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Synthesis and evaluation of the mucoadhesivity of a CD-chitosan derivative

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Abstract

Combining mucoadhesive characteristics of a biodegradable polymer such as chitosan with the potential to enhance drug release by increasing the solubility of poorly water-soluble drugs has great potential for pharmaceutical technology and drug delivery design. Polymeric delivery systems have been extensively researched in an attempt to achieve modified drug release. Cyclodextrins (CD) offer an alternative approach. These cyclic oligosaccharides have the ability to form non-covalent complexes with a number of drugs altering their physicochemical properties. In the continuing challenge to improve the properties of delivery systems, this paper focuses on the modification of chitosan by introducing β-cyclodextrin and to test the mucoadhesive strength and inclusion properties of this synthesised cyclodextrin-polymer. β-Cyclodextrin was successfully grafted onto a chitosan chain polymer with a cyclodextrin grafting yield of 7% and a CD-chitosan yield of 85%. Although the complexation of (+)-catechin by the grafted β-CD was found to be about five times weaker than that by the β-CD monoaldehyde and natural β-CD, the inclusion properties of the chitosan-CD remain promising. The mucoadhesive properties of chitosan-CD were compared to that of pectin (reference) and the parent chitosan with the use of a tensile separation test. The chitosan-CD showed mucoadhesive strengths of 12% stronger than pectin, but 13.5% weaker than the parent chitosan. The synthesised chitosan-CD-polymer exhibits characteristics of a possible mucoadhesive drug delivery system with some inclusion properties from β-cyclodextrin.

Keywords: Chitosan; Drug delivery; Bioabsorption; Mucosal adhesion

1. Introduction

Bioadhesion, which is classically defined as the ability of a material to adhere to a biological substrate, was presented for the first time as a pharmacotechnical tool nearly 20 years ago (Ch'ng et al., 1985; Gurny et al., 1984; Nagai and Machida, 1985; Park and Robinson, 1984). During this period, bioadhesive polymers have received considerable attention for controlled drug delivery (Gu et al., 1988; Juninger, 1990; Lenaerts and Gurny, 1989; Smart et al., 1984). The main reason for the interest is that bioadhesive polymers may fulfill the following desirable features of a controlled drug delivery system:

- prolonged residence time at the site of drug absorption, e.g., by controlling gastro-intestinal transit;
- increased contact to the biological substrate, an absorbing mucosa, resulting in a steep concentration gradient to favor drug absorption;
- localisation in specified regions to improve and enhance the bioavailability of the drug (e.g., targeting to the colon).

The only problem when preparing a bioadhesive delivery system is the choice of the right bioadhesive polymer which will be added to the normal formulation.

Chitosan is a mucoadhesive polycationic polymer at acidic pH values with numerous applications in the food, agricultural and cosmetic industries (Kotzé et al., 1999; Muzzareli, 1973), while cyclodextrins (CDs) are a group of cyclic oligosaccharides with a hydrophilic exterior and a hydrophobic internal cavity (Bibby et al., 2000) (Fig. 1). The following main reasons

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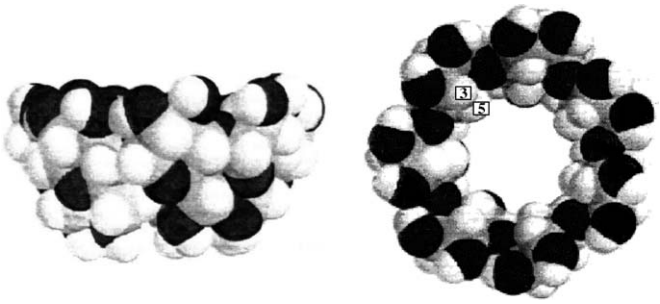

Fig. 1. A side view of the torus of the β-cyclodextrin molecule (left) and the β-cyclodextrin molecule, with the proton 3 and proton 5 hydrogen atoms in the cavity of the torus, as seen from the secondary hydroxyl side (right) (Bibby et al., 2000).

contributed to the grafting of CD molecules onto chitosan as a possible drug delivery system:

- polymer drug carriers allow a controlled release of a bioactive substance over a long period of time;
- both cyclodextrin and chitosan are biodegradable biopolymers;
- cyclodextrins are water-soluble cyclic oligosaccharides which can include various guest molecules into their hydrophobic cavity, allowing the solubilisation, stabilisation and transport of hydrophobic drugs;
- the existence of primary amine groups on the C-2 position of chitosan allows specific modifications on the repeat unit;
- it may lead to a molecular carrier exhibiting promising properties owing to the cumulative effects of transport properties of cyclodextrins and mucoadhesive properties of the polymeric matrix.

In terms of cyclodextrin inclusion complexes, the introduction of a guest molecule in the cavity classically takes place from the wider secondary hydroxyl groups side although the other situation may also be encountered, depending on the guest. It has been shown that steric hindrance effects due to substitution of cyclodextrin could result in an important decrease of the association constant of complexes (Lainé et al., 1995). In view of these data, the coupling strategy consisted in the monofunctionalisation of β-cyclodextrin on the primary face in order to specifically attach it on chitosan from the side which is "less involved" in the inclusion of guests.

In the continuing challenge to improve the properties of delivery systems, our aim of the current investigation was to modify chitosan by introducing β-CD and to investigate the mucoadhesive properties of this synthesised CD-polymer.

2. Experimental

2.1. Materials

Seacure 244 chitosan of medium molecular weight (M_v = 84,150 g/mol) from Pronova, Norway (lot 010-370-02R) was used. The degree of acetylation (DA) for the chitosan was 3% as provided on the analytical certificate from the supplier. The chitosan was not purified and used as provided by the supplier. β-Cyclodextrin monoaldehyde (CD), prepared from β-cyclodextrin (Roquette, France) according to a literature method (Cornwell et al., 1995), was a generous gift from CERMAV, France. Pectin, from apple USP, was purchased from Sigma (Steinheim, Germany). Other chemicals used were of analytical grade and was supplied by Sigma, Aldrich and Fluka. All water used was distilled and deionised.

2.2. Methods

2.2.1. Synthesis and characterisation of β-cyclodextrin-chitosan

Cyclodextrin-chitosan was synthesised as described previously (Auzély-Velty and Rinaudo, 2001). β-Cyclodextrin monoaldehyde (Cornwell et al., 1995) was coupled to chitosan by a reductive amination reaction. Chitosan dissolved in aqueous acetic acid was reacted with β-cyclodextrin monoaldehyde (0.11 equivalent per monomer unit) in the presence of sodium cyanoborohydride to provide CD-chitosan with a grafting yield of cyclodextrin of approximately 7%. The cyclodextrin-chitosan was precipitated with aqueous sodium hydroxide. The white precipitate was successively washed with water, 1:1 water–ethanol, and ethanol and dried to give β-cyclodextrin-chitosan with a yield of approximately 85% based on the original weight of chitosan included in the synthesis. The chemical integrity and purity of the synthesised β-cyclodextrin-chitosan derivative were checked by high resolution ^{1}H NMR.

2.2.2. Viscosity measurements

All polymers increase the viscosity of the solvent in which they are dissolved. Several important viscosity functions are used in viscosity studies. The relative viscosity, $\eta_r = \eta/\eta_0$, is the dimensionless ratio of solution viscosity, η, to solvent viscosity, η_0. The specific viscosity, $\eta_{sp} = (\eta - \eta_0)/\eta_0$, is related to the fluid viscosity increase due to all polymer solute molecules. The reduced viscosity, $\eta_{red} = \eta_{sp}/c$, is the fluid viscosity increase per unit of polymer solute concentration, c, expressed in g/mL. The intrinsic viscosity, $[\eta]$ (in mL/g), is the limit of the reduced viscosity as the polymer solute concentration approaches zero following the Huggins relationship:

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \quad (1)$$

The solvent used for viscosity experiments consisted of acetic acid (0.3 M) and sodium acetate (0.2 M) filtered through a Sartorius (Goettingen, Germany) cellulose nitrate membrane (pore size: 0.2 μm). All experiments were done on an Ubbelohde capillary viscometer (Schott, Germany) attached to a Schott AVS 360 digital counter (Schott, Germany) at 25 °C. The capillary of the viscometer had a diameter of 0.63 mm. The value of the intrinsic viscosity for chitosan allows to obtain the viscometric molecular weight (M_v) (Rinaudo et al., 1993). The concentrations were corrected for humidity content by thermogravimetric analysis (TGA) performed on a TGA 92 (Setaram, France) with the temperature stabilised at 130 °C.

2.2.3. Calorimetric titration

Isothermal titration microcalorimetry (ITC) was performed using a Model 4200 Microcalorimeter from Calorimetry Sciences Corporation (Utah, USA). In individual titrations, injections of 10 μL of (+)-catechin were added from the computer-controlled 250 μL microsyringe at an interval of 5 min into the natural β-CD, β-CD monoaldehyde or CD-chitosan solution (cell volume = 1.3 mL) containing the same solvent as (+)-catechin (pure water or 0.3 mol/L CH_3COOH/0.03 mol/L CH_3COONa), while stirring at 297 rpm at 25 °C. Identical injections of (+)-catechin into a cell containing only water produced heat signals corresponding to heat of dilution. The raw experimental data were presented as the amount of heat produced per second following each injection of (+)-catechin as a function of time. The amount of heat produced per injection was calculated by integration of the area under individual peaks by the instrument software, after taking into account heat of dilution. The experimental data were fitted to a theoretical titration curve using the instrument software, with ΔH^0 (the enthalpy change in kJ/mol), K_a (the association constant in L/mol) and n (complex stoichiometry) as adjustable parameters.

2.2.4. Mucoadhesive evaluation

The aluminium plates were prepared by adding 0.5 g of the polymer solution (1%, w/v, solution) on each plate to produce 0.05 g of polymer film after drying. The mucus (Sigma, UK, partially purified porcine gastric mucin type III) was prepared by adding 1.5 g to 5 mL (30%, w/v, solution) of distilled water. This solution was stirred until the consistency of the mucus was uniform, placed in a waterbath at 25 °C and left to reach the appropriate temperature. The experimental set-up for the tensile testing procedure is shown in Fig. 2. The aluminium plate was suspended from the microbalance (Hugo Sachs Elektronik, Force Transducer F30 Type 372, Germany) using a fine metallic thread that was free from elasticity. The plate was lowered until contact with the mucus was achieved and the tension between the plate and the microbalance declined, ensuring a 2 g downwards pressure on the mucus. The plate was left in this position for hydration of the polymer to occur after which it was again lifted at a rate of 0.25 mm/s.

The separation was registered by software (Chart for Windows v3.4, Powerlab system, UK) and the maximum detachment force was noted. The detection system was calibrated using standard calibration weights (Hugo Sachs Elektronik, 1 g). According to previous mucoadhesive studies performed, the optimal time of contact was determined at 120 s (Snyman et al., 2003). The experiments were done at 120 s hydration time and each experiment was done in six-fold. The basis for construction of the apparatus was vibration free and the waterbath was able to keep the temperature electronically at a constant 25 °C with regular temperature monitoring during experimental procedures.

3. Results and discussion

3.1. NMR characterisation

The DA value obtained from the supplier for chitosan was confirmed using ^{1}H NMR, considered to be the most sensitive method (Rinaudo et al., 1992). The degree of acetylation for chitosan raw material could be calculated from digital integration of the NMR signals arising from the anomeric protons (H-1) of the glucosamine and *N*-acetyl glucosamine units on the one hand, and from the integral of the –CH_3 signal at 1.98 ppm compared with the integral of the H-1 proton signals considered as an internal standard on the other (Fig. 3). The average of these separate calculations gave a reliable value for the DA of Seacure chitosan as 3% and correlates well with the DA obtained from the supplier. β-Cyclodextrin monoaldehyde was coupled to chitosan (0.11 equivalent per monomer unit) by a reductive amination reaction as described previously (Auzély-Velty and Rinaudo, 2001). The degree of substitution for β-cyclodextrin-chitosan could be calculated as approximately 7% from digital integration of the NMR signals arising from the anomeric protons of chitosan and cyclodextrin as shown in Fig. 4. From the degree of CD substitution on chitosan, the yield of the grafting reaction was then calculated as approximately 85%.

^{1}H NMR experiments were performed using a Bruker DRX400 spectrometer operating at 400 MHz. ^{1}D NMR spectra were collected using 16 K data points. Chemical shifts are given relative to external tetramethylsilane (TMS = 0 ppm) and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. Deuterium oxide was obtained from SDS (Vitry, France). Details concerning experimental conditions are given in the figure captions.

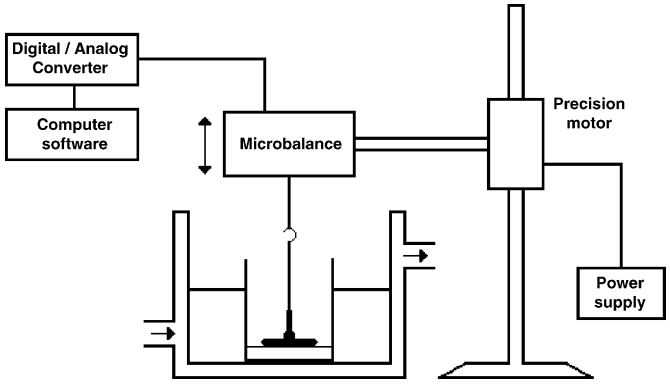

Fig. 2. The experimental set-up for the tensile testing procedure.

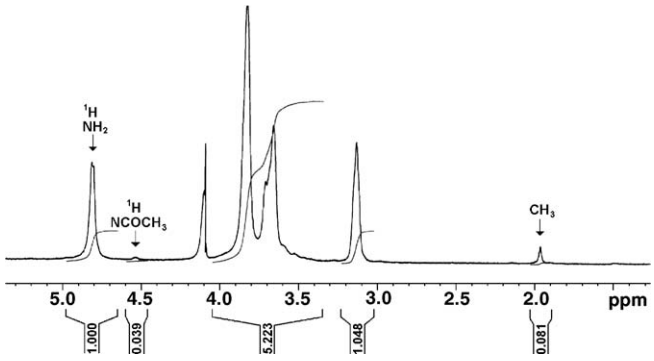

Fig. 3. ^{1}H NMR spectrum (400 MHz, 80 °C, 6 mg/mL in D_2O/DCl, p*D* 3.5) of Seacure chitosan raw material.

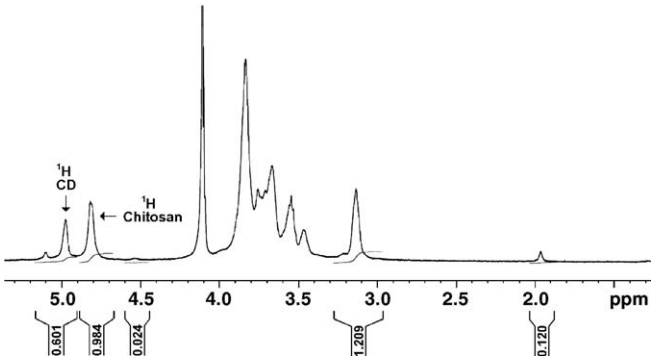

Fig. 4. ^{1}H NMR spectrum (400 MHz, 80 °C, 6 mg/mL in D_2O/DCl, p*D* 3.5) of synthesised chitosan-β-cyclodextrin derivative.

The NMR analysis for the Seacure 244 chitosan raw material and the synthesised derivative was performed in D_2O/DCl (p*D* 3.5) (Figs. 3 and 4).

3.2. Determination of intrinsic viscosities

Huggins plots (Fig. 5) were used to determine the intrinsic viscosity ([η]) for solutions of both Seacure chitosan raw material and synthesised β-cyclodextrin-chitosan. As shown in Fig. 5, the curves of both plots should be linear and the *y*-axes intercept is the intrinsic viscosity. The intrinsic viscosity ([η]) for Seacure chitosan was found equal to 454 mL/g and for β-cyclodextrin-chitosan to 283 mL/g.

From the data given in Fig. 5, one is able to determine the Huggins constant (k') (Eq. (1)) in both cases; one found 0.53 and 1.84, respectively, indicating an anomalous behavior for the CD-chitosan such as secondary interchain interactions. As the intrinsic viscosity reflects the specific hydrodynamic volume of the polymer chains in solution, these results seem to show that CD-chitosan is more compact than initial chitosan forming also intrachain interactions. This may be attributed to the presence of a few chemical cross-links between the chitosan chains resulting from the presence of traces of β-CD di- and trialdehyde as demonstrated recently by mass spectrometry and chromatography (paper in preparation by Auzély-Velty).

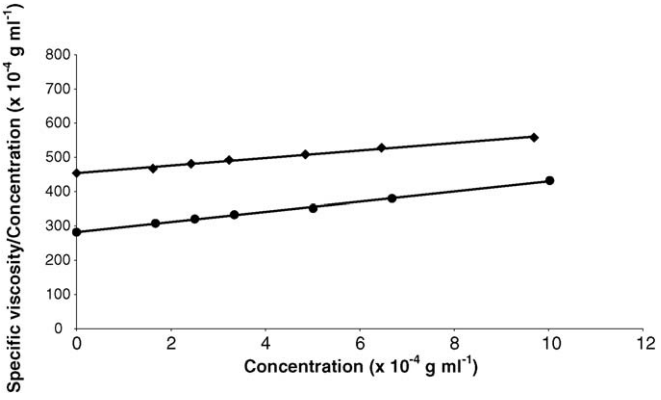

Fig. 5. Huggins plot (η_{red} vs. *c*) for Seacure chitosan (♦) and synthesised chitosan-β-cyclodextrin (●) with the intrinsic viscosity ([η]) calculated from the *y*-intercept.

3.3. AD–CD complex formation

The inclusion ability of β-CD-chitosan was investigated by isothermal titration calorimetry using water-soluble (+)-catechin as a model guest as it is well known in the laboratory. This polyphenolic compound of natural origin (see Fig. 6) forms a 1:1 inclusion complex with natural β-cyclodextrin with inclusion of the B-ring in the cavity (Kriz et al., 2003). Fig. 6 compares the data obtained for the calorimetric titration at 25 °C of the grafted β-CD and β-CD monoaldehyde with (+)-catechin. In the case of β-CD monoaldehyde, exothermic heat is produced after each injection of (+)-catechin. The magnitude of the released heat decreases rather rapidly with the first injections reflecting a moderate value of the association constant for the β-CD monoaldehyde/(+)-catechin complex. Indeed, the fact that the released heat is not maximum during the first injections, when CD cavities are in large excess with respect to added (+)-catechin, suggests that (+)-catechin is not completely complexed. In the case of the grafted β-CD, although the thermogram looks similar to that obtained with natural β-CD, it can be noticed that the amount of heat evolved for the first injections is much lower (even if the same concentrations of the host and guest molecules were used), suggesting a lower association constant for the grafted β-CD/(+)-catechin complex.

These preliminary conclusions derived from experimental observations were fully confirmed by the thermodynamic parameters calculated from the experimental data (see Table 1). Nevertheless, it can be noted that the experimental data for the grafted β-CD/(+)-catechin complex were fitted to a theoretical titration curve with only K_a, and *n* as adjustable parameters. Indeed, the value of ΔH^0 was imposed assuming that the grafted β-CD and β-CD monoaldehyde have similar mechanisms of binding and hence similar ΔH^0 values, otherwise totally inconsistent results were obtained. Table 1 shows that the complexation of (+)-catechin by the grafted β-CD is about five times weaker than that by the β-CD monoaldehyde, this one being also slightly weaker than that by the natural β-CD. The lower affinity for the grafted β-CD is the result of a less favorable change in entropy which might be related to the covalent binding to the polymer. Finally, it can be noted that the stoichiometry of the grafted β-CD/(+)-catechin complex is in the same order of magnitude of the β-CD monoaldehyde/(+)-catechin complex and not far from 1:1, indicating that most of the grafted CD cavities remain available for complexation.

3.4. Mucoadhesive evaluation

The mucoadhesive properties of the selected mucoadhesive polymers were measured with the use of a tensile separation test in which the polymer was brought into contact with mucus for a period of time during which interpenetration and hydration of the polymer film resulted in mucoadhesive bonding with the mucus chains. The tensile separation apparatus was based on an adaptation of the Wilhelmy plate method in which the mucoadhesion strength of the bond between mucus and the polymer was measured (Juninger, 1990). The selection of a reference substance

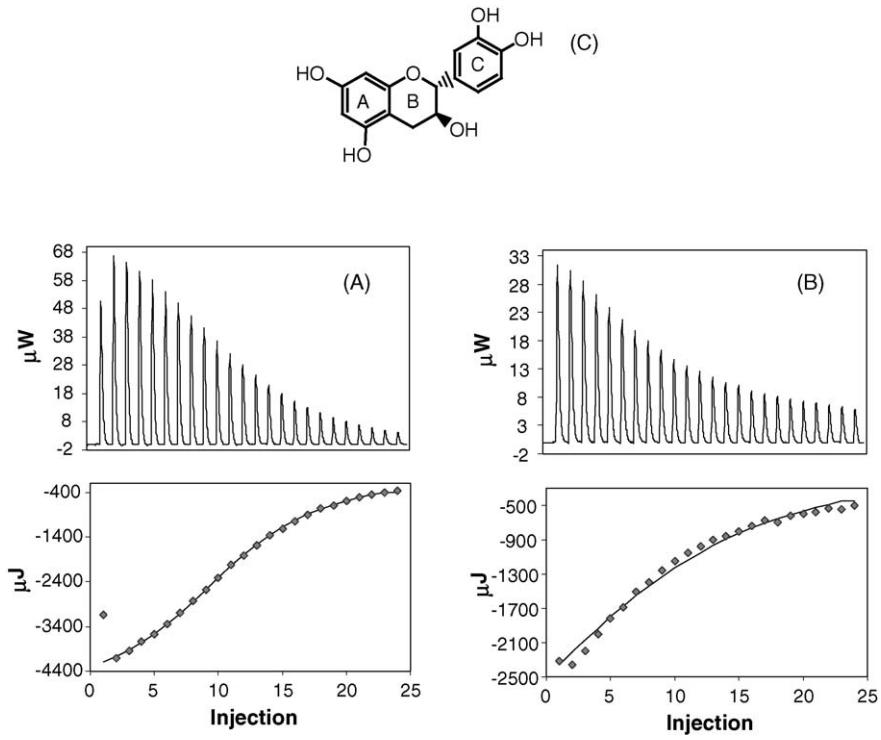

Fig. 6. Calorimetric titration of the binding of (+)-catechin to (A) the β-CD monoaldehyde and (B) the grafted β-CD. Upper halves: raw data obtained for 24 automatic injections, each of 10 μL, of (+)-catechin to the CD molecule (the concentration of the host and guest molecules in pure water or 0.3 mol/L CH_3COOH/0.03 mol/L CH_3COONa is indicated in Table 1). Lower halves: the integrated curve showing experimental points and the best fit for titration of the CD monoaldehyde and grafted CD. (C) is the chemical formula for catechin.

Table 1
Thermodynamic parameters for inclusion complex formation of (+)-catechin with the natural β-CD, β-CD monoaldehyde and the β-CD grafted on chitosan

CD derivative	CD cavity concentration (mM)	catechin concentration (mM)	K_a (M^{-1})	ΔH^0 (kJ/mol)	$T\Delta S^0$ (kJ/mol)	n (nAD:1CD derivative)
Natural β-CD	1	11	8040 (±250)	−42.2 (±0.8)	19.92	0.96 (±0.01)
β-CD monoaldehyde	1.1	11.8	5160 (±150)	−42.4 (±0.8)	21.22	0.94 (±0.02)
Grafted β-CD	1.1	11	1150 (±100)	−42.4 (imposed)	24.94	0.77 (±0.03)

was made according to data obtained from previous studies on mucoadhesion (Juninger, 1991). Pectin is a polysaccharide present in the cell walls of all plant tissues. Pectin functions as an intercellular cementing material and consists mainly of partially methoxylated polygalacturonic acids. Pectin exhibits relatively poor mucoadhesive properties and small deviations from the accepted values of mucoadhesive testing with different experimental set-ups are obtained. Therefore, pectin is suitable as a reference standard (Smart et al., 1984) for the determination of the mucoadhesive properties of synthesised β-cyclodextrin-chitosan. Comparative studies are possible between different polymers and the same mucus gel structure, given that other variables will be kept constant. A rank order of adhesion to mucus for various polymers can be made if one or more reference substances are used in the same studies. For example, a new mucoadhesive polymer may be tested by comparing it to existing data on other substances, and by "calibrating" the technique used.

In Fig. 7, a typical result of the tensile detachment testing of one of the polymers is shown. The peak value represents the maximum detachment force (MDF) measured in Newton, and the area under the curve is the total work of adhesion (TWA)

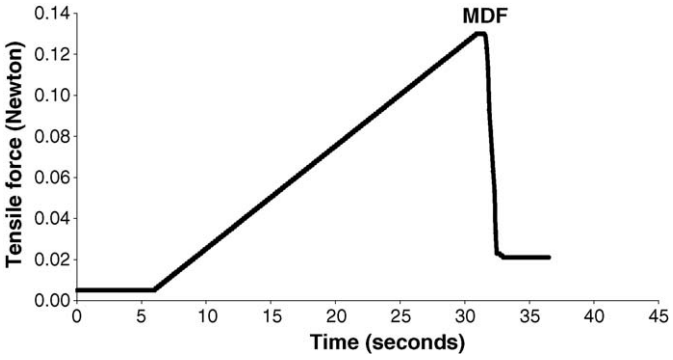

Fig. 7. Typical results of tensile separation testing.

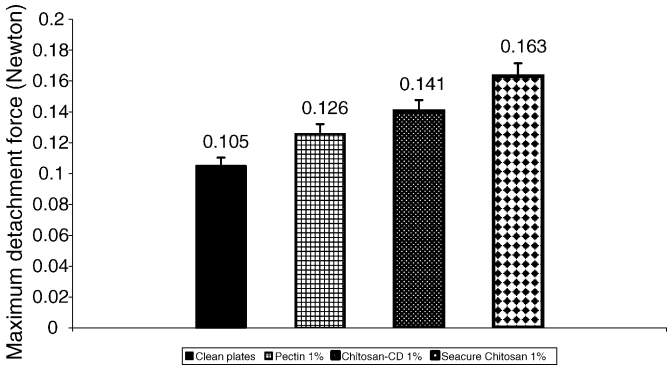

Fig. 8. The comparative MDF (N) for different polymers at 120 s hydration time with clean plates as control.

measured in Joule. The increase in the force applied against time was noted in the experiment and the maximum detachment force (MDF in Newton) was taken as an indication of the mucoadhesion strength of the polymer at the given time. The tensile force increases as the plate was lifted until the bond between the plate and the mucus is broken (MDF) after which the tensile force decreases. The amount of mucus still adhering to the plate can be seen by the difference in the baseline before and after testing of the polymer and is an indication that breakage of the interface occurred nearer to the side of the mucus. The comparative mucoadhesion profiles showing the MDF for pectin (1%), CD-chitosan (1%) and initial chitosan (1%) are shown in Fig. 8. The clean plate control was included to show the relative mucoadhesion. When the mucoadhesive polymer film touches the mucus, hydration of the film occurs and the polymer chains begin to penetrate the mucus layer. An interface between the film and the mucus forms as the positive charges interact with the negatively charged sialic groups on the mucus, forming the mucoadhesive bond. Compared to the clean plate control, a statistically significant increase ($p < 0.05$) on mucoadhesivity is found for pectin, chitosan and the chitosan derivative tested, even if mucoadhesivity of CD-chitosan is slightly depressed compared with the initial chitosan. No statistical significant difference ($p < 0.05$) was obtained between the mucoadhesivity of CD-chitosan and pectin.

4. Conclusion

Combining mucoadhesive characteristics with the potential to enhance drug release by increasing the solubility of poorly water-soluble drugs has great potential for pharmaceutical technology and pharmaceutical dosage form design. The use of β-CD in combination with chitosan resulted in a polymer which showed substantial mucoadhesive properties, although lower compared to that of chitosan alone. The decrease in mucoadhesion of β-cyclodextrin-chitosan may be explained by the change in conformation of the CD-chitosan (more compact as shown from intrinsic viscosity), which leads to less available positive charges for interaction with the negatively charged mucus. The molecular size of CD-chitosan might lead to a decrease of the interpenetration into the mucus layer with a subsequent decrease in mucoadhesivity. In fact, it is known that, besides molecular weight, mobility and flexibility of polymeric chains play an important role in mucoadhesion. Therefore, although a critical length (molecular weight) of the molecules is necessary to produce the interpenetrating layer, the chain conformation may impair interpenetration.

In conclusion, a monosubstituted β-CD derivative could be efficiently grafted on chitosan, yielding a CD-polymer with promising inclusion and mucoadhesive properties.

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