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Development and characterization of *in situ* gel system for nasal insulin delivery

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The objective of the present study was to develop a thermosensitive *in situ* gel system based on chitosan and poly vinyl alcohol (PVA) for nasal delivery of insulin. The hydrogel was prepared by mixing chitosan and PVA. The concentration of the components was optimized during formulation development. The prepared hydrogel was characterized for gelation temperature, gelation time, viscosity changes, degree of swelling, *in vitro* release and *in vivo* hypoglycemic effect. The prepared hydrogel was liquid at room temperature while underwent thermal transition from solution below or at room temperature to non-flowing hydrogel when incubated at 37 °C for approximately 12 minutes with increased viscosity. The *in vitro* release of insulin from gel network was observed spectrophotometrically which was good enough to maintain blood glucose level for six hour. Furthermore, the formulation when evaluated for their *in vivo* hypoglycemic effect, demonstrated its ability to reduce glucose level. The observed *in vitro* and *in vivo* results indicate that the proposed thermosensitive *in situ* gelling system has substantial potential as nasal delivery system for insulin.

1. Introduction

To replace the injectable form of insulin various alternative routes have been tried including oral, buccal, nasal, pulmonary, ocular, rectal and transdermal administration (Khafagy et al. 2007; Wu et al. 2007). Among the non-invasive routes, the nasal route may be a promising alternative for the delivery of insulin. Nasal route offers many advantages that include a large surface area available for drug absorption due to microvilli present on epithelial surface, highly vascularized subepithelial layer, avoidance of hepatic first pass metabolism, low dose requirement, rapid attainment of therapeutic blood level, quicker onset of pharmacological activity and fewer side effects (Khafagy et al. 2007). Additionally intranasal insulin delivery also provides a pharmacokinetic profile similar to that achieved after intravenous injection and, in contrast to subcutaneous insulin it bears a close resemblance with the endogenous insulin secretion (Hinchcliffe et al. 1999).

There are some limitations of nasal delivery as well like mucosal tissue irritation, rapid mucociliary clearance of the drug from the site resulting in a short duration of time period staying at the site of absorption, low permeability of the nasal membrane for high molecular weight compounds, and various pathological conditions such as cold or allergies which may alter the drug absorption from the nasal cavity. To overcome these limitations, formulation components should be minimum toxic to the nasal mucosa. Additionally they should increase the permeability of the macromolecules through nasal mucosa and should provide bioadhesion to minimize the effect of mucociliary clearance thereby improving absorption (Leung and Robinson 1990; Duchene and Ponchel 1993).

Previously nasal insulin delivery has been attempted by various researchers in the form of dry powder (Dyer et al. 2002; Haruta et al. 2003; Pringels et al. 2006, 2008), nanoparticles and nanocomplexes (Dyer et al. 2002; Simon et al. 2005; Bhumkar et al. 2007; Jain et al. 2008; Wang et al. 2009), microspheres and microparticles (Varshosaz et al. 2004; Krauland et al. 2006; Wang et al. 2006), nasal inserts (McInnes et al. 2007; Luppi et al. 2009), liposomes (Jain et al. 2007), gel (Varshosaz et al. 2006) and *in situ* gel (Wu et al. 2006, 2007). *In situ* gelling systems could be potential alternatives to the existing invasive delivery mode of insulin.

Hydrogels are cross linked macromolecular networks with the capacity to adsorb a substantial amount of water or biological fluids within its structure without dissolving. *In situ* hydrogels are gaining a great deal of interest due to potential applications in the controlled release of bioactive molecules and tissue engineering. There are two types of chitosan hydrogels; chemical hydrogels, formed by irreversible covalent links, and physical hydrogels, formed by various reversible links. Covalent cross linked gels show enhanced mechanical properties but cross linking agents can interact with incorporated bioactive compounds and are often associated with significant toxicity. Therefore physically cross linked hydrogels received considerable attention. Some hydrogels have the peculiarity of gelling within the desired site in the body as a result of polymer interactions (Schuetz et al. 2008). *In situ* gel systems are solutions before administration, but gel under physiological conditions according to temperature, pH etc. Thus these systems have the advantages of being easy to administer in solution form and to stay long at the site of absorption due to conversion into gel.

Recently chitosan has gained wide attention as a polymer for drug and vaccine delivery. Chitosan [2-amino-2-deoxy-(1 → 4)-β-D-glucopyranan] is a mucopolysaccharide obtained by the alkaline hydrolysis of chitin, a process which randomly deacetylates and shortens the chain length of the chitin molecules. It is commercially available in a range of grades with different molecular weight and degree of deacetylation. There are various favourable properties which make the chitosan a polymer of interest like nontoxicity (Rao and Sharma 1997), biodegradability (Chen and Chen 1998; Muzzarelli 1997), biocompatibility (Hirano and Noishiki 1985; Hirano et al. 1988) and mucoadhesiveness (Lehr et al. 1992). Higher viscosity of the chitosan solution and the presence of positively charged amino groups which bind to the negatively charged sialic acid residue on mucous membranes makes the chitosan mucoadhesive. Transient widening of the tight junctions makes it a suitable penetration enhancer.

PVA is a hydrophilic, non-toxic component of the investigated chitosan based *in situ* gelling systems. PVA based systems have been explored for use in biomedical applications such as drug delivery devices (Aleyamma et al. 1991; Tamura et al. 1986), contact lenses (Hyon et al. 1994) and artificial organs (Noguchi et al. 1991). Biocompatibility of PVA implants has been proved in rabbits (Kodama et al. 1996). The co-polymeric nature of PVA provides the polymer with unique gelling characteristics, which in turn are responsible for its adhesive properties (Korsmeyer et al. 1983). Chitosan and PVA blend has been used by Minoura et al. (1998) and Koyano et al. (2000) to study its surface properties and relationship of these properties with the cell attachment/growth behavior, Kim et al. (2002) for electrical charge sensitivity, Wang et al. (2004) for pH sensitivity and Tang et al. (2007) for rheological characterization of the blend. In earlier studies an *in situ* gelling system composed of quaternized chitosan and poly (ethylene glycol) for nasal insulin delivery was developed (Wu et al. 2006, 2007). However, glycerophosphate as one of the components of the proposed system presents some drawbacks such as turbidity of the hydrogel and interaction with the numerous bioactive components due to the presence of negatively charged moieties (Schutez et al. 2008). In the present study a clear and non toxic *in situ* gel system based on phosphate free additives (PVA) was developed and characterized for various *in vitro* characteristics. Also, we have envisaged the development of a chitosan-PVA hydrogel for insulin delivery without any further chemical reaction with crosslinking agents such as glutaraldehyde or paraformaldehyde. Reduction in normal blood glucose level *i.e.* a hypoglycemic effect was measured in normal wistar rat model to assess the potential of the developed system in non-invasive insulin delivery.

2. Investigations, results and discussion

2.1. Characterization of thermosensitivity

The delivery system studied was a solution with low viscosity at room temperature, which could be administered easily by simple devices used for liquid nasal formulation. After being incubated at 37 °C, the solution transformed to a non flowing hydrogel. The pre-gelled solution and gel are shown in Fig. 1.

The effect of temperature on gelling time was also recorded to observe the thermosensitivity of the proposed system. The gelling time was reduced at increasing temperature conditions. The time taken to convert to gel under various temperature conditions is shown in Fig. 2.

Chitosan is a cationic polymer having pH-dependent solubility. In acidic solution *i.e.* below its pKa value (6.2), it gets solubilized and its free amino group (–NH₂) gets converted into the protonated form (–NH₃⁺). In acidic solution due to the presence of

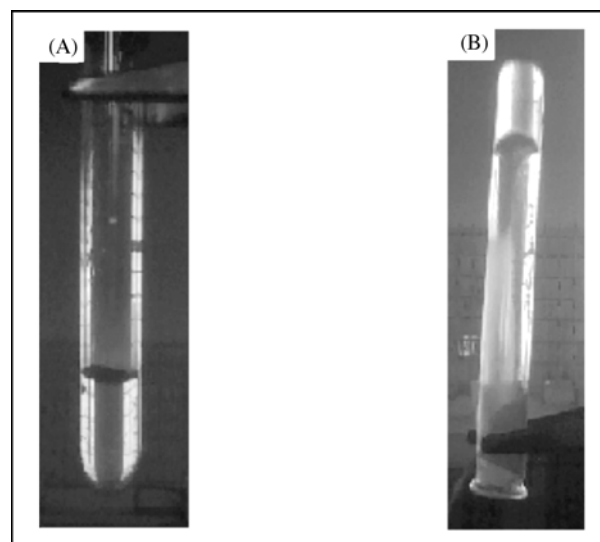


Fig. 1: The solution at room temperature (A) and the formed hydrogel at 37 °C (B)

the –NH₃⁺ it acts as a weak acid. Due to presence of this charge, electrostatic repulsion will be there and chitosan chains remain separated. Addition of PVA and NaHCO₃ leads to increase in pH of the solution. The increase in pH may cause deprotonation (–NH₃⁺ → –NH₂) resulting in a decrease in electrostatic repulsion (Tang et al. 2007). At low temperature and decreased electrostatic repulsion hydrogen bonds exist between chitosan, PVA and water due to the high hydrophilicity of PVA, which can lead to dissolution of chitosan chains. Low temperature also reduces the mobility of chitosan molecules which prevents the association of chitosan chains. At elevated temperature the hydrogen bonding interactions are reduced and the energized water molecules surrounding the chitosan are removed. The dewatered hydrophobic chitosan chains associate with each other. As a result, a gel is formed. Therefore, hydrophobic interactions are assumed to be the main driving force to form the gel consisting of chitosan and PVA at high temperature (Tang et al. 2007). When this solution was placed at 37 °C it converted into gel in approximately 12 min. Although it is difficult to stock the amount for time to convert to gel in the nasal cavity but the presence of positively charged –NH₃⁺ groups on chitosan will show bioadhesion due to binding with the negatively charged sialic acid group present on mucosal surface. Due to this, *in vivo* performance of the formulation may exceed *in vitro* performance.

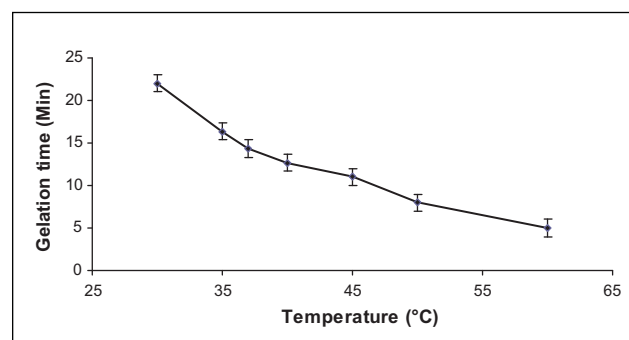


Fig. 2: Temperature-dependent gelling behavior of chitosan-PVA gels. Test tubes containing chitosan-PVA sol were incubated at 37 °C and after every minute gelling was checked by inverting the tube till the sol stopped flowing

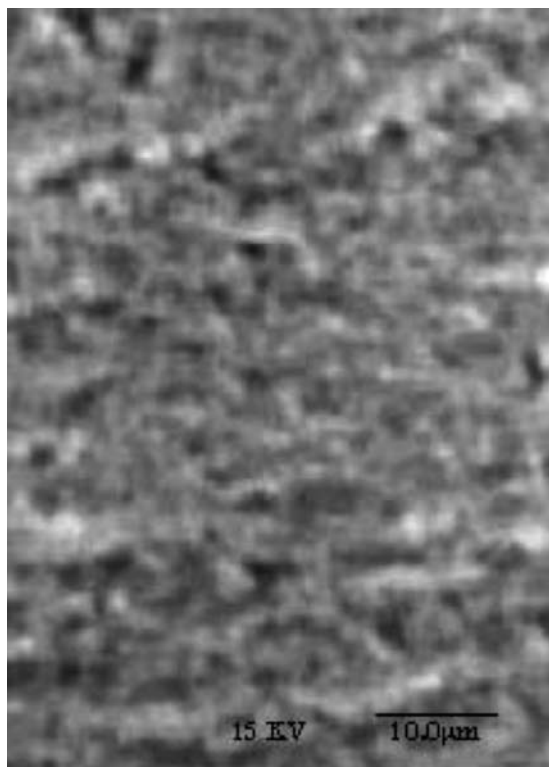


Fig. 3: SEM photomicrograph of Chitosan-PVA gel

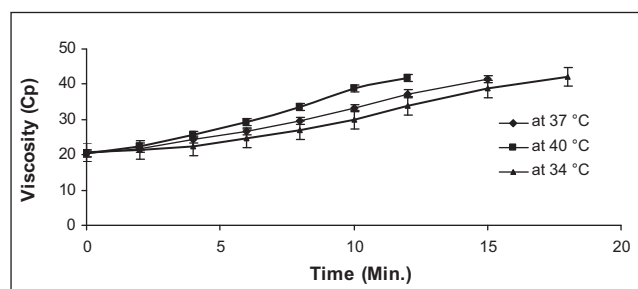


Fig. 4: Effect of temperature on gelation time and viscosity of the formulation

2.2. Morphology of hydrogels

The cross-sections of the chitsan-PVA gel were examined by scanning electron microscopy to investigate morphology and the compatibility between chitosan and PVA molecules (Fig. 3). A smooth and homogeneous morphology was observed suggesting high miscibility and blend homogeneity between chitosan and PVA. The good miscibility may be sustained by the hydrogen bonds and intermolecular interaction between chitosan and PVA in gel.

2.3. Rheological measurement

Rheological studies showed increased viscosity with time after incubating the formulation at 37 °C. The viscosity change with time was also measured at 34 °C and 40 °C, so that the effect of various physiological conditions like hyperthermia on viscosity of the formulation can be measured. At the same time point the viscosity of the formulation was different under different temperature conditions and time taken to convert into gel varied with temperature as shown in Fig. 4.

If hyperthermia causes increased viscosity of the formulation then it may cause obstruction of the nasal cavity which will result in interference with normal breathing. After converting

into gel at various temperatures it did not show any difference in viscosity. Hence it can be concluded that in physiological inconsistencies patients will not suffer from obstruction in normal breathing. The formulation also exhibited pseudoplastic character *i.e.* decrease in viscosity with increase in shear. The formulation showed pseudoplastic character because due to shearing action the disarranged chitosan molecules align themselves along their long axis in the direction of motion with reduced internal resistance (Kashyap et al. 2007).

2.4. Degree of swelling

Hydrogels are polymeric networks that can retain a significant amount of water within their structures, and swell without dissolving in water. Their high water content, hydrophilicity, expandability, selective permeability, soft rubbery consistency and low interfacial tension are among the advantages of hydrogels, enabling them to resemble soft living tissues and thus they are promising drug delivery vehicles. The degree of swelling studies was conducted in phosphate buffer solution (pH 7.4) maintaining the buffer solution at 37 °C. The formulations containing different amounts of chitosan and PVA were allowed to swell in buffer solution until equilibrium swelling. The maximum degree of swelling (%) was observed with formulation C (CS 3%, PVA 2%) and the minimum degree of swelling was observed with formulation A (CS 1%, PVA 4%). The results are shown in Fig. 5.

Hydrogels can absorb water from the nasal mucosa, thus resulting in a temporary dehydration of the epithelial membrane and opening of its tight junctions (Khafagy et al. 2007). The chitosan and temporary dehydration of the epithelial membrane by hydrogel may cause a synergistic effect on the tight junction opening and increased insulin absorption through the nasal mucosa may be observed. The minimum degree of swelling was observed with formulation A which may be attributed to the high concentration of PVA, which results in a more compacted gel structure (Tang et al. 2007). If only the concentration of PVA is responsible for the gel strength then, a maximum degree of swelling should be shown by formulation D (CS 4%, PVA 1%), because due to the minimum concentration of the PVA a less compact gel will be formed. The possible explanation for this inconsistency may be that chitosan has pH dependent solubility and at increased pH shows precipitation. But in the presence of PVA at increased pH it forms gel rather than precipitation. The concentration to prevent precipitation and to make a gel network may be minimum 2%, so that sufficient free groups $-NH_2$ and $-OH$ on the chitosan molecule are available to form hydrogen bonds with the water molecules.

2.5. In vitro release study

In vitro release of the insulin from various formulations was investigated using egg membrane. Egg membrane was selected

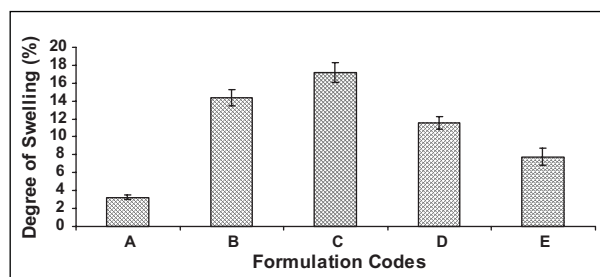


Fig. 5: Degree of swelling (%) of various formulations in phosphate buffer solution maintained at 37 °C

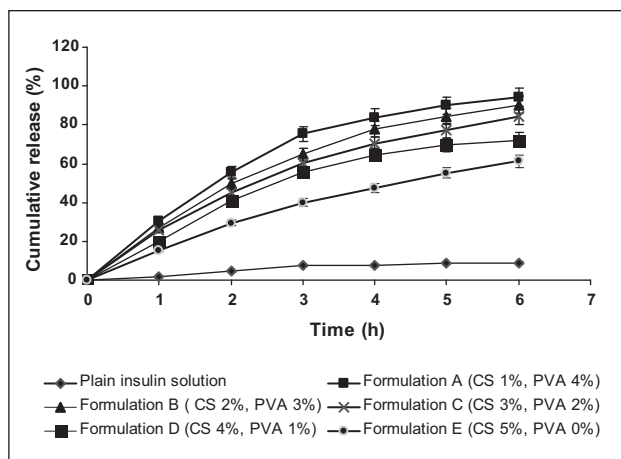


Fig. 6: Cumulative release (%) from various gel formulations in phosphate buffer (pH 7.4) at 37 °C

for *in vitro* release study because egg membrane mimic the behavior of biological membrane hence on the basis of release results, *in vivo* efficacy of the gel can be predicted more accurately. Comparative release was measured between plain insulin solution and various formulations. The results are shown in Fig. 6.

Initially the release rate of insulin from hydrogel was relatively rapid, and thereafter it was slowed down after several hours. This may be due to the fact that part of insulin may adsorbed on the surface or distributed in the tunnels of the hydrogel during the gelation process, and it diffused rapidly when the hydrogel came into contact with the release medium. In the later stage the insulin entrapped into the hydrogel releases slowly due to the swell and degradation of the hydrogel. These finding is in accordance with previous investigations (Wu et al. 2007).

The insulin release from plain insulin solution was about 9% while it was up to 60–94% by various gel formulations during a 6 h release study. Such a low release from plain insulin solution may be attributed to the high molecular weight of the insulin which prevents it to cross the egg membrane. While in case of various formulations the observed induced release may be attributed to the penetration enhancing effect of chitosan. From the experimental data it may be concluded that there are two constraints which affect the release pattern, first is chitosan concentration and the second is gel network. The effect of chitosan may be seen in terms of induced release through formulations in comparison to plain insulin solution. Results indicate that an 1% concentration of chitosan is sufficient for widening the tight junction if it is not then, an increased release pattern should be observed from formulation A (CS 1%, PVA 4%) to E (CS 5%, PVA 0%) while it is not. Another contributing constraint may be the gel network due to the different release profile by various formulations observed in the study. On the basis of the release pattern it can also be concluded that release through gel network is concentration dependent. Chitosan is believed to be essential for the formation of a 3-D structure. The PVA has proven to have a synergistic effect on the network by essentially improving the network density (Wang et al. 2004). The remaining drug releases through gel network during 24 h.

2.6. *In-vivo* hypoglycemic effect

To evaluate the effectiveness of the developed formulation, hypoglycemic effect in normal rats was measured. For that purpose formulation C (CS 3%, PVA 2%) was selected for the *in vivo* study. The selection of the formulation C was based on the highest degree of swelling which may synergize the penetration

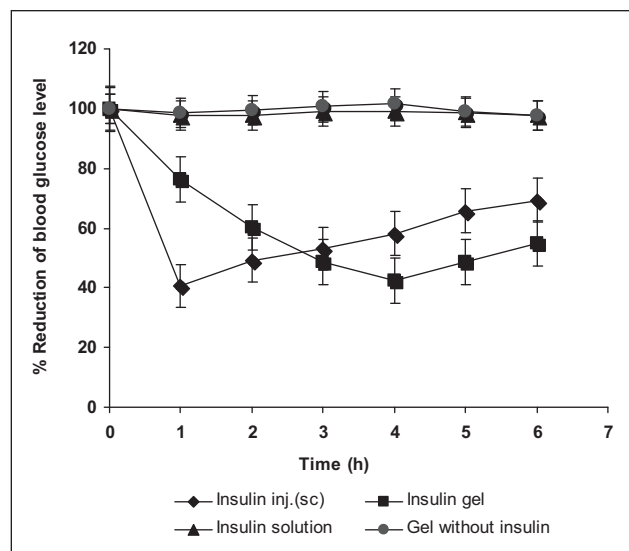


Fig. 7: Effect of various formulations on the blood glucose level after nasal administration to rats (n = 5)

enhancing effect of chitosan and moderate *in vitro* release. A comparative study was done with insulin solution given nasally. Additionally, insulin was injected subcutaneously (s.c.) as a positive control. The percentage reduction of blood glucose level with various formulations is shown in Fig. 7.

Nasal administration of insulin solution did not show any significant decrease in blood glucose level, only a slight fluctuation was observed which may be due to the stressful conditions during the experiment. The formulation without insulin did not decrease blood glucose concentration, which proved that the formulation itself was not active in adjusting blood glucose values. Insulin injection (s.c.) resulted in maximum reduction within the first hour after administration, while in case of the gel, maximum reduction was observed four hours after administration. It means that a slow release through gel network is obtained. This slow release phenomenon through gel network may also be useful to control the hypoglycemic shock which may be seen with injectable insulin. The relative bioavailability obtained was 8.8% which was better than the results of a previous investigation (Wu et al. 2007). The results suggest that the proposed system can be used as a potential nasal drug delivery system, which effectively enhances the absorption of a drug at the site of action.

The chitosan–PVA based insulin gel showed a prolonged hypoglycemic action as compared with plain insulin injection because of the sustained insulin release. The nasal insulin delivery from nanoparticles or hydrogel based systems has been investigated previously (Wang et al. 2006; Aikawa et al. 1998; Fernández-Urrusuno et al. 1999). The plasma glucose level fell sharply and then stayed at a low concentration for almost 2–3 h. Then the glucose level raised again and the blood glucose level increased nearly 100% of the initial blood glucose level after 4 h. It was due to the clearance of the formulation from the nasal cavity by the ciliary movement. Our chitosan-PVA based hydrogel system can maintain the low blood glucose concentration for a longer time (up to 6 h) because of the superior bioadhesivity characteristics of the gel. The developed gel formulation prolongs the contact between the formulation and the absorption sites in the nasal cavity. In addition, it also promotes direct absorption of the drug through the nasal mucosa. This may be attributed to the enhancing permeation effect of chitosan via opening of the tight junction of the nasal mucosa. Chitosan blended with PVA have already been reported to have good mechanical properties (Koyano et al. 2000) because of

the specific intermolecular interactions between PVA and chitosan in the blends. Hydrogels composed of such blends have a high blood compatibility and are good candidates for use as matrices for the controlled delivery of drugs or proteins.

3. Experimental

3.1. Materials

Chitosan (degree of deacetylation 85%) was purchased from Fluka Co. Ltd., Switzerland. PVA (Mw 30,000–70,000 Da) was purchased from Sigma Chem. Co. (St. Louis, Mo, USA). The insulin used was Huminsulin™, 40 IU/ml, Eli Lilly, USA. Rats were provided by animal facility lab, V.N.S. Institute of Pharmacy, Bhopal, M.P., India. All other chemicals were of analytical grade. Blood glucose level was determined using Accu check® Go (Roche Diagnostics, Germany).

3.2. Preparation of hydrogel

The hydrogel was prepared by the method reported by Tang et al. (2007) with slight modification. A clear solution of chitosan with different concentration (1%, 2%, 3%, 4%, 5% w/v) was prepared in 0.1 M HCl and chilled in an ice bath for 15 min. PVA was dissolved in preheated distilled water (80 °C) to get 1, 2, 3 and 4% w/v solution. This solution was also chilled on ice bath. Both the solutions were mixed under magnetic stirring for 10 min to make various formulations (A, 1% CS, 4% PVA; B, 2% CS, 3% PVA; C, 3% CS, 2% PVA; D, 4% CS, 1% PVA; E, 5% CS, 0% PVA) and the final pH of solutions were adjusted with 1.0 M NaHCO₃ to about to the neutral. Finally insulin was added in the formulated delivery system such that final solution contained 1 IU insulin per 200 µl of the solution.

3.3. Characterization of thermosensitivity

The gelation time at 37 °C was determined by test tube inverting method (Wu et al. 2006). The 2.0 ml formulation was added into a tube (10 ml) with a diameter of 1.0 cm and kept in a water bath at 37 °C. The tube was taken out every minute and inverted to observe the state of the formulation. The gelation point was determined by flow or non-flow criterion.

3.4. Morphology of hydrogels

The surface feature of the hydrogel after lyophilization was observed by scanning electron microscopy. The sample of the hydrogels were coated with gold, then observed and photographed on a scanning electron microscope (SEM, JEOL 6100, Japan).

3.5. Rheological measurement

To measure rheological properties change in viscosity with time was measured by Brookfield Viscometer (Model RVDVE 330, Brookfield Eng. Lab., USA) by putting the formulation in a water bath to maintain the temperature that mimic the body temperature.

3.6. Degree of swelling

Degree of swelling of the prepared gel was carried out in phosphate buffer pH 7.4. In this, gel of fixed weight corresponding to 10 ml of sol was taken in buffer solution and the gel was allowed to swell until equilibrium swelling reached (complete saturation) with buffer solution. At appropriate intervals, the wet weight of the swollen hydrogel was determined by blotted with a piece of filter paper to remove the adsorbed water on the surface and then weighed immediately on an electronic balance. The degree swelling (S_w) was calculated by dividing the weight gained by the original weight by using the formula given below:

$$S_w = (W_s - W_D)/W_D$$

where, W_s is the weight of swollen gel, and W_D the weight of dry gel.

3.7. In vitro release study

A 10 ml of sol containing 50 IU of insulin was filled in egg sac (separated by egg shell). The filled sac was suspended in release media (phosphate buffer saline, PBS, pH 7.4). Release media were maintained at 37 °C with magnetic stirring at 70 rpm. Sampling was done at different time points for 6 h and media were replaced with fresh media so that volume of the release media remains constant. The samples were analyzed using UV-Visible spectrophotometer 1700 (Shimadzu corporation) at 272 nm.

3.8. In vivo hypoglycemic effect

In vivo hypoglycemic effect of formulation was measured in wistar rats (200–250 g) fasted overnight. Rats were divided into 4 groups ($n = 5$). Group 1 served as positive control and was injected with insulin (1 IU/kg, subcutaneously). Group 2 was treated intranasally with insulin gel (10 IU/kg). The insulin formulation (200 IU/ml), were delivered through the right nostril using a PVC tube connected to a micropipette to give an insulin dose of 10 IU/kg. The preparation administered nasally was about 10–13 µl, depending on the weight of the rat. Group 3 was administered intranasally with plain insulin solution. Group 4 was treated with formulation without insulin to observe the effect of formulation component on blood glucose level. Blood samples (0.2 ml) were taken at various time points and blood glucose levels were determined using Accu check® Go (Roche Diagnostics, Germany). All the animals were procured from the animal laboratory facility of the institute. They were maintained under standard environmental conditions and housed individually in plastic cages in a controlled environment (22–24 °C and 12:12 light–dark cycle) with free access to food and water. All the experimental protocols were approved by Institutional Animal Ethics Committee, V.N.S. Institute of Pharmacy, Bhopal, India. The percent relative pharmacological bioavailability (Fr) was calculated according to formula (Wu et al. 2007) given below:

$$Fr = [AAC_{i.n.} \times Dose_{s.c.}] / [AAC_{s.c.} \times Dose_{i.n.}] \times 100\%$$

where “AAC”, “i.n.”, and “s.c.” represent area above the blood glucose levels-time curves, intranasal and subcutaneous respectively.

3.9. Statistical analysis

Student *t*-test and ANOVA were used to determine statistical significance. Differences were considered to be significant for values of $P < 0.05$.

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