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Poly(lactic-co-glycolic acid) - polyethylene glycol copolymer for long-acting injectable: Synthesis, characterization, and *in-vivo* study

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ABSTRACT

Aim: This work was aimed to develop a carrier system of poly(lactic-co-glycolic acid) (PLGA) by using polyethylene glycol (PEG) for longer action in various diseases by preparing a di-block copolymer by using Risperidone (RSP) as a drug. **Methodology:** PLGA-PEG copolymer was synthesized, which can be used to prepare nanoparticles like polymeric micelles (PMs) and gets circulated longer time in body. The preparation of copolymer was then confirmed with 1HNMR. Molecular weight was confirmed by mass spectroscopy. The PMs were prepared by single emulsion method. The PMs were characterized for particle size, zeta potential in vitro release, and in vivo study. The results of experiment show that copolymer can be prepared by ring-opening polymerization. Proton nuclear magnetic resonance (1HNMR) and Fourier-transform infrared spectroscopy were consistent with the structure. **Results:** The entrapment efficiency was around 80%, zeta potential was −0.5 mV, and particle size was found to be 163 nm (±5). RSP release from PMs showed initial burst and then sustained and controlled release. In-vivo studies were done for 7 days in rats and showed extention of 12 folds in half-life of RSP. Thus, PLGA-PEG PMs can be an effective carrier for drug delivery system.

Keywords: Drug delivery, poly(lactic-co-glycolic acid), polyethylene glycol, polymeric micelles, ring-opening polymerization, risperidone

INTRODUCTION

Recently, a strong interest on synthetic biodegradable polymers has inclined researchers which have been proved. Polymeric biomaterials play important roles in biomedical applications, which can be applied to improve *in-vitro* performance and *in-vivo* stability. Till date numerous work has been done to design and develop an unique carrier system but those carrier possessed few biological oriented issues and are easily cleared by circulation and excreted from the body.

In pharmaceutical field, polymers are designed with the advantages of low hydrophilicity and crystallinity to controls its *in-vitro*, *in-vivo* stability, and mainly the degradation rate and poor compatibility. Copolymerization of poly(lactic-co-glycolic acid) (PLGA) is one of the option adopted to achieve the above said advantages. Polyethylene glycol (PEG) incorporation enhance biocompatibility and degradation property. Mostly polymeric micelles (PMs) have been composed of both types of segments that are hydrophobic in inner core and hydrophilic in outer core. The inner core acts as reservoir of drug and outer helps to modulate pharmacokinetic behavior.[1] In the literature, various studies are reported but relatively low drug loading was achieved with PLA-PEG, poly-epsilon-caprolactone – PEG polymeric systems due to limited access for hydrophilic drug into hydrophobic polymer.[1]

Carrier systems based on polymers of lactic acid and glycolic acids are mostly used in the number of biomedical and pharmaceuticals applications. They have made outstanding awareness due to their admirable biocompatibility and physicochemical properties and mechanical strength. For various biomedical applications, PLGA has proven its controlled release in various drug delivery systems due to its biodegradability and biocompatibility. In addition, PLGA-PEG

copolymers have been approved Food and Drug Administration (FDA), are non-toxic after hydrolysis. As reported in earlier studies, PLGA-PEG diblock polymer had shown superior drug loading for various anti-cancer agents like doxorubicin. Moreover, copolymers of PLGA have been used to modify the physicochemical properties of various therapeutics such as triclosan, paclitaxel, ellagic acid, streptomycin, estradiol, cyclosporine, and gentamicin.[2]

When it comes to psychiatric drugs or psychiatric patients, non-adherence to pharmacotherapy leads to decompensation and relapse. Non-adherence results in generally negative outcomes for patients and increases the economic burden of treatment. Relapse and hospitalizations are major factors in the cost of treating schizophrenia.[3] They need long-term psychiatric treatment. One method that is believed to increase patient adherence is the utilization of long-acting injectable (LAI) antipsychotics.[4] Risperidone (RSP) LAI was the first FDA approved atypical LAI agent, introduced in 2004.[5] It is administered through intramuscular (IM) injection and requires oral supplementation for the first 3 weeks.[6] RSP is second-generation antipsychotic that has affinity for D2, 5HT2A, alpha 1, alpha 2, and H1 receptors. RSP reduces both negative and positive symptoms of schizophrenia. To overcome poor solubility and poor bioavailability, RSP has been tried with various drug delivery systems such as microspheres and PMs.[7]

In our study, we try to disperse more RSP into the PLGA-PEG polymer. PLGA 50:50 molar ratio was ring polymerized by PEG in the presence of stannous octoate. The copolymer was synthesized by ring-opening polymerization of PLGA in the presence of PEG using stannous octoate as a catalyst.[8-19] The objective of our experiment was to get long-lasting action of drug from PLGA-PEG pol9meric micelles. Various analytical methods were used to characterize the copolymer and release pattern was also studied. These finding indicate that more relapses result in higher health-care costs and that interventions to prevent relapse could reduce those costs.

MATERIALS AND METHODS

D-lactide and glycolide, PEG molecular weight 6000, and stannous octoate were purchased from Sigma. Poly (vinyl alcohol) was purchased from Aldrich. Dichloromethane (DCM) of high performance liquid chromatography (HPLC) grade was used.

Animals

Female Wistar rats weighed 200–250 g were supplied by National Institute of Bioscience, Pune. The animals were quarantined at temperature 25 ± 2°C and relative humidity of 70 ± 5% under 12:12 light-dark conditions for 1 week.

Synthesis of PLGA-PEG Block Copolymer

DL=lactide and glycolide and PEG in a round bottom flask were heated at 180°C with the catalyst stannous octoate. Molar ratio of DL-lactide and glycolide with PEG was 1:1. Stannous octoate used was in excess amount. The reaction mixture was heated for 2 h. Polymerization was carried out in round bottom flask. The polymer was recovered by mixing it with ice-cold water and then heating it at 80°C. The precipitate was filtered through Whatman filter paper and washed with ice-cold water.[20] The block copolymerization of PLGA-PEG occurred as per shown in Figure 1.

Characterization of Copolymer

^{1}H NMR

^{1}HNMR spectra of PLGA, PEG, and synthesized copolymer were recorded and analyzed using Bruker AM 400 MHz spectrometer. Samples were liquefied in dimethyl sulfoxide (DMSO) to procure NMR spectra.

Fourier-transform infrared (FTIR) study

Fourier transform spectra were obtained by (Jasco 4100) mixing of KBr and Copolymer in 9:1 ratio through mortar and pestle and the FTIR spectra were analyzed in the wavelength from 4000 to 400.

MALDI TOF mass spectroscopy

The molecular weight of block copolymer was determined by MALDI TOF mass spectroscopy with LC-mass spectrometry (MS)-MS series 6460 at the wavelength of 280 nm using pump 2080 PLUS 2087 by dissolving the substance in DMSO solvent.

Preparation of RSP-loaded PMs

The RSP-loaded PLGA-PEG PMs were synthesized by emulsification process. Seven milligram RSP and 50 mg PLGA-PEG dissolved in 5 ml of DCM and ultrasonicated for 1 min on ice to form oil phase emulsification. Twenty-milliliter PVA was then added to the oil phase and ultrasonicated for 1 min to form water phase emulsification. Then, this water phase emulsion was added to 100 ml water and stirred for 6 h to evaporate DCM and PMs were hardened. Then, PMs harvested by centrifugation (Allegra TM 64R centrifuge, BECKMAN COULTER) at 12,000 rpm for 20 min and washed 3 times and then lyophilized.

Characterization of RSP-loaded PLGA-PEG PMs

Determination of encapsulation efficiency

RSP-loaded PLGA-PEG PMs were centrifuged 15,000 rpm and supernatant was collected. This is then diluted with phosphate buffer saline (PBS) 7.4 and analyzed with ultraviolet spectrophotometer at the wavelength of 280 nm. The amount of RSP encapsulated within PMs was calculated by the

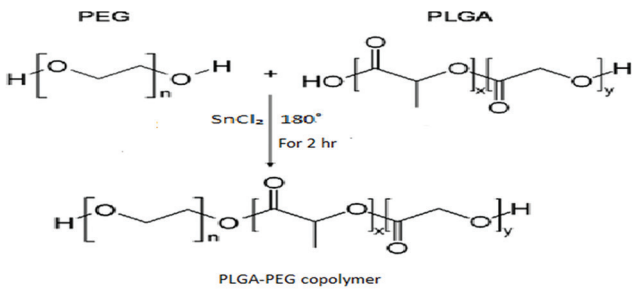

Figure 1: Copolymerization of poly (lactic-co-glycolic) acid - polyethylene glycol block copolymer

calculating difference between total amount added while preparation and amount of drug present in the supernatant.

$$\text{Encapsulation efficiency } (\%) = \frac{\text{Amount of drug entrapped in the micelles}}{\text{Total amountof initial drug}} \times 100$$

Morphological Characterization of PMs

Transmission electron microscopy (TEM) analysis

The surface morphology study of PMs was carried out by TEM (Philips CM 200). PMs was first applied on the carbon-coated copper grid and allowed to dry. The excess of sample was wiped out by Whatman filter paper. After that, the copper grid was stained with phosphotungstic acid (2%w/v) and allowed it to dry for 30 s and then images were captured.

Particle size analysis and zeta potential

Particle size and polydispersity index were measured by laser diffraction technique. (Malvern particle analyzer 2000 SM version 5.22, Malvern Instruments Corp., U.K). Zeta potential was measured by Lesser Doppler Electrophoretic Mobility (Zetasizer).

FTIR

The FTIR of RSP and RSP-loaded PLGA-PEG PMs was obtained by mixing it with KBr (9:1 ratio) to form pellets and then examined it in the wavelength range of 4000–400 cm^{-1}.

Differential scanning calorimetry (DSC) thermogram

DSC spectra of RSP, PLGA, PEG, and RSP-loaded PMs were analyzed using DSC-1 STARe System (Mettler Toledo, USA). Samples were weighed accurately and then placed on hot aluminum pan in a temperature range of 20–250°C.

In vitro Release Study

To study the release of drug from synthesized, RSP-loaded PLGA-PEG copolymer PMs for pH 7.5 formulation was kept in cellophane dialysis bag and compared with aqueous solution of RSP, RSP-loaded PLGA PMs, then the bag was allowed to float in pH 7.5 buffer solution in beaker. Then, the samples were removed at fixed intervals for 7 days. The time intervals were 15 min, 3 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 42 h, 72 h, 96 h, 120 h, 144 h, and 164 h. The sink condition was maintained throughout the study with freshly prepared PBS pH 7.4 by changing the media every day.

In vivo Study

The hypothetical studies were approved by Institutional Animal Ethics Committee (registered with CPCSEA/PCP/PCT 04/2017-18) of Poona College of Pharmacy Pune. Research plan was composed according to the guidelines of the committee for the purpose of control and supervision of experiment of animals (CPCSEA).

Healthy female Wistar rats weighing between 200 and 250 g (National Institute of Bioscience Pune, India) were used for pharmacokinetic study. Animals were housed together under standard conditions of relative humidity (55 ± 10%), temperature 24 ± 1°C, and 12:12 h duration light/dark cycles throughout the experiment. Animals had free access to commercial available standard pellet diet (containing 22.15% protein, 4% carbohydrate, 4.15% fat 1.8% vitamins, and 2.46% glucose) and filtered water. Animas were adapted before the initiation of the experiment and health status of animals was monitored daily during the acclimatization period.

To get the plasma time profile of aqueous solutions RSP, RSP-loaded PLGA micelles and RSP-loaded PLGA-PEG micelles, three groups were used (n = 6). Each animal was administered a preparation containing 1.5 mg/kg of RSP.

After the administration, blood samples were collected at fixed time intervals 0.25, 0.5, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 42 h, 72 h, 96 h, 120 h, 144 h, and 164 h in EDTA tubes. To 200 µl of plasma sample, 100 µl acetonitrile is centrifuged at 8000 rpm and then collected the supernatant and diluted to 1000 µl mobile phase from which 20 µl sample was injected to HPLC system.

Brain Tissue Distribution

To study, the tissue distribution animals were divided in three groups. They were treated with Group 1 aqueous solution of RSP, Group 2 RSP-loaded PLGA micelles, and Group 3 RSP-loaded PLGA-PEG PMs. After the administration rats were sacrificed and tissue was collected on day 3 and 7.

RESULTS

Characterization of Copolymer

^{1}HNMR

The chemical structure of PLGA-PEG copolymer was confirmed by ^{1}HNMR. The most prominent feature peak of methylene group of PEG was found to be at 3.4ppm. Conformed peaks of lactic acid and glycolic acid from PLGA were found to be at 2.5 ppm which shows the complexity of peaks from D-lactide, L-lactide, and glycolic acid in the polymer backbone. The overlapping of doublets between 1 ppm to 2 ppm denoted the formation of longer chain polymer as shown in Figure 2.

FTIR

The characteristic peaks of both polymers were appeared in the FTIR spectrum [Figure 3],those are 2948.8 cm^{-1} of CH and CH3, 1757.8 cm^{-1} of C=O stretching. 1468 cm^{-1} of binding vibrations of – CH2 and 1144 cm^{-1} of C-O ester stretching. At 3509 cm^1 shows terminal hydroxyl group of PEG which had been removed. At 3660 cm^{-1} OH stretch has been shifted because of hydrogen bonding, at 2972 cm^{-1} methylene group deformation of PEG as interpreted in Figure 3.

Mass Spectroscopy

In mass spectroscopy peaks at 116,201.9 and 341 was found which denotes the higher molecular weight of copolymer and lower molecular weight of copolymer. The strong peak denotes copolymer which molecular weights of the copolymer at 341 the peak as designated in Figure 4.

Morphological Characterization

To study, the morphological characterization PMs were prepared by single emulsion method. Size and shape of

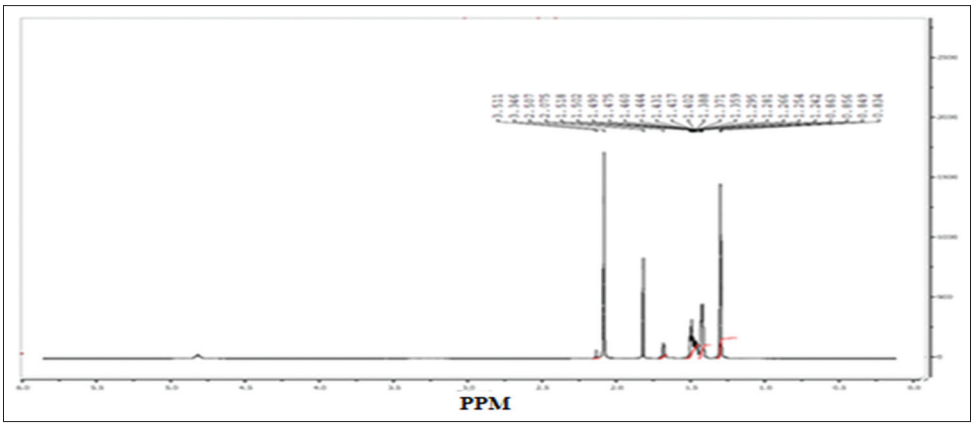

Figure 2: Nuclear magnetic resonance spectra of poly(lactic-co-glycolic acid) - polyethylene glycol block copolymer

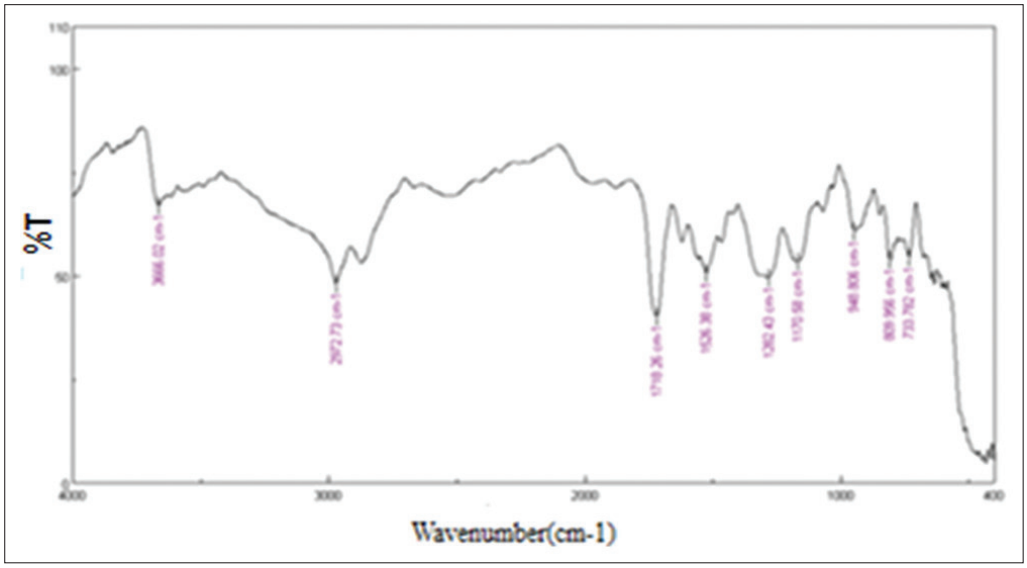

Figure 3: Fourier-transform infrared spectra of poly(lactic-co-glycolic acid) - polyethylene glycol block copolymer

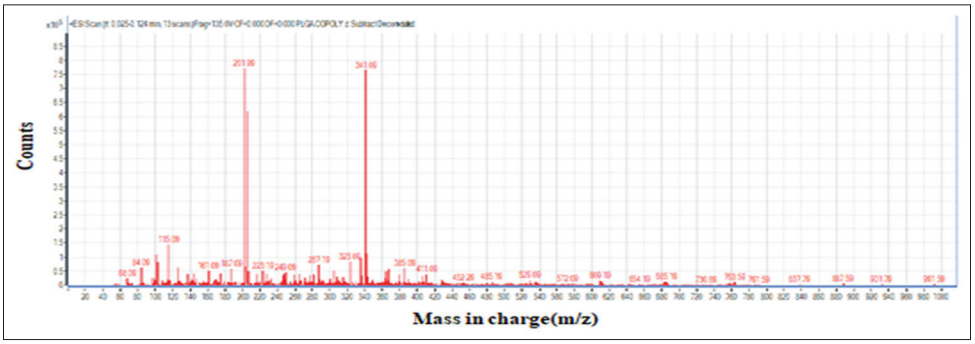

Figure 4: Mass spectra of poly(lactic-co-glycolic acid) - polyethylene glycol block copolymer

PMs were analyzed by TEM. The images of TEM showed spherically shaped and uniform size within 200 nm as labeled in Figure 5.

Encapsulation efficiency was calculated for RSP-loaded PLGA-PEG PMs which was found to be 83.5%. Particle size of PMs was achieved 163 nm by Malvern as shown in Figure 6. The zeta potential of PMs was found to −0.5(±0.1) mV shown in Figure 7.

FTIR of Formulation

FTIR studies gave characteristic peaks of PLGA-PEG copolymer. Weak band of C-H of aromatic ring was present at 2943.8 cm^{-1} 2948.8 cm^{-1} of C-H and C-H3 peaks were observed. 1757.8 cm^{-1} of C=O stretching was observed 1468 cm^{-1} of binding vibrations of $–CH_2$ and 1144 cm^{-1} of C-O ester stretching as depicted in Figure 8.

DSC Thermogram

DSC thermogram studies were done with pure RSP, PLGA, PEG, and lyophilized formulation and the peaks revealed endothermic peak at 60°C of PEG as it is crystalline in nature, at 170°C of drug because of crystallinity.

At 70°C of PLGA did not have sharp endothermic peaks as PLGA is amorphous in nature and PMs showed peak at 170°C because of the amorphous nature of formulation.

The shifting of melting endotherm of formulation, i.e., PLGA-PEG PMs strongly suggested that RSP was dispersed in PMs as represented in Figure 9.

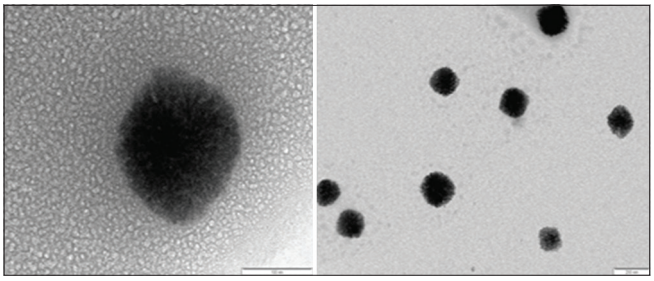

Figure 5: Transmission electron microscope analysis of polymeric micelles

In vitro Release Study

The *in-vitro* release studies were compared with aqueous solution of RSP, RSP-loaded PLGA micelles, and RSP-loaded PLGA-PEG micelles [Figure 10]. The release profiles showed 83% consistent release of the formulation in 7 days which was because of the PEG content of the copolymer. The chains of PEG adjust itself toward exterior which is an aqueous phase in micelles that is why they surround the micelles by encapsulating it. This coating of PEG works as wall to decrease the interaction of drug molecules by steric repulsion which gives enhances stability.

This *in-vitro* study the release occurred in two phases the first one was initial burst and second was sustained release. In first 24 hours formulation showed initial burst, significant amount of drug was released from formulation and after 24 hours the release of drug got sustained. The amount of drug released from formulation was nearly 83% in 7 days, whereas the drug loaded PLGA micelles showed 94% of release within 4 days only.

In vivo Study

The plasma time profile of RSP-loaded PLGA-PEG PMs after intramuscular injection was shown in Figure 10. PLGA-PEG PMs gave initial burst in blood circulation when compared

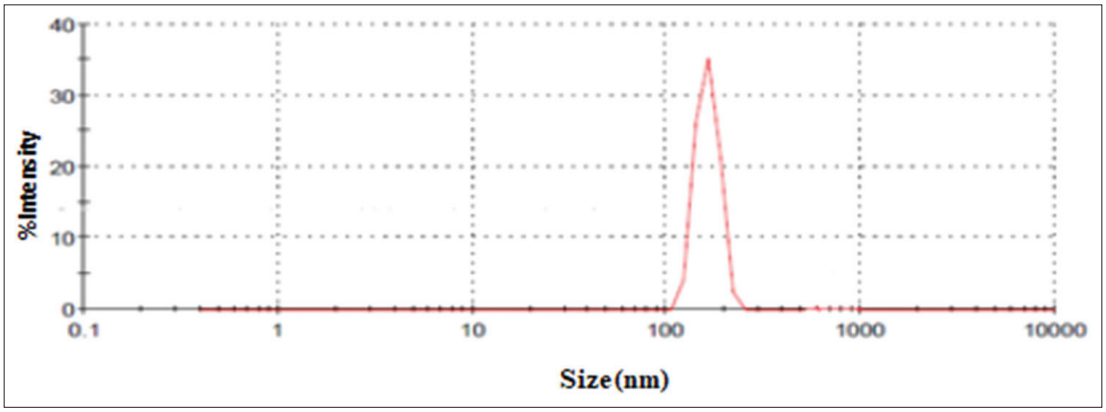

Figure 6: Particle size of polymeric micelles

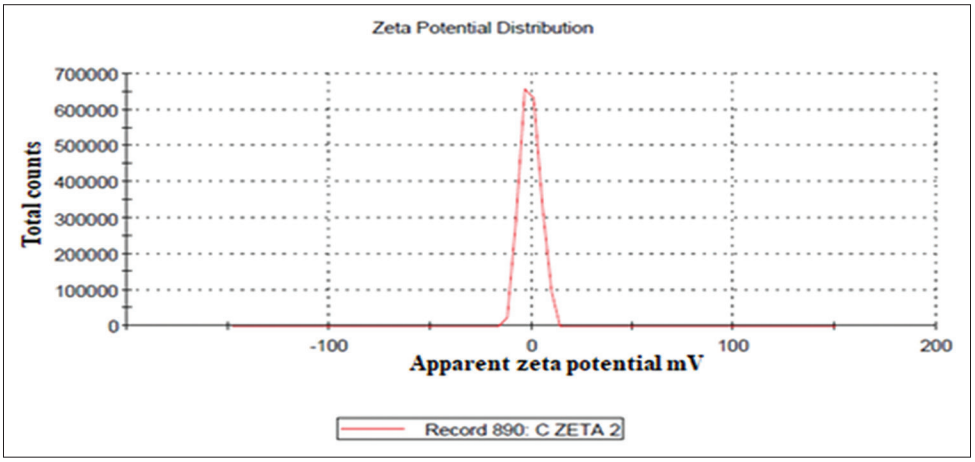

Figure 7: Zeta potential of polymeric micelles

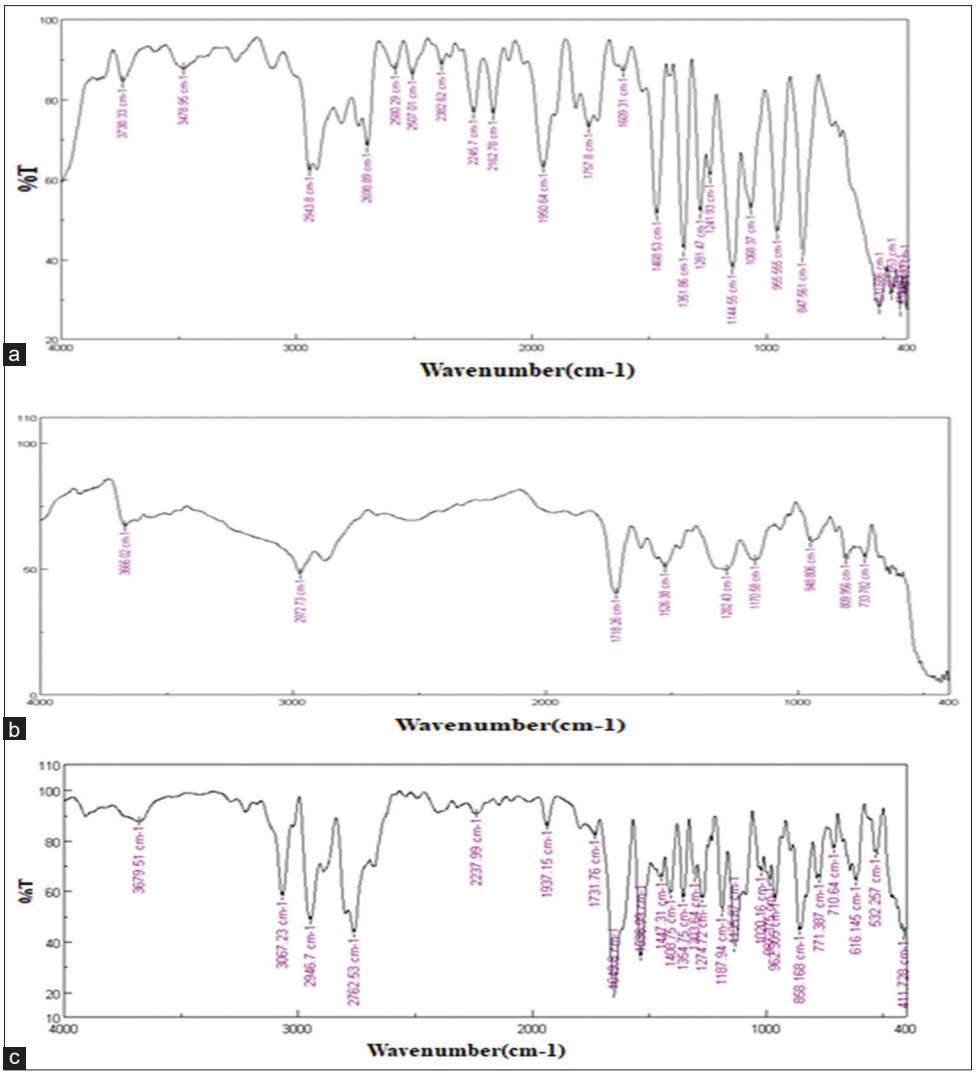

Figure 8: Fourier-transform infrared of (a) polymeric micelles, (b) copolymer, and (c) risperidone

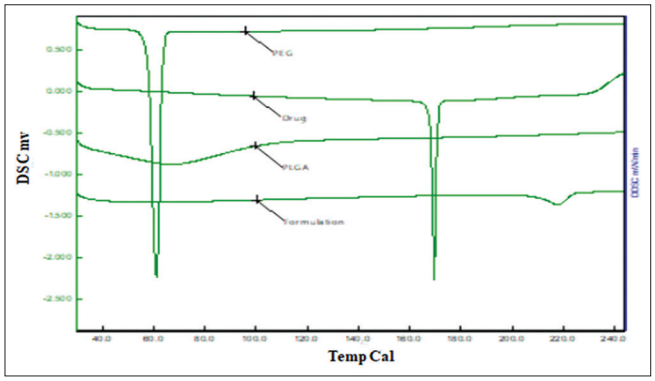

Figure 9: Differential scanning calorimetry thermogram

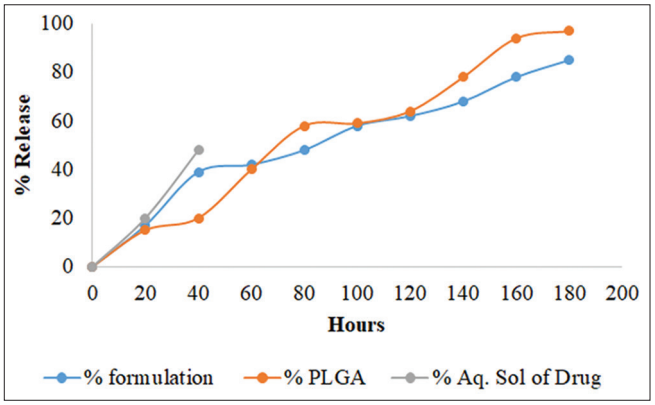

Figure 10: *In vitro* drug release of formulation, blank micelles/poly(lactic-co-glycolic acid) and %Aq. Sol of drug

with RSP-loaded PLGA PMs. 11-fold increased bioavailability showed by RSP-loaded PLGA-PEG PMs which show highest concentration of RSP in blood as compared to aqueous solution of RSP and RSP-loaded PLGA micelles (5.4 fold). The PMs with only PLG were removed from the blood in 4 days; on the contrary, the PLGA-PEG micelles remained in the blood for 7 days.

The rate of elimination of RSP-loaded PLGA-PEG PMs showed remarkable decrease with 18.17 fold. T_{max} of RSP-loaded PLGA-PEG PMs (72 h) showed difference when compared to aqueous solution of RSP (4 h) and RSP-loaded PLGA micelles (48 h). It could be seen that RSP-loaded PLGA-PEG PMs were

Table 1: Pharmacokinetic parameters

Parameters	Aq. solution of drug	PM's	Poly (lactic-co-glycolic acid) micelles
C_{max} (μg/ml)	49.46076	323.0182	269.23452
T_{max} (hour)	4	72	48
AUC_{0-t} (hr*ug/ml)	608.68	6943.01	1985.13347
Ke (hr^{-1})	0.064767934	0.010308085	0.015181948
MRT (hour)	16.42386105	92.50872389	66.80135536
$T_{1/2}$ (hour)	3.70201162	67.24306133	45.65601218

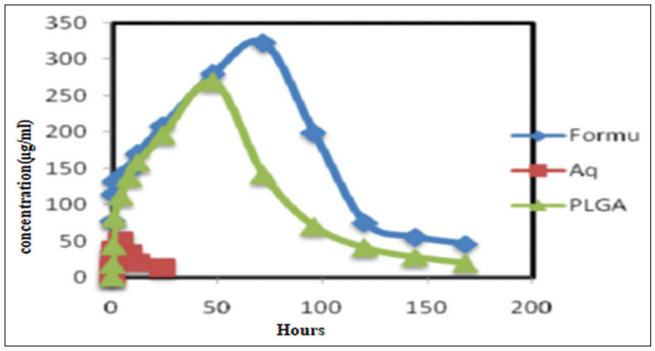

Figure 11: *In vivo* release study of polymeric micelles

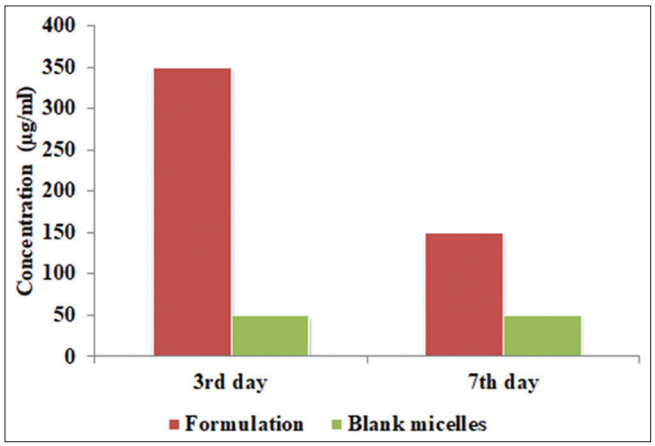

Figure 12: Tissue biodistribution of formulation and blank micelles

remained in bloodstream for 7 days compared with RSP-loaded PLGA micelles and aqueous solution of RSP [Table 1].

When RSP-loaded PLGA-PEG PMs administered intramuscularly, it will get deposited in gluteal muscles. Then, they will get metabolized by CP2D6S and CYP3A4 receptors. After metabolism active metabolites of RSP will be formed which are 9-hydroxy RSP. Then, the absorption of drug molecule will be taken by p-glycoproteins and will reach to systemic circulation.[21-23]

The Brain Distribution

The brain distribution profiles of RSP-loaded PLGA-PEG PMs, RSP-loaded PLGA micelles, and aqueous solution of RSP was studied after intramuscular administration. On the day 3 and 7, RSP-loaded PLGA micelles and aqueous solution showed negligible concentration in brain tissues whereas RSP-loaded PLGA-PEG PMs showed significant amount of RSP in brain tissue as interpreted in Figure 12. RSP-loaded PMs were eliminated gradually from brain tissues. RSP-loaded PLGA-PEG micelles were transported from bold to brain tissue with differential amount and profile as in PLGA micelles. The plasma levels of PLGA-PEG micelles were prominently higher than the PLGA micelles at same time point.

DISCUSSION

In the paper, PLGA copolymer was synthesized with PEG. As PLGA is very successfully used as a biodegradable polymer, however PLGA alone gives less stability issues so it gives problem in controlled release applications of DDS. So we synthesized PLGA-PEG copolymer by ring-opening polymerization which is di-block copolymer composed of one block of PLGA and other block of PEG. The ^{1}HNMR and FTIR were dependable with the structure of PLGA-PEG copolymer. The molecular weight was determined by mass spectroscopy.

Biodegradable polymers have a problem associated with stability, which is most important in controlled release application for DDS. When a carrier system has been developed, it should give controlled release. Controlled release is mostly dependent on the stability of the carrier system. The carrier system should be able to deliver its content with appropriate duration, bio-distribution, and concentration for therapeutic effect.

Attaching PEG to a drug or polymer is called PEGylation, it is known for increased circulating time in body. This important property can be useful in the concluding the degradation of polymer.

When PEG is attached with PLGA, PEG chains adjust themselves toward the external aqueous phase in micelles by giving it a surrounded encapsulated species. PEG acts a barrier and decreases interactions with drug molecules.[24]

Our study shows a strong and novel example of *in vitro* and *in vivo* that the antipsychotic effect of drug can be improved by its delivery by dispersing in PLGA-PEG copolymer. Our results show that development of novel drug delivery strategies that improvise the delivery of drug to tissue. Our study of *in-vivo* release shows that addition of PEG to PLGA can considerably improve the delivery to the brain tissues.

When two PMs were compared with release both showed initial release, but PLGA-PEG PMs showed sustained release for 7 days whereas PLGA micelles showed 4 day release. The presence of PEG chain can differentiate the release profiles.

Our experiment showed that playing with tow polymer can increase the biological half-life and change the drug

distribution in rats. Formation of copolymer can improve circulatory half-life of drug and can be a potential carrier system for DDS.

CONCLUSION

By this experiment, PLGA-PEG copolymer can be synthesized by ring-opening polymerization of PLGA with PEG. ^{1}HNMR and FTIR showed exact results with respect to structure of the copolymer. RSP-loaded PLGA-PEG PMs were prepared by single emulsion method. The entrapment efficiency was 83% and particle size was 163 nm. The release profiles showed initial burst and then sustained controlled release up to 7 days. By this experiment, we showed that PLGA-PEG PMs can be a good carrier system.

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