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Preparation and Evaluation of Trimetazidine Hydrochloride Microspheres using Chitosan

Basu S.K.[1]*, Kavitha K.[*2], Rupesh kumar M. [3]

[1]Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, Kolkata - 700 032, India.
[2]Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist, Karnataka - 571 422, India.
[3]Department of Pharmacology, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist, Karnataka - 571 422, India.

Corres author: kaviyaju@yahoo.co.in
Tel: +91-9916283206Fax: +91-8232-235111

ABSTRACT: Microspheres of Trimetazidine Hydrochloride (TZ) were prepared by coacervation method without the use of chemical cross–linking agents such as glutaraldehyde to avoid the toxic reactions and other undesirable effects of the chemical cross-linking agents. Alternatively, ionotropic gelation was employed by using sodium-tripolyphosphate (Na-TPP) as cross linking agent. Chitosan was used as polymer. All the prepared microspheres were subjected to various physico-chemical studies, such as drug-polymer compatibility by Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FTIR), surface morphology by scanning electron microscopy (SEM), frequency distribution, encapsulation efficiency by High Peformance Thin Layer Chromatography (HPTLC), in-vitro drug release characteristics and release kinetics. TLC and FTIR studies indicated no drug-polymer incompatibility. Surface smoothness of microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. As the drug to polymer ratio was increased, the mean particle size (MPS) of TZ microspheres was also increased. A maximum of 80% of drug entrapment efficiency was obtained by the method employed. All the MS showed zero order release kinetics followed by a Fickian diffusion mechanism. From the above data it was concluded that it may be possible to design a controlled drug delivery system for the prolonged release of TZ, improving therapy by possible reduction of time intervals between administrations.
Key words: Trimetazidine Hydrochloride; Microspheres; Coacervation; Chitosan; In-vitro release kinetics.

INTRODUCTION

The present study reports a novel attempt to prepare coacervates of chitosan carrier for the cardio vascular drug TZ. Oral ingestion has long been the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, least sterility constraints and flexibility in the design of the dosage form[1]. Trimetazidine 1 (2, 3, 4-trimethoxybenzyl-piperazine hydrochloride) has been reported to exert anti-ischemic properties without affecting myocardial oxygen consumption and blood supply[2, 3]. In idiopathic dilated cardiomyopathy with heart failure, TZ increased cardiac function and had both cardiac and extra cardiac metabolic effects. Additionally, the TZ-induced increase in ejection fraction was associated with greater β_1-adrenoceptor occupancy, suggesting a synergistic mechanism[4]. It improves left ventricular function in diabetic patients with coronary heart disease. Recently, it has been shown to be effective in patients with heart failure of different etiologies[5]. TZ inhibits the enzyme of fatty acid β-oxidation, long-chain 3-ketoacyl coenzyme A thio-lase (known as 3-KAT). Through the inhibition of myocardial fatty acid oxidation, glucose and pyruvate oxidation is increased (pyruvate dehydrogenase activity is increased) and lactate production is decreased at the time of effort- (or emotionally) induced ischemia – that is, the supply–demand imbalance is restored, independently of hemodynamic actions[6, 7]. A few studies performed in

small groups of patients with post-ischemic left ventricular dysfunction have shown that TZ may be beneficial in terms of left ventricular function preservation and control of symptoms[8]. Because of its short biological half-life and therapeutic use in chronic diseases it is considered as an ideal drug candidate in this study. The use of a controlled release dosage form assist physicians in obtaining optimal treatment through better patient compliance and safer systems with low peak/trough ratios.

Chitosan or β (1, 4) 2-amino-2-deoxy-D-glucose is a hydrophilic biopolymer with excellent biodegradable and biocompatible characteristics is a naturally occurring polymer. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been examined extensively used in the pharmaceutical industry for its potential in the development of drug delivery system. Chitosan has been extensively used by many researchers for the encapsulation and controlled delivery of various drugs[9-11].

Recently reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking is applied to avoid possible toxicity of reagents and other undesirable effects. Na-TPP is a poly anion, and can interact with cationic chitosan by electrostatic forces[12, 13]. Hence, microspheres of TZ for oral delivery were developed with the aim to improve patient compliance and to obtain improved therapeutic efficacy in the treatment of angina pectoris.

MATERIALS AND METHODS

Chemicals

TZ was obtained from (IPCA Laboratories Ltd, Mumbai, India) as a gift sample, Chitosan with a degree of deacetylation of > 85% and viscosity of 500cps at 1%(w/v) in 1 % (v/v) aqueous acetic acid at 20^0C was supplied from (Central Institute of Fisheries and Technology, Cochin, India) as a gift sample and was used as received. Type B gelatin, bloom strength 225 received from (Sigma Chemical Company, St. Louis, USA), Sodium tri polyphosphate (Na-TPP) from(Fluka Chemical Company, GmbH, Switzerland), light and heavy liquid paraffins, tween 80, acetone, glacial acetic acid, methanol and other chemicals were from (S.D. Fine Chem. Limited, Mumbai, India).

Preparation of Trimetazidine Hydrochloride Microspheres

The TZ microspheres were prepared by phase separation and coacervation technique by using chitosan as coating material. Chitosan was dissolved in dilute acetic acid solution (1% v/v) at concentrations of 1-4% w/v and adjusted to a certain solution pH (usually 5.0). TZ (100mg) was dissolved in the above polymeric solution. The drug in polymeric solution was emulsified in 200ml of liquid paraffin (1:1 mixture of light and heavy liquid paraffin) containing 1ml tween 80 (2% w/v). The emulsification time was allowed for 10min under mechanical stirring (500 rpm). Then 50ml Na-TPP (1%w/v) with pH in the range 4-5 was added drop wise. Stirring was continued for 15-60min to obtain cross-linked microspheres. Microspheres were collected by centrifugation and washed with double distilled water several times, then with acetone to remove water and dried at room temperature under vacuum. The prepared microspheres were stored in desiccator for further studies. TZ loaded microspheres with different polymer compositions (1:1, 1:2, 1:3 and 1:4) were named asT1, T2, T3 and T4 respectively.

Compatibility studies

Chemical interaction between the drug and the polymeric material, if any, during the preparation of the microspheres was studied by using Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FTIR).

Thin Layer Chromatography (TLC)

Thin Layer Chromatography was carried out in TLC chamber. The sample solutions of pure drug and prepared microspheres were prepared by dissolving in methanol and applied to silica gel G plates. The plates were then developed in the following solvents systems.
Solvent system 1: n-butanol: water: methanol: ammonia (20%) (14:0.2:0.2:2 % v/v/v/v)
Solvent system 2: Concentrated ammonia: alcohol (20:80 v/v).
The R_f value of the pure drug as well as prepared microspheres were determined by placing the plates in an iodine chamber and the R_f value of pure drug was compared with the R_f value of prepared microspheres.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared (FTIR) spectrum of the drug, drug loaded microspheres, blank microspheres and physical mixture of drug and empty microspheres were recorded using a FTIR (model 4100 type A, Perkin-Elmer, Norwak, CT, USA) spectrometer using KBr pellets (400-4000^{-1}) with a scanning speed of 2 mm/sec.

Scanning Electron Microscopy (SEM)

The shape and surface morphology of the TZ loaded microspheres were studied using (Jeol, JSM-840A scanning electron microscope, Japan). The gold coated (thickness $200A^0$; (Jeol, JFC-1100E sputter coater, Japan) microspheres were subjected to secondary imaging technique at 15^0 tilt, 15mm working distance and 20Kv accelerating voltage.

Frequency distribution analysis

Samples of microspheres were analyzed for frequency distribution with calibrated optical microscope fitted with a stage and an ocular micrometer. Small quantities of microspheres were spread on a clean glass slide and the average size of 200 particles, frequency distribution was determined in each batch using the calibration factor.

Determination of Percentage Drug Entrapment (PDE)

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula;

$$\text{PDE} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100$$

Theoretical drug loading

Theoretical drug loading was determined by calculation assuming that the entire drug present in the polymer solution used gets entrapped in microspheres, and no loss occurs at any stage of preparation of microspheres[14].

Practical drug loading

Practical drug loading was analyzed as follows. 20mg of microspheres were added to 100ml of glacial acetic acid (1%v/v) and methanol in the ratio of 3:2 and occasionally shaken for 30min. The solution was centrifuged and 1ml of the clear supernatant was diluted to 10ml with 0.1N HCl, the supernatant liquid was filtered through Watt Mann filter paper and analyzed for TZ by High Performance Thin Layer Chromatography (HPTLC) [15].

In-vitro drug release studies

Microspheres equivalent to 50mg TZ were subjected to in-vitro drug release studies in simulated gastric fluid (pH 1.2 buffer) from 0-2h, simulated intestinal fluid (pH 7.4 phosphate buffer) from 2-12h to assess their ability in providing the desired controlled drug delivery. Drug release studies were carried out using USP XXIII basket dissolution rate test apparatus (100 rpm, 37 ± 1^0C). 900ml of the dissolution medium was used in this study. At different time intervals, 5ml of the sample was withdrawn and replaced with same amount of pH 7.4 phosphate buffer. The sample was analyzed for TZ at 269nm using a UV/ VIS spectrometer against a reagent blank.

Kinetics of drug release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero-order (Q v/s t), first-order (log (Q_0-Q) v/s t), Higuchi's square root of time (Q v/s $t^{1/2}$) and Korsemeyer peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t.

RESULTS AND DISCUSSION

In this study, the formation of TZ microspheres by simple coacervation with chitosan as function of several variables was investigated.

Formulation optimization of Trimetazidine hydrochloride microspheres

The formulation conditions for the preparation of coacervates were first optimized. The electrostatic interaction between Na-TPP (anion) and chitosan (cation) may exist only at certain pH region (1.9-7.5). In this study the pH of chitosan and the cross-linker solutions were usually adjusted to 4-5. This is valuable for the selection of the preparation conditions of ionotropic gelation of chitosan microspheres. It can be seen that the solution pH may play an important role on the chitosan microsphere formation[16].

Compatibility studies

Chemical interaction between drug and the polymeric material, if any, during the preparation of the microspheres was studied by using a TLC and FTIR. The comparable Rf values of pure drug and microencapsulated drug in the TLC study indicated the compatibility of drug with polymer and other excipients used in the preparation of TZ microspheres[17]. No difference in the IR patterns of a physical mixture of the drug and blank microspheres, and drug loaded microspheres was observed (Fig. 1). Therefore, the FTIR studies ruled out the possibility of any drug polymer interaction during the preparation of microspheres[18].

Morphological characteristics (SEM)

The surface morphology of TZ loaded microspheres were studied by scanning electron microscopy (Fig. 2). Surface smoothness of MS was increased by increasing the polymer concentration, which was confirmed by SEM. At lower polymer

concentration (1% w/v) rough and wrinkled surface of MS was obtained (Fig. 2a) and at higher polymer concentration (4%) the MS with smooth surface was obtained (Fig. 2b).

Particle size distribution

The results of accuracy and precision of frequency distribution studies and histograms showed the normal frequency distribution of microspheres (Fig. 3). As the drug to polymer ratio was increased, the mean particle size (MPS) of TZ microspheres was also increased (Table 1). The significant increase may be because of the increase in the viscosity of the droplets (due to the increase in concentration of polymer solution). This increase is high enough to result in difficult dispersion and subdivision of droplets reported[19, 20].

Drug entrapment efficiency

The drug loading efficiency of TZ microspheres was determined by HPTLC method. A maximum of 80% of drug entrapment efficiency was obtained by the method employed. By increasing the polymer concentration the encapsulation efficiency was increased (Table 1).

In-vitro drug release studies

From the in-vitro release of TZ microspheres it was observed that the rate of release decreased as the concentration of the carrier was increased. This may be due to low permeability of polymer to the drug. The in-vitro release profiles are shown in Fig. 4. All the parameters were run 3 times (n=3). The difference in mean of drug release of batch series 'T' was significant ($p < 0.05$).

Release kinetics

The data obtained in In-vitro release study were fitted to zero order, first order, Higuchi square root of time and Korsemeyer-Peppas equations to understand the mechanism of drug release from the microspheres[21]. The slopes and the regression co-efficient of determinations (r^2) are listed in Table 2. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics followed by fickian diffusion. All the parameters were run 3 times (n=3). The difference in mean of Zero order, First order, Higuchi kinetics and Peppas Equation between batch series 'T' was indicating significant ($p < 0.05$).

Table 1. Particle size, Drug entrapment and encapsulation efficiency of TZmicrospheres.

Formulation	Mean particle size (µm) ± SEM	(%)Drug entrapment	Drug Encapsulation Efficiency (%)
T1	238.75±7.91	42.08	46.29
T2	312.65±7.93	30.48	62.48
T3	344.81±9.78	23.12	77.67
T4	412.76±11.08	19.99	79.96

Table 2. Diffusion exponent (n) of Peppas model and Regression co-efficient (r^2) of Trimetazidine Hydrochloride release data from microspheres according to different kinetic models

Formulation	Peppas Model (n)	Zero order	First order	Higuchi
T1	0.485	0.989 ± 0.004	0.921 ± 0.008	0.963 ± 0.008
T2	0.499	0.983 ± 0.002	0.951 ± 0.006	0.943 ± 0.006
T3	0.397	0.947 ± 0.009	0.981 ± 0.001	0.983 ± 0.004
T4	0.423	0.990 ± 0.003	0.910 ± 0.007	0.985 ± 0.003

SD=Standard deviation (n=3) The difference in mean of %Cumulative Release, Zero order, First order, Higuchi kinetics, Peppas Equation between batch series 'T' was significant ($p < 0.05$).

Fig. 1. Fourier Transform Infrared (FTIR) Spectrum
(A) Trimetazidine Hydrochloride , (B) Trimetazidine Hydrochloride loaded microspheres
(C) Physical mixture of Trimetazidine Hydrochloride and blank microspheres ,(D) Blank microspheres

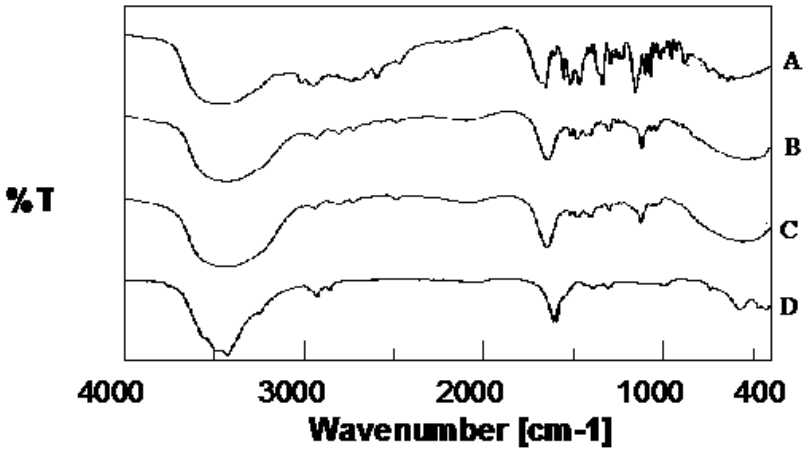


Fig. 2. Scanning Electron Micrographs (SEM) of Trimetazidine Hydrochloride Microspheres.
A. Microspheres prepared with 1:1 drug/polymer ratio
B. Microspheres prepared with 1:4 drug/polymer ratio

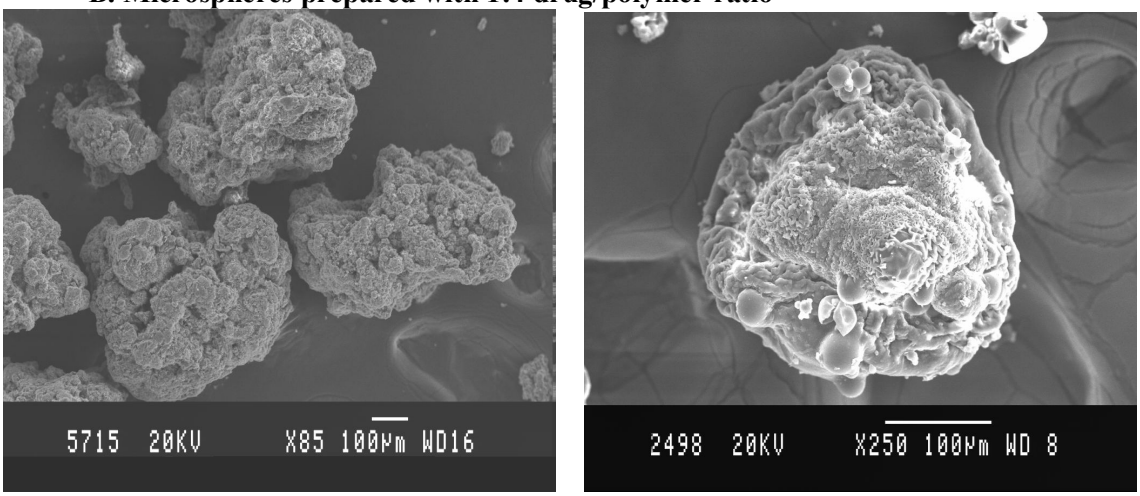


Fig. 3. Frequency distribution of Trimetazidine Hydrochloride microspheres.

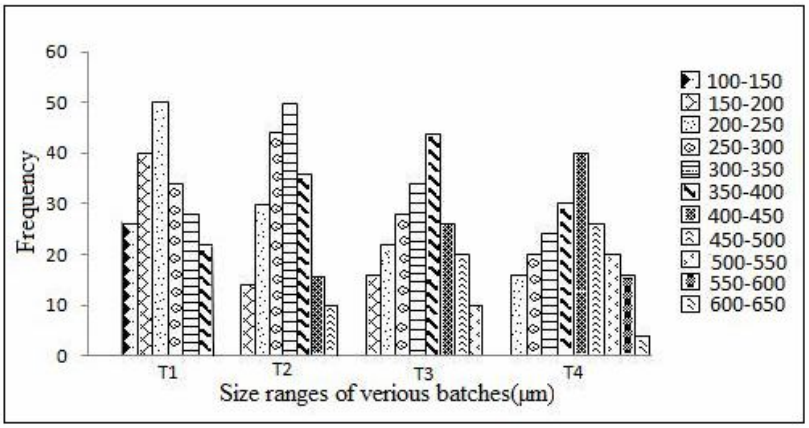
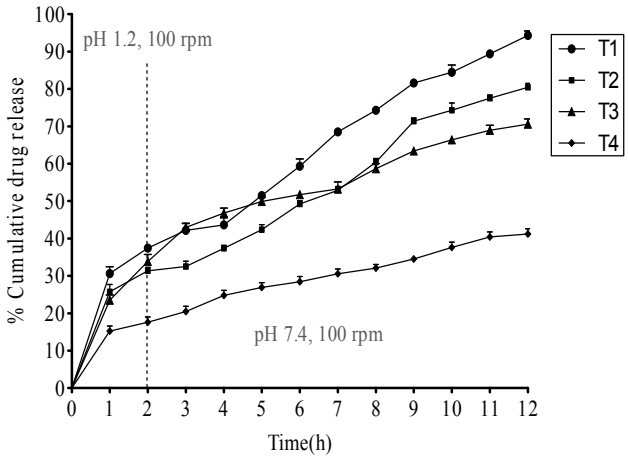


Fig. 4. In vitro release of Trimetazidine Hydrochloride microspheres.



CONCLUSION

The micro particulate drug delivery system proposed in this work based on chitosan, a natural biodegradable polymer, seems to hold promise for oral administration of TZ. The method of preparation of chitosan microspheres of TZ was found to be simple and reproducible. Chitosan, which is used as a carrier, is easily available and biocompatible. From the above data, it may be concluded that drug loaded microspheres are a suitable delivery system for TZ and may help to reduce the dose of the drug and frequency of administration.

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