RESEARCH ARTICLE

Formulation and evaluation of microspheres containing ropinirole hydrochloride using biodegradable polymers

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The present work relates with developing long acting sustain release microspheres of ropinirole hydrochloride (RPN) for treatment of Parkinson's disease, that will sustain drug release up to 1 month. Biodegradable microspheres of RPN were prepared by using two different polymers (poly lactic co glycolic acid [PLGA] 50:50 and PLGA 75:25) employing double emulsion (W/O/W) solvent evaporation method. Preliminary optimization of process parameter was done for concentration of polyvinyl alcohol (PVA) solution, stirring speed, temperature of PVA solution, ratio of the drug to polymer (D/P) and ratio of internal phase to external phase volume (IP/EP). All formulations were evaluated for particle size, percentage yield, entrapment efficiency (EE), shape etc. Formulation E3 and E4 shows maximum EE. % *in vitro* drug release per day of E3 and E4 batch was studied. The RPN was incorporated successfully in microspheres prepared with 0.5% w/v PVA at 8000 RPM stirring speed, 20°C processing temperature, 1:4 drug polymer ratio and 1:30 IP/EP ratio, which provides sustained release up to 4 weeks with better efficacy and patient compliance and can be employed as an alternative to existing oral medications.

Key words: *Entrapment efficiency, poly lactic co glycolic acid 50:50, polyvinyl alcohol, solvent evaporation, sustained release*

INTRODUCTION

Parkinson's disease is a chronic disorder for which lifelong treatment with antiparkinsons drugs is necessary. Antiparkinsons drugs are not readily accepted by patients, thus, non-compliance is common.[1,2] Microspheres made up of biodegradable polymers, can be injected with a syringe into the body and once injected, solidify to form a semi-solid depot.[3,4] They are considered as a useful therapeutic option in patients with parkinsonism who lack insight or are known to adhere poorly to oral medications. Long-acting injectable antiparkinsons drugs have demonstrated a number of advantages over oral medication, including stabilization of serum drug levels, avoidance of the first pass metabolism, assured medication delivery and well-controlled titration to lowest effective doses.

Long-acting injectables reduce variation between peak and trough levels and have lower peak concentrations that can reduce the incidence of adverse effects.[5,6] Depot formulations have been well tolerated and more efficacious than their oral equivalents. In view of the need to provide extended release of antiparkinson drugs for longer duration with better patient compliance, the present study has been directed toward the incorporation of antiparkinson drug, ropinirole hydrochloride (RPN) into polymeric biodegradable microspheres formulation.

MATERIALS AND METHODS

RPN was obtained as a gift sample from Hetero Drugs Pvt. Ltd., Hyderabad, India. Poly lactic co glycolic acid (PLGA) 50:50 (PUROSORB 5002 PDLG) was generously provided by PURAC Biopolymers, Netherland. PLGA 75:25 (RESOMER 752S) was gifted by Evonik Polymers, Mumbai. Dichloromethane (DCM) and

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polyvinyl alcohol (PVA) were gifted by Loba Chemical Pvt. Ltd, Mumbai. All other chemicals used were of analytical grade.

RPN microsphere preparation using double emulsion solvent evaporation technique

RPN was dissolved in 1-2 ml distilled water. It was sonicated (Oscar Ultrasonics, Mumbai) for 3 min. with organic phase containing 5 ml of DCM and PLGA polymer (300 mg, 400 mg, 500 mg) for 10 min, which forms (W/O) primary emulsion. The obtained primary emulsion (6 ml) was further emulsified with aqueous PVA solution (0.5, 1 and 2% w/v at different volumes viz. 300 ml, 180 ml, 120 ml) using high speed homogenizer (REMI, India) at varying speed (6000, 8000, 10,000 rpm), which forms W/O/W secondary emulsion. The resultant solution was stirred for 4 h to evaporate the solvent completely. Further, the microspheres were washed with distilled water, filtered by vacuum filtration and kept in vacuum desiccators for 12 h. Dried microspheres were collected in well-labeled glass vials and stored at 2-8°C temperature.

In order to optimize the formula of the microspheres, the following process and formulation variable parameters were studied; PVA concentration, stirring speed of homogenizer, process temperature of PVA solution, drug: polymer ratio (D/P ratio) and ratio of internal phase/external phase volume (IP/EP ratio).

Optimization of process parameters

PLGA loaded RPN microspheres were prepared by using double emulsion solvent evaporation by changing the concentration of EP of the secondary emulsion (PVA solution) as shown in Table 1. Based on the results obtained, concentration of PVA solution was fixed to 0.5% w/v and kept constant for further B1 to B6 batches. Further RPN microspheres batches were prepared by varying stirring speed (6000-10000 rpm) using high speed homogenizer (REMI, India) as shown in Table 2.

Batches C1 to C6 were prepared by fixing stirring speed to 8000 rpm to optimize temperature of EP (PVA solution) as shown in Table 3. Batches D1 to D6 were prepared by fixing temperature of PVA solution at 20°C to optimize drug/polymer (D/P) ratio as shown in Table 4.

Batches E1 to E6 were prepared by keeping PVA concentration 0.5% and stirring speed 8000 rpm, temperature of PVA solution 20°C and D/P ratio 1:4 to optimize IP (primary emulsion): EP volume of secondary emulsion ratio as shown in Table 5.

Table 1: Selection of PVA concentration

Formulation code	Drug (mg)	PLGA 50:50 (mg)	PLGA 75:25 (mg)	PVA concentration (%)	Mean particle size (µm)	EE	Yield (%)	Shape
A1	100	300		0.5	25	44.3	75.3	Spherical
A2	100		300	0.5	30	46.7	78.5	Spherical
A3	100	300		1	18	30.4	72.8	Spherical
A4	100		300	1	21	32.4	70.2	Spherical
A5	100	300		2	12	25.8	68.9	Irregular
A6	100		300	2	16	27.2	68.4	Irregular

PVA: Polyvinyl alcohol, PLGA: Poly lactic co glycolic acid, EE: Entrapment efficiency

Table 2: Selection of stirring speed

Formulation code	Drug (mg)	PLGA 50:50 (mg)	PLGA 75:25 (mg)	Stirring speed (rpm)	Mean particle size (µm)	EE (%)	Yield (%)	Shape
B1	100	300		6000	33.4	47.2	68.8	Spherical
B2	100		300	6000	27.8	48.2	68.5	Spherical
B3	100	300		8000	17.8	50.5	76.8	Spherical
B4	100		300	8000	17.6	49.6	76.5	Spherical
B5	100	300		10000	10.9	26.2	66.7	Irregular

PLGA: Poly lactic co glycolic acid, EE: Entrapment efficiency

Table 3: Selection of PVA temperature

Formulation code	Drug (mg)	PLGA 50:50 (mg)	PLGA 75:25 (mg)	Temperature of PVA solution (°C)	Mean particle size (µm)	EE (%)	Yield (%)	Shape
C1	100	300		15	31.4	31.2	70.2	Irregular
C2	100		300	15	30.8	32.3	76.2	Irregular
C3	100	300		20	22.1	40.3	76.3	Spherical
C4	100		300	20	20.4	41.5	77.7	Spherical
C5	100	300		30	16.8	36.4	70.2	Irregular
C6	100		300	30	17.2	36.9	70.4	Irregular

PVA: Polyvinyl alcohol, PLGA: Poly lactic co glycolic acid, EE: Entrapment efficiency

Table 4: Selection of drug polymer ratio

Formulation code	Drug (mg)	PLGA 50:50 (mg)	PLGA 75:25 (mg)	D/P ratio	Mean particle size (µm)	EE (%)	Yield (%)	Shape
D1	100	500		1:5	26.38	56.7	72.4	Spherical
D2	100		500	1:5	27.2	56.8	72.6	Spherical
D3	100	400		1:4	16.8	48.5	78.3	Spherical
D4	100		400	1:4	16.7	47.3	79.4	Spherical
D5	100	300		1:3	10.3	30.8	74.6	Irregular
D6	100		300	1:3	9.5	27.8	73.7	Irregular

PLGA: Poly lactic co glycolic acid, EE: Entrapment efficiency, D/P: Drug/polymer

Table 5: Selection of ratio of internal phase volume to external phase volume

Formulation code	Drug (mg)	PLGA 50:50 (mg)	PLGA 75:25 (mg)	IP/EP ratio	Mean particle size (µm)	EE (%)	Yield (%)	Shape
E1	100	400		1:50	16.7	41.4	76.7	Spherical
E2	100		400	1:50	16.2	40.8	76.2	Spherical
E3	100	400		1:30	20.4	52.5	80.8	Spherical
E4	100		400	1:30	23.6	51.8	81.2	Spherical
E5	100	400		1:20	26.3	56.5	78.2	Spherical
E6	100		400	1:20	26.3	52.7	78	Spherical

PLGA: Poly lactic co glycolic acid, EE: Entrapment efficiency, IP/EP: Internal phase/external phase

EVALUATION OF MICROSPHERES

Percent yield

Microspheres recovered at the end of preparation were weighed and the % yield was calculated by using the following equation.

$$\%\,\text{Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100 \quad (1)$$

Entrapment efficiency

To calculate the entrapment efficiency % (EE), 10 mg prepared microspheres were dissolved in 2 ml DCM. Diluted up to 10 ml with distilled water. This solution was subjected to centrifugation at 1000 rpm. Supernatant was filtered through 0.45 µm filter. The absorbances of these solution was noted using ultraviolet (UV) spectrophotometric method at λ max 249 nm and the %EE was calculated using the following equation

$$\%\,\text{Entrapment efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Amount of total drug used}} \times 100 \quad (2)$$

Particle size

The mean particle size of freshly prepared microsphere samples of each batch was determined by laser light scattering (model Mastersizer 2000, Malvern Instruments, Malvern, UK).

Surface morphology

Surface Morphology of E3 batch was studied by using scanning electron microscope (SEM) (Model: JEOL JSM-6360) with an accelerating voltage of 10 kV.

In vitro RPN release from microspheres

The *in vitro* drug release for batch E3 and E4 was performed in phosphate buffer, pH 7.4 release media under sink conditions on shaker incubator (Chromous Biotech.) at a constant temperature (37°C). Microspheres equivalent to RPN 56 mg were dispersed in 5 ml distilled water in dialysis bag, which is suspended in the volumetric flask containing 50 ml of release media and kept for shaking in shaker incubator. At the set points, 3 ml sample was removed by syringe for analysis through 0.45 µm mdi nylon filters for which the concentration was analyzed at 249 nm by UV spectrophotometer.[7-9]

RESULTS AND DISCUSSION

The most suitable method for encapsulation of hydrophilic drugs into PLGA microspheres is the double emulsification solvent evaporation technique.[10] The drug RPN, being water soluble, the double emulsification solvent evaporation method was selected.[11] Multiple studies demonstrate that PLGA can easily be formulated into the drug carrying devices as microspheres.[12-15]

Table 1 shows that the particle size of RPN loaded PLGA microspheres were inversely proportional to the concentration of PVA solution used as EP. Previous investigations suggests that increasing the PVA concentration in the continuous medium reduces the particle size of microspheres.[16,17] Increasing the PVA concentration leads to more PVA molecules overlaying the surface of the droplets and provided protection to the droplets against coalescence, which resulted in the production of smaller emulsion droplets (25-30 µm).

Further, highest % yield and %EE of 78.5% and 46.7% respectively was obtained for A2 batch prepared using PVA solution at 0.5% concentration. Thus for further batches 0.5% w/v PVA solution as aqueous phase was fixed.

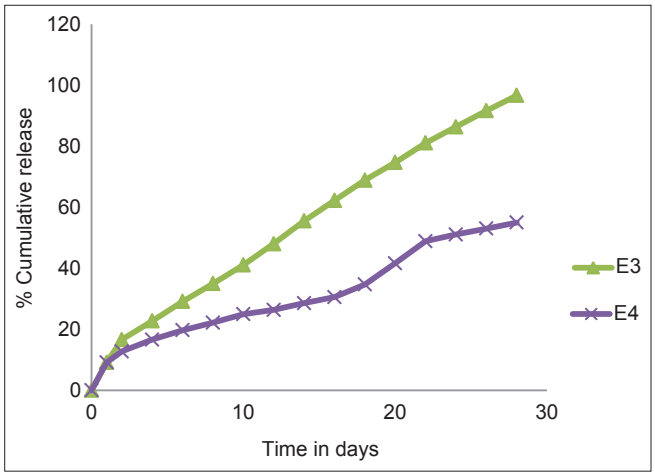

Figure 1: *In vitro* dissolution profile of formulation E3 and E4

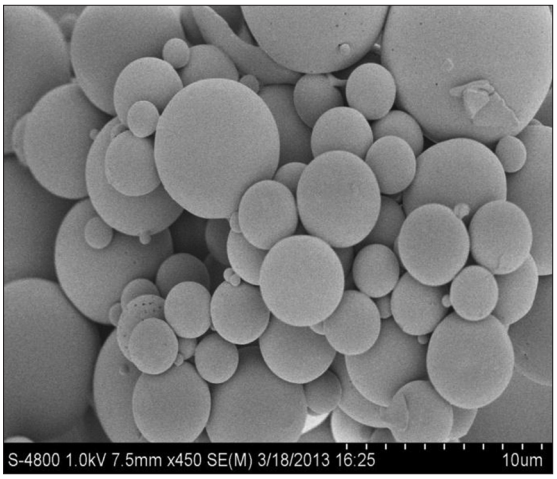

Figure 2: Scanning electron microscope microgarphs of microspheres

Table 2 indicates that an increase in stirring speed from 6000 rpm to 10000 rpm decreases the microsphere size significantly from 33.4 µm to 10.9 µm, but the %EE was reduced to 26.2%. The speed was therefore fixed to an optimum level of 8000 rpm for further batches.

At a higher temperature of PVA solution (30°C), it was observed that particle size was 17.2 µm and at 15°C of PVA solution particle size of microspheres was observed to be 31.4 µm to. This is due to the fact that the W/O/W double emulsion at high temperatures is less viscous, thus it is much easier for the emulsion to be broken up into smaller droplets at the same input power of mixing.[18]

The EE was decreased at both higher temperature of external aqueous phase (30°C) and lower temperature (15°C) as compared to 20°C where EE of about 41% was observed. For further batches 20°C temperature of PVA solution was fixed. It was observed [Table 4] that EE was decreased from 56.6% to 27.8% by changing D/P ratio from 1:5 to 1:3. The D/P ratio was fixed to 1:4 for further batches as at this ratio best EE% (78-79%) was achieved. Table 5 reveals that EE and particle size decreases as the ratio of IP to EP was increased from 1:20 to 1:50. An increase in the volume of the EP of the secondary emulsion led to a decrease in the particle size of microspheres. The droplet size of the secondary emulsion may decrease because of a decrease in the frequency of collision of droplets with an increase in the volume of the EP of the secondary emulsion.[16]

IP to EP was further fixed to 1:30 to produce optimum particle size and to achieve a better entrapment (52%). These optimized batches E3 and E4 were evaluated for percentage *in vitro* drug release by using dialysis bag method. Drug released through E3 formulation containing PLGA 50:50 is 96% at the end of 28 days and more uniform than E4 formulation containing PLGA 75:25, where only 52% drug was released at the end of 28 days as shown in Figure 1. Polymer composition is the most important factor to determine the hydrophilicity and rate of degradation of a delivery matrix. A systematic study of the polymer composition with its degradation has been shown by many groups.[19,20]

PLGA 50:50 (PLA/PGA) exhibited a faster degradation than PLGA 75:25 due to preferential degradation of glycolic acid proportion assigned by higher hydrophilicity.[20,21] SEM micrographs of E3 formulation shows that PLGA 50:50 microspheres containing RPN are spherical in shape, having a smooth surface and size ranges from 20 to 25 µm as shown in Figure 2.

CONCLUSION

For a short-term release requirement (up to 1 month), an amorphous polymer PLGA 50:50 is recommended. For a longer-term release requirement (1-6 months), the choice of an amorphous polymer PLGA 75:25 would be appropriate. From this study is concluded that the RPN can be incorporated successfully in microspheres prepared with 0.5% w/v PVA at 8000 RPM stirring speed, 20°C processing temperature, 1:4 drug polymer ratio and 1:30 IP/EP ratio, which provides sustained release up to 4 weeks with better efficacy and patient compliance and can be employed as an alternative to existing oral medications.

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