Formulation and In-Vitro Evaluation of Propranolol Hydrochloride Loaded Polycaprolactone Microspheres

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ABSTRACT

Controlled drug delivery system can overcome some of the problems of conventional drug delivery system such as reduction in dosing frequency, minimization of systemic side effects, improved patient convenience and maintenance of therapeutic activity for full dosing interval. In the present study, controlled release microspheres of propranolol hydrochloride were prepared using polycaprolactone as matrix material by the emulsion solvent evaporation method to overcome the problems associated with the conventional dosages form of the drug. The effects of preparative parameters such as polymer concentration, PVA concentration, stirring speed, on size and morphology of dummy microspheres were investigated. It was found that by adding 0.75% polymer solution in to 0.4% PVA solution at stirring speed 1100 rpm, spherical microspheres with relatively smooth surface " ~ 48.75 ± 1.79 µm in diameter could be produced. The drug loaded microspheres were evaluated for surface morphology, entrapment efficiency, particle size, in vitro drug release study, and release kinetics and stability studies. The drug loaded microspheres were spherical and smooth. The entrapment efficiency increased with the increase of the polymer concentration, attaining the highest entrapment 74.46 ± 0.18% at 50 mg of propranolol hydrochloride (1:3 Drug to polymer ratio). The entrapment efficiencies of formulation F4 and F5 (71.12 ± 0.19% and 72.46 ± 0.18%) were found to be less as compared to F3 formulation (74.46 ± 0.18%). The mean particle size of the microspheres significantly increased with increasing polycaprolactone concentration (1:1 to 1:5) and was in the range 32.76 ± 3.09 µm to 72.15 ± 3.57 µm. The drug release profiles of the microspheres were extended over a period of 10 hrs; release was influenced by polymer concentration. Drug release followed the Higuchi model. The propranolol hydrochloride loaded polycaprolactone microspheres prepared under optimized conditions showed controlled release characteristics and were stable under the conditions studied.

Keywords: Controlled drug delivery, Propranolol hydrochloride, PCL, Microspheres.

INTRODUCTION

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery technologies are patent protected formulation technologies that modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance.

Current efforts in the area of drug delivery include the development of controlled drug delivery system which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. The two main advantages of controlled drug delivery systems are: maintenance of therapeutically optimum drug concentrations in the plasma through zero-order release without significant fluctuations; and elimination of the need for frequent single dose administrations. For controlled release system, oral route of administration has most attention. This is because there is more flexibility in dosage form dosing for oral route than there is for parenteral route. Oral route has been the most popular and successfully used for delivery of drug because of convenience and ease of administration greater flexibility in dosage form design and ease of production and low cost of such a system.

Controlled release delivery systems provide desired concentration of drug at the absorption site allowing maintenance of plasma concentrations within the therapeutic range and reducing the dosing frequency. These products typically provide significant benefits over immediate-release formulations, including greater effectiveness in the treatment of chronic conditions, reduced side effects, and greater patient convenience due to a simplified dosing schedule. Majority of per oral controlled release dosage forms fall in the category of matrix, reservoir, or osmotic systems. In matrix systems, the drug is embedded in a membrane and osmotic pressure of core formulation polymer matrix and the release takes place by partitioning of drug into the polymer matrix and the release medium.

The basic rationale for controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery systems or by modifying the molecular structure and/ or physiological parameters inherent in a selected route of administration.

For the controlled release of drug solid biodegradable microspheres are potential which incorporates a drug dispersed or dissolved throughout particle matrix. The term microcapsule is defined as a spherical particle with
size varying from 50 nm to 2 mm, containing a core substance.

Propranolol hydrochloride is a nonselective beta-adrenergic blocking agent, has been widely used in the treatment of hypertension, angina pectoris, and many other cardiovascular disorders. It reduces the oxygen requirement of the heart at any level of effort by blocking catecholamine induced increase in the heart rate, systolic blood pressure, the velocity and extent of myocardial contraction. Propranolol hydrochloride is commercially available in the form of conventional formulation with the dose 10-40 mg in 3-4 times daily. However, propranolol hydrochloride usefulness is limited due to its short half life that ranges from 3 to 5 hours. Hence it requires three-four times a day dosing which produce the patient uncompliance. To reduce the frequency of dose administration and to improve patient compliance, a controlled/sustained release formulation is desirable.

The objective of present study was to prepare and evaluate the controlled release microspheres containing propranolol hydrochloride using PCL (polycaprolactone) as the matrix material by emulsion solvent evaporation method in order to achieve slow in vitro release and to overcome the rapid elimination of drug and increase half-life of the drug or maintain drug plasma concentration in the therapeutic range for desired period of time. Major advantages of the suggested novel preparation technique include short processing times, no exposure of the ingredients to high temperatures, the ability to avoid toxic organic solvents and high encapsulation efficiencies. This management generates a number of favorable outcomes in drug therapy including reduced undesirable side effects, improved patient compliance and reduced overall cost of therapy.

**MATERIALS AND METHODS**

**Materials**

Propranolol hydrochloride was procured as a gift sample from Torque Pvt. Ltd, Punjab, India. Poly-ε caprolactone (PCL) was obtained as a gift sample from Sigma Aldrich Co. USA. Dichloromethane (DCM) and polyvinyl alcohol (PVA) was purchased from Loba chemie Pvt. Ltd, Mumbai, India. HPLC water was used throughout the study. All chemicals and reagents were of analytical grade and were used without further purification.

**Optimization of parameters influencing size, morphology of microspheres**

**Preparation & characterization of dummy microspheres**

Microspheres were prepared by solvent evaporation method. 0.25% solution of PCL (prepared in DCM) was added to 0.4% PVA solution (prepared in deionised water). Resultant emulsion was then stirred at 1100 rpm (25°C) for 2 hours. Microspheres were formed, filtered and washed 3 times with water to remove PVA. Washed microspheres were air dried overnight and stored at room temperature. The effect of various physical parameters such as polymer concentration, stabilizer concentration and stirring speed, on the morphology of microspheres were studied in detail.

**Effect of polymer concentration on size of microspheres**

Different concentrations of PCL (0.25%, 0.5%, 0.75%, 1%, 1.25% prepared in DCM) was added to 0.4% PVA solution the emulsion was then stirred at 1100 rpm. Microspheres were filtered and washed 3 times with water to remove PVA. Washed microspheres were air dried overnight and stored at RT.

**Effect of PVA concentration on size of microspheres**

0.75% solution of PCL (prepared in DCM) was added to solution of different concentrations of PVA (0.1%, 0.3%, 0.4%, 0.6%, and 0.8%) and stirred at 1100 rpm. The microspheres were filtered and washed 3 times with water to remove PVA and then air dried and stored at RT.

**Effect of stirring speed on size of microspheres**

0.75% PCL solution was added in 0.4% PVA solution and stirred at different speeds (700 rpm, 900 rpm, 1100 rpm, 1300 rpm, and 1500 rpm). The emulsion was filtered and washed 3 times with water to remove stabilizer. Microspheres were air dried on and stored at RT.

**Preformulation study**

1. Capillary method was used to determine the melting range of the drug. A capillary sealed at one end filled with small amount of drug was placed in the melting point measuring apparatus. The melting range was noted down.

2. For absorption maxima (λmax)

10 mg of propranolol hydrochloride was accurately weighed and transferred in to 100 ml of volumetric flask, sufficient quantity water dissolve propranolol hydrochloride and the volume was made up to 100 ml with water to obtain a stock solution of 100 µg/ml. This solution was scanned between 200 nm to 400 nm in a double beam UV-Visible-spectrophotometer (Shimadzu UV spectrophotometer, Japan) to get spectrum.

3. Solubility of drug was determined in different solvents and estimated by Shimadzu UV spectrophotometer, Japan.

4. Partition coefficient

The partition coefficient of the drug was determined by taking equal volumes of n-octanol and aqueous phases in a separating funnel. A drug solution was prepared and 1ml of the solution was added to octanol: water (50:50) was taken in a separating funnel and shaken for 10 minutes and allowed to stand for 1 hour and is continued for 24 hrs. Then aqueous phase and octanol phase was separated, centrifuged for 10 min at 2000 rpm. The aqueous phase and octanol phase were assayed after partitioning using UV Spectrophotometer at their respective λ max to get partition coefficient. Triplicate readings (n=3) were taken and average was calculated.
Drug estimation was done by UV spectroscopy (Shimadzu UV spectrophotometer, Japan)\textsuperscript{13}.

5. Drug excipients interaction

The compatibility of drug with various excipients was ascertained by Fourier Transform Infrared Spectroscopy (FTIR, PerkinElmer USA) techniques. FTIR was used as a tool to detect any physical and chemical interaction between drug and excipients. Drugs and various excipients were mixed thoroughly in a ratio of 1:1. Samples were stored at 40°C and 75% RH in closed vials for 21 days. After 21 days samples were scanned by FTIR. The spectra of pure drug and drug with excipients (PCL, PLGA, and PVA) were compared to check any incompatibility and physical changes.

Formulation of Drug loaded microspheres

Microspheres were prepared using solvent evaporation method. Briefly drug (1): polymer (3) microspheres were prepared by dissolving 150 mg of polymer in 20 ml DCM, and 50 mg of drug in water. Now polymer solution (20ml) and drug solution was added to the 100 ml solution of 0.4% PVA and stirred at 1100 rpm at 25°C for 2 hrs. The emulsion of microspheres was filtered and washed three times with water. The washed microspheres were air dried overnight and stored at room temperature. Microspheres of drug polymer ratio 1:1, 1:2, 1:4, and 1:5 were also prepared by the same procedure\textsuperscript{16}. Ingredients used for PCL microspheres are given in table 1.

Table 1: Ingredients used for drug loaded PCL microspheres

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Qty (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1 (1:1)</td>
</tr>
<tr>
<td>Propranolol hydrochloride</td>
<td>50</td>
</tr>
<tr>
<td>Poly-ε-caprolactone (PCL)</td>
<td>50</td>
</tr>
<tr>
<td>Poly vinyl alcohol (PVA)</td>
<td>400</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>20ml</td>
</tr>
<tr>
<td>Water</td>
<td>100ml</td>
</tr>
</tbody>
</table>

Characterization and evaluation of drug loaded microspheres

Percentage yield (% production yield)

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer used to prepare that batch multiplied by 100.

All the experimental units were analyzed in triplicate (n=3)\textsuperscript{12}.

Surface morphology

Surface morphology and surface appearance of microspheres were examined by scanning electron microscope (FESEM, S-4300, Hitachi, Japan). Samples for SEM were freeze dried, mounted on metals with double sided tape. Pictures were taken and examined for surface morphology and surface appearance of microspheres\textsuperscript{17}.

Determination of drug content and entrapment efficiency

100 mg of microspheres were crushed in glass mortar and crushed microspheres equivalent to 10 mg of the drug were dissolved in 10 ml of distilled water, vortexed for 5 min and filtered through whatman filter paper. The filtered samples were suitably diluted with distilled water; the drug content was assayed by UV spectrophotometer at 290 nm. All the experimental units were analyzed in triplicate (n=3).

The drug entrapment efficiency (DEE) was calculated by the equation,

\[ \text{DEE} = \left( \frac{\text{Pc}}{\text{Tc}} \right) \times 100 \]

Where:

\( \text{Pc} \) - Practical content,
\( \text{Tc} \) - Theoretical content\textsuperscript{11-12}.

Particle size

The size was measured using a microscope with the help of projection microscope, and the mean particle by means of a calibrated stage micrometer with eye piece micrometer.

Procedure: Calibrate the eye piece micrometer using stage micrometer and find out the length of one division of eye piece micrometer. Prepare the slide by using a small quantity of treated microspheres and mount a drop of glycerin and cover with cover slip. Replace the stage micrometer with the prepared slide. Measure the diameter of the microspheres by observing the no. of divisions covered by microspheres\textsuperscript{18}.

FTIR study

FTIR spectra of propranolol hydrochloride, PCL blank microspheres, propranolol hydrochloride loaded PCL microspheres were obtained in KBr pellets using a PerkinElmer spectroscope in the ranges 400 to 4000 cm\textsuperscript{-1} 19.

In vitro drug release studies

In vitro drug release studies were carried out for prepared microspheres by using USP dissolution rate test apparatus-ll (37±0.5°C). An accurately weighed amount of microspheres equivalent to 10 mg of the drug were filled into hard gelatin capsules and placed in basket separately. The dissolution medium was 0.1 N HCL as stimulated gastric fluid (SGF) (900ml, pH 1.2) for first 2 hrs, followed by phosphate buffer as stimulated intestinal fluid (SIF) (900ml, pH 7.4) for the rest of 8 hrs. 5 ml of the samples were withdrawn at specified time intervals (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, hrs.) and equal volume of fresh medium was replaced immediately. After a suitable
dilution, samples were analyzed by UV spectrophotometry\textsuperscript{11}.

Statistical analysis

One way ANOVA analysis was applied on final in vitro dissolution readings. Microsoft excel 2007 software was used to calculate the value of F-ratio to determine the statistical difference in the results\textsuperscript{20}.

Release models and kinetics

To determine the drug release mechanism and to compare the release profile amongst various microspheres formulations, the in-vitro release data was fitted to various kinetic equations. The kinetic models included zero order, first order, Higuchi model, and Korsmeyer-Peppas model. The plots were drawn by use of software Origin Pro 8 as per the following details.

- Cumulative percent drug released as a function of time (zero order kinetic plots).
- Log cumulative percent drug retained as a function of time (first order kinetics plots).
- Log cumulative percent drug released as a function of log time (Korsemeyer plots).
- Cumulative percent drug released versus square root of time (Higuchi plots).

Stability Studies

The optimized formulations were placed in screw-cap, amber glass containers and stored at ambient humidity and different temperatures such as 25 ± 2°C, 35 ± 2°C and 40 ± 2°C for a period of 3 months. The samples were analyzed for physical appearance and for drug content at regular intervals of 30 days\textsuperscript{21}.

RESULTS AND DISCUSSION

Identification of drug

The drug was identified by different methods including melting range by capillary fusion method i.e. 163.6°C, \( \lambda_{\text{max}} \) by UV spectrophotometric study i.e. 290nm, and functional group by FTIR spectroscopy. All the parameters were found within limit and complied the requirements of official’s compendia

FTIR study

FTIR spectra of propranolol hydrochloride, was obtained in KBr pellets using a Perkin-Elmer spectroscope in the ranges 400 to 4000 cm\(^{-1} \) (Fig. 1). The observed FTIR spectrum of the drug was matched with reference spectra as shown in Table 2. The study confirmed that the test sample was propranolol hydrochloride. All the tests confirmed the identity and purity of the drug.

Drug excipients compatibility study

The compatibility of the drug with various excipients was ascertained by Fourier Transform Infrared Spectroscopy (FTIR, Perkin Elmer USA). All characteristic peaks of drug were observed in the FTIR spectra of physical mixture of drug and excipients (results are not shown). The results showed no chemical interaction and changes took place in FTIR spectra of drug and various excipients alone or in combination. Thus FTIR studies indicated compatibility between propranolol hydrochloride and PCL, PVA.

![Figure 1: FTIR spectrum of Propranolol hydrochloride.](image)

<table>
<thead>
<tr>
<th>Reported peaks (cm(^{-1}))</th>
<th>Observed peak (cm(^{-1}))</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500-3300</td>
<td>3322.85</td>
<td>N-H Streching</td>
</tr>
<tr>
<td>3400-3300</td>
<td>3281.28</td>
<td>O-H Streching</td>
</tr>
<tr>
<td>2955-2800</td>
<td>2926.51</td>
<td>C-H Streching</td>
</tr>
<tr>
<td>1680-1620</td>
<td>1629.29</td>
<td>C=C Streching</td>
</tr>
<tr>
<td>1260-1000</td>
<td>1241.36</td>
<td>C-O Streching</td>
</tr>
</tbody>
</table>

Characterization and evaluation of dummy microspheres

The effect of various physical parameters such as polymer concentration, stabilizer concentration and stirring speed, on the size and morphology of microspheres were studied in detail.

Effect of polymer concentration on size of microspheres

The mean size of the microspheres increased from 28.86 \( \mu \m) to 76.05 \( \mu \m) as the concentration of polymer increased from concentration 0.25% to 1.25%. We found that we can control the size of the microspheres by adjusting the concentration of the polymer solution. The mean size increased with increasing polymer concentrations (0.25% to 1.25%), which produce a significant increase in the viscosity, leading to the formation of larger size emulsion droplets and finally a higher microsphere size. The mean size was also influenced by the content and type of polymer used and its ratio in the formulation\textsuperscript{15}. As the viscosity of the medium increases, this results in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This results in the formation of larger particles\textsuperscript{22}.

Effect of PVA concentration on size of microspheres

The mean size of the microspheres decreased from 79.95 \( \mu \m) to 30.03 \( \mu \m) as the concentration of PVA increased from 0.1% to 0.8%. PVA is a macromolecular stabilizer

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with two functions as follows: one is decreasing interfacial tension between dispersing phase (inner organic phase) and continuous phase (outer aqueous phase) to make the large droplets liable to rupture; the other is coating on the surface of the microspheres to protect them from aggregation and agglutination\textsuperscript{23}.

**Effect of Stirring speed on size of microspheres**

According to the literature, the effect of stirring speed on the size and shape of microspheres is not consistent. In most cases, however, particle size decreases with increasing stirring speed. In the present study, a dramatic decrease of microsphere size from 77.22µm to 28.47µm occurred when the stirring speed in the o/w emulsion increased from 700 to 1500 rpm. As the stirring speed increases, the shear stress increases and the established balance between tangential stresses at the droplet interface impacted by the homogenizer and interfacial tension is going to be altered. The larger tangential stress leads to a reduction in droplet size, while the stirring speed affects the relative viscosity of the emulsion. Typically, the viscosity reduction at a higher rotational speed is responsible for a decrease in particle size. While stirring speed was found to be the dominant factor for the sizing of microspheres\textsuperscript{24}.

From all above experiments the best formulation S3 was selected on the basis of particle size and surface morphology.

**Characterization and evaluation of drug loaded microspheres**

**Percentage yield (% production yield)**

The production yield was found to be between 66.33 ± 0.58% - 76.40 ± 0.40% for F1-F5 formulations as shown in Table 3. The highest production yield was found in formulation F4 (1:4) 76.40 ± 0.40%. The yield of formulation F5 (1:5) was decreases i.e. 75.44 ± 0.51% due to flocculation and aggregation of microspheres as the viscosity increases with increase in concentration of polymer\textsuperscript{25}.

**Surface morphology**

Scanning electron microscopy (SEM) of propranolol hydrochloride-loaded microspheres showed spherical shaped microparticles with a relatively smooth and non-porous surface, as depicted in Fig. 2 (a, b, c, d, and e) respectively for formulations F1, F2, F3, F4, and F5.

**Drug content and entrapment efficiency**

Several batches of PCL-microspheres containing propranolol HCl were prepared varying the drug-to-polymer ratio. Values of the drug entrapment efficiency into PCL microspheres were presented in table 3.

As it can be seen, the entrapment efficiency increased with the increase of the polymer concentration, attaining the highest entrapment 74.46 ± 0.18% at 50 mg of propranolol hydrochloride (1:3 Drug to polymer ratio). The entrapment efficiency was found to be 64.32 ± 0.12%, 66.22 ± 0.08%, and 74.46 ± 0.18% for F1, F2 and F3 formulation respectively. The contribution of a high polymer concentration to the loading efficiency can be interpreted in three ways. First, when highly concentrated, the polymer precipitates faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary. Second, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets. Third, the high polymer concentration results large size of microspheres which result in loss of drug from surface during washing of microspheres is very less as compare to small microspheres\textsuperscript{26}. It has been reported that choice of organic solvents influenced the entrapment efficiency of water soluble drugs. The use of solvents with greater aqueous solubility, e.g., dichloromethane (2.0% w/w aqueous solubility) resulted in greater drug entrapment than observed whenever the organic solvents of low aqueous solubility e.g., chloroform (0.8% w/w aqueous solubility) were employed. Organic solvents of greater aqueous solubility caused rapid precipitation of polymer at droplet interface, and thus created a barrier to drug diffusion out of the forming microspheres. The entrapment efficiencies of formulation F4 and F5 (71.12 ± 0.19% and 72.46 ± 0.18%) were found to be less as compared to F3 formulation (74.46 ± 0.18%). This may be due to the ability of drug to partition in to aqueous phase prior to microspheres solidification\textsuperscript{27}.

**Particle size Analysis**

The mean particle size of the microspheres significantly increased with increasing polycaprolactone concentration (1:1 to 1:5) and was in the range 32.76 ± 3.09 µm to 72.15 ± 3.57 µm (Table 3). The viscosity of the medium increases at a higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This results in the formation of larger particles\textsuperscript{28}. With increase in polymer concentration viscosity of the polymer solution increases, this in turn decreases the stirring efficiency. The polymer rapidly precipitates leading to hardening and avoiding further particle size reduction during solvent evaporation\textsuperscript{29}.

**FTIR study**

The FTIR spectra of the pure drug (Fig. 1), PCL and the drug loaded microspheres were recorded (Fig.3 and 4). The identical peaks corresponding to functional groups and polymer confirms that neither the polymer nor the method of preparation has affected the drug stability. Propranolol hydrochloride had characteristic bands at 3322.85 (N-H stretch), 3281.28 (O-H stretch), 2926.51 (C-H stretch), 1629.29 (C=C stretch), 1241.36 (C-O stretch). These bands were not shown in PCL microspheres (Fig. 3), but still in propranolol hydrochloride loaded microspheres (Fig. 4), which indicated that propranolol hydrochloride was physically entrapped in the polymer matrix and there was no chemical interaction between propranolol hydrochloride and polymer.
The different microspheres formulations were subjected to \textit{in-vitro} release studies using USP dissolution rate test apparatus-II (37±0.5°C). The cumulative percent release was found to be 91.73 ± 0.01, 84.20 ± 0.03, 77.82 ± 0.03, 71.07 ± 0.02 and 59.78 ± 0.03% for formulations F1 to F5 after 10 hrs. as shown in Table 3. It was observed that for each formulation the drug release decreased with increase in the amount of polymer. This may be due to the fact that smaller microspheres were formed at lower polymer concentration and have larger surface area exposed to dissolution medium, giving rise to faster drug release\textsuperscript{30}. The time course of drug release from F1, F2, F3, F4 and F5 microspheres were shown in (Fig. 5).

**Figure 2**: SEM of drug loaded PCL microspheres (a) F1, (b) F2, (c) F3, (d) F4, AND (e) F5.

**Figure 3**: FTIR spectra of empty PCL microspheres.

**Figure 4**: FTIR spectra of propranolol hydrochloride loaded PCL microspheres.

**In vitro drug release studies**

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**Figure 5**: \textit{In vitro} release rate profiles of propranolol hydrochloride from PCL microspheres in 0.1 N HCL and phosphate buffer saline at 37±0.5°C after 10 hours.

Fig. 5 shows a plot of the % cumulative amount of drug released (% CDR) against time. The amount of drug release in the first two hours was found to be higher than that in later time intervals in the formulations F1 and F2 due to the burst effect. This is due to that rate of solvent diffusion from the internal phase to the external phase was fast which causes heterogeneous distribution of drug molecules within microspheres, and accumulation of drug molecules at the ridges of the surface of microspheres. This leads to a burst effect in drug release\textsuperscript{31}. This effect was minimized in the formulations containing higher polymer content (F3 to F5). Release of drugs from polymeric microspheres in which the drug particles are dispersed in the polymer matrix with no contact between

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each other occurs by the permeation of solvent through the polymer matrix to the drug particle, dissolution of drug particle and diffusion of the drug through polymer to the release medium. Fig. 5 showed that F1 formulation higher release rates (91.73 ± 0.01%) at all time-points compared with the other formulations. It was possible that in the F2, F3, F4, and F5 formulation, the majority of the drug particles were discrete and isolated within the polymer matrix and that drug release was limited by the diffusional barrier of the polymer matrix.

The data obtained from in vitro dissolution readings demonstrated that only about a 1/4th of the drug would be released in gastric tract and the rest would be available for release in the intestinal fluid. The total absorptive area of the small intestine is about 200 m² while an estimate for stomach is only 1 m². Therefore the absorption of drug is more in intestine than in stomach region.

Statistical analysis

One way ANOVA analysis was applied on final in vitro dissolution readings. Microsoft excel 2007 software was used to calculate the value of F-ratio. The calculated value of F was 1.5 which is less than the table value of 2.54 at 1% level with d.f. being ν₁ = 4 and ν₂ = 55 and hence could have arisen due to chance. This analysis supports the null-hypothesis of no differences in sample means.

Release models and kinetics

From the release data different models were used to find out the kinetics of drug release. The data obtained for the in vitro release were fitted into equations for the zero order, first order, Higuchi, Korsmeyer Peppas models. The interpretation of data was based on the value of a resulting regression coefficient. The data obtained were shown in Table 4.

Table 3: Characterization of physicochemical parameters of microspheres

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug: polymer ratio</th>
<th>Production yield* (%)</th>
<th>Entrapment efficiency* (%)</th>
<th>Particle size* (µm)</th>
<th>Cumulative % drug release* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:1</td>
<td>66.33 ± 0.58</td>
<td>64.32 ± 0.12</td>
<td>32.76 ± 3.09</td>
<td>91.73 ± 0.01</td>
</tr>
<tr>
<td>F2</td>
<td>1:2</td>
<td>74.00 ± 0.67</td>
<td>66.22 ± 0.08</td>
<td>40.17 ± 4.11</td>
<td>84.20 ± 0.03</td>
</tr>
<tr>
<td>F3</td>
<td>1:3</td>
<td>69.83 ± 0.76</td>
<td>74.46 ± 0.18</td>
<td>52.65 ± 2.34</td>
<td>77.82 ± 0.03</td>
</tr>
<tr>
<td>F4</td>
<td>1:4</td>
<td>76.40 ± 0.40</td>
<td>71.12 ± 0.19</td>
<td>64.74 ± 2.44</td>
<td>71.07 ± 0.02</td>
</tr>
<tr>
<td>F5</td>
<td>1:5</td>
<td>75.44 ± 0.51</td>
<td>72.46 ± 0.18</td>
<td>72.15 ± 3.57</td>
<td>59.78 ± 0.03</td>
</tr>
</tbody>
</table>

*All values are given as Mean ± SD; n= 3

Table 4: In-vitro drug release models for different Propranolol hydrochloride microspheres formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi model</th>
<th>Korsmeyer Peppas model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>K (mg/hr.)</td>
<td>R</td>
<td>K (h⁻¹)</td>
</tr>
<tr>
<td>F1</td>
<td>0.964</td>
<td>8.572</td>
<td>0.963</td>
<td>-0.103</td>
</tr>
<tr>
<td>F2</td>
<td>0.970</td>
<td>7.963</td>
<td>0.968</td>
<td>-0.078</td>
</tr>
<tr>
<td>F3</td>
<td>0.971</td>
<td>6.994</td>
<td>0.960</td>
<td>-0.056</td>
</tr>
<tr>
<td>F4</td>
<td>0.962</td>
<td>6.369</td>
<td>0.961</td>
<td>-0.046</td>
</tr>
<tr>
<td>F5</td>
<td>0.955</td>
<td>5.450</td>
<td>0.981</td>
<td>-0.035</td>
</tr>
</tbody>
</table>

The regression coefficient values of different microspheres formulations namely F1-F5 was found to be between 0.964-0.955, respectively for zero order model; 0.963-0.981, respectively for first order model and 0.982-0.982, respectively for Higuchi model. The R values were much closer to one for the Higuchi kinetics. From the correlation coefficient values it was concluded that the drug release from different microspheres formulations follow Higuchi model. Higuchi model explained the matrix diffusion mechanism of drug release. The mechanism of drug release of the all microspheres formulations was studied by fitting the release data to Korsmeyer equation. The n values for formulations F1-F5 was found to be between 0.529-0.785, respectively. The n value for Korsmeyer-Peppas model was found to be between 0.5-1 indicative of non-fickian diffusion.

Selection of the best formulation on the basis of dissolution profile

Percentage drug release of the all formulations in 0.1N HCL and Phosphate buffer saline (pH 7.4) was studied for 10 hrs. The release was found to be 91.73 ± 0.01, 84.20 ± 0.03, 77.82 ± 0.03, 71.07 ± 0.02 and 59.78 ± 0.03% for PCL microspheres. The drug release from the microspheres prepared at 1:3 drug to polymer ratio was the most constant and controlled which released 77.82 ± 0.03% of drug over 10 hrs.

Stability Studies

The optimized formulation F3 (1:3), when subjected to stability studies at 25 ± 2 °C, 35 ± 2 °C, and 40 ± 2 °C, showed no significant changes in the physical properties and drug content, which confirmed that the formulation (F3) was stable at the end of 90 days (Table 5).
CONCLUSION

Controlled release propranolol hydrochloride microspheres with a matrix structure were prepared successfully by the emulsion solvent evaporation technique. The preparation method was simple and inexpensive. The obtained microspheres were fine and free flowing, the method followed was economical to get reproducible microspheres and the drug polymer ratio had an impact on the drug encapsulation efficiency and in-vitro release.

A higher encapsulation efficiency of drug was obtained at 1:3 drug: polymer ratio. The results indicated formation of spheres with different and reproducible size ranges, uniform shape and smooth outer surfaces. The different size of the produced spheres will affect significantly the drug loading efficiency, the release profiles and the dose of the released drug. The smaller size formulation showed the burst release of the drug. In comparison, the large formulation will exhibit an almost lower loading efficiency.

The drug release from the microspheres prepared at 1:3 drug to polymer ratio was most constant and controlled. Fast percolation of drug from microspheres into the dissolution medium was due to the heterogeneous distribution of drug within the matrix, the small particle size, cracks present on the surface, the hydrophilicity of the drug and the low hydrophobicity of the polymer, the low polymer-drug ratio. With the increase in the P: D volume, the fraction of the emulsion increased and larger particles were produced due to more coalescence of globules and the high viscosity of the polymer. This resulted in a homogeneous distribution of drug within the polymer matrix, reduction of the burst effect and the release of drug at a low and uniform rate. The decrease in release rate with increasing content of the polymer can be explained by a decreased amount of the drug present close to the surface and also by the fact that the amount of uncoated drug decreases with increase in polymer concentration.

The kinetic of propranolol hydrochloride release from polycaprolactone microspheres was mainly governed by the diffusion mechanism. The n value for Korsemeyer-Peppas model was found to be between 0.5-1 indicative of non-fickian diffusion. The release of propranolol hydrochloride can be controlled by proper designing of the formulation and selection of a suitable method of preparation. In the present study formulation F3 (1:3) met the target requirements.

Stability studies for three months revealed that the formulations were stable at the end of 90 days and showed no significant changes in the physical properties and drug content.

From all the parameters studied, it can be concluded that polycaprolactone is better choice of polymer for the formulation of controlled release microspheres of propranolol hydrochloride for oral administration.

Thus, the formulated microspheres seem to be a potential candidate as oral controlled delivery system.

REFERENCES

7. Neetika B, Arash D, Manish G, An overview on various approaches to oral controlled drug delivery system via

Table 5: Stability studies of formulation f3 at various storage temperatures and ambient humidity.

<table>
<thead>
<tr>
<th>Sampling interval (days)</th>
<th>Storage condition</th>
<th>Drug content*</th>
<th>Drug content*</th>
<th>Drug content*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 ± 2 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35 ± 2 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 ± 2 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>68.05 ± 0.72</td>
<td>68.05 ± 0.72</td>
<td>68.05 ± 0.72</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>67.10 ± 1.10</td>
<td>65.19 ± 0.72</td>
<td>64.23 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>66.86 ± 1.09</td>
<td>65.90 ± 1.24</td>
<td>64.47 ± 1.90</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>65.19 ± 2.15</td>
<td>64.23 ± 1.66</td>
<td>63.99 ± 2.19</td>
<td></td>
</tr>
</tbody>
</table>

*All values are given as Mean ± SD; n= 3


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