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Original Article

TRANSDERMAL PATCHES OF CHITOSAN NANOPARTICLES FOR INSULIN DELIVERY

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ABSTRACT

Objectives: To develop a nanoparticle system with Chitosan for transdermal delivery of insulin.

Methods: Chitosan and Tripolyphosphate (TPP) were used to prepare the insulin loaded chitosan nanoparticles based on ionotropic gelation method and characterized using Zeta-sizer Nano ZS, Scanning electron microscopy and Optical Microscope**.** Transdermal drug delivery system of the formulated Insulin-chitosan Nanoparticles was prepared using solvent casting method.

Results: The results indicated that the nanoparticles were in the size range of 465 and 661 nm and exhibited quasi circular structure with better encapsulation efficiency. Controlled release Transdermal patches of insulin–chitosan nanoparticles were prepared using the polymer combinations HPMC, PVP K30 and PEG 400 with Tween 80 as plasticizer. The release rate of drug through patched increased simultaneously as the concentration of hydrophilic polymer was increased.

Conclusion: The encapsulated insulin drug was very effectively released by patch F3.

Keywords: Chitosan encapsulated Insulin, Encapsulation efficiency, Transdermal patches, *In vitro* Permeation Study.

INTRODUCTION

Drug delivery refers to technologies for delivering a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. It involves scientific site-targeting inside the body or systemic pharmacokinetics facilitation of the drug. Drugs have been used to improve health and extend lives. Side effects limit our ability to design optimal medications for many diseases [1]. Drug delivery systems control the rate at which a drug is released and the location in the body where it is to be released. The Lipinski's rule of five, formulated by Christopher A. Lipinski is based on the observation that most drugs are relatively small and lipophilic molecules. [2, 3]. The rule briefs on the molecular properties important for a drug's pharmacokinetics in human body. However, the rule does not predict if a compound is pharmacologically active.

Affecting more than 371 million people in the world, Diabetes Mellitus is ranked as the one of the top ten diseases causing high mortality rates in the present century. Insulin is a peptide hormone whose name gets derived from the Latin ''insula'' for "island" from the cells that produce the hormone in the pancreas. It has a molecular weight of 5.8kDa. Insulin regulates carbohydrate and fat metabolism in the body. Human recombinant Insulin is the most opted Insulin for Pharmacological research. [4]. Its Monomeric form is the most active form while Hexamer is the most stable form available [1]. There are three main classes of diabetes mellitus viz, insulin-dependent diabetes mellitus (IDDM), non insulin-dependent diabetes mellitus (NIDDM) and Gestational Diabetes [5]. Other than these there are intermediate conditions like Impaired Glucose Tolerance (IGT) and Impaired Fasting Glycaemia (IFG), which can lead to NIDDM. Heinemann *et al.* depicted new methods of insulin delivery [6]. The insulin compounds such as Exubera and AIR have been examined and used in design experiments which could be a promising one in the field of Diabetology. Passive transmission of insulin is not possible through skin due to its large molecular weight, but, with treatment with different delivery aids like microneedles and patches (with polymers) has been experimenting successfully both *in vitro* and *in vivo* [7, 8].

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4) linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) produced by the deacetylation of chitin [9]. The degree of deacetylation (%DD) can be determined by NMR spectroscopy, which ranges from 60 to 100%. The amino group in chitosan has a pKa value of around 6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the degree of acetylation (%DA) value which makes chitosan water soluble and a bio adhesive that readily binds to negatively charged surfaces such as mucosal membranes. Chitosan greatly enhances the transport of polar drugs across epithelial surfaces, and is biodegradable [10]. The properties of Chitosan allow it to be used in drug delivery. It is mucoadhesive in nature and reactive (so it can be produced in many different forms). It has a positive charge under acidic conditions, so it can be used in transdermal drug delivery effectively. The positive charge comes from the protonation of free amino groups. Lack of positive charge means Chitosan is insoluble in neutral and basic environments. However, protonation of amino groups enhances the solubility [11].

One major example of insulin drug delivery has been the transport of drugs through a transdermal patch as indicated by the following procedure in this report [9]. In a recent market report, it was suggested that the growth rate for transdermal delivery systems will increase 12% annually. The transdermal route of drug administration is recognized as one of the significant route for the local and systemic delivery of the drugs. Transdermal products for heart disease, depression, sexual dysfunction, urinary incontinence, etc. are at various stages of clinical development [12]. The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetration across the skin, which is associated primarily with the outermost stratum corneum layer of the epidermis. Consequently the daily dose of drug that can be delivered from a transdermal patch is 5-10 mg effectively limiting this route of administration to potent drug. A Transdermal patch is a flexible pharmaceutical preparation of various sizes, containing one or more active substances called drug. They are intended to be applied on the unbroken skin in order to deliver the active substance(s) into the systemic circulation after passing through the physiological skin barrier. [13]. The exact mechanism of drug penetration from the transdermal patch through the Stratum corneum was explained by Trommer *et al.* [14]. So, the main objective of our study is to passively delivery insulin as nano formulation along with Chitosan. The encapsulated insulin in Chitosan polymer is to be analyzed for its efficiency in facile controlled-release delivery system as transdermal patches.

MATERIALS AND METHODS

The preparation of insulin-Chitosan nanoparticles required pure Insulin Powder (Sigma Aldrich), Chitosan (LMWC, Sigma Aldrich), Sodium Tri Poly Phosphate (TPP) (Sigma Aldrich), 2% Acetic Acid and insulated (40 IU/ml)-(Human DNA Recombinant) for

Optimization. The transdermal patch was synthesized from hydroxy Propyl Methyl Cellulose (HPMC) (Sigma Aldrich), Poly Ethylene Glycol (PEG) (Sigma Aldrich), Poly Vinyl Pyrolidine (PVP) (LobaChem), Ethanol and Isopropanol.

Characterization

The characterizations was done using Zeta-Sizer Nano ZS (Malvern Instruments, Malvern, UK), Scanning electron microscopy (VEGA3:TESCAN) and Optical Microscope.

Ionotropic gelation

Different Concentration of Chitosan Solutions was prepared by dissolving in 2% acetic acid and kept for stirring until the clear transparent solution of Chitosan was obtained. Subsequently, 1 ml of insulin was added to the Chitosan solution and stirred for an hour in an ice bath.

Synthesis of insulin-chitosan nanoparticles

0.25% of TPP solution were prepared by dissolving 2.5 mg/ml in deionized water (pH 5) and added dropwise in the Insulin-Chitosan solution. The change to opalescent suspension determines the formation of insulin encapsulated Chitosan Nanoparticles. All optimization procedures were done in triplicate and the same procedure was repeated at different concentrations of Chitosan and tabulate in table 1. The obtained Insulin Chitosan Nanoparticle solution is stored at 4 °C.

Table 1: Composition of Chitosan, TPP, and insulin in the formulation of insulin Chitosan nanoparticles

Trials	Chitosan	Insulin(mg/ml)	TPP
1	0.1%	20	0.25%
2	0.15%	20	0.25%
3	0.2%	20	0.25%
4	0.25%	20	0.25%

Size determination

Samples were measured in folded capillary cells integrated with gold electrodes using Zeta-sizer Nano ZS (Malvern Instruments, Malvern, UK) at 25 °C. Three measurements were conducted and automatically determined by the software. The results were expressed as mean±standard deviation (SD).

Morphological studies

The morphologies of uncoated and Chitosan coated nanoparticles containing Insulin were observed using Optical and Scanning Electron Microscopy (VEGA3: TESCAN).

Encapsulation efficiency

Nanoparticles were separated from the aqueous phase by ultracentrifugation (Sigma 3k30, Germany) at 15000 rpm and 4°C for 20 minutes. The supernatants were collected and evaluated for insulin residue by UV spectroscopy.

$$EE\% = \frac{100 \times (\text{amount of total drug} - \text{amount offree drug})}{\text{amount of total drug}}$$

Transdermal patches

Transdermal drug delivery system of the formulated Insulin-chitosan Nanoparticles has been formulated by using solvent casting method. Monolithic systems prepared by using Hydroxypropyl methylcellulose (HPMC), Poly Vinyl Pyrrolidine (PVP) K-300, Mucoadhesive agents and Permeation enhancer. Tween 80– Plasticizer. The various concentrations of polymers in formulations are depicted in table 2.

Evaluation of patches

All the transdermal patches were visually inspected for color, clarity, flexibility and smoothness. The dried patches were weighed on a digital balance. Film thickness was measured by a screw Micrometer at five different random points on the film. Folding endurance was determined by repeatedly folding the film at the same place until it broke. All patches were cut at 2x2 cm^2. Constant dry weight of the patch was measured as W1. The patch was immersed in 100 ml of distilled water for 3 days at 37 °C. Excess water was removed and weighed as W2. The patch was kept in desiccators and dried to remove water and re weighed as W3.

$$\%\text{dissolution} = \frac{(W1 - W3)}{W1} \times 100$$

$$\text{SwellingIndex(SI)} = \frac{(W2 - W3)}{W3} \times 100$$

The patches were allowed to swell for 1 hour in glass tubes and the surface pH was then noted. Patches of specified area (1 cm sq.) were dissolved in 5 ml of Tris Hcl and the volume was made up to 10 ml with phosphate buffer (pH 7.4). A blank was prepared using a drug-free patch treated similarly. Absorbance was read at 270 nm in a UV-Vis spectrophotometer.

Table 2: Various concentrations of polymers used in the formulation of patches

Trials	HPMC (%w/v)	PEG (%w/v)	TWEEN 80 (%w/v)	PVP (%w/v)	DRUG (ml)
F1	80	20	-	20	2
F2	70	30	-	20	2
F3	60	40	-	20	2
F4	70	20	10	20	2
F5	80	10	10	20	2
F6	70	10	20	20	2
F7	70	20	20	20	2
F8	80	-	20	20	2

***In vitro* permeation study**

The *in-vitro* permeation studies were conducted using a vertical type Franz diffusion cell. The excised goat skin was mounted between the half-cells with dermis in contact with phosphate buffer pH 7.4. The temperature of approximately 32 °C was maintained (Skin temperature). Samples were withdrawn (2 ml each time) periodically from the receiver cell and an equal volume of phosphate buffer was added to keep the volume constant. The insulin content was determined by UV Spectroscopy at 270 nm. Each experiment was carried out for 10 hours to achieve a steady permeation rate.

RESULTS AND DISCUSSION

Formation of insulin encapsulated chitosan nanoparticles

In this study, low molecular weight chitosan and Tripolyphosphate (TPP) were used to prepare the insulin loaded chitosan nanoparticles based on ionotropic gelation method in different trials from S1 to S4 [15]. The formation of insulin-chitosan nanoparticles was depicted by the opalescent suspension as shown in fig. 1 [16]. Chitosan nanoparticles with various sizes encapsulated with insulin were formed. Two of the trial (S2 and S3) formulation among them showed desired size range of 465 and 661 nm (table 3). The size of the nanoparticles formed was characterized by Zeta-sizer whose results are displayed in fig. 2. The particle size was greatly influenced by the concentrations of chitosan solution (CS) and TPP solutions. The more the concentration of CS, greater the size of the nanoparticle formed. The increased size could be attributed due to the dense spatial distance among the molecules of chitosan at higher concentrations, the high molecular weight of the drug molecules and drug surface adsorption during incubation [17, 18].

Morphology of nanoparticles

The morphologies of insulin encapsulated chitosan nanoparticles were determined by optical microscopy and SEM analyzes are shown in fig. 2 and 3. The nanoparticles possess a Quasicircular shape and uniform size which coincides with the results obtained by Ma *et al.* (2002) [19].

Table 3: Size and Encapsulation efficiency Insulin-Chitosan nanoparticles

Trials	Chitosan (%)	Insulin (ml)	TPP (%)	NP Size (nm)	EE %
S1	0.1	1	0.25	-	-
S2	0.15	1	0.25	465	77.3±0.50
S3	0.2	1	0.25	661	78.9±0.25
S4	0.25	1	0.25	4367	78.7±0.25

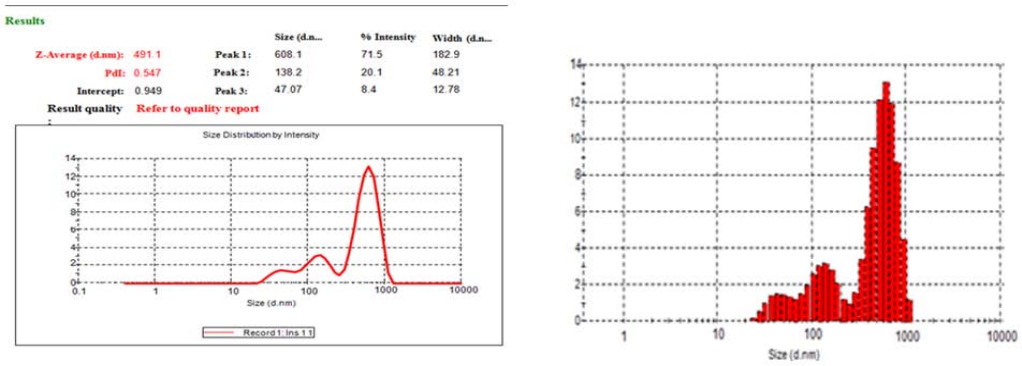

Fig. 1: Zeta sizer showing particle size distribution of Synthesized Insulin nanoparticles using 0.2% Chitosan

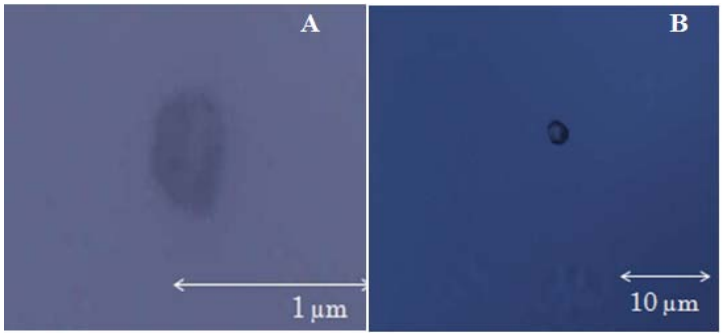

Fig. 2: Morphological studies using Optical microscopy A-20 X resolution (left), B-50 X resolution (right)

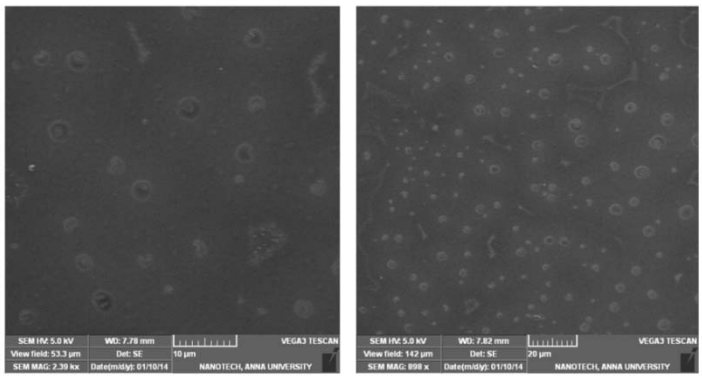

Fig. 3: SEM micrographs of chitosan insulin nanoparticles

Encapsulation efficiency

Encapsulation efficiency (EE) was significantly dependent on the preparation method and as tabulated in table 1, the encapsulation efficiency of the formulated nanoparticles was found to be high. The formulation showing the minimal particle size of 465 nm showed good encapsulation efficiency of 77.3%. The reason for the high encapsulation efficiency may be due the strong affinity between chitosan and TPP. Chitosan being a cationic polymer binds readily with the cross-linking anionic polymer like TPP. Insulin being hydrophilic gets attached with either of the polymers and thus getting entrapped efficiently [20]. The increase in encapsulation is propositional to the increase in the amount of polymer ratio taken for encapsulation [1]. The efficiency seen in our study is close to the values obtained in previous studies [4].

Transdermal patches-insulin entrapped chitosan nanoparticles

The transdermal patches containing insulin chitosan nanoparticles were prepared by solvent casting technique and were characterized by thickness, weight, folding endurance, content uniformity, opacity, percentage dissolution, swelling index and Surface pH [21]. The selection of polymer combinations produced smooth, uniform, flexible and films of desired thickness for the transdermal delivery systems of insulin. The physicochemical characteristics of the films are shown in table 4. Film thickness was found to vary between 0.26 to 0.34 mm. The formulation F1, F2, F3, F4 and F5 showed minimal folding endurance where as the maximal value was found in F7 and F8. The presence of tween 80 increased the folding endurance of the films. For the various formulations, drug content was found to be between 2.89 to 3.10 mg per film.

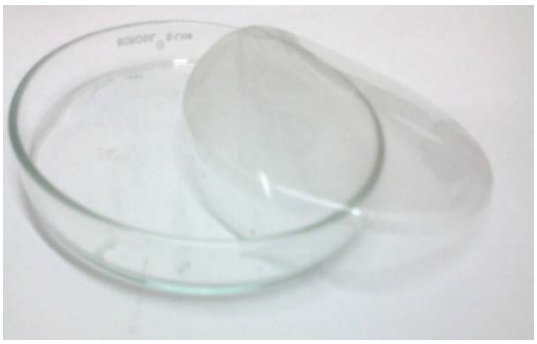

Fig. 4: Formulated transdermal patch containing Insulin-chitosan nanoparticles

Table 4: Evaluations for transdermal patches

Trial	Mean thickness (mm) n = 5	Mean weight (g) n = 5	Folding endurance n = 5	Opacity	% Dissolution	Swelling Index	Surface pH	Drug content (mg/film)
F1	0.252±0.012	0.275	<100	Opaque	7.87±0.92	49.22±3.21	6.8±0.05	3.07±0.42
F2	0.376±0.015	0.343	<100	Opaque	7.66±1.21	48.34±3.72	6.9±0.031	2.98±0.37
F3	0.322±0.015	0.277	<100	Opaque	4.45±1.37	43.67±2.83	6.7±0.056	3.03±0.53
F4	0.282±0.019	0.296	<100	Translucent	4.56±1.08	40.34±3.53	6.8±0.024	2.88±0.39
F5	0.243±0.035	0.292	<100	Translucent	5.44±0.97	54.43±4.39	6.7±0.036	2.78±0.41
F6	0.322±0.030	0.292	>100	Translucent	2.32±0.63	47.87±2.87	6.7±0.041	2.93±0.32
F7	0.232±0.014	0.318	>100	Translucent	3.56±0.68	53.43±3.91	5.6±0.08	3.02±0.44
F8	0.272±0.012	0.262	>100	Translucent	5.45±1.03	53.67±3.74	5.7±0.07	2.99±0.49

***In vitro* permeation study**

The *in vitro* permeation study was performed on excised goat skin using Franz diffusion cell [22]. It was found that only 60% of the drug permeated through goat skin from F8 (without permeation enhancer) at the end of 8 hours. The transdermal films (F2 and F3) containing PEG as a permeation enhancer showed a significant increase in permeation rate. Hence, the combination of HPMC and PEG in the films increased the drug permeability. The synergistic effect may be due to functioning of glycols in combination as co-solvents to produce saturated or nearly saturated solution of active medicament in the formulation thereby maximizing the thermodynamic activity of the penetrant. Fig. 5 shows percentage release of the drug over a time scale of 10 hours for those patches that showed considerably better release patterns.

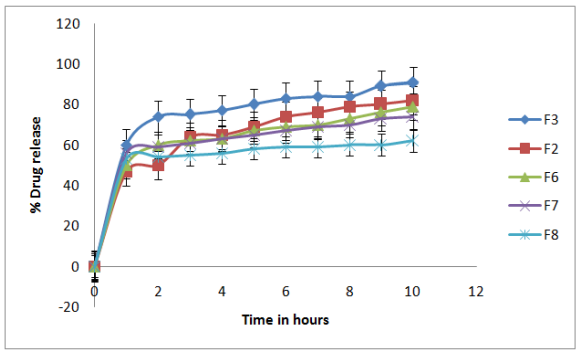

Fig. 5: Permeation of Insulin-Chitosan Nanoparticles across excised goat skin from different formulation

The drug permeated from film F3 at the end of 8 hours was found to be 83%. F3 showed maximal permeation followed by F2 and F6. The formulations can be arranged in order of permeation rate as: F3>F2>F6>F7>>F8. The patches showed good release of insulin based on the concentration of PEG that aided the process. F3 patched showed the maximum drug release at the end of 8 hours since it contained the maximum concentration of PEG, along with a good moderate swelling index of 43.67%.

CONCLUSION

Transdermal patches of chitosan encapsulated insulin using polymers such as HPMC, PEG and PVP were made and found to give the desired results that we predicted. The patches were smooth with good swelling index and ranged from 0.2 to 0.3 mm in thickness. The folding endurance was increased with the aid of tween 80. The release kinetics showed that F3 patch was best among the others because of its permeation rate up to 83% for 8 hours and swelling index of 43.67%. F2 and F6 stand in line behind F3 based on their decreasing release percentage of the drug in the given time scale. Hence, we would like to conclude that the encapsulated insulin drug was very effectively released by patch F3. Further, *in vivo* studies have to be performed to correlate with *in vitro* release data for the development of suitable controlled release patches for Insulin.

CONFLICT OF INTEREST

Declared None

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