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Key Parameters for the Development of Long Term Delivery of Antipsychotic Drug

Shailendra Patil
Department of Pharmaceutical Sciences, Dr. H.S. Gour Central University, Sagar, MP, India
Email ID : shailpatil27@rediffmail.com

Asmita Gajbhiye
Department of Pharmaceutical Sciences, Dr. H.S. Gour Central University, Sagar, MP, India
Email ID : asmitapatil27@rediffmail.com

Govind Prasad Agrawal
Department of Pharmaceutical Sciences, Dr. H.S. Gour Central University, Sagar, MP, India
Email ID : gpagrawal2005@rediffmail.com

Abstract — Use of biodegradable polymers as drug carriers mainly depends on desired degradation mechanisms and mechanical properties. Schizophrenia requires medication for months to years. Presently, all the existing atypical preparations have to be given daily. Non-compliance is a major problem in these patients. Risperidone is an atypical antipsychotic and drug of choice for the treatment of schizophrenia.

In the present study, risperidone (RSP) long acting microspheres were prepared by emulsion solvent evaporation method using biodegradable polymer i.e. poly-(lactic-co-glycolic acid) (PLGA) 50:50(Mw-18,27and 66 Kda) and 75:25(Mw-18,27and 67 Kda). Various formulation and process parameters, which play a pivotal role, are drug polymer ratio, PVA concentration, stirring rate and time, temperature of external phase, solvent removal time and o/w phase ratio were optimized. Microspheres were characterized for particle size, encapsulation efficiency, surface morphology and *in-vitro* drug release. The optimized formulations exhibited initial drug release followed by sustained release up to 28 days time period. Microspheres containing PLGA(50:50, Mw 27Kda) had shown maximum percent of drug release (94.54±2.54), whereas the formulation containing PLGA(75:25, Mw 67Kda) had released 69.23±2.53 percent of drug in 28 days time period. Formulation containing PLGA (75:25, Mw 67Kda) was found to be the best among all the formulations of risperidone, in terms of drug release profile for 28 days.

Results of this study suggest that biodegradable microspheres of PLGA can be used for long term delivery of atypical antipsychotic drug for 4 weeks, which may lead to improvement in the patient compliance, therapeutic efficacy and results in cost-effective treatment of schizophrenia.

Keyword - Biodegradable microspheres, Long acting injection, Psychosis Risperidone, Solvent evaporation method.

1. INTRODUCTION

A major challenge of PLGA microspheres delivery is to achieve an ideal controlled-release profile, namely a zero-order release in most cases [1]. However, due to the hydrophobic property of PLGA, a triphasic release profile is commonly observed in PLGA microspheres, consisting of an initial burst, a lag period and a finally erosion accelerated release phase [2]. However, for long-term sustained release microspheres made from high molecular weight PLGA, the release profiles were difficult to be modified only through adjusting sizes of microspheres or formulation and preparation parameters.

In many cases, the proper managing of disease is dependent on achieving consistent pharmacokinetic profile of a therapeutic drug. This depends on the constant compliance of the patient, ensuring that the medication is taken exactly as prescribed. For many patient populations, daily oral dosing inherently presents complications, as is observed with schizophrenic and depressive patients. Patients receiving continuous i.e. extended release therapies have been shown to have lower rates of relapse, because of the constant dosing of medicine, allowing a consistent and stable pharmacodynamic profile to be achieved. In schizoaffective patient populations, the incidence of relapse has been repeatedly linked to an overall poorer prognosis, illustrating the importance of patient compliance for the management of diseases [3].

Schizophrenia is defined as a clinical syndrome that may include a collection of diseases that share a common presentation. Genetic factors are the most important in the etiology of the disease, with unknown environmental factors potentially modulating the expression of symptoms. The dopamine and other neurotransmitter systems are certainly involved in the treatment or modulation of psychotic symptoms [4].

Risperidone, an "atypical" antipsychotic agent, has been used in the treatment of psychotic disorders. It has been approved by USFDA as an "atypical" antipsychotic agent due to its less cause of extrapyramidal effects than conventional antipsychotics. It is practically insoluble in water, and undergoes significant `first-pass' metabolism; oral bioavailability is 70%. The active metabolite of risperidone is 9-hydroxy risperidone. The half-life of risperidone and its metabolite 9-hydroxy risperidone is 3 and 21 h, respectively [5-7]. Animal studies suggest that the blood-brain barrier may be preferentially penetrable to risperidone over 9-hydroxy risperidone, with blood/brain ratios of 0.22 and 0.04, respectively. This difference in penetrability may provide clinical importance to risperidone [8]. Therefore, maintenance of risperidone (owing to its short half-life) in plasma level using long acting microspheres via i.m. route may enhance the bioavailability and biodistribution of risperidone and improve the pharmacotherapy of psychotic disorders. Risperidone injectable formulation was recently approved by the USFDA as the first atypical long acting antipsychotic medication. Compared to oral formulation risperidone loaded PLGA microspheres via i.m. route will reduce the frequency of administration, dose (without first pass effect) and adverse effects. In the recent years many research groups have shown interest to utilize drug loaded polymeric microspheres/microparticles to achieve controlled drug delivery that were prepared by the solvent emulsification/evaporation method [9].

In the present study, we have optimized the various formulation and process parameters and characterized risperidone loaded poly (lactide-co-glycolide) microspheres formed by single emulsion solvent evaporation method [10] in order to sustain the release of risperidone for 28 days with an initial release and studied the drug release profile of risperidone loaded PLGA microspheres.

2. EXPERIMENTAL

Preparation of risperidone-PLGA microspheres

PLGA microspheres entrapping risperidone were prepared by o/w solvent evaporation method as reported by Tsung and Burgess [10] with slight modification. PLGA (100 mg) and risperidone (50 mg) were dissolved in dichloromethane (DCM; 1 ml) to form an organic phase. The organic phase was added drop wise using syringe, in a beaker containing 100 ml aqueous solution of poly vinyl alcohol (PVA) and homogenized for 5 min to form the emulsion. The dispersion was stirred using a stainless steel stirrer with a half moon paddle at 1500 rpm. Thereafter, the risperidone PLGA microspheres were solidified, while DCM was being evaporated for 4 hrs under stirring at 100 rpm (Remi, India). The hardened microspheres were filtered, washed repeatedly with double distilled water to remove PVA and lyophilized (Heto dry winner, DW 1.0-60e). The microspheres were kept in tightly closed glass vial at 4˚C until further use.

Determination of encapsulation efficiency

The entrapment efficiency of RSP PLGA microspheres was determined after removal of surface adsorbed drug [11].The surface adsorbed risperidone was removed by taking the microspheres (10 mg) in a dialysis bag (Cellulose membrane, molecular weight cutoff 12000) containing 1 ml of acetate buffer (pH 5.5). The bag was tied and kept in 10 ml acetate buffer (pH 5.5) for 2 hrs with stirring. The free drug dialyzed out in buffer. The residual microspheres were collected and washed with double distilled water. These microspheres were dissolved in acetone (1 ml). The resulting solution was diluted with 2 ml of 0.01N hydrochloric acid and volume was made up to 10 ml with PBS (pH 7.4). It was centrifuged at 3000 rpm for 10 min. The supernatant was collected by means of a syringe fitted with 0.45 µm filter and analyzed spectrophotometrically at 298 nm to determine entrapped RSP in the PLGA microspheres. The entrapment efficiency of RSP PLGA microspheres was calculated.

Scanning electron microscopy

The shape and surface morphology of the risperidone PLGA microspheres was observed using optical microscopy and scanning electron microscopy (SEM). Samples were mounted on metal stubs and sputter coated with gold for 5 min under an argon atmosphere for 150s to achieve a 20 nm film (sputter coater, Polaron SC-7640) prior to examination under SEM (Carl-Zeiss, EVO 40 SEM). These microspheres were incubated in dissolution media (PBS pH 7.4) for 28 days and then observed for any signs of degradation.

Particle size

Particle size of microspheres was examined by a laser diffraction based particle size analyser (Malvern: Mastersizer 2000). Microspheres were suspended in a 1% aqueous solution of Tween 80 and sonicated for 60 s prior to particle size determination. Polydispersity was calculated by the following formula:

Polydispersity = $(D_{0.9} - D_{0.1}) / D_{0.5}$

Where $D_{0.9}$, $D_{0.5}$ and $D_{0.1}$ are the particle diameters determined at the 90th, 50th and 10th percentile of undersized particles, respectively. The average particle size of RSP PLGA microspheres was recorded.

Determination of risperidone content

Risperidone content in various formulations were determined by dissolving the accurately weighed amount of microspheres (10 mg) in 1 ml of acetone and resulting solution was diluted with 2 ml of 0.01 N hydrochloric acid and volume was made up to 10 ml with PBS (pH 7.4). It was centrifuged at 3000 rpm for 10 min and the supernatant was collected by means of a syringe fitted with 0.45 µm filter and analyzed spectrophotometrically at 298 nm.

Drug release studies

An accurately weighed 15 mg of RSP PLGA microspheres (RSP_1M66, RSP_1M27, RSP_2M67 and RSP_2M27) were transferred to a 15 ml screw-cap centrifuge tube and incubated with 10 ml of 50 mM phosphate buffer (pH 7.4) containing 0.02% w/v tween 80 and 0.05% w/v sodium azide at 37˚C in water bath oscillator (50 rpm) [12]. At predetermined time intervals [0.16 (4 hrs), 1, 4, 7, 14, 21, 28 days], the samples were centrifuged and 4 ml of the supernatant was withdrawn using a pipette fitted with a micro filter at its tip, and replaced with fresh buffer to maintain constant volume and sink conditions. The RSP PLGA microspheres were vortexed for resuspension and put back in to the oscillator to agitate the suspension continuously throughout the release experiment, in order to prohibit microspheres from aggregation and sedimentation. The concentration of risperidone in the supernatant was detected by UV spectrophotometrically at 298 nm.

3. Results and Discussion

Risperidone is given daily (orally) for longer duration ranging from months to years for the treatment of schizophrenia. It would be beneficial to develop alternative delivery system containing risperidone to treat schizophrenia. In the present study it was planned to develop long acting risperidone PLGA microspheres based injectable formulation for 28 day time periods. Solvent evaporation method was used for the preparation of microspheres. Various process parameters were optimized to observe the effect on encapsulation efficiency and drug release. The RSP_1 microspheres comprise of polymer PLGA (50:50) of different molecular weights (18 Kda, 27 Kda and 66 Kda) and RSP_2 comprise of polymer PLGA (75:25) of different molecular weights (18 Kda, 27 Kda and 67 Kda).

Effect of drug polymer ratio

In order to optimize drug: polymer ratio, various proportions 1:1, 1:2 and 1:3 were used in the preparation of microspheres. In case of RSP_1 microspheres, it was found that as the molecular weight of PLGA (50:50) increased, the particle size also increased ranging from 24.45±0.35 µm to 62.52±0.27 µm at 1:1, 25.45±0.35 µm to 63.52±0.27 µm at 1:2 and 26.25±0.15 µm to 65.52±0.67 µm at 1:3 drug : polymer ratio. The drug entrapment efficiency also increased with increasing molecular weight of PLGA and was found in the range of 40.02±2.71% to 83.73±3.80% at 1:1, 43.53±2.71% to 85.04±2.80% at 1:2 and 44.18±1.52% to 86.48±2.18% at 1:3 drug : polymer ratio (Fig. 1).

In case of microspheres RSP_2, it was found that as the molecular weight of PLGA (75:25; 18 Kda, 27 Kda and 67 Kda) increased, the particle size also increased ranging from 28.46±0.32 µm to 67.58±0.39 µm at 1:1, 29.56±0.32 µm to 68.58±0.59 µm at 1:2 and 31.46±0.22 µm to 68.99±0.39 µm at 1:3 drug: polymer ratio. The drug entrapment efficiency also increased upon increasing the molecular weight of PLGA and was found in the range of 52.06±3.35% to 87.05±2.58%, 55.76±2.35% to 90.33±2.78% and 56.73±2.45% to 92.65±2.08% at 1:1, 1:2 and 1:3 drug: polymer ratio respectively (Fig. 1).

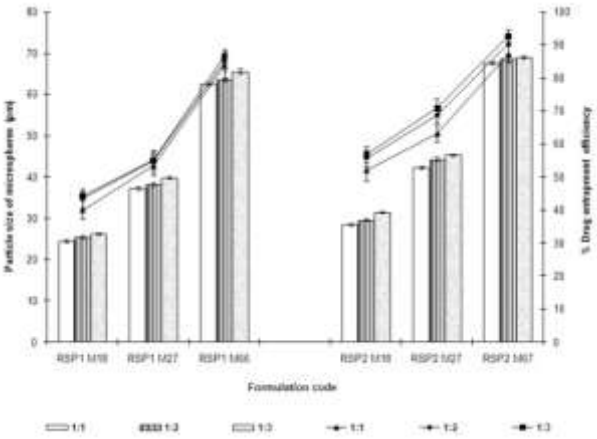

Fig.1. Effect of drug: polymer ratio on particle size and drug entrapment efficiency of RSP microspheres prepared using PLGA of varying molecular weights.

For both RSP_1 and RSP_2 microspheres, these observations indicate that, for a particular molecular weight of polymer used in the preparation of microspheres, on increasing the drug: polymer ratio from 1:1 to 1:2 and 1:2 to 1:3, the mean particle size and drug entrapment efficiency increased.

On comparison of the RSP_1 and RSP_2 microspheres, it is clear that the microspheres prepared from PLGA (75:25) had bigger particle size as well as higher drug entrapment efficiency than the microspheres prepared from PLGA (50:50) of similar molecular weights. However, the microspheres prepared using highest molecular weight polymer i.e. 66 and 67Kda, had shown bigger particle size and the higher drug entrapment efficiency.

The increase in particle size upon increasing the drug: polymer ratio may be due to higher viscosity of polymer solution at its higher concentration for which it was more difficult to form small emulsion droplets at the same input power of mixing. So, the obtained microspheres became larger with increasing polymer concentration [13]. The higher entrapment efficiency observed in this study may be due to the increasing polymer concentration resulting in increased internal cavity of microspheres entrapping more drug. The drug: polymer ratio 1:2 was selected as an optimum drug: polymer ratio for the preparation of PLGA microspheres, as there was insignificant difference in the entrapment efficiency of the formulation prepared with drug: polymer ratio 1:3.

Effect of PVA concentration

The varying concentrations (0.5 %, 1.0% and 1.5% w/v) of PVA were used to study the effect on particle size and drug entrapment efficiency. In case of RSP_1 microspheres, particle size increased with increase in molecular weight of PLGA (50:50). It ranges from 32.55±0.55 µm to 50.22±0.87 µm at 0.5% w/v PVA, 30.54±1.55 µm to 48.12±0.32 µm at 1.0 w/v PVA and

29.25±1.25 µm to 46.22±1.67 µm at 1.5 w/v PVA concentration. The drug entrapment efficiency also increased with increasing molecular weight of PLGA ranging from 46.21±2.91% to 86.42±2.80% at 0.5 % w/v PVA, 42.53±1.91% to 81.73±1.80% at 1% w/v PVA and 35.93±1.31% to 78.73±1.58% at 1.5% PVA concentration (Fig. 2).

In case of microspheres of RSP_2, particle size increased with increase in molecular weight of PLGA (75:25). It ranges from 41.46±0.92 µm to 63.78±0.79 µm at 0.5% w/v PVA, 38.16±1.84 µm to 51.68±1.19 µm at 1.0% w/v PVA and 34.96±1.92 µm to 50.78±1.79 µm at 1.5% w/v PVA concentration. The drug entrapment efficiency also increased with increasing molecular weight of PLGA ranging from 52.06±2.35% to 92.33±1.85% at 0.5% w/v PVA, 48.06±1.35% to 87.53±2.85% at 1.0 w/v PVA and 46.73±1.75% to 83.33±1.65% at 1.5 w/v PVA concentration (Fig. 2).

For both RSP_1 and RSP_2 microspheres, these observations indicate that, for a particular molecular weight of polymer, mean particle size and entrapment efficiency decreased with increase in the PVA concentration from 0.5 to 1.0% and 1.0 to 1.5% w/v.

On comparison of the RSP_1 and RSP_2 microspheres, it is clear that the RSP_2 microspheres had bigger particle size as well as higher drug entrapment efficiency than the RSP_1 microspheres of similar molecular weights. In each case, the microspheres prepared using highest molecular weight polymer i.e. 66 and 67 Kda, had shown the best results in respect of drug entrapment efficiency.

The decrease in the particle size on increasing the PVA concentration may be due to decreased shear stress and viscosity [14]. However, the decrease in the entrapment efficiency might be explained by the fact that the solubility of RSP is increased in aqueous PVA solution [15]. The PVA concentration (0.5%) was selected as an optimum concentration for the preparation of PLGA microspheres on the basis of particle size and better entrapment efficiency.

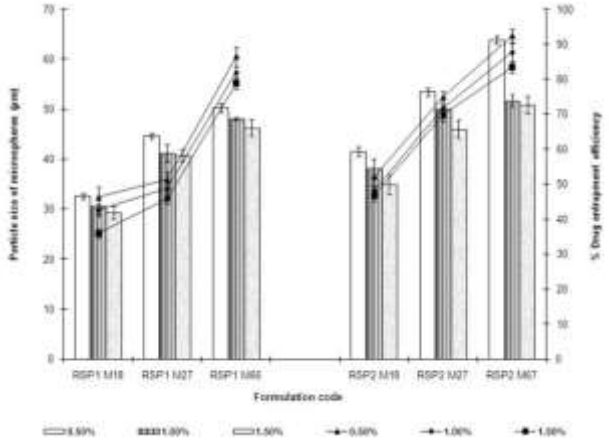

Fig.2. Effect of PVA concentration on particle size and drug entrapment efficiency of RSP microspheres prepared using PLGA of varying molecular weights.

Effect of temperature of external phase

In order to optimize the temperature of external phase, it was varied from 10±2˚C, 25±2˚C and 40±2˚C in the preparation of microspheres. In case of RSP_1 microspheres, it was found that as the molecular weight of PLGA (50:50) increased, the particle size was increased ranging from 24.46±1.65 µm to 57.89±1.92 µm at 10±2˚C, 22.35±2.25 µm to 56.39±4.47 µm at 25±2˚C and. 21.76±4.53 µm to 54.26±7.36 µm at 40±2˚C temperature of external phase. The drug entrapment efficiency also increased with increasing molecular weight of PLGA and was found to be in the range of 50.93±1.61% to 88.86±1.84% at 10±2˚C, 38.64±1.43% to 69.21±1.59% at 25±2˚C 35.62±2.11% to 64.26±2.58% at 40±2˚C temperature of external phase (Fig. 3). However, for a particular molecular weight of polymer used, on increasing the temperature of external phase, the mean particle size and drug entrapment efficiency was decreased.

In case of RSP_2 microspheres, it was found that as the molecular weight of PLGA (75:25) increased, the particle size was increased ranging from 42.88±2.32 µm to 67.84±1.76 µm at 10±2˚C, 38.89±3.13 µm to 66.29±3.47 µm at 25±2˚C and 30.85±5.16 µm to 64.28±7.23 µm at 40±2˚C temperature of external phase. The drug entrapment efficiency also increased upon increasing the molecular weight of PLGA and was found in the range of 55.42±1.35% to 92.43±1.75%, 45.73±1.56% to 88.42±1.64% and 42.06±2.75% to 86.06±2.65% at 10±2˚C, 25±2˚C and 40±2˚C respectively (Fig. 3). However, for a particular molecular weight of polymer, on increasing the temperature from 10±2˚C to 25±2˚C and 25±2˚C to 40±2˚C, the mean particle size and entrapment efficiency decreased.

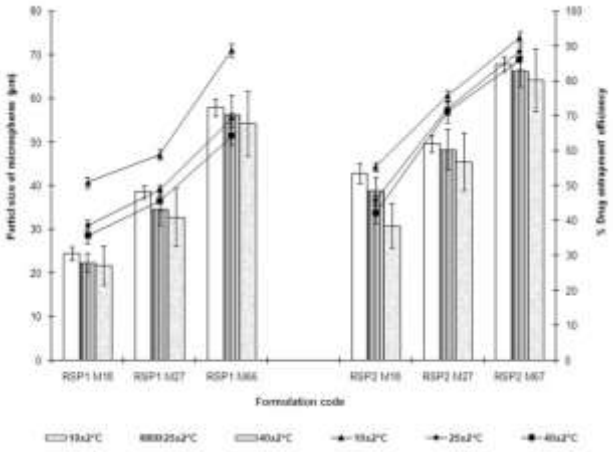

Fig.3. Effect of temperature of external phase on particle size and drug entrapment efficiency of RSP microspheres prepared using PLGA of varying molecular weight.

On comparison of the RSP_1 and RSP_2 microspheres, it is clear that the RSP_2 microspheres had bigger particle size as well as higher drug entrapment efficiency than the RSP_1 microspheres of similar molecular weights. However, in case of the microspheres prepared using

highest molecular weight polymer i.e. 66 and 67Kda respectively had shown the higher drug entrapment efficiency.

The decrease in particle size of microspheres with increasing temperatures may be due to the formation of microspheres at high temperature starts from low viscosity solutions but the spheres harden much rapidly. This probably results in the formation of microspheres of non uniform and smaller size [16]. It was found that the encapsulation efficiency was considerably reduced by increase in temperature of external phase. This observation may be ascribed to the formation of more porous microspheres due to an increase in the rate of diffusion of dichloromethane into the external aqueous phase at elevated temperature since the miscibility and rate of evaporation of the dichloromethane would be more at higher temperature. Thus, we have selected 10±2˚C as an optimum temperature of external phase for preparation of microspheres.

Effect of stirring rate

The stirring rate varying from 500 rpm, 1500 rpm and 2500 rpm was used in the preparation of microspheres to study the effect on particle size and drug entrapment efficiency. In case of RSP_1 microspheres, it was found that as the molecular weight of PLGA (50:50) increased, the particle size also increased and found to be in the range of 22.35±1.45 µm to 54.22±1.87 µm at 500 rpm, 21.85±2.35 µm to 50.42±1.85 µm at 1500 rpm and 20.45±1.75 µm to 46.62±1.57 µm at 2500 rpm. The drug entrapment efficiency also increased with increasing molecular weight of PLGA and was found in the range of 46.26±1.72% to 89.53±1.59%, 44.93±2.21% to 87.06±1.64% and 41.27±1.26% to 85.87±1.56% at 500, 1500 and 2500 rpm respectively (Fig. 4).

In case of RSP_2 microspheres, it was observed that as the molecular weight of PLGA (75:25) increased, the particle size increased ranging from 33.98±2.73 µm to 66.54±1.65 µm at 500 rpm, 32.96±1.74 µm to 62.68±1.83 µm at 1500 rpm and 31.19±1.85 µm to 64.08±1.83 µm at 2500 rpm. The drug entrapment efficiency increased from 53.73±1.74% to 94.73±1.68%, 52.86±1.35% to 91.66±1.65% and 48.07±1.64% to 90.67±1.75% at 500, 1500 and 2500 rpm respectively (Fig. 4).

For both RSP_1 and RSP_2 microspheres, it is clear that for a particular molecular weight of polymer, on increasing the stirring rate from 500 rpm to 1500 rpm and 1500 rpm to 2500 rpm, both the mean particle size and entrapment efficiency decreased with increase in the stirring rate from 500 to 1500 to 2500 rpm.

On comparison of the RSP_1 and RSP_2 microspheres, it is clear that the RSP_2 microspheres had bigger particle size as well as higher drug entrapment efficiency than the RSP_1 microspheres of similar molecular weights. However, in each case the microspheres prepared using polymer of highest molecular weight i.e. 66 and 67Kda respectively, had shown the best results in respect to drug entrapment efficiency.

A slower stirring rate (500 rpm) produced aggregated non–spherical particles, while a faster rate (2500 rpm) produced much smaller but irregular shape particles. This may be attributed to increased energy transferred to the emulsion medium at higher stirring rate where the polymer solution got dispersed into smaller droplets and the size of microspheres was reduced. The higher stirring rate results in high shear and kinetic energy and thus prevents the particle agglomeration. Thus, the stirring rate of 1500 rpm was selected as optimum speed for preparing the microspheres.

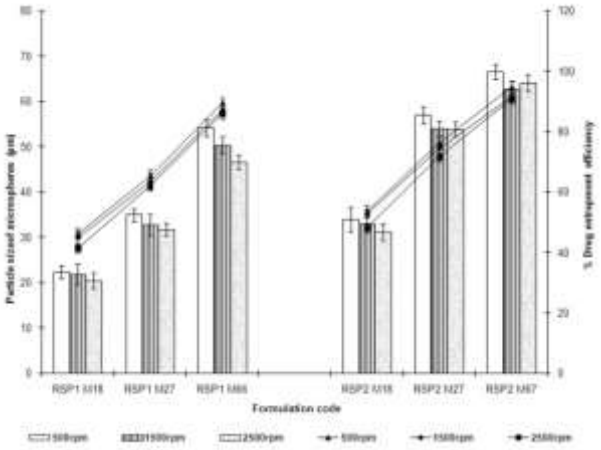

Fig.4. Effect of stirring rate on particle size and drug entrapment efficiency of RSP microspheres prepared using PLGA of varying molecular weights.

Effect of stirring time

In order to optimize the stirring time, for the preparation of microspheres, it was varied from 3 min to 5 min to 10 min. In case of RSP_1 microspheres, it was found that, as the molecular weight of PLGA (50:50) increased, the particle size increased ranging from 26.55±1.25 µm to 44.52±1.77 µm at 3 min, 23.55±1.45 µm to 42.25±1.28 µm at 5 min and 21.56±1.85 µm to 39.26±1.47 µm at 10 min stirring time. Drug entrapment efficiency increased from 35.61±2.35% to 81.53±2.08%, 45.51±2.54% to 86.21±2.78% and 35.63±2.71% to 84.53±1.88% at 3, 5 and 10 min stirring time.

In case of RSP_2 microspheres, it was found that, as the molecular weight of PLGA (75:25) increased, the particle size also increased ranging from 35.95±2.37 µm to 60.54±1.65 µm at 3 min, 32.95±2.23 µm to 58.54±1.56 µm at 5 min and 32.04±2.03 µm to 56.54±1.67 µm at 10 min stirring time. Also the drug entrapment efficiency increased from 38.73±2.57% to 87.06±2.75%, 46.43±2.35% to 90.16±2.15% and 38.06±2.65% to 88.41±2.05% at 3 min, 5 min and 10 min stirring time respectively.

For both RSP_1 and RSP_2 microspheres, it is clear that for a particular molecular weight of polymer, on increasing the stirring time from 3 min to 5 min and 5 min to 10 min, the mean particle size decreased and entrapment efficiency first increased and then decreased again.

On comparison of the RSP_1 and RSP_2 microspheres, it is clear that the microspheres prepared from PLGA (75:25) had bigger particle size as well as higher drug entrapment efficiency than the microspheres prepared from PLGA (50:50) of similar molecular weights. However, the microspheres prepared using highest molecular weight polymer i.e. 66 and 67 Kda respectively, had shown the higher drug entrapment efficiency. This may be due to diffusion of drug in to the continuous phase mostly occurred during the first few minutes of emulsification, therefore, as the time the polymer phase stayed in the non solidified state was extended, the drug got diffused out and encapsulation efficiency became relatively low. As the polymers have relatively more solubility in methylene chloride, it takes longer time to solidify which results in low encapsulation efficiency [17]. Thus, 5 min stirring time was considered optimum for preparation of the microspheres of suitable particle size and maximum drug entrapment.

Effect of solvent removal time

In order to optimize the solvent removal time, it was varied from 2hr, 4hr and 8hr and the effect of solvent removal time on particle size and drug entrapment was observed. In case of RSP_1 microspheres, it was found that, as the molecular weight of PLGA (50:50) increased, the particle size also increased ranging from 25.58±2.26 µm to 58.49±1.37 µm at 2hr, 24.38±2.47 µm to 55.89±1.38 µm at 4hr and 24.26±2.54 µm to 55.54±1.36 µm at 8 hr solvent removal time. The drug entrapment efficiency also increased from 44.86±2.11% to 82.56±1.88%, 43.57±1.42% to 81.86±1.56% and 43.28±1.61% to 81.15±1.72% at 2, 4 and 8 hr solvent removal time respectively.

Similarly, in case of RSP_2 microspheres, it was found that, as the molecular weight of PLGA (75:25) increased, the particle size also increased ranging from 29.46±1.38 µm to 64.34±1.23 µm at 2hr, 28.26±1.26 µm to 63.64±1.67 µm at 4 hr and 28.06±1.34 µm to 63.08±1.82 µm at 8 hr solvent removal time. The drug entrapment efficiency also increased from 46.73±1.95% to 91.06±1.75%, 45.62±1.45% to 90.83±1.95% and 45.12±1.78% to 90.03±1.16% at 2, 4 and 8 hr solvent removal time respectively.

For both RSP_1 and RSP_2 microspheres, it is clear that for a particular molecular weight of polymer, on increasing the solvent removal time from 2hr to 4hr and 4hr to 8hr, both the mean particle size as well as drug entrapment efficiency were decreased.

On comparison of the RSP_1 and RSP_2 microspheres it is obvious that the microspheres prepared from PLGA (75:25) had bigger particle size as well as higher drug entrapment efficiency than the microspheres prepared from PLGA (50:50) of similar molecular weights. However, in case of the microspheres prepared using highest molecular weight polymer i.e. 66 and 67Kda respectively, had shown better drug entrapment efficiency.

The solvent removal time is a temperature dependent process, i.e. higher the temperature, lesser will be the time required. The solvent is removed at high temperature i.e. 30-40˚C, lesser time will be required but the microspheres formed will not possess the properties required for sustained release. However, the hollow core is formed due to rapid expansion of methylene chloride entrapped within the solidified microspheres [18] which results in the formation of unstable microspheres. Thus, we have selected 4 hrs as an optimum time for solvent removal at 20˚C.

Effect of o/w phase ratio

Effect of o/w phase ratio varying from 2:100, 5:100 and 10:100 was studied to observe its effect on particle size and drug entrapment efficiency. In case of RSP_1 microspheres, it was found that, as the molecular weight of PLGA (50:50) increased, the particle size also increased ranging from 30.46±1.28 µm to 43.49±1.56 µm at 2:100, 28.86±1.83 µm to 42.46±1.46 µm at 5:100 and 18.26±1.26 µm to 39.49±1.28 µm at 10:100 o/w phase ratio. The drug entrapment efficiency increased from 48.56±1.19% to 71.93±1.38% at 2:100, 50.21±1.38% to 74.23±1.54% at 5:100 and 52.49±1.11% to 75.26±1.78% at 10:100 o/w phase ratio (Fig. 5).

In case of RSP_2 microspheres it was found that as the molecular weight of PLGA (75:25) was increased, the particle size also increased ranging from 32.48±2.76 µm to 54.54±1.93 µm at 2:100, 28.98±2.76 µm to 48.67±1.93 µm at 5:100 and 20.28±2.25 µm to 42.84±1.28 µm at 10:100 o/w phase ratio. The drug entrapment efficiency also increased from 52.06±1.45% to 95.43±1.65%, 53.73±1.55% to 97.06±1.75% and 54.16±1.65% to 98.16±1.35% at 2:100, 5:100 and 10:100 o/w phase ratio respectively (Fig. 5).

For both RSP_1 and RSP_2 microspheres, it is clear from the observations that for a particular molecular weight of polymer, on increasing the o/w phase ratio from 2:100 to 5:100 and 5:100 to 10:100, the mean particle size decreased while drug entrapment efficiency was increased.

On comparison of the RSP_1 and RSP_2 microspheres, it is clear that the microspheres prepared from PLGA (75:25) had bigger particle size as well as higher drug entrapment efficiency than the microspheres prepared from PLGA (50:50) of similar molecular weights. However, the microspheres prepared using highest molecular weight polymer i.e. 66 and 67 Kda, had shown bigger particle size and the higher drug entrapment efficiency.

The reason for decrease in particle size on increasing the o/w phase ratio may be attributed to the faster solidification of the polymer resulting in the formation of small size microspheres. The entrapment efficiency increased when the o/w phase ratio increased, as the solidification is fast and water could not flow in to the dispersed phase substantially but the dispersed phase shrank to make the microspheres dense. These microparticles are small in size and their encapsulation efficiency is high [14]. The faster solidification of

microspheres may be due to larger volume of continuous phase in comparison to disperse phase which provided a high concentration gradient of the organic solvent across the phase boundary by diluting the solvent. Thus, we have selected 2:100 as an optimum o/w phase ratio for preparation of microspheres.

On the basis of optimized parameters, microspheres of PLGA (50:50 and 75:25 ratio) Mw 27kda and 66/67 kda were prepared and characterized for the various parameters.

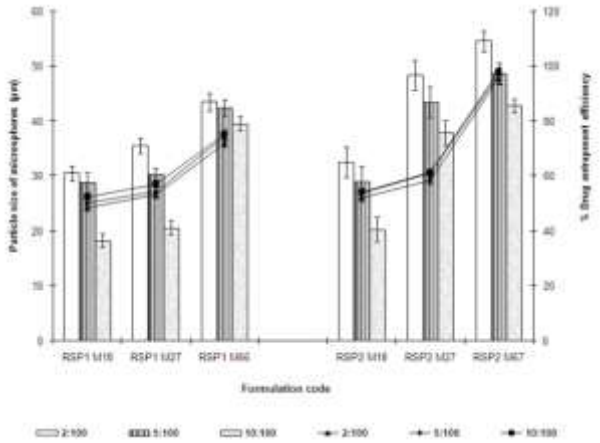

Fig.5. Effect of o/w phase ratio on particle size and drug entrapment efficiency of RSP microspheres prepared using PLGA of varying molecular weights.

Particle size analysis

Particle size is one of the important characteristics of microspheres, because of its effect on degradation rate, drug loading and initial burst release of microspheres [19]. The mean particle size of optimized microspheres of risperidone was below 64 µm and the values of $D_{0.1}$, $D_{0.5}$ $D_{0.9}$ and polydispersity were found to be 10.32, 26.51, 47.26 and 1.825, respectively. This type of particle size distribution of the microspheres is very well accepted for an injection. The size of the microspheres formed may however be a function of many factors such as stirring speed, viscosity of the dispersed phase and dispersion medium, temperature of external phase, amount of drug, drug – polymer ratio etc. Therefore, it was possible to prepare microspheres of desired size by varying some of these parameters.

Determination of encapsulation efficiency

The encapsulation efficiency is highly influenced by various parameters, such as polymer concentration in the oil phase, viscosity and molecular weight of polymers [20]. The percent entrapment efficiency of RSP PLGA microspheres was found to be 94.43±1.75 %, 86.23±1.15 %, 98.04±1.34% and 90.93±1.42% for RSP_1M66, RSP_1M27, RSP_2M67, and RSP_2M27 microspheres respectively. The high entrapment efficiency of risperidone may be attributed to their poor aqueous solubility. High molecular weight of PLGA or higher concentration of polymer in oil phase increased the drug encapsulation of PLGA microspheres, which may be attributed to an increase in the viscosity of the oil phase which prevented the drug from diffusion to surrounding media. However, an increase in the concentration of PVA in the external phase leads to decrease in the encapsulation efficiency. It might be due to the increased solubility of risperidone in aqueous PVA solution which acts as surfactant [15].

The higher entrapment efficiency was observed in RSP PLGA microspheres due to its hydrophobicity. The polymer PLGA 50:50 consists of 50% glycolide, which is hydrophilic in nature, and 50% lactide, which is hydrophobic in nature. Whereas PLGA 75:25 (consisting of 75% lactide and 25% glycolide), being more hydrophobic hence showed higher entrapment efficiency of this hydrophobic drug.

Scanning electron microscopy

All RSP PLGA microsphere formulations were observed under microscope for morphological characteristics and were found to be spherical (Fig.6 - 9). The microspheres incubated in the dissolution media (PBS pH 7.4) were observed initially and after 28 days for the changes in morphology due to erosion or polymer degradation. The 28-day samples showed signs of surface erosion and degradation. The microspheres appeared irregular in shape and slightly fragmented (Fig. 10 – 11).

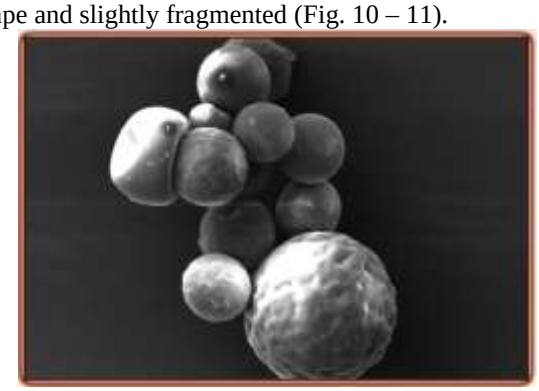

Fig.6. SEM photomicrograph of RSP_1M66 microspheres.

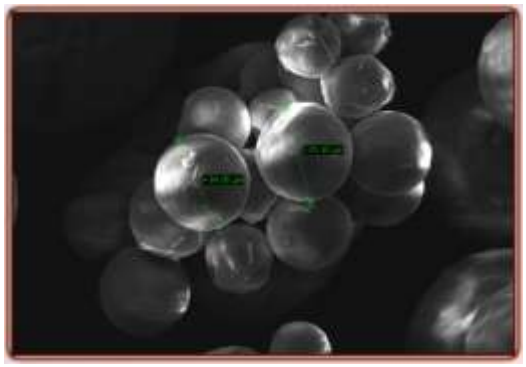

Fig.7. SEM photomicrograph of RSP_1M27 microspheres.

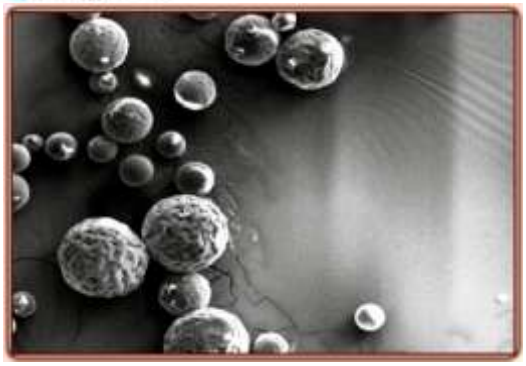

Fig.8. SEM photomicrograph of RSP_2M67 microspheres.

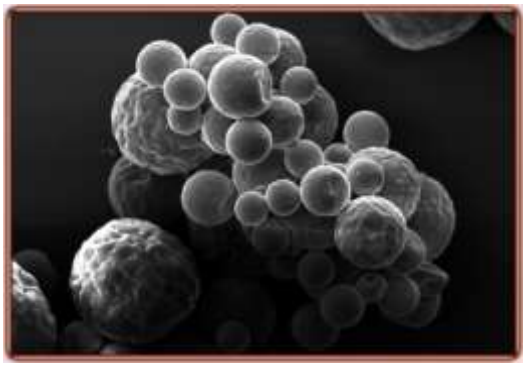

Fig.9. SEM photomicrograph of RSP_2M27 microspheres.

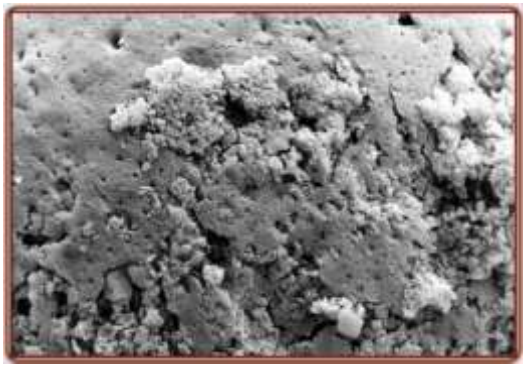

Fig.10. SEM photomicrograph of degraded RSP-PLGA microspheres prepared using PLGA (75:25) after 28 days.

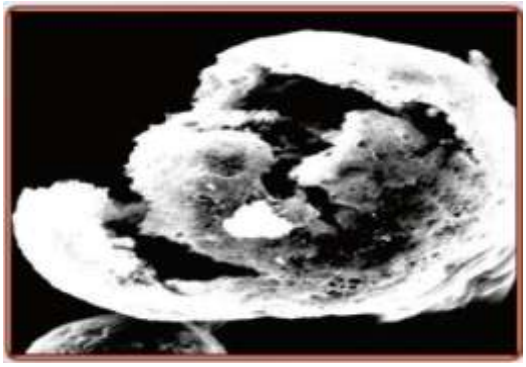

Fig.11. SEM photomicrograph of degraded RSP-PLGA microspheres prepared using PLGA (50:50) after 28 days.

Determination of risperidone content

The risperidone content in PLGA microspheres was determined spectrophotometrically. It was found to be 2.79±0.03 mg, 2.51±0.03 mg, 2.92±0.03 mg and 2.69±0.03 mg for RSP_1 M66, RSP_1 M27, RSP_2M67 and RSP_2M27 respectively.

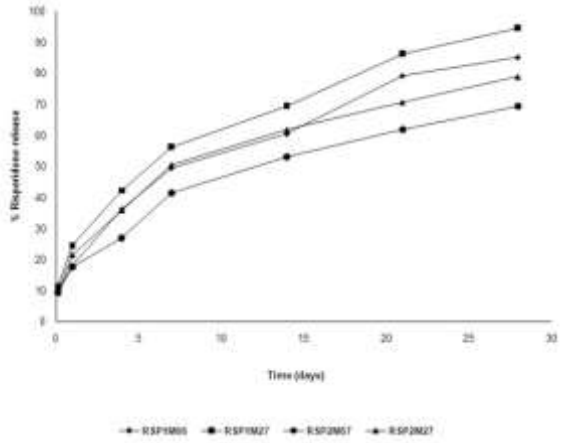

Fig.12. *In vitro* release profile of risperidone from various formulations of RSP-PLGA microspheres.

Drug release studies

The *In vitro* release of risperidone from PLGA microspheres from various formulations RSP_1M66, RSP_1M27, RSP_2M67, and RSP_2M27 was studied in PBS (pH 7.4) containing 0.02% tween 80 and 0.05% sodium azide as antimicrobial agent [12]. All formulations of polymeric microspheres showed an initial burst release followed by slower drug release pattern. The various microspheres of risperidone have shown the percent cumulative drug release as planned i.e. RSP_1M66 (10.26±0.59 to 85.16±2.61), RSP_1M27 (11.32±0.48 to 94.54±2.54) RSP2M67 (9.18±0.42 to 69.23±2.53) and RSP_2M27 (9.53±0.48 to 78.86±2.12) (Fig. 12). The sustained drug release observed after the initial release may be due to the passive diffusion of drug through the pores in the polymer matrix and depends on the porosity of the microspheres formed.

The general mechanism of drug release from biodegradable polymer matrixes can be probably due to the release of drug from the pores created on erosion of the polymer matrix. For PLGA matrices, diffusion of drug through pre-existing pores and channels in the microsphere matrix that subsequently become interconnected and enlarged on chemical degradation of the polymer back bone has been regarded as a predominant drug release mechanism. The above factors led to the increased porosity of the microparticles and mass erosion [21].

Another probable mechanism could be hydrolytic cleavage of ester groups within the polymers, occurring with time, hence, the erosion of microspheres leads to increased drug release from the polymer matrix. Thus drug release from the PLGA matrix systems is believed to be by hydrolytic degradation of the polymer matrix and also by diffusion of drug through the polymer matrix.

This is further influenced by a number of factors e.g. the molecular weight, crystallinity, and the ratio of lactide to glycolide of the PLGA copolymer. In order to study the drug release kinetics from the polymeric microspheres, the data obtained from *in vitro* drug release study were fitted to different mathematical models i.e. zero order, first order, matrix (Higuchi matrix), Peppas–Korsmeyer and Hixson–Crowell using the software PCP-Disso v3 developed by Bharti Vidyapeeth, Deemed University, Pune [22].

Correlation coefficients of risperidone cumulative release *vs.* time profiles were fitted to different mathematical models using the software PCP Disso v3 developed by Bharati vidyapeeth deemed university, Pune. Among the different PLGAs formulations, the formulation RSP_1M66, RSP_1M27, RSP_2M67, and RSP_2M27 had followed Korsmeyer-Peppas model and their correlation coefficients (r^2) were 0.9944, 0.9997 0.9977 and 0.9998 respectively. It reveals that the mechanisms of drug release of the microspheres formulations was through diffusion and degradation of polymer matrix.

Among all the formulations of risperidone PLGA microspheres, it was found that the formulation RSP_1M27 had shown maximum percent of drug release (94.54±2.54), whereas the formulation RSP_2M67 had released 69.23±2.53 percent of drug in 28 days time period, which was minimum. It reveals that the formulation RSP_2M67 is the best among all the formulations of risperidone, in terms of drug release profile for 28 days.

4. Conclusion

The aim of the present work was to optimize different process and formulation parameters for devising the long acting risperidone microspheres. Of the two polymers ratio and various molecular weight studied, the PLGA (75:25, Mw 67Kda) microspheres showed the highest entrapment efficiency of risperidone. The burst release was highest from the PLGA (50:50, Mw 27Kda) microspheres and least from the (75:25, Mw 67Kda) microspheres. In *in vitro* studies, formulation containing PLGA (50:50, Mw 27Kda) had shown maximum percent of drug release (94.54±2.54), whereas the formulation containing PLGA (75:25, Mw 67Kda) had released 69.23±2.53 percent of drug in 28 days time period, which was least. It reveals that the formulation containing PLGA (75:25, Mw 67Kda) is the best among all the formulations of risperidone, in terms of drug release profile for 28 days. These findings reveal that biodegradable microspheres can be exploited as a potential drug delivery system for risperidone in the effective treatment of schizophrenia.

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AUTHOR'S PROFILE

Dr. Shailendra Patil has about 15 years experience of administration, research and teaching experience at both UG and PG levels. He is a well renowned scientist who has published more than 15 papers in journals of international and national repute and presented more than 15 papers in the various conferences / seminars and symposia at national and international level. He has also received the best presentation awards at national level. He has delivered invited lectures and chaired many sessions in several National and International conferences and symposia in India. Presently, he is working as Professor and Head of Pharmaceutics in Sagar Institute of Pharmaceutical Sciences, Sagar, M.P., India.