Formulation and in vitro Evaluation of Eudragit L100 Microspheres of piroxicam

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Abstract

The aim of this study was to formulate and evaluate controlled release preparations of a water-insoluble drug using Copolymers synthesized from acrylic and methacrylic acid esters (Eudragit L 100) as the retardant material. Microspheres were prepared by solvent evaporation method using an alcohol / liquid paraffin system. Span60 was used as the droplet stabilizer. The prepared microspheres were characterized for their micromeritic properties and drug loading, as well by, differential scanning calorimetry. The in vitro release studies were performed first in pH1.2 and then pH 6.8, phosphate buffer. The prepared microspheres were yellow, and spherical in shape. The drug-loaded microspheres showed 71-85% of entrapment and release was extended up to 7 h. The differential scanning calorimetry thermographs showed stable character of drug in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. The best-fit release kinetics was achieved with Higuchi plot. The release of drug was influenced by the drug to polymer ratio and particle size that was found to be diffusion controlled.

Keywords: Microsphere, drug release rate, eudragit, piroxicam.

Introduction

The population of patient with chronic diseases or complications of other diseases have recently been increasing. These situations necessitate taking drug for a long period and/or multiple medicines simultaneously, which can lead to increase in non-compliance. The problem would be worse for drugs with short biological half-life. One method to solve such problems is to find a dosageform capable of releasing the drug gradually. Microsphere has been used as one of the methods to deliver drugs in a controlled manner (1). Off-white to light tan or light yellow, odourless powder. Very slightly soluble in water, in dilute acids, and in most organic solvents; slightly soluble in alcohol and in aqueous alkaline solutions. Store in airtight containers. Protect from light (2) and for prevent of its side effect in gastric preparation of Entric coated microsphere of it seem a relevant idea. Microencapsulated techniques have mostly been used for lipophilic drugs since hydrophilic drugs showed low loading efficiency. The objective of the present investigation was to prepare controlled release microspheres of drug by improving biological half-life and entrapment efficiency (3). In this study, microspheres were prepared by solvent evaporation technique using Eudragit L 100 as a matrix polymer (4). Eudragit L 100 is a derivative of methacrylic acid that is soluble in intestinal fluid from pH 5.5 and used for entric coating of piroxicom (5). Liquid paraffin and Ethanol system were used for the preparation of microspheres. Span60 was used as a droplet stabilizer to prevent droplet coalescence in the oil medium (6). The effect of various processing and formulation factors such as drug to polymer ratio, stirring speed and surfactant concentration on the mean particle size of microspheres was investigated. The prepared spherical microspheres were evaluated for micromeritic properties and drug content, and also by DSC, as well as for in vitro drug release studies (7).

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MATERIALS AND METHODS

Materials

Piroxicam and Eudragit L 100 was obtained as a gift from the department of pharmaceutics of Shahid Beheshti University of medical science. All other reagents and solvents used were of pharmaceutical or analytical grade and were obtained from Shahid Beheshti laboratory of Iran.

Method

Microspheres were prepared by solvent evaporation technique. Different amounts of Eudragit were dissolved in 8 ml ethanol by using a magnetic stirrer. The resulting dispersion was then poured into 250 ml beaker, containing the mixture of 100 ml liquid paraffin light and 0.2% Span 60, while stirring. A mechanical stirrer with a 4 blade paddle was used. Stirring (at 500 rpm) was continued for 4 hour, until alcohol evaporated completely. After evaporation of alcohol, the microspheres formed were filtered using filter paper. The residue was washed 4-5 times by 50 ml of dichloromethane. Microspheres were dried at room temperature for 24 h. Formulations containing 750 mg of Eudragit L100. All batches were prepared in triplicate.

Microspheres dried at room temperature were then weighed and the yield of microspheres preparation was calculated using the following formula (7):

Percent yield = (the amount of microspheres obtained (g)/ the theoretical amount (g) of non volatile material) X 100

Morphology and particle size of microsphere

About 200 particles of each Serial of preparations were transferred on a lam and their size and shape were investigated by a scaled optical microscope with magnification ratio of 10 and by its SEM image. Its SEM image also was obtained and is shown in figure 1.

Drug entrapment efficiency

About 20 mg of accurately weighed drug-loaded microspheres were added to 20 ml of chloroform and ethanol (1:1). The resulting mixture was shaken in a mechanical shaker for 1 h. The solution was filtered with a paper filter and 2 ml of this solution was appropriately diluted to 25 ml using chloroform, and analyzed spectrophotometrically at 335 nm by UV-Visible Spectrophotometer.

%EE= (Percent of real loaded drug/Theoretical loaded drug) X 100 (9).

Results were bring in the follow in table 1.

Differential Scanning Calorimetry (DSC)

The DSC analysis of pure piroxicam and drug-loaded microspheres was carried out using a Diamond DSC to evaluate any possible drug-polymer interaction. The analysis was performed at a rate 10.00 C min^-1 from 00 C to 2700 C temperature range under nitrogen flow of 30 ml min^-1(10).

Drug release studies

The in vitro release studies of drug-loaded microspheres were carried out at 37 °C. The in vitro piroxicam release was analyzed by the use of a basket apparatus (USP I). Drug-loaded microparticles were suspended in 300 ml phosphate buffer systems of different pH (first in pH 1.2 and then in pH 6.8). The dissolution medium was kept under stirring at 50 rpm. All the experiments were carried out at 37 C for 6 h. Aliquots of the dissolution medium (300 ml) were withdrawn at predetermined time intervals. Piroxicam concentrations were analyzed by the UV-spectrophotometer via calibration curve in relevant medium. Accurately weighed samples of microspheres (approx. 500 mg) were added to dissolution medium and at preset time intervals 5 ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. The samples were analyzed spectrophotometrically at 335 nm. The concentration of piroxicam in test samples was corrected and calculated using a regression equation of the calibration curve.

Release Kinetic

Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of piroxicam release from microspheres. The kinetics models used were zero order, first order, and Higuchi models. The rate constants were also calculated for the respective models.

Results and Discussion

From effect of various processing and formulation parameters on mean particle size it was observed that when continuous phase was aqueous, selection of a suitable dispersed phase respect to solubility of polymer and piroxicam is restricted and a stabilized emulsion couldn’t be formed. It was observed that when the speed of stirrer was below 500 rpm, there was no formation of spherical microspheres and foliate or mass crops were obtained. This could be due inadequate agitation to disperse the inner phase in the total mass. Therefore, particles were found to settle at the bottom of vessel. At stirring speeds of 700-1000 rpm, the resulting high turbulence caused frothing and adhesion to the container wall and paddle. Therefore, the mean
particle size of microspheres decreased. The desired spherical microspheres were obtained at stirring speeds of 500 When 0.2% of span60 was incorporated, and without it, microspheres were not formed because it prevents droplet coalescence in the oil medium. Span60 in contrast to tweens had relevant HLB in this medium. Spherical microspheres were formed when 8ml of Ethanol was used as dispersed phase because of its required properties and viscosity. When the drug: polymer ratio was 1:1, there was formation of microspheres with small and irregular size, and as the polymer concentration was increased, solution viscosity also increased, resulting in large particles. Thus, mean particle size also increased until this ratio reached to 1:7.5.

**Differential Scanning Calorimetry (DSC)**

The piroxicam may have been dispersed in crystalline or amorphous form or dissolved in the polymeric matrix during formation of microspheres. Any abrupt or drastic change in the thermal behavior of either the piroxicam or constant, therefore likely there was no obvious chemical interaction among Eudragit and piroxicam. After 200°C where the polymer is in the form of liquid a mild interaction between polymer and drug or degradation may be occurred.

**Drug release behavior**

The pure piroxicam showed a fast release as 90 % was released within 15 min. When it was encapsulated, sustained release up to 7 h was observed. The smaller particle size, the larger surface area available for piroxicam release is available.

**Release Kinetics**

The release mechanism of piroxicam from formulation was determined by comparing their respective correlation coefficient. It would appear that the mechanism of piroxicam release from microspheres was diffusion-controlled. When the release rate constants of piroxicam microspheres were compared, it was found to follow the Higuchi model as has indicated below in figures 3 and 4.

**CONCLUSION**

Microspheres were prepared successfully using the solvent evaporation method. Polymer: piroxicam ratio, stirring speed and the content of Span60 influenced the sphericity of the microspheres. The yield and entrapment efficiency were high for all formulations. It was observed that with increase in polymeric particle concentration, the mean particle size of the microspheres increased but increasing the polymer may indicate a possible drug-polymer interaction. As is shown in figure 2 the thermogram of pure piroxicam shows an endotherm at 204.70 C, which corresponds to its melting point. This endotherm was also observed for microspheres at 221.70 C but it was less sharp and this suggests that there is a significant reduction in piroxicam crystallinity in the polymer matrix (12, 13). In the physical mixture of piroxicam and polymer both peaks of piroxicam and polymer are observed but in microsphere the ratio of piroxicam is lower and molecular dispersion has occurred so crystalline length of piroxicam is very short and its peak has approximately been disappeared. Furthermore the peak of polymer has been broadened. Comparative thermograms of piroxicam and its physical mixture with polymer and prepared microsphere have been shown in fig 2 that indicate pure polymer has 2 broad peaks and maybe there is a polymorphic transformation in the structure of the polymer but their location have remained stirring speed, resulted in a decrease in the mean particle size of microspheres. The assessment of the release kinetics revealed that drug release from piroxicam microspheres followed Higuchi Model. It was suggested that mechanism of piroxicam release from microspheres was diffusion-controlled. Controlled release with initial peak level achieved with these formulations may reduce dose frequency and side effects as well as improve the patient compliance.

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**References**

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<table>
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<th>Batch Code</th>
<th>Yield (%)</th>
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Table 1. Physical characteristics of the microspheres.
Fig. 2. DSC Thermograms of Microspheres and drug, polymer and their physical mixture (up to down: Microsphere, Eudragit L100 only, physical mixture of drug and Eudragit, Drug only.

Fig. 3. Depicting the cumulative percent of release vs time.

\[ y = 5.0583x + 12.03 \]
\[ R^2 = 0.9868 \]

\[ y = 0.178x + 1.7342 \]
\[ R^2 = 0.9501 \]

Fig. 4. Best fitted trend line to higuchi model for release.