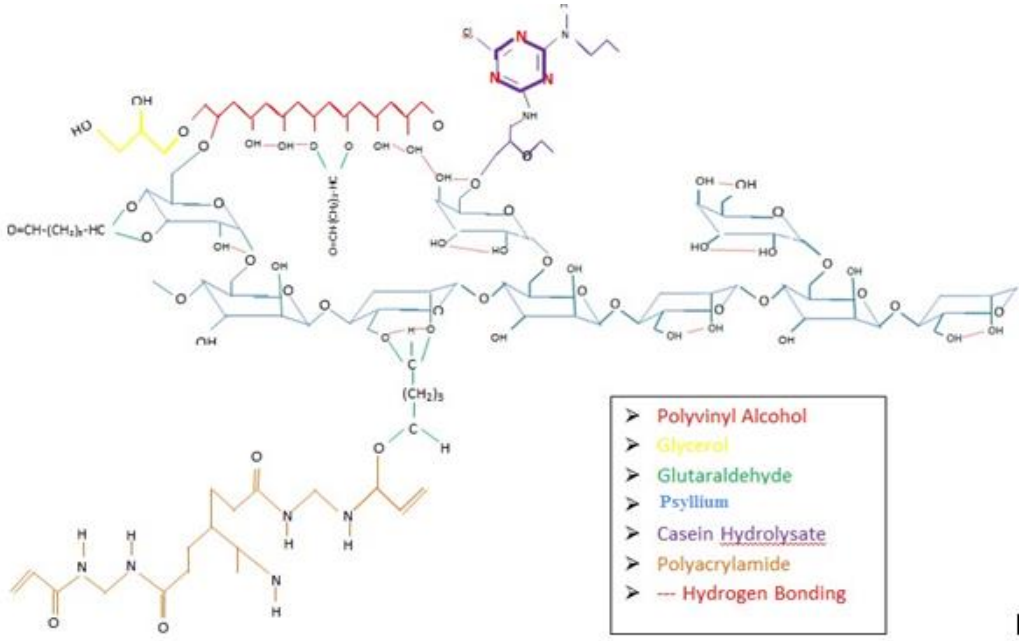

Figure 2. Schematic diagram of mechanism of action for hydrogel A and B.

FTIR of Hydrogel A and B

FTIR spectrum of hydrogel A and B is shown in Figure 3 Absorbance peak at 3190.26 cm^{-1} of hydrogel A (3305 cm^{-1} in hydrogel B) shows the symmetric vibration of -NH_2 of acrylamide unit and –OH stretching band of PVA and *Psyllium*. Peak at 2935.66 cm^{-1} of Hydrogel A (2937.59 cm^{-1} of Hydrogel B) and 2931 cm^{-1} indicates the presence of C-H broad alkyl stretching band of PVA and *Psyllium* while vibrational bands of CH and CH_2 groups of acrylamide unit. Vibrations of COO- group of casein hydrolysate and *Psyllium* can be seen at peak 1656.85 cm^{-1} of Hydrogel A (1668.43 cm^{-1} in case of Hydrogel B) whereas peaks at 1417.68 cm^{-1} and 1654 cm^{-1} of Hydrogel A (1419.61 cm^{-1} and 1037.70 cm^{-1} in case of Hydrogel B) suggests the stretching vibration of N-H and C=O groups of acrylamide, *Psyllium* and casein hydrolysate. Characteristic absorption peak of PVA and broader absorption bands of C-O and C-O-C are indicated at 1078.21 cm^{-1} of Hydrogel A (1103.28 cm^{-1} in case of Hydrogel B) that was due to glutaraldehyde and PVA cross-linkage. In addition, C-O-C stretching vibration of *Psyllium* was also found at 920.05 cm^{-1} in Hydrogel 'B' (Chaud et al. 2002; Magalhães et al. 2012; Kenawy et al. 2014; Reis et al. 2006; Vaghela et al. 2014; Mushtaq et al. 2020). (Figure 3).

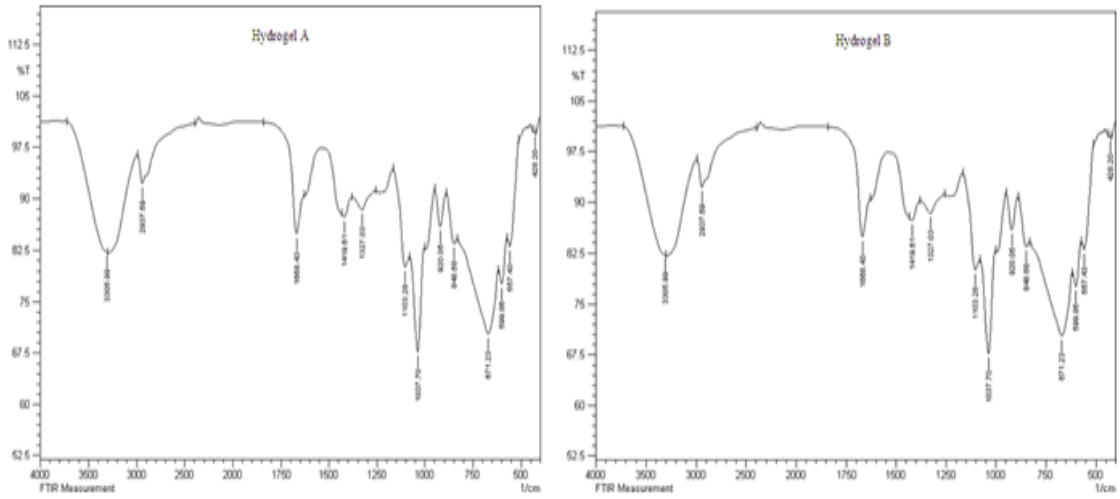

Figure 3. Comparison of FTIR of Hydrogel A and B.

SEM of Hydrogel A and B

Figure 4 shows the surface characteristics of hydrogel A and B that was examined via SEM with magnification of 500x, 1.00x and 2.00x at 10.0 KV. (A) With 500x magnification shows irregular porous surface with polymerization of acrylamide and strong cross-linkages of *Psyllium* and glutaraldehyde with acrylamide in the presence of PVA. (B) With 1.00x magnification shows bulging appearance of inter-penetrated network with dynamic porosity (2-4 µm) for controlled water uptake while (C) with 2.00x magnification shows large number and regular size pores for Fickian diffusion mechanism. Rithe et al. reported that the presence of an appropriate amount of glutaraldehyde (1%) aids in attaining the structure containing fine pores and spaces and enhanced surface area (Chandrika et al. 2016; Wu et al. 2007; Rithe et al. 2014). Excessive addition of cross-linkers transforms the hydrogel and its physicochemical properties.

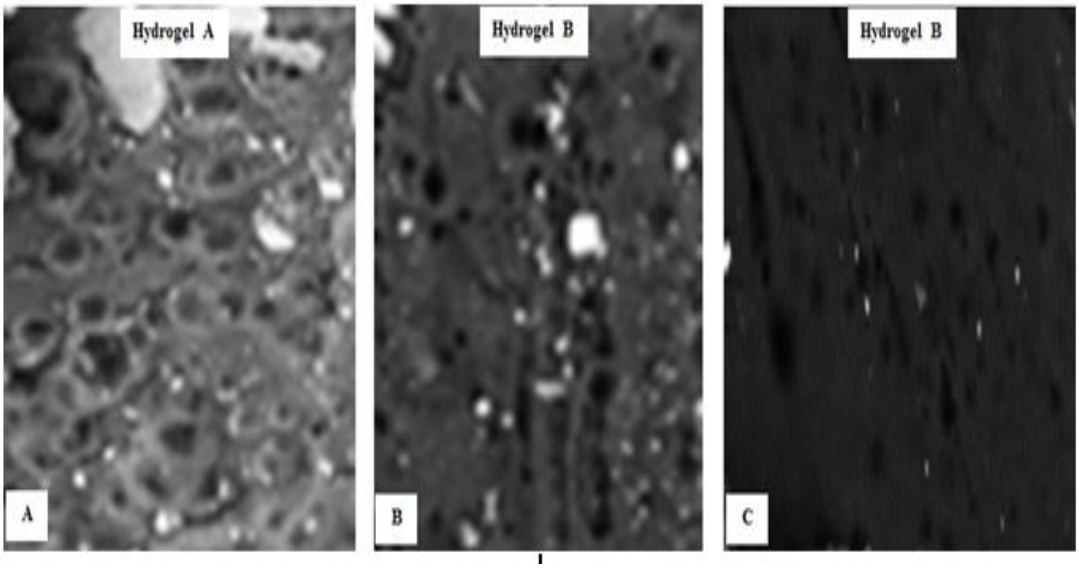

Figure 4. Morphology of Hydrogel A obtained via use of SEM at (A) 500x. Morphology of Hydrogel B (B) 1.00x and (C) 2.00x at 10.0 KV.

Swelling property of hydrogel A and B

Swelling ratio: The fundamental feature of any type of hydrogel is its swelling in solvent. The hydrophilic amino acids of casein hydrolysate and hydrophilic nature of the *Psyllium* can interact with the charged or water molecules. More the concentration used, more will be the crosslinking and more will be the interaction with the charged/water molecules. The swelling degree of hydrogels was recorded in buffers of different pH. The buffers were acidic (phosphate buffer of pH 5.4); alkaline (Tris-Cl buffer of pH 7.5) were used to obtain the swelling degree of hydrogels. All the experiments were run in triplicates (Figure 5). In different pH solutions different swelling were observed. This difference was due to the protonation and deprotonation of various functional groups in the polymeric chain. The fabricated hydrogel have free -NH_2 and -OH groups that accept proton in the acidic pH as in the acidic pH amount of proton is high and hence major cause of swelling of hydrogel in acidic pH [38]. Maximum swelling of hydrogel ‘A’ and ‘B’ at pH 5.4 was 3.76 g and 5.25 g was observed respectively. Swelling at pH 7.5 was 2.66 g and 3.12 g in hydrogel ‘A’ and ‘B’ respectively. This swelling variability was due to change in the monomeric ratio. This capability of swelling decrease sharply due to decrease in ionization degree and decreased interaction with the free protons which are not available in the basic pH (Wu et al. 2007; Danish et al. 2020).

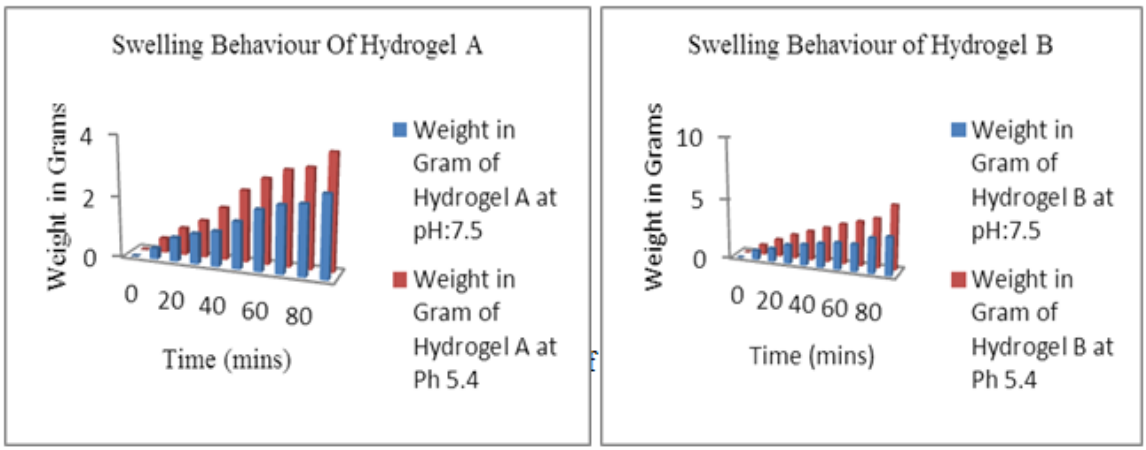

Figure 5. He buffers were acidic (phosphate buffer of pH 5.4); alkaline (Tris-Cl buffer of pH 7.5) were used to obtain the swelling degree of hydrogels.

Swelling kinetics: Diffusion is the process by which hydrogels undergo swelling process (Figure 6). A similar trend as compared to swelling ratio was observed. Hydrogels 'A' and 'B' show maximum swelling at acidic PH.

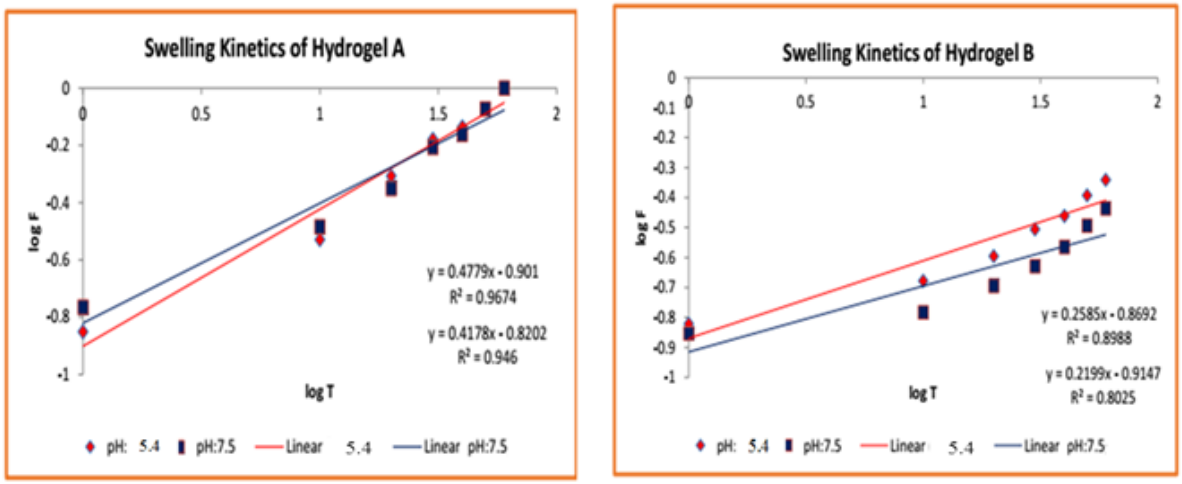

Figure 6. Swelling kinetics of Hydrogel A and B.

Diffusion coefficients: The swelling of fabricated hydrogels follows the Fick's law of diffusion. In Figure 7, the calculated values of diffusion coefficients for *Psyllium* based hydrogels 'A' and 'B' decrease with an increase in pH, Martinez-Ruvalcaba have reported similar results (Martínez-Ruvalcaba et al. 2007). The diffusivity or diffusion coefficient represents the diffusion across the porous structure of hydrogel. When the diffusion coefficient of a substance decreases the diffusion also decrease. The estimated diffusion coefficient of hydrogels was high at acidic pH and reduced with the subsequent increase in pH suggesting lower diffusion at basic pH. As the pH of the medium goes up the hydrophobicity of formulated hydrogel decreases, this results in lesser diffusion and small values of the diffusion coefficient (Figure 7).

Ph	Hydrogel weight (g)	Swelling fractional constant (F)	Log of F	Slope n	Intercept k	R-squared value (R_2)	Type of diffusion
5.4	0.10	0.141	-0.851	0.4779	-0.901	0.9674	Fickian diffusion Hydrogel
	0.21	0.296	-0.529				
	0.35	0.493	-0.307				
	0.47	0.662	-0.179				
	0.52	0.732	-0.135				
	0.60	0.845	-0.073				
	0.71	1	0				
7.5	0.1	0.172	-0.764	0.4181	-0.8178	0.9517	Fickian diffusion hydrogel
	0.19	0.328	-0.484				
	0.27	0.466	-0.332				
	0.36	0.621	-0.207				
	0.40	0.690	-0.161				
	0.49	0.845	-0.073				
	0.58	1	0				

pH	Swelling fractional constant (F)	Log of F	Slope n	Intercept k	R-squared value (R2)	Type of diffusion
5.4	0.151	-0.821	0.2585	-0.8692	0.8988	Fickian diffusion Hydrogel
	0.210	-0.678				
	0.254	-0.595				
	0.312	-0.506				
	0.346	-0.461				
	0.405	-0.393				
	0.456	-0.391				
7.5	0.140	-0.854	0.2199	-0.9147	0.8025	Fickian diffusion hydrogel
	0.165	-0.783				
	0.202	-0.695				
	0.235	-0.629				
	0.272	-0.565				
	0.321	-0.493				
	0.366	-0.915				

Figure 7. Swelling Kinetics of Hydrogel A and B at Different pH (Ph 5.4 and 7.5).

In-vitro drug release by Hydrogel 'A and B'

In-vitro drug release from the fabricated hydrogel 'A' and 'B' in different pH solutions was measured by taking absorbance at 280 nm as shown in Figure 8. The amount of drug released from the hydrogel was higher in pH 5.4 then in pH 7.5. Amount of insulin released in pH 5.4 and 7.5 was 3.332 ug and 2.269 ug in hydrogel 'A' and 3.881 ug and 2.188 ug in hydrogel 'B' per 0.5g of gel respectively after 150 min. The drug release from polymeric matrix follows the Fickian diffusion mechanism. It shows a sustained drug release from the hydrogel which is in requirement with the drug delivery system. Gorle et al. (2016) published similar results by formulating a transdermal patch formed by xanthum gum. The published data indicate that the patch was an effective formulation for the delivery of insulin.

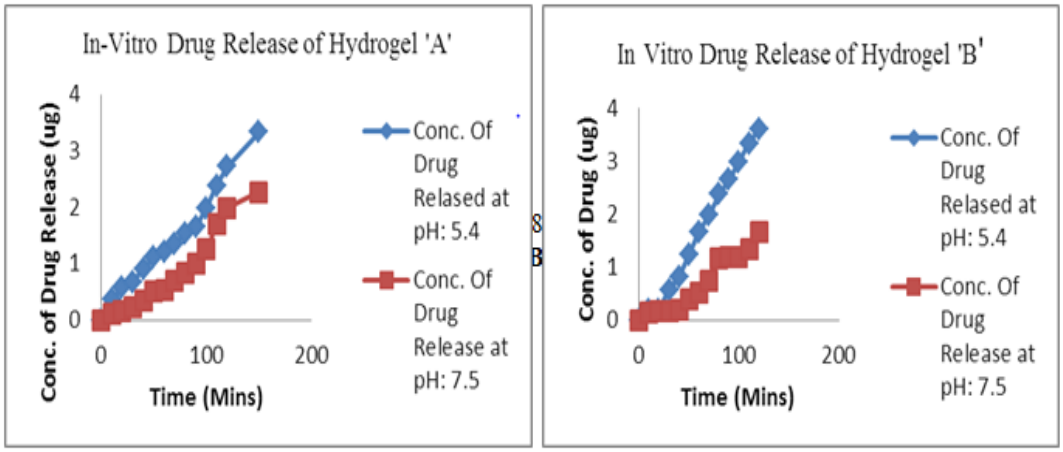

Figure 8. *In-vivo* drug delivery by hydrogel A and B.

The working of the transdermal patches of *Psyllium* based insulin loaded hydrogels was determined on the avian skin, as model. The outermost layer (ectoderm) of chicken skin which acts as physical barrier was used to study the drug release *in-vivo*. Figure 8 shows drug release from *Psyllium* based hydrogel via the chicken skin into the buffer. Approximately 3.673 ug and 2.982ug of insulin was released by Hydrogel 'A' and 3.132 ug and 2.269ug of insulin was released by Hydrogel 'B' per 0.5g of gel at pH 5.4 and 7.5 respectively in 150mins. Linear trend line

suggests sustained release of insulin from *Psyllium* fabricated hydrogel. Kinetics studies confirmed that the drug release followed zero order kinetics (Gorlea 2016; Varelas et al. 1995). Our results are in agreement with the findings reported by Baljit et al. (2010) where hydrogels containing insulin followed the same mechanism of release of drug (Figure 9).

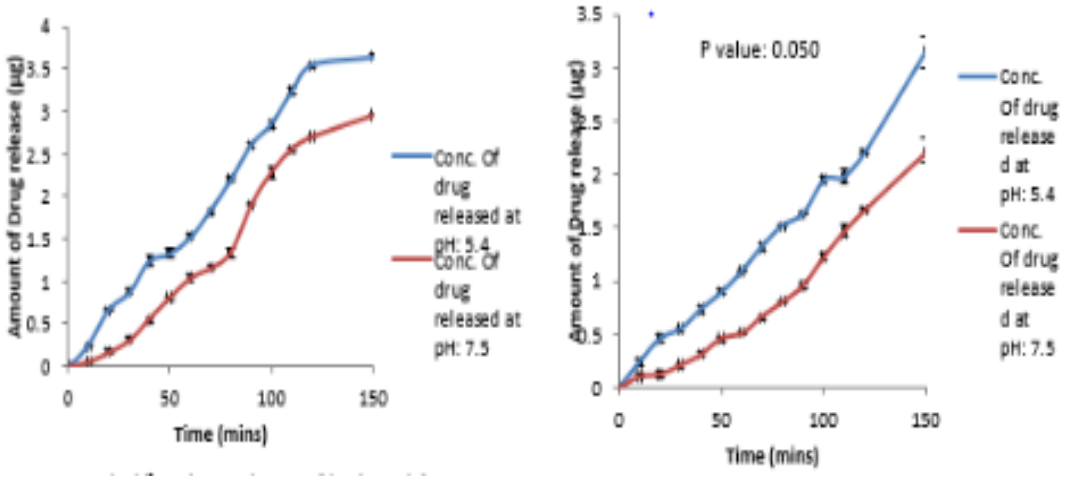

Figure 9. *In-vivo* drug release of hydrogel A and B.

Biocompatibility assays

Anti-microbial activity: Disc diffusion method was used to observe the anti-microbial and contact inhibition of these characterized gels. The anti-microbial activity was mainly due to casein hydrolysate, a component of hydrogels A and B. No growth of *E. coli* strain (DH5α) was observed on or in the contact inhibition areas. The contact area was calculated in various hydrogels was 20 to 25mm. the results were quite similar to the findings of Wu et al. (2007) who reported casein hydrolysate hydrogels with effective antibacterial properties. The proposed mechanism of this inhibition was due to the polycationic nature of the polymers in the hydrogel which interferes with the negatively charged residues in the cell wall of bacteria.

CONCLUSION

Psyllium represents a double potential in drug delivery system due to its glucose lowering and gel forming nature making it admirable vehicle for the treatment of diabetes mellitus as indicated from the drug release dynamics in different release medium and avian skin. Fickian diffusion mechanism at pH 5.4 and 7.5 buffer system is followed in drug release because of entering of water into the hydrogel goes parallel with the drug release showing its good swelling properties. Anti-albumin denaturation activity indicates the biological activity of the released drug is not affected from the hydrogels. These *Psyllium* based insulin loaded hydrogels (A and B) could be safe, biocompatible and efficient in transdermal drug delivery system

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