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Application of novel chitosan derivatives in dissolution enhancement of a poorly water soluble drug

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Solid dispersions of the poorly water soluble drug dexamethasone and newly synthesized chitosan derivatives (chitosan succinate, CS, and chitosan phthalate, CP) were prepared by spray drying. The resulting microspheres were evaluated in terms of their drug loading or encapsulation efficiency as well as drug release profile. Differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and infrared spectroscopy (IR) were used to evaluate the solid dispersion for possible interactions between drug and polymers. The pure drug was evaluated in the same manner for comparison purposes. High loading levels (>74%) were achieved using CP and CS as polymer matrices. Drug release rate was accelerated significantly upon the formation of the solid dispersions; the drug release rate was increased with increasing percentage of the chitosan derivatives in the microspheres. IR studies showed no chemical interaction while the X-ray studies showed a significant change in the crystallinity of the drug upon formation of solid dispersions.

1. Introduction

Natural polysaccharides and their derivatives represent a group of polymers widely used in pharmaceutical dosage forms. Various kinds of natural gums (such as: agar, sodium alginate, pectins, carragenans, cellulose, chitosan, starches, xanthan gum etc.), are used in food industry and are safe for human consumption. These polysaccharides are obtained usually as plant exudates containing various sugars other than glucose and having significant quantities of oxidizable groups adjacent to their normal polyhydroxy format (Bhardwaj et al. 2000; Angelova and Hunkeler 1999).

Natural gums have been examined as matrices for the sustained release of drugs. And they are often preferred over synthetic materials due to their safety, low cost, and wide availability. It is noted that many old materials compete successfully today after almost a century of efforts to replace them (Bhardwaj et al. 2000).

The use of natural polymers as drug carriers has received considerable attention in dosage form design. Chitosan, which is a partially deacetylated chitin, and its various synthetic derivatives have recently attracted much attention because of its natural origin, safety profile, biocompatibility, biodegradability, bioadhesiveness, and gel forming properties at low pH ranges (Aiedeh et al. 1998; Orienti et al. 1996; Aral and Akbuga 1998; Lopez and Bodmeier 1996; Rege et al. 1999; No et al. 2000; Lehr et al. 1992).

Chitosan is soluble in solutions of pH values less than 6.0. As cationic polymer, its solubility is strongly dependent on the pH of the surrounding environment. This property is disadvantageous for biomedical applications of chitosan because at physiological pH (7.4) most chitosans precipitate from solution (Thanou et al. 1999; Kotze et al. 1999a).

Owing to the presence of reactive amine groups, chitosan is readily modifiable. Its derivatives may provide some advantages over the unmodified chitosan because of their significantly different physicochemical properties. (Aideh and Taha 2001; Alexeev et al. 2000; Taha et al. 2000).

In recent years, several derivatives of chitosan have been synthesized to obtain different favorable properties over chitosan, like: *N*-trimethyl chitosan and *N*-ethylene phosphonic chitosan, which possesses water solubility over a wider pH range. (Thanou et al. 1999; Kotze et al. 1999b; Heras et al. 2001; Illuman et al. 2000).

Dexamethasone is an important steroidal drug used extensively in medicine for its anti-inflammatory and immunosuppressive properties (Swartz et al. 1978). However, it is characterized by a low water solubility (0.08 mg/ml 25 °C), and consequently low and irregular bioavailability (Swartz et al. 1978).

The aim of the present study was to investigate the effect of new chitosan derivatives namely chitosan succinate (CS) and chitosan phthalate (CP) on the dissolution behavior of dexamethasone to improve its release properties at physiological pH values and potentially its bioavailability. Dexamethasone loaded chitosan microspheres with different drug/polymer ratios, were obtained by spray-drying technique (Pavanetto et al. 1993, 1994). Drug-polymers physical mixtures of corresponding compositions were also prepared and evaluated for drug release.

2. Investigations, results and discussion

2.1. Synthesis and characterization of chitosan phthalate and chitosan succinate polymeric conjugates

The conjugation reactions were carried out using succinic and phthalic anhydrides in the presence of pyridine (Aiedeh and Taha 1999). Both anhydrides are strong electrophiles and react readily with the nucleophilic amine groups of chitosan. Pyridine was added as an acylation catalyst.

Probably, the amino groups were selectively acylated due to their superior nucleophilic character in comparison to the surrounding hydroxyl groups (Scheme).

The IR spectra of the prepared polymers, CP and CS, are shown in Figures 1 and 2. The amide carbonyl stretching vibrations in the CP and CS spectra appear in the range of 1650–1670 cm^{-1}, while the carboxylic carbonyl stretching vibrations appear in the range of 1710–1735 cm^{-1}. Both observations indicate the formation of the amide bond with the phthalate and succinate moieties.

The average degrees of chitosan substitution by phthalate and succinate moieties were 8.1 and 12%, respectively. Phthalic anhydride resulted in a lower substitution degree than succinic anhydride because of both its lower electrophilicity and larger molecular size.

Table 1 shows the solubilities of CP and CS in water. In contrast to chitosan, both semisynthetic polymers exhibit the highest solubilities in alkaline media; this is probably due to ionization of the carboxylic acid moieties under alkaline conditions yielding the carboxylate anions. The hydrophilic ionic species facilitate efficient polymeric hydration and dissolution in aqueous media. In addition, CP and CS have some degree of solubility in acidic media, which can be attributed to the protonation of the remaining amine groups within the CP and CS chitosan derivatives.

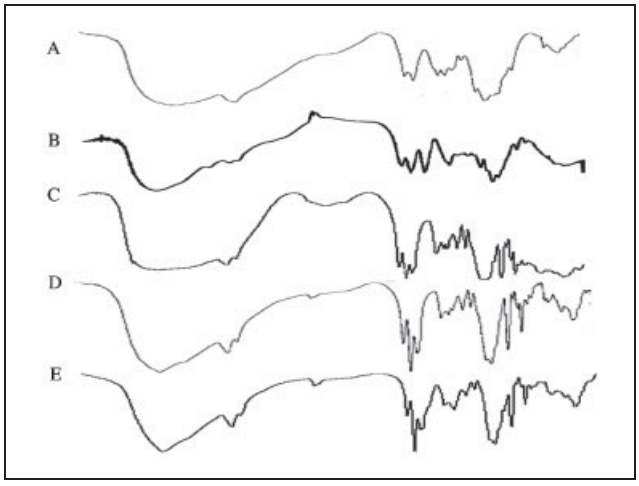

Fig. 1: IR spectra of chitosan phthalate: A. chitosan, B. chitosan phthalate, C. dexamethasone, D. physical mixture and E. spray dried with CP

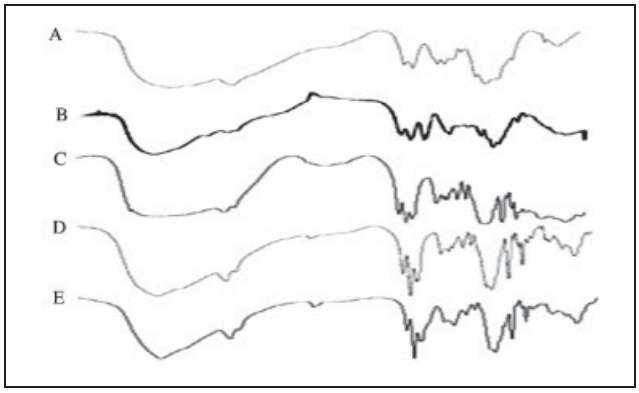

Fig. 2: IR spectra of chitosan succinate: A. chitosan, B. chitosan succinate, C. dexamethasone, D. physical mixture and E. spray dried with CS

Scheme

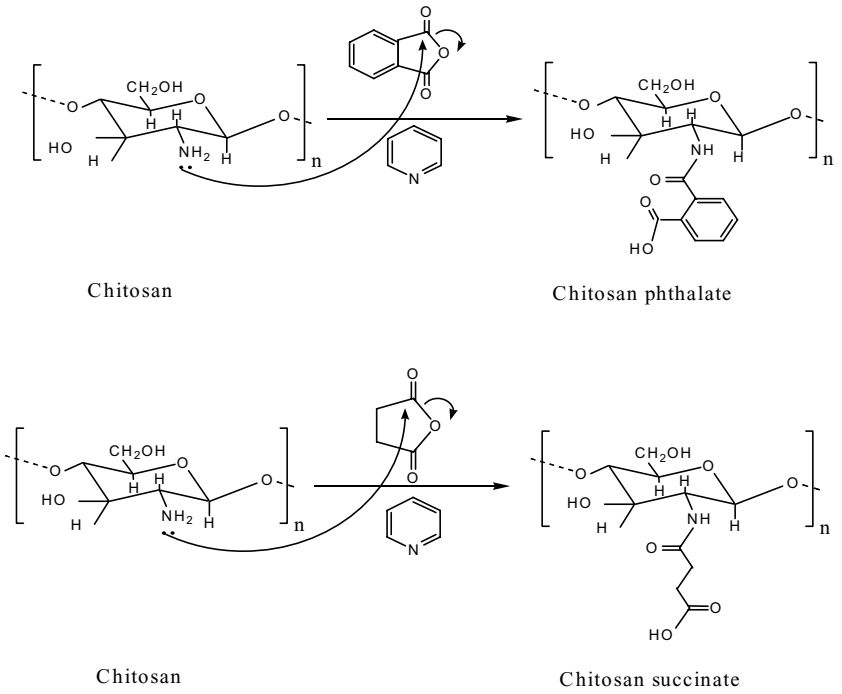

Table 1: Solubility of modified chitosans

Polymer	Solvent	Solubility (g%)	Solubility classification
Chitosan	0.1 N NaOH	0	Insoluble
	0.1 N HCl	3.67	soluble
	Water	0	Insoluble
Chitosan phthalate	0.1 N NaOH	6.67	Soluble
	0.1 N HCl	0.66	Slightly soluble
	Water	0.56	Slightly soluble
Chitosan succinate	0.1 N NaOH	7.33	Soluble
	0.1 N HCl	0.86	Slightly soluble
	Water	1.67	Sparingly soluble

Table 2: Encapsulation efficiency of chitosan succinate and chitosan phthalate spray dried particles

Preparation (mg drug/g polymer)	Theoretical drug content (mg)	Average encapsulation efficiency (%)	Standard deviation
CP 100 mg/1 g	10	82.33	2.97
CP 50 mg/1 g	5	86.53	2.08
CP 25 mg/1 g	2.5	95.47	2.66
CS 100 mg/1 g	10	74.37	1.91
CS 50 mg/1 g	5	81.13	2.10
CS 25 mg/1 g	2.5	88.53	3.23

It is obvious that the solubility of CS is higher than that of CP in aqueous media regardless of pH conditions because of the superior hydrophilic character of the succinic moieties since the hydrophobic aromatic rings within the phthalate moieties are expected to hinder water penetration.

2.2. Preparation and characterization of polymeric microspheres

Alkaline aqueous/acetonic solutions of CP or CS combined with dexamethasone were spray-dried in a temperature range of 55–120 °C. The spraying/drying process resulted in the formation of homogenous drug-loaded spherical polymeric microspheres of 2–10 μm diameter range (Fig. 3).

Table 2 shows the average encapsulation efficiencies of dexamethasone-loaded microspheres. The encapsulation process showed high loading efficiency (74–95%) indicating the suitability of the two polymers for this loading procedure.

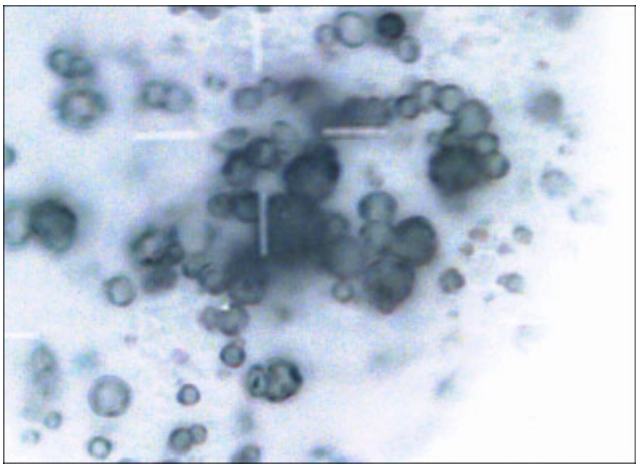

Fig. 3: Light microscopical photograph (6000X) of spray dried particles

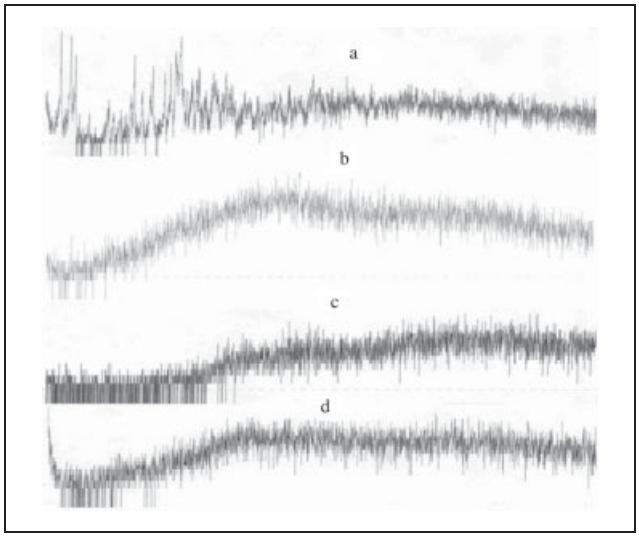

Fig. 4: X-Ray powder diffraction profiles of (a) dexamethasone pure powder, (b) chitosan phthalate-dexamethasone physical mixture, (c) chitosan succinate-dexamethasone physical mixture and (d) spray dried high loaded chitosan pthalate microspheres

The encapsulation efficiencies in CP microspheres were significantly higher than their CS counterparts which may be explained on the basis of relative preference of dexamethasone for CP over CS due to the presence of the hydrophobic aromatic rings within the phthalate moieties.

The IR spectra of pure dexamethasone powder (Figs. 1 and 2) show two carbonyl peaks at 1670 and 1710 cm^{-1}. These two peaks were also observed in the IR spectra of the physical mixtures and the drug loaded microspheres indicating that no chemical interaction and/or modification occurred on the drug upon spray-drying.

The X-ray diffraction pattern of the pure drug (Fig. 4) indicates a crystalline structure. However, the physical mixtures and drug loaded microspheres showed a change in the solid state of the drug from crystalline to amorphous.

The DSC profile of the pure drug (Fig. 5) showed an endothermic melting peak at 260 °C. At this high temperature a significant loss of CP and CS weight was evident by the TGA profiles since the thermal degradation of chitosan begins around 250 °C (Sakurai et al. 2000).

On the other hand, the glass transition of the polymers was not clear from the DSC profiles, chitosan is well known for the difficulty in measuring its glass transition temperature (Dong et al. 2004). Consequently, it was not possible to draw conclusions based on the behavior of Tg of either CP and CS in pure, physical mixture or microsphere form.

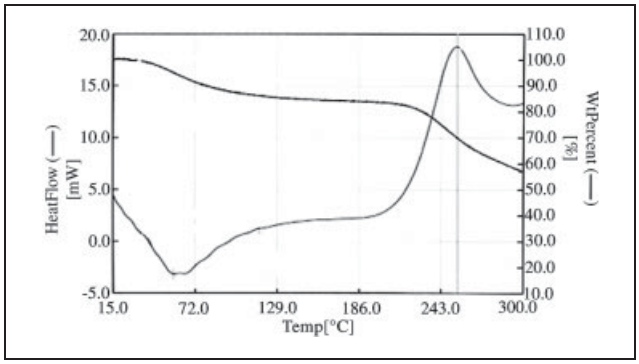

Fig. 5: TGA of "medium" drug loaded CP microspheres

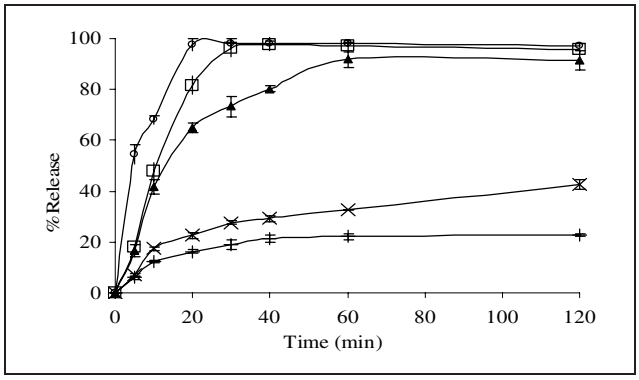

Fig. 6: Drug release from chitosan phthalate spray dried particles in comparison with that of drug alone and the physical mixture. ▲ 100 mg/1 gm CP microspheres, □ 50 mg/1 gm CP microspheres, ◇ 25 mg/1 gm CP microspheres, × 100 mg/1 g CP, + dexamethasone powder

2.3. In vitro release profiles of polymeric microspheres

Dissolution of the pure drug powder was the slowest of all samples with only 20% dissolved after 2 h at pH 7.4. This was expected considering the hydrophobic nature of dexamethasone (Swartz et al. 1978). However, the dissolution rate was enhanced significantly at an level of 0.05 upon incorporation of the drug in a physical mixture with either CP or CS (Figs. 6 and 7). The increase in dissolution rate is probably due to two factors the change in the solid state of the drug from a crystalline material in the case of drug alone to an amorphous high energy state with a higher solubility and a faster dissolution rate in the physical mixtures as seen in Fig. 5 and the hydrophilic properties of the polymeric matrices created around the drug particles by mixing. Both CP and CS are ionized at pH 7.4 and consequently the water penetration is enhanced and dexamethasone is wetted more effectively by the dissolution media.

On the other hand, upon the comparison of the two physical mixtures in terms of drug release, it is observed that the drug release from the Dex-CS mixture is significantly faster than that from Dex-CP mixtures. This is due to the difference in the solubility of the two polymers, CS matrices encourage dissolution more effectively in comparison with CP matrices where the hydrophobic aromatic rings within the phthalate moieties hinder effective water penetration.

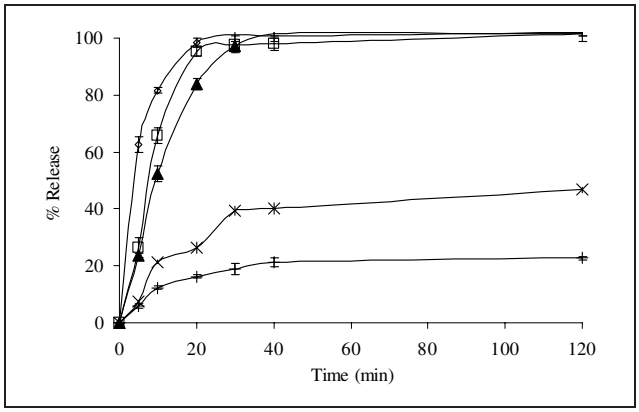

Fig. 7: Drug release from chitosan phthalate spray dried particles in comparison with that of drug alone and the physical mixture: ▲ 100 mg/1 gm CS microspheres, □ 50 mg/1 gm CS microspheres, ◇ 25 mg/1 gm CS microspheres, × 100 mg/1 g CS, + drug alone

The drug release from dexamethasone-loaded CS microspheres was faster than that from their corresponding dexamethasone-loaded CP microspheres, this is similar to the observation made with the physical mixtures and can be explained by the same factors.

On the other hand, dexamethasone-loaded microspheres prepared from either CP or CS showed a more pronounced enhancement of the dissolution of dexamethasone in comparison to the drug powder as well as the physical mixtures (Figs. 6 and 7), which may be attributed to the more intimate interaction between the drug and its hydrophilic polymeric matrices in case of spray dried particles in comparison to the physical mixtures.

The level of drug loading in both types of polymeric microspheres affected the drug release significantly. The lower the loading level of the drug the faster the dissolution rate is within the same type of the polymeric matrix. This is expected to result from the slower water penetration and uptake in the matrices with the higher drug loading because of the hydrophobic nature of the drug distributed with in the matrix. A larger proportion of CP or CS would enhance wetting and dissolution of dexamethasone from particles containing a lesser proportion of the hydrophobic drug.

The dissolution rate trends described above were validated by calculating the apparent release rate constants for all of the prepared mixtures and microspheres by plotting the Higuchi equation:

$$Q = K \cdot t^{1/2} \quad (1)$$

Where Q is the percentage of release of the drug, t is the release time, and K is the apparent release rate constant, the release rate constant obtained using this methods are presented in Table 3.

3. Experimental

3.1. Materials

Dexamethasone from Sicor (Milano, Italy), low molecular weight chitosan (Mwt, 70000) obtained from Aldrich Chemical Company (USA), reagent grade succinic anhydride, phthalic anhydride and other reagents and solvents were all purchased from Fluka (Switzerland) and were used without further purification.

3.2. Preparation of chitosan succinate (CS) and chitosan phthalate (CP) conjugates

The synthesis reaction was carried out according to Aiedeh and Taha (1999). Briefly, chitosan (5.00 g, corresponding to approximately 31 mmol glucosamine) was dissolved in HCl aqueous solution (0.37%, 300 ml) at ambient temperature. Subsequently, a solution of the particular anhydride (31.25 mmol; succinic 3.15 g, phthalic 4.6 g) in pyridine (25 ml) was added dropwise to the polymeric solution with vigorous stirring. The reaction pH was maintained at 7.0 by dropwise addition of NaOH solution

Table 3: Apparent release rate (K) obtained from the Higuchi equation for the spray dried particles, physical mixture and pure drug powder

Preparation	Apparent rate constant (%/h$^{1/2}$)
Drug Powder	2.21
DEX-CS Physical Mixture	6.54
DEX-CP Physical Mixture	4.23
100 mg/1 g – CP microspheres	13.78
50 mg/1 g – CP microspheres	18.11
25 mg/1 g – CP microspheres	22.04
100 mg/1 g – CS microspheres	18.69
50 mg/1 g – CS microspheres	21.67
25 mg/1 g – CS microspheres	22.49

(1.0 M). After 40 min the reaction was terminated by the addition of NaCl aqueous solution (20%, 500 ml). The resulting precipitate was filtered, washed with acetone and diethyl ether, and desiccated to give CS or CP.

3.3. Infrared (IR) spectroscopy

IR spectra of Chitosan, CP, CS, drug-polymer physical mixtures and drug loaded microspheres were determined between 500–4000 cm^{-1} using the KBr disc method in a Nicolet Impact 400 IR spectrophotometer (Nicolet Technologies, USA).

3.4. Determination of the degree of substitution

The chitosan conjugates (CS or CP, 0.1 gm) were completely hydrolyzed in NaOH solution (3.0 M, 30 ml) for 48 h. The concentrations of phthalic and succinic acids in the hydrolysis solutions were determined by UV spectrophotometry at $\lambda = 232$ nm for phthalic acid and $\lambda = 228$ nm for succinic acid. Unmodified chitosan was treated in the same way and the resulting solution was used as a blank.
The degree of substitution (g %) is defined as the ratio of the measured amount of phthalic or succinic acid in the hydrolysis solution to the amount of chitosan conjugate.

3.5. Polymeric solubility

Excess of the particular polymer was placed in a 30 ml screw-capped bottle containing either HCl solution (30 ml, 0.1 M, pH 1.2), NaOH solution (30 ml, 0.1 M, pH 13.0) or distilled water (30 ml, pH 5.5).
The polymeric suspension was then shaken using a mechanical shaker (KS 500, Janke and Kunkel-IKA, Germany) at room temperature for 48 h. The suspension was then filtered and left overnight to dry under vacuum. The dissolved amount was then calculated by weight difference.

3.6. Preparation of chitosan succinate and chitosan phthalate microspheres

Chitosan phthalate and chitosan succinate microspheres were produced using a spray-drying technique successfully used to produced microparticles (Pavanetto et al. 1993, 1994) using a Pulvis Minispray GA32 (Yamato Scientific, Japan).
Blank spray-dried microspheres have been prepared from solutions of CP or CS (1.0 g) in NaOH (0.1 N)/acetone (100 ml, 50/50 v/v) prepared by adding 50 ml of acetone to a 50 ml polymeric solution in 0.1 N NaOH.
For the preparation of dexamethasone-loaded microspheres, different amounts of drug were solubilized in acetone (50 ml) and mixed with the polymeric solution (50 ml) prepared by dissolving the polymer (1.0 g) in NaOH (0.1 N). Three different drug/polymer ratios were compounded for both CP and CS polymers: high (100 mg/1.0 g), medium (50 mg/1.0 g) and low (25 mg/1.0 g).
The experimental conditions were optimized in such a way to obtain microspheres of narrow particle size distribution via employing the following settings: inlet air temperature of 120 to 122 °C, outlet air temperature of 55 °C, spraying flow rate of 6 ml/min.

3.7. Preparation of physical mixtures

Physical mixtures with the same composition as the spray-dried microspheres were prepared. Dexamethasone and CP or CS were mixed in a weight ratio corresponding to the highest loading level microspheres (100 mg/1.0 g) in cylindrical glass containers using a Turbula apparatus (W.A. Bachofen, Basel, Swithzerland) for 2 h at a tumbling rate of 30 rpm.

3.8. Differential scanning calorimetry and thermogravimetric analysis (DSC/TGA)

DSC and TGA studies were conducted simultaneously using Rheometric Scientific DSC/TGA apparatus (model STA 1500H, U.K.)

3.9. Powder X-ray diffraction

Powder X-ray diffraction was carried out using a Philips Xpert PW3040/00 diffractometer (Philips, Holland) with graphite monochromator Cu−Kα radiation.

3.10. Light microscopy

The shape and size characteristics of chitosan phthalate and chitosan succinate microparticles were examined using a light microscope (Olympus-Vonex, USA).

3.11. Determination of the microspheres' drug content

The amount of dexamethasone entrapped in the microspheres was determined by digesting the microspheres in NaOH aqueous solution (1.0 N, 1 L) and measuring the absorbance of the resulting solution spectrophotometrically at $\lambda = 254$ nm. The procedure was repeated on unloaded microspheres and the resulting solution was used as blank.

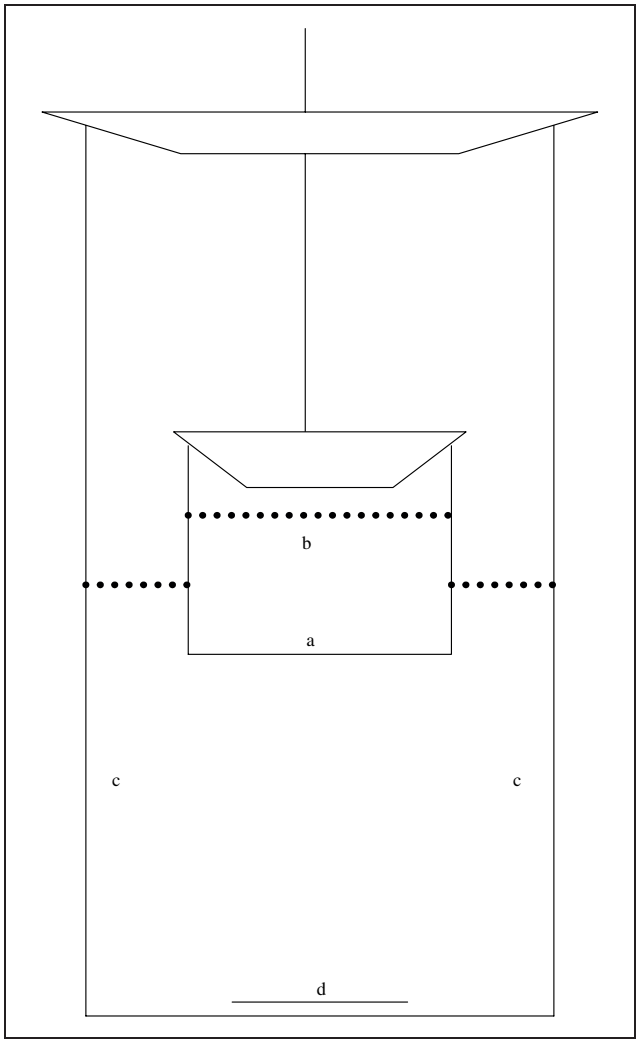

Fig. 8: Diffusion cell used for the evaluation for the in vitro release of dexamethasone powder, physical mixtures and dexamethasone loaded polymeric microspheres. a. dialysis membrane, b. donor compartment, c. receiver compartment, d. magnetic stirrer

3.12. In vitro release studies

In vitro release tests were carried out for all preparations of drug-loaded CP and CS microspheres and corresponding drug/polymer physical mixtures. Practically, the particular microspheres (100 mg), or the corresponding drug/polymer physical mixture, were suspended in 5 ml of aqueous phosphate buffer solutions (pH 7.2). The suspension was placed in a donor cell separated by a dialysis membrane (surface area 12.57 cm) from a receiving compartment containing 100 ml of the same aqueous buffer utilized to suspend the microspheres (Fig. 8).
The receiving buffer was replaced after time intervals suitable to guarantee sink conditions throughout the runs (Orienti et al. 1996). The system was stirred and thermostated at 37 °C. The drug was spectrophotometrically detected at $\lambda_{max} = 254$ nm in the receiving phase over time. The unloaded CP or CS microspheres were utilized as blank.

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