Preparation and Characterization of Biodegradable Microspheres Containing Sertraline Hydrochloride

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Abstract

Four batches of sertraline HCl microspheres were prepared using a poly (D-L-lactide-co-glycolide) (PLGA) polymer (Mw. 9, 27, 30 and 83 KDa) as a delivery system. The microspheres were prepared by a dispersion/solvent extraction-evaporation method and characterized for drug loading by UV, particle size by laser diffractometry and surface morphology by scanning electron microscopy (SEM). The in vitro sertraline HCl release was studied. Spherical microspheres with a mean diameter of 21 to 26 µm loaded with 24.6 – 38.2% were produced. The in vitro drug release was shown to be depend on polymer molecular weight and also on the drug loading. Differential scanning calorimetry (DSC) was employed to investigate the physical state of sertraline HCl inside the microspheres and stability and polymer interaction study were performed in solution.

Key words: Sertraline HCl , Microspheres , PLGA polymer

الخلاصة

اربعة صيغ من الكرات المجهرية للسيرترالين هايدروكلورايد (Sertraline HCl) حضرت بأستخدام بوليمر متعدد احامض اللبن والكلايكولك(PLGA) (الوزن الجزيئي 9, 27, 30 و 83 كيلو دالتون) كنظام لايصال الدواء .الكرات المجهرية حضرت بطريقة الانتشار/ استخلاص المذيب -تبخير ,ووصفت من حيث :كمية التحميل للدواء بواسطة الاشعة فوق البنفسجية ,حجم الكرات بواسطة جهاز مشتت الليزر(Laser diffractometry) ,والشكل السطحي بواسطة المسح بالمجهر الالكتروني (SEM) . تم دراسة التحرر للسيرترالين هايدروكلورايد خارج الجسم .كرات مجهرية ذو معدل قطر 21- 26 µm محملة بنسبة 24.6- 38.2 %قد انتجت . التحرر خارج الجسم للدواء قد اظهر انه يعتمد على الوزن الجزيئي للبوليمر وكذلك على كمية تحميل الدواء . تحليل المسح التفريقي للسعرات DSC) قد استخدم للتقصي عن الحالة القيزياوية للسيرترالين هايدروكلورايد داخل الكرات المجهرية ,كذلك تم دراسة الاستقرارية وتفاعل البوليمر داخل المحلول .

Introduction

Sertraline HCl is the second most potent inhibitor of serotonin reuptake and the second most selective blocker of serotonin over noradrenaline uptake. It has been approved in 1997 in France, and is currently widely prescribed in Europe and the United states[1]. It has been also used for the treatment of depression, obsessive-compulsive disorder (OCD), depression relapse and social phobia[2,3]. It is the only selective serotonin reuptake inhibitor (SSRI) that binds to dopamine transporters[4]. Sertraline HCl exhibits linear pharmacokinetics[5]. After single doses between 50 and 200 mg, t1/2 is similar for single dose and steady-state conditions[6]. The elimination rate constant is higher in young males than in females or subjects 65 years old or older[7]. The hepatic metabolism is the most important pathway, with only 0.2% of an oral dose being excreted unchanged in the urine[8]. The N-demethylation is the main metabolic step in the biotransformation of sertraline[9]. Drug absorption from the GIT is slow, but complete with maximum plasma concentrations (Cmax) attained within 6-8 hr and compared to other SSRIs, a relevant portion of oral sertraline is excreted in the feces (~50%) [10]. Increasing evidence from randomized controlled trials of SSRIS show their efficacy in treating pediatric depression. The number of prescriptions for sertraline HCl use in pediatric populations has exploded recently with figures ranging from 600,000 children and adolescents[11 -13].

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Received : 30 /4/2008
Accepted : 28/6/2008

The oral administration of this drug to children is hard to control compared to adults. In addition, to frequent administration, chances of drug misuse following oral administration are high. This necessitates administration of the drug via a different route. Therefore, there is a strong need for a non-oral controlled delivery dosage form for this drug. This paper investigates the feasibility of formulating sertraline HCl into biodegradable microspheres using PLGA polymer to be used as injectable dosage form. In addition, drug stability and drug-polymer interactions were studied. Finally, in vitro release efficacy from the formulations was also assessed.

Experimental

Materials

Poly(D,L-Lactic-co-glycolic acid) (PLGA); Resomer® 502H (9 kD), 503 H (27 kD), 503 (30 kD), PLGA 50:50 and 6535DL (83 kD) PLGA 65:35 ;were supplied by Boehringer Ingelheim (Ingelheim, Germany). Polyvinyl alcohol (M.wt. 30000-70000; PVA) and sertraline HCl were supplied by Sigma (St. Louis, MO, USA). All other chemicals were obtained commercially as analytical grade reagents.

Preparation of Microspheres

Four batches of sertraline HCl microspheres (MS) were formulated with polymers at increasing molecular weight. The microspheres were prepared by dispersing the homogenous suspension of polymer and drug into a 0.35% PVA solution continuous phase followed by solvent extraction / evaporation /dilution as already described[14]. In detail sertraline HCl was sieved through a 150 mesh sieve and amount corresponding to 25, 40 and 45% loading of the sieved powder was dispersed in methylene chloride and properly sonicated. The polymer in the proportion of 12% was then added to the resulting suspension and after its complete dissolution the suspension was slowly injected into phosphate buffer saline (pH 8.9) containing 0.35% poly vinyl alcohol (PVA) and mixed at 900 rpm. at 4°C. The microspheres hardening and complete evaporation of the solvent were accomplished increasing slowly the temperature up to 20°C in on hour. At the end of the process the microspheres have been recovered by filtration through a 0.65µm harvesting filter and freeze-dried overnight .Table 1 outlines the preparation parameters for sertraline HCl microspheres. The s/o/w method is represented in figure 1.

Polymer	M.W. (kDa)	Method	pH of CP	Target Load % w/w	Drug Content % w/w	Encapsulation Efficiency (%)	Particle size (µ)
503H	27	s/o/w	8.8	45	38.2	84.9	19.6
502H	9	s/o/w	8.8	25	24.6	98.4	21.0
503	30	s/o/w	8.8	40	34.3	85.8	26.0
6535DL	83	s/o/w	8.8	40	34.5	86.3	24.0

Table 1 - Preparation parameters and particle size of Sertraline HCl MS.

Particle Characterization:-

Particle Size Distribution

The prepared particles were sized by laser diffractometry using a Malvern 2600 laser sizer (Malvern 2600 particle sizer, Malvern, UK). The average particle size was expressed as the volume mean diameter Vmd in microns (µ).

Surface Morphology

Surface morphology was examined by scanning electron microscopy (SEM) (Hitachi Model S800 Japan) after palladium/gold coating of the microspheres sample on an aluminum stub.

Drug Content

10 mg of microspheres were dissolved in dimethyl sulfoxide (DMSO). The Sertraline HCl was extracted, since polymer and drug were soluble in DMSO. In detail, triplicate samples of 10 mg of the microspheres were quantitatively transferred to 12 ml glass test tube. The microspheres was solubilized in 2 ml

of DMSO, then 10 ml of 0.1M acetate buffer a wrist action shaker for 1 hr. The sample were centrifuged at 3000 rpm and the aqueous layer was analyzed spectrophotomery at 273 nm. Absorbance measurements were made at a pH 5 was added and the tubes were agitated by selected wavelength (λ max = 273 nm).Absorbance measured values were fitted against a calibration curve based on a Lambert–Beer law[15].

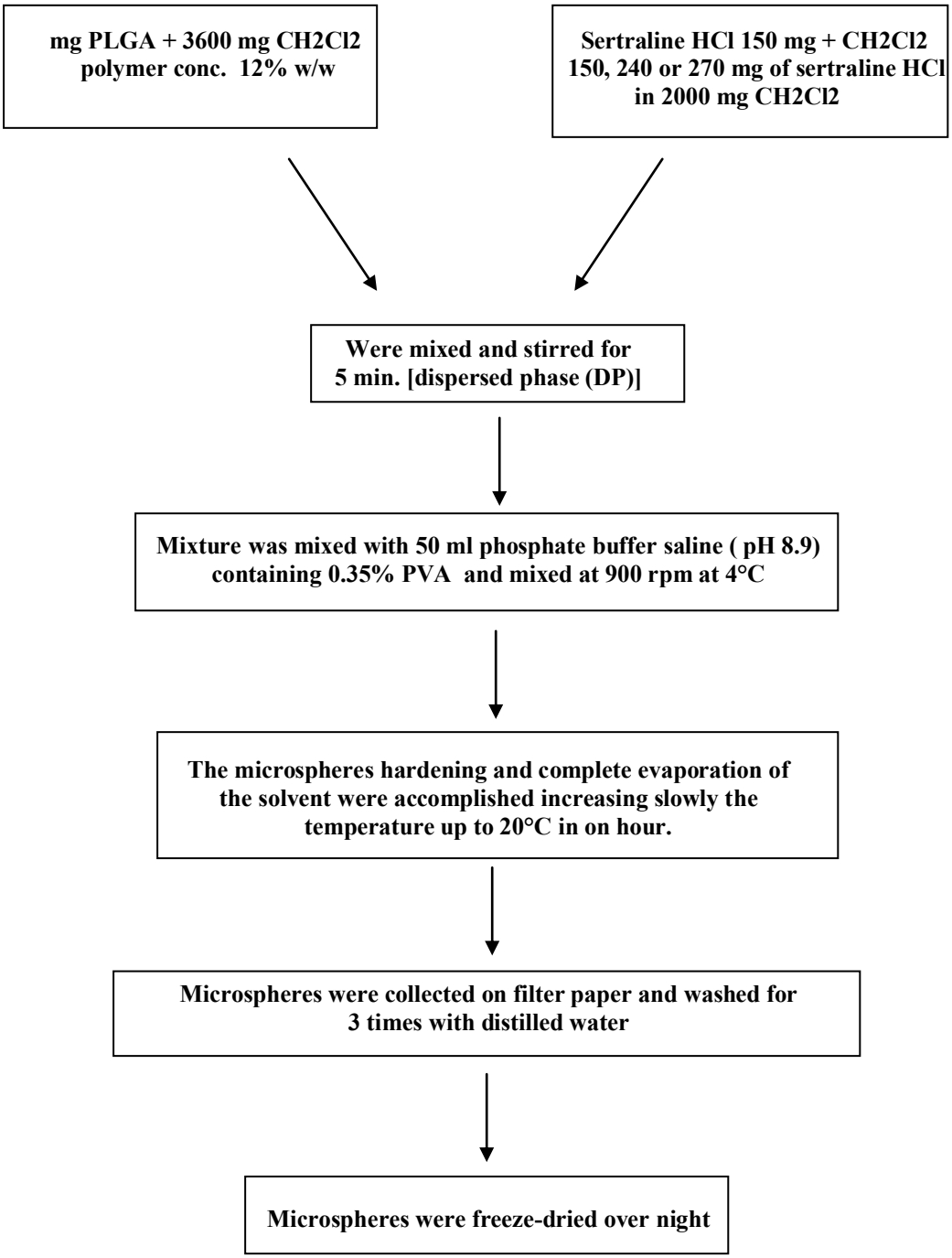


Figure 1: Preparation of PLGA Sertraline hydrochloride microspheres using dispersion/solvent extraction-evaporation method.

DSC Analysis

Sertraline HCl thermotropic behavior inside the microspheres was investigated by a DSC 2920, DE differential scanning calorimeter. Samples of sertraline loaded microspheres and blank microspheres were scanned at 5 °C/min heating rate in the range –10°C to 300°C. In addition, DSC scans were run on the drug, polymers and physical mixtures of the drug with polymers used in the preparation of microspheres. Further measurements were carried out on drug powder after suspension and sonication for 20 seconds in dichloromethane, then evaporation of dichloromethane and on the drug, post-sieving, to detect any structural modification due to the preparation process. All the samples were freeze-dried dried over night before the analysis[16].

Drug-polymer Interaction

Drug-polymer interaction studies were carried out in solutions containing lactic acid, glycolic acid and with mixtures of lactic acid and glycolic acid (50:50 LA : GA) at 37°C. Sampling was performed periodically (5, 10, 20, 30, 40 and 50 days) followed by UV analysis at 273 nm. All analysis were performed in duplicate[17].

In Vitro Drug Release Study

Long-term (48 days) in vitro drug release was carried out in 0.1M acetate buffer, pH 5 at 37 °C. The pH of this buffer is close to that of an acidic microenvironment that form within the PLGA matrix[18]. Briefly, 10 mg of microspheres were suspended in 10 mL of the buffer. At each time point(1, 3 , 8, 10, 15, 20, 27, , 34, 41, and 48 days) 1 mL of supernatant was withdrawn from each tube after centrifugation (2min, 3000 rpm) and an equivalent volume of fresh buffer was then added to replace the amount collected. Analyses were carried out using UV spectrophotometry at 273 nm on triplicate or duplicate samples.

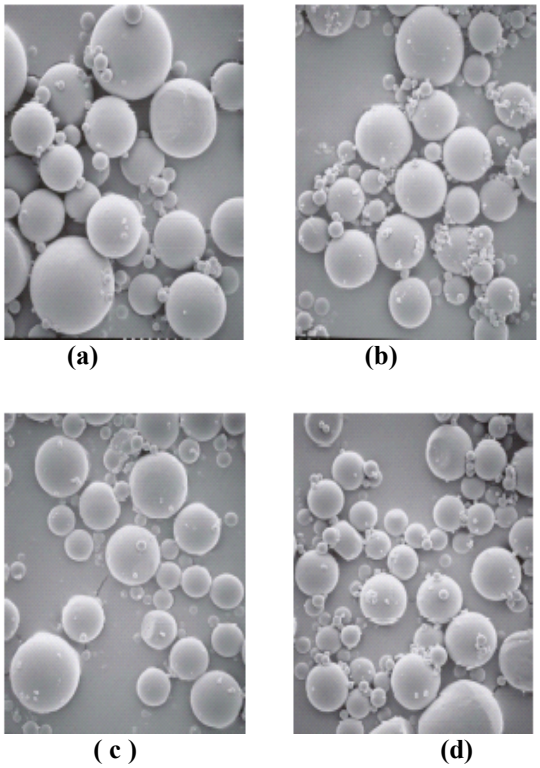
Results and Discussion

Preparation of Microspheres

Preparation of sertraline HCl loaded microspheres was accomplished by the s/o/w method already described in the experimental section. The reason for choosing such procedure is the low solubility shown by the drug into most of the solvents commonly used in microsphere formulation. Various preparation conditions and materials were investigated in order to obtain the best results concerning loading and drug release. Microspheres morphology and size distribution and in vitro release behavior to test the feasibility of sertraline formulation. The results shown in Table 1 reveal the remarkable encapsulation efficiencies (84.9% - 98.4%). A critical step at this point was the complete drug dispersion that is fundamental to have a uniform distribution of the drug inside the microspheres and higher encapsulation efficiency. PLGA polymers were employed with increasing molecular weight (9-83 kDa.) and different glycolic/lactic ratio (50:50 and 35:65) in order to investigate the effect of these parameters on the release behavior of such formulations. The best batches resulted PLGA based preparations and especially microspheres with PLGA Resomer 503H and 502H polymers showed the best results in term of encapsulation efficiency and drug content.

Microspheres Characterization

SEM analysis on sertraline HCl microspheres showed that the microspheres were successfully fabricated with a spherical shape, a certain fragility and relatively low porosity (figure 2). The average particle size was approximately 22µm which is suitable for intramuscular or subcutaneous injections[19].



(a) **(b)**

(c) **(d)**

Figure 2: SEMs of Sertraline hydrochloride loaded microspheres.
503 (a), 502H (b), 6535DL (c) , 503H (d)

Drug Content and Encapsulation Efficiency

Dispersion /solvent extraction-evaporation method has been used succefully in the incorporation of hydrophobic drugs with good yield value loading percentage[20]. Loading

efficiencies ranged from 84.9-98.4 as illustrated in table 1. Yield value are function of the efficiency of preparation method and values up to 70% were accepted[21]. Sertraline HCl , being a water insoluble molecule is better dispersed in organic solvent then emulsified in aqueous solution of the surfactant where minimum amount of the drug would be in the aqueous continous phase[22]. The loading efficiency of 502H microspheres was the highest among other batches(table 1). This result may be due to its lowest target load (25%), since a higher target load of bioactive material is likely to decrease the entrapment efficiency of drug in PLGA[23-25]. The drug content ranged from 24.6-38.2 % .

Drug-polymer Interaction

There was no detectable decrease in sertraline HCl concentration in 0.1M acetate buffer, pH 5.0 for the entire duration of study (50 days) at temperatures 37 °C. There is no significant change in drug levels when incubated with solutions of lactic acid, glycolic acid and a 50:50 mixture corresponding to the molar amount that would be obtained on complete hydrolysis of the PLGA polymers , 502H and 503H.

The DSC Analysis

The DSC analysis confirmed a high drug-polymer affinity. The comparison of thermal profiles of drug, polymer, physical mixture and drug loaded microspheres revealed that the drug was present as a dispersion in the polymeric matrix for all the microsphere batches as demonstrated by the lack of sertraline HCl melting peaks **(Figure 3 a-d)**. Differences in glass transition temperature (Tg) between drug loaded microspheres and raw polymer suggest that the drug has a plasticizing effect on the internal structure of the polymer[26]. The drop in the Tg was greater for microspheres prepared from high molecular weight polymers. Tg values of all the systems studied are shown in Table 2.

Table 2 : The Tg of the Sertraline bpowder, microsphere and the physical mixture of sertralin with polymer.

RG503H	**Tg (°C)**	**RG502H**	**Tg (°C)**
Sertraline-503H Phys. mix.	43.09	Sertraline-502H phys. mix.	34.90
Pure polymer	45.60	Pure polymer	33.62
503H MS	37.79	502H MS	32.27
RG503	**Tg (°C)**	**6535DL**	**Tg (°C)**
Sertraline-503 phys. mix.	46.25	Sertraline-6535 DL phys. Mix.	43.01
Pure polymer	47.05	Pure polymer	46.25
503 MS	30.15	6535 DL MS	33.80

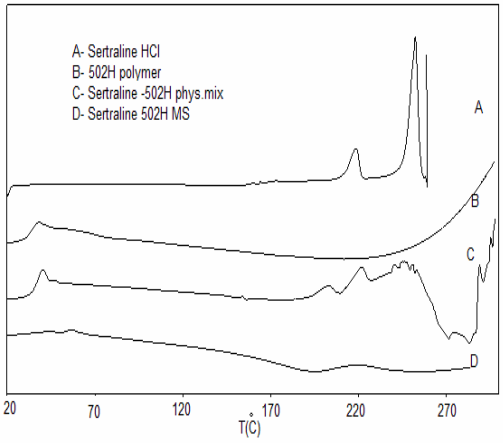


Figure 3a : DSC scan of Sertaline HCl, Sertaline HCl-502H polymer physical mixture, 502H polymer and Sertaline HCl 502H microspheres.

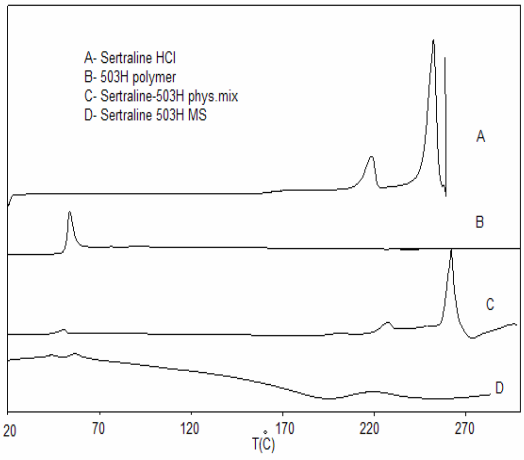


Figure 3b : DSC scan of Sertaline HCl, Sertaline HCl-503H polymer physical mixture, 503H polymer and Sertaline HCl 50 microspheres.

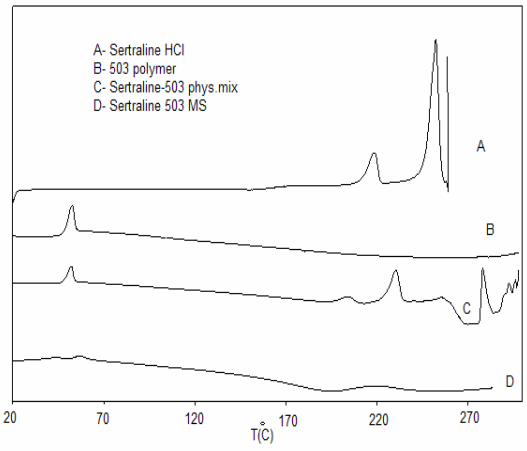


Figure 3c : DSC scan of Sertaline HCl, Sertaline HCl-503 polymer physical mixture, 503 polymer and Sertaline HCl microspheres.

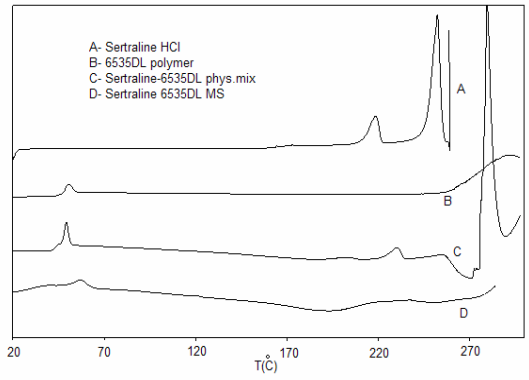


Figure 3d : DSC scan of Sertaline HCl, Sertaline HCl-6535DL polymer physical mixture, 6535DL polymer and Sertaline HCl 6535DL microspheres.

In Vitro Drug Release

A pathway for sertraline HCl release was provided by microsphere degradation where water-soluble degradation products (i.e. monomers and oligomers) leave the microspheres matrix for the surrounding aqueous medium. Since oligomers are close to the surface they can leach out faster than that located deeper within the matrix, carboxylic acid oligomers trapped within the matrix autocatalyze further ester bond hydrolysis, resulting in the increasing rate of mass loss[27]. Four batches of microspheres were subjected to long-term in vitro release (48 days) at 37°C in 0.1M acetate buffer, pH 5.0. The data in figure (4) showed complete sertraline HCl release from 503H and 502H microspheres throughout 35 days with no significant variation between them($P< 0.01$). On the other hand, 503 and 6535DL microspheres gave total drug release about 82% and 59% respectively within 35 days. The high drug release from 503H and 502H microspheres can be attributed to the highest loading percent of the drug for 503H microspheres and to the low molecular weight for 502H polymer, and these two effects may fasten the hydrolysis of microspheres[28,29]. In paired comparison (503H vs 503), where the overwhelming majority of structure are chemically identical, and the difference between them is whether the polymer end groups are a carboxylic function (503H) or a long-chain fatty ester(503), the more hydrophilic polymer , the greater amount of drug bound . In a similar study, release of bone morphogenetic protein-2 from hydrophilic PLGA microspheres was higher than that from hydrophobic one[30]. The slow release of sertraline HCl from DL6535 microspheres might be due to the slow hydration and degradation of the high molecular weight polymer[31]. This result was expected and similar results reported by researchers[32-34].

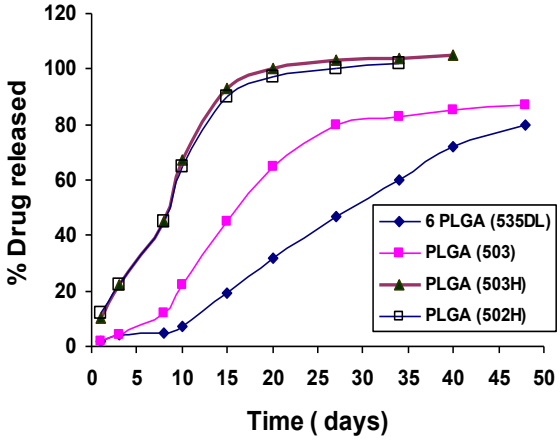


Figure 4: In vitro release of sertraline HCl from different polymer microspheres in acetate buffer pH 5 at 37°C.

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