Formulation and Evaluation of Pralidoxime-PLGA Microspheres as Antidote against Organophosphorous Poisioning

Shyam Bihari Sharma[1], Suman Jain[1], K. Ganesan[2], J. Acharya[2]

[1]School of Studies in Pharmaceutical Sciences, Jiwaji University, Gwalior, Madhya Pradesh, India, [2]Defence Research and Development Organisation, Gwalior, Madhya Pradesh, India

Abstract

Objective: The objective of the present work is to formulate and evaluate Pralidoxime chloride (PAM)-controlled release microspheres using poly (lactic-co-glycolic acid) (PLGA, 50: 50) as polymer and poly vinyl alcohol as surfactant. **Materials and Methods:** PAM-controlled release microspheres were prepared by solvent evaporation method. The prepared microspheres were characterized by flow properties such as angle of repose, compressibility index, particle size, and encapsulation efficiency and drug release profiles. **Results:** All the prepared microsphere were spherical and exhibited good flow properties. PAM was greatly encapsulated with PLGA and poly vinyl alcohol. The Fourier transform infrared spectroscopy and differential scanning calorimetry studies were conducted for pure drug, polymer, and optimized formulations, the studies revealed that they were no incompatibilities between drug and polymers used in the present study. Scanning electron microscopy analysis showed that the microspheres were uniform and spherical in nature with good surface characteristics among all the PAM-controlled release microspheres formulations. **Conclusions:** The optimized formulation (F 6) prepared with appropriate proportion of polymer PLGA and surfactant poly vinyl alcohol was found to extend the release of drug up to 28 h.

Key words: Antidote, cholinesterase reactivator, microspheres, oxime, PLGA, poly vinyl alcohol, solvent evaporation method

INTRODUCTION

Organophosphate poisoning results from exposure to organophosphates (OPs), which cause acetylcholinesterase (AChE) inhibition, leading to acetylcholine (ACh) accumulation in the body. Organophosphate poisoning most commonly results from exposure to nerve agents or insecticides. OPs are one of the most common causes of poisoning in the world, and are often used intentionally in agricultural suicides.[1,2]

Organophosphates (OPs) intoxication accounts for the highest number of poisoning cases across the globe. It is estimated that about three million people are exposed to OP each year, with up to 3000 fatalities.[3,4] Severe poisoning affects peripheral and central nervous systems (CNS) and eventually causes paralysis of body extremities and respiratory muscle. The major cause of death in severe poisoning cases is respiratory failure due to depression of CNS respiratory center, neuromuscular weakness (mainly the diaphragm muscle), and excessive bronchosecretions.[5]

Despite the wide use of OP[6] and high OP intoxication incidences worldwide,[3] the management of severe acute OP poisoning cases is still a challenge with the available drugs. Pralidoxime was reported to be of great benefit in reversing respiratory symptoms of OP poisoning,[7] but its use is limited due to a poor blood brain barrier (BBB) penetration.[8] OP, on the contrary, can freely pass the barrier resulting in various CNS poisoning effects. Improved CNS distribution of oximes can potentially improve the drug effectiveness in the management of OP poisoning through reversal of the

nicotinic and muscarinic receptor effects both peripherally and centrally.

Efforts to overcome the BBB have focused on altering either the barrier integrity and characteristics or the drug properties. Microspheres might be a better technique to circumvent the BBB since no BBB or drug manipulation is necessary. Poly (lactic-co-glycolic acid) (PLGA) microspheres have proved to improve the BBB penetration of a number of drugs that are poorly distributed in the CNS.[9] The present study is aimed at fabrication and analyses of Pralidoxime-loaded PLGA microspheres, using different drug/polymer ratios, for potential use in the delivery of the drug into the CNS.

Microspheres, microparticles, and microcapsules are frequent constituents of multiparticulate drug delivery systems, and their application is suited for convenient and tolerable drug administration through numerous routes due to their structural and functional characteristics.[10] These carrier systems are widely employed to disguise the taste and odor of therapeutic molecules, prolong drug release, improve drug stability, and increase bioavailability.[11-13] Compounds are difficult to administrate due to features such as insolubility, volatility, reactivity, hygroscopicity, and physical state. Microspheres can help to structure such molecules.[14] They may also help to protect the encapsulated contents against degradation caused by external environmental variables including oxygen, light, heat, and humidity, which can harm any labile chemical.

Microsphere might also be used to allow for the controlled release of encapsulated contents, which could be controlled by chemical, physical, and mechanical aspects. It may also control the encapsulated product's release at the desired time, rate, dose, and site of action.

Pralidoxime is an OP antidote with poor CNS distribution due to a high polarity. In the present study, Pralidoxime-loaded PLGA microspheres were prepared and evaluated as a potential delivery system of the drug into the CNS. The microspheres were prepared using emulsion solvent evaporation method. Different drug/polymer ratios were used (0.5:1, 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 1:2, 1:2.5, and 1:3) and the fabricated microspheres were characterized for encapsulation efficiency using UV-VIS Spectroscopy; particle size distribution, polydispersity index, and zeta potential using photon correlation spectroscopy (PCS); and *in vitro* drug release profile using UV-VIS Spectroscopy.

MATERIALS AND METHODS

Pralidoxime chloride (PAM) was obtained as a gift sample from DRDE, Gwalior and Sigma Aldrich, USA. PLGA (PLGA, 50:50) was obtained from Nomisma Healthcare, Sigma-Aldrick, USA Chemical Co., and poly vinyl alcohol was commercially procured from LOBA Chemie Pvt. Ltd. Mumbai. Ethyl acetate, Ethanol, Acetone, and Dist. water were commercially procured from SD Fine Chemicals Ltd. Mumbai.

Preparation of PAM-controlled release microspheres

Pralidoxime PLGA (50:50)-controlled release microspheres of PAM were prepared by solvent evaporation method using PLGA as polymer and poly vinyl alcohol as surfactant. All nine experimental formulations (F1-F9) were produced in triplicate. PAM and PLGA (50:50) in ratio of 0.5:1, 1:1, 1.5:1, 2:1, etc., were dissolved in 1:2 mixture of solvent system of water and ethyl acetate using a vortex shaker to form a homogeneous solution of drug and polymer. This solution was poured slowly in a thin stream of the aqueous solution of poly vinyl alcohol in varying concentration using high pressure homogenizer to prepare the emulsion. The emulsion formed was stirred on magnetic stirrer for 4 h at a speed of 500 rpm at $25 \pm 2°C$. After stirring, the solidified microspheres were recovered by filtration, washed with phosphate buffer (pH 7.4) to remove all non-encapsulated drug, and further with distilled water to wash off surfactant solution. The contents were subjected to a vacuum oven for complete solvent evaporation. Recovered microspheres were stored in desiccator at 2–8°C. All formulations were evaluated for their entrapment and loading efficiency. Formulations with remarkable encapsulation and loading efficiency were, further, subjected for particle size analysis, scanning electron microscopy (SEM), and *in vitro* drug release studies. The composition of pralidoxime-controlled release microcapsules is given in Table 1.

Characterization and evaluation of PAM-controlled release microspheres

The prepared microspheres are generally characterized and evaluated for micromeritic properties such as particle size, bulk density, tapped density, angle of repose, compressibility index, and other important characters such as drug entrapment efficiency, drug loading, surface morphology, and *in vitro* drug release behavior using appropriate kinetic models. Nine formulations as microspheres were prepared with varying polymer and drug combinations to make a wide variety in formula for effective comparison and final selection of the potential batch.

S. No	Formulation code	PLGA W/W (%)	Polymer-drug ratio	PVA (%)
Table 1: Formulations of the prepared microspheres				
1.	F1	0.1	0.5:1	0.2
2.	F2	0.1	1:1	0.4
3.	F3	0.1	1.5:1	0.6
4.	F4	0.2	2:1	0.8
5.	F5	0.2	2.5:1	1.0
6.	F6	0.2	3:1	1.5
7.	F7	0.3	1:2	2.0
8.	F8	0.3	1:2.5	2.5
9.	F9	0.3	1:3	3.0

PLGA: Poly (lactic-co-glycolic acid)

Particle size determination

Particle size is one of the most important parameters of microspheres. The particle size of the pralidoxime-loaded microspheres was determined in triplicates by a PCS on a submicron particle size analyzer (Malvern Instruments) at a scattering angle of 90°. Approximately, 1 mg of each sample was dissolved in 1 mL of deionized water. The dissolved sample was sonicated for 30 min. The sample was placed in a Zetasizer and the particle size was then observed.

Bulk density (ρB) determination

Apparent bulk density was determined by pouring pre-sieved drug excipient blend into a graduated cylinder and measuring the volume and weight "as it is." It is expressed in g/cm³. Bulk density of the formulations was calculated by volume (Vb) of 5 g of microspheres observed in a 10 ml measuring cylinder and dividing weight of sample (W) by volume of the microspheres (Vb) using the following formula:

$$\text{Bulk density } \rho B = W/Vb$$

Tapped density (ρT) determination

Tapped density is used to investigate packing properties of microcapsules. It was determined by placing a graduated cylinder, containing a known mass of drug excipient blend, on mechanical tapping apparatus. The tapped volume (Vt) was measured by tapping the powder to constant volume. It is expressed in g/ml. The tapped density was measured by employing the conventional tapping method using a 10 mL measuring cylinder and the number of tappings was 100 as sufficient to bring a plateau condition. Vt was observed. Tapped density was calculated dividing Weight of sample (W) by Tapped volume Vt) using the following formula:

$$\text{Tapped density } \rho T = W/Vt$$

Angle of repose determination

The powder flow parameters were investigated to establish if the material flow was good or bad.

Angle of repose was determined to predict flow ability of the microparticles. There is an empirical relationship between angle of repose and the ability of the powder to flow. Angle of repose of the microspheres is the maximum angle possible between the surface of the pile of microspheres and the horizontal plane, which was obtained by fixed funnel method. It was determined by passing microspheres through the glass funnel on a horizontal surface. The height (h) of the heap formed was measured and the radius (r) of the cone base was also observed and calculated using the following equation as tan θ = h/r.

$$\text{Angle of repose} \theta = \tan^{-1}(h/r)$$

Where,
h = height of the tip of powder from the base,
r = radius of the cone and

Compressibility index determination

It is indirect measurement of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials since all of them can influence the consolidation index. It is also called as compressibility index or Carr's Index. It is denoted by (Ci) and is calculated using the formula:

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density-Bulk density}}{\text{Tapped density}} \times 100$$

It is expressed in percentage. A high Carr index is indicative of the tendency to form bridges. Value of Carr's index describes flow property of powder given below in Table 2.

Imortant characterization of PAM microspheres

Percentage yield

Suitability of preparation under variable influencing factors yield of product must be considered as an important parameter. The microspheres were evaluated for percentage yield after their preparation. The prepared microspheres with a size range of 1–1000 μm were collected and weighed from different formulations. After preparation and subsequent drying of microspheres, final weight was measured in triplicate. Percentage yield of different formulation was determined by weighing the microspheres after drying. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.[15] The yield was calculated as per equation given below.

$$\%\text{Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \times 100$$

Drug entrapment efficiency

Entrapment efficiency is the percentage of drug entrapped in Pralidoxime loaded microspheres related to the initial quantity of the drug used in the formulation. The various formulations of the microspheres were subjected for drug content. 10 mg of the microspheres from all batches were accurately weighed and crushed. The powder of microspheres was dissolved in 10 ml of 0.1 N HCl and centrifuge at 1000 rpm. This supernatant solution is, then, filtered through Whatmann filter paper No. 44. After filtration, from this solution, 0.1 ml was taken out and diluted up to 10 ml with 0.1 N HCl. The absorbance of the resulting solution was measured at wavelength maximum of 294 nm using double-beam UV-Visible Spectrophotometer with 1 cm path length

Batch	Avg. size (μm)	Bulk density (gm/cc)	Tapped density (gm/cc)	Angle of repose (o)	Carr's index (%)
F1	289.11±1.17	0.321±0.18	0.366±0.05	15.23±0.39	10.26±0.25
F2	306.09±1.19	0.315±0.08	0.372±0.08	16.78±0.33	11.49±0.20
F3	275.19±0.88	0.352±0.08	0.324±0.09	17.44±0.61	12.02±0.21
F4	261.25±1.21	0.378±0.19	0.340±0.01	16.76±0.45	14.57±0.53
F5	227.03±0.89	0.358±0.12	0.413±0.02	18.29±0.76	15.29±0.34
F6	205.15±1.11	0.384±0.08	0.425±0.07	16.53±0.31	17.33±0.11
F7	235.21±1.26	0.323±0.10	0.341±0.03	19.71±0.54	12.83±0.63
F8	311.54±1.34	0.298±0.15	0.356±0.04	21.14±0.34	11.95±0.23
F9	334.14±0.96	0.311±0.07	0.321±0.24	20.53±0.54	15.23±0.15

Table 2: Micromreitic properties of microspheres

sample cells. The amount of pralidoxime loaded in the microspheres was determined by measuring the amount of the drug encapsulated per mL of microsphere suspension, which was done in triplicates. Entrapment efficiency was calculated using the following formula:

$$Encapsulation\ Efficiency\ (EE)\ (\%) = \frac{Amt\ of\ pralidoximed\ entrapped}{Total\ amt\ of\ pralidoxime\ added} \times 100$$

The drug entrapment efficacies of different formulations were in range of 61.90–79.52%w/w. The maximum percentage drug entrapment was found in formulation F6 (79.52% w/w).

Determination of zeta potential

The zeta potential is representative of particle charge. Zeta potentials were measured by electrophoresis. The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. Approximately, 1 mg of each sample was dissolved in 1 mL of deionized water. The dissolved sample was sonicated for 30 min. The samples were placed in a zetasizer and the zeta potential was, then, observed. The zeta potential of the drug-loaded microspheres was measured in triplicates by a PCS using a zetasizer (Malvern Zetasizer Nano ZS90, UK).

Polydispersity index measurement

The polydispersity index of the pralidoxime-loaded microsphere was determined in triplicates by a PCS using a zetasizer (Malvern Zetasizer Nano ZS90, UK). Approximately, 1 mg of each sample was dissolved in 1 mL of deionized water. The dissolved sample was sonicated for 30 min. The sample was placed in a zetasizer and the polydispersity index was then observed.

In vitro drug release study of microspheres

The drug release rate from prepared microspheres was carried out using the USP type II (Electro Lab.) dissolution paddle assembly. The *in vitro* drug release studies were conducted in blood pH using paddle type dissolution apparatus. Accurately weighed quantity of microspheres was taken into 900 ml of dissolution medium (pH 7.4) which was maintained at 37 ± 0.50 C with paddle rotating at 100 rpm. Aliquot of sample was withdrawn at various intervals such as 30, 60, 120, 180, 240, 300, 360, 420, and 480 min which are filtered. Up to 24 h of samples were taken at regular intervals. Aliquots were withdrawn and same portion of fresh medium was refilled immediately. To maintain a consistent volume throughout the experiment, fresh medium was replaced with the same volume. One ml sample was withdrawn at predetermined intervals and filtered and equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. Finally, the collected samples are diluted and analyzed spectrophotometrically at 294 nm. The dissolution studies were carried in triplicate manner. After that, the percentage drug release and the concentration of drug present in the dissolution medium were calculated. The results obtained from *in vitro* drug release studies were plotted in four different kinetic models such as Zero-order rate kinetics, First-order rate kinetics, Higuchi's diffusion model, and Korsmeyer-Peppa's exponential. The *in vitro* drug release profile of various PAM-controlled release microcapsules is shown in Figures 1 and 2.

RESULTS AND DISCUSSION

Preparation of PAM-controlled release microspheres by solvent evaporation method

In the present investigation, PAM-controlled release microcapsules were prepared by solvent evaporation method. PLGA (PLGA, 50: 50) and polyvinyl alcohol were used as controlled release coating polymeric material for the preparation of microspheres. The compositions of various PAM-controlled release microspheres are given in Table 1.

Evaluation of physical parameters of PAM-controlled release microspheres

The prepared microspheres were evaluated for angle of repose, compressibility index, % drug content, encapsulation efficiency, and particle size. Angles of repose values for various microspheres ranged from $15.23 \pm 0.39°$ to $21.14 \pm 0.34°$, indicating that microspheres have good flow characteristics. The compressibility indexes for all microspheres were ranged from 10.26 ± 0.25 to 17.33 ± 0.11 indicating good flow of microsphere characteristics. The average particle size was assessed using a simple microscopic method, and all of the formulations were between 205.15 ± 1.11 and 334.14 ± 0.96. The % yield for prepared microspheres was found to be in the range of $56.40 \pm 0.31–69.75 \pm 0.17$. The drug entrapment of PAM-controlled release microspheres was found
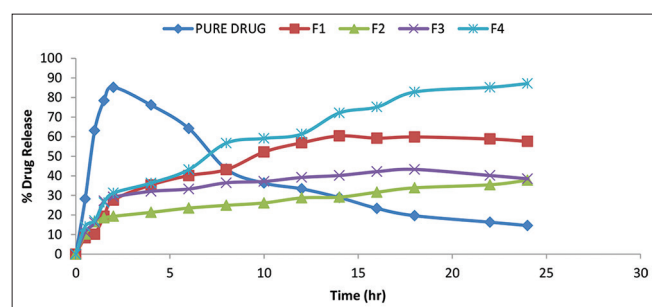
to be in the range of $61.90 \pm 0.45–79.52 \pm 0.62\%$. The physical parameters evaluated and other important characters for various microspheres are given in Tables 2 and 3.

In vitro drug release study of PAM-controlled release microspheres

All of the microspheres were tested *in vitro* dissolution test apparatus equipped with USP type II (Electro Lab.) dissolution paddle assembly and 900 ml of 0.1 N HCl (pH = 1.2) as the dissolution medium. The *in-vitro* studies results indicate that, when the drug: polymer ratio was increased, the drug release from microspheres was decreased which may be due to increased path length for diffusion of drug molecule from microspheres. Formulations F4 to F6 containing PLGA in sufficient amount ranging from 200 to 300 mg released 87.13–92.21% drug before 24 h. Formulation F6 showed about 92.21% of drug release over a period of 24 h and was found to be suitable for extending drug release up to 28 h. The drug release profiles for various microspheres are shown in Figures 1 and 2.

SEM studies

SEM image of drug-loaded microspheres (a) and blank microspheres (b) are displayed in Figure 3. The surfaces of some of the microspheres were quite smooth and no pores were observed. SEM has been used to confirm the formation of spherical structures of the microspheres. The microspheres were coated with gold color and subjected to SEM, which showed that the microspheres are spherical in shape. It was found that blank microsphere appears smaller as compare to larger drug-loaded microsphere.

Differential scanning calorimetry (DSC) thermograms

DSC thermograms of PLGA, Drug and PLGA microsphere, are displayed in Figure 4. The result shows the sharp peak of PLGA and drug was obtained at 63° and 190°C. In microsphere sharp, PLGA and drug peak also occurred at the same thermal condition. This indicates that the drug will retain their characteristics in formed polymeric microsphere.

Figure 1: *In vitro* drug release profiles of pralidoxime chloride-controlled release microcapsules (F1 to F4) (mean ± S.D; *n* = 3)

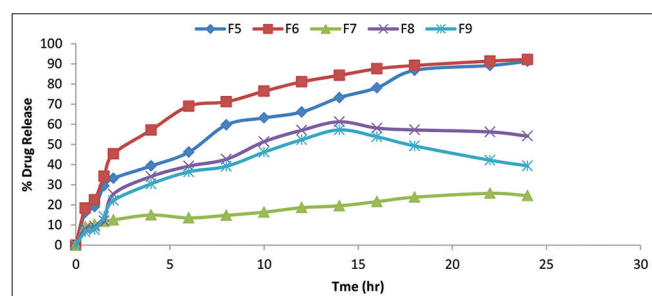**Figure 2:** *In vitro* drug release profiles of PAM controlled release microcapsules (F5 to F9) (mean ± S.D; *n* = 3)

| Table 3: General characterization of microspheres				
Batch	Drug entrapment	PDI	Zeta potential (mv)	% yield
F1	69.52±0.19	0.216±0.021	−47.09±0.56	68.66±0.36
F2	65.23±0.35	0.143±0.032	−43.04±0.87	63.50±0.46
F3	71.42±0.49	0.161±0.001	+44.33±1.02	56.40±0.31
F4	70.00±0.63	0.223±0.011	−42.44±1.14	57.33±0.16
F5	68.57±0.47	0.136±0.026	+44.29±0.29	61.33±0.68
F6	79.52±0.62	0.128±0.014	+52.09±0.64	69.75±0.17
F7	75.23±0.51	0.207±0.027	+48.22±1.17	61.33±0.53
F8	66.19±0.53	0.112±0.041	−45.23±0.86	56.57±0.36
F9	61.90±0.45	0.241±0.019	+51.71±0.68	58.50±0.29

*Mean±SD, (*n*=3), PDI: Poly dispersity index
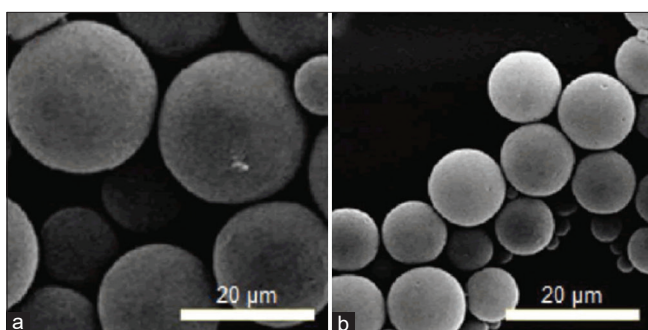
Figure 3: Scanning electron microscopy of (a) drug loaded microspheres and (b) blank microspheres
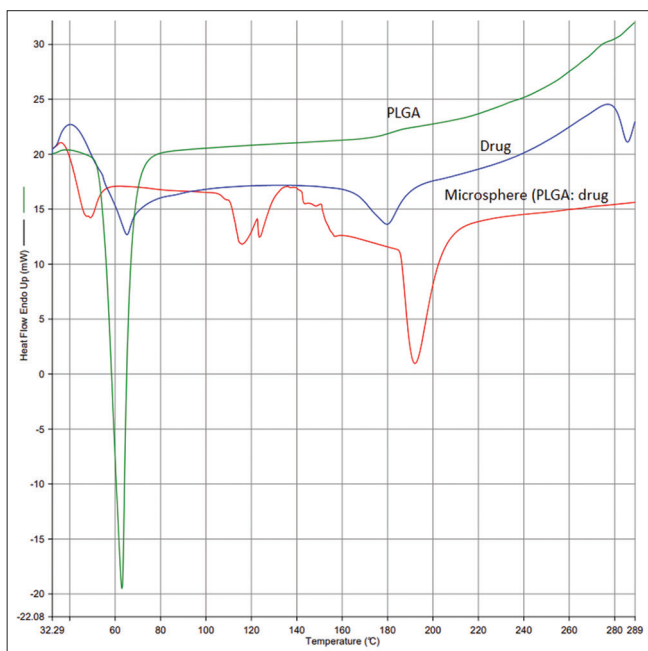

Figure 4: Thermogram of poly (lactic-co-glycolic acid, drug, and microsphere)

CONCLUSIONS

The concept of formulating microspheres containing PAM offers a suitable, practical approach to achieve a prolonged therapeutic effect by continuously releasing the medication over an extended period of time. Thus, the microspheres of PAM were successfully prepared by solvent evaporation method using the different concentration of polymer PLGA and poly vinyl alcohol.

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REFERENCES

1. Pandit V, Seshadri S, Rao SN, Samarasinghe C, Kumar A, Valsalan R. A case of organophosphate poisoning presenting with seizure and unavailable history of parenteral suicide attempt. J Emerg Trauma Shock 2011;4:132.
2. Yurumez Y, Durukan P, Yavuz Y, Ikizceli I, Avsarogullari L, Ozkan S, *et al*. Acute organophosphate poisoning in university hospital emergency room patients. Intern Med 2007;46:965-9.
3. Li Y, Sun C, Qiu Z, Ma S, Yu X, Wang Z, *et al*. Clinical toxicology in China: Current situation and future development. Clin Toxicol (Phila) 2009;47:263-9.
4. Eddleston M, Buckley NA, Eyer P, Dawson AH. Management of acute organophosphorus pesticide poisoning. Lancet 2008;371:597-607.
5. Occupational Health Services. MSDS for Methyl Parathion. Secaucus, NJ: OHS Inc.; 1991.
6. Balali-Mood M, Balali-Mood K. Neurotoxic disorders of organophosphorus compounds and their managements. Arch Iran Med 2008;11:65-89.
7. Tush GM, Anstead MI. Pralidoxime continuous infusion in the treatment of organophosphate poisoning. Ann Pharmacother 1997;31:441-4.
8. Ellyn RI, Wills JH. Oximes antagonistic to inhibitors of cholinesterase. J Pharm Sci 1964;53:995-1007.
9. Choonara YE, Pillay V, Ndesendo VM, du Tiot LC, Kumar P, Khan RA, *et al*. Polymeric emulsion and crosslink-mediated synthesis of super-stable nanoparticles as sustained-release anti-tuberculosis drug carriers. Colloids Surf B Biointerfaces 2011;87:243-54.
10. Bale S, Khurana A, Reddy AS, Singh M, Godugu C. Overview on therapeutic applications of microparticulate drug delivery systems. Crit Rev Ther Drug Carrier Syst 2016;33:309-61.
11. Khamanga SM, Parfitt NN, Nyamuzhiwa T, Haidula H, Walker RB. The evaluation of eudragit microcapsules manufactured by solvent evaporation using USP apparatus 1. Dissolut Technol 2009;16:15-22.
12. Belgamwar VS, Kawtikwar PS, Phutane GP, Belgamwar AV. Design and evaluation of microcapsules containing ciprofloxacin hydrochloride for sustained release. Indian Drug 2008;45:553-7.
13. Shabaraya AR, Narayanacharyulu R. Design and evaluation of chitosan microspheres of metoprolol tartrate for sustained release. Indian J Pharm Sci 2003;65:250-2.
14. Wieland-Berghausen S, Schote U, Frey M, Schmidt F. Comparison of microencapsulation techniques for the water-soluble drugs nitenpyram and clomipramine HCl. J Control Release 2002;85:35-43.
15. Patel A, Ray S, Thakur RS. *In vitro* evaluation and optimization of controlled release floating drug delivery system of metformin hydrochloride. DARU J Pharm Sci 2006;14:57-64.