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DESIGN AND *IN VIVO* EVALUATION OF GASTROINTESTINAL MUCOADHESIVE PATCH SYSTEM (GMPS) LOADED WITH CHITOSAN NANOPARTICLES

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ABSTRACT

A novel gastrointestinal mucoadhesive patch system was designed to improve the oral bioavailability of macromolecular therapeutics using chitosan nanoparticles for effective delivery of macromolecules (using FITC-Dextran as model drug). The patch system consisted of mucoadhesive layer containing the drug and backing layer made of water-impermeable polymer. The gastrointestinal mucoadhesive patch (GMP) was formulated using 10% ethyl cellulose in 80:20 dichloromethane:methanol along with triethyl citrate as plasticizer. The mucoadhesive layer was formulated using 2% carbopol dispersion in water along with 30% propylene glycol as a plasticizer. Pharmacokinetic study was done in rabbits for FITC-Dextran solution, patch containing FITC-Dextran incorporated in to the mucoadhesive layer filled into enteric coated capsules and patch containing FITC-Dextran incorporated chitosan nanoparticles loaded in to the mucoadhesive layer filled inside enteric coated capsules. FITC-Dextran was found to be 1.16 times more bioavailable orally from chitosan nanoparticle loaded in mucoadhesive layer as compared to other prepared formulations.

Keywords: Patch system, FITC-Dextran, Chitosan nanoparticles, Mucoadhesion, Gastrointestinal, Pharmacokinetics.

INTRODUCTION

Biomolecules like protein and peptides are gaining significant importance as therapeutic entities in a variety of diseases [1] due their high selectivity and potency [2, 3]. The evolution of biotechnology and DNA recombinant technology led to the development of many new such molecules as therapeutic agents [4]. However, the major problem associated with the formulation for these molecules are high biochemical and structural complexity [2, 5, 6]. Hence, most of the bioactive molecules are administered parenterally which is associated with very poor patient compliance and additionally needs a trained medical staff for the proper administration of dose [7]. Oral route is relatively much more patient compliant and is the most widely exploited route for drug delivery [3]. But the major limiting factors for oral delivery of protein and peptide drugs are pre-systemic degradation due to proteases and harsh gastrointestinal pH on the gut wall as well as poor penetration across intestinal membrane [8]. Various strategies for overcoming these problems and developing oral protein delivery systems includes use of absorption enhancers, enzyme inhibitors, mucoadhesive polymers, particulate carrier delivery systems and designing formulations that allow protection of protein drugs from the harsh environment in the GI tract [4, 9].

A multilayered patch system is one of the promising approaches for incorporating the desired functionalities of large molecular weight [10]. Since the past couple of years, various intestinal patch systems have been developed for the oral delivery of proteins [11]. Gastro-intestinal patches provide mucoadhesion, protection against harsh gastric pH and enzymes, and permeation enhancing effect. This delivery system has been employed for delivery of G-CSF [12]. Another report suggests the preparation of intestinal patches by sandwiching a film of cross-linked bovine serum albumin microspheres between a film of ethyl cellulose and Carbopol/pectin [13]. Recently the new generation of polymers called the thiolated polymers have also been used to fabricate the gastro-intestinal patches [10, 14].

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This study was focused on developing a suitable delivery system for therapeutic proteins by combining the approach of nanotechnology with gastrointestinal mucoadhesive patch system (GMPs). GMPs were prepared containing a backing layer of ethyl cellulose and a drug-in-mucoadhesive layer made up of carbopol incorporated with model protein FITC-Dextran loaded chitosan nanoparticles. It is reported that chitosan has mucoadhesive action and is capable of enhancing the intestinal permeability of different compounds through tight junction epithelium [15-17]. Chitosan has been especially exploited for its tight junction opening properties in formulation of nasal drug delivery system for protein and peptide drugs [18]. Mucoadhesive poly (acrylates) like carbopol have the ability to enhance the intestinal absorption of peptides by inhibiting the luminal and membrane bound enzymes and by further opening up the intercellular junctions [19, 20]. It was assumed that the GMPs would protect the model protein from harsh gastrointestinal pH and proteases and increase the residence time of drug in intestine and in the mean time chitosan nanoparticles would facilitate the protein drug absorption by enhancing the intestinal permeability of drug and further providing mucoadhesion. Hence, the overall result would be the better systemic bioavailability of the macromolecular therapeutic. FITC-Dextran was used as a model macromolecular compound which was incorporated into the developed formulation as it resembles protein drugs in term of its size and molecular complexities. It has already been used as a model macromolecular drug and as fluorescence marker for studying delivery of protein/peptide based drugs [10]. The prepared chitosan nanoparticles were evaluated on the basis of particle size, zeta potential, entrapment efficiency and SEM analysis. GMPs were evaluated on the basis of FT-IR analysis, mucoadhesion strength, tensile strength, *in-vitro* release, unidirectional studies, *ex-vivo* permeation studies and pharmacokinetic studies in rabbits.

MATERIALS AND METHODS

Fluorescein Isothiocyanate Dextran (FITC-Dextran; FD_4. Mol. Wt. 4000 Da) was obtained as gift sample from TdB Consultancy, Uppsala, Sweden. Chitosan low molecular weight (85 % deacetylated, viscosity 20-300 cP 1% in 1% acetic acid) and sodium tripolyphosphate (TPP; Technical grade, 85%) were purchased from Sigma-Aldrich Co., MO, USA. Carbopol® 934 P NF was obtained as a gift sample from Lubrizol Advanced Materials India Pvt. Ltd., Mumbai, India. Ethyl cellulose N 22 Pharm USP was purchased from Signet Chemical Corp. Pvt. Ltd., Mumbai, India. Triethyl citrate was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Propylene glycol was purchased from S.D. Fine Chemicals, Mumbai, India. Sodium hydroxide and Potassium dihydrogen ortho-phosphate (analytical grade) were obtained from Qualigen Fine Chemicals, Mumbai, India. All the solvents and chemicals used for the study were of chromatographic grade and purchased from Qualigen Fine Chemicals, Mumbai, India. Heparin was purchased from Biological E. Ltd, Hyderabad, India. Deionised water for HPLC was prepared in-house using a Milli-Q water purifier system (Millipore Elix, Germany).

Preparation and *in vitro* Characterization of Chitosan Nanoparticles

Chitosan nanoparticles were prepared by ionic gelation of low molecular weight chitosan hydrochloride and sodium tripolyphosphate (TPP) [21-23]. Various concentrations of chitosan (0.2, 0.5, 1 µg/mL) and TPP (0.5 and 1%) were taken in order to determine an optimum ratio for nanoparticle preparation. Chitosan solution was prepared in 1% acetic acid and pH was adjusted to 5 using 0.2 M sodium hydroxide TPP solution was prepared in deionised water and FITC-Dextran was dissolved in to it. TPP solution was added dropwise to chitosan solution with constant stirring. The resultant suspension was centrifuged at 10,000 rpm for 30 min at 25ºC [24]. The pellets were re-suspended with deionised water and stored at room temperature until further studies.

The average particle size, the particle size distribution and zeta potential of chitosan nanoparticles were determined using ZetaSizer Nano ZS 90 (Malvern Instruments, UK). The drug entrapment efficiency was determined by estimating the amount of FITC-Dextran in the supernatant obtained after centrifuging the nanoparticle suspension at 10,000 rpm for 30 min and substracting it from the total amount added [24]. The samples were analyzed using the spectrofluorometer (Elico SL 140).

$$\text{Entrapment efficiency} \quad \frac{C_0 - C_1}{C_0} \times 100$$

C_0 = Total amount of FITC-Dextran initially added
C_1 = Amount of FITC-Dextran present in the supernatant as determined by spectrofluorometric analysis.

Preparation of GMPs

The patches were prepared in two separate layers and then adhered together. First, ethyl cellulose backing layer was prepared. A 10% solution of ethyl cellulose N 22 Pharm USP was made by dissolving it in a mixture of dichloromethane: methanol (80:20). Triethyl citrate was added as a plasticizer in a concentration of 3% to the solution of ethyl cellulose and the final mixture poured on a glass petridish (4.9”diameter) and kept at room temperature till a semi-dried film was formed. Meanwhile the drug-in-mucoadhesive layer was prepared. Carbopol dispersion (2%) was prepared by slowly adding a powder of Carbopol 934P to vigorously stirring water. Propylene glycol (30%) was added to carbopol dispersion as a plasticizer. The previously prepared suspension of FITC-Dextran loaded chitosan nanoparticles was then added to this dispersion and the entire mixture stirred for 15 min. The resulting dispersion was poured on a semi-dried film of ethyl cellulose and dried for 16 h in oven at 40°C. GMPs equivalent to the FITC-Dextran concentration of 2 mg was cut and filled in to an enteric coated capsules of size#3. The

enteric coated capsules had a coating of Eudragit L100 & hence the capsules would release the GMPs only when the gastrointestinal pH goes above 5.5, near the jejunum region. GMPs without chitosan nanoparticles were prepared by directly adding FITC-Dextran in to the carbopol dispersion and then subsequently pouring it on semi-dried ethyl cellulose backing layer.

Evaluation of GMPs

FT-IR Analysis of FITC-Dextran Loaded GMPs

FT-IR spectra were taken for the determination of stability of the FITC-Dextran after preparation of the patch. FT-IR spectra also give an indication of the compatibility of the FITC-Dextran with the excipients used for preparing the GMPs. Previous report on mucoadhesive patches provides the data regarding the use of FITC along with the components used in our formulation (Chitosan, carbopol and ethyl cellulose) [25]. Thus, in this study, FTIR spectra of only FITC-Dextran, blank patch and FITC-Dextran loaded chitosan nanoparticles incorporated in GMPs were obtained using a spectrophotometer (Shimadzu FTIR affinity 1 equipped with IR solution version 1.21) by potassium bromide (KBr) pellet method. In this method, 1 part of sample was mixed with 99 parts of KBr in mortar and pestle to form powder. The scanning range was 400–4000 cm^{-1}, and the resolution was 1 cm^{-1} .The spectra of FITC-Dextran, blank patch and FITC-Dextran loaded chitosan nanoparticles incorporated in GMPs were compared for any significant change in peaks.

Evaluation of Mucoadhesion Strength

The mucoadhesion strength was estimated to determine the adhesion force between the intestinal wall and the patch and thus inferring about the total retention of the patch in the gastrointestinal tract. The mucoadhesion strength of GMPs was measured using Texture Analyser QTS, Brookfield Instruments, UK [26]. This study was performed using small intestine of rabbit which was obtained after euthanizing the animal under carbon-dioxide asphyxiation. The small intestine was excised; mucosa was cleaned, opened longitudinally and cut in to pieces. A portion of the intestinal segment was then placed on sample holder. The piece of GMPs was cut and pre-hydrated with simulated intestinal fluid for 2 min. The hydrated piece was adhered to a 10 mm cylindrical probe with a double-faced tape. The cylindrical probe was moved down near to the mucosa and kept in contact with mucosa for 30 sec. A predetermined compressive force of 0.5 N was applied during this period. The probe was then removed at a speed of 10 mm/min. The adhesion force and adhesiveness was measured from the load *versus* time curve.

Determination of Tensile Strength

Tensile strength of GMPs is an indication of the physical strength and durability of the patch. The tensile strength of GMPs was measured using Texture Analyser QTS, Brookfield Instruments, UK. GMPs were cut in to pieces and both of its ends were attached to a dual grip jig type of probe. The initial distance between the two clamps was 15 mm. The upper clamp was moved upwards at a speed of 20 mm/min. The patch was stretched until it teared into two pieces. The percentage elongation and tensile strength was measured at this point.

***In vitro* Drug Release**

The release rate of FITC-Dextran from the GMPs was investigated in pH 7.4 phosphate buffer saline. GMPs equivalent to 2 mg of FITC-Dextran was placed in a Franz diffusion cell and the receptor compartment was filled with phosphate buffer saline (pH 7.4). The temperature of the cell was maintained at 37°C during the entire duration of experiment. Samples were withdrawn at specific time intervals from the receptor compartment. The samples were then analyzed and exact amount of FITC-Dextran released during each time interval was determined using the spectrofluorometer.

Unidirectional Release Study

Ideally for the GMPs, the entire amount of FITC-Dextran should be released only from the mucoadhesive side and nothing should be released from the backing layer. The unidirectional release studies were performed to determine the extent of FITC-Dextran releasing from the water impermeable ethyl cellulose backing layer of the GMPs using a reported assembly [13]. A custom designed diffusion cell was built. The cell provided an orifice of 0.785 mm^2 between the two compartments. The patch was inserted between the two compartments in such a way that the mucoadhesive layer was exposed to receptor compartment and ethyl cellulose backing layer exposed to donor compartment. Both the donor and receptor compartments were filled with phosphate buffer (pH 7.4). Samples were withdrawn at 2 h, 3 h, 4 h, 5 h, 6 h and 12 h from the donor compartment and analyzed using spectrofluorometer.

Ex vivo Permeation Study

The *ex vivo* permeation study of FITC-Dextran from the designed formulation was determined using rabbit intestinal mucosa. Intestinal mucosa of rabbit was excised and mounted on a Franz diffusion cell. FITC-Dextran loaded patch was adhered on rabbit intestinal mucosa. The receptor compartment was filled with phosphate buffer (pH 7.4) and the cell was placed on a magnetic stirrer. The temperature of the cell was maintained at 37°C during the entire duration of experiment. Samples of 0.5 mL volume from the receptor compartment were withdrawn at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 12 h and the amount of drug permeated was estimated using spectrofluorometer.

Pharmacokinetic Study

Male New Zealand white rabbits weighing 1.00–

1.25 kg were obtained from the animal house of B. V. Patel PERD Centre, Ahmedabad. Animal housing and handling were performed in accordance with Good Laboratory Practice (GLP) mentioned in CPCSEA guidelines. Animal house is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India, vide registration no. 1661/PO/a/12/CPCSEA, dated 21/11/2012. All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee prior to initiation of the experiment. The animals were housed singly per cage and were placed in the experimental room where they were allowed to acclimatize for a week before experiment. A 10% air exhaust conditioning unit was maintained along with a relative humidity of 60±5% and a temperature of 25±3ºC in the animal house facility. A 10:14 h light:dark cycle was also regulated for the experimental animals. Eighteen animals (six animals per group) were used in the study to determine the oral bioavailability of the FITC-Dextran (Dose: 2 mg per animal). The first group was dosed with FITC-Dextran solution in phosphate buffer (pH 7.4, concentration of 0.4 mg/mL) and second with patch containing FITC-Dextran (2 mg) directly incorporated in to the mucoadhesive layer was administered by filling them in to enteric coated capsules. The third group was dosed with patch containing FITC-Dextran (2 mg) incorporated chitosan nanoparticles loaded in to the mucoadhesive layer filled inside enteric coated capsules. The blood samples of 0.5 mL were withdrawn from the marginal ear vein of each rabbit at 0, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h and 8 h, post dose, collected into heparinized microcentrifuge tubes and centrifuged at 4000 rpm for 7 min at 4º C. The resulting plasma samples were kept frozen at -80ºC prior to HPLC analysis. The amount of FITC-Dextran in plasma was determined by previously reported HPLC method with some modifications [10].

The HPLC system consisted of PU-980 HPLC pump (Jasco, Hachioji, Tokyo, Japan), FP-920 spectrofluorometer (Jasco, Hachioji, Tokyo, Japan), AS-950 autosampler (Jasco, Hachioji, Tokyo, Japan) and the data were analyzed by Borwin software version 1.5. Chromatographic separation was achieved by using C_{18} column (Lichrospher Hibar 250×4.6 mm, 5µm) column maintained at room temperature. The mobile phase consisted of acetonitrile: phosphate buffer, pH adjusted to 7.4 (35:65, *v/v*). The mobile phase was prepared daily and degassed before use. The flow-rate was maintained at 0.8 mL/min. The detection was done on fluorescence detector at an excitation wavelength of 490 nm and emission wavelength of 511 nm. Samples were quantified by determining the response (Peak area$_{Drug}$/Peak area$_{IS}$).

The maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) were directly determined from the plasma concentration *versus* time curves. The area under the curve from 0 to t (AUC_{0-t}) was calculated following linear trapezoidal rule by summing the area from 0 to t h. Elimination rate constant (K_{el}) was determined by taking the absolute value of the slope of any three points lying on a straight line of the curve after the C_{max}, i.e. during the elimination phase. Elimination half life ($t_{1/2}$) was determined using the relationship $t_{1/2} = 0.693/K_{el}$. The volume of distribution (V_d) was calculated by dividing amount of drug dosed by the total plasma concentration. The total clearance (Cl_T) was calculated using the relationship $Cl_T = 0.693Vd/t_{1/2}$.

Statistical Analysis

Statistical data analysis was performed using the Student's *t*-test with $p \leq 0.05$ as the minimal level of significance unless indicated otherwise.

RESULTS AND DISCUSSION

Preparation and *in vitro* Characterization of Chitosan Nanoparticles

The formulation development of chitosan nanoparticles was carried out first by varying the concentration of chitosan solution, sodium tripolyphosphate concentration and acetic acid concentration to get the desired particle size distribution.

The optimized preparation conditions of chitosan nanoparticles were 0.2% chitosan in 1% acetic acid along with 1% TPP. The developed nanoparticles had z-average particle size of 200.60 ± 15.40 nm and zeta potential of +14.70 ± 0.8 mV. Figure. 1 (a) and (b) is a representation of the obtained data for average particle size and zeta potential, respectively. The entrapment efficiency determined by back calculation method was found to be 32.00 ± 1.40%.

Preparation of GMPs

The gastrointestinal mucoadhesive patch (GMP) was formulated using 10% ethyl cellulose N 22 Pharm in 80:20 dichloromethane:methanol along with triethyl citrate as plasticizer as this gave the most optimum film properties. It was also observed that carbopol (2%) dispersion in water with propylene glycol (30%) gave the best film for mucoadhesive layer.

Evaluation of GMPs

FT-IR Analysis of FITC-dextran Loaded GMPs

Previous report suggests the use of ethyl cellulose, carbopol and Chitosan along with FITC in preparation of GMPs and hence individually these excipients were presumed to be compatible with FITC, and no preferential interaction with individual excipient was studied [25]. The results of FT-IR analysis showed no significant changes in major peaks of pure FITC-Dextran and FITC-Dextran loaded chitosan nanoparticles incorporated in GMPs as shown in Figure. 2. This suggested that FITC-Dextran remained stable during the process of preparation of GMPs and was compatible with the excipients used for preparing the GMPs.

Evaluation of Mucoadhesion Strength

The adhesion force for GMPs was -8 gm and the

adhesiveness was -127.28 g.s. The values of the test results indicated that GMPs possessed sufficient mucoadhesiveness and were capable of adhering to the intestinal mucosa upon oral administration. It could also be assumed that the patch would adhere only to the intestine by its mucoadhesive layer and not backing layer, since backing layer did not have any mucoadhesive properties. Figure. 3 represents the load *versus* time curve for mucoadhesion test.

Determination of Tensile Strength

The results for the tensile strength as shown in Table 1 suggest that the GMPs have sufficient tensile strength to withstand the folding and other pressures during normal handling of GMPs. Figure. 4 represents the load *versus* distance curve for tensile strength of GMPs.

***In vitro* Drug Release**

The results of the *in vitro* release showed that over 97% of the FITC-Dextran release took place within a period of 5 h. This suggests that the release of FITC-Dextran containing chitosan nanoparticles from the patches was immediate and carbopol matrix did not interfere or retard the release rate of FITC-Dextran containing chitosan nanoparticles from the patches. Further the presence of free FITC-Dextran in the receptor compartment implies that FITC-Dextran had released from the chitosan nanoparticles under passive diffusion conditions as is expected to occur physiologically. The carbopol layer maintained its integrity and did not dissolve in the medium of receptor compartment demonstrating the efficacy of the ethyl cellulose coating. Figure. 5 shows the *in vitro* release profile of the FITC-Dextran from the GMPs.

Unidirectional Release Study

The release from the backing layer was checked for the period of 12 h. A cumulative release of 3.6% was observed from the GMPs after a period of 12 h. The values of unidirectional release upon comparison with *in vitro* release studies mentioned earlier, inferred that 1.8% of FITC-Dextran was released from the backing layer and 98% of FITC-Dextran (5 h) was released from the mucoadhesive side. This result indicated that a minute amount of FITC-Dextran may have been lost for the purpose of saturation of the ethyl cellulose layer prior to its release in to the donor compartment. Hence a separate study to determine the amount of FITC-Dextran retained in the ethyl cellulose layer was not performed. The results of the unidirectional release indicates that a negligible amount of FITC-Dextran was released from the backing layer and that the ethyl cellulose film proved to be a good barricade against the leakage of the FITC-Dextran from GMPs. Figure. 6 depicts the unidirectional release profile of FITC-Dextran from GMPs.

***Ex vivo* Permeation Study**

The results of *ex vivo* permeation study as shown in Figure. 7 inferred that 13% of the total amount of FITC-Dextran in GMPs was found to be permeating throughout the 12 h sampling period indicating that GMPs serves as a good delivery system.

Pharmacokinetic Study

Pharmacokinetic parameters of the different animal groups are shown in Table 2. The mean plasma concentration *versus* time profile of FITC-Dextran upon oral administration as solution, as a patch containing FITC-Dextran directly incorporated in to the mucoadhesive layer and as a patch containing FITC-Dextran incorporated chitosan nanoparticles loaded in to the mucoadhesive layer is shown in Figure. 8. FITC-Dextran was not detectable in plasma upon oral administration as solution which was in good accordance with a previous report describing that their values of the bioavailability after peroral administration as a solution were virtually zero [27]. However, the oral bioavailability of FITC-Dextran was good from the two different patches. The results showed a significant difference in pharmacokinetic profile of FITC-Dextran among the animals of group 2 and 3. FITC-Dextran was found to be 1.16 times more bioavailable orally from chitosan nanoparticle loaded in mucoadhesive layer as compared to other prepared formulation.

Statistical Analysis

Student's *t*-test was applied to determine the difference in oral bioavailability of FITC-Dextran in different study groups. The bioavailability of FITC-Dextran was significantly higher from the patch containing FITC-Dextran incorporated chitosan nanoparticles loaded into the mucoadhesive layer as compared to the patch containing FITC-Dextran directly incorporated into the mucoadhesive layer with $p \leq 0.05$ as calculated from AUCs of the respective groups. The results are expressed as means ± SEM.

Gastrointestinal mucoadhesive patch system (GMPs) was formulated with the objective of increasing the permeation of macromolecules through intestinal mucosa which would result in improved oral bioavailability. This aim was achieved by using various strategies one of which was the use of Carbopol as a mucoadhesive polymer. Carbopol is reported for its ability to temporarily open up tight junction epithelium, thus allowing rapid absorption of peptide drugs. Carbopol formulations showed a resulting drop in transepithelium electrical resistance (TEER) across the Calu-3 cell lines thus suggesting the paracellular transport enhancement by carbopol [28]. It also causes the depletion of extracellular Ca^{+2} levels and thus disruption of Ca^{+2} salt bridges of tight junction epithelium and thus destabilize zona occludens-1 (ZO-1) and zona adherens [28, 29]. In addition, carbopol also shows an inhibitory effect on brush border proteases due to its higher affinity for bivalent cations Ca^{+2} and Zn^{+2} as compared to the proteolytic enzymes [20]. Besides these factors, a prolonged period of adhesion to the intestinal mucosa due to use of carbopol as

mucoadhesive agent might have also led to the increased systemic bioavailability of FITC-Dextran.

Another probable reason for the enhanced absorption of FITC-Dextran from GMPs as compared to solution form, might be the increase in local drug concentration at the site of absorption. The concentration at the interface between the patches and intestinal membrane is much higher than the one produced between the solution and intestinal membrane [30]. Thus the flux of absorption would have been higher in case of patches resulting in higher absorption of drugs.

The use of chitosan nanoparticles mediated delivery approach was another attempt to increase the permeation of macromolecules from intestinal mucosa. Chitosan has a property of binding tightly to the epithelium and inducing a redistribution of F-actin and tight junction protein, ZO-1 [17]. Researchers have investigated the implication of chitosan nanoparticles in improving the intestinal absorption of insulin [15]. They clearly demonstrated that chitosan nanoparticles increased the intestinal absorption of insulin. The developed formulation was designed using various strategies which eventually resulted in improved oral bioavailability of the model macromolecular therapeutic (FITC-Dextran).

Figure 1. Representation of the obtained data for (a) average particle size and (b) zeta potential of the developed chitosan nanoparticles

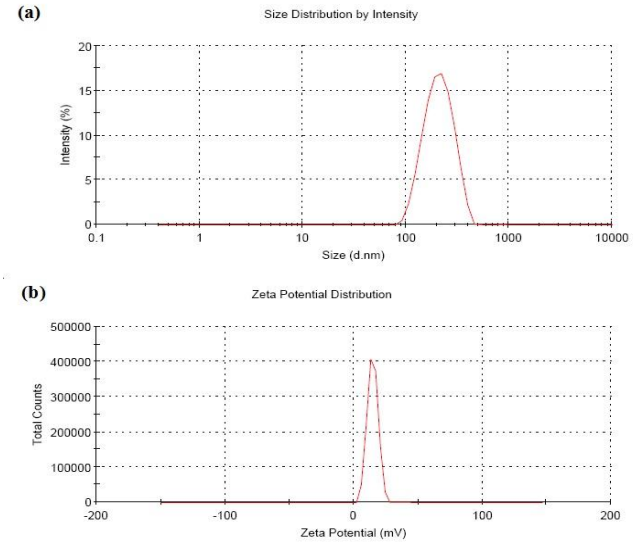

Figure 2. FT-IR spectra of FITC-Dextran, blank patch and FITC-Dextran loaded chitosan nanoparticles incorporated in GMPs

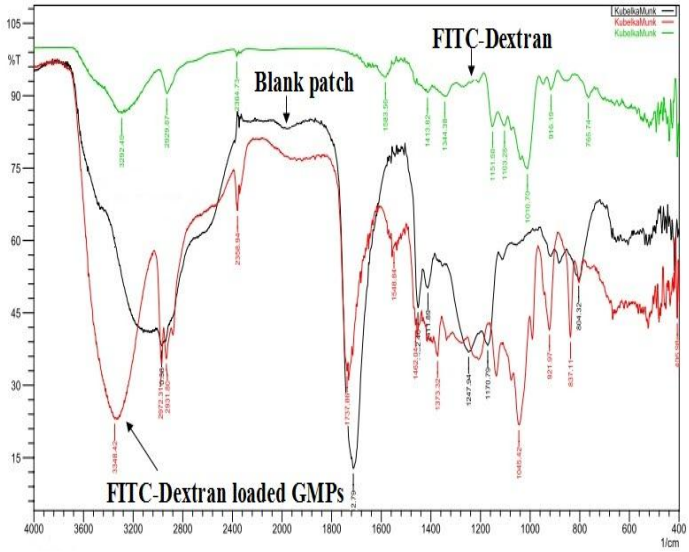

Figure 3. Representation of load *versus* time curve for the mucoadhesion study

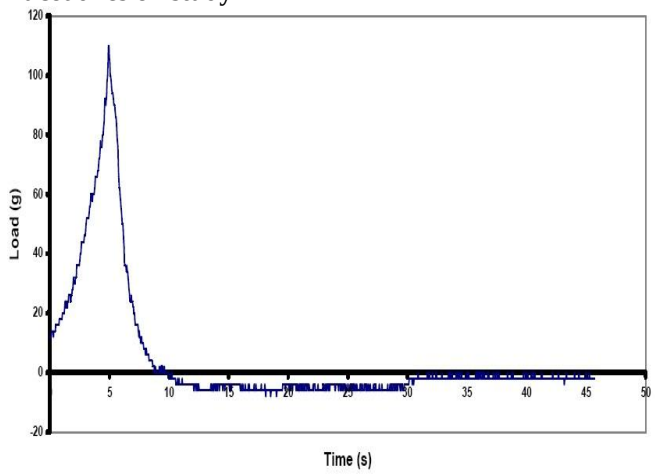

Figure 4. Representation of load *versus* distance curve for tensile strength of GMPs.

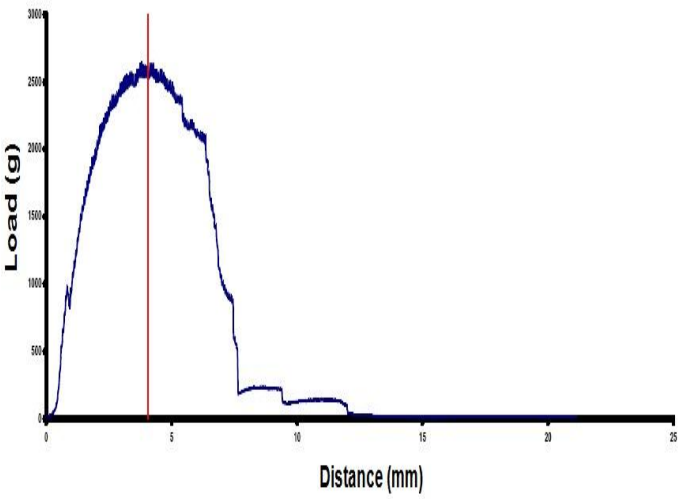

Figure 5. Unidirectional release profile of FITC-Dextran from GMPs containing chitosan nanoparticles, data expressed as mean±SEM (n=6)

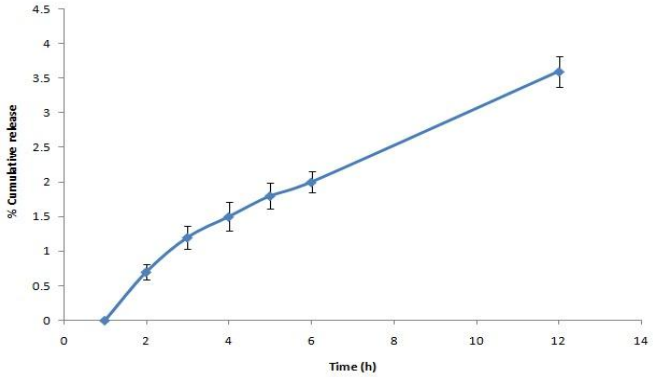

Figure 6. *In vitro* release profile of FITC-Dextran from GMPs containing chitosan nanoparticles, data expressed as mean±SEM (n=6)

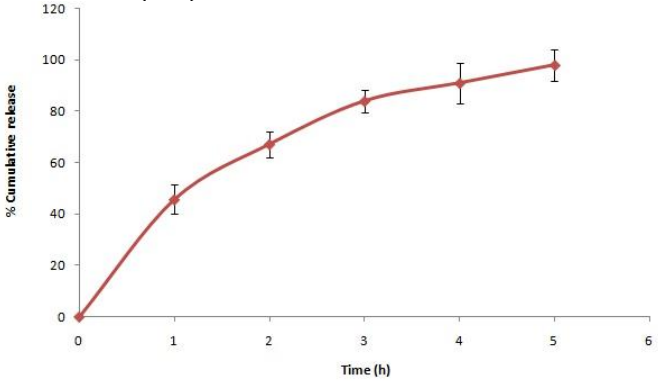

Figure 7. *Ex vivo* permeation study of FITC-Dextran from GMPs, data expressed as mean±SEM (n=6)

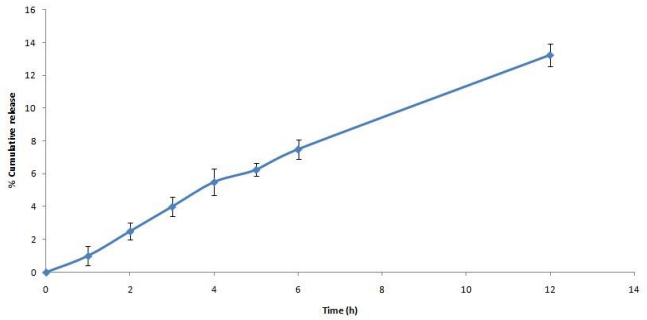

Figure 8. Mean (±SEM) plasma concentration of FITC-Dextran from different formulations after oral administration in rabbits

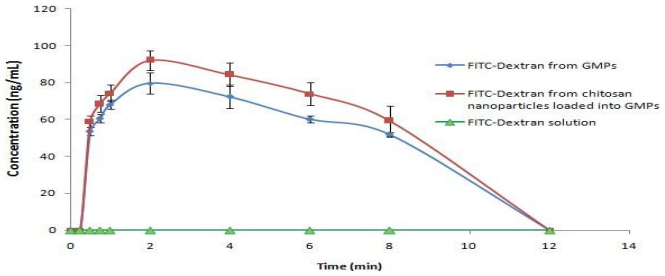

Table 1. Tensile strength data

Parameters	Values
Force at break	2642 ± 197 g
Increase in length at breaking	4.07 ± 0.84 mm
Tensile strength	7.34 ± 1.04 g mm^{-2}
Elongation at break (%)	27.133 ± 2.35%

Data expressed as mean±SEM, n=6

Table 2. Pharmacokinetic parameters of FITC-Dextran from different formulation upon oral administration in rabbits

Parameters	FITC-Dextran solution	FITC-Dextran GMPs	FITC-Dextran nanoparticles + GMPs
AUC_{0-t} (ng h/mL)	Not Detected	507.35 ± 20.25	592.19 ± 28.15*
T_{max} (h)		2.00±0.00	2.00±0.00
C_{max}(ng/mL)		79.65 ± 5.85	92.09 ± 5.23
K_{el} (1/h)		0.0014 ± 0.0003	0.002 ± 0.001
V_d (L)		25.79 ± 1.23	22.69 ± 1.65
$t_{1/2}$ (h)		7.82 ± 0.85	6.58 ± 1.11
Cl_T (L/h)		0.04 ± 0.01	0.03 ± 0.01

Data are expressed as mean±SEM, (n=6); *$p \leq 0.05$ as compared to FITC-Dextran GMPs

CONCLUSION

Oral delivery of drugs is the most preferred route of administration. However, oral administration of macromolecular drugs is a difficult task. The present study aims in developing a suitable intestinal patch delivery system using chitosan nanoparticles for effective delivery of macromolecules using FITC-Dextran as a model drug. The presence of water-insoluble backing layer and chitosan nanoparticle mediated drug delivery approach made the existing formulation different and better from the previously reported GMPs. The superiority of the developed formulation was confirmed from various *in vitro* studies as well as from the pharmacokinetic studies in rabbits. Therefore, FITC-Dextran loaded chitosan nanoparticles incorporated in GMPs might be a promising approach for the oral delivery of macromolecules.

CONFLICT OF INTERESTS

There is no conflict of interests regarding the publication of this paper.

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