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Poly(palmitoyl-L-hydroxyproline ester) microspheres as potential oral controlled drug delivery system

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Abstract

A new type of carrier using poly(palmitoyl-L-hydroxyproline ester) (PPH) [IUPAC name: poly((1-palmitoyl-4,2-pyrrolidinediyl)carbonyloxy)], a poly(amino acid) is described. The polymer is synthesized by conventional method and the microspheres were prepared by solvent evaporation technique for application in drug delivery system. Microspheres with different sizes were prepared by varying certain formulation and technological parameters and their distributive stabilities under physiological conditions were studied. The microspheres were characterized by DSC, optical and laser particle size analysis. A model drug, rifampicin (antituberculosis drug) was entrapped in the microspheres and the in vitro release studies were performed in pH 7.4 and pH 1.5 buffer media. The pH value seemed to have some influence on the dissolution rate of the rifampicin-containing microspheres. Dissolution experiments using rifampicin indicated the possibility of using PPH microspheres with other hydrophobic drugs.

Keywords: Microspheres; Rifampicin; Solvent evaporation; Drug release; Poly(palmitoyl-L-hydroxyproline ester)

1. Introduction

One of the approaches to the controlled release of drugs involves incorporation of drug molecules into the matrix of polymeric microspheres or microcapsules (Madan et al., 1972; Mathiowitz et al., 1990; Shantha and Rao, 1993) and to deliver it at the targeted site over a long period of time. Orally administered drug has limitations such as gastrointestinal transit time (Davis et al., 1984) and too short residence time of the drug at the site of absorption. Several other bioadhesive systems like 'stick' dosage forms are available which provides longer transit time due to adhesion of the device to the gastric mucosal wall (Ch'ng et al., 1985). However possibility of irritation to the mucosa, associated with such systems, makes it toxic. Our interest is to design the microspheres

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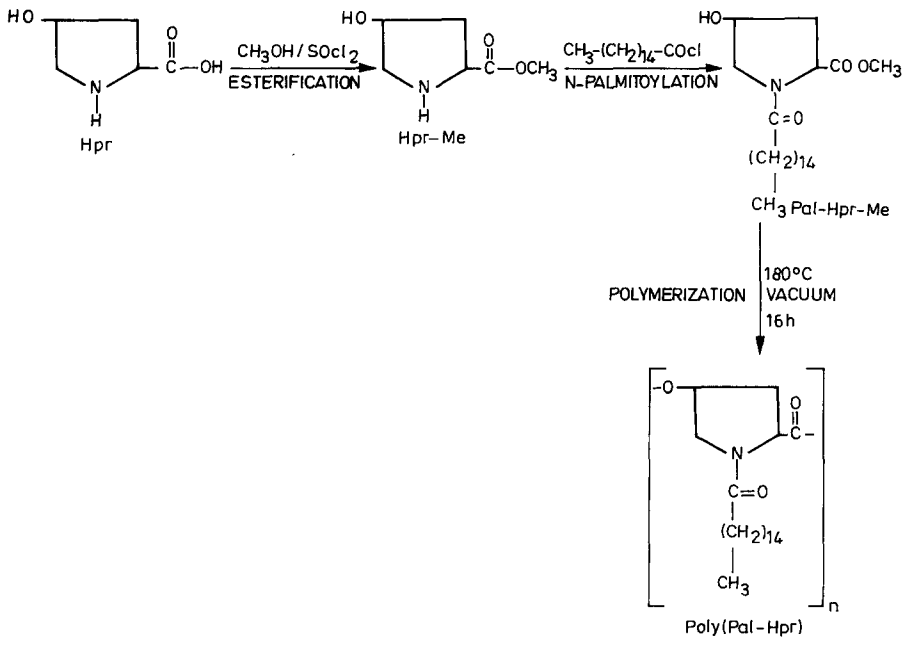

Fig. 1. Steps involved in the synthesis of PPH.

which has high distributive stability and which will be dispersed widely throughout the gastrointestinal tract, providing a possibility of achieving longer-lasting and more reliable release of drug. Furthermore, such a stable dispersion of microspheres in the gastric fluid avoids a high local concentration of drug in the gastric mucus (Hunter et al., 1980, 1982) which ultimately leads to mucosal injury (Hey et al., 1979).

The possible toxicity associated with the metabolites of a polymer that degrades slowly within the human body is another major problem (Smith and Hunneyball, 1986; Lherm et al., 1992) which can be avoided by the use of naturally occurring nutrients or metabolites such as amino acids as monomeric starting material. In the present study, poly(palmitoyl-L-hydroxyproline ester) a polymer synthesized from L-hydroxyproline was utilized and such non-peptide poly(amino acid) has been identified as a potent biodegradable polymer (Kohn and Langer, 1985). The present study centers on the development of methods to produce poly(Pal. Hpr. ester) microspheres of various sizes and to evaluate its application in delivering hydrophobic drugs. Rifampicin, an hydrophobic drug, was chosen as a model drug for our studies. Rifampicin is used mainly for the treatment of tuberculosis in combination with other drugs. Nevertheless, rifampicin may cause certain adverse actions like heartburn, epigastric distress, cramps, diarrhoea, anorexia, nausea, vomiting and other CNS side effects which can be avoided by incorporating in a controlled releasing matrix.

2. Materials and methods

L-Hydroxyproline was purchased from Sigma Chemical (St. Louis, MO). Aluminum isopropoxide and palmitoyl chloride were from Aldrich Chemical (Wisconsin, USA) and were used as such without any purification. 1,6-Diaminohexane is from E. Merk (Germany). Tween-80 was purchased from SRL (India).

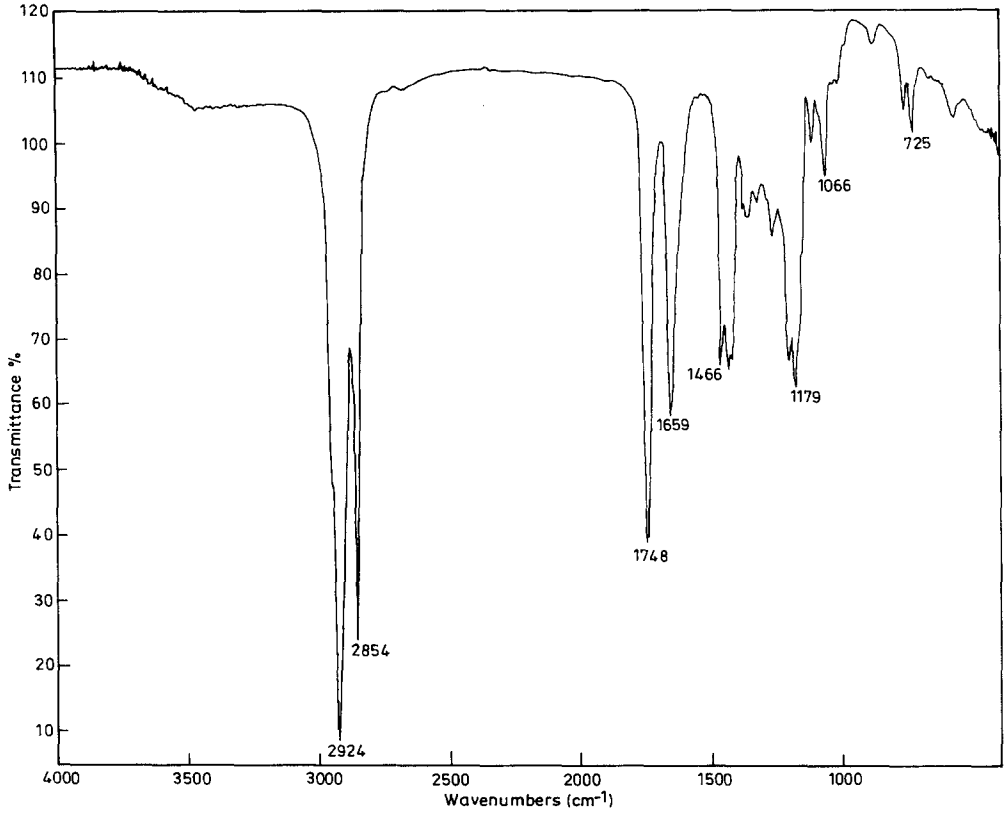

Fig. 2. FTIR spectrum of poly(Pal. Hpr. ester).

2.1. Preparation of poly(Pal. Hpr. ester)

Poly(palmitoyl-L-Hydroxyproline ester) (PPH), was prepared by *N*-palmitoylation of L-hydroxyproline ester followed by transesterification of palmitoyl-L-hydroxyproline methyl ester in the presence of aluminum isopropoxide as catalyst under vacuum at 180°C for 16 h similar to the method developed by Kohn and Langer (1987). The resulting polymer was purified by dissolving it in tetrahydrofuran (THF) (0.5 g/ml) and reprecipitating it by the addition of isopropanol. FT-IR and NMR characterization were carried out for the polymer.

2.2. Microsphere preparation

Poly(pal. Hpr. ester) microspheres were prepared by solvent evaporation method. Free microspheres as well as microspheres loaded with rifampicin, an antituburculosis drug were prepared. Microspheres of various sizes have also been prepared by varying the concentration of the dispersing agents added. In a typical procedure, about 100 mg of polymer and 30 mg of the drug were dissolved in 3 ml of dichloromethane and dripped into 50 ml of an aqueous solution containing 0.25% of polyvinylalcohol and 2% of Tween-80. The resulting solution was stirred at 30°C at 300 rpm for 20 min until all the solvent evaporates, leaving behind the microspheres. The microspheres were filtered, washed several times with distilled water to remove the adhered surfactant and dried in vacuum.

Addition of hydrophobic diamines like 1,6-diaminohexane has a profound effect on the size of the microspheres which was also carried out. The concentration of diaminohexane in aqueous dis-

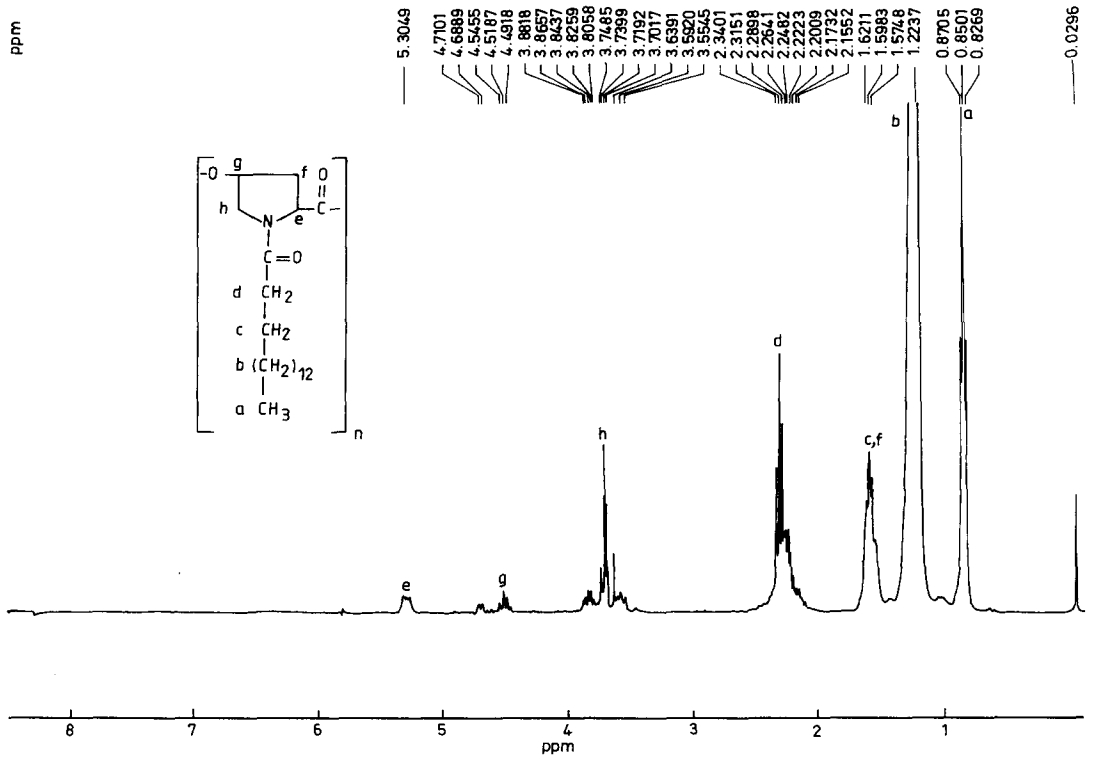

Fig. 3. ^{1}H-NMR spectrum of poly(Pal. Hpr. ester).

persing solution was increased gradually prior to the addition of polymer solution.

2.3. Microsphere characterization

The microsphere morphology was studied by optical microscopy (Hertel and Reuss, West Germany). All the particles were sized by a laser diffraction technique (Malvern Particle size analyzer). Size distributions were displayed in terms of percentage versus particle size. Differential scanning calorimetry (DSC) of the microspheres was carried out using V4.1C Dupont 2000 instrument. The PPH microspheres were sealed in an aluminum sample pan and DSC thermogram was obtained at the heating rate of 10°C min^{-1} with a sampling size of 5.755 mg in nitrogen atmosphere.

2.4. Distributive stability test

In order to determine the distributive stability (Ohya et al., 1994) of the microspheres, they were added to phosphate buffered saline (PBS) of pH 7.4, and the suspension (4 mg/ml) was poured into a quartz cell. The change in the turbidity of the suspension was monitored periodically by observing the transmittance at 600 nm using a UV-Visible spectrophotometer (Shimadzu 2100S).

2.5. Percentage loading of drug

First, 2.5 mg of microspheres loaded with rifampicin was dissolved in about 25 ml of chloroform at room temperature. The assay was carried out using an UV spectrophotometer for quantitative determination of rifampicin (λ_{max} in chloroform = 351 nm).

2.6. In vitro release of rifampicin

The in vitro release of drug at intestinal (pH 7.4) and gastric (pH 1.5) media were carried out by suspending about 25 mg of microspheres in 50 ml of the corresponding buffer. The contents were equilibrated at 37°C and perfect sink condition was observed throughout the release studies. At periodic intervals of time, aliquot of 3 ml were withdrawn from the release medium and assayed spectrophotometrically at 335 nm in pH 7.4 buffer and at 337 nm in pH 1.5 medium. After each removal of aliquot a fresh buffer of equal volume were added to the release medium. The experiment was carried out in triplicate.

3. Results and discussion

An outline of the steps involved in the synthesis of PPH is summarized in Fig. 1. The polymer obtained was found to be soluble in all chlorinated hydrocarbons, THF, ether and hexane.

The structure of the polymer was confirmed by IR and NMR spectra (Figs. 2 and 3).

FTIR (KBr): 2924 cm^{-1} (s), 2854 cm^{-1} (m) (CH stretching), 1748 cm^{-1} (s) (C=O str), 1659 cm^{-1} (s), 1466 cm^{-1} (m) (amide band) and 1179 cm^{-1} (s), 1066 cm^{-1} (m) and 725 cm^{-1} (w) (C–O–C str)

^{1}H-NMR ($CDCl_3$): 0.8501 (3H, t, CH_3), 1.2237 (24 H, s, 12 CH_2), 1.62 (2 H, m, CH_2), 2.24 (4 H, br, m, $2CH_2$), 3.79 (2 H, br, m, CH_2), 4.5 (H, t, CH), 5.3 (H, br, m, CH)

One of the interesting and advantageous feature that can be envisioned from the structural point of view of the polymer is that it does not have any reactive functional group. Hence the carrier-drug interaction which is common with some carriers (Arias et al., 1995; Lessen and Zhao, 1996), is not feasible in the case of PPH. Such an inert matrix would be an ideal carrier for controlled release system.

The optical micrographs of PPH microspheres formed by solvent evaporation method are shown in Fig. 3. As it is observed the microspheres are spherical with relatively a wide distribution of particle sizes. The waxy nature of the polymer results in soft microspheres which adheres to each other in the dried condition (Fig. 4a). However, dispersion in aqueous medium causes the microspheres to be separated from each other (Fig. 4b).

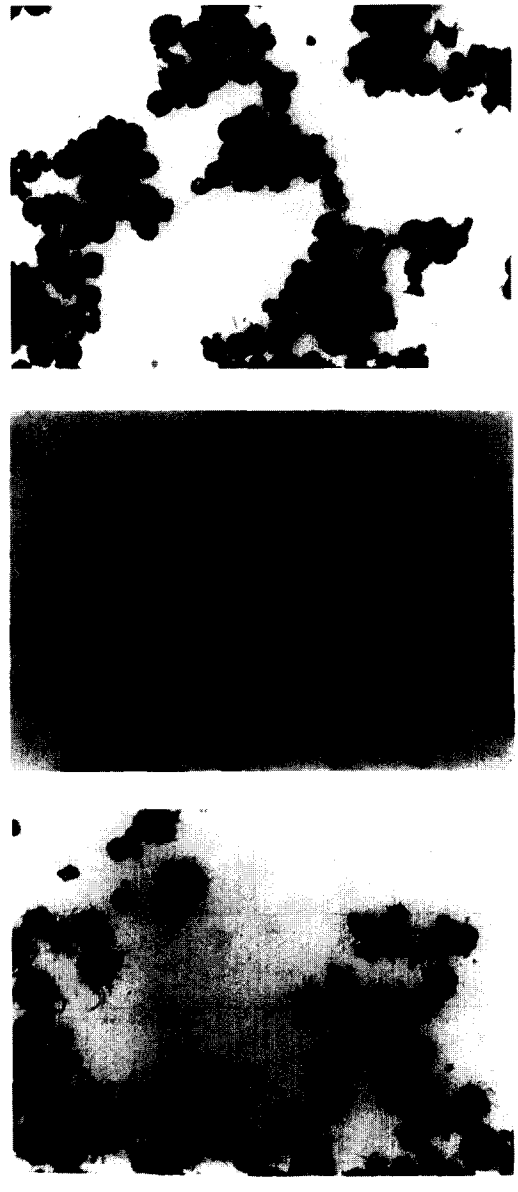

Fig. 4. Light micrographs of poly(Pal. Hpr. ester) microspheres prepared at 300 rpm by solvent evaporation method. (a) Dried microspheres; (b) microspheres after dispersion in water; (c) microspheres after incubation in basic medium (pH 8) for 12 h.

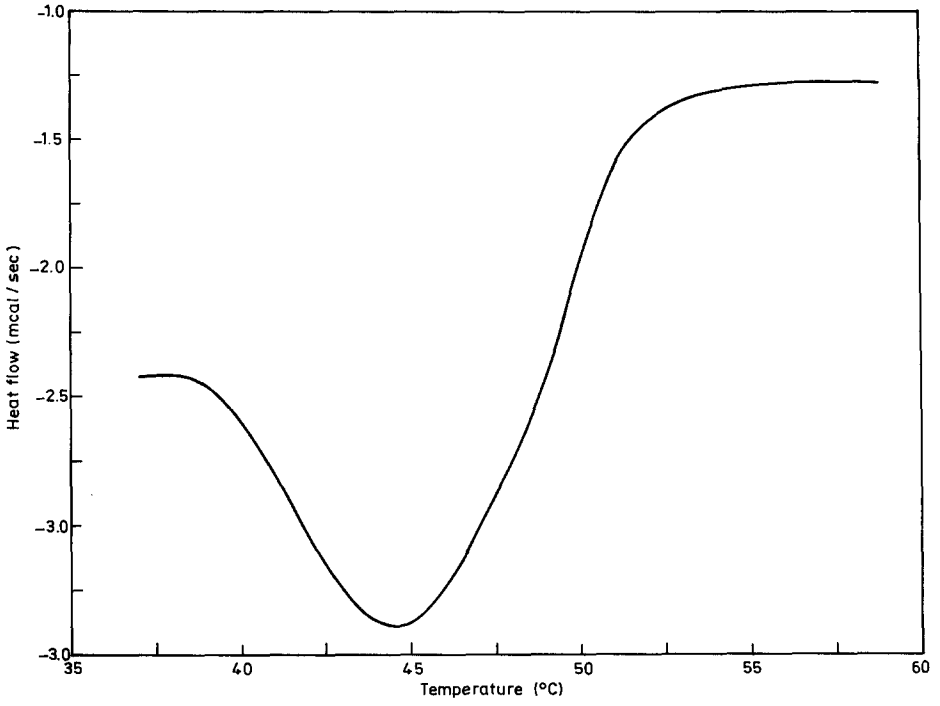

Fig. 5. DSC thermogram of empty PPH microspheres.

Incubation of microspheres in a basic medium for more than 12 h causes the microspheres to degrade slowly with a surface erosion and fibre formation.(Fig. 4c).

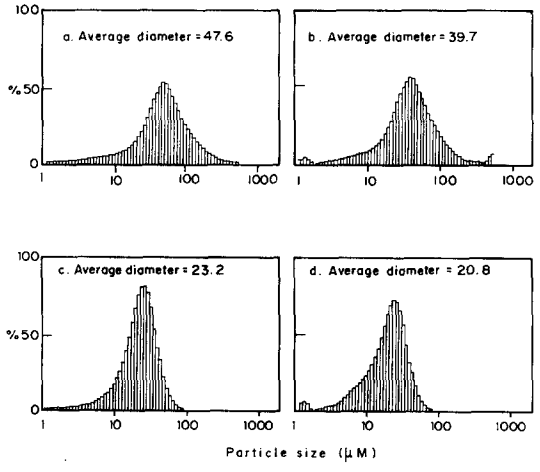

Fig. 6. Particle size distribution curve for the microspheres after addition of hexamethylenediamine to the dispersing solution. (a) 1% (v/v) of HMD addition; (b) 2% of HMD addition; (c) 3% of HMD addition; (d) without HMD addition.

The DSC thermogram of the empty PPH microspheres shows the melting transition of the material to occur around 45°C (Fig. 5).

In the preparation of poly(Pal. Hpr. ester) microspheres, the effect of addition of 1,6-diaminohexane and Tween-80 (poly(oxyethylene) sorbitan monooleate) a surfactant have been studied. Addition of Tween-80 during the preparation stabilizes the emulsion. A high speed stirring technique which in general utilized for particle size reduction in solvent evaporation method (Niwa et al., 1993; Scholes et al., 1993) cannot be applicable for soft natured PPH microspheres which results in col-

Table 1
Influence of HMD concentration on microsphere size

HMD concentration (% v/v)	Mean diameter (μm)
0	47.6
1	39.7
2	23.2
3	20.8

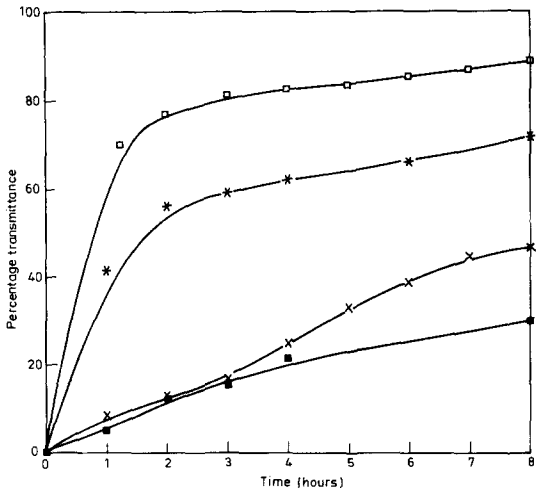

Fig. 7. Effect of mean particle size on the distributive stability of the microspheres in phosphate buffered saline (PBS) solution. (□) 47.6 μm, (*) 39.7 μm, (x) 23.2 μm, (■) 20.7 μm.

lapsed structure. However addition of a water miscible hydrophobic diamine like 1,6-diaminohexane (HMD) was found to disintegrate the emulsion into microemulsions which results in microspheres of smaller size. This effect can be attributed to the interfacial turbulence between the organic phase and the aqueous phase arising from the rapid diffusion of HMD across the interface, which spontaneously produces a larger interfacial area and results in finer droplets/particles. The size distributions of microspheres in buffer (pH 7.4) are shown Fig. 6. At a constant stirring speed without addition of HMD, the mean diameter of the microspheres were found to be 47.6 μm. Presence of about 1% (v/v) of HMD in the dispersing solution decreases the particle size to 39.8 μm at the same stirring speed (Table 1). Similarly increase in the concentration of HMD to 2% and 3% decreases the mean particle size of the microspheres to 23.2 μm and 20.8 μm respectively. However further increase in the concentration of HMD does not have any significant effect on the particle size which indicates the saturation limit. Such formulation and technological variables have influenced the particle size distribution revealing the importance of the characteristics of the emulsion formed prior to the particles.

The results of distributive stability test of PPH microspheres of various sizes are shown in Fig. 7. The lower distributive stability of microspheres results in increasing the transmittance of the suspension. Apparently, microspheres with the mean particle size range of 20–25 μm have high distributive stability compared to that of microspheres of larger size. Such smaller size microspheres with high dispersion stability can be an ideal candidate for oral drug delivery system.

The loading efficiency of rifampicin entrapped PPH microspheres was found to be 6.8%. The dissolution profiles of rifampicin from microspheres at pH 7.4 (simulated intestinal fluid) and pH 1.5 (simulated gastric fluid) media are shown in Fig. 8. In both the media a more or less zero order release rate is observed for the first 12 h, which predicts the diffusion mechanism to be the rate controlling. There is a continuous release of the incorporated drug from the microspheres. The release was about 7% for the first 2 h both in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). For the first 12 h, about 40% of drug was released in SIF, whereas in SGF only about 15% of the drug was released. Thus the release rates from the microspheres changes above and below pH 7. As discussed earlier, PPH microspheres has a tendency to undergo surface erosion with fibre formation in the basic medium. This observation precludes a release mechanism where

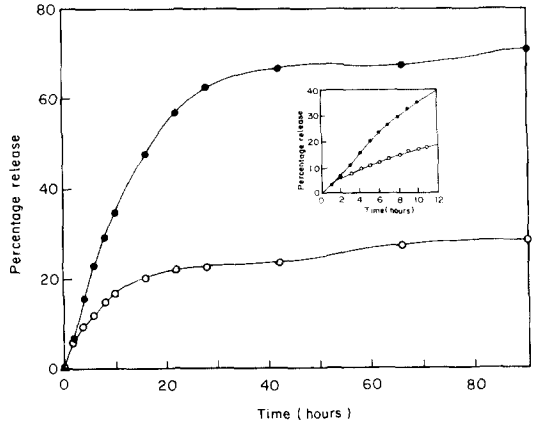

Fig. 8. Release of rifampicin from PPH microspheres. (●) pH 7.4 buffer; (○) pH 1.5 buffer. Each datum point represents the average of three determinations.

the rate-limiting control is by diffusion alone in acidic medium (pH 1.5) which results in lower dissolution rate, and is by both diffusion and surface erosion of microspheres in pH 7.4 buffer (SIF) which results in faster dissolution of drug.

4. Conclusions

In conclusion, a new type of polymeric microspheres with a lipidic side chain has been evaluated as oral drug delivery system. Alteration in the experimental conditions has made it possible to predict the optimal conditions for the microsphere formation and to anticipate the size of the obtained microspheres. From the above study it can also be presumed that decrease in the size of the microspheres increases their dispersion stability under gastrointestinal conditions thereby proving its worthiness as oral drug delivery system. These microspheres can be used with a wide variety of hydrophobic drugs as a new drug delivery system. Hydrophobic drugs such as ibuprofen, indomethacin and nifidipine have been successfully encapsulated in the microspheres, the results of which will be discussed in our future publications.

An attempt is also being made to reduce the particle size to submicron range. When particle size is properly selected, then microspheres could be promising and effective as an injectable carrier for lipophilic antitumour agents in order to enhance the tumour delivery and efficacies with reducing toxicities.

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