Acta Poloniae Pharmaceutica – Drug Research, Vol. 73 No. 1 pp. 175–181, 2016 ISSN 0001-6837
Polish Pharmaceutical Society

DEVELOPMENT OF OLANZAPINE LOADED PNA MICROGELS FOR DEPOT DRUG DELIVERY IN TREATMENT OF SCHIZOPHRENIA: I*N VITRO* AN *IN VIVO* RELEASE PROFILE

FAHAD PERVAIZ[1]*, MAHMOOD AHMAD[1], TALIB HUSSAIN[2], ARFAT IDREES[1], AYESHA YAQOOB[1] and KHIZAR ABBAS[3]

[1]Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan
[2]Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan
[3]College of Pharmacy, GC University, Faisalabad, Pakistan

Abstract: Schizophrenia is a lifelong debilitating illness requiring extended treatment with antipsychotic agents. Non-adherence to therapy is a very common and severe problem in these patients, which can be improved by prescribing depot injectable or implant formulations. The purpose of this study was to develop PNA microgels based in situ gelling system for sustained release of olanzapine. PNA [poly(N-isopropylacrylamide-co-acrylic acid)] microgels were prepared using a previously developed method employing emulsion polymerization technique, applying one of the optimized formulations. Olanzapine loaded PNA microgels were characterized by viscosity measurements, cytotoxicity assay and TEM analysis. *In vitro* release of olanzapine from PNA microgels was determined on different pH and temperature range. *In vivo* studies were performed on male Sprague-Dawley rats with average weight of 315 g (n = 6). Olanzapine loaded PNA microgels were successfully prepared with drug loading efficiency of 2.14 ± 0.52%. The fluid like viscosity of microgels formulation at lower pH value (pH 5.0) and room as well as body temperature made it favorable for injection form. *In vitro* release was characterized by a high initial burst release up to 38.6% of the drug release within 2 h. *In vivo* release data also indicated similar initial high burst release that might indicate toxicity when administered in injectable dosage form but subcutaneous injection of PNA microgels proved fruitful results as this initial burst release followed a sustained release for 72 h. Hence, PNA microgels can be formulated for short term depot injection, which can potentially provide the release of olanzapine for 72 h.

Keywords: schizophrenia, olanzapine, PNA microgels, cytotoxicity assay, TEM analysis, sustained release, depot injection

Schizophrenia is severe debilitating mental illness leading to devastating disability in about 0.6% of the world population (1). Cost of treatment in such large population is in billions of dollars annually but still patients have significantly reduced life expectancy and suffer from mental disability (2). Schizophrenia has characteristic episodes of relapse and remission, which requires a long term pharmacotherapy with antipsychotic medication (3). There is no cure available for the chronic debilitating disease like schizophrenia but medication therapy can certainly reduce the symptoms of the patients and improve the quality of life. Most of the patients require a lifelong treatment with medications. Other interventions for the treatment includes psychosocial training and rehabilitation care process (4). Atypical antipsychotics can be described as drugs producing antipsychotic effects with minimal extrapyramidal symptoms on normal treatment doses (5). Atypical antipsychotic drugs are very potent antagonists of 5-HT_{2A} receptors, but in comparison having a low antagonistic effect on D_2 receptors (6). Olanzapine is considered as an innovative drug entity and widely used in about four million patients around the world, due to its safety and efficacy profile (7). Olanzapine has shown to have affinity with dopamine D_1 and D_2, muscarinic and serotonin $5HT_2$ receptors (8). Nonadherance to antipsychotic agents can lead to frequent relapses, decreased mental and functional capacity, re-hospitalization and inability to maintain relationships in schizophrenic patients (9). Nonadherance to med-

* Corresponding author: e-mail: fahad_bwp@yahoo.com; phone: +92-3216805365

ication can be improved by introduction of depot parenteral drug delivery system for sustained delivery of antipsychotic medication (10), which will significantly reduce the dosage frequency and thereby improve adherence.

Microgels have attracted attention in recent years as *in situ* gelling system for biomedical application (11). Microgels are hydrogels in colloidal size range and consisted of cross-linked polymers. Microgels are *in situ* gelling systems, which response to various stimuli presents *in situ* e.g., solvent composition, pH of dispersion medium, temperature of medium, ionic strength and osmotic pressure (12). Microgels in concentrated dispersion show a better phase transition behavior as compared to linear polymers, as small sized microgels combine to form a larger network of gel (13-15). All these properties of microgels, make them a potential and suitable candidate as an *in situ* gelling system for drug delivery (16). Parenteral depot delivery of olanzapine based on poly(N-isopropylacrylamide-co-acrylic acid) (PNA) *in situ* gelling has not been reported to our knowledge.

A dual temperature/pH responsive microgels based on PNA were prepared according to previously developed and optimized method (16). PNA microgels based *in situ* gelling system was applied for depot injection drug delivery of olanzapine.

EXPERIMENTAL

Materials

Pure drug samples of olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3][1,5]benzodiazepine) and fluoxetine were kindly gifted by Miracle Pharmaceuticals (Islamabad, Pakistan). N-isopropylacrylamide (NIPAM), N,N'-methylenebisacrylamide (MBA), sodium dodecyl sulfate (SDS), acrylic acid (AA), potassium persulfate, Dulbecco's modified Eagle's medium (DMEM), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich (Steinheim, Germany).

Methods

Preparation of olanzapine loaded PNA microgels

PNA microgels were prepared using a method as stated by Xiong Wi et al., employing an optimized formulation developed by authors (16). Initially, 2.6 g of NIPAM (monomer), 0.2 g of MBA (monomer and cross linker) and 0.116 g of SDS (surfactant) were added to 280 mL of water containing 10% AA (co-monomer). This solution was transferred to three necked flask fitted with reflux condenser. The solution was bubbled with nitrogen for at least 40 min to remove any oxygen from the solution. After purging with nitrogen, the solution was heated at 70°C on a magnetic stirrer at 300 rpm. Potassium persulfate solution (0.14 g/10 mL) was rapidly added to above solution to initiate the process of polymerization. The polymerization process was carried out for 5 h at 70°C. Residual monomers were removed by dialysis against water for 2 weeks. After dialysis, the microgels were separated and dried by using a freeze dryer. Formulation was sterilized with γ irradiation at 25 kGy and degradation was prevented by using dry ice.

Loading of olanzapine to microgels was done according to previously described procedure (17). The freeze dried PNA microgels were immersed in 100 mL of 0.2 mg/mL solution of olanzapine for 4 days. Drug loaded PNA microgels were washed with phosphate buffer saline (PBS) repeatedly for at least three times and freeze dried or carried to dissolution medium for release studies.

Drug loading efficiency

Drug loading efficiency of PNA microgels was calculated using the following formula:

$$\text{Loading efficiency } \% = \frac{W_{drug}}{W_{dried\ microgel}} \times 100$$

where W_{drug} is weight of drug in dosage form and $W_{dried\ microgel}$ is weight of drug loaded freeze dried PNA microgel.

Transmission electron microscopic analysis

Transmission electron microscope (TEM) model JEOL JEM-1010 (Korea) was used to analyze the morphology of olanzapine loaded PNA microgels. A dispersion of microgels was deposited on a carbon coated formvar grid. These grids were air dried and observed with TEM at accelerating voltage of 100 kV.

Measurement of viscosities

Viscosity of olanzapine loaded PNA microgels was evaluated at various pH and temperature range. The samples of microgels (8%) were dispersed separately in citric acid-phosphate buffer solutions of pH 5.0, 7.0 and different temperature ranges of 20-45°C using thermostat. All viscosity measurements were taken on programmable viscometer (Brookfield DV-II pro, USA).

Measurement of cytotoxicity with MTT assay

MTT method was used to evaluate the cytotoxicity of olanzapine loaded PNA microgels. Briefly describing, NH 3T3 cells (mouse embryon-

ic fibroblast cells) were seeded at 5000 cells per µL in each well of 96-well plate. Well plate was maintained in DMEM with 100 U/mL of penicillin, 100 µg/mL streptomycin and 10% heat inactivated FBS. Well plate was incubated for 1 day in 5% CO_2 atmosphere at 37°C and 100 µL of olanzapine loaded microgel dispersion was added to culture medium. After adding microgels, this culture medium was incubated for 24 and 72 h. After incubation time point, the culture medium was replaced with MTT solution (5 mg/mL) and incubated for 4 h in dark at 37°C. Then, MTT solution was replaced with DMSO (200 µL) and stirred at room temperature. Samples were analyzed with optical rotation measured at 490 nm.

In vitro release studies

In vitro release of olanzapine from PNA microgels was carried out in USP apparatus I with phosphate buffer saline (PBS) pH 7.4 at 100 rpm and calibrated to 37°C ± 0.5°C. At predetermined time intervals, the samples (10 mL) were collected, fil-

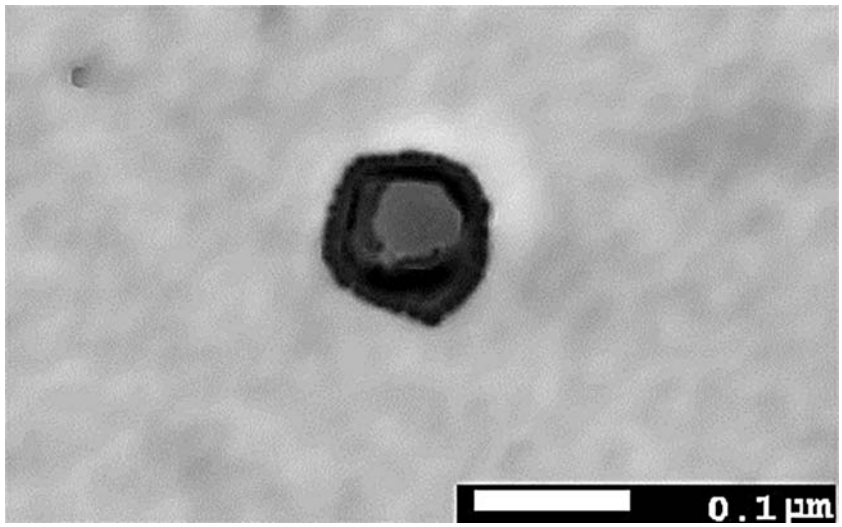

Figure 1. Transmission electron microscopic (TEM) image of olanzapine loaded PNA microgels

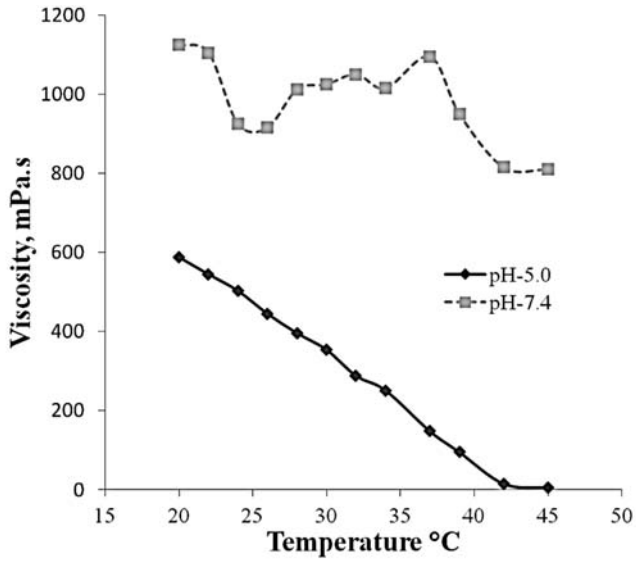

Figure 2. Viscosity of olanzapine PNA microgel dispersion at various pH and temperature range

tered (0.45 μm membrane filter) using Sartorius syringe filtration assembly and were replaced with fresh BPS already maintained at specified temperature. The samples were analyzed for olanzapine concentration using ultraviolet spectrophotometer (Shimadzu, Japan) at 226 nm. A calibration curve demonstrating absorbance of known concentration of olanzapine was constructed in BPS media and analyzed to determine amount of olanzapine released (18).

The release pattern was evaluated by applying different kinetic equations including zero order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell to determine correlation coefficient r^2 value for interpretation of release pattern along with mechanism of drug release from PNA microgels.

***In vivo* studies of olanzapine loaded PNA microgels**

In vivo studies were performed on male Sprague-Dawley rats with average weight of 315 g. Rats were divided into two groups (n = 6) and provided with free access to food and water at all the time during study. Control group of animals was administered with oral dose of olanzapine (7 mg/kg) and test group was administered with subcutaneous injection of olanzapine (7 mg/kg) loaded PNA microgels suspended in normal saline. All animals were observed for 72 h prior to study for any abnormality. Animal studies were approved by Pharmacy Research Ethics Committee, Department of Pharmacy, the Islamia University of Bahawalpur and all experiments were performed in accordance

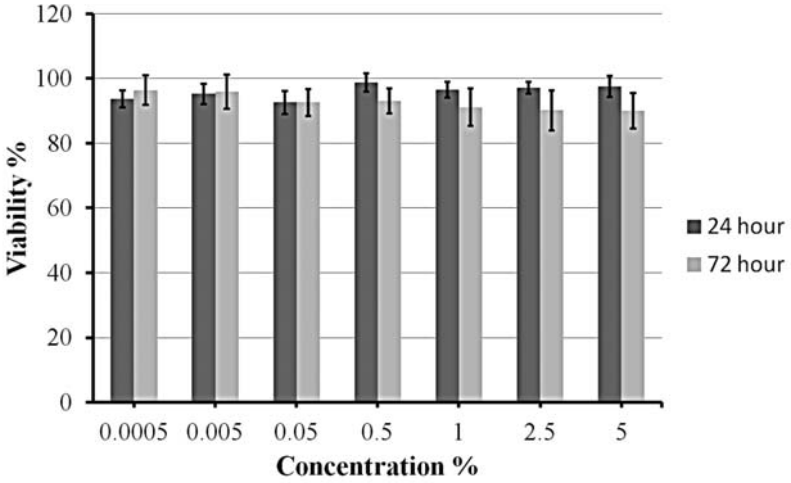

Figure 3. MTT assay of olanzapine loaded PNA microgels after incubation for 24 h and 72 h

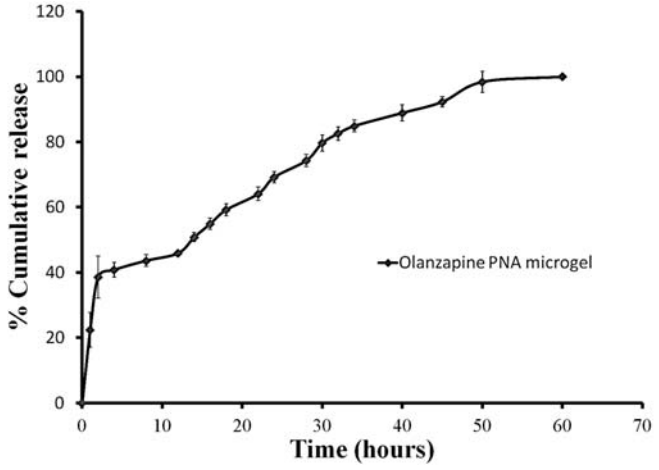

Figure 4. *In vitro* release profile of olanzapine from PNA microgels observed on tissue conditions

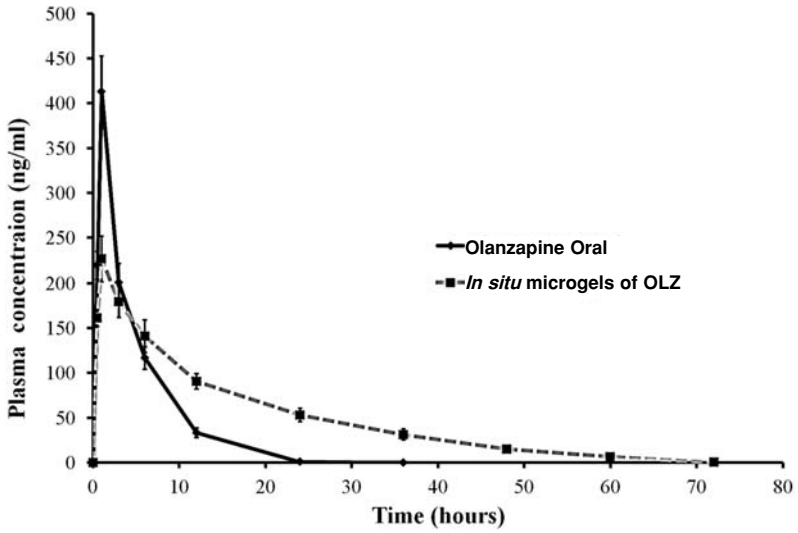

Figure 5. *In vivo* release profile of olanzapine from oral olanzapine (7 mg/kg) and subcutaneous PNA microgels (7 mg/kg) in rats (n = 6)

with international guidelines for animals (19). Blood samples (0.5 mL) from tail vein were collected at 0, 1, 3, 6, 12, 24, 36, 48, 60 and 72 h. All blood samples were immediately centrifuged at 4500 × g for 10 min and plasma was separated and stored at -80°C in dark. Plasma samples were brought to room temperature before analyzing with a previously developed and validated reverse phase HPLC method. Thermo Kinetica 5.0 (Thermo Fisher Scientific, USA) was used to evaluate the pharmacokinetic parameters of olanzapine in rat plasma.

Statistical analysis

Results were statistically investigated for significant difference by using t-test at 5% significance level and considered significantly different if value of $p \leq 0.05$. All statistical analysis was performed using Microsoft Excel-2010 software package.

RESULTS AND DISCUSSION

Olanzapine loaded PNA microgels were successfully prepared with drug loading efficiency of 2.14 ± 0.52%. Loading efficiency was found low because of the nature of drug loading process; however, the results were consistent with a previous study using the same method of drug loading (17). Transmission electron microscopic studies revealed the morphology of olanzapine loaded PNA microgels as shown in Figure 1. TEM study indicates the micron size of the PNA gel particles with spherical shape and slightly irregular outer surface.

Viscosity measurements of olanzapine PNA microgels

Viscosity of olanzapine PNA microgel dispersion was measured at pH 5.0 and pH 7.4, applying temperature range of 20-45°C as shown in Figure 2. The PNA microgels formulation is both pH and temperature sensitive, therefore showing a variable behavior in viscosity when temperature was increased on pH 7.4. At pH 7.4, viscosity was high even at 20°C, showing conversion to gel form on this pH value. Viscosity was decreased as temperature increases when measured in pH 5.0 dispersion. The nature in viscosity variation make this formulation favorable for injection form, as at pH 5.0 injection will not turn into gel on room temperature or even at higher temperatures. The dual nature of microgel prevents clogging of syringe and premature gelation of formulation when compared with only temperature sensitive *in situ* gels.

MTT cytotoxicity assay

Olanzapine loaded PNA microgels did not show any significant sign of cytotoxicity when evaluated with MTT assay, as shown in Figure 3. A range of microgels concentrations (up to 50 mg/mL) were applied, but did not show a cytotoxic effect on any of the concentrations. After incubation for longer period (72 h), there was little but no significant effect on metabolic proliferation of NH 3T3 cells. Alone MTT assay cannot predict the cytotoxicity of dosage form; further animals studies are required to prove the safety parameter regarding

PNA microgel, so careful and precise animal studies can predict the actual toxicity of PNA microgels. A previous study also showed the non toxic behavior with PNA microgels (17).

In vitro release studies

In vitro release profile of olanzapine from PNA microgels was observed on simulated tissue conditions, as shown in Figure 4. *In vitro* release was characterized by a high initial burst release up to 38.6% of the drug release within 2 h that was attributed to the hydrophilic characters of the gel material, which resulted in excellent swelling on penetration of water in the gel matrix. This high initial burst release may indicate that this microgel dosage form might not be suitable candidate for injectable dosage form due to potential of producing toxic effects but efficiently controlling the loading of drug by limiting minimum toxic concentration to 40% of loaded drug can overcome the stated problem. *In vitro* release pattern for olanzapine from PNA microgel was consistent with *in vitro* release of other hydrophobic drugs from microgels in a previous study (17). This initial release was followed by an extended sustained release phase, which lasted for 50 h. Release mechanism was best described by Korsmeyer-Peppas model ($r^2 = 0.9681$, n = 0.397), n value indicates that release of olanzapine from PNA microgels was controlled by Fickian diffusion (20). The initial burst release demonstrated such release pattern but the release profile was also found somewhat consistent with the Higuchi model ($r^2 = 0.944$) indicating the release pattern from these PNA microgel followed diffusive release as a predominant pattern of drug release (21). *In vitro* release kinetics of olanzapine from PNA microgels is described in Table 1.

In vivo studies of olanzapine loaded PNA microgels

In vivo release profile of subcutaneous injection of olanzapine loaded PNA microgels was compared with oral olanzapine dose on healthy rats. *In vivo* release data indicated an initial high burst absorption of released drug from the subcutaneous injection of PNA microgels but this initial burst release was followed by a sustained release for 72 h (Fig. 5). Oral dose of olanzapine also provided an initial rapid absorption but plasma levels were maintained for only 24 h (Fig. 5). PNA microgels can be formulated for short term depot injection, which can potentially provide the release of olanzapine for 72 h. Pharmacokinetic parameters were evaluated for both oral olanzapine and olanzapine loaded PNA microgels as described in Table 2. Half life of the *in situ* gel (8.66 ± 0.18) was higher as compared to oral

Table 1. *In vitro* release kinetics of PNA microgels based olanzapine *in situ* gel formulation.

Model	Zero order		Higuchi		Korsmeyer-Peppas			Hixson-Crowell	
Parameter	K_0	R^2	K_H	R^2	K_{KP}	R^2	n	K_{HC}	R^2
Value	2.269	0.4584	14.057	0.9448	20.027	0.9681	0.397	0.015	0.8401

Table 2. Non-compartmental pharmacokinetic parameters of olanzapine (Mean ± S.D) in rats (n = 6) after oral dose of olanzapine (7 mg/kg) and subcutaneous dose of PNA microgels of olanzapine (7 mg/kg).

Parameter	Unit	Olanzapine (oral)		PNA microgels of olanzapine		p-value	Difference based on p-value
		Mean	± S.D.	Mean	± S.D.		
$t_{1/2}$	h	2.54	0.15	8.66	0.18	1.85E-10	highly significant
T_{max}	h	1	5.09	1.33	0.82	0.36322	non significant
C_{max}	ng/mL	412.7	18.7	226.58	10.08	5.77E-06	highly significant
$AUC_{0\text{-}t}$	ng/mL × h	1956.7	119.2	3522.90	226.90	3.45E-05	highly significant
$AUC_{0\text{-}inf}$	ng/mL × h	1959.5	119.5	3525.20	227.39	3.45E-05	highly significant
$MRT_{0\text{-}inf}$	h	4.6	0.08	16.24	0.26	8.53E-10	highly significant

Where $t_{1/2}$ is half life, T_{max} is time for maximum concentration, C_{max} is maximum plasma concentration, AUC is area under plasma *vs*. time curve, MRT is maximum residence time, h = hours and S.D is standard deviation.

olanzapine (2.54 ± 0.15), which indicates a more sustained release pattern of olanzapine from PNA microgels (22). Mean residence time (MRT) was also higher for PNA microgels when compared with oral olanzapine. Area under the plasma *versus* time curves (AUC) for subcutaneous injection of PNA microgels was higher as compared to oral olanzapine because subcutaneous route of administration provides better bioavailability than oral route (23). When pharmacokinetic parameters were compared statistically, all parameters were having highly significant difference except T_{max} (time for maximum plasma concentration). T_{max} for oral olanzapine and PNA microgels of olanzapine was found with non-significant difference, which indicates that sustained formulation was exhibiting an initial burst release comparable with oral absorption.

CONCLUSIONS

Olanzapine loaded PNA microgels prepared by radial polymerization containing drug loading efficiency of 2.14 ± 0.52% proved fruitful method of delivery as depot injection. The favorable characteristics of viscosity at low pH such as pH 5.0 made this formulation ideal candidate for injectable delivery because it will not turn to rigid gel instead of microgel on room temperature or even at higher temperatures. *In vitro* release was characterized by Fickian diffusion due to high swellability and initial burst release of 38.6% of the total drug loaded within first 2 h of the study. Similar kind of release behavior was shown by *in vivo* subcutaneous injection of PNA microgels that indicated a potential of toxicity if loaded dose is not controlled efficiently. Both *in vitro* and *in vivo* release data indicated initial burst release followed by a steady sustained release for 72 h. Hence, PNA microgels can be formulated for short term depot injection, which can potentially provide the release of olanzapine for 72 h.

REFERENCES

1. Goldner E.M., Hsu L., Waraich P., Somers J.M.: Can. J. Psychiatry 47, 833 (2002).
2. Piccinelli M., Homen F.G.: Gender differences in the epidemiology of affective disorders and schizophrenia, World Health Organization, Geneva 1997.
3. Gilbert P.L., Harris M.J., McAdams L.A., Jeste D.V.: Arch. Gen. Psychiatry 52, 173 (1995).
4. Mary A., Glen L.: in Koda-Kimble and Young's Applied Therapeutics: The Clinical Use of Drugs, Koda-Kimble M.A., Alldredge B.K., Corelli R.L., Ernst M.E. Eds., p. 1921, Lippincott Williams & Wilkins, Philadelphia 2012.
5. Meltzer H.Y.: Psychopharmacology (Berl) 148, 16 (2000).
6. Meltzer H.Y., Huang M.: Prog. Brain Res. 172, 177 (2008).
7. Tollefson G.D., Taylor C.C.: CNS Drug Rev. 6, 303 (2000).
8. van der Zwaal E.M., Luijendijk M., Adan R.A., la Fleur S.E.: Eur. J. Pharmacol. 585, 130 (2008).
9. Rabin C., Liang Y., Ehrlichman R., Budhian A., Metzger K., Majewski-Tiedeken C. et al.: Schizophr. Res. 98, 66 (2008).
10. Kane J.M., Eerdekens M., Lindenmayer J.-P., Keith S.J., Lesem M., Karcher K.: Am. J. Psychiatry 160, 1125 (2003).
11. Khan A.: J. Colloid Interface Sci. 313, 697 (2007).
12. Bradley M., Ramos J., Vincent B.: Langmuir 21, 1209 (2005).
13. Romeo G., Fernandez-Nieves A., Wyss H.M., Acierno D., Weitz D.A.: Adv. Mater. 22, 3441 (2010).
14. Han C.K., Bae Y.H.: Polymer 39, 2809 (1998).
15. Mohanty P.S., Richtering W.: J. Phys. Chem. B 112, 14692 (2008).
16. Xiong W., Gao X., Zhao Y., Xu H., Yang X.: Colloid Surface B 84, 103 (2011).
17. Zhang J.T., Keller T.F., Bhat R., Garipcan B., Jandt K.D.: Acta Biomater. 6, 3890 (2010).
18. Hussain T., Saeed T., Mumtaz A.M., Javaid Z., Abbas K. et al.: Acta Pol. Pharm. Drug Res. 70, 749 (2013)
19. N.I.H.: Guide for the care and use of laboratory animals, National Academies, Washington, D.C. 1985.
20. Costa P., Sousa Lobo J.M.: Eur. J. Pharm. Sci. 13, 123 (2001).
21. Idrees A., Rahman N.U., Javaid Z., Kashif M., Aslam I. et al.: Acta Pol. Pharm. Drug Res. 71, 287 (2014).
22. Vismari L., Pires M.L.N., Benedito-Silva A.A., Calil H.M.: Rev. Bras. Psiquiatr. 24, 74 (2002).
23. Jundt J., Browne B., Fiocco G., Steele A., Mock D.: J. Rheumatol. 20, 1845 (1993).

Received: 1. 12. 2014