Poly (D, L-Lactide) Nanosuspensions of Risperidone for Parenteral Delivery: Formulation and *In-Vitro* Evaluation

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Abstract: Risperidone, an "atypical" antipsychotic drug, having large scope for prolonged psychotic treatments through novel parenteral drug delivery systems. Polymeric nanoparticles suspensions containing risperidone made of poly (D, L-Lactide) were designed by nanoprecipitation method using polymeric stabilizer (Pluronic® F-68 or Pluronic® F-127). The prepared nanosuspensions were characterized for particle size by photon correlation spectroscopy and scanning electron microscopy. The free dissolved drug in the nanosuspension was determined by bulk equilibrium reverse dialysis bag technique. *In vitro* release studies were carried out using dialysis bag diffusion technique. The particle size of the prepared nanoparticles in the nanosuspensions ranged between 78-184 nm. Nanoparticles of risperidone in the nanosuspensions were obtained with high encapsulation efficiency (91 - 94 %). The drug release from the risperidone nanosuspension was sustained in some batches for more than 24 h with 75% drug release whereas release from risperidone solution showed release within 1.5 h. The release pattern of drug is analyzed and found to follow first order equation and Fickian diffusion kinetics. These studies suggest the feasibility of formulating risperidone loaded poly (D, L-Lactide) nanoparticles suspension for the treatment of psychotic disorders.

Keywords: Nanoprecipitation method, poly (D, L-Lactide) nanoparticles suspensions, reverse dialysis technique, release kinetics, risperidone, stability study.

INTRODUCTION

Carrier-mediated drug delivery has emerged as a powerful methodology for the treatment of various pathologies. Among the biodegradable and biocompatible polymeric carriers, nanoparticulate systems are interesting for controlled drug delivery and drug targeting [1-3]. These polymeric nanoparticles have been investigated especially in drug delivery for drug targeting because of their particle size (ranging from 10 to 1000 nm) and long circulation in blood [4]. Carrier size under 1 μm, an intravenous (i.v) injection (the diameter of the smallest blood capillaries is 4 μm) is enabled, minimizing any possible irritant reactions [5].

Poly (D, L-Lactide) (PDLLA), biodegradable and biocompatible polymer has been extensively used for developing an array of drug delivery systems. It is an United States Food and Drug administration (USFDA) approved polyester [6]. The rapid clearance of circulating nanoparticles from the blood stream coupled with their high uptake by liver and spleen can be overcome by making the particle surface hydrophilic with poloxamers and poloxamines [7]. PDLLA nanoparticles loaded with pentamidine showed more activity against visceral leishmaniasis [8].

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% (coefficients of variation [CV] =25%). 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 [9-11].

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 [12]. Therefore, maintenance of risperidone (owing to its short half-life) in plasma level using long circulating nanoparticles via i.v 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. Long acting risperidone has been available in the high doses of 25 and 50 mg for gluteal injection every 2 weeks. It has the drawback of lack of initial drug release (< 1% of dose) for first 3 weeks after injection and hence, requires oral antipsychotic supplementation for first 3 weeks. Repeated injections (4 injections: 1 injection every 2 weeks) are required to attain steady state level of drug in 6-8 weeks. This injection will not produce any effect during first 3 weeks of treatment of psychosis. The repeated injections result into higher incidences of adverse effects [13]. Moreover, the above injection cannot be used for the short-term management (less than a month's duration) of manifestations of psychotic disorders; oral formulations of risperidone are the only available preparation for the therapy. The risperi-

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done from the conventional oral formulations will be metabolized rapidly (owing to first pass effect) to 9-hydroxy risperidone (an active metabolite) which will remain in the plasma for longer period because of its long half-life. However, the penetrability of 9-hydroxy risperidone to blood-brain barrier is less than risperidone which may result in decreased therapeutic effect. This can be minimized by controlling the release of risperidone and avoiding the metabolite formation. Compared to oral formulation risperidone loaded PDLLA nanoparticles via i.v route will reduce the frequency of administration, dose (without first pass effect) and adverse effects during the short-term management of manifestations of psychotic disorders.

In the recent years many research groups have shown interest to utilize drug loaded polymeric nanoparticles to achieve controlled drug delivery that were prepared by different methods like nanoprecipitation and solvent emulsification/evaporation [14]. However, there is no literature report about the release mechanisms of drug loaded PDLLA nanoparticles as yet.

We have prepared and characterized risperidone loaded poly (ε–caprolactone) (PCL) nanoparticles suspensions by nanoprecipitation method [15]. In the present study, we have developed risperidone loaded PDLLA nanoparticles suspensions in order to sustain the release of risperidone and studied the release mechanism of risperidone nanoparticles intended for i.v administration.

MATERIALS AND METHODS

Materials

Risperidone was obtained from APL Research centre, Hyderabad, India as gift sample. Poly (D, L-Lactide) (PDLLA 100) (molecular weight of 75,000 - 1,20,000), Pluronic® F-68, Pluronic® F-127 and dialysis bag with a 12,000 molecular weight cut off were purchased from Sigma-Aldrich Chemicals Private Limited, Bangalore, India. All other chemicals were of analytical grade.

Methods

Formulation of Nanosuspensions

Nanosuspensions (containing nanospheres) were prepared by nanoprecipitation according to the method developed by Fessi *et al.* 1992 [16]. PDLLA was dissolved in acetone at 45° C. Volume changes due to evaporation of acetone was adjusted at room temperature, and then risperidone was added and dissolved. This organic solution was injected at the rate of 48ml/min in distilled water containing Pluronic® F-68 or Pluronic® F-127 under magnetic stirring at room temperature. Acetone and some proportion of water were eliminated at 40° C under reduced pressure and the final volume of the suspension was adjusted to 10 ml. This final nanosuspension (containing nanoparticles) was used for the characterizations of the nanoparticles [17]. Nanosuspension formulas were established (Table **1**) with different risperidone and polymer concentration level in order to obtain higher encapsulation efficiency, desired particle size and suitable drug release kinetics. Concentration of Pluronic® F-68 and Pluronic® F-127 were also optimized and selected to get stable nanosuspensions.

Particle Size Analysis and Polydispersity of Risperidone Nanosuspensions

Particle size and polydispersity of nanoparticles suspensions were measured by photon correlation spectroscopy (PCS) using Mastersizer 2000 (Malvern Instruments, Malvern, UK), which allows sample measurement in the range of 0.020-2000.00 µm. In order to analyze, nanosuspensions were diluted with filtered (0.22 µm) ultrapure water.

Polydispersity was determined according to the equation below:

$$\text{Polydispersity} = \frac{D\,(0.9) - D\,(0.1)}{D\,(0.5)}$$

Where D (0.9) corresponds to particle size immediately above the sizes of the 90% of the nanoparticles. D (0.5) corresponds to particle size immediately above the sizes of the 50% of the nanoparticles. D (0.1) corresponds to particle size immediately above the sizes of the 10% of the nanoparticles [17].

Characterization by Scanning Electron Microscopy

External morphology of nanoparticles of nanosuspension were determined using scanning electron microscope (SEM) Zeiss Leo-430, 20kV. Samples of nanosuspension were pre-

Table 1. Formulas of Nanosuspensions

Batches	Risperidone (mg)	PDLLA (mg)	Pluronic® F-68 (mg)	Pluronic® F-127 (mg)	Acetone (ml)	Water (ml)
NS_0	10	120	-	-	25	50
NS_1	10	120	250	-	25	50
NS_2	10	200	250	-	25	50
NS_3	10	280	250	-	25	50
NS_4	10	120	-	250	25	50
NS_5	10	200	-	250	25	50
NS_6	10	280	-	250	25	50

pared by placing one drop on a copper grid and dried under vacuum pressure. Then nanoparticles were coated with gold-palladium and their surface morphologies were observed.

Determination of Total Drug Content of Nanosuspension

The total drug amount in nanosuspension was determined spectrophotometrically [18]. A 0.50-ml aliquot of nanosuspension was evaporated to dryness under reduced pressure at 35 °C. The residue was dissolved in dichloromethane and filtered with a 0.45 μm filter, and risperidone content was assayed spectrophotometrically at 279 nm (λ_{max}).

The calculation was performed as follows:

$$\text{Total drug content} = \frac{\text{Vol. total}}{\text{Vol. aliquot}} \times \text{Drug amount in aliquot}$$

Where Vol. total / Vol. aliquot is the ratio of the aliquot to the total nanosuspension volume.

Encapsulation Efficiency of Nanoparticles in the Nanosuspension

The total drug content in nanosuspension was calculated as described earlier. Free dissolved drug amount present in the nanosuspension was determined by bulk equilibrium reverse dialysis bag technique, as described by Singh and Muthu 2007 [15]. Briefly, a dialysis bag (cellulose membrane, molecular weight cut off 12,000 D) containing 1 ml of distilled water with 25mg Pluronic® F-68 or Pluronic® F-127 was placed directly into 10 ml of nanosuspension. After equilibrium (6 h), dialysis bag was withdrawn from the nanosuspension. Sample collected from dialysis bag was assayed spectrophotometrically at 275 nm (λ_{max}) for risperidone content. Because of the change of solvent the λ_{max} of risperidone in aqueous systems {distilled water and phosphate buffered saline (pH 7.4)} was different from that obtained in dichloromethane.

The calculation was performed as follows:

Free dissolved drug = Total volume X Drug amount in the dialysis bag sample

Where Total volume is the sum of total volume of the nanosuspension and volume of the dialysis bag sample

Encapsulation Efficiency was Calculated as Indicated Below:

Encapsulation efficiency (%) =

$$\frac{\text{Total drug content} - \text{Free dissolved drug}}{\text{Drug amount used}} \times 100$$

Determination of Risperidone Solubility in Phosphate Buffered Saline (pH 7.4)

Risperidone solubility in over-saturation condition (C >10 x Cs) was obtained by dispersing 300 mg of drug in 100 ml of phosphate buffered saline (pH 7.4) [19]. The suspensions were stirred (100rpm), at 37 ° C ± 0.5 ° C for 24 h (adequate time for equilibration), filtered through a syringe filter (0.45μm), and then assayed spectrophotometrically at 275 nm. The experimental values were the average of three replicates and standard deviations did not exceed 3% of the mean value.

C = Concentration to be maintained for risperidone solubility determination per ml

Cs = Approximate saturation solubility concentration of risperidone per ml

In-Vitro Drug Release Studies

The dialysis bag diffusion technique was used to study the in-*vitro* drug release of risperidone nanosuspensions [15, 20, 21]. Nanosuspensions (1ml of sample) were placed in the dialysis bag (cellulose membrane, molecular weight cut off 12,000 D), hermetically sealed and immersed into 50 ml of phosphate buffered saline (pH 7.4). The entire system was kept at 37 ± 0.5 ° C with continuous magnetic stirring at 200 rpm/min. Samples were withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. The amount of drug dissolved was determined with UV spectrophotometry at 275nm.

Risperidone has low solubility in phosphate buffered saline (pH 7.4) (Cs_1 = 140 μg/ml at 37 ° C). Sink condition were maintained for release studies ($C_1 < Cs_1$ x 0.2). Therefore, final concentration of risperidone after the complete release in phosphate buffered saline (pH 7.4) was maintained less than 28 μg/ml in compliance with the sink conditions.

C_1 = Final concentration of risperidone in medium after the complete release of the drug in the phosphate buffered saline (pH 7.4).

Cs_1 = Saturation solubility of risperidone in the phosphate buffered saline (pH 7.4).

Since risperidone is insoluble in water, risperidone solutions (1 mg/ml) in 50% w/w mixture of PEG 400 and water were used as controls for the in-*vitro* drug release studies.

Stability Studies

Risperidone loaded PDLLA nanosuspensions with and without stabilizer were stored at 25 °C for 2 months and average particle size and encapsulation efficiency were determined [22].

Statistical Analysis

Results are given as mean ± standard deviation (S.D). Mean values of nanoparticles size and encapsulation efficiency were compared using the Student's t-test. Differences are considered significant at a level of $P<0.05$.

RESULTS AND DISCUSSION

Formulation Considerations of Nanosuspensions

In the present work risperidone loaded PDLLA nanosuspensions and nanosuspension without stabilizer were prepared by the nanoprecipitation method (Table **1**). The nanoprecipitation protocols utilize an organic phase composed of volatile solvents, whose elimination is readily achieved by evaporation. Risperidone was dissolved in acetone along with the different quantities of PDLLA and introduced into aqueous medium containing surfactants (Pluronic®) which resulted in the formation of nanoparticles (polymeric matrix with drug).

Drug was adsorbed on the nanoparticles and/or dispersed into the matrix of nanoparticles. This may be due to precipitation of the drug when put into aqueous phase because of its limited solubility in the water [17]. The speed of nanoparticles solidification by nanoprecipitation method enables the drug to be rapidly entrapped, thus preventing its diffusion into the outer phase [23].

The main requirements for a particulate formulation intended for parenteral use include biocompatibility of its ingredients and suitable drug carrier size. Pluronic® had been used for stabilization of polymeric nanosuspensions [24]. Pluronic® is regarded as non-toxic for parenteral use, therefore no special purification step is required for its elimination from the final formulation [18].

Particle Size and Polydispersity of Risperidone Nanosuspensions

The mean particle size of risperidone loaded nanoparticles in the nanosuspensions is shown in Table **2**. The particle size distribution curves for all the samples are unimodal. The nanoparticles size was found to depend directly on PDLLA amount. The smallest particles of 79.4 ± 2.6 nm were found in batch NS_1 (120 mg PDLLA content) and largest particles of 184.7 ± 4.6 nm were seen in batch NS_3 (280 mg PDLLA content) (Table **2**). Difference between particle size of these batches were significant ($P<0.05$). Similar trend was found in batches NS_4 to NS_6. The data in Table **2** suggest that increase in polymer concentration results in large nanoparticles.

Risperidone loaded PDLLA nanosuspensions containing different stabilizer (Pluronic® F-68 and Pluronic® F-127) showed no significant ($P>0.05$) difference in particle size when same quantity of PDLLA was used (Table **2**).

Polydispersity of the nanoparticles in the nanosuspensions decreased with an increase in PDLLA concentration and particle size of the nanoparticles (Table **2**).

Particle size must be strictly controlled in nanoparticulate formulation intended for intravascular delivery. Since the estimated diameter of the smallest blood capillaries in the human body is 4-7 µm, the particle size should optimally be kept in the submicronial range to prevent capillaries occlusion [25].

The results of this study show that the nanoparticles size is influenced by concentration of the polymer. The effect of the polymer concentration on the increased nanoparticles size may be due to the higher resultant organic phase viscosity, which leads to larger nanodroplet formation [26].

External Morphological Study

The external morphological study using SEM revealed that all nanoparticles of nanosuspensions were spherical in shape. The nanoparticles size as observed by SEM correlated well with the size measured by PCS (Fig. **1**).

Determination of Total Drug Content, Free Dissolved Drug Content of Nanosuspension and Encapsulation Efficiency of Nanoparticles in the Nanosuspensions

The total drug content in the nanosuspensions varied from 9.90 ± 0.04 to 9.99 ± 0.05 mg (NS_1 to NS_6) (Table **3**). The amount of free dissolved drug in the nanosuspensions ranged from 0.51 ± 0.02 mg to 0.70±0.01 mg (NS_1 to NS_6). This was due to the limited solubility of risperidone in the aqueous phase. Free dissolved drug in the nanosuspension decreased with increase in the PDLLA concentration (Table **3**).

Encapsulation efficiency was also inversely related to free dissolved drug. However, it was directly related to amount of PDLLA used. Although encapsulation efficiency increased with higher amount of PDLLA but it was not significant ($P>0.05$) (Table **3**). During the calculation of encapsulation efficiency, drug adsorbed on the surface of the nanoparticles was considered as encapsulated drug of the nanoparticles.

In-Vitro Drug Release Studies

Figs. (**2** and **3**) show the percentage release of risperidone from different batches (NS_1 to NS_6) in phosphate buffered saline (pH 7.4). The release profile of risperidone control solution (risperidone in PEG 400) indicates very rapid diffusion of risperidone with nearly 50% release in 30 min and 75% in 1.5 h.

Table 2. Particle Size and Polydispersity of Risperidone Nanosuspensions (mean ± *S.D*, *n*=3)

Batches	Particle Size (nm)	Polydispersity
NS_0	80.4 ± 2.2	0.7811 ± 0.012
NS_1	79.4 ± 2.6	0.7893 ± 0.029
NS_2	124.3 ± 4.4	0.7719 ± 0.023
NS_3	184.7 ± 4.6	0.6315 ± 0.056
NS_4	78.1 ± 3.1	0.7453 ± 0.013
NS_5	127.9 ± 3.2	0.6711 ± 0.037
NS_6	183.4 ± 3.9	0.6118 ± 0.048

Table 3. Total Drug Content, free Dissolved Drug in the Nanosuspensions and Encapsulation Efficiency of the Nanoparticles in the Nanosuspensions (mean ± *S.D*, *n*=3)

Batches	Total Drug Content (mg)	Free Dissolved Drug (mg)	Encapsulation Efficiency (%)
NS_0	9.89 ± 0.03	0.69 ± 0.02	92.00 ± 0.48
NS_1	9.90 ± 0.04	0.70 ± 0.01	92.00 ± 0.45
NS_2	9.93 ± 0.04	0.56 ± 0.05	93.70 ± 0.28
NS_3	9.99 ± 0.05	0.55 ± 0.04	94.40 ± 0.96
NS_4	9.91 ± 0.03	0.72 ± 0.02	91.90 ± 0.50
NS_5	9.92 ± 0.02	0.51 ± 0.02	94.10 ± 0.43
NS_6	9.98 ± 0.01	0.53 ± 0.01	94.50 ± 0.26

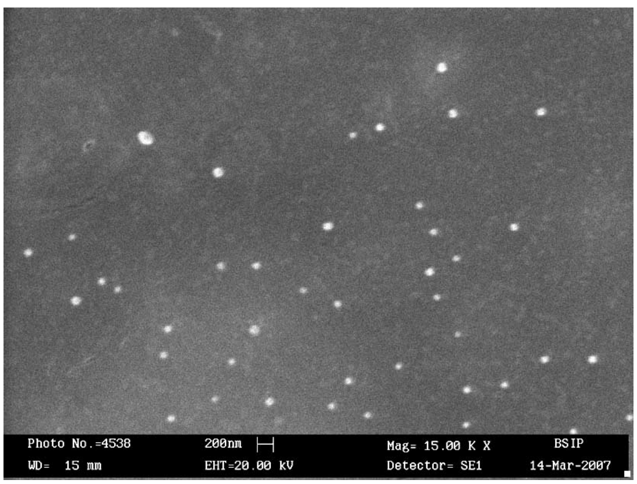

Fig. (1). SEM micrograph of risperidone loaded PDLLA nanoparticles (Batch No: NS_1) magnified 15,000 x

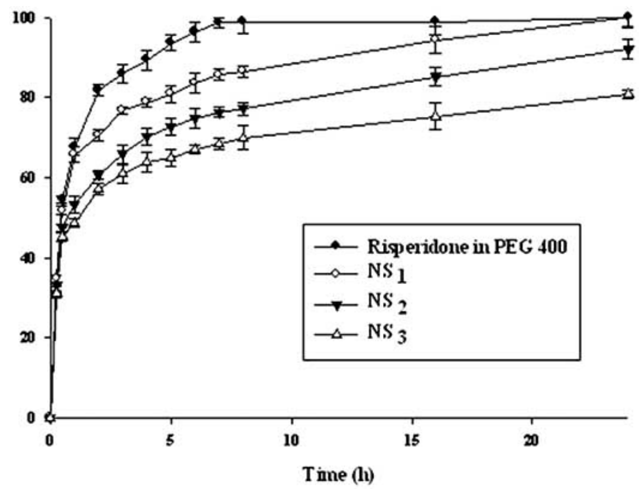

Fig. (2). *In-vitro* drug release from risperidone nanosuspensions containing Pluronic® F-68 and control risperidone solution (1 mg/ml) in phosphate buffered saline (pH 7.4).

The nanosuspensions (batches from NS_1 to NS_6) initially showed burst release followed by sustained release up to 24 h**.** After 24 h of dialysis in phosphate buffered saline (pH 7.4), the percentages of risperidone released were 100, 92 and 81 % for batches NS_1, NS_2 and NS_3, respectively (Fig. **2**).

The effect of surfactant on risperidone release was also studied. The $t_{75\%}$ for batches NS_1 and NS_4 was same (5 h) in phosphate buffered saline (pH 7.4). Similar drug release profiles were observed with other batches (Figs. **2** and **3**). No significant (P>0.05) difference in the *in vitro* release profile of risperidone loaded PDLLA nanosuspensions was observed with two different surfactant, i.e. Pluronic® F-68 and Pluronic® F-127 studied (Figs. **2** and **3**). The PDLLA content also affected the drug release kinetics. The increase in PDLLA content in the nanosuspensions resulted in slower kinetics. The $t_{50\%}$ in phosphate buffered saline (pH 7.4) was about 0.5 h, 1 h and 1.5 h for batches NS_1, NS_2 and NS_3 containing 120, 200 and 280 mg of PDLLA, respectively (Fig. **2**). Similar results were obtained for batches NS_4 to NS_6 (Fig. **3**).

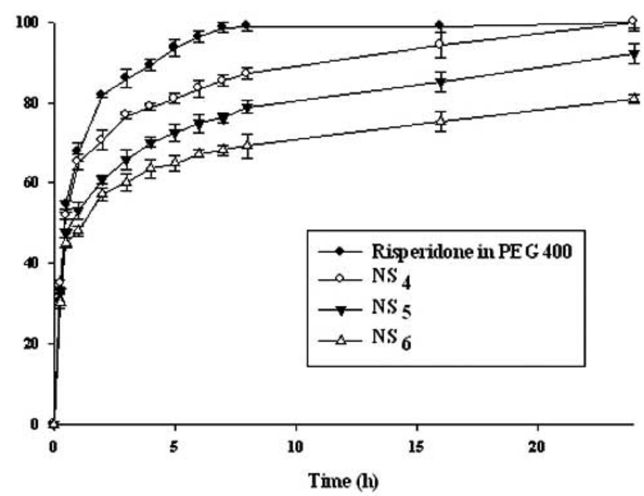

Fig. (3). *In-vitro* drug release from risperidone nanosuspensions containing Pluronic® F-127 and control risperidone solution (1 mg/ml) in phosphate buffered saline (pH 7.4).

The release of drug from polymeric nanosuspensions was studied by dialysis bag diffusion technique. This is a very popular method to study the release of drugs from colloidal suspensions [20]. Since the aim of the study was to administer the nanosuspensions by the i.v route, the release studies were carried out with phosphate buffered saline (pH 7.4) to mimic the *in vivo* conditions.

The release rate of the risperidone from the nanosuspensions and its appearance in the dissolution medium was governed by the partition coefficient of the drug between the polymeric phase and the aqueous environment in the dialysis bag and by the diffusion of the drug across the membrane as well. Dialysis bag retained nanosuspensions and allowed the diffusion of the drug immediately into the receiver compartment.

The nanoparticles size was also associated with changes in drug release kinetics. The smaller sized nanoparticles prepared with lower amounts of PDLLA exhibited higher drug release rates. This may be attributed to a corresponding increase in the total nanoparticles surface, resulting in a larger drug fraction exposed to the leaching medium. Smaller nanoparticles size also leads to a shorter average diffusion path of the matrix-entrapped drug molecules [27].

Initial burst effect on release of risperidone from different nanosuspensions batches (NS_1 to NS_6) (Figs. **2** and **3**) may be due to the free dissolved drug observed with nanosuspensions and free drug adsorbed on the nanoparticles in the nanosuspensions. The release of drug adsorbed on the nanoparticles surface was considerably faster compared to the encapsulated drug. Initial burst release of risperidone from PDLLA nanosuspensions within 1 h was approximately 50 % (Figs. **2** and **3**), although the determined free dissolved risperidone was only 5-7% (Table **3**). The remaining 43-45% may be from drug adsorbed on the surface of the nanoparticles. The higher amount of PDLLA content in the risperidone nanosuspensions increases the size of the polymeric nanoparticles (Table **2**), improves the encapsulation of the drug (Table **3**) and results in slower drug release (Figs. **2** and **3**).

In order to investigate the mode of drug release from PDLLA nanoparticles the release data were analyzed with the following mathematical models: zero-order kinetics ($Q = k_0 t$); first order kinetics ($\ln (100-Q) = \ln Q_0 - k_1 t$); Higuchi equation ($Q = k_H t^{1/2}$) [28,29]. The examination of the coefficient of determination (r^2) indicated the drug release followed first order kinetics from the nanoparticles (batches from NS_1 to NS_6), as the r^2 values for first order kinetics (ranged between 0.9925 to 0.9953) was always higher in comparison to zero-order kinetics (ranged between 0.9403 to 0.9486) as well as Higuchi´s square root of time (ranged between 0.9731 to 0.9849) (Table **4**). Further, to understand the drug release mechanism, the data were fitted to Peppas exponential model $Mt/M\infty = Kt^n$, where M_t/M_∞ is fraction of drug released after time 't' and 'K ' is kinetic constant and 'n' is release exponent which characterizes the drug transport mechanism [30]. The values 'n' were in the range of 0.1290 - 0.1536, which was further indicative of the drug releases following Fickian diffusion control mechanism (Table **4**).

The initial burst release will not allow to perform the real release kinetics of medicated nanoparticles. During release studies using dialysis bag, risperidone molecule diffuses

Table 4. *In Vitro* Drug Release Kinetics of Risperidone from PDLLA Nanoparticles Suspensions

Batches	Drug Release Kinetics, Correlation Coefficients (r^2)			Release Exponent (n)
	Zero Order	First Order	Higuchi	
NS_1	0.9463	0.9941	0.9881	0.1290
NS_2	0.9403	0.9932	0.9809	0.1527
NS_3	0.9486	0.9925	0.9849	0.1324
NS_4	0.9462	0.9953	0.9872	0.1284
NS_5	0.9460	0.9930	0.9731	0.1536
NS_6	0.9433	0.9927	0.9825	0.1360

Table 5. Effect of Time of Storage (at 25 °C) on Particle Size and Entrapment Efficiency of Risperidone Loaded Pdlla Nanoparticles in the Nanosuspensions with and Without Stabilizer (mean ± *S.D*, *n*=3)

Batches	Particle Size (nm)			Encapsulation Efficiency (%)		
	0 day	1 month	2 months	0 day	1 month	2 months
NS_0	77.4 ± 2.0	123.1 ± 9.1	380.1 ± 25.9	92.10 ± 0.56	89.17 ± 0.51	89.01 ± 0.44
NS_1	79.2 ± 3.1	80.2 ± 2.6	79.9 ± 2.3	92.19 ± 0.34	91.71 ± 0.34	91.31 ± 0.34
NS_4	78.6 ± 3.2	81.3 ± 3.4	81.6 ± 3.2	92.08 ± 0.57	91.88 ± 0.57	91.24 ± 0.57

from the nanoparticles to the water phase of the nanosuspension, then to the outer aqueous phase (release medium). The content measurement was performed on the outer aqueous phase. The changes in the diffusion rates of these steps will be less after the burst release due to free dissolved drug and adsorbed drug and during the steady state of drug release. Therefore, drug release kinetics was calculated from 3 to 24 h (after burst release) and during the phase of steady state drug release.

Stability Studies

During 2 months of storage at 25 °C, the nanoparticles size of batch NS_0 (nanoparticles containing a suspension without any stabilizer) increased up to 380 nm. But other nanoparticles stabilized by a stabilizer (batch NS_1 and NS_4) retain an acceptable particle size changes during 2 months of storage period. Similar results were observed by Fessi et al 1989 [22]. However, encapsulation efficiency was not affected by the changes or the absence of surfactant during the storage period (Table **5**).

CONCLUSION

This study confirms that nanoprecipitation technique is suitable for the preparation of risperidone nanoparticles suspension with high encapsulation efficiency. By co-dissolving the drug with the polymer, high encapsulation efficiency (up to 94%) was achieved as the polymer precipitated into solid particles. *In vitro* release profiles revealed no effect of surfactant on release studies. The changes in nanoparticles size and drug release kinetics were shown to be affected by polymer concentrations. *In vitro* release of risperidone followed first order and Fickian diffusion kinetics better than Higuchi equations. This formulation approach can be used to improve the therapeutic efficacy of poorly soluble drugs. The controlled drug release from the risperidone nanosuspensions suggests that frequency of administration and dose dependant adverse effects of this molecule could be reduced, if administered via i.v route.

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