PREPARATION, CHARACTERIZATION AND IN-VITRO EVALUATION OF PIROXICAM MICROSPHERES

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ABSTRACT
Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used particularly in elderly patients for both rheumatic as well as non-rheumatic conditions. Elderly patients are more prone to the adverse effects of these drugs. Adverse effects are seen in almost every organ system, but GI tract is most frequently affected as they cause GI irritation, bleeding and also peptic as well as duodenal ulceration. Piroxicam is one of these drugs which are commonly used for treatment of ankylosing spondylitis, osteoarthritis and rheumatoid arthritis which need long term treatment. In order to reduce the GI side effects of piroxicam, microspheres of the drug were prepared with different polymers of time dependant eudragit polymers. Different techniques of microencapsulation were experienced to prepare controlled release formulations of piroxicam. Solvent evaporation technique was the technique of choice for this purpose. The obtained microspheres were evaluated using scanning electron microscopy (SEM) as well as IR spectroscopy. The obtained results showed the presence of intermolecular hydrogen bonding between the drug and polymer. SEM indicated that microspheres have rough and porous surface due to solvent evaporation during preparation. Drug percent yield was measured and ranged from 74.24% to 92.03%. Dissolution studies which were performed at pH 1.2, 6.8 and 7.4 (representing stomach and intestinal conditions respectively) showed that the drug release was continuous and faster at pH 7.4 and the cumulative drug release reached 89% within 8 hrs.

KEYWORDS: Piroxicam, ankylosing, dependant eudragit.

1. INTRODUCTION
Non-steroidal anti-inflammatory drugs are the most prescribed medications for treating conditions such as arthritis but have serious side effects in almost every organ system, whereas GI tract is the most frequently affected. Piroxicam is a member of the oxicam group of non-steroidal anti-inflammatory drugs. It is indicated for acute or long term use in the relief of signs and symptoms of osteoarthritis and rheumatoid arthritis. Piroxicam is a non-selective COX enzyme inhibitor with a proven higher risk of serious gastrointestinal complications compared to other non-steroidal anti-inflammatory drugs. Its bioavailability is between 45-75% after oral administration. Piroxicam is a class 2 drug according to biopharmaceutical classification system thus it has low solubility and high permeability, so dissolution is the rate limiting step of bioavailability. Microencapsulation is one of the most interesting fields in the area of pharmaceutical technology by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material. It is a promising technique that is used to reduce GI-disorders of acidic drugs (NSAIDs), allow controlled release of active compounds (sustained or delayed release), targeted release of encapsulated materials, improve the bioavailability of water insoluble drugs as well as patient compliance and also allows masking of unpleasant taste. The eudragits are a family of polymers based on acrylic and methacrylic acids suitable for use in orally administered drug delivery systems. It has different grades of pH and time dependant release. They have been used in the microencapsulation of drugs. The main objective of this research is preparing microspheres of piroxicam using emulsion solvent evaporation method and evaluating the physico-chemical properties as well as the release of the drug from the prepared microspheres.

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.[8]

2.2.2. Preparation of piroxicam microspheres
Piroxicam microspheres were prepared by emulsification solvent evaporation method.[9] Drug and eudragit polymer were used in ratios of 1:1, 1:3 and 1:5 to obtain significant different characteristics. Eudragit polymers used are Rs100 and Rl100. The required amounts of the polymer were dissolved in a mixture of dichloromethane and ethanol (2:1 v/v). The calculated amounts of piroxicam powder were dissolved in the polymeric solution. The prepared dispersion was slowly poured into 200 ml of 0.2 % w/v sodium lauryl sulphate aqueous solution and was emulsified by vigorous stirring at 1300 rpm at room temperature using magnetic stirrer. The dispersed drug and polymer 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

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<tr>
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<tbody>
<tr>
<td>Eudragit Rs100</td>
<td>A1</td>
<td>1:1</td>
<td>1300</td>
<td>0.2%</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>1:3</td>
<td>1300</td>
<td>0.2%</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>1:5</td>
<td>1300</td>
<td>0.2%</td>
<td>22.5</td>
<td>200</td>
</tr>
<tr>
<td>Eudragit Rl100</td>
<td>B1</td>
<td>1:1</td>
<td>1300</td>
<td>0.2%</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>1:3</td>
<td>1300</td>
<td>0.2%</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>1:5</td>
<td>1300</td>
<td>0.2%</td>
<td>22.5</td>
<td>200</td>
</tr>
</tbody>
</table>

3. Evaluation of the prepared microspheres
3.1. Drug-polymer interaction (FTIR study)
IR spectrophotometer was used to indicate interaction (if any) between drug and polymer. 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.[12]

3.2. Percentage yield
The prepared microspheres were collected and weighed.[13] The actual weight of obtained microspheres divided by the total amount of all materials that was used for the preparation of the microspheres:

% yield of prepared microspheres = (Actual weight of the product/Total weight of excipients and drug) X 100.

3.3. 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 20 KV.[14]

3.4. Flow properties of microspheres
The flow properties of microspheres were investigated by determining the angle of repose, bulk density, tapped density, Carr’s index as well as Hausner’s ratio. The angle of repose was determined by the fixed-based funnel method. Bulk and tapped densities were measured in 10 mL of a graduated cylinder. The cylinder was tapped from a height of 2 inches until a constant volume was obtained. The volume occupied by the sample after tapping was recorded and bulk density, tapped density, Carr’s index and Hausner’s ratio was calculated.[15]

3.5. Entrapment efficiency
The entrapment efficiency % of the prepared microspheres was evaluated using the method of Gangadhar et al. with certain modification.[16] 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.[17, 18] The actual drug loading and encapsulation efficiency (EE %) were calculated using the following equations:

Theoretical drug loading (%) = (Drug (total) / (Drug (total) + polymer)) X 100
Actual drug loading (%) = (Drug (entrapped) / (Drug (total) + polymer)) X 100
Encapsulation efficiency (%) = (Actual drug loading/Theoretical drug loading) X 100
3.6. In vitro drug release study
In vitro drug release from the microspheres was performed in different pH media (1.2, 6.8 and 7.4) at 37 ±0.5°C. 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. Scanning electron microscope
Eudragit microspheres of piroxicam were successfully prepared by emulsion solvent evaporation technique in different drug to polymer ratios. Piroxicam microspheres were spherical in appearance, although some were found to be elongated. The surface morphology of the prepared microspheres was investigated using scanning electron microscope. Microspheres have rough and porous surface due to solvent evaporation during preparation. Also SEM photomicrographs indicated that microspheres were hollow in nature. Fig. 1 shows the surface morphology of the prepared microspheres using SEM.

4.2. Entrapment efficiency and percentage yield
The percent entrapment efficiency was changed according to polymer type and drug to polymer ratio. It ranged from 60.61-80% for eudragit Rs,000 and 43.03-61.18% for eudragit Rl,000 (Table 2). Entrapment efficiency increased by increasing amount of drug and decreasing amount of polymer. The high drug loading typically results in lower encapsulation efficiency due to high concentration gradient resulting in the drug to diffuse out of the polymer/solvent droplets to the external processing medium. The viscosity of the polymer solution was very high from formula A3-A1 and from F3-F1 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] At high polymer concentration entrapment efficiency decreases, this may be due to dilution of the drug in polymer. Also the percentage yield of microspheres of all formulations was found in the range of 74.24-92.03 % as illustrated in Table 2.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Entrapment efficiency (%)</th>
<th>Percentage Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>80±0.85</td>
<td>83.74±0.22</td>
</tr>
<tr>
<td>A2</td>
<td>70.32±2.59</td>
<td>86.42±0.37</td>
</tr>
<tr>
<td>A3</td>
<td>60.61±2.26</td>
<td>92.03±0.18</td>
</tr>
<tr>
<td>B1</td>
<td>61.18±2.95</td>
<td>91.65±0.28</td>
</tr>
<tr>
<td>B2</td>
<td>55.7±3.2</td>
<td>88.99±0.06</td>
</tr>
<tr>
<td>B3</td>
<td>52.2±0.05</td>
<td>74.24±0.11</td>
</tr>
</tbody>
</table>

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

4.3. IR analysis
The IR spectra of pure drug, eudragit polymer, and physical mixture of the drug and eudragit polymer as well as prepared microspheres are presented in (Fig. 2). Piroxicam is found in three different forms, enolic form, ketonic form and zwitter ionic form. Piroxicam 1724cm⁻¹ band wasn’t observed in this IR spectrum suggesting that piroxicam is present in its enol form having intramolecular hydrogen bond in its structure. Physical mixture spectrum indicates only the summation of piroxicam and eudragit polymer spectra and revealed that there was no interaction between them. IR absorption peaks at 1632 and 1529 cm⁻¹ are due to stretching vibration of the carbonyl group and the second amide band of piroxicam respectively. These peaks were shifted to 1733 and 1600 cm⁻¹ in case of microspheres IR spectrum as intramolecular hydrogen bonding disappeared and the IR peaks were shifted to higher values.[20, 21] Piroxicam which is present as enol or zwitter ionic forms showed the NH or OH stretching vibration at 3339 cm⁻¹, NH or OH stretching vibration wasn’t detected in microspheres chart which may indicate that drug is present in amorphous shape. The intermolecular hydrogen bonding occurred in amorphous
shape might be stronger than those containing crystalline drug, therefore the NH or OH stretching might be weakened resulting in a weak as well as broad peak that was completely covered by bond stretching from eudragit polymer. [22]

Fig. 2 IR spectrum of (a) piroxicam, (b) Eudragit Rs100 polymer, (c) physical mixture of piroxicam and eudragit Rs100, (d) prepared piroxicam microspheres

4.4. Flow properties of microspheres
The prepared formula showed good flow properties that appeared from the values of angle of repose, Carr’s index and Hausner’s ratio. Flow properties were improved as illustrated in Table 3.

Table .3: Flow properties of different formulations of microspheres and free drug.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Angle of repose(ᶿ)</th>
<th>Bulk density (gm/ml)</th>
<th>Tapped density (gm/ml)</th>
<th>Carr’s index (%)</th>
<th>Hausner’s ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free drug</td>
<td>29±48±0.08</td>
<td>0.20±0.01</td>
<td>0.35±0.03</td>
<td>42±5.90</td>
<td>1.7±0.15</td>
</tr>
<tr>
<td>A1</td>
<td>14±10±0.21</td>
<td>0.30±0.00</td>
<td>0.38±0.01</td>
<td>20.33±1.84</td>
<td>1.26±0.03</td>
</tr>
<tr>
<td>A2</td>
<td>22±52±0.21</td>
<td>0.22±0.00</td>
<td>0.28±0.01</td>
<td>22.7±2.19</td>
<td>1.28±0.02</td>
</tr>
<tr>
<td>A3</td>
<td>12±37±0.15</td>
<td>0.50±0.00</td>
<td>0.58±0.01</td>
<td>13.28±1.46</td>
<td>1.15±0.02</td>
</tr>
<tr>
<td>B1</td>
<td>24±43±0.05</td>
<td>0.32±0.01</td>
<td>0.40±0.00</td>
<td>21.07±1.4</td>
<td>1.27±0.02</td>
</tr>
<tr>
<td>B2</td>
<td>14±49±0.16</td>
<td>0.50±0.00</td>
<td>0.57±0.01</td>
<td>11.7±1.79</td>
<td>1.14±0.02</td>
</tr>
<tr>
<td>B3</td>
<td>13±42±0.17</td>
<td>0.51±0.01</td>
<td>0.57±0.00</td>
<td>11.1±2.02</td>
<td>1.13±0.02</td>
</tr>
</tbody>
</table>

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

4.5. In vitro release results
The in vitro release study was carried out in different pH media to mimic the GIT environment as shown in fig. (3-6). Drug release for the initial 2 hrs (in 0.1 N HCl), the drug release was found to be faster from microspheres having a higher drug payload. For different drug/polymer ratio of microspheres, increasing drug percent resulted in more drug release at different pH media. At high drug payloads the hindrance to diffusion of the drug from the polymer matrix would be rather low making the diffusion facile at high loadings which are reflected in the faster release of the drug. For all formulations amount of drug released increased with time for about 8 hrs. In case of intestinal fluid, the release is faster than
the release in the gastric fluid. At pH 6.8 the release was continued for 4 hrs then pH was modified for 7.4 using NaOH and release lasted for another 2 hrs. The release was continuous in the intestinal fluid at pH 6.8 and 7.4 respectively. Being a weakly acidic drug having a PKa of 6.3, at the pH of simulated gastric fluid (pH 1.2), the extent of the ionized drug is of the order 2.5*10^-6. At the PH of the intestinal fluid (pH 7.4), the ratio of ionized to unionized form is of the order 15.1. The poor ionization in gastric fluid would account for the incomplete release seen in this medium as opposed to intestinal fluid. The solubility of piroxicam is known to increase rapidly with pH values above the pKa of the drug. [23, 24] Eudragit RS100 and RL100 polymers are insoluble in aqueous media and digestive juices, so in the digestive juices it swells and becomes permeable which causes diffusion of the drug from microspheres. Piroxicam is a class 2 drug according to biopharmaceutical classification system which means it has low solubility and high permeability. Piroxicam shows better dissolution at pH 6.8 and 7.4 than pH 1.2 releasing more than 60% within 2 hrs.

Fig. 3 represents the dissolution of piroxicam at different pH values.

Fig. 3. Dissolution profiles of piroxicam in a free form at different pH values.

Fig. (4-6) displays the cumulative release of piroxicam from its different microsphere formulations at different pH values. For pH 1.2 all formulations showed a delayed release except for formula A1 which shows a burst release giving more than 15% within the first 15 minute. Cumulative release of piroxicam from different preparations depends on drug to polymer ratio as increasing amount of polymer caused decreasing release of the drug because of better coating of the drug as well as the delaying effect of the chosen polymers.

Fig. 4. Dissolution profiles of piroxicam from its microspheres of different formulations at pH 1.2.

Fig. 5. Dissolution profiles of piroxicam from its microspheres of different formulations at pH 6.8.

Fig. 6. Dissolution profiles of piroxicam from its microspheres of different formulations at pH 7.4.
The cumulative release of piroxicam at intestinal fluid (pH 6.8 and 7.4) is faster than release at pH 1.2. Formula F1 shows the highest release within 8 hrs with release up to 89% while formula A3 shows the lowest release within 8 hrs with release up to 67%. Release from eudragit RL100 formulations is higher than that of eudragit RS100 formulations because it possesses more quaternary compound than eudragit RS100 so eudragit RL100 is more permeable. Eudragit RL100 and RS100 are insoluble in aqueous media and digestive juices, but swell and are permeable, which means that the drug can be released by diffusion.\(^{(28)}\)

**CONCLUSION**

The obtained results showed that, the microspheres of piroxicam prepared using emulsion solvent evaporation method have rough and porous surface as well as have better flowability than free drug. The IR analysis of piroxicam microspheres revealed presence of intermolecular hydrogen bonding between drug and eudragit polymer. In-vitro release study indicated that the release of the drug from microspheres increased as drug to polymer ratio increased. Microspheres highest drug release formula is F1 having drug release up to 8 hrs where it released 89% of piroxicam drug, while formula with lowest drug release is A3 having drug release up to 67% during 8 hrs.

**REFERENCES**

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