# Bioadhesive Hydrophobic Chitosan Microparticles for Oral Delivery of Insulin: *In Vitro* Characterization and *In Vivo* Uptake Studies

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**ABSTRACT:** Hydrophobically modified polymeric matrices for drug delivery were developed by *N*-acylation of chitosan with long($C_{18}$) and medium chain($C_8$) fatty acid chlorides like octanoyl and oleoyl chloride. Chemical modifications of chitosan were confirmed by IR spectra and trinitrobenzenesulphonic acid assay. Modified chitosan particles were prepared by ionotropic gelation with sodium tripolyphosphate. Hydrophobic modification was confirmed by contact angle measurements. Scanning electron micrographs showed the presence of compact microparticles. Swelling studies showed that oleoyl chitosan exhibited low swelling profile than octanoyl chitosan at acidic pH. *In vitro* release profile at pH 7.4 showed that about 90% of insulin was released by 5th hour. ELISA studies proved that the microparticles were capable of maintaining biological activity of insulin. Mucoadhesion studies proved that oleoyl derivative was more mucoadhesive than octanoyl derivative. *In vivo* uptake studies of fluorescent-labeled microparticles on rat intestinal sections showed that oleoyl chitosan microparticles exhibited significant uptake than octanoyl chitosan. These results suggests that oleoyl moiety would resist degradation by the gastric enzymes and will enhance mucoadhesivity through hydrophobic interactions and also the permeability by loosening the tight junctions, thus making it a useful carrier for oral peptide delivery applications. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2902–2910, 2011

**Key words:** hydrophobic chitosan; mucoadhesion; octanoyl chitosan; oleoyl chitosan; oral insulin delivery

## INTRODUCTION

During the past few decades hydrophobically-modified natural polymers are gaining increasing importance for controlled drug delivery applications.[1] Mucosal delivery of insulin is one of the most intensively studied subjects, among which, achieving oral delivery of insulin has been an elusive goal for many investigators. The use of colloidal carriers made of hydrophilic polysaccharides, has arisen as a promising alternative for improving the transport of macromolecules such as peptides, proteins, oligonucleotides, and plasmids across biological surfaces. Modification of natural polymers permits the design of biocompatible polymers with desired characteristics, in particular, controlled hydration or controlled acid or proteolytic degradation, for controlled drug delivery applications.[2]

Chitosan, copolymer of β (1→4) linked glucosamine and *N*-acetyl glucosamine, have drawn considerable attention in the pharmaceutical and biomedical fields in view of its excellent biocompatibility, biodegradability, and reactive surface functional groups for easy modification.[3–5] Moreover, it has a unique feature of adhering to the mucosal surface and transiently opening the tight junctions between epithelial cells.[6] Despite its favorable biological properties, chitosan is very rarely used in oral administration of drugs because of its fast dissolution in the stomach and limited capacity for controlling the release of drugs. To overcome this limitation, various chemical modification of chitosan has been carried out, viz., hydrophobic,[7–9] hydrophilic,[10,11] thiolation, etc.[12,13]

Several modification techniques for imparting hydrophobicity to the chitosan structure, such as phthaloylation,[14,15] alkylation,[16,17] and acylation reactions,[18–21] are available in the open literature. Phthalyl chitosan-poly(ethylene oxide) (PCP) semi-interpenetrating network microparticles, oral insulin-delivery matrices developed by Rekha and Sharma, showed improved release properties compared to chitosan.[22] The authors in another work have also tried to establish the role of hydrophilic/hydrophobic balance on gastrointestinal absorption of peptide

drugs along with its surface charge. Lauryl succinyl chitosan particles developed by them were found to be highly mucoadhesive, which could be due to the hydrophobic interaction of the lauryl groups to the hydrophobic domains of mucosa as well as its negative zeta potential.[23] The hydrophobic moiety is expected to enhance mucoadhesivity through hydrophobic interactions and also the permeability by loosening the tight junctions.[24]

One of the facile approaches that have drawn much attention is the introduction of hydrophobic groups onto chitosan through acylation reaction. Fatty acids have been shown to enhance the permeability of peptide drugs. They act primarily on the phospholipids component of the membrane thereby creating disorder and leading to increased permeability.[25,26] It is established by various groups that sodium salts of medium chain fatty acids such as caprylate ($C_8$), caprate ($C_{10}$), and laurate ($C_{12}$) are able to enhance the paracellular permeability of hydrophilic compounds.[27] N-acylation of chitosan with various fatty acid ($C_6$–$C_{16}$) chlorides increased its hydrophobic character and made important changes in its structural features. Tein et al.[28] described the N-acylation of chitosan with fatty acyl chlorides ($C_8$–$C_{16}$) to introduce hydrophobicity for use as matrix for drug delivery. The best mechanical characteristics and drug release properties were found for palmitoyl chitosan (substitution degree 40–50%) tablets with 20% acetaminophen as a tracer, which appears to be due to hydrophobic interactions between side chains. Therefore the objective of the present work is to develop hydrophobic derivatives of chitosan with medium ($C_8$) and long chain ($C_{18}$) fatty acid chlorides like octanoyl and oleoyl chloride and compare its efficiency for oral insulin delivery. Hydrophobic interactions are believed to enhance the stability of substituted chitosan by reducing the hydration of the matrix thereby resisting the degradation by gastric enzymes.[29] The derivatization was confirmed by TNBS assay, IR spectra, contact angle measurements, and scanning electron microscopy. Mucoadhesion, swelling in vitro release, and uptake studies were carried out to evaluate the efficacy of hydrophobic derivatives for oral insulin delivery.

## EXPERIMENTAL

### Materials

Chitosan from CIFT (Cochin), MW-2,70,000, 85% deacetylated, Octanoyl chloride and Oleoyl chloride (Merck, India), Human Insulin (400 IU mL$^{-1}$) from USV, (Mumbai), Human Insulin ELISA kit from Mercodia (Sweden). All other reagents were of AR grade. Fluorescein isothiocyanate (Sigma Aldrich). Acetone and Methanol were used as such without further purification.
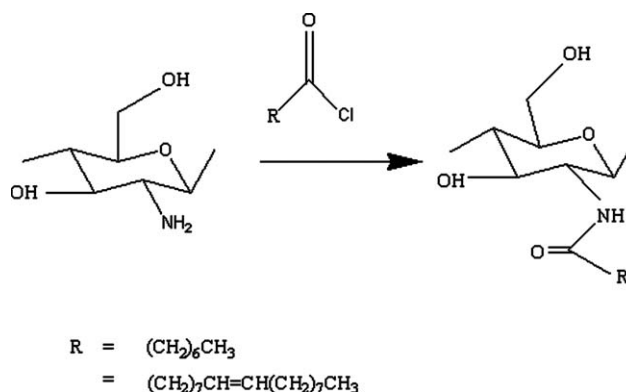


Scheme 1 Acylation of chitosan with octanoyl and oleoyl chloride.

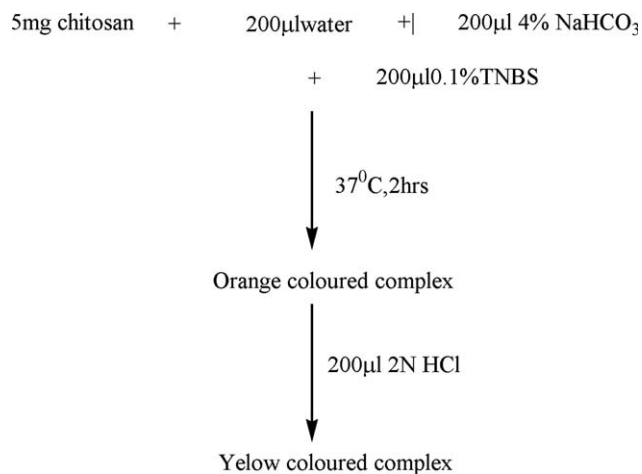### Preparation of octanoyl and oleoyl chitosan

The method for the preparation of acylated chitosan was adopted from elsewhere (Scheme 1).[28] About 1 g of chitosan was dissolved in 1% acetic acid solution and the pH adjusted to 6.5 using 1N NaOH. About 1 mL of octanoyl chloride was added and the reaction mixture diluted with deionized water. The reaction was carried out for 6 h, followed by neutralization with NaOH and precipitation with acetone. The precipitate was washed with methanol at 50–60°C to remove unreacted acid chlorides and was dried with pure acetone at 37°C (COT 1). Similarly, different volumes of octanoyl chloride (2–4 mL) were used to obtain different degrees of substitution (COT2, COT3, and COT4). Oleoyl derivatives were obtained under same conditions substituting with oleoyl chloride (COL). COT1, COT2, COT3, and COT4 represents octanoyl chitosan obtained by using 1, 2, 3, and 4 mL of octanoyl chloride respectively, and COL1, COL2, COL3, and COL4 represents oleoyl chitosan obtained by using 1, 2, 3, and 4 mL of oleoyl chloride respectively.

### Preparation of microparticles

Following the modification, fragments were used for particle formation via ionotropic gelation with sodium tripolyphosphate. Briefly 8 mL 1% TPP solution was added drop wise to 30 mL chitosan solution under magnetic stirring for 30 min. The particles were washed several times with water to remove unreacted tripolyphosphate and dried at 4°C.[30]

### Trinitro benzene sulfonic acid assay

The degree of substitution or the extent of derivatisation of chitosan was determined using TNBS assay (Scheme 2). Briefly, to 5 mg of chitosan solution, 200 μL water, 200 μL 4% NaHCO$_3$, and 200 μL 0.1%

5mg chitosan   +   200μlwater   +|   200μl 4% NaHCO$_3$

+   200μl0.1%TNBS

↓ 37$^0$C,2hrs

Orange coloured complex

↓ 200μl 2N HCl

Yellow coloured complex

**Scheme 2** Trinitrobenzene sulfonic acid assay for the determination of free amino groups of chitosan.

TNBS was added. The solution was incubated for 2 h at 37°C. Following incubation, 200 μL of 2$N$ HCl was added. The absorbance was measured at 344 nm using UV-visible spectrophotometer.[31] Chitosan of same concentration was used as control system and glucosamine was used as standard.

### Infrared spectroscopy

FTIR spectra of chitosan (CS) and modified chitosan derivatives, octanoyl chitosan and oleoyl chitosan were measured in the 4000–400 cm$^{-1}$ region using NICOLET 5700 FTIR spectrophotometer.

### Determination of contact angle

Contact angle was measured to determine the extent of hydrophobicity. The goniometric static drop method was used to evaluate the changes in surface energy. In this process a drop of double distilled water was placed on each test sample by means of a microsyringe. The advancing contact angles were measured by means of an A-100, Rame-Hart, contact angle Goniometer (Mountain Lakes, NJ).

### Scanning electron microscopy

The surface morphology of the particles was studied using scanning electron microscope (HITACHI S-2400). The particles were sputter coated with gold and observed under microscope.

### Swelling studies

Swelling studies of the microparticles were carried out separately at pH 1.2 and pH 7.4. About 25 mg of octanoyl, oleoyl and chitosan microparticles were suspended in buffer solutions of respective pH. At specific time intervals, the samples were removed from the buffer solution, excess water on the surface was removed, and the weight of swollen particles were determined.[32] The degree of hydration of the samples were calculated with the following equation:

$$\text{Degree of swelling} = [(W_s - W_d)/W_d] \times 100\%$$

where $W_s$ is the weight of the swollen particles and $W_d$ is the weight of the dried particles.

### Drug loading and release studies

To evaluate the potential application of the polymer to oral insulin delivery, the insulin incorporation and release properties of microparticles were examined. A known amount of dried microparticles was loaded with insulin (400 IU mL$^{-1}$) by diffusion filling method and dried at 2–4°C.[33] *In vitro* release study of insulin was performed in simulated gastric fluid (SGF, pH 1.2—Place 50 mL 0.2 M KCl in a 200 mL standard flask. To this solution add 85 mL–0.2M HCl and make it up to 200 mL with distilled water) for the first 2 h and then in simulated intestinal fluid (SIF pH 7.4 Place 50 mL of 0.2 M monobasic potassium phosphate in a standard flask. To this solution add 39.1 mL 0.2 M NaOH and make it up to 200 mL with distilled water), respectively, as per U.S. Pharmacopoeia (USP) under ambient conditions. The insulin-loaded particles were suspended in pH 1.2 buffer (SGF) for the first 2 h and then at pH 7.4 (SIF). The samples were aliquoted at 1 h interval for 6 h and the respective buffer was replaced with the same amount of fresh buffer. The released insulin was estimated by means of Lowry method. The amount of insulin in the test solution was calculated from the insulin standard maintained during the assay.[34] The biological activity of the loaded insulin was analyzed by means of enzyme-linked immunosorbent assay (ELISA) as per standard protocol. The percentage of drug loading was calculated as follows % of drug loading = $C_i - C_f/C_i \times 100$ where $C_i$ and $C_f$ are the initial amount of insulin loaded and insulin content in the supernatant solution, respectively.

### Mucoadhesion studies

Mucoadhesion studies were carried out on freshly excised rat intestinal tissue. The intestinal tissue of about 5 cm was flushed with normal saline to remove the luminal contents and cut open longitudinally. Then the tissue was mounted and fixed on a semi cylindrical polypropylene support and washed with saline to remove free mucin. A known amount of particles was spread on the tissue and kept in humid conditions for 5 min. It was then washed with phosphate buffer saline at a rate of 1 mL min$^{-1}$ for 20 min. The dislodged particles were collected and dried. The percentage of particles adhered were
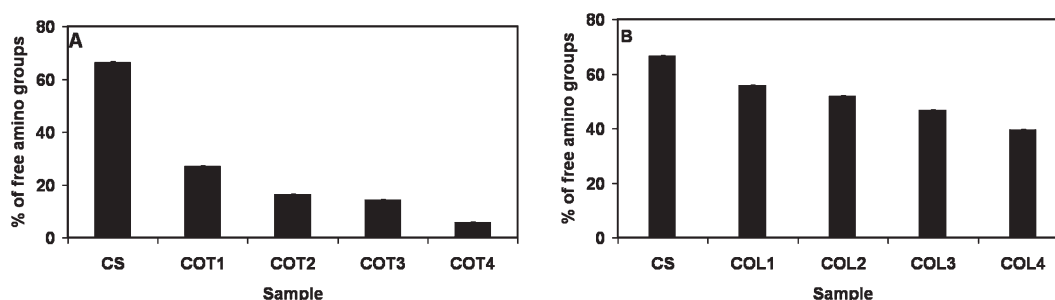
**Figure 1** Degree of substitution of amino groups, of chitosan by (A) Octanoyl chloride and (B) Oleoyl chloride. COT1 (59%), COT2 (75%), COT3 (78%), COT4 (91%) represents octanoyl chitosan obtained by using 1, 2, 3, and 4 mL of octanoyl chloride, respectively. COL1 (16%), COL2 (22%), COL3 (29%), and COL4 (40%) represents oleoyl chitosan obtained by using 1, 2, 3, and 4 mL of oleoyl chloride, respectively. The degree of alkylation is given in brackets.

calculated by comparing the weight of particles adhered to weight of particles applied.[35]

## Uptake studies

The animal experiments were done as per the requirements of the Animal Ethics Committee of this Institute. Animals were housed in rooms at controlled temperature and relative humidity. Uptake studies were carried out on streptozotocin induced diabetic rats. The rats were fasted for 20 h and FITC-insulin and FITC-insulin loaded microparticles were given orally to fasting adult male Wistar rats (weight range 180–200 g). The rats were sacrificed after 3 h and the intestine was isolated and flushed with normal saline to remove the free mucosa. The isolated tissue was frozen in liquid nitrogen in the presence of isopentane. The frozen tissues were mounted using Jung tissue freezing medium (Leica Instruments) and were sectioned into 10-μm thick specimens using Leica 3050 microtome. The sections were mounted on 0.1% poly lysine-coated slides and were examined under a fluorescent microscope.[23]

## RESULTS AND DISCUSSION

Acylation of chitosan was carried out at pH 6.5. The higher derivative of chitosan especially oleoyl chitosan was not soluble in any of the common organic solvents. This poses a problem in particle preparation. So COT2 and COL 2 microparticle were used throughout the studies.

## Determination of degree of substitution (TNBS assay)

Trinitrobenzenesulphonic acid reacts with amino functional group to give an orange colored complex. The extent of derivatisation was evaluated by determining the remaining underivatised primary amino groups in chitosan. The free amino groups in chitosan is taken as hundred percent and the percentage of free amino groups in the derivatives was calculated with respect to this. TNBS assay results showed a significant reduction in the percentage of free amino groups, increasing with extent of derivatization. Octanoyl derivative was found to be more highly substituted than oleoyl (Fig. 1). This is attributed to the presence of long alkyl chain length (steric hindrance) and high molar mass of oleoyl chloride. The degree of alkylation of chitosan is represented in brackets. COT1 (59%), COT2 (75%), COT3 (78%), COT4 (91%) represents octanoyl chitosan obtained by using 1, 2, 3, and 4 mL of octanoyl chloride, respectively. COL1 (16%), COL2 (22%), COL3 (29%), COL4 (40%) represents oleoyl chitosan obtained by using 1, 2, 3, and 4 mL of oleoyl chloride, respectively.

## IR spectroscopy

A comparison of IR spectra of native chitosan and modified chitosan, octanoyl chitosan (COT 2), and oleoyl chitosan (COL 2) are shown in Figure 2. Acylation is characterized by the formation of an amide bond between carboxylic and amino group of chitosan. There was a considerable shift (decrease) in the absorption peaks of OH and $NH_2$ groups of native chitosan ($3421$ cm$^{-1}$) as compared to modified chitosan ($3277$ cm$^{-1}$). The peaks at 1655 and 1547 cm$^{-1}$ (amide II band) confirms the presence of both amide and amino group in the modified chitosan and the peak at 2920 cm$^{-1}$ indicates the presence of alkyl group. Thus infrared spectra confirmed the modification of amino groups of chitosan.

## Contact angle

Contact angle measurements confirmed the hydrophobic nature of modified chitosan (in the range of 90°–112°) compared to native chitosan (88.6°) (Fig. 3). As the acyl chain length increased contact angle also increased. There was a significant increase
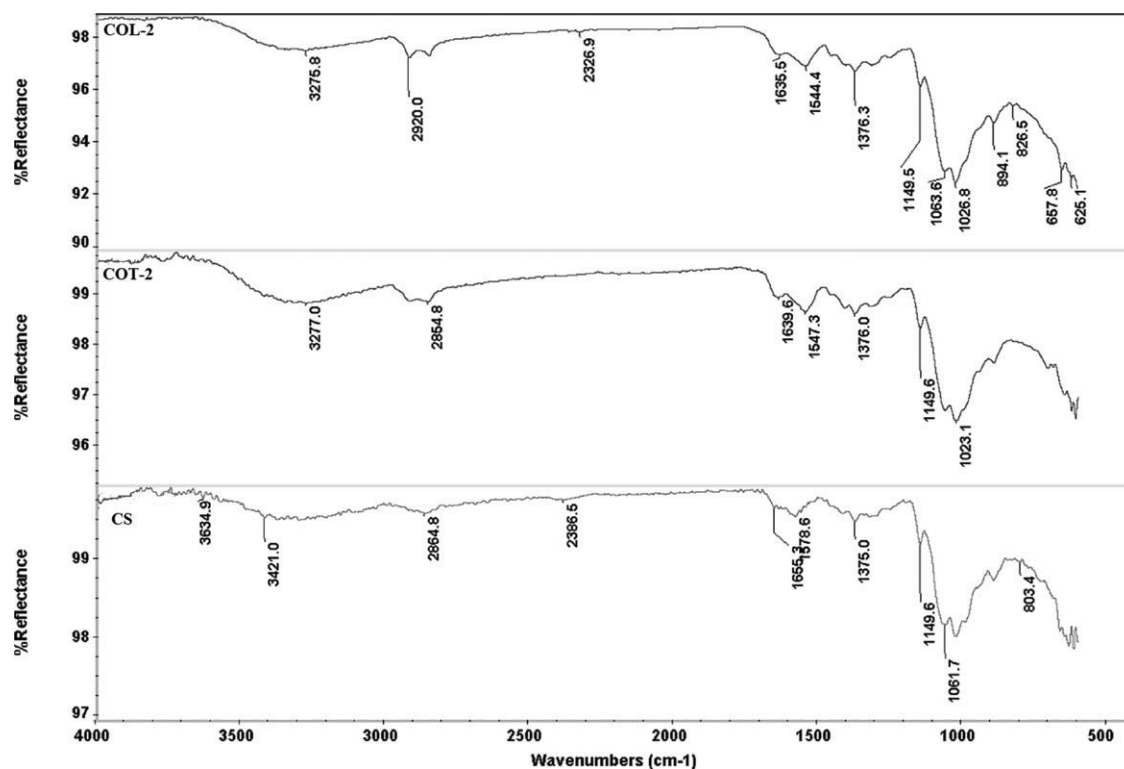
**Figure 2** IR spectra of chitosan, octanoyl (COT) and oleoyl chitosan (COL).

in contact angle values with increase in hydrophobic nature of chitosan especially octanoyl chitosan. The higher derivative of chitosan especially oleoyl chitosan was not soluble in any of the common organic solvents. This poses a problem in film formation and hence the determination of contact angle becomes difficult.

### Scanning electron micrograph

Scanning electron micrographs of these microparticles (particle size of about 10 μm) indicate that they are irregularly shaped. Oleoyl derivative was found to be more compact than octanoyl derivative (Fig. 4).

### Swelling studies

Chitosan microparticles exhibited significant swelling due to the protonation of free amino groups at acidic pH. Octanoyl and oleoyl chitosan microparticles showed comparatively low swelling ratio compared to native chitosan microparticles due to the decrease in free amino groups (data not shown). The swelling percentage of hydrophobic chitosan microparticles decreased with increase in alkylchain length. This may be due to the addition of hydrophobic group onto hydrophilic amino group and also due to steric repulsion of attached acyl groups. Swelling is very high at pH 1.2 than at pH 7.4. The

inherent hydrophobicity of chitosan microparticles at pH 7.4 reverted faster swelling at neutral or alkaline conditions. The particles showed almost similar swelling profile at pH 7.4.

### Release studies

Biological activity of loaded insulin was estimated using ELISA technique. ELISA measures biologically
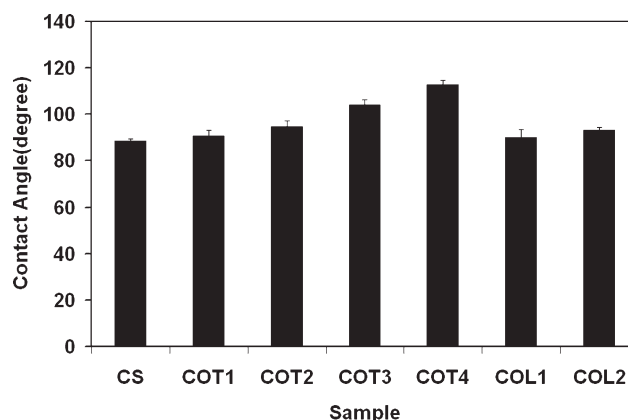


**Figure 3** Contact angle data of octanoyl and oleoyl chitosan. COT1, COT2, COT3, COT4 represents octanoyl chitosan obtained by using 1, 2, 3, and 4 mL of octanoyl chloride respectively, and COL1, COL2, COL3, COT4 represents oleoyl chitosan obtained by using 1, 2, 3, and 4 mL of oleoyl chloride, respectively.
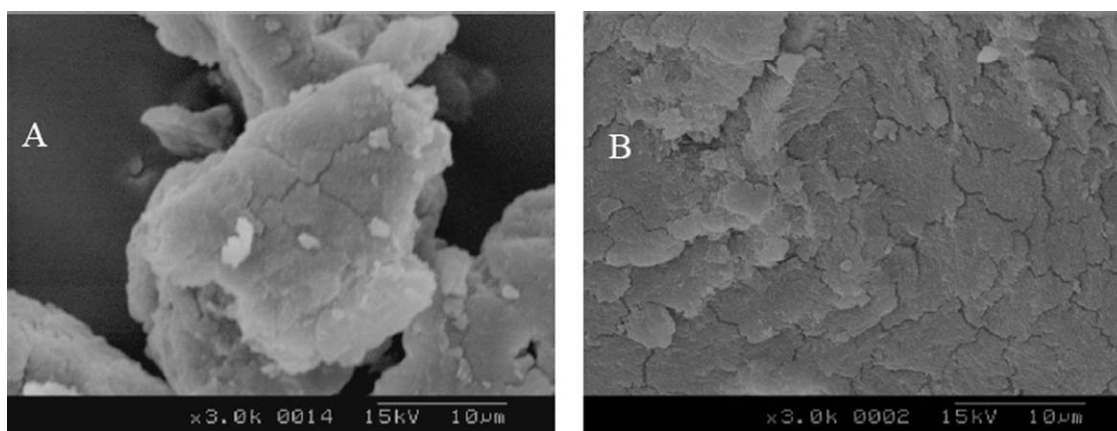
**Figure 4** Scanning electron micrographs of (a) octanoyl and (b) oleoyl chitosan.

active insulin with a high degree of specificity, using a pair of mouse monoclonal antibodies.[36] ELISA studies shows that the concentration of insulin in chitosan, octanoyl chitosan (COT 2), and oleoyl chitosan (COL 2) microparticles was found to be 32, 5.971, and 8.4 IU/100 mg. Full biological activity of proteins is dependent on preserving the integrity of its three-dimensional structure. Compared to lowry-protein analysis, ELISA results suggest that hydrophobic chitosan microparticles are capable of preserving biological activity of encapsulated-insulin.

Even though significant amount of drug is physically adsorbed onto the surface of the particles, the compact nature of the microparticles allows the entrapment of insulin by some hydrophilic–hydrophilic interaction. In line with TNBS Assay, swelling-studies and contact angle measurements it is clear that the particles retained some hydrophilic nature. Because of its partly hydrophilic nature some amount of insulin are diffused into the microparticles. Diffusion of insulin can occur through the unhydrated polymer matrix but will generally be facilitated as the polymer gradually swells in contact with the body fluids. As the alkyl chain lengthincreases, swelling ability decreases which in turn increases the ability of the particles to hold the drug intact. *In vitro* insulin release studies of these microparticles were carried out in SGF for the first 2 h and then at SIF. Release profile of CS (chitosan), COT 2 and COL 2 are shown in Figure 5.

According to Peppas[37], there are three primary mechanisms by which the release of active agents can be controlled: erosion, diffusion, and swelling followed by diffusion. Erosion may take place via hydration or hydrolysis of the bulk, the polymer being slowly degraded starting at the periphery of the matrix. For oral peptide delivery systems minimal release in the gastric pH is appropriate as it may save the loaded insulin and increase the bioavailability compared to that of a matrix which does

not exhibit this property. Tein et al.[28] has reported that release profile of chitosan with shorter alkyl chain length substitution showed burst release as compared to chitosan with longer alkyl chain length. It is suggested that the release of drug is controlled by diffusion, or by swelling followed by diffusion, depending on both the acyl chain length and the degree of acylation. In the case of native chitosan at pH 1.2 the free $NH_2$ will be completely protonated, resulting in matrix swelling due to hydration and intermolecular repulsion and that is why chitosan release about 62.5% insulin in the first hour at gastric pH. This limits its use as drug delivery matrix. But in the case of oleoyl chitosan only 22.5% was released in the first hour and in the case of octanoyl chitosan 48.1% was released. As expected oleoyl chitosan showed a slow release profile as compared to octanoyl chitosan. This may be probably due to decreased water entrance (no swelling) and unfavorable diffusion of drug and the release profile
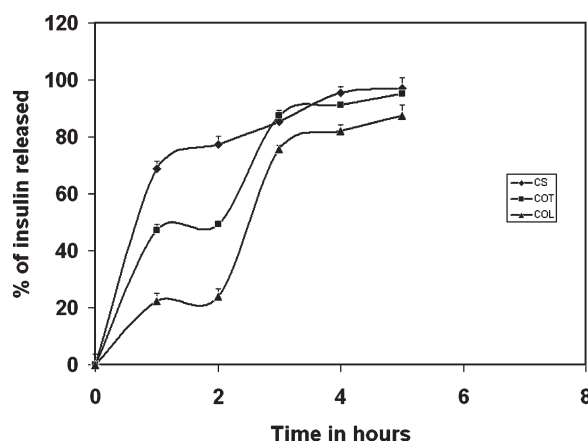


**Figure 5** Release profiles of native chitosan, octanoyl, and oleoyl chitosan at pH 1.2 (SGF) for the first 2 h and then at pH 7.4 (SIF).
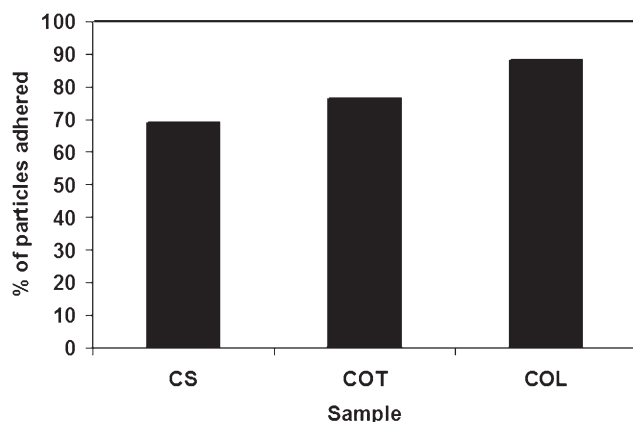
**Figure 6** Mucoadhesion profile of chitosan, octanoyl (COT), and oleoyl chitosan (COL).

may be based on diffusion alone. Octanoyl chitosan showed a burst release in the first hour followed by a sustained release. This is because most of the insulin molecules are adsorbed on the surface of chitosan microparticles and the release mechanism may be controlled by swelling followed by diffusion. At pH 7.4, about 82.1% insulin is released from oleoyl chitosan microparticles and about 90% insulin was released from octanoyl chitosan microparticles in the fifth hour. This establishes that the control of drug release was improved by hydrophobic stabilization of matrices and the substitution degree, which is in agreement with previous reports by Noble et al.[38] and Martin et al.[39] The hydrophobic chitosan microparticles, especially oleoyl chitosan exhibited a slow release profile in gastric pH, minimized the loss, and increased the bioavailability of insulin.

### Mucoadhesion studies

As per the interpenetration theory of mucoadhesion, hydrated polymeric chains diffuse into the intestinal mucus forming strong bonds via molecular interactions. This interaction is composed of attractive interactions like Van derWaal forces, hydrogen bonding, electrostatic attraction, and hydrophobic interaction.[40] This leads to intimate contact which could enhance the permeability of macromolecular drugs either by transcytosis or by paracellular pathway. The mucosal surfaces are coated with mucin, a high molecular weight, hydrated glycoprotein. Mucins have hydrophobic groups such as the naked blocks of the protein backbone chains.[41,42] The interaction of mucins to hydrophobic surfaces has been explored by various groups and it is reported[43] that the mucin shows strong affinity to hydrophobic surfaces. They enhance the residence time of the formulation in contact with the mucosal surfaces which increases the bioavailability of drug at the

desired site. Oleoyl chitosan was found to be more mucoadhesive than octanoyl chitosan (Fig. 6). This is attributed to the presence of some hydrophobic interaction with long alkyl chain of fatty acids and hydrophobic components of mucus glycoprotein Hydrophobic interactions differ from other interactions such as electrostatic ones, hydrogen or Vander Waals bonds by the fact they are not directly due to cohesive interactions between molecules but more to the specific structure of water molecules close to the polymer chains.[44] Adhesion of the matrices into the mucosa can lead to the release of insulin into the mucosal surface which can reduce the proteolytic degradation. No covalent bonds were observed, but aggregates constituted from the hydrophobic moieties which act as crosslinks of a physical network. The driving forces for such associations are thought to be entropic in nature, stemming from the desire to minimize the disruption of the water molecules surrounding the hydrophobic sites. Fatty acids are known to primarily act on the phospholipid component of the membrane thereby creating disorder by opening the tight junctions, resulting in increasing permeability.[45]

### Uptake studies

Three possible mechanisms can be suggested for intestinal uptake of insulin nano/micro particles and/or insulin released from nano/microparticles: (i) uptake via a paracellular pathway, (ii) transcytosis or receptor-mediated transcytosis and transport via the epithelial cells of the intestinal mucosa, (iii) lymphatic uptake via the M cells of the Peyer's patches mostly abundant in the ileum.[46] Figure 7 shows the fluorescent microscopic images of derivatised chitosan microparticles in rat intestinal sections, duodenum, jejunum, and ileum. The FITC-labeled insulin microparticles can be seen in the form of green dots localized in the intracellular spaces of intestinal sections, agreeing well with the third hypothesis of absorption mechanisms. The chitosan microparticles described in this study is less than 10 μm (as determined from SEM) and they are taken up by M cells and transported to the dome of Peyer's patches. Peyer's patches are the predominant sites for particle uptake and have 2–200 folds higher uptake of particles than the nonpatch tissue.[47] After taken up by M cells in Peyer's patches, the microparticles slowly degrade *in vivo* and release entrapped antigens. Therefore, microparticles have considerable potential as a controlled release antigen delivery system for the induction of long-term immune responses at mucosal surfaces. Importantly, insulin has been reported to be absorbed into the rat ileal epithelium in the presence of permeation enhancers and protease inhibitors. It has been
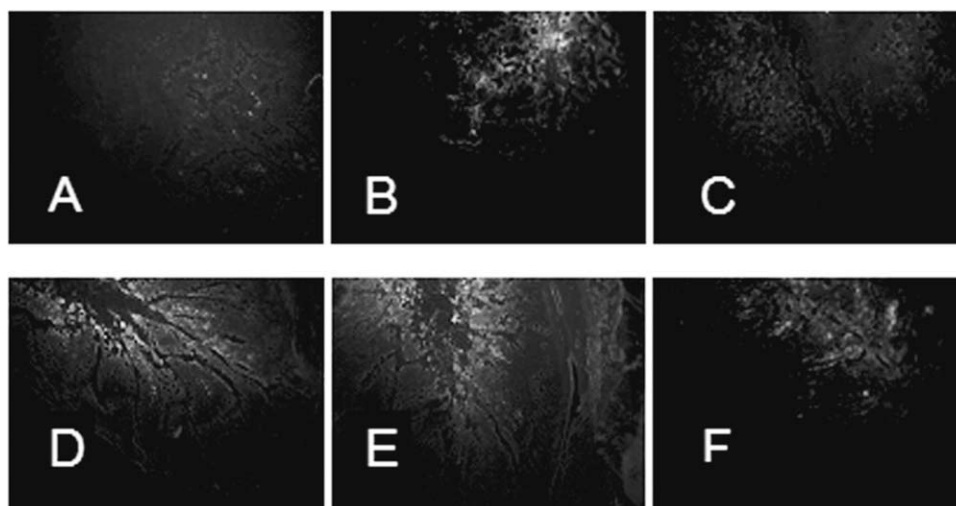
**Figure 7** Fluorescent microscopic images of FITC-insulin-loaded microparticle of octanoyl and oleoyl chitosan. Images A and D represents duodenum, B and E represents jejunum and C and F represents ileum of rat intestinal sections of FITC-insulin-loaded octanoyl and oleoyl chitosan microparticles, respectively.

demonstrated that cationic polymers such as chitosan are able to reversibly open the tight junctions between enterocytes allowing the transport of macromolecular drugs.[48]

Fatty acids are known to have absorption enhancing capacity.[49] Substitution of amino groups of chitosan by fatty acids increases the absorption enhancing property. Studies have shown that one of the principal factors determining the efficiency of interaction of particles with M cells is the hydrophobicity.[50] The particles of oleoyl chitosan exhibited significant uptake than octanoyl chitosan. This may be due to the steric association of longer alkyl chain and hydrophobic interaction that leads to intimate contact with the mucosal epithelium which could enhance the permeability of macromolecular drugs either by transcytosis or by paracellular pathway.[51,52] In vivo studies proved that the oleoyl chitosan microparticles can be targeted to the Peyer's patches. Since uptake by M-cells is considered as the first step in oral delivery, our results suggest that these hydrophobic chitosan microparticles are promising as an efficient oral delivery system.

## CONCLUSIONS

Hydrophobic modification of chitosan was successfully carried out which was confirmed by IR spectra and TNBS assay. The contact angle of modified chitosan was higher than that of unmodified chitosan, indicating that aliphatic acyl groups are present on the particle surface to a certain extent. SEM micrographs showed the presence of irregularly shaped microparticles. Swelling ability decreased with increase in alkyl chain length. Hydrophobic

oleoyl chitosan microparticles suppressed insulin release and promoted sustained release in pH 7.4 phosphate buffer and are shown to protect insulin from enzymatic degradation in vitro. These microparticles were found to retain the biological activity of insulin in presence of hydrophobic components. Mucoadhesion studies confirmed that the hydrophobic groups of mucins can strongly interact with the hydrophobic moieties of modified chitosan. With increasein alkyl chain length, mucoadhesive nature and uptake was found to increase due to the steric association of hydrophobic moiety of chitosan and mucus glycoprotein component. These results indicate than oleoyl moiety, could resist degradation by the gastric enzymes and could enhance mucoadhesivity through hydrophobic interactions and the through hydrophobic interactions and also the permeability by loosening the tight junctions thus making it a useful carrier for oral peptide delivery.

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