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Physicochemical Characterization of Poly(L-lactic acid) Microspheres Bearing Bromhexine Hydrochloride

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This work embodies the details of a project on characterization of controlled-release dosage form of bromhexine hydrochloride to prevent gastric irritation. The sustained release formulations of bromhexine hydrochloride were prepared using poly(L-lactic acid) with the method of emulsion/solvent evaporation technique. The physicochemical characterizations of microspheres were investigated by X-ray powder diffraction and DSC analysis techniques. The DSC thermograms of drug loaded microspheres were different from bromhexine hydrochloride. Based on the data, it was evident that a crystalline fraction of the drug exists in microspheres when drug:polymer ratio was 1:1. X-ray diffraction patterns indicate a possible interference between the drug and the polymer. In conclusion, the sustained release formulations dosage form of bromhexine hydrochloride prepared with 1:1 drug:polymer ratio is the best formulation.

Key Words: Bromhexine hydrochloride, Microspheres, poly(L-lactic acid), XRD, DSC, SEM.

INTRODUCTION

Bromhexine hydrochloride (BRX-HCl), *N*-(2-amino-3,5-dibromophenylmethyl)-*N*-methylcyclohexyl-amine, possesses mucolytic and mucokinetic activities[1-3]. Conventional oral dosage forms of the drug (*e.g.* tablet, syrup) exist in the market. Drug causes gastric irritation upon oral administration. In this study, preparation and characterization of sustained release (SR) formulations of BRX-HCl was aimed to reduce the administered dose and dosage frequency and hence, overcome gastric side effects.

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As poly(L-lactic acid) has been used for the preparation of biocompatible and biodegradable microspheres with low toxicity[4-10], this polymer was chosen to form sustained release microspheres of BRX-HCl. The influence of drug:polymer ratio on the drug loading capacity and particle size were investigated. The amount of drug released from the SR microspheres was determined *in vitro*. The particles were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), X-ray powder diffraction analysis and particle size measurements.

EXPERIMENTAL

The bromhexine hydrochloride was supplied by Sifar Pharmaceuticals Co., Istanbul, Turkey. Poly(L-lactic acid) (PLA R-104, mean molecular weight: 50,000, ICN Pharmaceuticals, Costa Mesa, CA, USA) was utilized as a biodegradable polymer for the preparation of microspheres. Poly(vinyl alcohol), dichloromethane, polysorbat 20 and hydrochloric acid were provided from Merck, Germany. All other chemicals were of analytical grade and used without further purification.

Preparation of microspheres: BRX-HCl microspheres were prepared employing different drug:polymer ratios (1:1, 1:5 and 1:10) by emulsion/solvent evaporation technique[11-13]. Required amounts of poly(L-lactic acid) were dissolved in dichloromethane and the drug was dispersed in this solution. It was then poured into the 2 % aqueous solution of poly vinyl alcohol to form emulsion and stirred at a constant rate of 8,000 rpm. This process was carried out while keeping poly vinyl alcohol solution in the ice-bath. The resultant emulsion was left overnight by continuously stirring to evaporate organic phase. The microspheres formed were centrifuged at 3,000 rpm. The supernatant was decanted while the subnatant composed of microspheres were washed three times with distilled water and stored at -35 °C. Subsequently, the microspheres were lyophilized.

Determination of drug loading capacity: Analysis on drug loading capacity was performed with BRX-HCl microspheres of different drug:polymer ratios (1:1, 1:5 and 1:10). 25 mg of BRX-HCl microspheres were accurately weighed and dissolved in 25 mL dichloromethane. 0.5 mL of this solution was transferred into a volumetric flask and the total volume was completed to 10 mL with dichloromethane. The absorbance of each formulation was determined spectrophotometrically (Shimadzu, Japan) at 252 nm. Each measurement was repeated three times. Using the calibration curve for BRX-HCl, the total content of the drug encapsulated in the microspheres was calculated as "drug loading capacity" for each formulation.

Drug dissolution and release studies: Both the dissolution of the plain drug and drug release from the microspheres were examined utilizing USP paddle method under *in vitro* conditions[9]. Aymes dissolution apparatus

(Istanbul, Turkey) was used. These in vitro tests (6 replicates) were carried out in 0.1 N HCl containing 0.5 % polysorbat 20 (500 mL, 37 ± 0.5 °C, 100 rpm). 5 mL of aliquots were withdrawn at predetermined time intervals and the sink condition was maintained. The released amount of drug was determined spectrophotometrically at 311 nm.

Particle size measurements: Size volume distributions for each formulation were obtained using Malvern Mastersizer, UK. The results were presented as the mean values of triplicate samples ± standard deviation.

Morphology of microspheres: Surfaces of representative microspheres were analyzed under a scanning electron microscope (Jeol 840 AJXA, Tokyo, Japan). Samples were examined under vacuum after being coated by gold.

Differential scanning calorimetry: 2-5 mg of samples were placed in crimped aluminum pans and scanned at a rate of 10 °C/min using a differential scanning calorimeter (Perkin Elmer DSC-2, Norwalk, CT, USA). The scanning range was 30-250 °C. Thermograms were recorded for the drug itself, polymer and the microspheres. The transition and melting temperature were determined utilizing the computerized procedure of the TADS software package.

X-ray powder diffraction analysis: X-ray powder diffraction patterns were recorded using a Philips PW1710 diffractometer (The Netherlands) at a voltage of 50 KV and a current of 40 mA for the generator, with CuK_{α} radiation (λ = 1.5418 Å).

Scanning electron microscopy: Scanning electron micrographs of microspheres were taken. Each sample was mounted on stubs using conductive double-sided carbon tape and sputter-coated with gold/palladium in sputter coater (Polaron SC7620, UK) for 90 s at 9 mA. The samples were examined and digital images were captured using a Jeol JSM-5500 (Tokyo, Japan) scanning electron microscope at an accelerating voltage of 5 kV.

RESULTS AND DISCUSSION

Drug loading capacity of PLA microspheres: The solvent evaporation method employed to produce PLA microspheres was found to be successful for encapsulating BRX-HCl. Although it was low, the highest drug loading capacity (20.14 ± 1.15 %) was obtained with the drug: polymer ratio of 1:1 (Formulation, F1) (Table-1). The reason behind low drug loading capacity may be the high aqueous solubility of BRX-HCl. Indeed, high levels of drug loading can be achieved with drugs which are soluble in organic solvents and insoluble in an aqueous phase. In addition, it was found that the loading capacity was affected by the amount of polymer. More specifically, the loading capacity was enhanced by reducing the amount of poly(L-lactic acid) in the formulation.

TABLE-1
INFLUENCE OF DRUG:POLYMER RATIO ON DRUG LOADING CAPACITY AND PARTICLE SIZE

Formulation code	Drug:polymer ratio	Drug loading capacity (%)	Yield (%)	Particle size (μm)
F1	1:1	20.14 ± 1.16	26.12 ± 0.89	4.23 ± 1.20
F2	1:5	7.38 ± 0.61	19.8 ± 1.02	4.92 ± 0.86
F3	1:10	4.53 ± 0.66	3.21 ± 0.46	6.25 ± 1.18

Drug dissolution and release studies: The microspheres prepared with the drug:polymer ratio of 1:1 and 1:5 were selected for release studies as they possess higher drug loading capacity. The amounts of drug released from formulations F1 and F2 after 6 h were found to be 25.48 ± 2.58 and 17.24 ± 1.82 %, respectively. These data were compared to the dissolution data for the plain drug (Fig. 1). 100 % of the plain drug dissolved within 45 min (Fig. 2). As the dissolution and release profiles show, drug release from microspheres is slower than the drug dissolution, suggesting that the release of BRX-HCl is controlled by diffusion.

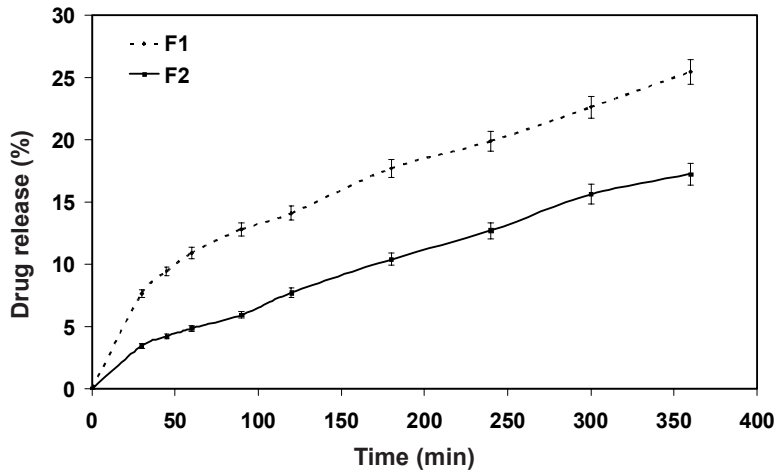

Fig. 1. Release profiles of bromhexine hydrochloride from poly(L-lactic acid) microspheres with different drug:polymer ratio

The release rate significantly decreased as drug loading increased from 17.24 % (formulation F2) to 25.48 % (formulation F1). Attempts were made to fit the release data to several kinetic models. Fig. 3 shows that the fraction of BRX-HCl released (F %) correlated linearly with the square root of time for about 0.5 h at which times 7.65 and 3.42 % BRX-HCl had been released from F1 and F2, respectively according[14] to eqn. 1:

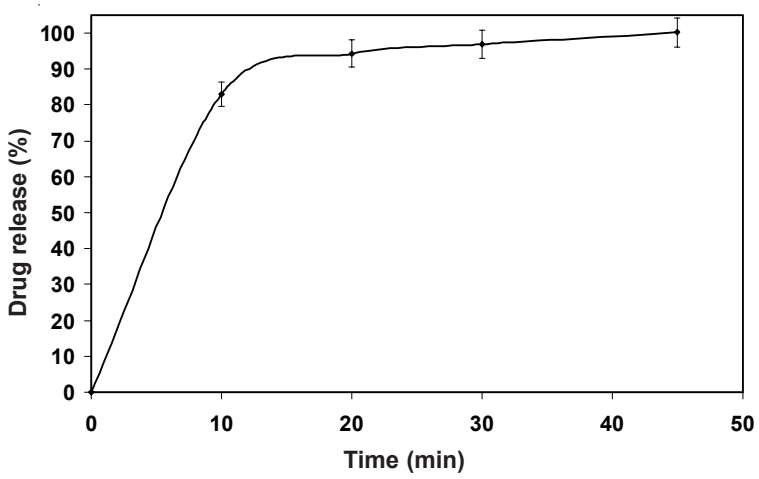

Fig. 2. Dissolution profile of bromhexine hydrochloride

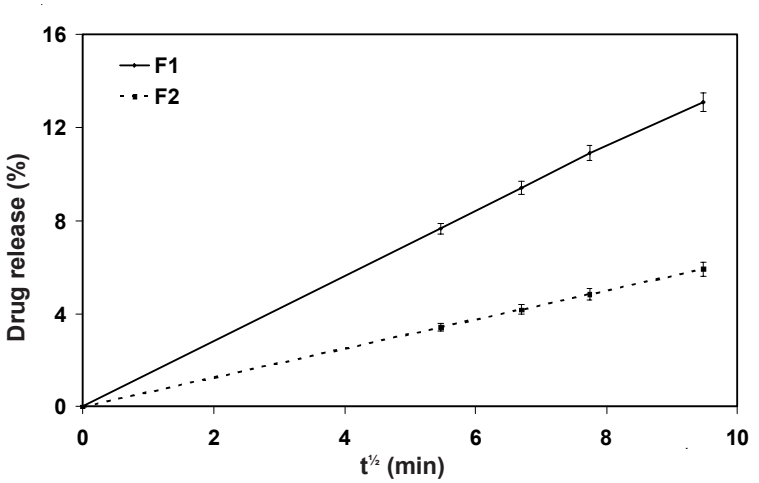

Fig. 3. Plot for the domination of $t^{1/2}$ kinetics of BRX-HCl release from poly(L-lactic acid) microspheres at early times as function of drug:polymer ratio

$$F = k_m t^{1/2} \quad (1)$$

where k_m is the release rate constant determined from the slope. Although the particles approximate spherical shape, the kinetics at early times follow non-linear pattern. The release of BRX-HCl from the microspheres was subsequently dominated by first order kinetics as shown in Fig. 4 according[15] to eqn. 2:

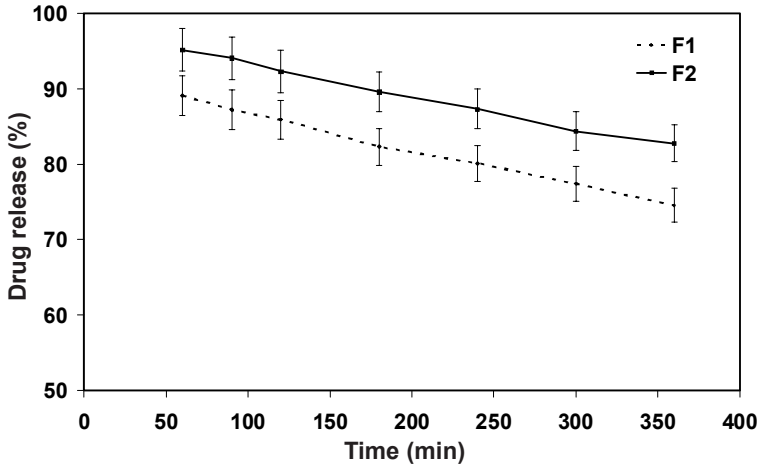

Fig. 4. Plot for the domination of first order release kinetics of BRX-HCl from poly(L-lactic acid) microspheres at later times

$$\log f = \log\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D.t}{r^2}\right)t \quad (2)$$

where f is the percent drug remaining to be released, r is the radius of the spherical core of the microspheres in which the remaining drug is dissolved in poly(L-lactic acid) and D is the diffusion coefficient. Eqn. 2 refers to the release of dissolved BRX-HCl from a microsphere. Therefore, it can be assumed that after *ca.* 25.48 % of BRX-HCl had been released from formulation F1, the remaining drug was mostly dissolved in poly(L-lactic acid) in the spherical core of the microspheres. Same trend is seen in formulation F2. It is apparent that the kinetics at early times may be dominated by release from dispersed drug. Similarly, release of BRX-HCl occurs from both dispersed and dissolved drug at later times and the kinetics of this time period may be dominated by release of dissolved drug. Moreover, particle size and polymer ratio influence the rate of release from microspheres. The higher the amount of polymer, the smaller the particle size and slower the release rate.

Particle size measurements: The size range of microspheres was 4.0-6.5 μm. Results in Table-1 indicate that the mean size of the microspheres increased from 4.23 ± 1.20 to 6.25 ± 1.18 μm as the polymer content in the microspheres was reduced. However, the particle size distribution was narrow, irrespective of the microsphere composition.

Morphology of microspheres: Morphological analyses of drug loaded microspheres were performed using scanning electron microscopy. Electronmicrographs of microparticles showed a spherical shape with porous surface regardless of polymer ratios used in this study (Fig. 5).

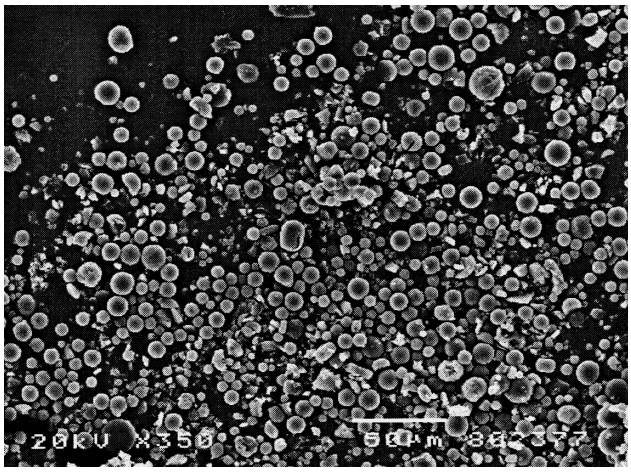

Fig. 5. SEM picture of BRX-HCl bearing poly(L-lactic acid) microspheres prepared with the drug:polymer ratio of 1:1

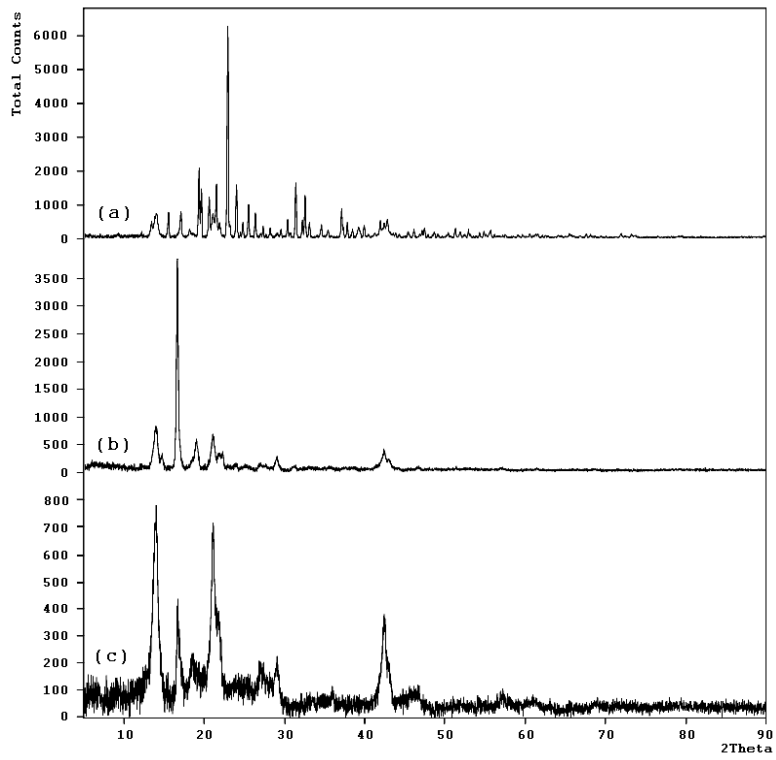

Fig. 6. X-ray powder diffraction pattern of bromhexine hydrochloride (a), poly(L-actic acid) (b) and poly(L-lactic acid) microspheres with bromhexine hydrochloride (c)

Differential scanning calorimetry: The DSC curves of BRX-HCl and poly(L-lactic acid) showed endotherms around 243 and 174 ºC, respectively. These endotherms are characteristic for the melting behaviour of BRX-HCl and poly(L-lactic acid)[16,17]. The melting peak of BRX-HCl was absent on DSC thermogram of poly(L-lactic acid) microspheres containing BRX-HCl, indicating that the drug was dispersed in the microspheres as an amorphous form or dissolution of BRX-HCl in the poly(L-lactic acid) matrix[18].

X-ray powder diffraction analysis: The crystal peak of BRX-HCl and poly(L-lactic acid) is clearly observed by X-ray powder diffraction data shown in Fig. 6. However, the diffraction patterns of the poly(L-lactic acid) microspheres containing BRX-HCl were similar to that of poly(L-lactic acid)microspheres not containing BRX-HCl. These poly(L-lactic acid) microspheres did not contain any peaks associated with the crystals of drug, suggesting that the drug was amorphous in the polymer matrix. These results agree with the DSC results.

Conclusion

Microencapsulation of BRX-HCl in poly(L-lactic acid), using solvent evaporation technique enables preparation of sustained release dosage forms. Microspheres of BRX-HCl showed sustained release effect upto 6 h *in vitro*, regardless of polymer ratio. The release rate of BRX-HCl from the poly(L-lactic acid) microspheres can be controlled by appropriate formulation adjustments. In fact, the drug:polymer ratio is an important parameter as well as particle size which influences the quality of the microspheres and the fraction of total amount of drug dispersed and dissolved in poly(L-lactic acid).

Rapid matrix release kinetics of early times was followed by a slower release obeying first order kinetics. Thus, an optimized formulation of poly(L-lactic acid) microspheres containing BRX-HCl can be used as an alternative way to deliver the drug by oral route *via* gastric ports to the patients as it will improve patient compliance and presumably, reduce the side effect. The small particle size range (4.0-6.5 mm) of the microspheres may also present another opportunity to deliver the drug *via* different route. poly(L-lactic acid) microspheres of BRX-HCl may be considered for parenteral administration.

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