FORMULATION, CHARACTERIZATION AND BIOPHARMACEUTICAL EVALUATION OF INDOMETHACIN MICROSPHERES

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ABSTRACT

The purpose of the present study was to obtain a polymeric, multi unit system for the extended release of indomethacin. In this study three different microencapsulation techniques, namely, coacervation phase separation by non solvent addition, coacervation phase separation by solvent evaporation, and emulsification solvent evaporation technique were used to prepare indomethacin loaded ethylcellulose microspheres. The microspheres were evaluated based on average particle size by optical microscopy, surface morphology by scanning electron microscopy (SEM) and in vitro drug release. The drug - polymer interactions and the effect on drug crystallinity due to the incorporation of indomethacin in polymer matrix have been evaluated by differential scanning calorimetry (DSC) and thin layer chromatography studies. The in vitro release studies were performed in phosphate buffer medium pH 7.2. The microspheres fabricated by emulsion solvent evaporation method had average particle size of about 35µ and observed to have a spherical shape with smooth surface. TLC and DSC revealed that the drug stability is not affected due to the selected coating materials and presence of drug as a molecular dispersion in the polymer. The microspheres prepared by the solvent evaporation technique released 92% of the drug in a prolonged manner over a period of 10 h. In vivo study in rabbits was performed and pharmacokinetic treatment of data also revealed the mean residence time (MRT) of the microspheres to be comparatively higher than the pure drug. Other associated pharmacokinetic parameters were also reported. The particle characteristics, in vitro and in vivo behaviors demonstrated that the emulsification solvent evaporation technique was potentially suitable for the preparation of ethyl cellulose microspheres for sustained delivery of indomethacin.

Key words: Indomethacin, Ethylcellulose, Microspheres, Drug release, Bioavailability.

INTRODUCTION

Indomethacin, a non-steroidal anti-inflammatory drug has been successfully used in the treatment of soft tissue problems associated with trauma, osteoarthritis and rheumatoid arthritis. However, drug therapy with this agent is associated with several adverse effects (Tripathi, 2004).

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The frequency and the severity of the adverse effects are well correlated with the plasma concentration of the drug. Clinical study revealed that conventional dose dumping of indomethacin from capsules induces several adverse effects like epigastric pain, peptic ulcer, vertigo, and headache. On the other hand, sustained release indomethacin capsules maintain adequate therapeutic plasma level of the drug avoiding peaks and troughs and there by minimize the emergence of adverse effects and increase patient’s compliance by reducing the frequency of administration. Moreover, a multi unit system spreads in a large area of the absorbing mucosa and prevents exposure to a high drug concentration, when compared to single unit dosage form on chronic dosing (Davis et al., 1984). Although many
polymers are used in the pharmaceutical formulations, the most widely used are the cellulose derivatives. Hence the present investigation aimed to present indomethacin in sustained release dosage form using ethylcellulose by various microencapsulation techniques and to characterize the microspheres based on particle size, drug release, and in vivo analysis.

**MATERIALS AND METHODS**

**Materials**

Indomethacin was a gift sample from Maral labs, Chennai, India), ethylcellulose was purchased from Loba Chemicals, India. All the other chemicals used were of analytical grade and used as received.

**Methods**

**Method I: Coacervation - phase separation from carbon tetrachloride by the addition of petroleum ether** (Anita et al., 2002)

This method consists of decreasing the solubility of the encapsulating polymer by addition of a third component to the polymer solution in an organic solvent (Jalil et al., 1990). Ethyl cellulose (500mg) was dissolved in 50ml of carbon tetrachloride to form a homogenous polymer solution. Core material (500mg) i.e. (1:1) was then added to the polymer solution and dispersed thoroughly with the aid of a mechanical stirrer (200 ± 10 rpm) for 10 min. Coacervation was then induced by addition of 30ml of petroleum ether slowly over a period of 20 minutes while stirring at the same speed. At a particular point, this method yields two liquid phases: the polymer containing coacervate phase and the supernatant phase depleted in polymer (Jain et al., 2000). The system was then cooled for 20 minutes, with stirring to rigidize the coating of the microcapsules. The encapsulated product was collected by vacuum filtration and air dried to obtain discrete microcapsules.

**Method II: Coacervation phase separation technique induced by solvent evaporation**

The solvent evaporation technique to produce microcapsules is applicable to a wide variety of liquid and solid core materials (Lachman et al., 1991). Ethyl cellulose (500mg) was dissolved in 10ml of solvent acetone and the drug (500mg) was dispersed into it. This was then suspended in 25ml of light liquid paraffin at 1000 rpm ± 10 rpm. The phase separation and consequent coating of the drug by polymer was achieved by solvent evaporation at room temperature. The microspheres were filtered through a 150 mesh sieve and washed several times with n-hexane to make them free from liquid paraffin and then air dried.

**Method III: Emulsification solvent evaporation technique**

This process involves oil in water (o/w) emulsification (Wu Xs et al., 1995). The polymer solution was prepared by taking 500mg of ethylcellulose which was dissolved in 22.5 ml of dichloromethane and 500mg of drug was dissolved in it. A 1% poly vinyl alcohol (PVA) solution was taken in a beaker, and the prepared drug solution was added drop by drop into it and homogenized at 4000 rpm ± 10 rpm for 5 min to form an emulsion. The emulsion was then stirred with the help of magnetic stirrer for 2 h and 40°C to get the microspheres by solvent evaporation of dichloromethane. The rate of solvent removal by evaporation method strongly influences the characteristics of the final microspheres. Very rapid solvent evaporation may cause local explosion inside the droplets and lead to formation of porous structure on the microsphere surface (Arshady., 1991). The microspheres were filtered off and the filtered microspheres were washed with phosphate buffer pH 7.2 and dried at room temperature.

**Determination of Drug Content**

The amount of indomethacin present in different sized microspheres was determined by the following method (Babay et al., 1988). Accurately weighed amount of the dried indomethacin loaded ethyl cellulose microspheres (100mg) was dissolved in 100ml of chloroform and was assayed spectrophotometrically at 320 nm using UV-Vis spectrophotometer (UV-1601, Shimadzu).

**Scanning Electron Microscopy (SEM)**

The sample for the SEM analysis were prepared by sprinkling the microspheres on to one side of double adhesive stub and the stubs were then coated with gold using JEOL-JFC 1100E sputter coater. The SEM micrographs of the microspheres were taken using JEOL - JFC 5300 scanning microscope.

**Drug Stability studies by Qualitative Thin Layer Chromatography (TLC)**

Qualitative TLC was used for the physical characterization of drug loaded microspheres (Tamilvannan et al., 1999). The method was carried out using 12 x 6.5cm sheets with a thickness of 0.25mm. An accurately weighed amount of pure drug indomethacin was dissolved in 2ml of dichloromethane. Likewise 100mg equivalent indomethacin microspheres and 100mg of pure ethyl cellulose were separately dissolved in 2ml of dichloromethane. These dissolved materials were applied using a sample applicator (1 µl capillary) directly on to the TLC sheet leaving 2 cm from the edge. The sheet was developed with benzene-ether-glacial acetic acid -methanol (120:160: 18:1) system in TLC chamber for 20 min. After development, the sheet was air dried and examined under UV-light. The experiment was repeated thrice for significance. The mean Rf values obtained from indomethacin and drug loaded microspheres were compared.

Differential Scanning Calorimetry (DSC)

DSC scans of indomethacin and drug loaded microspheres were performed in an atmosphere of nitrogen. Weighed amount (∙ 4mg) of sample were kept in hermetically sealed aluminum pans and were heated at a scan speed of 10°C min⁻¹ over a temperature range of 50°C - 250°C in a differential scanning calorimeter (Perkin - Elmer, DSC - 7, Calibrated with Indium) at a chart speed of 10 mm/min.

In Vitro Drug Release Studies

The in vitro release of drug loaded microspheres was carried out at 37°C using phosphate buffer pH 7.2. Each batch of microspheres containing 50 mg of indomethacin was individually added to 100ml of medium (one volume of phosphate buffer pH 7.2 and four volumes of distilled water) in an iodine flask. The flask was shaken (50 rpm) in an incubator at 37°C. One ml of sample was withdrawn at regular intervals of time and the same volume of medium was replaced. After suitable dilution, indomethacin content in the medium was estimated at 320nm by using UV-Vis spectrophotometer (UV-1601, Shimadzu). Since microspheres from method III yielded spherical microspheres with reproducible and satisfactory in vitro profiles, the microspheres of method III were subjected to the in vivo study.

In Vivo Study (Ghosh, 1984)

Four healthy rabbits weighing 2 - 2.5 kg each were released and fed with standard rodent pellet diet. Microspheres equivalent to animal dose/kg (5.2 mg/kg of indomethacin) of drug suspension was prepared with 0.05% carboxy methyl cellulose (CMC). This was administered orally to four rabbits using vinyl tubing (40 cm length, 6.0 mm i.d., 8.0 mm o.d.), which was introduced into rabbit stomach. Thereafter, the tubing was rinsed twice with 5ml warm water (37°C). Similarly animal dose of pure drug was given to set of the 4 rabbits. Food was with held for a period of 2 hours. Samples of blood (1ml) were collected at ½, 1, 2, 3, 4 h, up to 12 h. The samples were withdrawn from the marginal ear vein and heparin was added to prevent blood clotting. The plasma was separated immediately by centrifuging the blood samples at 5000 rpm for 10 min. The supernatant fluid after suitable dilution was analyzed for indomethacin at 320 nm respectively using UV-Vis spectrophotometer. Standard plasma indomethacin samples were prepared by adding known amounts of indomethacin to the blank rabbit plasma.

Data Analysis

A non-compartmental pharmacokinetic analysis was applied to the data. The terminal elimination rate constant, Area under the plasma concentration - time curve after administration (AUC), the area under first moment curve after administration (AUMC), and the mean residence time (MRT) were some of the parameters calculated.

RESULTS AND DISCUSSION

Particle size and surface morphology

The average particle size was determined by optical microscopy. The microspheres were observed to be 51.02µ, 44.4µ and 35.6µ for method I, method II and method III respectively (Figure 1). The morphology of microspheres produced was investigated by scanning electron microscopy. Figure 2-5 shows typical scanning electron micrographs of the microspheres prepared by different methods of encapsulation. The surface of ethylcellulose microcapsules in method I was rough and irregular (Figure 2). Microspheres prepared by method II were uniform, spherical with a smooth surface (Figure 3). The microspheres prepared by method III - emulsification by solvent evaporation technique (figure. 4 and 5), appear well formed with a spherical and regular shape with smooth surface indicating the optimization of solvent evaporation rate in indomethacin microspheres.

![Figure 1. Particle size distribution of microspheres prepared by different methods](image-url)
Stability analysis by TLC and DSC

The compatibility of the drug with the polymer and any possible drug decomposition or drug polymer interactions was checked through qualitative thin layer chromatography. Qualitative TLC analysis (Table 1) shows that the Rf values of prepared microparticles by method I, II and III were almost the same as that of the pure drug and polymer. Therefore, it reveals that the drug stability is not affected due to the selected coating materials.

Table 1: Qualitative TLC analysis

<table>
<thead>
<tr>
<th>Spot nature</th>
<th>Method I</th>
<th>Method II</th>
<th>Method III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Drug</td>
<td>0.850</td>
<td>0.861</td>
<td>0.852</td>
</tr>
<tr>
<td>Microspheres</td>
<td>0.852</td>
<td>0.852</td>
<td>0.849</td>
</tr>
<tr>
<td>Pure ethylcellulose</td>
<td>0.239</td>
<td>0.231</td>
<td>0.231</td>
</tr>
<tr>
<td>Microspheres</td>
<td>0.241</td>
<td>0.235</td>
<td>0.235</td>
</tr>
</tbody>
</table>
DSC thermograms of indomethacin in the microparticles prepared by various methods are presented in figure 6. Indomethacin showed sharp endothermic peak at 160°C which was close to the reported melting point (160°C - 161°C) of the drug. The microparticles prepared by method I, II and III did not show any endothermic peaks this suggests that, in these methods, the drug was dispersed at a molecular level in the polymer, at least so at its melting temperature. There is no detectable endotherm if the drug is present in a molecular dispersion or a solid solution state in the polymeric microspheres loaded with enough amount of drug. Thus the result suggests that the drugs were molecular level at polymer melting temperature.

![DSC thermograms](image)

**Figure 6:** DSC thermograms of drug (A), microspheres made by method I (B), Method II (C) and method III (D)

**In vitro drug release**

Almost 95% of indomethacin from the pure drug was released within 3 h, whereas the drug release from the microparticulate dosage form extended over a period of time depending on method of preparation (Figure 7). The microspheres prepared by method III were found to be release indomethacin in a more sustained manner with a cumulative release of 92% at the end of 10th hour. The cumulative release of drug from microparticles from method I and II were 98 and 97 % at the end of 9th and 10th hour respectively. Though the in vitro studies indicated an initial burst effect, the overall release rates were slow. Therefore, characterization of the crystal form of the drug in the polymer might disclose a plausible reason for the slow release (Dhanikula et al., 1999). The slow and constant rate of drugs release may be due to diffusion of drugs from polymer as well as due to erosion of polymer (Dhanaraju et al., 2003).
In vivo Systemic Availability

The bioavailability studies were carried out on healthy rabbits to determine the drug release characteristic in plasma concentration Vs time curve as shown in figure 8. The mean peak level 3.25 ± 0.41 µg/ml was obtained after administration of indomethacin (pure drug) and thereafter the plasma indomethacin level reduced rapidly with a terminal elimination half life of 0.104 h. The mean peak level after microspheres administration (as described in experimental section) was 2.75 ± 0.20 µg/ml. This peak level appeared 4 h after administration when compared to pure drug, which showed peak concentration at 3h. As shown in the Table-2, the AUC obtained after pure drug administration was 13.0225 h µg/ml. All the other associated parameters are also shown in Table 2. From these data, the MRT was calculated and the values are shown in Table 2. Compared with the MRT of the oral indomethacin (pure drug), which was 4.38h MRT of the microspheres (5.57 h) was increased suggesting a sustained release of indomethacin from the microspheres.

Table 2: Pharmacokinetic parameters of indomethacin after oral administration in rabbits

<table>
<thead>
<tr>
<th>Preparation</th>
<th>t½ (h)</th>
<th>AUC (µg/ml)h</th>
<th>AUMC h(µg/ml)h</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Drug</td>
<td>0.104</td>
<td>13.03 ± 0.29</td>
<td>57.08 ± 2.91</td>
<td>4.38</td>
</tr>
<tr>
<td>Microspheres</td>
<td>0.078</td>
<td>18.14 ± 1.14</td>
<td>101.19 ± 3.02</td>
<td>5.571</td>
</tr>
</tbody>
</table>

Figure 7. in vitro release of indomethacin from microspheres prepared by various methods

Figure 8: Comparison of AUC after oral administration
CONCLUSION

Ethylcellulose microspheres of indomethacin were prepared by three different microencapsulation techniques. The batch of microspheres prepared by emulsification solvent evaporation technique was found to be uniform in size and shape with a controlled release pattern. The in vivo studies showed the microspheres to have better bioavailability than the pure form of drug. Physical characterization using thin layer chromatography and differential scanning calorimetry ruled out the possibility of drug interaction and further confirmed the presence of drug in the soluble or crystalline dispersion form at its melting point. The result of the present studies reveals that the ethylcellulose microspheres of indomethacin may constitute a useful alternative to the conventional dosage forms.

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