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RESEARCH ARTICLE

STUDY ON FORMULATION AND EVALUATION OF ROPINIROLE HYDROCHLORIDE LOADED MICROSPHERES USING POLYMERS BLEND OF ETHYL CELLULOSE AND CARBOPOL 934P

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Abstract

Microspheres of Ropinirole hydrochloride (RH) loaded with ethyl cellulose and carbopol 934P were prepared using solvent evaporation method. Formulations (RH1-RH5) with different ratios were evaluated for various parameters percent yield, shape, particle size, drug content, entrapment efficiency, swelling ratio, powder X-ray diffraction studied (PXRD) and *in-vitro* release studies. All the particles of prepared microspheres were in micrometric range (73.04-120.16 µm). Entrapment efficiency and percent drug content was found to be highest for formulation RH3. *In vitro* release studies revealed that all the formulations showed sustained release pattern with highest release in RH3 formulation (96.09%) and selected for further studies. Various kinetic models were applied and best fit with highest correlation coefficient (89.1± 1.70) was observed in koresmeyer peppas model, indicating diffusion controlled principle. Swelling ratio was highest for formulation RH 3 and revealed initial swelling followed by diffusion of drug from microspheres. Scaning Electron Microscpy (SEM) images of RH 3 showed that microspheres were smooth, non- porous, homogenous in size and spherical in shape. PXRD studies indicated that drug was molecularly dispersed throughout the polymeric matrix. It is concluded that microspheres has improved residence time by enhancing its sustainability in body.
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Keywords: Entrapment efficiency, *in vitro* release studies, microparticles, Parkinson's therapy, SEM, swelling ratio.

Introduction

Ropinirole HCl (4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one monohydrochloride) is an orally administered non-ergoline dopamine D2 receptor agonist used for treating Parkinson's disease and restless legs syndrome (Adler et al., 1997; Tulloch, 1997). It is highly water soluble drug (133 mg ml^{-1}) (Patil et al., 2013), but possess low bioavailability due to less GI residence time and short elimination half life (6 hrs) (Moffat et al., 2004). Moreover, it is a potent drug (2 mg) required to be administered 3–4 times in a day that affects the drug plasma concentration triggering on-off phenomenon associated with the Parkinson's disease (Agnihotri et al., 2012). Thus, it is utmost requirement to reduce the dosing frequency and to maintain the optimum drug concentration.

To hamper these problems; drug with sustain release effect has to be formulated. These sustained release formulations releases the drug slowly and maintained an effective drug concentration at the target site for a longer period of time and improve patient compliance at target site by decreasing frequent dosing. Various approaches are available in literature for delivering a therapeutic substance to the target site in a sustained release fashion. One such approach is using microspheres (an accurate and more reliable drug release method) as carriers for drugs (Das and Rao, 2006). Microspheres, the best suitable oral drug delivery system because this leads to increased bioavailability, reduced side effects, maintained improved efficacy by decreasing dose frequencies.

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Polymers like Ethyl cellulose (EC) and Carbopol 934P was used for the preparation of microspheres because these can modify the drug release by increasing their residence time in GIT tract and thus, increases the therapeutic efficacy of the drug. EC is a non-ionic inert hydrophobic, non-biodegradable and biocompatible polymer with minimal toxicity (Garud and Garud, 2012) whereas Carbopol is an anionic polymer, hydrophilic in nature, readily absorbs water, gets hydrated and swells and hence, a potential candidate for use in controlled release drug delivery (Obeidat, 2009). It was noticed from literature that the different concentrations of polymers blend have great influence on particle size, drug content, *in vitro* release studies and entrapment efficiency of the formulations.

Thus, in present investigation, microspheres of Ropinirole Hydrochloride (RH) were prepared by solvent evaporation technique (Deveswaran et al., 2010) using polymers blend of Ethyl Cellulose (EC) and Carbopol 934P using different ratios *viz;* 1:3, 2:3, 3:3, 4:3 and 5:3. Developed EC/Carbopol 934P microspheres were evaluated for various parameters like percent yield, shape, particle size, drug content, entrapment efficiency, swelling ratio, SEM, PXRD and *in-vitro* release studies.

Materials and Methods

Ropinirole Hydrochloride (RH) was procured as gift sample from Ind Swift Ltd., Derabassi and was used without further purification. Ethyl Cellulose and Span 80 was procured from Himedia Laboratories Pvt Ltd, Mumbai whereas Carbopol 934P from Ranbaxy Laboratories Ltd., Gurgaon.

Preparation of microspheres by solvent evaporation technique (Manekar et. Al., 1992)

RH microspheres were prepared using polymers blend (EC/Carbopol 934P) in different ratios *viz;* 1:3, 2:3, 3:3, 4:3 and 5:3, by solvent evaporation technique. Weighed amounts of RH, EC and carbopol 934P were dissolved in ethanol separately and mixed with stirring for 15 min and was transferred to a mixture of 90 ml light liquid paraffin and Span 80 with constant stirring for 2 hr at 500-700 rpm on a mechanical stirrer at room temperature until ethanol evaporated completely. The formed microspheres were filtered and washed 4–5 times with petroleum ether (50 ml). The product was then dried at room temperature for 24 h and stored in desiccator for further use.

Evaluation of microspheres

Determination of %yield

Thoroughly dried microspheres were collected and weighed accurately. The % yield was calculated using the formula given below (El-Kamel et al., 2001)

% Yield = (Total weight of floating micro particles / Total weight of drug and polymer) × 100

Determination of Particle Size and their distribution

Particle size of drug-loaded microspheres was determined by optical microscopy *(*Josephine et al., 2011). A small amount of formulation was placed on a clean glass slide, mounted on microscope. Microspheres seen in the four virtual quadrants of focused region of glass slide under microscope were counted by tally marking and their projected diameters were determined. Particle size was calculated by using following Edmondson's equation:

$$D\ mean = \Sigma nd / \Sigma n$$

Where, n= number of microspheres observed and d= mean size range.

Encapsulation efficiency of microspheres

For determination of encapsulation efficiency, microspheres were crushed in glass mortar and powdered, then suspended in 10 ml of ethanol for 24 h. Solution was filtered and analyzed spectrophotometrically at 250 nm. The drug efficiency was calculated by the following formula (Prajapati et al., 2008)

% Encapsulation Efficiency = Practical content/Theoretical Content x 100

Drug content

The microspheres were powdered and suspended in phosphate buffer (pH 7.4) (Anand et al., 2004). The resultant dispersion was kept for 20 min on the sonicator bath for uniform mixing and filtered through whatman filter paper. The filtrate obtained was examined using a UV visible spectrophotometer at 250 nm.

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In vitro drug release studies

The drug release from microspheres was determined using USP paddle type dissolution testing apparatus (maintained at 37°C) (Kundawala et al., 2011; Madan et al., 2013). Paddle speed was adjusted to 50 rpm using phosphate buffer pH 7.4 as dissolution media for 24 h. At predetermined time intervals, aliquots of samples (10 ml) were withdrawn and replaced with an equal volume of plain dissolution medium and analysed by UV spectrophotometer at 250 nm and repeated.

Determination of swelling ratio

The swelling ratio of RH microspheres was determined thrice by using shaking method immersing 100 mg of microsphere sample in 25 ml phosphate buffer (pH 7.4) at room temperature for 24 h with gentle shaking. At specific time points (1, 2, 3, 4, 6, 8, 10, 12, 22, 24 h) samples were removed and weight of swollen microspheres was determined by the following equation (Sevgi and Omer, 1998; Mladenovska and Raick, 2007)

$$E_{sw} = W_{sw}\text{-}W_o / W_o \text{ X } 100$$

Where, E_{sw} is the swelling ratio ; W_o is initial dry weight of microspheres and W_{sw} is the weight of the swollen microspheres.

Scanning Electron Microscopy (SEM*)*

Samples of the microspheres were mounted onto the stubs using double-sided adhesive tapes. The stubs were then coated with platinum to a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. Afterwards, the stub containing the microspheres was placed in the SEM chamber and the surface morphology of the microspheres was studied.

Powder X-Ray Diffraction (PXRD) Studies

X-ray diffraction study was carried out to characterize the physical form of Ropinirole HCl. PXRD patterns were traced using Nickel filtered CuK (gamma) radiation, a voltage of 35 kV and a current of 20 mA. The samples were analyzed over 2θ range of 2–40° with scan step size of 0.020° (2θ) and scan step time of 1 s.

Curve Fitting (Kinetic modelling)

In vitro studies indicate about the percentage release of drug from microspheres but provide slight insight about the mechanism of release of drug. To find the way of release the in *vitro* release data were fitted to different kinetic models (Saffari et al., 2008)

Cumulative percentage drug release Vs. Time (zero order rate kinetics)

- ❖ Log cumulative percentage drug retained Vs. Time (first order rate kinetics)
- ❖ Cumulative percentage drug release Vs. √T (Higuchi's classical diffusion equation)
- ❖ Log of cumulative percentage drug release Vs. log Time (Peppas exponential equation)
- ❖ Cube √percent drug unreleased Vs. Time (Hixon crowell)

Results and Discussion

Percentage yield

Percentage yield was calculated and was found to be higher for formulation RH 3 (98.34%) which is quite satisfactory for formulating microspheres.

Particle size and its distribution

Particle size of all the five formulations was found to be in range of 73.04 to 120.16 µm (shown in Figure 1). It was observed that with increased amount of polymer, size of the particle increases. RH5 (120.16 µm) formulation have shown highest particle size which is in micrometric limit required to deliver the drug at target site in desired concentration when given by oral delivery mode. The order of particle size of formulations was found to be RH 5 > RH 4 > RH 3 > RH 2 and RH 1.

Drug content

Drug content of microspheres was estimated by UV Spectophotometric method. % drug content of each formulation was evaluated and compiled in Table 1. Value of drug content ranges from 84.1% to 96.8% and was found to be higher in formulation RH3. Drug content increases with increased concentration of polymers (RH1-RH3) but decreases after due to increased amount of polymer/drug ratio/bulkiness.

Encapsulation Efficiency

It is clear from table 1 that, with the increased concentration polymer, encapsulation efficiency of the drug increases (RH1-RH3) which may be attributed to the increased availability of the polymer for encapsulating the drug i.e. Ropinirole HCl. Drug

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loading was further found to decreased (RH4-RH5) with further increasing the concentration of encapsulating polymer which might be due to high concentration of EC/carbopol 934P in the aqueous phase, retarding their drug loss. Moreover, increased quantity of polymers may increase the bulkiness and decrease the amount of drug per microsphere and lowers its efficacy. Out of all the formulations encapsulation efficiency was found to be higher for formulation RH 3 (83.35%).

Swelling index for microspheres

Results from figure 2 showed that swelling ratio increases with the passage of time as well as with the increase in concentration of polymers blend. Enhanced swelling index was due to the high molecular weight of polymers. Moreover, polymers decrease the release of drug by reducing pore size of polymer matrix greatly due to imbibition of dissolution media. However, small projections were appeared on the surface of the microspheres and grew larger with the passage of time and expanded out of the microspheres. From the graph, it was clear that RH3 formulation have shown highest swelling ratio out of all the formulations.

RH3 formulation have optimum amount of polymer required to entrap the sufficient amount of drug inside the microsphere formulation and showed satisfactory swelling properties as it fulfils the requirement of slow and continuous release of drug through microspheres.

Comparative In-vitro release studies of RH loaded microspheres.

In vitro release data after 24 hr was shown in figure 3. From the graph it was found that RH1, RH2, RH3, RH4 and RH5 have shown 99.35%, 98.82%, 96.09%, 87.08% and 83.75% release of drug respectively. It was observed that as the polymer quantity increases, the dissolution profile decreases. This is due to enhanced concentration of polymer increases the size of the microsphere, thereby decreases the overall surface area for the erosion leading to reduced dissolution. Formulation RH 3 showed higher drug release 96.09% of drug in 24 h and was found to be the best formulation among all formulations.

Additionally, in dissolution profile the lowest curve of formulation also represent that formulation RH 3 showed maximum sustained release and was selected for further studies (SEM and PXRD).

Kinetic modelling studies of RH loaded EC/Carbopol 934Pmicrospheres

Kinetic models were applied to best formulation (RH3) to find out the mechanism of drug release from microspheres. The interpretation of data was based on the value of the resulting regression coefficient obtained through the graphs as shown in figure 4. The regression parameters obtained from the plots are tabulated in table 2. The values of R^2 in Zero order, First order, Higuchi, Hixon crowell equation and Korsemeyer Pappas equation were found to be higher in korsemeyer Pappas. The order for the models found in RH loaded formulations is

Korsemeyer Pappas> Hixon crowell> First order> Higuchi> Zero order

From the results, it was found that the release kinetics of RH loaded microspheres containing blend of polymers were best explained by Korsmeyer Peppas model in all the formulations and described drug release kinetics in the most befitting manner. The observed value of R^2 was found to be less than 0.455 which indicates non fickian diffusion as possible mechanism of drug release.

Scanning electron microscopy (SEM)

It was clear from figure 5 that microspheres prepared by polymers blend (EC/carbopol 934 P) were smooth, non- porous, homogenous in size and spherical with nearly regular surface. SEM image also revealed that there is no aggregation or lumps of microspheres, thus indicating topological physical stability of microspheres.

Solid-State Characterization: Power X-ray Power Diffraction

Diffraction pattern of Ropinirole HCl shows sharp diffraction peaks at (2θ) 10.24°, 11.40°, 12.42°, 13.46°, 19.90°, 21.47°, 22.52°, 24.80°, 26.26°, 30.23°, 35.21°, 36.16°, 37.17°, 38.17° and 40. 16° with corresponding to linear count of 3000; verify the crystalline character of the drug. But, these characteristic peaks are not found in drug loaded sample showed the diffused pattern (Figure 6) indicating that the drug is dispersed at molecular level in the polymer matrix. The diffused pattern observed in drug loaded microspheres may be due to the amorphous nature of polymers and inclusion of drug in holes of the polymer.

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Table 1. Evaluation of RH loaded Microspheres containing polymers blend

Parameters	RH 1	RH 2	RH 3	RH 4	RH 5
Percent yield (%)	76.13	81.16	98.34	92.34	90.51
Shape	Spherical	Spherical	Spherical	Spherical	Spherical
Particle size (µm)	73.04	85.37	105.12	112.15	120.16
Drug content (%)	84.1	87.3	96.8	91.4	89.5
Encapsulation efficiency (%)	70.07	77.45	83.35	80.87	78.42

Table 2. Kinetic modelling regression parameters for RH 3

Regression parameters	Zero order	First order	Korsemeyer peppas model	Higuchi model	Hixon crowell model	Best fit model
Slope	2.94	0.076	0.618	19.17	0.139	Korsemeyer peppas model

Figure 1. Particle size distribution of drug-loaded microspheres

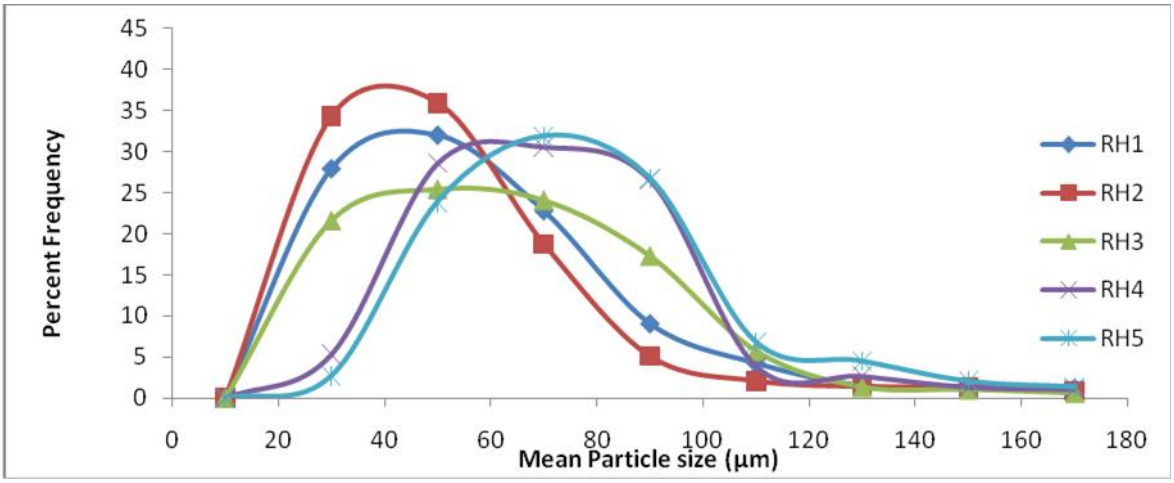

Figure 2. Swelling study of the prepared microspheres in PB 7.4. Data shows mean (n=3) ±SD

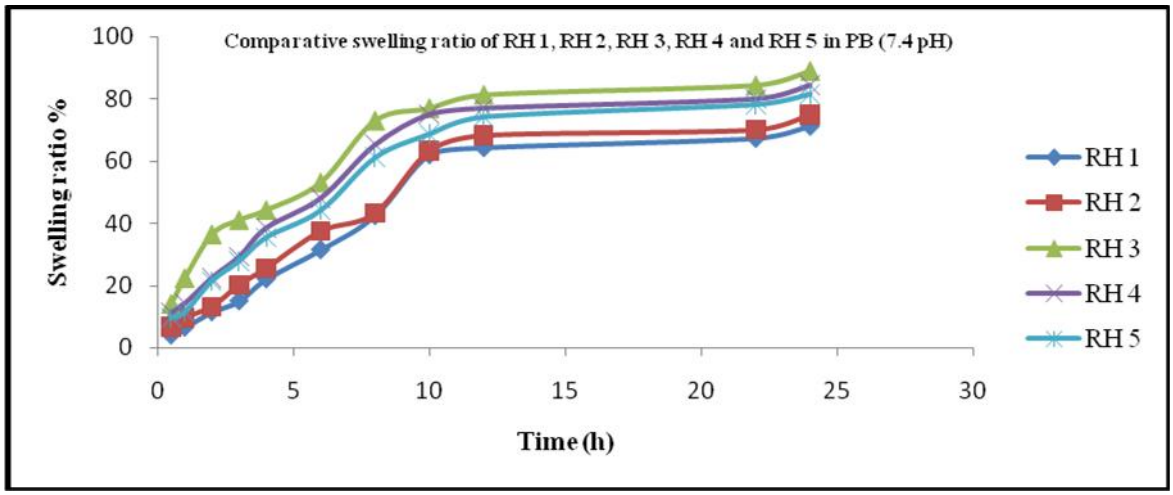

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Figure 3. Comparative *in vitro* release profiles of RH loaded microspheres. Data shows mean (n=3) ± SD

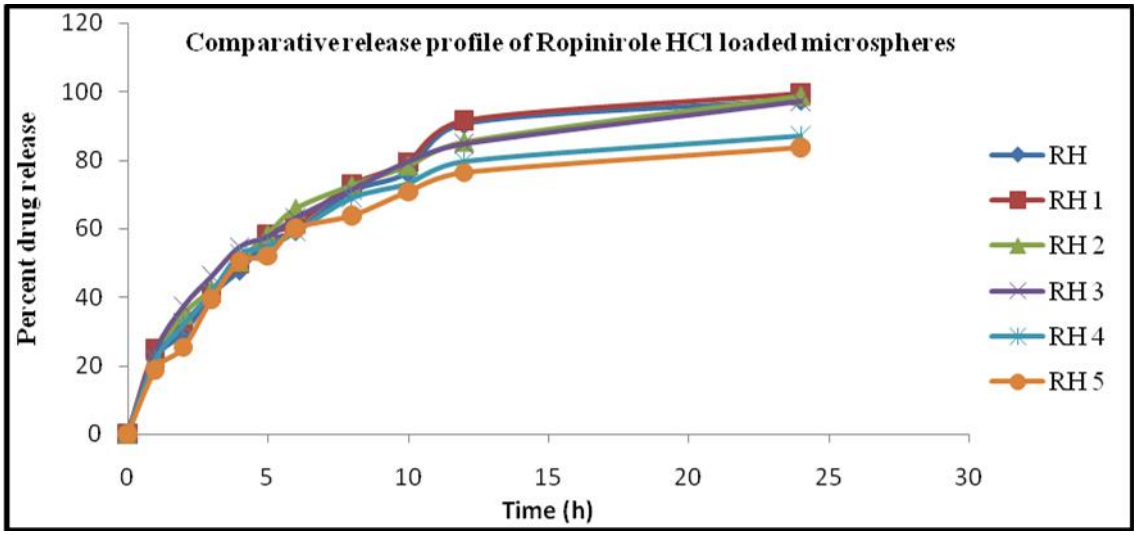
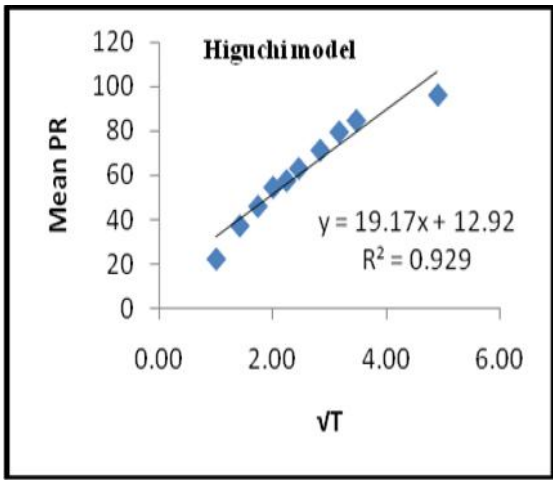
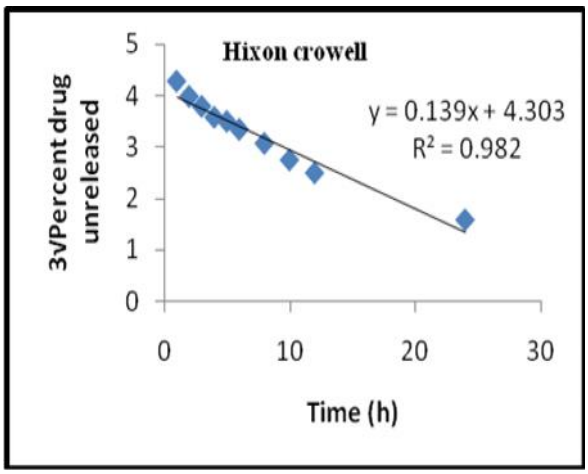


Figure 4 : Plots of developed kinetic models for formulation RH 3





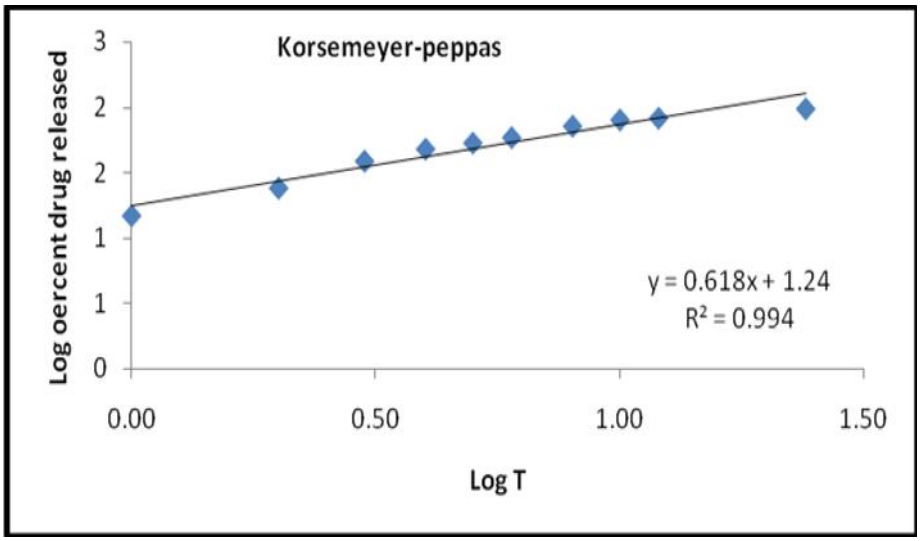


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Figure 5; SEM photomicrograph of formulation RH 3 prepared with: EC/Carbopol 934P (120 X)

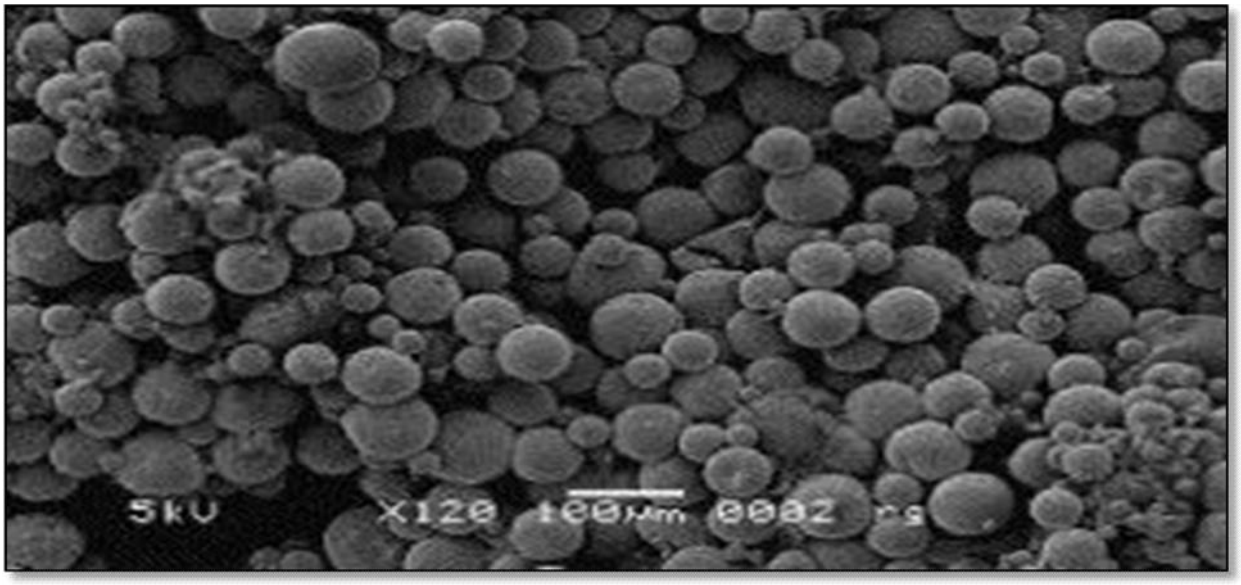
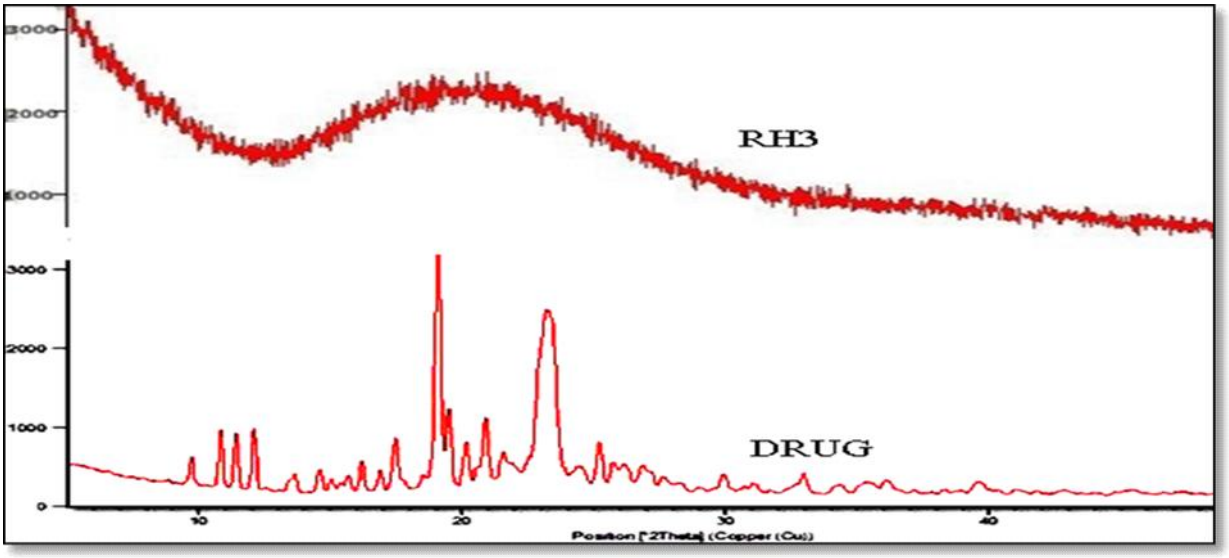


Figure 6: X-ray Diffraction pattern of formulation RH 3 and EC/Carbopol 934P



Conclusion

Out of five prepared formulations, RH3 formulation was selected as the best formulation because it has shown sustained release pattern with optimum swelling index. Further, this formulation lies in micrometric range with maximum entrapment efficiency. From the results it was concluded that microspheres can be a good alternative to tablets, which offer dose flexibility, prolonged GI residence and can prevent dose dumping.

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