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Preparation and Characterization of Terbutaline Sulphate-loaded Chitosan/Carbopol Nanoparticles

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ABSTRACT

Objective: The objective of the present study is to prepare and evaluate terbutaline sulphate loaded chitosan/carbapol nanoparticles for treatment of asthma via nasal route. **Methods:** Nanoparticles were prepared by ionic gelation method with various concentrations of chitosan and carbopol. The developed Terbutaline sulphate-loaded chitosan/carbopol nanoparticles were analysed for particle size, dispersion homogeneity, zeta potential, entrapment efficiency, in vitro drug release and fourier transform infrared ray spectroscopy (FT-IR). **Results:** The sizes of all of the formulations which showed opalescent colloidal dispersions visually were in nanorange which was between 575.31 nm and 601.12 nm and entrapment efficiency ranged from 34.71 % to 40.04 % and zeta potential from -27.04 mV to -33.62 mV. All the CS/CP nanodispersions showed an initial burst release followed by a more gradual sustained release. The FT-IR studies suggested that an interaction between CS and TS during the formation of nanoparticles. **Conclusion:** CS/CP nanoparticles were successfully prepared by ionic gelation method and may also be useful for a variety of other therapeutic delivery systems.

Keywords: *Carbopol; Chitosan; Nanoparticles*

INTRODUCTION

Asthma is a predisposition to chronic inflammation of the lungs in which the airways (bronchi) are reversibly narrowed. Asthma affects a total of 300 million people across the world[1]. Terbutaline sulphate (TS) is an effective bronchodilator and relatively short acting ß$_2$-adrenergic agonist. It is incompletely absorbed from the gastrointestinal tract and also subject to extensive first pass metabolism[2].

Nanoparticles have been paid more and more attention as drug delivery vehicles in the last two decades due to advantages such as improving drug solubility, extending drug circulation time, enhancing its bioavailability, and reducing the drug toxicity and side effects. As the development of nanoparticle drug delivery system, biocompatible, and biodegradable properties of carrier should be taken in account[3].

Chitosan (CS) is one of the most common polymers used for preparation of polymeric nanoparticles due to its polycationic, muco-adhesive property, biocompatibility and biodegradability[4]. Chitosan can spontaneously form polyelectrolyte complexes when mixed with polyanions such as carpabol in appropriate aqueous conditions through the electrostatic attraction between the negatively charged carboxylic groups of carbapol and the positively charged amino groups of chitosan[5].

Carbapol is a cross-linked polymer of long chains of poly (acrylic acid) cross-linked with allyl sucrose[6]. The combination of CS with carbopol, poly (acrylic acid) and alginate led to the development of sustained delivery formulations[7]. More specifically,

Kao et al[8] found that the pilocarpine-loaded CS/CP nanoparticles sustained the drug release and prolonged therapeutic effect when compared with eye drops or pilocarpine-loaded into gels and liposomes.

The purpose of this study was the preparation of TS-loaded CS/CP nanodispersion using the ionic gelation method and evaluation the particle size, zeta potential and entrapment efficiency, FT-IR and in vitro release of these nanodispersions.

MATERIALS AND METHODS

Materials

Terbutaline sulphate was kindly supplied by European MEDIZEN pharmaceutical industries Company (Alexandria, Egypt). Chitosan (low Mw, viscosity, 20 cps, degree of deacetylation 85%) were purchased from Sigma Aldrich Chemical Co. (Germany). Carbapol, disodium hydrogen phosphate, potassium dihydrogen phosphate and acetic acid were purchased from El Nasr Pharmaceutical Company (Cairo, Egypt). All other reagents were of analytical grade. All other reagents were of analytical grade. Cellophane membrane (Spectra/PorV®Membrane, molecular porous MWCO: 6–8000) was obtained from Spectrum Laboratories (Los Angeles, CA).

Methods

Preparation of TS-loaded CS/CP nanodispersions

Polymeric nanodispersions with different CS:CP ratios (1:1, 2:1 and 3:1) were prepared using ionic gelation method[8]. Briefly, Aqueous solutions of CS of various concentrations (0.25, 0.5, 0.66, 0.75, 1 and 2 mg/ml) and TS (0.5 %) were first dissolved by sonication in specified volume of 1% v/v acetic acid. Aqueous solutions of CP of various concentrations (0.25, 0.5, 0.66, 0.75, 1 and 2 mg/ml) were prepared then five millilitres of CP aqueous solution were added dropwise to 5 ml of CS solution under magnetic stirring at room temperature. The pH of carbopol and chitosan solutions was initially adjusted to 4.5. **Table 1** summarized the composition of the nanoparticles formation.

Evaluation of TS-loaded nanoparticles

Particle size, polydispersity index and zeta potential

The mean particle size, size distribution and zeta potential of freshly prepared nanoparticle dispersions were determined using a Malvern Zetasizer 2000 (Malvern Instruments Ltd., UK). The measurements were performed after diluting samples by 100-fold with distilled water at ambient temperature.

Entrapment efficiency (EE %)

The amount of drug entrapped in the nanoparticles (NPs) was determined by calculating the difference between the total amount of TS used to prepare the NPs and the amount of non-entrapped drugs remaining dissolved in the aqueous dispersion medium. Five milliliters of TS-loaded nanodispersions were centrifuged at 13500 rpm and 4 ºC for 60 min (cooling ultracentrifuge 3–30 K; Sigma, Germany) to separate the drug loaded nanoparticles from the supernatant containing unloaded TS. The supernatant was analyzed for the free drug spectrophotometerically at 275 nm (UV spectrophotometer; Shimaduz, USA). The drug entrapment efficiency (EE%) of NPs was calculated as indicated below[9].

$$EE\ \% = \frac{A - B}{A}\ X\ 100$$

Where A is the total amount of drug in the nanodispersion and B is the free amount of drug in the supernatant.

In vitro drug release of TS from CS/CP nanodispersions

The in vitro drug release test was performed using the USP dissolution apparatus (USP rotating basket dissolution apparatus (Schleuniger pharmaton, Switzerland)). Samples of one ml of TS-loaded nanodispersions were placed in glass cylindrical tubes (2.5 cm in diameter and 10 cm in length) with one end tightly covered with a cellophane membrane soaked overnight in phosphate buffer saline (PBS) pH 6 (pH of nasal mucosa) and the other end attached to the shaft of the USP dissolution tester apparatus, instead of the baskets. The formulations were immersed in 50 ml PBS (pH 6). A sink condition was maintained in this volume. The release study was carried out at 37 º 0.5 ±C, and the stirring shafts rotated at a speed of 50 rpm. Aliquots of 3 ml of the release medium were collected at predetermined time intervals (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 hrs) and replaced with equal volumes of PBS. The collected samples were analyzed for drug content spectrophotometrically at 275 nm against the samples withdrawn at a respective time intervals. The experiment was performed in triplicate, and the percentage of released TS was calculated.

Kinetic studies of release

To investigate the mechanism of drug release, the in vitro drug release data were analyzed mathematically according to various models: zero order, first order, Higuchi models, and Korsemeyer-Peppas. The correlation coefficient (r) was calculated by linear regression analysis of the release plots.

Fourier transform infra-red (FTIR) spectroscopy

FTIR spectra of TS, CS, CS/CP physical mixture and freeze-dried TS-loaded CS/CP nanoparticles were recorded on a Genesis II Mattson FT/IR spectrometer (Madison, WI). The scanning was

Table 1. Composition of TS-loaded CS/CP nanodispersions prepared by ionic gelation method

Formulation Code	Composition		Total polymer concentrations (%)	CS:CP Ratio	Physical Appearance
	CS (mg/ml)	CP (mg/ml)			
C1	0.25	0.25	0.05	1:1	√
C2	0.5	0.5	0.1	1:1	√
C3	1	1	0.2	1:1	√
C4	2	2	0.4	1:1	√
C5	0.66	0.33	0.1	2:1	√
C6	0.75	0.25	0.1	3:1	√
C7	2	1	0.3	2:1	√

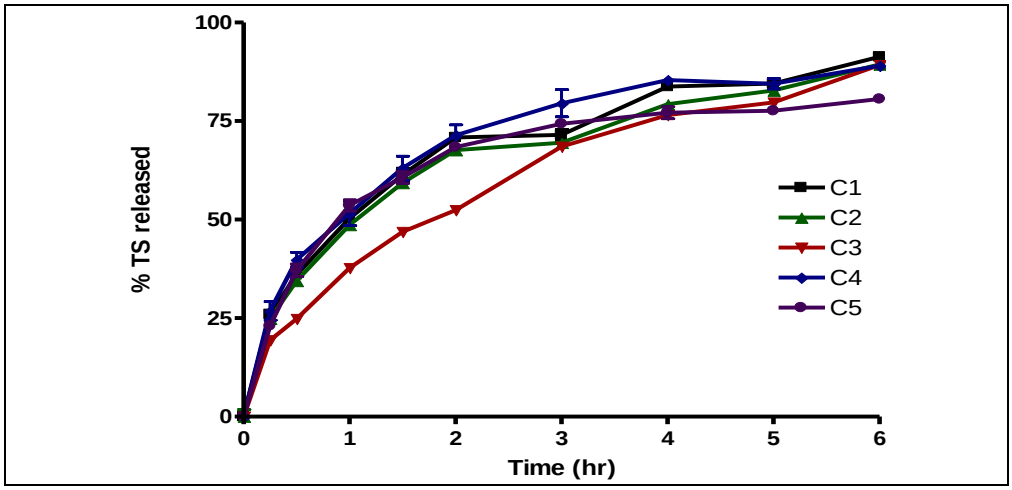

Figure 1. Release profiles of TS from CS/CP nanodispersions in PBS (pH 6) at 37ºC

done in the range of 4000-400 cm^{-1} at a speed of 2 mm/s and a resolution of 4 cm^{-1} at room temperature. The band width was measured at 50% of height of the peaks.

Statistical analysis

Student's t-test was used to analyse data of two groups obtained in different experiments at the 0.05 level of significance using GraphPad Instate 3 software.

RESULTS AND DISCUSSION

Preparation of chitosan/carbopol (CS/CP) Nanodispersions

Nanoparticles were produced using an ionic gelation process that involves carbapol as a negatively charged molecule and chitosan as a positively charged molecule[8]. From **Table 2** it was obvious that, the particle size and entrapment efficiency were not significantly ($p>0.05$) affected when two polymers concentration increased proportionally from 0.5 mg/ml (C1) to 1 mg/ml (C2), 2 mg/ml (C3) and 4 mg/ml (C4). But by comparing C2 and C5 which contain the same amount of total polymers concentration (1 mg/ml) and different CS: CP ratio; 1:1(C2) and 2:1 (C5), it was found that C2 has higher particle size and lower entrapment efficiency than C5. This result may be attributed to higher CS: CP ratio in C5 than in C2 resulted in significantly ($p<0.05$) increase in particle size from 579.91 (C2) to 601.12 (C5). Increasing the CS:CP ratio led to increasing the formation of hydrogen bond between CS and TS which resulted in increasing the particle size[10] and resulted in bulk of the NPs and less entrapment efficiency[11].

Table 2. Physicochemical characterisation of TS-loaded CS/CP nanodispersions (mean ± SD, n=3).

Formulation Code	Particle size (nm)	Zeta potential (mV)	Polydispersity index (PDI)	Entrapment fficiency (EE%)	Diffusion exponent (n)
C1	575.31±19.65	-27.04±2.87	0.618	34.71%±2.01	0.394
C2	579.91±6.71	-30.21±2.07	0.741	40.04%±3.25	0.384
C3	585.52±19.16	-32.61±1.45	0.824	39.04%±2.26	0.440
C4	590.21±15.2	-33.62±1.87	0.703	35.02%±2.46	0.383
C5	601.12±1.12	-30.51±1.98	0.769	35.23%±1.56	0.375

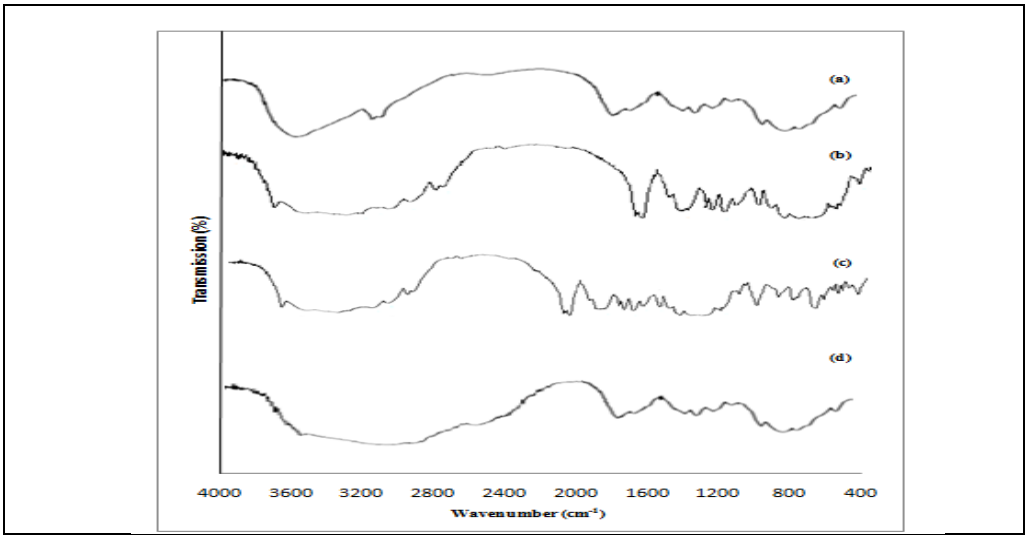

Figure 2. FTIR spectra of (a) TS, (b) CS, (c) physical mixture of TS and CS, (e) lyophilized TS-loaded nanoparticles.

Besides that, increasing the CS concentration from 0.25 mg/ml (C1) to 0.75 mg/ml (C6), both of them containing 0.25 mg/ml CP lead to aggregation of particles and the same result occur in formulation C3 and C7 both contains 1 mg/ml CP, increase concentration of CS from 1 mg /ml (C2) to 2 mg/ml (C7) lead to aggregation of particle also. When the available quantity of CS was high, the probability of formation of hydrogen bond was high which enabled NPs to form larger particles and large flocculating aggregates[12].

All TS-loaded (CS/CP) nanoparticles showed negative values of ZP which ranged from -27.04 (C1) to – 33.62 mV (C5) (**Table 2**). CS/CP nanoparticles were formed by the interaction between protonized $–NH3^+$ in CS and the polyanion groups in CP, the negatively charged zeta potential were due to more available negative charge on the surface of nanoparticles. This is due to formation of CS/CP nanoparticles with a CS core and a CS/CP membrane were formed[8].

In-vitro drug release of TS from CS/CP nanodispersions in PBS (pH 6)

From the release profiles of TS from CS/CP nanodispersions, illustrated in **Figure 1**, it could be noted that, all the CS/CP nanodispersions showed an initial burst release followed by a more gradual sustained release. The initial burst of TS was probably due to the free in the nanodispersion, which was not entrapped in the NPs, and that was adsorbed onto the surface of the NPs[13]. During this phase, the structure integrity of the particles is believed to be maintained, and drug release is probably controlled by diffusion rather than particle degradation, while the second sustained phase is probably characterized by pore formation and particle deformation[14]. All formulae of different nanoparticle formulations followed Korsmeyer-Peppas model with n-values ranged from 0.375 to 0.440 indicating anomalous (Fickian) mechanism.

FTIR

The FTIR spectrum of the physical mixture of TS and CS **(Figure 2(c))** exhibited the characteristic bands of the drug, which indicates the absence of chemical interaction between them. However, observing the FTIR analysis of TS-loaded nanoparticles **(Figure 2(d))**; the characteristic peak of CS at 3329.14 cm^{-1} (OH) became broader indicating the enhancement of hydrogen bonding.

CONCLUSION

In the present study, CS/CP nanoparticles can be prepared easily using the dropping method (i.e. adding positively charged CS dropwise into negatively charged Carbopol). The mean particle size of all TS-loaded CS/CP nanoparticles was in the nanometer range except C6 and C7. All nanoparticles exhibited a negative zeta potential, where CS is localized in the core with excess charge units of CP being segregated to the surface of the particles. The expected sustained-release effects of TS-loaded nanoparticles were verified by in-vitro test. CS/Carbopol nanoparticles may also be useful for a variety of other therapeutic delivery systems.

Conflict of Interest

The authors declare that they don't have any kind of conflict of interest.

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