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Research Article

FORMULATION AND EVALUATION OF TRIMETAZIDINE HYDROCHLORIDE LOADED GELATIN MICROSPHERES

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ABSTRACT

Trimetazidine hydrochloride-loaded Gelatin microspheres were prepared by the ionic cross-linking technique using TPP as cross-linking agent. The process induced the formation of microspheres with the incorporation efficiency of 47% to 77%. The effect of Gelatin concentration, cross-linking agents and conditions was evaluated with respect to entrapment efficiency, particle size, surface characteristics and *in vitro* release behaviors. Infrared spectroscopic study confirmed the absence of any drug-polymer interaction. Differential scanning colorimetric analysis revealed that the drug was molecularly dispersed in the Gelatin microspheres matrices showing rough surface, which was confirmed by scanning electron microscopy study. The mean particle size and entrapment efficiency were found to be varied by changing various formulation parameters. The *in vitro* release profile could be altered significantly by changing various formulation parameters to give a sustained release of drug from the microspheres. The kinetic modeling of the release data indicate that trimetazidine hydrochloride release from the Gelatin microspheres follow anomalous transport mechanism after an initial lag period when the drug release mechanism was found to be fickian diffusion controlled.

Keywords: Microspheres, Drug delivery, Targeting, Drug release, Gelatin

INTRODUCTION

Oral controlled release (CR) dosage forms (DFs) have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems[1-3]. They have varied applications and are prepared using assorted polymers[4]. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes[5-8]. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[9-12]. Gelatin (obtained by deacetylation of chitin) is a cationic polymer that has been proposed for use in microsphere systems by a number of authors[13-17]. Gelatin was selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive and biodegradable properties. Hence, there is a need to develop an oral drug delivery system that is convenient for patients. Various synthetic and natural polymers like alginate, Gelatin and polyesters have been used to develop drug delivery systems for entrapping and delivering drugs orally[18].

Objectives

The objective of the present investigation was to develop an extended and controlled release composition and formulation of trimetazidine using Gelatin polymer along with sodium tripolyphosphate to reduce dose/dosing frequency in the angina pectoris, which otherwise demands prolonged chemotherapy and to identify the modulation of drug release from the formulated matrix devices and demonstrate its utility in pharmaceutical drug carrier systems.

MATERIALS

Gelatin was obtained from shreeji laboratories. Trimetazidine was obtained from Nivedita Chemicals (Mumbai, India). Sodium tripolyphosphate (TPP) and all other reagents were of analytical grade.

METHODS

Preparation of microspheres

The preparation of the microspheres followed the method described by Ko et al with some modifications. Gelatin solutions of varying concentrations were prepared by dissolving them in dilute acetic acid (1% v/v). Tween 80 was added into the solution as a surfactant. The core material, trimetazidine hydrochloride, dissolved in CH_2Cl_2 (2:10), was mixed with the aqueous phase (Gelatin solution) in a homogenizer at 5000 rpm for 20min. The volume ratio of CH_2Cl_2: aqueous phase was 1:10. The emulsion was cross linked by dropping through a spray gun into the TPP solution (10%). After cross linking was allowed for varying time, microspheres were washed with distilled water repeatedly and vacuum dried for 12 h. Three different formulations with drug: polymer ratios (1:1, 1:2, 1:3) are prepared and coded as F1, F2 and F3.

Evaluation parameters

Drug polymer interaction (FTIR) Study [19]

IR spectroscopy was performed on Fourier transformed infrared spectrophotometer (840, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm-1. FTIR study was carried on Trimetazidine hydrochloride, physical mixture, formulations and empty microspheres.

Scanning electron microscopy (SEM)

Scanning electron photomicrographs of drug-loaded Gelatin microspheres were taken. A small amount of microspheres was spread on gold stub. Afterwards, the stub containing the sample was placed in the scanning electron microscopy (SEM) chamber. A scanning electron photomicrograph was taken at the acceleration voltage of 20 KV.

In Vitro release

Dissolution studies of Trimetazidine from microspheres was performed according to USP XXII type I dissolution apparatus in pH 1.2 for first 2 h and subsequent rest of the release study was performed in phosphate buffer of pH 7.4. The temperature was maintained at 37±0.5°C and the rotation speed was 100 rpm. The 5 ml of sample was withdrawn at various time intervals and replenished with an equal volume of fresh dissolution media. The

drug content in the sample was analyzed spectrophotometrically at 270 nm. A study was performed concurrently with placebo microspheres to record for any interference by the microsphere components.

Particle size measurement [20]

The size of the prepared microspheres was measured by the optical microscopy method using a calibrated stage micrometer for randomly selected samples of all the formulations.

Percentage yield [20]

Percentage practical yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of microspheres recovered from each batch in relation to the sum of starting material. The percentage yield of prepared microspheres was determined by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

The prepared microspheres were then characterized for their various properties

Determination of drug content

Practical drug content was determined by taking a weighed quantity of Gelatin microspheres (approximately 100 mg) in a 100-mL volumetric flask. Sufficient quantity of water was added to make the volume 100 ml. The suspension was shaken vigorously and then left for 24 hours at room temperature with intermittent shaking. Supernatant was collected by centrifugation and drug content in supernatant was determined by UV spectrophotometry at suitable wavelength (270 nm) using a shimadzu UV visible spectrophotometer (SHIMADZU, Spectrascan-2200, Japan).

Determination of percentage drug entrapment (PDE) [20]

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

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

Theoretical drug content was determined by calculation assuming that the entire drug present in the Gelatin solution used gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres.

X-Ray Power diffractometry (X-RD) study [21]

X-ray diffractometry of the Trimetazidine hydrochloride, physical mixture of Trimetazidine hydrochloride and polymer, Trimetazidine hydrochloride microspheres and blank microspheres were performed by a diffractometer using model (Joel JDX-8030, Japan) equipped with a graphite crystal monochromator (Cu-Kα) radiations to observe the physical state of Trimetazidine hydrochloride in the microspheres.

RESULTS AND DISCUSSION

In the present work controlled release microspheres of Trimetazidine hydrochloride were formulated using Gelatin polymer by ionic cross-linking emulsion technique. Three batches prepared with different polymer ratios were evaluated for physical properties like FTIR, SEM, particle size, Percentage yield, percentage drug content, encapsulation efficiency, *in vitro* dissolution, release kinetics and XRD of Trimetazidine hydrochloride microspheres.

Table 1: Percentage yield, drug content, encapsulation efficiency and average particle of Trimetazidine microspheres

Batch Code	% yield	Drug content (%)	Encapsulation efficiency (%)	Average particle size
F1	42	28.87	47.74	81±5.24
F2	59	26.65	59.95	100±5.84
F3	68	26.65	76.50	197±8.49

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

Batch Code	Zero order	first order	Higuchi	Peppas model (n)
F1	0.984±0.0008	0.938±0.05	0.946±0.0005	0.475±0.08
F2	0.968±0.0006	0.956±0.06	0.953±0.0002	0.394±0.05
F3	0.981± 0.0005	0.949±0.09	0.947±0.0006	0.412±0.06

The FTIR Spectra of Trimetazidine hydrochloride, Gelatin, physical mixture of Trimetazidine hydrochloride and Gelatin, formulations and blank microspheres are shown in the Fig 1. From this it is clear that the peaks at Alkane C-H stretch (2920.0), secondary amine N-H stretch (3446.6), aromatic C=C stretch (1602.7), tertiary amine C-N stretch (1363.6), ether –O- stretch (1288.4) cm^{-1} are present in both the pure and formulations without any change in their positions indicating no chemical interaction between Trimetazidine hydrochloride and polymers.

The Controlled release microspheres of Trimetazidine hydrochloride prepared by ionic cross-linking were found to be almost spherical and free-flowing. SEM was performed on the prepared microspheres of 1:1, 1:2 and 1:3 to access their surface and morphological characteristics as shown in Fig 2.

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulation may be due to microspheres lost during the washing process. Percentage yield, drug content, encapsulation efficiency shown in Fig 3 and average particle shown in Fig 4 were given in Table 1.

Keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity, which influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle. If there was increase in the amount of polymer concentration, there was increase in relative viscosity so as a result increases in mean particle size. The maximum particles range between 50- 100.

Trimetazidine release from the microsphere was studied for 12 h the drug released at constant rate in all these preparation and showed controlled release. The release of trimetazidine hydrochloride in two different media (pH 1.2 and 7.4) is shown in Fig 5.2 and 5.3. The percent of drug release at pH 1.2 was higher in comparison to that at pH 7.4 exhibiting pH-sensitivity of the microspheres. At the end of 4 h, 95.02% of drug load was depleted from formulation in dissolution media (0.1 N HCL, pH 1.2), in comparison to 66.88% in media (pH 7.4).

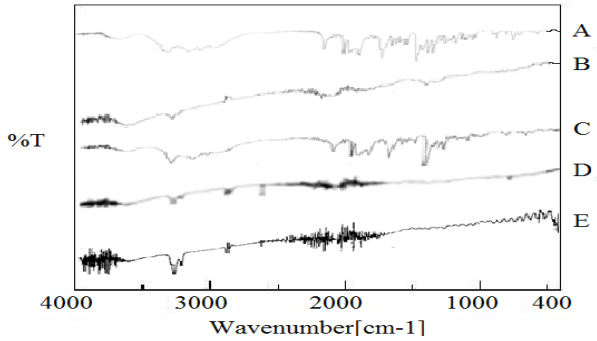
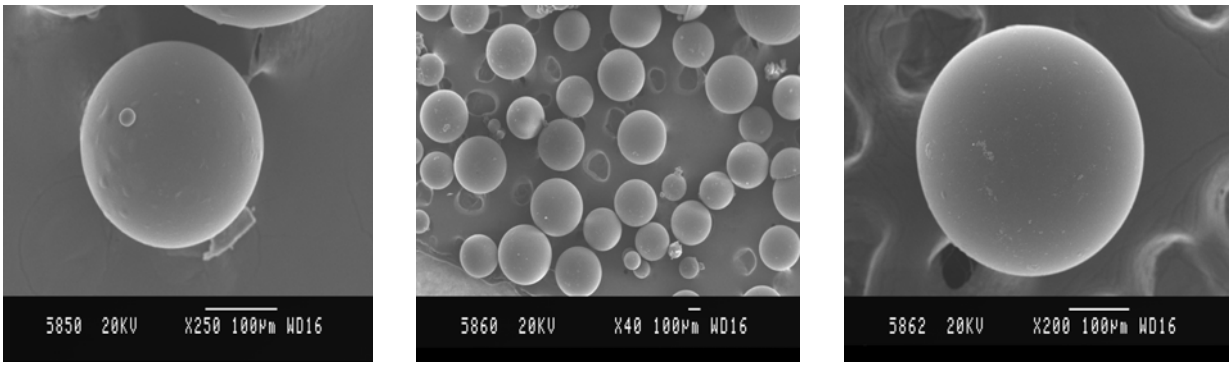


Fig. 1: Fourier transform infrared (FTIR) spectrum



(a) (b) (c)

Fig. 2: Scanning Electron Micrographs (SEM) of Trimetazidine hydrochloride microspheres

The results was similar for microspheres prepared with high concentration of Gelatin with a release of 83.92% for formulation at pH 1.2 and sustained release at pH 7.4. An initial burst release of drug was observed from all the batches that can be attributed to two reasons, the leaching of drug on the microspheres outer surface and faster ingress of dissolution medium and subsequent diffusion of drug. However, on changing the pH from lower to higher level, the drug released slowed (Fig 5.1). At the end of 12 h, 93.82% of drug was released from formulation. Similar pattern was observed in the case of other formulations. Fig 5. Data obtained for *in vitro* release studies was utilized for release kinetics. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range of 0.5 to 1 indicating Non-Fickian of drug through Trimetazidine hydrochloride microspheres were given in Table 2.

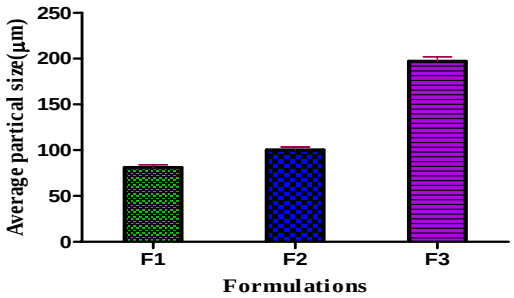


Fig. 3: Average diameter of Trimetazidine hydrochloride microspheres

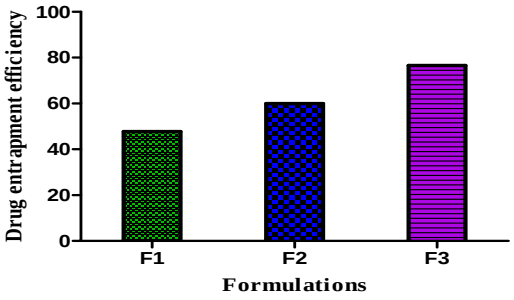
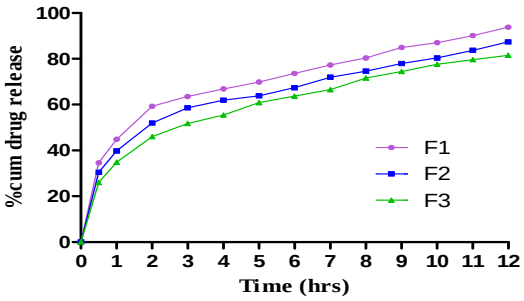
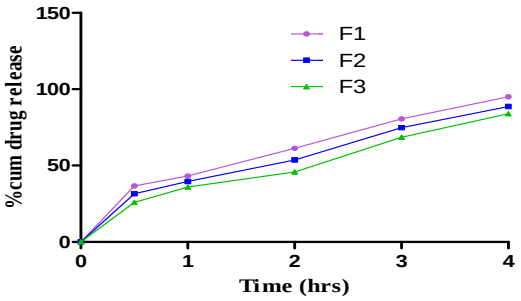


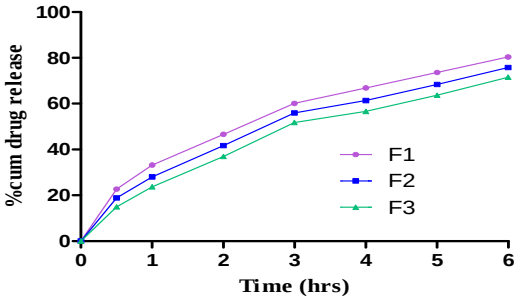
Fig. 4: Drug entrapment efficiency of Trimetazidine hydrochloride microspheres



(a) In vitro drug release was tested for first two hours in pH 1.2 and change to pH 7.4



(b) In vitro drug release profile in pH 1.2 for 4 hrs



(c) **In vitro drug release profile in pH 7.4 for 6 hrs**

Fig. 5: *In vitro* release of Trimetazidine hydrochloride microspheres

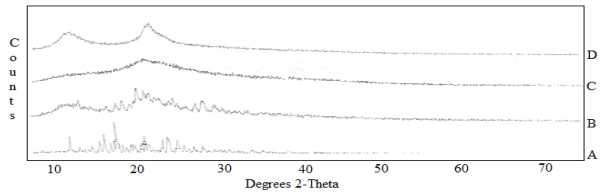


Fig. 6: X-ray Thermograms (XRD)

Wide angle X-ray diffraction patterns of the Trimetazidine hydrochloride, physical mixture of Trimetazidine hydrochloride and polymer, formulation and blank microspheres were shown in Fig 6. The XRD data indicates that the Trimetazidine hydrochloride is still present in its lattice structure in the physical mixture where as it is completely amorphous inside the Trimetazidine hydrochloride microspheres. This may be due to the conditions used to prepare the Trimetazidine hydrochloride microspheres lead to cause complete TMH amorphization.

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