BIOLOGIA (PAKISTAN)
June, 2021, 67 (I), 1-12

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Synthesis and Characterization of Various Porous Hydrogels for In-Vitro and In-Vivo Drug (Insulin) Delivery

USMAN AHMAD*[1], AMTUL JAMIL SAMI[1], MADEEHA KARAMAT[1], MADEEHA KHALID[1], FAIZAN NAEEM[2] & MUHAMMAD JAMIL YOUSAF[3]

[1]Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan
[2]Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Islamabad
[3]Directorate Colleges Gujranwala

ARTICLE INFORMAION

Received: 10-06-20
Received in revised form: 20-10-20
Accepted: 20-02-21

***Corresponding Author:**

Usman Ahmad:
ahmadusman167@gmail.com

Original Research Article

ABSTRACT

Transdermal drug delivery seems to be overwhelming and alternative to other means of drug delivery via oral or intravenous. To treat the diabetes mellitus and to get rid of the traditional injection treatment which is a painful process and can cause infections and allergy, the drug delivery through the skin cells can substitute only if the skins cells permeability for the drug is increased, which is now done by chemical, ultrasonic and electrical methods. Drug delivery through the largest organ (skin) by dermal patches of psyllium based hydrogels was observed. In this article we have fabricated different psyllium based dermal patches of hydrogels for insulin delivery. Psyllium, a natural polysaccharide, is a dietary fiber and widely utilized in gel forming. To study the structural aspects of these various polymeric webs were categorized with FTIR, SEM and release related experiments such as swelling kinetics, swelling ratio and drug kinetics and the impact of pH on the release of insulin from medicated hydrogels has been concentrated to evaluate the medication discharge instrument in-vitro and in-vivo. Fickian diffusion mechanisms have been read for the release of insulin at diverse pH (5.4 and 7.5).

Keywords: Psyllium, Insulin, Hydrogel, Drug Release, Transdermal Patches.

INTRODUCTION

Diabetes or hyperglycemia (high blood glucose) is a hereditary or multifactorial disease. It is also a major reason of kidney failure, adult blindness, lower limb exclusion and cardiac diseases. The worldwide diabetes occurrence in 2019 was 9.3% (463 million people) reported by the International Diabetes Federation. In order to control the blood glucose level at constant level, diabetic patients usually have to inject insulin 2 to 4 times per day exogenously via a subcutaneously needle syringe, insulin pen or catheter attached to insulin drives. These traditional practices are usually not convenient because it can cause allergic reactions, infections, pain, needle “phobia” and also nullifying the standard of life of patients (Buysschaert & Lambert, 1989; Lenhard & Reeves, 2001). The delivery of insulin through the skin shows alternative way because it provides advantages over escaping degradation in the gut or first pas liver effects. But first it has to surpass the impermeability of the skin (stratum corneum) which is now attained by different kinds of enhancers like chemical, ultrasonic or electrical (Bastaki, 2005; Benson, 2002). Another alternative for drug delivery through skin cells is hydrogels. Hydrogels have been proved to be a hopeful candidate for such a system (Peppas *et al*., 2004; Kim *et al*., 2003).

Hydrogels (also called smart or intelligent gels) are three-dimensional systems. They are hydrophilic polymers that become swollen with the help of a component (water) or other body fluids (Campoccia *et al*., 1998). Hydrogels are usually stable, but after swelling in solvent it gradually breaks down but stability can be improved by the increasing amount of cross-linker. Hydrogels can be physically cross-linked by hydrophobic/hydrophilic interactions or hydrogels bonding as well. Others can be a chemical cross-linked by means of covalent interaction within the network (Wichterle & Lim 1960). Hydrogels have a broad variation of

Author Contribution: U. A., Prepared all the gels and did their optimization and characterization; **A. J. S.,** Being head of the lab, provided all the lab equipment and materials; **M. K.,** Performed FTIR; **M. K.,** Performed antimicrobial activity; **F. N.,** Performed all the statistical analysis along with graph preparation; **M.J.Y**., contributed in structural characterization of hydrogels.

functions such as in cell culture, tissue engineering, direct 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 damaged heart valves.

Hydrogels are being used to deliver drugs orally or dermally which were mostly protein in nature and were previously intravenously administered. As those drugs which cannot pass through the harsh conditions of the stomach (pH 2.0) because they can easily be denatured (Campoccia *et al*., 1998). 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 the stomach and can deliver the protein of interest to the small intestine where its absorption takes place (Wichterle & Lim 1960).

As for the diabetic patients the controlled glucose level in the blood is meant for survival of such patients which can be acquired via oral, buccal, nasal, respiratory, rectal, and ophthalmic and transdermal paths. The exogenous insulin delivery should mimic the secretion of insulin physiologically. By various complications of the above stated methods, skin is now gaining pronounced deal of concern as substitute for transdermal delivery of drugs especially insulin for diabetic patients. However, stratum corneum (outermost skin layer) is seemed to be a major hurdle in drug delivery. But permeability of the skin can be increased by different types of enhancers like chemicals, ultrasonic and electrical (e.g. iontophoresis or electroporation) (Zakzewski *et al*., 1998; Brand *et al*., 2000).

The drug delivery through the skin is also carried out for topical drugs for skin remedies. The Transfer of drugs via the skin into the body is now being practiced for the safe and easy means for those drugs which are being degraded in the acidic environment of the gastrointestinal tract. Hydrogels which are acting as a possible contestant 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 (Brand *et al*., 2000). 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 growth of thermo-sensitive Poloxamer 407/Carboxymethyl cellulose sodium (P407/CMCs) composite hydrogel preparation by double purposes of humidity & drug delivery for acute dermatitis therapy. Hydrogels based iontotherapeutic devices are also being investigated for insulin, calcitonin and vasopressin through transdermal delivery (Wang *et al*., 2016)

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 (Constantin *et al*., 2020). This effect of dietary fibers based food lies in their viscous nature which can either trap glucose or reduces its absorption in intestine and thus controls the rise of sugar in the blood (Sharpe *et al*., 2014). The Soluble form of these dietary fibers shows more beneficiary impacts on gastric emptying, macronutrient incorporation, and reduces the glucose reactions during or after the meal (Mark *et al*., 2008). Plantago plants are the source of psyllium husk which is normally utilized as dietary fibers. They are very helpful in reducing the sugar level by interrupting its absorption in the intestine because these fibrous husks increase the intestinal motility. This has been clearly demonstrated when both the psyllium and glucose were fed simultaneously (Wang *et al*., 2016). More flow in the intestine will lead to less absorption of glucose in ileum and hence less increase in blood sugar level, this show sugar tolerance in diabetes (Valenta *et al*., 2004). This reaction may be because of breaking down of gastric exhausting, expanded intestinal section, or a transformation of the discharge and impact of digestive enzymes (Jenkins et al., 1978).

The grinding of Plantago seeds produces psyllium husks mucilage which accounts for 25% to total seeds yields. When this fibrous mucilage absorbs water, it can form clear, colorless mucilaginous gel. They can absorb approximately tenfold of H_2O. In literature, the network of polysaccharides and the gel structure of *Plantago ovate* seeds have been elaborated. These mucilaginous gels have several medicinal aspects in lowering blood glucose level, treating diarrhea, lowering cholesterol level and obesity (Vuksan *et al*., 2000). In assessment of the pharmacological significance of psyllium polysaccharides to diminish glucose assimilation and medication conveyance techniques built on hydrogels, psyllium, if appropriately intended to define the hydrogels, may proceed as the twofold potential chosen people to make novel medication dissemination frameworks..

That is why, current study is an effort to create psyllium based different hydrogels (C, D and E) by various cross-linked combinations of psyllium, polyvinyl liquor, casein hydrolysate, polyethylene glycol, collagen, agrose and hyaluronic corrosive in the presence of glutaraldehyde and N, N-methylenebisacrylamide as a cross-linker & ammonium persulfate (APS) as a trigger and a while later use as medication transport frameworks. It likewise considers the in vitro and in-vivo release elements of insulin in a different pH discharge channel, to estimate the conveyance component and appropriation coefficients.

MATERIALS AND METHODS

Chemicals

Plantago psyllium husk was acquired from Qarshi industries (Lahore, Pak). Acrylamide was obtained from Merck Chemicals, Polyvinyl alcohol and acetone was bought from MERCK Chemicals. Bradford reagent, glycine, Casein hydrolysate and pepsin were bought from Sigma-Aldrich. Bromophenol blue dye, Glutaraldehyde, Bovine serum albumin, Sodium bicarbonate and iso-butanol, Muller- Hinton agar and formalin were bought from Fisher Sci., Bio basic Inc., Bio-World, Riedel-deHaën, TM media and ACROS Organics respectively. Collagen was separated from heart muscles of chicken bought from a traditional market of Lahore, Pakistan.

The Fabrication of Hydrogels/Membranes

The optimum conditions for the modification of psyllium based hydrogels have been discussed somewhere else (Sami *et al*., 2017, 2018a, b, c). To carry out the reactions, psyllium ispaghol husk (1g) was combined with 200ml of d.H_2O to erupt homogenously and placed for overnight. At that point ispaghol (1%) and Poly-Vinyl liquor (PAA) (2%) were consolidated in a container and put on magnetic agitator for 120 minutes to which 1% casein hydrolysate. After that glutaraldehyde (20%) was consolidated as a cross-linker. To this level, all hydrogels have comparable cosmetics however contrasts as per different segments, with the goal that they are divided into hydrogel C, D and E, accordingly the extra division on the base of rates utilized.

Hydrogels C, D & E (ISP-PVA-CH-PAA-AG-PEG hydrogel)

In hydrogel C & D, 0.7% agarose, 4.5% Polyethylene glycol (5 and 7ml), 20% glycerol (5 and 1ml), 20% acryl/bis-acrylamide, 10% APS (500 and 600µl, respectively) and TEMED (15µl) were combined after glutaraldehyde cross-linking for 5hours. Mixture was inserted in molds & at first incubated at 50°C for 10minutes then at 30°C for all night to dry the hydrogels' scaffolds. In hydrogel E, 1% agarose, 20% PAA, TEMED and 10% APS were utilized with rising concentration of 4.5% PEG and declining concentration of glycerol with incubation for all night at 30°C.

Characterization

These formulated hydrogels were characterized as described by Sami *et al*., 2017.

Fourier Transform Infra-Red Spectroscopy (FTIR)

Fourier Transformed Infra-Red spectroscopy (FTIR) of parental molecules & prepared hydrogel was done on Shimadzu IR prestige-21, Kyoto Prefecture Japan. 4cm−1 at a range of 500–4000cm−1 wave number was the spectral resolution.

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 (SEM) S-3700N Hitachi with attached EDX was utilized both for drug loaded and without drug that confirms the presence of the drug on hydrogels.

Swelling Properties

Swelling Ratio and Kinetics

Formulation Buffer solutions of 5.4 and 7.5 pH (0.1 M HCl-KCl buffer with 5.4 and 0.1 M phosphate buffer with pH 7.5) were processed . Individual flask having Each solution of a 20 ml buffer was carried. Every type of hydrogel was weighed up & submerged in buffer of 20 ml solutions of diverse pH (5.4 & 7.5). The gels' weight was documented after every 10 minutes until equilibrium was achieved. Triplicates of every experiment were performed. By the following formula, the swelling extent of each buffer was determined.

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

Where,
Ws = Weight of hydrogel that has swelled
Wd = dry hydrogel weight
Q = swelling extent (Singh, B. 2007).

To define the swelling kinetics of drug (insulin) loaded ispaghol based hydrogels at different pH conditions (pH= 5.4, 7.5) weight of hydrogels and documented data were assigned to the following equation (Korsmeyer *et al*., 1983) (Rithe *et al*., 2014).

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

as,
F (%) = fraction of uptake of swelling,
M^t = sample weight at time 't'
M^∞ = sample weight at equilibrium,
K = constant
n = diffusional exponent which in the long run closed the vehicle component of hydrogel (Peppas *et al*., 1983).

Folding Endurance

The strength of folding of hydrogels (2.5 diameters) was determined by repetitively folding at similar place. Until hydrogel was shattered, the number of folds was calculated by following formula:

$$Fd = log10d$$

Here,
Fd = folding endurance; d = double folds number

Biocompatibility Assays

Anti-Microbial Activity

Disk diffusion method is used for the evaluation of antimicrobial action of insulin loaded ispaghol based hydrogel. *E. coli* (O.D 600 = 0.6, 50ul), newly grown culture was dispersed on the LB agar plates (Sami *et al*., 2018b). The cleaned hydrogels were then arranged on the plate. Bring forth of the considerable number of plates were done at 37°C for the night the gels were cleared next morning subsequent to agonizing, and the contact limitation was perceived on the agar plate.

Fabrication of Drug Loaded Hydrogels

Estimation of Insulin

For the formation of drug loaded with hydrogel, the amount of insulin was measured by taking absorbance of number of standard solutions (BSA) by using UV visible spectrophotometer and standardized graph was plotted and concentration of insulin was measured from this standard graph as the concentration with equivalent solution absorbance (Peppas *et al*., 1983).

Fabrication of Drug (Insulin) Loaded Hydrogels

Swelling equilibrium method is the method by which insulin was loaded onto hydrogels. In the drug mixture of known concentration where insulin diffuses into the hydrogels due to relaxation of the polymeric chain, hydrogels were soaked. Swelling of hydrogels occurs due to absorption of insulin. This process was carried out at 37◦C for overnight. The hydrogels were kept at the same temperature unless it dried to get the release device.

Drug Release from Hydrogels

Out of different methods, two separate techniques for tranquilize release were utilized for figuring the medication release from hydrogels

In-Vitro Drug Release

Medication discharge was determined utilizing extraction strategy in pH 5.4 and pH 7.5 cradles. In a 20 ml conical flask 0.1 M Tris-Cl buffer was held that was acted as discharge medium. Hydrogel fused with insulin was submerged in buffer at 37°C and the medication discharge was observed by taking absorbance at 280 nm after every thirty minutes for 6 hours.

Drug Release Through Chicken Skin Model

The model of chicken skin was sorted out to inspect the drug movement by methods for skin model. The skin of chicken was taken from a customary market. Extreme fats were isolated and the skin was washed broadly with refined water. By then, it was isolated into the 3×3cm territories. A benefactor zone was made using a polypropylene tube. At one terminal of the vessel, the skin fix alongside sedate filled hydrogel was mounted; the uttermost edge was made sure about. A gathering chamber was figured utilizing a Petri dish containing Tris-Cl support containing pH of 7.5 and other with pH cradle 2. The giver segment with hydrogel fix and the skin was plunged in the support of gathering segment. The getting depression with cushion was put on the attractive stirrer at the

speed of 50 rpm. The segments of cradle from the getting hole were taken after like clockwork and optical thickness was seen at 280 nm. The drug discharged was resolved from the standard diagram of insulin discharge. To look at the release vitality of the drug from the skin model, underneath condition was used:

$$Q_0 - Q_t = K_0$$

Where,

Qt = the total measure of drug discharged at't' time; *Q0* = the starting amount of drug in the solution (mostly zero), & *K0* = the constant of the zero order release. Assembled data from in vitro drug conveyance was plotted as cumulative% of drug discharge versus time t (Rithe *et al*., 2014).

RESULTS AND DISCUSSION

Physiochemical characteristics of devised insulin drug loaded hydrogels were studied to manage their usage as the specified drug conveyance system for transdermal patches. Glutaraldehyde cross-links the psyllium and monomers that lead to formulation of polymeric network of hydrogels. This 3D polymeric hydrogel was used to study the drug discharge both in-vitro and in-vivo (Benson, 2002).

Hydrogel C, D and E

Hydrogel 'C' has opposition with bowing limit (adaptability) because of presence of psyllium, Poly-Vinyl liquor (PVA), agarose, PEG and 20% acrylamide cross-linkages having amazing hydrogen holding. Hydrogel 'D' has more wholeness and force with twisting limit (adaptability) because of presence of Psyllium, Polyvinyl Alcohol, glycerol, 20 % Polyacrylamide, agarose and PEG cross-linkages having ground-breaking hydrogen holding and extending capacity while hydrogel 'E' has more flawlessness and force with bowing limit (adaptability) because of quality of Psyllium, Polyvinyl Alcohol, glycerol, 20 % Polyacrylamide, 1% agarose and 4.5% PEG cross-linkages having incredible hydrogen holding and colossal extending capacity.

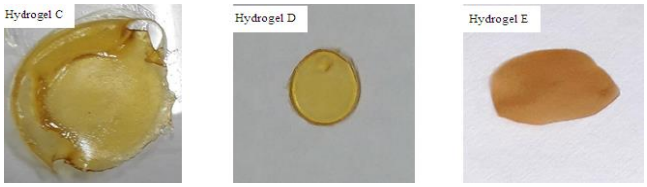

Fig.1: Hydrogel C (Psyllium-PVA-Casein-hydrolysate-PAA-agarose-PEG) with 20% polyacrylamide & 0.7% agarose. Hydrogel D (Psyllium-PVA-Casein hydrolysate-PAA-AG-PEG) with 20% polyacrylamide), Hydrogel E (Psyllium-PVA-CH-PAA-AG-PEG) with 1% agarose

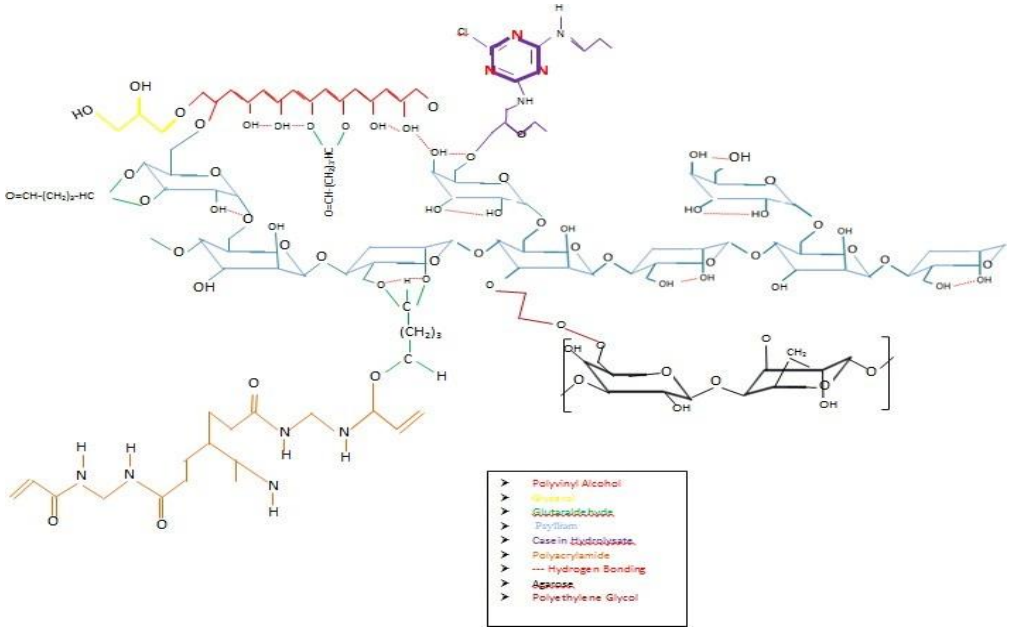

Fig 2: Schematic diagram of mechanism of action for hydrogel C, D and

Fourier Transform Infrared Spectroscopy (FTIR) of Hydrogel C, D and E

Fourier Transform Infrared Spectroscopy (FTIR) range of hydrogel C, D and E is appeared in Fig. 3. Absorbance peaks at 3327.21cm-1, 3329.14cm-1 and 3344.57cm-1 of hydrogel C, D and E correspondingly shows the vibration of – OH extending band in agarose-psyllium, acrylamide and PVA because of cross-linkages. Peaks at 2941.44 cm-1 and 2883.58cm-1 and 2885.5cm-1 and 2933.73cm-1 of hydrogel C, D and E separately, connote the event of C-H expansive alkyl extending band of PVA and vibrational groups of CH and CH2 gatherings of acrylamide unit. Vibrations of COO-gathering of casein hydrolysate, C=O bonds in PVA, glutaraldehyde and acrylamide unit can be seen at top 1668.43cm-1, 1664.57cm-1 and 1668.43cm-1 for hydrogel C, D and E separately though top at 1417.68cm-1, 1452.40cm-1 and 1429.25cm-1 in hydrogels C, D and E individually recommends the extending vibration of CN and C=O gatherings of acrylamide. Event of agarose is appeared through tops at 1037.70cm-1, 920.05cm-1 and 850.61cm-1 of hydrogel A that shows glycosidic bond, C-O-C scaffold and CH precise distortion while in hydrogel B and C tops are at 1109.07cm-1 and 1101.35cm-1 correspondingly. Unmistakable retention pinnacle of PVA and more extensive ingestion groups of C-O and C-O-C is assigned at 11.07.14cm-1, 921.97cm-1 and 921.97cm-1 in hydrogel C, D and E correspondingly that was a direct result of glutaraldehyde and PVA cross-linkage (Chandrika *et al*., 2016; Wu *et al*., 2007).

Scanning Electron Microscopy (SEM) of Hydrogel C, D and E

Fig. 4 represented the shallow properties of hydrogel C, D and E that was examined by means of SEM with various amplifications (X500 and X1.00k) at 10.0KV (a) With X500 the enhancement exhibits web-like organization having ordinary structure and dynamic porosity. Amassing is shown by the thick structure because of acrylamide, agarose and psyllium interlinkages though pore size contrasts for example, huge pores were a direct result of the presence of agarose while little pores were a result of polymerized acrylamide. (b) With X1.00k amplification shows the greater measured pores with web-like shapes. Hydrogel E was examined by means of SEM with amplification of X500 and X1.00k at 10.0KV.) With X500 amplification and (b) with X1.00k amplification show the dense state of hydrogel E when contrasted with hydrogel C and E that resembled web setup with typical shape and dynamic porosity. (a While, the expansion of 1% agarose veils the general structure giving unpredictable shape less observable porosity. Hydrogel E is incredibly cross-connected making the little measured pores.

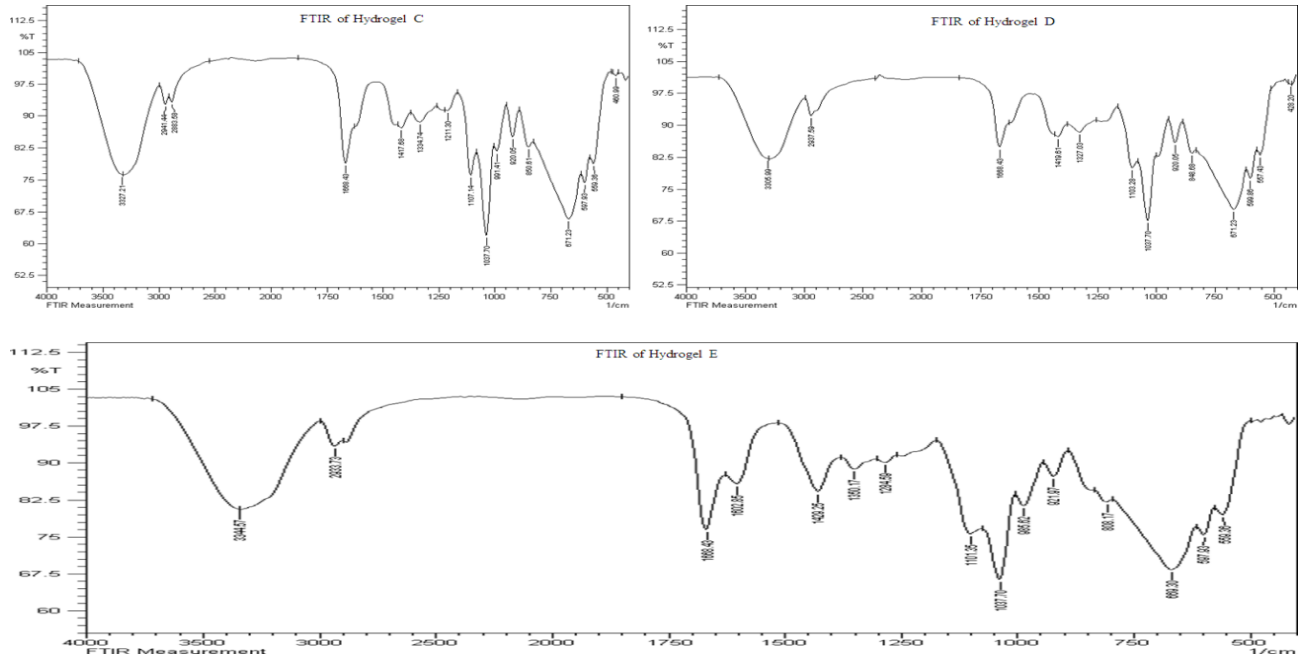

Fig. 3: Comparison of FTIR of Hydrogel C, D and E

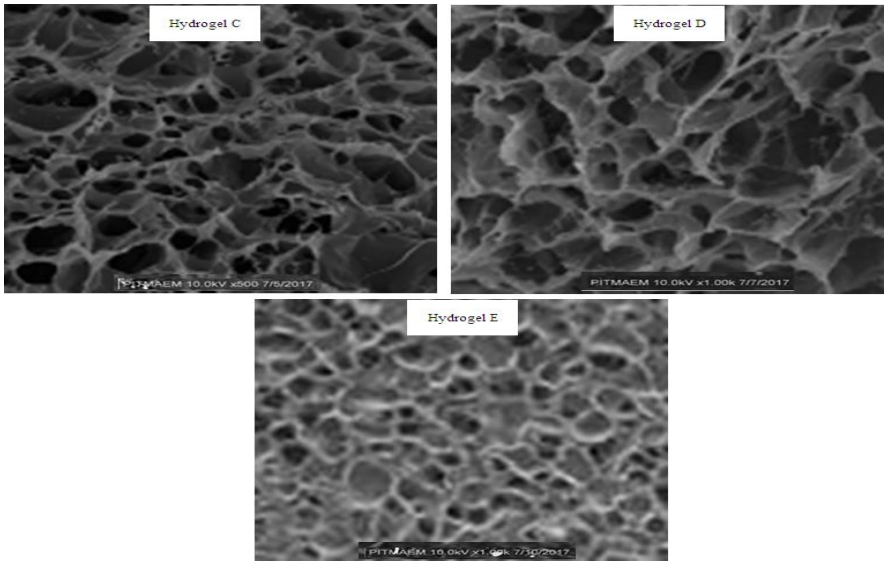

Fig. 4: Morphology of Hydrogel C, D and E obtained via use of SEM at (a) X500 and (b) X1.00k at 10.0KV

Swelling Property of Hydrogel C, D and E

Swelling of hydrogel C, D & E also follows the same diffusion mechanism. The documentation of swelling degree of hydrogels happens in the buffers having different pH. The acidic (phosphate buffer of pH 5.4); alkaline (Tris-Cl buffer of pH 7.5) buffers were utilized to acquire the swelling degree of hydrogels. Triplicates operating procedures are applied in all the experiments (Fig. 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 –NH2 and -OH groups that accept a 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. (Leelaprakash & Dass, 2011). Maximum swelling at pH 5.4 (4.65g, 6.25g and 3.35g) was observed in hydrogel C, D and E respectively while swelling at pH 7.5 was 3.06g, 4.89g and 2.29g per 0.5g of gel respectively. This capability of swelling decreases sharply due to decrease in ionization degree and decreased interaction with the free protons which are not available in the basic pH (Magalhães *et al*., 2012).

Swelling Kinetics of Hydrogel C, D and E

Swelling of hydrogel is due to diffusion process. An equal pattern when contrasted with expanding proportion was distinguished. Hydrogels indicated most elevated growing at acidic pH as shown in Fig. 6.

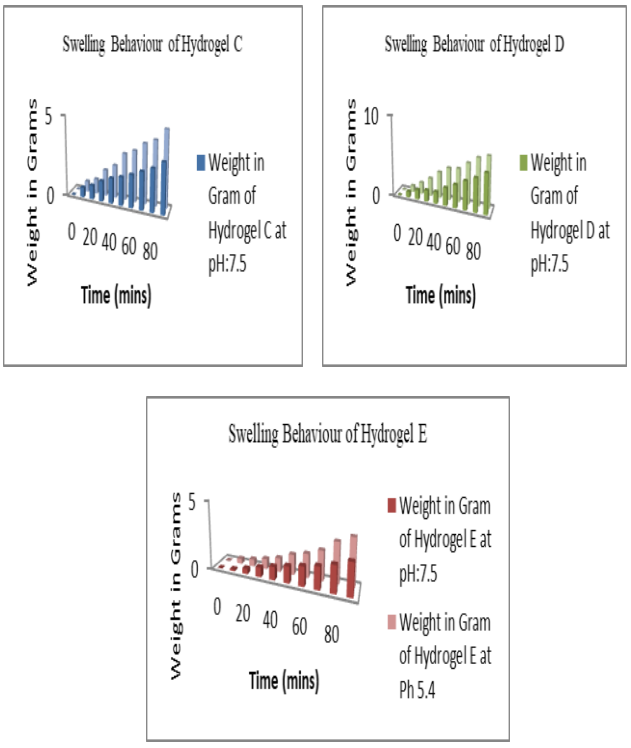

Fig. 5: Swelling Behaviour of Hydrogel C, D and E

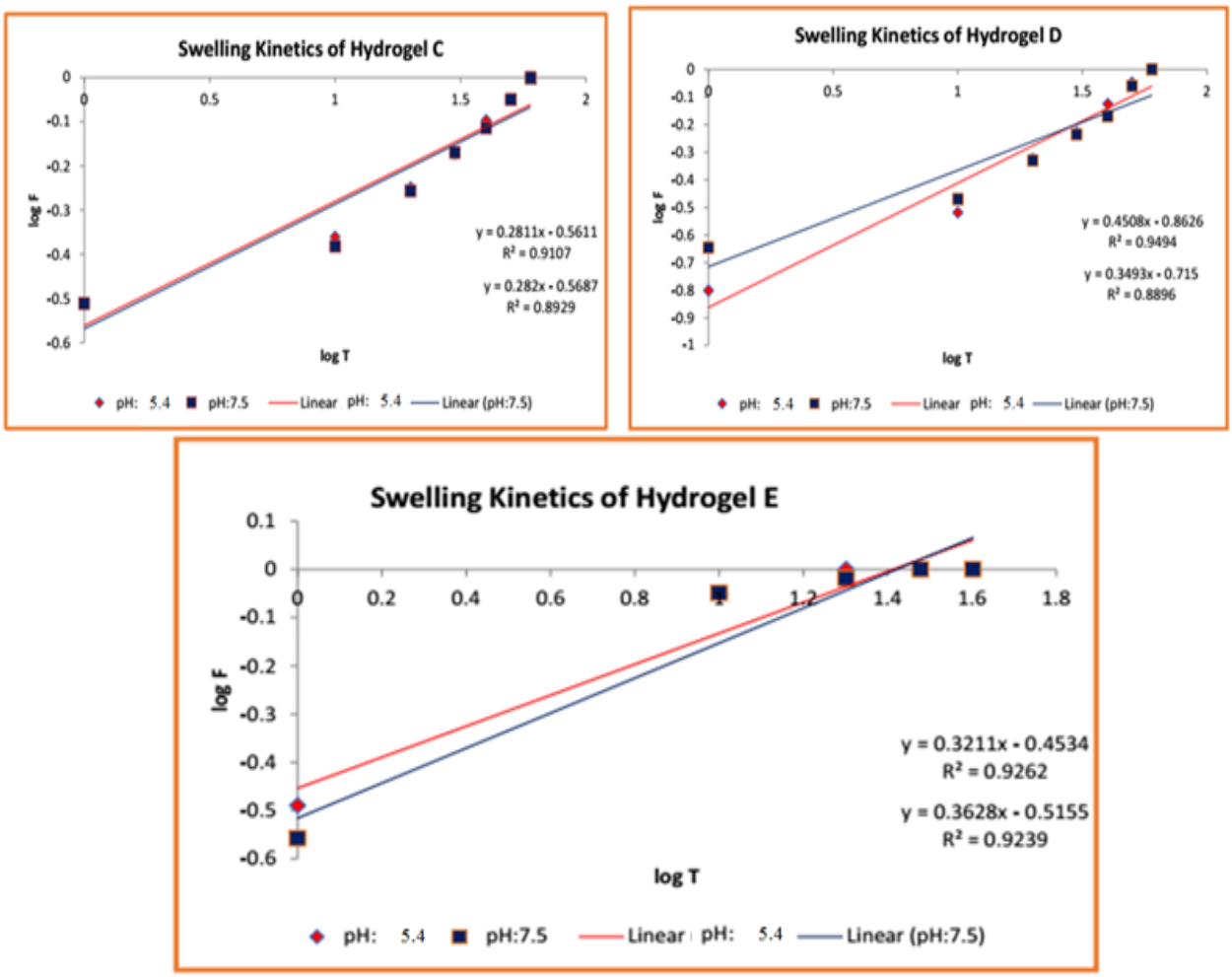

Fig. 6: Swelling Kinetics of Hydrogel C, D & E

In-Vitro Drug Release by Hydrogel 'C, D & E

In vitro drug release from the fabricated hydrogel 'C, D & E' in different pH solutions was measured by taking absorbance at 280nm as shown in fig. 7. The quantity of drug discharge from the hydrogel 'C, D and E' was higher in pH 5.4 then in pH 7.5. Amount of drug released in pH 5.4 was 2.532ug, 2.452ug, 2.252ug, respectively, and in 7.5 the drug released was 1.329ug, 1.929ug and 1.529ug per 0.5g of gel, respectively after 150mins. The drug discharge from polymeric matrix obeys the Fickian diffusion system. It shows a continuous drug release from the hydrogel which is in requirement with the drug delivery system. Gorle & Jayabalan (2015) published same outcomes by formulating a transdermal fix arranged by xanthum gum. The published information reveal that the patch was an efficient preparation for the delivery of insulin.

In-Vivo Drug Delivery by Hydrogel 'C, D & E' via Chicken Skin

The working of the transdermal patches of psyllium based insulin loaded hydrogel 'C, D and E' was determined on the avian skin cells, as a model. The outermost layer of skin of chicken was used to study the drug release in-vivo. Fig. 8 showed medicates discharge from Psyllium based hydrogel by means of the chicken skin into the support. Roughly 2.932ug, 2.452ug, 2.652ug and 2.269ug, 1.829ug, 2.029ug insulin was discharged per 0.5g of gel after 150 mins in pH: 5.4 and pH: 7.5 arrangements separately.

The Direct pattern line recommends supported arrival of insulin from psyllium manufactured hydrogel. Dynamic investigations built up that the medication discharge followed the zero request energy.

Table I: Swelling Kinetics of Hydrogel C, D & E at Different pH

pH	Hydrogel weight (g)	Swelling fractional constant (F)	Log of F	Slope n	Intercept k	R-squared value (R2)	Type of diffusion
5.4	0.17	0.309	-0.510	0.2811	-0.5611	0.9107	Fickian diffusion Hydrogel
	0.24	0.436	-0.361				
	0.31	0.564	-0.249				
	0.37	0.673	-0.172				
	0.44	0.8	-0.097				
	0.49	0.891	-0.050				
	0.55	1	0				
7.5	0.2	0.308	-0.511	0.282	-0.5611	0.8929	Fickian diffusion hydrogel
	0.27	0.415	-0.382				
	0.36	0.554	-0.256				
	0.44	0.677	-0.169				
	0.50	0.769	-0.114				
	0.58	0.892	-0.050				
	0.65	1	0				

Hydrogel C

pH	Swelling fractional constant (F)	Log of F	Slope n	Intercept k	R-squared value (R_2)	Type of diffusion
5.4	0.158	-0.801	0.4508	-0.8626	0.9494	Fickian diffusion Hydrogel
	0.303	-0.519				
	0.474	-0.324				
	0.592	-0.228				
	0.75	-0.125				
	0.895	-0.048				
	1	0				
7.5	0.226	-0.646	0.3493	-0.715	0.8896	Fickian diffusion hydrogel
	0.339	-0.470				
	0.468	-0.330				
	0.581	-0.236				
	0.677	-0.169				
	0.871	-0.060				
	1	0				

Hydrogel D

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	0.12	0.324	-0.489	0.3211	-0.4534	0.9262	Fickian diffusion hydrogel
	0.33	0.892	-0.050				
	0.37	1	0				
	0.37	1	0				
	0.37	1	0				
	0.37	1	0				
7.5	0.13	0.277	-0.558	0.3628	-0.5155	0.9239	Fickian diffusion hydrogel
	0.42	0.894	-0.049				
	0.45	0.957	-0.019				
	0.47	1	0				
	0.47	1	0				
	0.47	1	0				

Hydrogel E

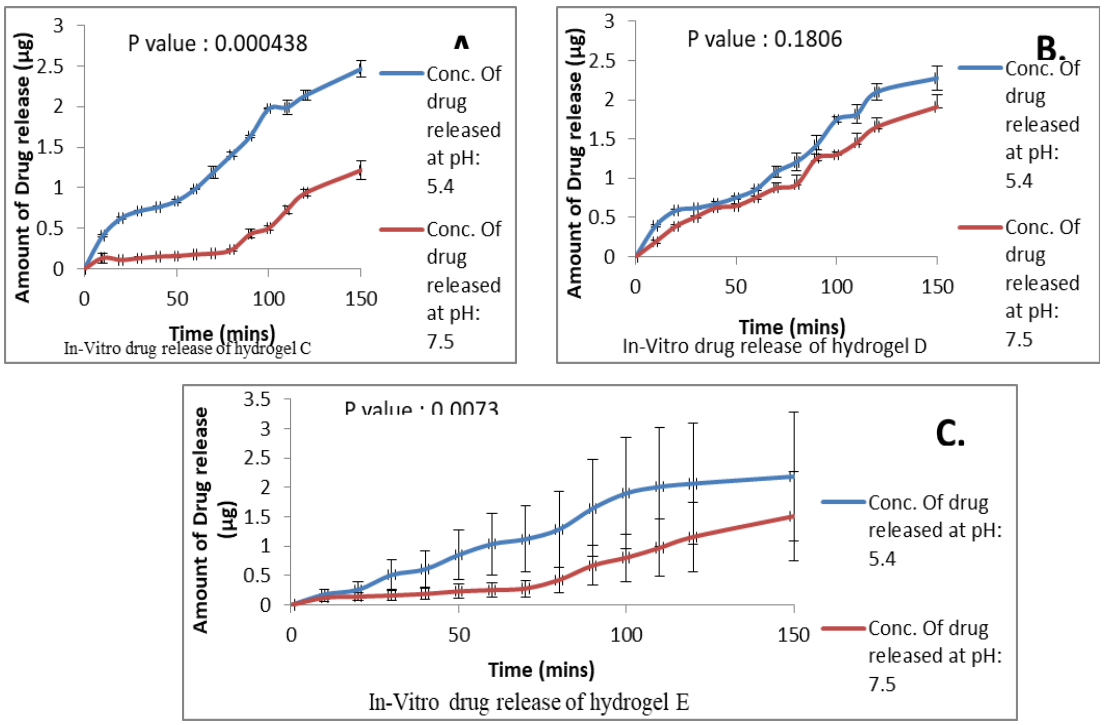

Fig. 7: In-Vitro Drug Release by Hydrogel 'C, D & E

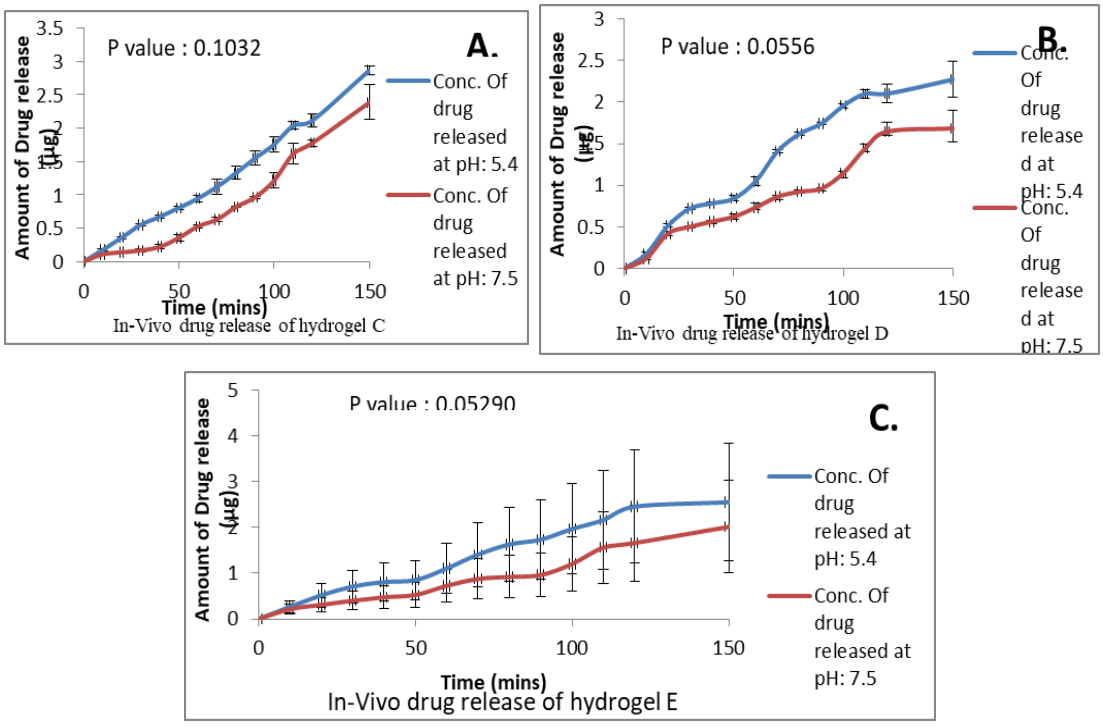

Fig. 8: In-Vivo Drug Release by Hydrogel C, D and E

Our outcomes were in concurrence with the results announced by Singh (2007) where drugs containing hydrogels followed a similar component of arrival of medication.

Insulin Determination Assay

The quantitative determination of insulin released from the hydrogels was also determined by enzyme-linked immunosorbent assay (ELISA). Insulin concentration was normalized to total protein content determined with the BCA Protein Assay (Pierce®: Thermo Sci.). These experiments were conducted in triplicate.

Biocompatibility assays

Anti-microbial activity

Disc diffusion method was used to observe the anti-microbial and contact inhibition of all characterized gels. The anti-microbial activity was mainly due to casein hydrolysate, a component of different hydrogels (A-E). No growth of *E. coli* strain (DH5α) was observed on or in contact inhibition areas. The contact area was calculated in various hydrogels was 20 to 25mm. The results in this examination were extremely similar to the consequences of Wu *et al*., (2007) who reported casein hydrolysate hydrogels with ground-breaking antibacterial properties. The adduced part of this limitation was relied upon to the polycationic thought of polymers in the hydrogel which intrudes with the unfavorably charged stores in the cell mass of microscopic organism.

CONCLUSION

Psyllium represents a double potential in a drug delivery system due to its glucose lowering and gel forming nature, making it an admirable vehicle for the handling of diabetes mellitus as shown from the drug release dynamics in various release media and avian skin. The Fickian diffusion mechanism at pH 5.4 and 7.5 buffer system is followed in drug release because 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. The psyllium based insulin loaded hydrogels could be safe, biocompatible and efficient in a transdermal drug delivery system.

REFERENCES

Bastaki, S. 2005. Diabetes mellitus and its treatment. *Diabetes Metab J.*., 13: 111–134.

Benson, H.A.E., 2002. Transdermal Drug Delivery: Penetration Enhancement Techniques. *Curr. Drug Deliv*., 2: 23–33.

Brand, R.M. Hannah, T.L. and Hamel, F.G., 2000. A combination of iontophoresis and the chelating agent 1, 10 phenanthroline act synergistically as penetration enhancers. *AAPS Pharm Sci.,* 2: 35.

Buysschaert, M. and Lambert, A.E., 1989. The insulin pen. *Lancet.,* 1: 965.

Campoccia, D. Doherty, P. Radice, M. Brun, P. Abatangelo, G. and Williams, D. F., 1998. Semi-synthetic resorbable materials from hyaluronan esterification. *Biomaterials.*, 19 (23): 2101-2127.

Chandrika, K.P. Singh, A. Rathore, A. and Kumar, A. 2016. Novel cross linked guar gum-g-poly (acrylate) porous superabsorbent hydrogels: characterization and swelling behaviour in different environments. *Carbohydr. Polym.,* 149: 175–185.

Constantin, M. Bucatariu, S. Ascenzi, P. Butnaru, M. and Fundueanu, G., 2020. Smart drug delivery system activated by specific biomolecules. *Mater. Sci. Eng..,* 108: 110466.

Gore T. F. and Jayabalan, M. 2015. Hybrid amphiphilic bimodal hydrogels having mechanical and biological recognition characteristics for cardiac tissue engineering. *RSC. Adv*., 5: 38183-38201.

Jenkins, D.J. Wolever, T.M. Leeds, A.R. Gassull, M.A. Haisman, P. and Dilawari, J. 1978. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br Med J.*, 1(6124): 1392–4.

Kim, B. Flamme, K.L. and Peppas, N.A., 2003. Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery. *J Appl Polym Sci*., 89: 1606–13.

Korsmeyer, R.W. Gurny, R. Doelker, E. P. and Buri, N.A., 1983. Peppas, Mechanism of solute release from porous hydrophilic polymers, *Int. J. Pharm.* 15: 25–35.

Leelaprakash, G. and Dass, S.M. 2011. In vitro anti-inflammatory activity of methanol extract of Enicostemma axillare, *Int. J. Drug Dev. Res.*, 3 (3): 189–196.

Lenhard, M.J. and Reeves, G.D., 2001. Continuous subcutaneous insulin infusion: a comprehensive review of insulin pump therapy. *Arch Inter Med*., 161: 2293–2300.

Magalhães, A. S. G. Almeida Neto, M. P., Bezerra, M. N. Ricardo, N. M. and Feitosa, J. 2012. Application of FTIR in the determination of acrylate content in poly (sodium acrylate-co-acrylamide) superabsorbent hydrogels. *Quím. Nova*. *35*(7): 1464-1467.

Mark, R. Prausnitz, M.R. and Langer R 2008. Transdermal drug delivery. *Nat. Biotechnol.,* 26: 1261-1268.

Peppas, N.A. and Franson, N.M. 1983. The swelling interphase number as a criterion for prediction of diffusional solute release mechanisms in swellable polymers, *J. Polym. Sci. B: Polym. Phys*. 21: 983–997.

Peppas, N.A. Wood, K.M. and Blanchette, J.O., 2004. Hydrogels for oral delivery of therapeutic proteins. *Expert Opin Biol Ther*., 4(6): 881–7.

Rithe, S.S. Kadam, P.G. and Mhaske, S.T. 2014. Preparation and analysis of novel hydrogels prepared from the blend of guar gum and chitosan: cross-linked with Glutaraldehyde, *Adv. Mater. Sci. Eng.* 1 (2): 230-238.

Sami, A. J. Butt, Y. N. and Nasar, S. 2018a. Elimination of a Carcinogenic Anionic Dye Congo Red from Water Using Hydrogels Based on Chitosan. *J Bioprocess Biotech*., 8:5.

Sami, A. J. Khalid, M. Iqbal, S. Afzal, M. and Shakoori, A. R. 2017. Synthesis and application of chitosan-starch based nanocomposite in wastewater treatment for the removal of anionic commercial dyes. *Pak. J. Zool.,* 1, 49.

Sami, A.J. Khalid, M. Nasar, S. Butt, Y. N. 2018b.Sustained release of ibuprofen by a novel formulated hydrogel containing graphene oxide. *J. Mol. Pharm. Org. Process Res.,* 5(1): 1-6.

Sami, A.J. Madiha, K. Jamil, T. Aftab, S. Mangat, S.A., Shakori, A.R. Iqbal, S., 2018c. Formulation of novel chitosan guargum based hydrogels for sustained drug release of Paracetamol. *Int. J. Biol. Macromol..,* 108, 324-332.

Sharpe, L. A. Daily, A. M. Horava, S. D., and Peppas, N. A. 2014. Therapeutic applications of hydrogels in oral drug delivery. *Expert Opin. Drug Deliv.,* 11 (6): 901-915.

Singh, B. 2007. Psyllium as therapeutic and drug delivery agent. *Int J Pharm.* 334: 1–14.

Valenta, C. and Auner, B.G., 2004. The use of polymers for dermal and transdermal delivery. *Eur. J. Pharm. Biopharm.,* 58: 279–289.

Vuksan, V. Sievenpiper, J.L. Owen, R. Swilley, J.A. Spadafora, P. and Jenkins, D.J. 2000. Beneficial effects of viscous dietary fiber from Konjac-mannan in subjects with the insulin resistance syndrome: results of a controlled metabolic trial. *Diab Care*., 23: 9–14.

Wang, W. Wat, E. Hui, P.C. Chan, B. Kan, C.W. and Wang, X., 2016. Dual-functional transdermal drug delivery system with controllable drug loading based on thermosensitive poloxamer hydrogel for atopic dermatitis treatment. *Sci Rep*., 6:1-10.

Wichterle, O., and Lim, D. 1960. Hydrophilic gels for biological use. *Nature.,* 185(4706): 117-118.

Wu, Y.B. Yu, S.H. Mi, F.L. Wu, C.W. Shyu, S.S. and Peng, C.K. 2007. Chao, Preparation and characterization on mechanical and antibacterial properties of chitosan/cellulose blends. *Carbohydr. Polym.,* 57: 435–440.

Yetisen, A. K. Naydenova, I. Vasconcellos, F. Blyth, J. and Lowe, C. R., 2014. Holographic sensors: three dimensional analyte-sensitive nanostructures and their applications. *Chem. Rev..,* 114 (20): 10654-10696.

Zakzewski, C.A. Wasilewski, J. Cawley, P. and Ford, W., 1998. Transdermal delivery of regular insulin to chronic diabetic rats: Effect of skin preparation and electrical enhancement. *J Control Release*., 50: 267–272.