MICROENCAPSULATION OF PIROXICAM USING PH SENSITIVE POLYMERS

Esmat E. Zein, Ahmed A. Donia and Sania El-kayad*

Faculty of Pharmacy, Pharm. Technology Dept. Tanta University, Tanta, Egypt.

*Corresponding Author: Dr. Sania El-kayad
Faculty of Pharmacy, Pharm. Technology Dept. Tanta University, Tanta, Egypt.

ABSTRACT

Different techniques are utilized to reduce gastrointestinal side effects of non-steroidal anti-inflammatory drugs (NSAIDs) including microencapsulation by pH sensitive polymers. Enteric coated piroxicam microspheres were prepared using emulsion solvent evaporation method using Eudragit L100 and Eudragit S100 as pH sensitive polymers for enteric coating of piroxicam. Entrapment efficiency and percent yield was measured for the prepared formulations and it was found that it depends on polymer type as well as drug polymer ratio. IR analysis was used to predict any interaction between drug and the used polymers. It revealed presence of intermolecular hydrogen bonding between piroxicam and Eudragit polymers. In vitro dissolution test was carried out using three different PH media (1.2, 6.8 and 7.4) to determine release of drug at different pH values. All formulations showed burst release at pH 1.2 but Eudragit L100 polymer containing formulations showed the highest initial rapid burst release. Formula B2 was found to be the best formula as it has only 6% burst release within 2 hrs at pH 1.2.

KEYWORD: Eudragit L100 and Eudragit S100

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are well known for their gastrotoxic and duodenotoxic effects. A growing proportion of elderly patients require NSAIDs therapy for the treatment of osteoarthritis or rheumatoid arthritis.

Piroxicam is one of the most potent (NSAIDs) used in musculoskeletal as well as joint disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis.[3] Piroxicam suffers from the same gastrointestinal side effects as all (NSAIDs).

Early studies using pH-sensitive polymers have been interested in pH variation in the gastrointestinal (GI) tract due to the most pronounced and huge pH fluctuation (pH 1–8) in the human body and have focused on the development of controlled delivery formulations for oral administration (e.g., enteric coating materials to protect stomach and/or acid-labile drugs in the stomach, colon specific delivery systems with a pH modulated release property, and taste masking materials for bitter drugs).[2-4]

pH-sensitive polymers are those of which solubility or conformation in aqueous solution is reversibly or irreversibly changeable by environmental pH. They are a class of polyelectrolytes that have ionizable groups in their structures of backbone, side group, or end group and demonstrate pH-dependent physico-chemical properties.[5]

One of the most representative pH-sensitive polymers developed for enteric coating purpose is methacrylic acid copolymers with methyl methacrylate or ethyl acrylate (Eudragits®) that already have a long history of use. Eudragit L100 and Eudragit S100 are polymers of choice for preparation of pH sensitive microspheres of piroxicam.

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the drug to the target tissue in the optimal amount in the right period of time thereby causing little toxicity as well as minimal side effects. One such approach is using microspheres as carriers for drugs.[6]

The main objective of this research is preparation of piroxicam microspheres using pH sensitive polymers of Eudragit L100 and Eudragit S100 to reduce its GI side effects.

2. MATERIALS AND METHODS

2.1. Materials

Piroxicam was purchased from Pfizer, New York, USA. Dichloromethane, ethanol, and sodium lauryl sulphate
were purchased from ISO-CHEM Company, China. All other chemicals and reagents were of analytical grade and used as received.

2.2. Methods

2.2.1. Construction of calibration curves

A suitable and accurately weighed quantity of piroxicam was dissolved in methanol to obtain a stock solution. Standard solutions were prepared by dilution of the stock solution with phosphate buffer (pH 6.8, 7.4) and SGF without pepsin (pH1.2). Ultraviolet absorbance of the solutions was determined spectrophotometrically (Thermo, Evo300pc, USA) at the wavelength of maximum absorbance at 354 nm for pH 6.8, 353 nm for pH 7.4 and 334 nm for pH 1.2.[9]

2.2.2. Preparation of piroxicam microspheres

Piroxicam microspheres were prepared by emulsification solvent evaporation method.[9] Drug and Eudragit polymers were used in ratios of 1:1, 1:3 and 1:5 in order to obtain significant different characteristics. Eudragit polymers used were L_{100} and S_{100}. The required amounts of the polymer were dissolved in a mixture of dichloromethane and ethanol (2:3 v/v). The calculated amounts of piroxicam powder were dissolved in the polymeric solutions. The prepared dispersions were slowly poured into 200 ml of 0.2 % w/v sodium lauryl sulphate aqueous solution and were emulsified by vigorous stirring at 1300 rpm at room temperature using magnetic stirrer. The dispersed drug and polymers were immediately transformed into fine droplets, which were subsequently solidified into rigid microspheres due to solvent evaporation.[10] Stirring was continued for 3-4 hrs until all solvent was evaporated. The formed microspheres were allowed to settle, filtered and washed several times with distilled water.[11] The microspheres were dried and stored in air tight containers until further analysis. The compositions of the drug as well as the other additives in addition to the conditions of the preparation are illustrated in Table 1.

Table 1: The composition and the conditions of preparation of different microspheres formulations

<table>
<thead>
<tr>
<th>Polymer used</th>
<th>Formula No.</th>
<th>Drug :polymer ratio</th>
<th>Stirring rate(rpm)</th>
<th>Surfactant conc.(w/v)</th>
<th>Internal phase volume(ml)</th>
<th>External phase volume(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit L_{100}</td>
<td>A1</td>
<td>1:1</td>
<td>1300</td>
<td>0.2%</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>1:3</td>
<td>1300</td>
<td>0.2%</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>1:5</td>
<td>1300</td>
<td>0.2%</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>Eudragit S_{100}</td>
<td>B1</td>
<td>1:1</td>
<td>1300</td>
<td>0.2%</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>1:3</td>
<td>1300</td>
<td>0.2%</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>1:5</td>
<td>1300</td>
<td>0.2%</td>
<td>50</td>
<td>200</td>
</tr>
</tbody>
</table>

3. Evaluation of the prepared microspheres

3.1. Surface morphology (SEM)

Scanning electron microscopy has been used to determine the surface morphology and texture of the prepared microspheres. 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 25 KV.[12]

3.2. Drug-polymer interaction (FTIR study)

IR spectrophotometer was used to indicate interaction (if any) between drug and polymers. IR spectroscopy was performed using Fourier- transform infrared spectrophotometer, (Jasco, Japan). Samples were mixed with potassium bromide (spectroscopic grade) and compressed into disks using hydraulic press before scanning between 4000 and 400 cm^{-1}. FTIR study was carried out on pure drug, Eudragit polymer, physical mixture of drug and polymer as well as prepared microspheres formulations.[13]

3.3. Percentage yield

The prepared microspheres were collected and weighed.[14] The actual weight of obtained microspheres divided by the total amount of all materials that was used for the preparation of the microspheres. Percentage yield of the microspheres was calculated as follow:

\[
\% \text{ yield of prepared microspheres} = \frac{\text{Actual weight of the product}}{\text{Total weight of excipients and drug}} \times 100
\]

3.4. Entrapment efficiency

The entrapment efficiency (%) of the prepared microspheres was evaluated using the method of Gangadhara et al.[15] with certain modification. About 25mg of the obtained microspheres were crushed into powder and were completely dissolved in 100ml of Phosphate buffer solution (pH 7.4) and agitated in a mechanical shaker for 6hrs then kept for 24hrs. Five ml of the obtained solution was filtered then the concentration of the drug was determined spectrophotometrically at 353nm after appropriate dilution.[16,17] The actual drug loading and encapsulation efficiency (EE %) were calculated using the following equations:

\[
\text{Theoretical drug loading} \% = \frac{\text{Drug (total)}}{\text{Drug (total) + polymer}} \times 100
\]

\[
\text{Actual drug loading} \% = \frac{\text{Drug (entrapped)}}{\text{Drug (total) + polymer}} \times 100
\]

\[
\text{Encapsulation efficiency} \% = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100
\]

3.5. In vitro drug release study

In vitro drug release from the prepared microspheres was performed in different pH media (1.2, 6.8 and 7.4) at 37 ±0.5. The release of piroxicam from microspheres was
determined using type 2 dissolution apparatus (Copley, NG 42JY, Nottingham, UK). Microspheres equivalent to 20 mg were weighed accurately and added to 900 ml of dissolution medium. The contents were rotated at 100 rpm. The pH of the dissolution medium was kept at 1.2 for 2 hrs and 6.8 for 4 hrs then the pH of the dissolution medium was adjusted to 7.4 using 0.1N NaOH and dissolution was continued for another 2 hrs. Five ml samples were withdrawn from the dissolution medium at various time intervals and replaced with 5 ml fresh media to keep sink conditions. The concentration of the drug released was analyzed using UV spectrophotometer.

4. RESULTS AND DISCUSSION

4.1. Entrapment efficiency and percentage yield

The percent entrapment efficiency varies according to different variable parameters such as polymer type and drug to polymer ratio. For polymer type, the higher viscosity of Eudragit S100 solution than Eudragit L100 leads to the formation of larger droplets under the same stirring conditions and thus less entrapment efficiency. A greater size of Eudragit S100 micro particles prepared under the conditions as Eudragit L100 micro particles was attributed to the nature of the polymer.[18] The results of entrapment efficiency and percentage yield is illustrated in Table 2.

Table 2: Characterization of piroxicam microspheres

<table>
<thead>
<tr>
<th>Formula</th>
<th>Entrapment efficiency (%)</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>70.94±0.02</td>
<td>90.65±0.3</td>
</tr>
<tr>
<td>A2</td>
<td>60.17±0.01</td>
<td>87.35±0.5</td>
</tr>
<tr>
<td>A3</td>
<td>50.96±0.03</td>
<td>90.98±0.4</td>
</tr>
<tr>
<td>B1</td>
<td>55.42±0.02</td>
<td>80.94±0.3</td>
</tr>
<tr>
<td>B2</td>
<td>64.86±0.01</td>
<td>81.57±0.2</td>
</tr>
<tr>
<td>B3</td>
<td>58.59±0.01</td>
<td>80.46±0.4</td>
</tr>
</tbody>
</table>

Each result is the mean of 3 determinations ± S.D

From Table 2, it is obvious that Formulations containing Eudragit L100 polymer indicates that entrapment efficiency decreases from formula A1-A3 depending on drug to polymer ratio. Increasing polymer concentration increases viscosity of the solution which was responsible for the formation of larger polymer/solvent droplets. It caused a decreased rate of entrapment of drug due to slower hardening of the larger particles allowing time for drug diffusion out of the particles which tends to decrease the encapsulation efficiency.[19] For formula B1-B3 containing Eudragit S100 polymer increasing polymer concentration increases encapsulation efficiency until formula B2 then increasing polymer concentration decreases encapsulation efficiency. The contribution of a high polymer concentration to the encapsulation efficiency can be due to better coating of drug resulting from precipitation of polymer on the surface of the dispersed phase which leads to preventing of drug diffusion across the phase boundary. Then further increase in the viscosity of the polymer solution of formula B3 than B2 results in decreasing encapsulation efficiency.

4.2. Scanning electron microscope and IR analysis

Scanning electron microscope results indicate that Eudragit microspheres of piroxicam were successfully prepared using emulsion solvent evaporation method. The prepared microspheres were spherical in shape with voids and pores on its surface due to solvent evaporation as shown in Figure 1.

The FTIR spectrum of piroxicam, Eudragit L100, Eudragit S100, physical mixture of piroxicam and Eudragit L100 and S100 polymers as well as the prepared microspheres are presented in Figure 2. Piroxicam has two possible
tautomeric forms, the 1724 cm\(^{-1}\) band was not observed in the obtained IR spectrum suggesting that piroxicam is present in its enol form to interact with intramolecular hydrogen bonding in the piroxicam structure.\(^{[20,21]}\) Physical mixture spectrum indicates only the summation of piroxicam and Eudragit polymer spectra revealing that there is no interaction between them. The IR absorption peaks at 1632 cm\(^{-1}\) and 1529 cm\(^{-1}\) are due to the stretching vibration of the carbonyl group and the second amide band respectively. IR spectrum of Eudragit L\(_{100}\) and Eudragit S\(_{100}\) shows broad peak at 3463 cm\(^{-1}\) and 3500 cm\(^{-1}\) respectively due to hydroxyl group stretching vibration which differs according to the difference between the two polymers in the hydroxyl group ratios that gives different possibility in the hydrogen bonding formation. The IR peaks at 1632 and 1529 cm\(^{-1}\) of pure piroxicam shifted to 1640 and 1526 cm\(^{-1}\) for the piroxicam microspheres respectively. This is due to the carbonyl stretching peak at 1632 cm\(^{-1}\) which previously formed the intramolecular hydrogen bond in the piroxicam structure, once the interaction occurred between piroxicam and Eudragit L\(_{100}\) or Eudragit S\(_{100}\) the intramolecular hydrogen bonding disappeared and the peak at 1632 cm\(^{-1}\) shifted to the higher wave number of 1640 cm\(^{-1}\). Moreover, the peak at 1529 cm\(^{-1}\) assigned the second amide band of piroxicam shifted to 1526 cm\(^{-1}\), due to the intermolecular interaction between piroxicam and Eudragit polymers. Other new peaks at 1600 and 1329 cm\(^{-1}\) also might be due to the complex formation of piroxicam and Eudragit polymers.\(^{[22]}\) IR spectrum of piroxicam shows a characteristic peak at 3354 cm\(^{-1}\) which may be due to NH or OH stretching vibration while microspheres spectrum of both Eudragit polymers have peak at 3454 cm\(^{-1}\) which may indicate presence of intermolecular interaction between piroxicam and the used Eudragit polymers. The results of the IR analysis is presented in (Fig. 2).

**Fig. 2** IR spectrum of (a) piroxicam, (b) Eudragit L\(_{100}\) polymer, (c) Eudragit S\(_{100}\) polymer, (d) physical mixture of piroxicam and Eudragit L\(_{100}\), (e) physical mixture of piroxicam and Eudragit S\(_{100}\), (f) prepared piroxicam and Eudragit L\(_{100}\) microspheres, (g) prepared piroxicam and Eudragit S\(_{100}\) microspheres.

**4.3. In vitro release results**

Stimuli-responsive polymers show a sharp change in properties upon a small or modest change in environmental condition, e.g. temperature, light, salt concentration or pH. This behavior can be utilised for the preparation of so-called ‘smart’ drug delivery systems, which mimic biological response behaviour to a certain extent. The possible environmental conditions to be used for this purpose are limited due to the biomedical setting of drug delivery as application. Different organs, tissues and cellular compartments may have large differences in pH, which makes the pH a suitable stimulus. The pH is an important signal, which can be addressed through pH-responsive materials.\(^{[23]}\)

pH sensitive polymers named as polyacids or polyanions, such as, poly(acrylic acid) (PAA) or poly(methacrylic) acid (PMAA) are polyanions that have in their structure a great number of ionizable acid groups, like carboxylic acid or sulfonic acid.\(^{[24]}\) The carboxylic groups accept protons at low pH values and release protons at high pH values.\(^{[25]}\) Thus, when the pH increases the polymer swells due to the electrostatic repulsion of the negatively charged groups. The pH in which acids become ionized depends on the polymer’s pKa (depends on the polymer’s composition and molecular weight). Thus, in an oral drug delivery system, the poly (acrylic acid) polymer retains the drug on the presence of acid pH (stomach), delivering it in alkaline pH (small intestine). The drug delivery occurs due to the ionization of pendant groups of carboxylic acids, forcing the polymer to swell.\(^{[26]}\) Eudragit L\(_{100}\) and Eudragit S\(_{100}\) are important polymers of these groups.

Eudragit polymers are copolymers derived from esters of acrylic and methacrylic acid, whose physicochemical properties are determined by functional groups (R). Eudragit polymers are available in a wide range of different physical forms (aqueous dispersion, organic solution granules and powders). To protect the active ingredient from the gastric fluid and to improve drug effectiveness– Eudragit L and S polymers are preferred choice of coating polymers. They enable targeting specific areas of the intestine. In addition, the different grades can be combined with each other, making it possible to adjust the dissolution pH, and thus to achieve
the required GI targeting for the drug. Targeted drug release in the colon is required for local treatment of intestinal disorders such as Crohn’s disease, ulcerative colitis or intestinal cancer. It is also required for drugs that are poorly soluble in the upper gastrointestinal tract. Moreover, the gastroresistance of the coating ensures that the oral dosage form is patient compliant.[27]

Eudragit L100 and S100 polymer are anionic copolymer based on methacrylic acic and methyl methacrylate which show dissolution at pH 6 and pH 7 respectively. These make them a suitable candidate for preparation of piroxicam microspheres to reduce gastric ulceration side effects of piroxicam.

Dissolution was carried out at different PH media (1.2, 6.8, and 7.4) in order to determine the effect of changing pH on the release of the drug from different microspheres formulations. Piroxicam shows different solubilities at different pH values as its solubility is pH dependent (Fig. 3).

![Fig. 3. Dissolution profiles of piroxicam in a free form at different pH values.](image)

The in vitro release of the drug from the microspheres prepared by the solvent evaporation method was reported to be biphasic in nature and rapid with a burst effect.[28] Eudragit L100 formulations show initial rapid burst release with more than 50% of drug released within 2 hrs at pH 1.2 (Fig.4). At pH 6.8 there is a rapid release of the drug as more than 75% released after 15 min. Formula A3 shows the highest amount released of the drug while A1 has the least amount released depending on drug to polymer ratio (Fig.5).

![Fig. 5. Dissolution profiles of piroxicam from its microspheres of different formulations of eudragit L100 polymer at pH 6.8.](image)

![Fig. 6. Dissolution profiles of piroxicam from its microspheres of different formulations of eudragit S100 polymer at pH 1.2.](image)
5. CONCLUSION

Enteric coated microspheres of piroxicam using Eudragit L100 and Eudragit S100 were prepared using emulsion solvent evaporation technique in order to reduce its gastrointestinal side effects. Entrapment efficiency and percent yield were measured to evaluate the prepared formulations. IR analysis indicated presence of intermolecular hydrogen bonding between drug and Eudragit polymers. All formulations showed burst release at pH 1.2 during in vitro dissolution test except for formula B2 which show only 6% burst release within 2 hrs at pH 1.2 so it is considered the best formula.

6. REFERENCES


