

Original Article**PREPARATION, CHARACTERISATION AND EVALUATION OF ROPINIROLE HYDROCHLORIDE LOADED CONTROLLED RELEASE MICROSPHERES USING SOLVENT EVAPORATION TECHNIQUE****KOYEL KAR^{a*}, R. N. PAL^b, N. N. BALA^c**

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*Received: 19 Mar 2018 Revised and Accepted: 27 Apr 2018***ABSTRACT**

Objective: The major objective of the research work was to design, characterise and evaluate controlled release microspheres of ropinirole hydrochloride by using non-aqueous solvent evaporation technique to facilitate the delivery of the drug at a predetermined rate for a specific period of time.

Methods: Ropinirole hydrochloride microspheres were prepared by using different low-density polymers such as eudragit RL 100, eudragit RS 100 and ethylcellulose either alone or in combination with the help of non-aqueous solvent evaporation technique. All the formulated microparticles were subjected to various evaluation parameters such as particle size analysis, micrometric properties, drug entrapment efficiency, percentage drug loading, percentage yield and *in vitro* drug release study. The compatibility of the drug and polymers was confirmed by physical compatibility study, fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and x-ray diffraction study (XRD). The formation of the most optimized batch of the microsphere (F12) was confirmed by scanning electron microscopy (SEM), DSC, FTIR, and XRD. *In vitro* drug release study and *in vitro* drug release kinetics study of the formulated microspheres were also carried out.

Results: Drug-polymer compatibility studies performed with the help of FTIR and DSC indicated that there were no interactions. Results revealed that non-aqueous solvent evaporation technique was a suitable technique for the preparation of microspheres as most of the formulations were discrete, free-flowing and spherical in shape with a good yield of 55.67% to 80.09%, percentage drug loading of 35.52% to 94.50% and percentage drug entrapment efficiency of 36.24% to 95.07%. Different drug-polymer ratios, as well as the combination of polymers, played a significant role in the variation of over-all characteristics of formulations. Based on the data of various evaluation parameters such as particle size analysis, percentage drug loading, percentage drug entrapment, percentage yield, rheological studies and *in vitro* drug release characteristics, formulation F12 was found to fulfil the criteria of ideal controlled release drug delivery system. F12 showed controlled release till the 14th hour (97.99%) and its *in vitro* release kinetics was best explained by zero-order kinetics and followed Korsemeyer-Pappas model (Non-Fickian mechanism). SEM of F12 revealed the formation of spherical structures. The FTIR study of F12 confirmed the stable nature of ropinirole in the drug-loaded microspheres. DSC and XRD patterns showed that ropinirole hydrochloride was dispersed at the molecular level in the polymer matrix.

Conclusion: The controlled release microparticles were successfully prepared and from this study, it was concluded that the developed microspheres of ropinirole hydrochloride can be used for controlled drug release to improve the bioavailability and patient compliance and to maintain a constant drug level in the blood target tissue by releasing the drug in zero order pattern.

Keywords: Ropinirole hydrochloride microspheres, Eudragit RS 100, Eudragit RL 100, Ethylcellulose, Controlled release, Solvent evaporation technique

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INTRODUCTION

The oral route is the most sought-after for the administration of drug molecules to the systemic circulation due to their ease of administration, better treatment, patient compliance and cost-effectiveness [1]. But the limitation of the conventional dosage form has been recognized for some time now which includes frequent medication, plasma drug level fluctuation, inability to maintain the drug content at the site of action and variation in absorption or metabolism resulting in toxic effects. However, these problems can be resolved by designing a new drug delivery system [2]. Recently, extensive efforts have been dedicated to developing controlled release drug delivery system which is designed to release the drug constantly over an extended period of time.

Controlled drug delivery by encapsulating the drug inside the polymeric carriers has achieved great progress in the last two decades due to the following reasons. A steady-state plasma concentration can be achieved by this system. It also enhances the drug release and decreases side-effects by drug localization at the site of action and by controlling the drug release [3].

Microspheres developed using bio-degradable polymers are widely used to achieve controlled release of drugs. The main advantage of bio-degradable polymer is that they can be broken down in a biologically friendly manner after their use [4].

Several microencapsulation techniques have been developed but the appropriateness of the technique depends on the nature of drug and polymer. Among various methods developed for formulation of microspheres, the solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of the drug [5].

Ropinirole hydrochloride is a non-ergoline dopamine D₂ receptor agonist used in the treatment of Parkinson's disease. It has the ability to stimulate the dopamine receptor in the striatum of the brain. Ropinirole hydrochloride would be a right candidate for controlled release drug delivery due to its low molecular weight (260.37 D) and optimum log P value (2.3). Moreover, its low and non-linear oral bioavailability due to first pass metabolism along with disadvantage of its conventional therapy reported as "off phenomenon" demands better alternative for its delivery [6, 7].

The rationale behind this study was to prepare the microspheres of ropinirole hydrochloride encapsulated in the eudragit and ethylcellulose polymer to control the release of the drug. Eudragit RS 100 and eudragit RL 100 are water-insoluble pH independent polymers whereas ethylcellulose is a pH-dependent polymer. In addition, the drug release kinetics for the formulations developed was also evaluated.

MATERIALS AND METHODS

Materials

Ropinirole hydrochloride was generously gifted by Central Drug Laboratory, Kolkata, India. Various polymers like eudragit RS 100 and eudragit RL 100 were purchased from Yarrow chem product, Mumbai, India; while Loba Chemie, Mumbai, India provided ethyl cellulose and magnesium stearate. Span 80 was purchased from Merck specialities Private Limited. The pure dug was of standard quality complying with official monographs. All the chemicals used for the analysis were of analytical grade complying with the official monograph. Deionised water (distilled) was used throughout the experimental procedure.

Methods

Drug-polymer compatibility study through physical compatibility study, FTIR, and DSC

Before the preparation of microspheres, compatibility of the drug with polymers present in microspheres was performed. The physical compatibility study was conducted by sealing the physical mixtures (1:1) into 15 ml USP type III flint glass vials and stored in a stability chamber for 30 d at 40 °C and 75% relative humidity conditions to note the initial state (table 2) [7]. The Fourier transform infrared

radiation (FTIR) spectral measurements were taken at ambient temperature using IR spectrophotometer (IR Prestige-21, Shimadzu make Japan). In this method peak of the pure drug was matched with that of polymer in the range of 4000-400 cm⁻¹ for 100 scans (fig. 1, fig. 2 and fig. 3) [8]. The differential scanning calorimetry (DSC) thermograms were obtained using DSC (Perkin Elmer (Singapore); MODEL-Pyris diamond) to investigate the presence of additional peaks or absence of peaks indicating possible polymer interactions or phase transformations (fig. 4, fig. 5 and fig. 6) [9].

Preparation of ropinirole hydrochloride microspheres

Ropinirole hydrochloride microspheres were prepared by solvent evaporation technique [10-12]. Different amount of eudragit RS 100, eudragit RS100 and eudragit RL100 combinations and eudragit RS 100 and ethyl cellulose combinations were dissolved in 15 ml acetone separately by using a magnetic stirrer (Remi Equipments, Model 2MIH Mumbai, India). The core material, ropinirole hydrochloride, was added to the polymer solution and mixed for 15 min followed by addition of magnesium stearate (50 mg) and then mixed thoroughly. The resulting dispersion was added in a thin stream to a mixture of 100 ml light liquid paraffin and span 80 with constant stirring at 500 round per minute (rpm) using a mechanical stirrer (Remi Motors, Model No. R0123R, Mumbai, India). The stirring was continued for 3 h until the acetone is evaporated completely. The microspheres formed were filtered by using whatmann filter paper. The residue was washed 4-5 times with 50 ml portions of n-hexane. The product was then dried at room temperature for 24 h. The formulation containing drug: polymer ratio were coded as F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, and F12. The composition of various formulations was mentioned in table 1.

Table 1: Composition of ropinirole hydrochloride microspheres

Formulation code	Ropinirole hydrochloride (mg)	Drug: eudragit RS 100	Drug: eudragit RS 100 and eudragit RL 100	Drug: eudragit RS 100 and ethylcellulose	Magnesium stearate (mg)
F1	2	1:3	-	-	50
F2	2	1:4	-	-	50
F3	2	1:5	-	-	50
F4	2	1:6	-	-	50
F5	2	-	1:2.5:0.5	-	50
F6	2	-	1:3:1	-	50
F7	2	-	1:3.5:1.5	-	50
F8	2	-	1:4:2	-	50
F9	2	-	-	1:2.5:0.5	50
F10	2	-	-	1:3:1	50
F11	2	-	-	1:3.5:1.5	50
F12	2	-	-	1:4:2	50

F = formulation code of microspheres

Evaluation of prepared controlled release microspheres

Determination of particle size of microspheres

The particle size of prepared microspheres from each batch was determined by the optical microscopy method using an ocular micrometer which was previously calibrated with a stage micrometer (table 3) [13].

Determination of percentage drug entrapment efficiency

Drug-loaded microspheres (50 mg) were powdered and suspended in 50 ml distilled water and diluted with methanol. It was stirred for 15 min and filtered. Ropinirole content in the filtrate was determined by UV spectrophotometer (shimadzu 1700, Japan) at 250 nm against phosphate buffer (pH 7.4) (table 3) [14]. The drug entrapment efficiency was calculated by the equation:

$$\% \text{ EntrapmentEfficiency} = \frac{\text{PracticalDrugContent}}{\text{InitialDrugContent}} \times 100$$

Determination of percentage drug loading

Drug-loaded microspheres (50 mg) were powdered and suspended in 100 ml methanol solutions and kept for 24 h. It was stirred for 5 min and filtered. Ropinirole content in the filtrate was determined UV spectrophotometer (shimadzu 1700, Japan) at 250 nm (table 3) [15].

The percentage drug loading was calculated by the equation:

$$\text{PercentageDrugContent} = \frac{\text{DrugcontentInMicrosphere}}{\text{WeightOfMicrosphere}} \times 100$$

Determination of percentage yield

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below (table 3) [15].

$$\text{PercentageYield} = \frac{\text{WeightOfObtainedMicrospheres}}{\text{TotalWeightOfDrugAndPolymer}} \times 100$$

Rheological properties

The angle of repose, bulk density, tapped density; Carr's index and Hausner's ratio were determined to assess the flow-ability of the prepared microspheres.

Angle of repose

It was measured by fixed funnel standing method. The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of the granules. The granules were allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured (table 4) [16]. It was calculated by using the following formula:

$$\tan \theta = \frac{h}{r}$$

h = height of pile, θ = angle of repose, r = radius of base pile and <25-excellent flow, 25–30-good flow, 30–40-passable,>40-very poor flow.

Bulk density

Apparent bulk density (ρ_b) was measured by pouring the pre-weight (M) blend into a graduated cylinder. The bulk volume (V_b) of the blend was determined (table 4) [16-18]. Then the bulk density was calculated by using the formula:

$$\rho_b = \frac{M}{V_b}$$

Tapped density

The measuring cylinder containing a known mass (M) of the blend was tapped for a fixed time, and the minimum volume (V_t) occupied in the cylinder was measured (table 4) [17, 18]. The tapped density (ρ_t) was calculated by using the following formula:

$$\rho_t = \frac{M}{V_t}$$

Carr's index

Carr's index of the powder was determined for the determination of flow of the powder (table 4). It is calculated by using tapped density and bulk density [17, 18].

$$\text{Carr's Index} = \frac{\frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Bulk Density}} \times 100}{\text{Tapped Density}}$$

In Carr's index, the value below 15% indicates a powder with usually give rise to good flow characteristics, whereas above 25% indicates poor flow-ability.

Hausner's ratio

It gives information about flow-ability of the powder. It is determined by comparing the tapped density to the bulk density (table 4) [17, 18]. It is calculated by the following formula:

$$\text{Hausner's Ratio} = \frac{\frac{\text{Tapped Density}}{\text{Bulk Density}} \times 100}{\text{Bulk Density}}$$

Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25).

In vitro drug release study at pH 7.4

In vitro release studies were performed by using USP-II type dissolution apparatus (paddle type). An accurately weighed sample (75 mg) of microspheres was suspended into 900 ml of phosphate buffer (pH 7.4) maintained at a temperature of 37 °C±0.5 °C and stirred at a speed of 100 rpm (round per minute). At predetermined time intervals, a 5 ml aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium kept at 37 °C. The absorption of the filtered withdrawn sample was measured by UV spectrophotometer (Shimadzu 1700, Japan) with suitable dilution and the corresponding concentration was determined from the respective

calibration curve. The temperature was maintained at 37 °C throughout the studies (fig. 7 and fig. 8) [19, 20].

In vitro drug release kinetics

Kinetic models are best-known tools to describe the drug release pattern from immediate and modified release dosage forms. In order to investigate the kinetics and mechanism of drug release from prepared microspheres of different drug and polymers ratios, the release data were examined using zero order kinetic, first-order kinetic, higuchi kinetic and korsmeyer-peppas model (table 5) [21, 22].

Scanning electron microscope (SEM)

Scanning electron microscopy was carried out to study the morphological characteristics of ropinirole hydrochloride microspheres of F12 formulation batch. It was carried out by sprinkling the microspheres on one side of an adhesive stub. The dried microspheres were coated with gold (100 Å) under an argon atmosphere in a gold coating unit and scanning electron micrographs (JEOL MAKE UK; MODEL-JSM 6360) were observed (fig. 9) [23].

Fourier-transform Infra-red spectroscopy (FTIR)

The IR spectrum was recorded using an FTIR spectrophotometer (IR Prestige-21, Shimadzu make Japan) by the KBr pellet method. The spectra obtained for ropinirole hydrochloride and formulation batch (F12) were compared (fig. 10). In this method peak of the pure drug was matched with that of the formulations in the range of 4000-400 cm⁻¹ for 100 scans [23].

Differential scanning calorimetry (DSC)

The DSC thermogram of pure drug and formulation batch (F12) was generated and investigated on a Perkin Elmer (Singapore); MODEL-Pyris diamond TG/DTA. A sample weight of 10±2 mg was used in each experiment. The samples were heated from 30 °C to 400 °C at a heating rate of 10 °C/min in the nitrogen atmosphere (flow rate 150 ml/min) to investigate the presence of additional peaks or absence of peaks indicating the uniform dispersion of drug in the polymer (fig. 11) [24].

X-ray diffraction studies (XRD)

X-ray diffraction measurements of the formulation batch (F12) and pure drug were recorded with a Model Ultima-III Rigaku make (Japan) diffractometer equipped with Ni-filtered Cu K α radiation ($\lambda = 0.1548$ nm) (fig. 12). The drug and microspheres were mounted on the sample holder and were scanned at a rate of 5 °/min in the angle 2 θ range of 5° to 60° [25].

RESULTS AND DISCUSSION

Preparation of microspheres

Controlled release microspheres of ropinirole hydrochloride were prepared using polymers such as eudragit RS100, eudragit RL100, and ethyl cellulose either alone or in combination in various drug and polymer ratios as given in formulation composition of microspheres (table 1) with the help of non-aqueous solvent evaporation technique using acetone as the solvent system. After the introduction of drug and polymer solution in the acetone solution, an emulsion got formed. Agitation provided by stirrer broke the poured polymer solution into discrete droplets, forming an emulsion where polymer and drug were still in their solution form in the organic solvent. As the stirring continued, the acetone started to evaporate leading to co-precipitation of the drug and polymer at the interface of emulsion droplet. This co-precipitation of drug and polymer resulted in a shell around droplet. In a previous study, it was reported that when the diffusion rate of solvent from emulsion droplet was too slow, microspheres coalesced together [26]. According to a previous report, diffusion of the organic solvents completed in the time span of 20 min leads to the hardening of microspheres [27].

Drug-polymer compatibility study

Physical compatibility study

Initially, the ropinirole hydrochloride and its physical mixture with polymers were white in color. Stability study between the drug and the polymer was conducted after the 15th and 30th day. No visible interaction between the drug and polymer was noticed. According to a previous study, change in temperature and humidity may lead to

the physical changes [28]. So, proper storage conditions were maintained for 30 d.

FTIR

The pure drug of ropinirole hydrochloride showed sharp characteristic peaks at 1703 and 1759 cm⁻¹ for ketone group (C=O), 3415 cm⁻¹ for secondary amine group, 1596 cm⁻¹ for tertiary amine group and 2881 and 2979 cm⁻¹ for the alkyl group. All the above characteristics peaks of the drug also appear in the spectra of the drug with polymer-like eudragit RS100, eudragit RL100 and ethylcellulose. It shows that there was no alteration in the properties of the drug and polymers during the formulation. Hence the drug and polymers were compatible with each other.

DSC

The DSC thermograph of ropinirole hydrochloride exhibits an endothermic peak at 248.36 °C corresponding to its melting point. The mixture of drug and polymers like eudragit RS 100 and eudragit RL 100 showed endothermic peaks at 244.08 °C and 245.72 °C respectively which showed that the drug was compatible with both the polymers and there was no major interaction of drug and polymers. A slightly broadened peak was observed in the thermogram of the physical mixture ropinirole hydrochloride and ethylcellulose. In a previous study, it was reported that the presence of ethylcellulose in the physical mixture depressed the melting point of the drug slightly and broadened its melting point endotherm [29].

Table 2: Physical compatibility study

Composition	Description		
	Initial	15 d	30 d
Ropinirole hydrochloride	white	No colour change	No colour change
Ropinirole hydrochloride, eudragit RS 100 and eudragit RL 100	white	No colour change	No colour change
Ropinirole hydrochloride and ethylcellulose	white	No colour change	No colour change

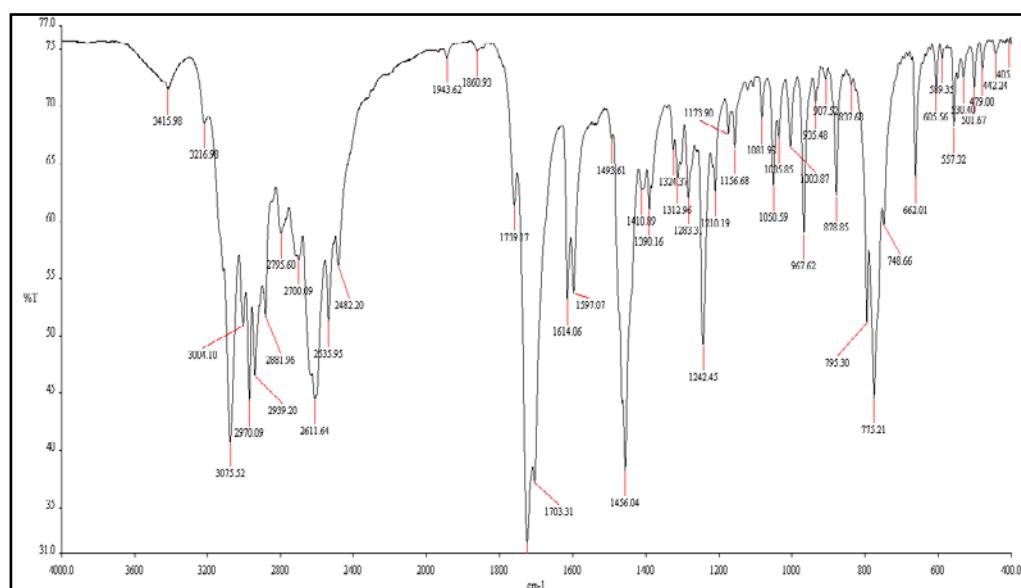


Fig. 1: FTIR spectra of pure ropinirole hydrochloride

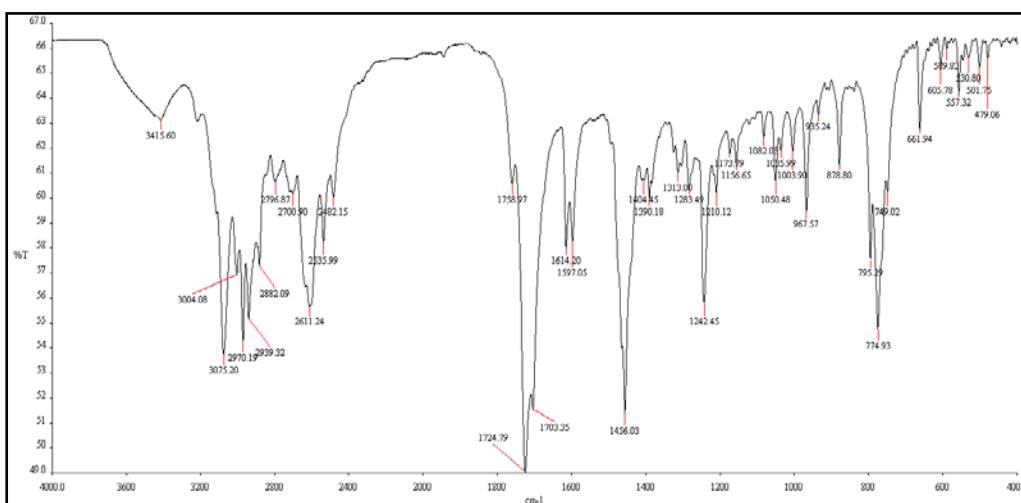


Fig. 2: FTIR spectra of a mixture of ropinirole hydrochloride, eudragit RL 100 and eudragit RS 100

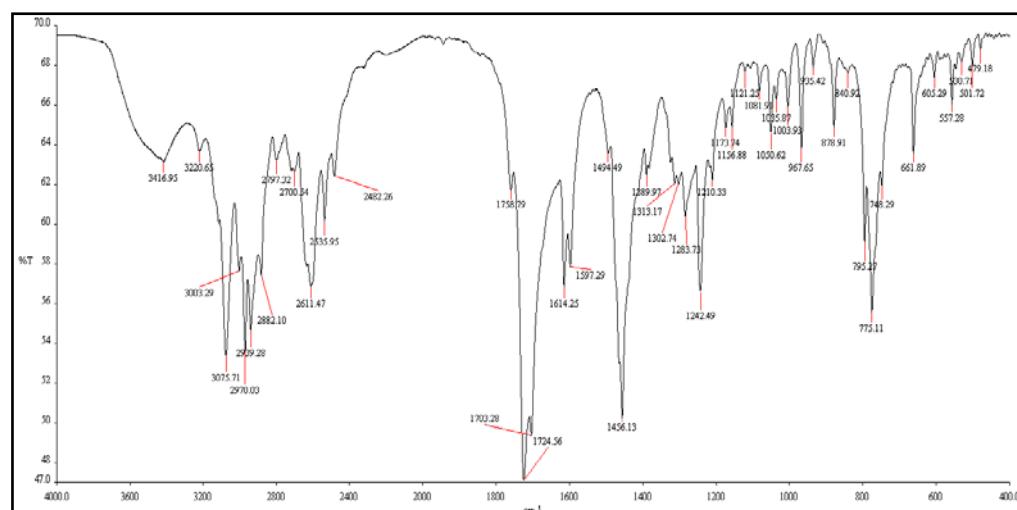


Fig. 3: FTIR spectra of a mixture of ropinirole hydrochloride and ethylcellulose

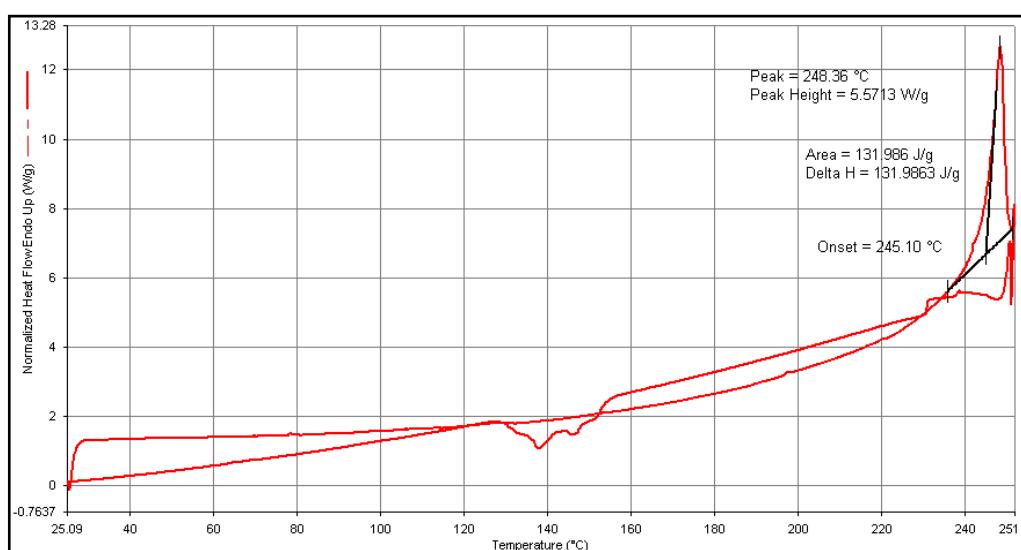


Fig. 4: DSC thermogram of pure ropinirole hydrochloride

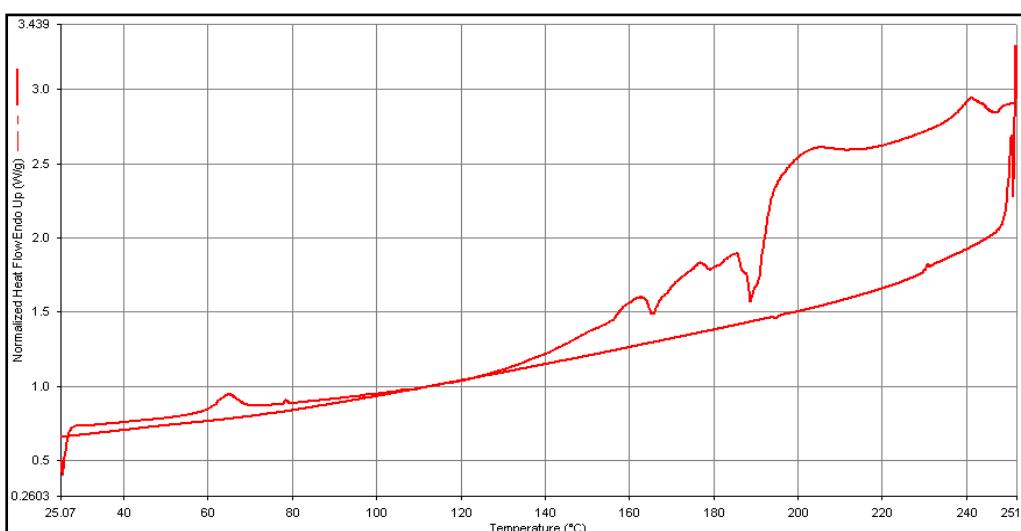


Fig. 5: DSC thermogram of a mixture of ropinirole hydrochloride, eudragit RL 100 and eudragit RS 100

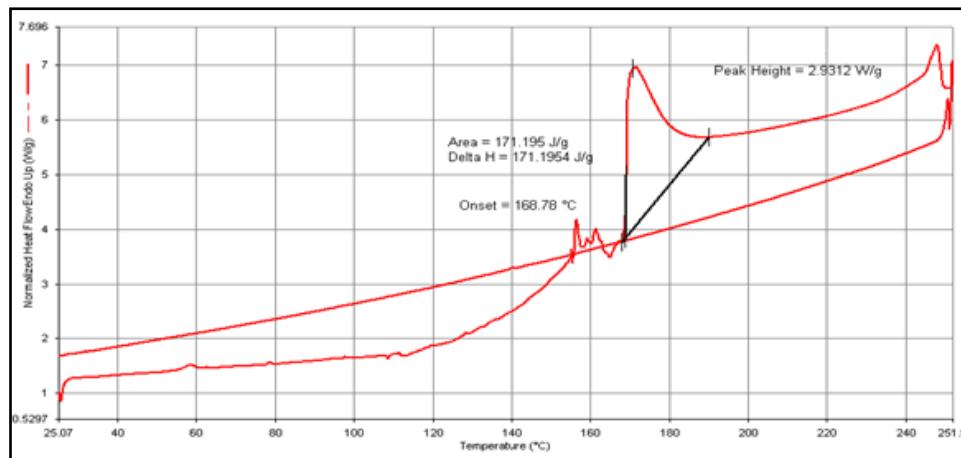


Fig. 6: DSC thermogram of a mixture of ropinirole hydrochloride and ethylcellulose

Determination of particle size of microspheres

All the microspheres obtained from non-aqueous solvent evaporation technique were discrete and spherical in shape. The optical microscopic study of particle size distribution revealed that the microspheres were uniform in size in each formulation with average diameter ranged from 90.01 ± 3.00 - 130 ± 4.16 μm . This may be due to the particle size is directly proportional to viscosity, where an increase in the viscosity upturns the droplet size. Hence, the adequate viscosity should be maintained. The particle size was maximum for the F12 batch with a maximum concentration of eudragit RS100 and ethylcellulose. According to a previous study, it was reported that the particle size increased with increase in ethyl cellulose concentration [30].

Determination of percentage drug entrapment efficiency

The percentage drug entrapment efficiency ranged from 36.24% to 95.07%. The effect of the combination of the polymers over encapsulation efficiency was convincing. The entrapment efficiency was found to be abruptly increasing when a combination of polymers was used and the maximum entrapment efficiency of 95.07% was noted for the batch F12 with a high drug-polymer ratio of 4:2 containing a combination of eudragit RS100 and ethylcellulose. This ratio of polymers was found to be efficient of encapsulating maximum drug than any other batches. The addition of the polymer ethylcellulose along with eudragit RS100 increased the entrapment efficiency compared to the combination of eudragit

RS100 and eudragit RL100. It was reported in the literature that the encapsulation efficiency depends on the solubility of the drug in the solvent and continuous phase. An increase in the concentration of polymer in a fixed volume of organic solvent resulted in an increase in encapsulation efficiency [31].

Determination of percentage drug loading

The percentage drug loading ranged from 35.52% to 94.50%. Higher drug loading was observed in F12 containing a combination of eudragit RS 100 and ethylcellulose in the ratio 4:2. A higher percentage of loading was obtained by the addition of ethylcellulose polymer and by increasing the amount of polymer with respect to the drug. A study reported that various factors such as the drug to albumin ratio, the concentration of surfactant, stirring rate of the emulsion and average size of microspheres could affect drug loading. They found that drug loading could be increased by increasing the drug to albumin ratio, decreasing surfactant concentration and increasing the stirring rate [32].

Determination of percentage yield

Percentage yield of all the formulations prepared from solvent evaporation technique was found in the range 55.67 to 80.09 % which is sufficiently high. As experimental result revealed percentage yield value was directly related to polymeric concentration rather than the combination of polymers used. According to a previous study, as the polymer concentration was increased, percentage yield was also increased [33].

Table 3: Particle size analysis, drug entrapment efficiency, percentage drug loading and percentage yield of microsphere formulation F1-F12

Formulation code	Average particle size (μm) [*]	Percentage drug entrapment efficiency*	Percentage Drug loading efficiency*	Percentage yield*
F1	90.01 \pm 3.00	36.24 \pm 1.03	35.52 \pm 0.57	55.67 \pm 0.34
F2	92.05 \pm 3.35	47.92 \pm 0.44	46.75 \pm 0.67	63.34 \pm 0.28
F3	95.01 \pm 4.42	60.25 \pm 0.92	59.03 \pm 1.03	70.00 \pm 0.62
F4	100.56 \pm 3.05	83.56 \pm 0.66	82.30 \pm 0.45	74.00 \pm 1.15
F5	94.62 \pm 2.00	45.69 \pm 1.06	44.45 \pm 0.64	56.88 \pm 0.64
F6	95.64 \pm 2.48	53.72 \pm 0.43	52.45 \pm 0.55	64.92 \pm 1.10
F7	101.26 \pm 4.16	67.45 \pm 0.33	66.84 \pm 0.72	72.36 \pm 0.55
F8	102.34 \pm 4.58	89.52 \pm 0.25	88.95 \pm 0.89	76.02 \pm 0.82
F9	94.72 \pm 2.64	39.65 \pm 0.69	38.10 \pm 0.99	58.65 \pm 0.98
F10	110.64 \pm 1.67	59.98 \pm 0.73	58.04 \pm 1.02	66.66 \pm 1.05
F11	112.26 \pm 3.89	78.84 \pm 1.06	77.54 \pm 0.46	73.05 \pm 0.87
F12	130.00 \pm 4.16	95.07 \pm 0.48	94.50 \pm 0.56	80.09 \pm 0.99

*Each reading is an average of three determinations (mean \pm SD) (n=3) n = no of observation code of microspheres

Rheological properties

Flow properties of batches were evaluated by measuring the angle of repose and Carr's index. Microspheres showed the desired flow-

ability due to the optimal presence of moisture, spherical shape, and diminished cohesiveness. The pure drug showed the angle of repose of 35.19 and exhibited poor flow, whereas the angle of repose of all the formulations showed excellent flow-ability and ranged from

30.10 to 32.30. The bulk density and the tapped density of all the formulations were within short range, i.e. 0.66 g/cm^3 to 0.66 g/cm^3 and 0.73 g/cm^3 to 0.76 g/cm^3 respectively. The Carr's index of all the formulations exhibited excellent flow properties and ranged from 13.15 to 9.58 as compared to the pure drug, which was 24.39 indicating poor flowability before formulating to microspheres. Hausner's ratio of the pure drug was 2.16, which indicated poor flowability, but the formulated microspheres exhibited good flow properties that ranged from 1.15 to 1.10. According to a previous study, it was reported that in the evaluation of flowability of dry solid, the substance showed excellent flowability and performance, when the angle of repose has the value less than 25° , while when

compressibility index has a value below 9%, no aid was needed for enhancing the flowability of the powder. Thus, the angle of repose and compressibility index is indicative of good flowability of microspheres, showing no need for the addition of glidants to enhance flowability [34, 35]. The better flow property of microspheres indicated that the microspheres produced were non-aggregated. The improved rheological properties of formulated microspheres, when compared to that of the pure drug alone, suggested that they could be easily handled and filled into a capsule. Excellent flow properties of prepared microsphere suggested less polydispersity, complete drying and particle size uniformity.

Table 4: Rheological characteristics of microsphere formulations F1 to F12

Formulation code	Angle of repose ($^\circ$)*	Bulk density (g/cm^3)*	Tapped density (g/cm^3)*	Carr's Index*	Hausner's ratio*
F1	30.10 ± 2.27	0.66 ± 0.003	0.76 ± 0.007	13.15 ± 0.36	1.15 ± 0.072
F2	32.24 ± 1.16	0.66 ± 0.003	0.76 ± 0.007	13.15 ± 0.36	1.15 ± 0.072
F3	31.33 ± 3.13	0.65 ± 0.001	0.77 ± 0.007	15.58 ± 0.14	1.18 ± 0.010
F4	33.42 ± 1.14	0.67 ± 0.013	0.75 ± 0.015	10.66 ± 0.52	1.11 ± 0.004
F5	32.37 ± 1.15	0.65 ± 0.001	0.73 ± 0.014	10.95 ± 0.66	1.12 ± 0.002
F6	30.12 ± 3.32	0.67 ± 0.013	0.76 ± 0.007	11.84 ± 1.03	1.13 ± 0.001
F7	35.66 ± 3.02	0.68 ± 0.007	0.79 ± 0.002	13.92 ± 0.44	1.16 ± 0.008
F8	34.69 ± 2.49	0.66 ± 0.003	0.75 ± 0.005	12.00 ± 0.59	1.13 ± 0.001
F9	32.40 ± 2.33	0.67 ± 0.013	0.77 ± 0.007	12.98 ± 0.72	1.14 ± 0.004
F10	33.51 ± 1.78	0.64 ± 0.005	0.78 ± 0.020	17.94 ± 0.74	1.21 ± 0.020
F11	34.15 ± 2.87	0.66 ± 0.003	0.76 ± 0.007	13.15 ± 0.42	1.15 ± 0.012
F12	32.30 ± 1.98	0.66 ± 0.003	0.73 ± 0.014	9.58 ± 1.05	1.10 ± 0.005

*Each reading is an average of three determinations (mean \pm SD) (n=3) n = no of observation, F= formulation code of microspheres

In vitro drug release study at pH 7.4

Different release profiles were observed with each combination of polymers. For formulation F1 to F4, eudragit RS100 was used alone and hence drug release was observed till the 13th hour of dissolution study. The formulation containing eudragit RS 100 showed less drug release because it is the least permeable polymer due to the presence of less quaternary groups than that in eudragit RL 100. According to a previous study, it was reported from the release data of different formulations, that the cumulative percentage release of drug from the microspheres depends upon the type, amount and combination of polymer used [36]. For formulation F5 to F8, a combination of eudragit RS100 and eudragit RL100 was used releasing the drug until the 12th hour because a higher concentration of eudragit RS100 was used compared to eudragit RL100 which is more permeable. Formulations F9 to F12 were prepared using a combination of ethylcellulose and eudragit RS100. F12 showed 7.35% of cumulative drug release at first hour.

The rate of release of formulation F12 was very much controlled because of the combination of polymers of ethylcellulose and eudragit RS100. When both these polymers were combined, they form a polymeric matrix of high density leading to the formation of a strong polymeric network due to the presence of ethylcellulose. In a previous study, it was reported that the non-toxic, non-allergic and non-irritant behaviour of ethylcellulose make this polymer one of the best for the formulation of microspheres [36, 37]. From the present study, it was observed that the formulations prepared from this polymer possess lower densities and was expected to be retained in the site-specific delivery for a prolonged period of time. Most of the formulations prepared from ethyl cellulose and eudragit RS100 did not show any burst effect or lag time which indicates homogeneous drug distribution. The release of F9 to F11 was higher than that of F12 because at lower polymer concentration smaller microspheres were formed. According to a previous study, due to the small size of microspheres larger surface area was exposed to dissolution medium giving rise to faster drug release [37].

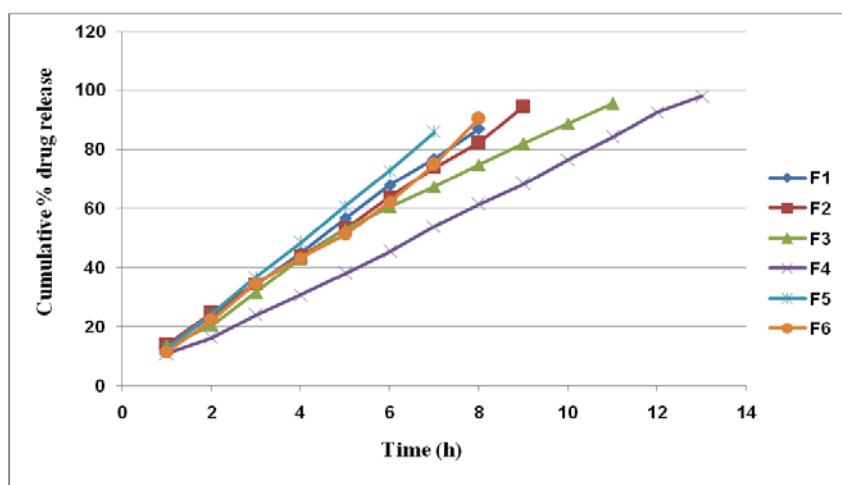


Fig. 7: Cumulative % drug release profiles of formulations F1-F6. Each reading is an average of six determinations (mean \pm SD) (n=6) n=no. of observation

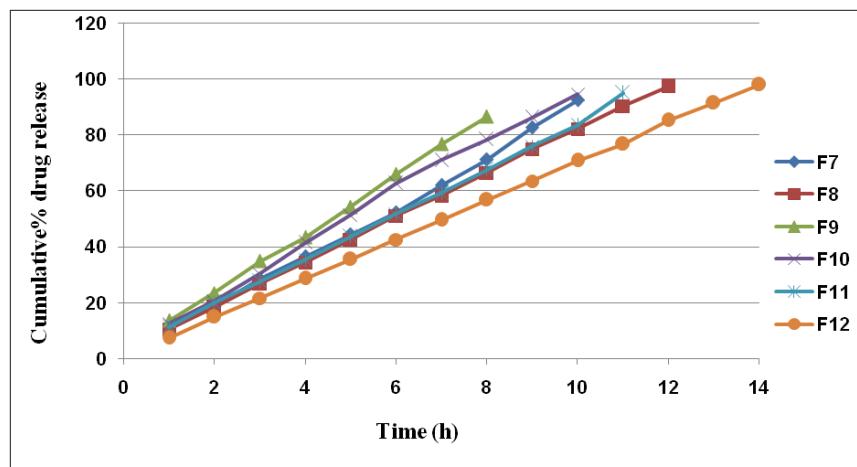


Fig. 8: Cumulative % drug release profiles of formulations F7-F12. Each reading is an average of six determinations (mean±SD) (n=6) n = no. of observation

In vitro drug release kinetics

The results of the *in vitro* drug release study obtained from the different batches were plotted using kinetic models. Zero-order kinetics, first-order kinetics, Higuchi's matrix and Korsmeyer-Peppas model were used to evaluate the release mechanism from ropinirole hydrochloride microspheres. The kinetic model showing highest correlation coefficient (R^2) was considered as the most appropriate model for the dissolution data. Korsmeyer model is widely used; when the release mechanism is not well known or when more than one type of release phenomena could be involved. Korsmeyer-Peppas equation: $M_t/M_\infty = K_m t^n$, where M_t/M_∞ is the fractional drug release in time 't', K_m = constant incorporating of

structural and geometric characteristics of the controlled release device, n = diffusional release exponent indicative of release mechanism. The 'n' value could be used to characterize different release mechanisms as follows $n = 0.5$ means Fickian diffusion, $0.5 < n < 1.0$ non-Fickian diffusion, and $n = 1.0$ Case II diffusion. The interpretation of data was based on the value of the resulting regression coefficients. The best fit with the highest correlation coefficient was observed in the Korsmeyer-Pappas model and zero-order release kinetics followed by Higuchi model. The 'n' value of the formulations indicated that the drug release followed anomalous (non-fickian) diffusion. In a previous study it was reported, that the variation in polymer concentration greatly affects the release kinetics and the diffusion mechanism [38].

Table 5: *In vitro* dissolution kinetics data of formulations F1 to F12

Formulations	Zero-order model	First order model	Higuchi model	Korsmeyer-pappas model	n
	R^2	R^2	R^2	R^2	
F1	0.999	0.941	0.982	0.999	0.926
F2	0.999	0.853	0.978	0.998	0.876
F3	0.990	0.903	0.992	0.994	0.858
F4	0.999	0.797	0.969	0.992	0.897
F5	0.999	0.930	0.979	0.999	0.988
F6	0.992	0.854	0.963	0.996	0.969
F7	0.997	0.861	0.966	0.997	0.942
F8	0.999	0.823	0.978	0.998	0.915
F9	0.999	0.933	0.977	0.997	0.906
F10	0.996	0.900	0.985	0.996	0.920
F11	0.998	0.831	0.972	0.998	0.890
F12	0.999	0.795	0.976	0.999	0.977
Best fit model	zero order kinetic release and non-fickian diffusion controlled mechanism				

R^2 = Regression correlation coefficient, n= diffusion coefficient, F= formulation code of microspheres

Characteristics of the optimized microsphere batch

On the basis of all the evaluation parameters, formulation F12 was selected as the most optimized formulation, and its statistical evaluation was done using analysis of variance (ANOVA) at $P \leq 0.05$ significance level and it was found to be statistically significant with P value < 0.05 .

Scanning electron microscope (SEM)

Scanning electron microscopy was done to determine the surface microscopy and internal texture of F12 microspheres.

Photomicrograph of the microspheres before and after the release of drugs was taken. The quality of the microspheres (with respect to surface properties) and the nature and size of pores developed on the surface was studied. It revealed that the F12 microspheres possessed a rough and porous surface. The micrograph taken after 14 h release studies also revealed porosity developed at the surface and no visible major surface irregularity and the structure remained retained as 14 h drug release study was carried out. According to a previous study, it was reported that the changes that occur during *in vitro* dissolution studies may have implications to the performance of the microspheres [39].

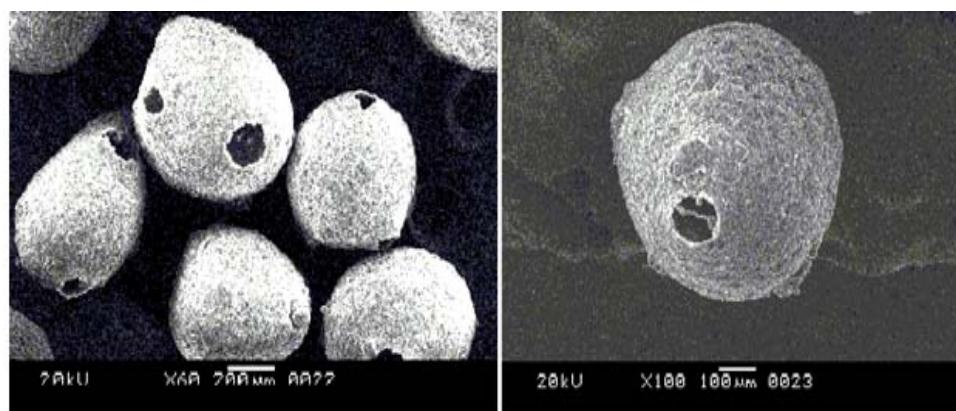


Fig. 9: SEM images of the optimized F12 microspheres before and after dissolution study

Fourier-transform infrared spectroscopy (FTIR)

The pure drug showed sharp characteristic peaks at 1703 and 1759 cm^{-1} for ketone group (C=O), 3415 cm^{-1} for secondary amine group, 1596 cm^{-1} for tertiary amine group, 2881 and 2959 cm^{-1} for the alkyl

group, 1071.77 cm^{-1} for-C-O-stretching and 781.93 cm^{-1} for-C-H-rocking vibration. All these characteristics peaks also appeared in the F12 microspheres without any change. This indicated that ropinirole hydrochloride did not undergo any chemical changes while forming the microspheres.

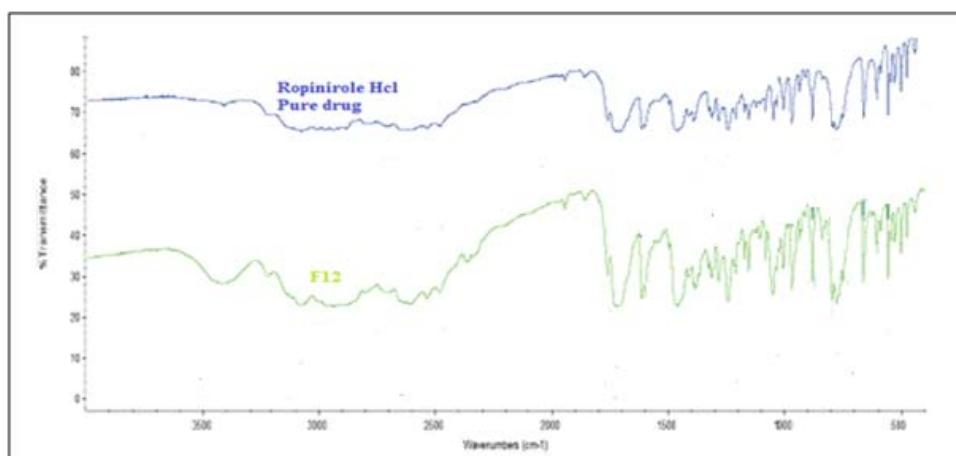


Fig. 11: FTIR spectra of pure drug and formulation F12 microsphere

Differential scanning calorimetry (DSC)

The DSC thermogram of pure drug exhibited a single sharp endothermic peak at 248.36 $^{\circ}\text{C}$ corresponding to its melting

transition temperature. In case of F12 microspheres, no characteristic peak was observed at 248.36 $^{\circ}\text{C}$ which indicated that the drug was uniformly dispersed at the molecular level in the microspheres.

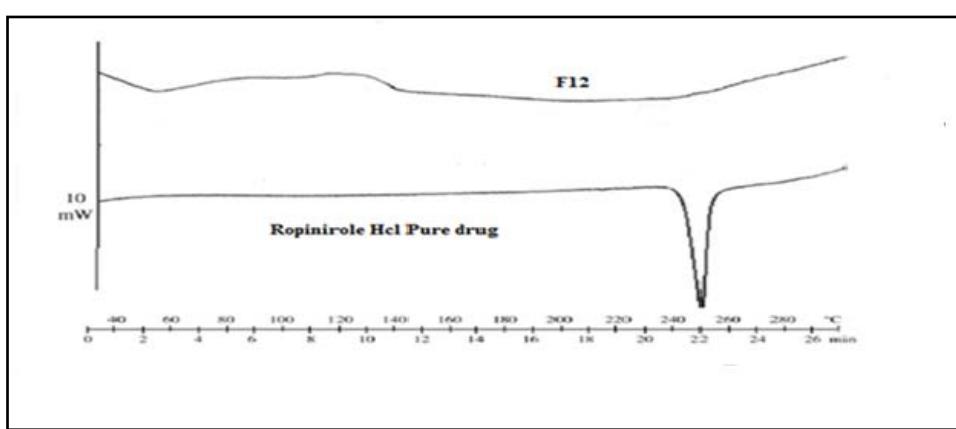


Fig. 10: DSC thermograms of pure drug and formulation F12 microsphere

X-ray diffraction studies (XRD)

The drug peaks of pure drug were observed at 2θ of 5.5° , 18.2° , 22.4° , 24.8° and 25.3° which was due to crystalline nature of

ropinirole hydrochloride, while in the case of drug-loaded microspheres of F12, these peaks of the drug were not observed which indicated that the drug particles were dispersed at molecular level in the polymer matrix.

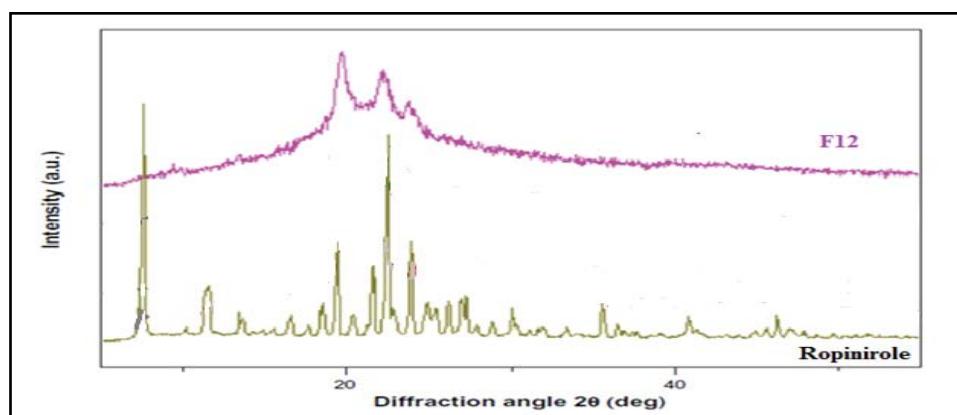


Fig. 12: XRD spectra of pure drug and formulation F12 microspheres

CONCLUSION

The present studies, therefore, shows the successful formulation of controlled release microspheres of ropinirole hydrochloride by the solvent evaporation method using eudragit RS100, eudragit RL100, and ethylcellulose as suitable polymers. This study has been a satisfactory attempt to formulate a micro-particulate system of ropinirole hydrochloride with a view of controlled delivery of the drug. In the formulation, the combination of cost-effective and biocompatible polymers of eudragit RS100 and ethylcellulose had been successfully used and there is a scope of scale-up of the batches to the commercial level. Physical compatibility study, FTIR and DSC studies used for drug-polymer compatibility study confirmed the absence of any physiochemical interaction between the drug and polymers. The formulation was found to be efficient with good percentage entrapment efficiency, percentage drug loading and percentage yield. The surface structure, particle size and flow analysis revealed that the microspheres showed good flow and packability, indicating that it can be successfully handled and filled into a capsule dosage form. *In vitro* drug release showed a prolonged and controlled release of ropinirole hydrochloride employing phosphate buffer as release media. The drug release was found to be polymer concentration dependent. It was best explained by zero-order kinetics and followed non-fickian diffusion mechanism. SEM analysis showed a rough and porous surface with the spherical appearance of microspheres. FTIR studies showed that ropinirole hydrochloride did not undergo any chemical changes while forming the microspheres. DSC thermograms and XRD spectra revealed that the drug particles were dispersed uniformly at the molecular level in the polymer matrix. Hence, the controlled release microsphere formation of ropinirole may provide a convenient dosage form for achieving best performance regarding flow, drug entrapment, and release. Further, their potential to improve the bioavailability of ropinirole in human needs to be investigated in further studies.

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AUTHORS CONTRIBUTIONS

Koyal Kar carried out the research work and wrote the manuscript. Dr. R. N. Pal and Dr. N. N. Bala contributed to the article with a critical revision. All authors read and approve the final manuscript.

CONFLICT OF INTERESTS

The authors have none to declare

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