

RESEARCH ARTICLE

BIODEGRADABLE SOLID LIPID MICROPARTICLES LOADED WITH DILTIAZEM HYDROCHLORIDE FOR ORAL DELIVERY: PREPARATION AND *IN-VITRO/IN-VIVO* EVALUATIONPilaniya K¹, *Pilaniya U², Saharan Shyoparakash², Saharan P², Chandrawanshi KH³¹Shivdan Singh Institute of Technology and Management, Aligarh-202001, U.P. (INDIA)²QA/QC Officer, Indovax Private Limited, Siswala, Hisar-125001, Haryana (INDIA)³Mittal Institute of Pharmacy, Bhopal-462038, M.P. (INDIA)*Corresponding Author's E mail: pilaniya.urmila@yahoo.com

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ABSTRACT:

Diltiazem, a benzodiazepine, voltage sensitive Ca^{2+} channel blocker with a high therapeutic potential but with a very short biological half life was encapsulated within microparticles. The encapsulation efficiency of prepared SLM reached $70.48 \pm 1.67\%$ w/w. Further, the particle size ($99.52 \pm 1.06 \mu m$), surface morphology (spherical) and drug loading efficiency ($17.62 \pm 2.14\%$ w/w) were investigated. Various formulation and process parameters like drug polymer ratio (3:1), nature and concentration of emulsion stabilizer in the external aqueous i.e. aqueous PVA solution (0.1%), phase viscosity of external aqueous phase (0.5%), volume of external aqueous phase and stirring rate (1000 rpm for 2 h) were optimized. The *in-vitro* release of diltiazem from the microparticles as observed with the initial burst release of 17% followed by the slow release to avoid dose dumping. The analytical methodology employed for characterization included UV and IR Spectroscopy, DSC, physicochemical stability studies which proves that the prepared solid lipid microparticles appear to have promising abilities for oral administration of diltiazem hydrochloride with improved half life, improved bioavailability and minimized local and systemic GI disturbances.

Keywords: Benzodiazepine, solvent evaporation method, biodegradable polymer, sustained release, encapsulation efficiency.

INTRODUCTION

Diltiazem is a calcium ion influx inhibitor (calcium entry blocker or calcium ion antagonist). The antihypertensive, antianginal and antiarrhythmic effects of diltiazem is believed to be related to its specific cellular action of selectively inhibiting transmembrane influx of calcium in cardiac muscle, coronary arteries, and systemic arteries and in cells of the intracardiac conduction system¹. Given orally, 90–100% of diltiazem is absorbed, but due to high first pass metabolism, bioavailability is much lower (40–60%), half life is 6–9 hours (with chronic dosages) and not cleared by hemodialysis²⁻³.

Solid lipid microparticles (SLMs) were developed in early 1990s and have since been considered to be promising drug carrier systems⁴, especially with a view to give the incorporated active substance a sustained release profile⁵⁻⁶. These are monolithic spherical structures with the drug distributed through out the microsphere matrix either as a molecular dispersion or as particle dispersion⁷. Solid lipids are advantageous pharmaceutical excipients being low cost, natural and biodegradable products with physiological, non toxic properties⁸⁻⁹. The drug solubility and miscibility in melted lipid¹⁰, chemical and physical structure of lipid materials, and their polymorphic state determine the loading capacity of drug in the lipid particles¹¹⁻¹².

Furthermore, the use of synthetic polymer matrix materials often goes along with detrimental effects on incorporated peptides during manufacturing of the formulations or during the erosion of the polymers after application¹³⁻¹⁴. Lipid materials, e.g. triglycerides and cholesterol¹⁵, may have the potential as biocompatible and biodegradable carriers for peptides and proteins¹⁶⁻¹⁷. In our study, we investigated the potential of physiological lipids such as monoglyceride as well as an alternative to polymers as a matrix material for controlled release devices for the diltiazem hydrochloride¹⁸⁻¹⁹. The aim of this study was to investigate various methods of preparing lipid microparticles with respect to their suitability to encapsulate diltiazem hydrochloride²⁰⁻²¹ and to characterize the resulting systems with respect to particle size²², modification of the lipid matrix and the *in-vitro* release behavior⁵.

MATERIALS AND METHODS

Materials

Diltiazem hydrochloride was received from Vipro Lifescience, (Gujarat, India), SOFTEMUL® - AS (glycerol monostearate), poly (vinyl alcohol), diethyl ether, span 60 grade from Pure Chemicals Co (Chennai, Tamil Nadu).

Preparation methods of lipid microparticles

Solvent evaporation method widely used for the preparation of polymeric microparticles. In this method drug and polymer were dissolved in organic solvent, this mixture was kept on a magnetic stirrer. Aqueous solution of 0.5% w/v of polyvinyl alcohol containing Span 60 (0.1% w/v) as an emulsifier were stirred with the help of mechanical stirrer. Then, both the mixtures were added and stirred at 1000 rpm. Microparticles were filtered, washed with acetone and vacuum dried over night at room temperature.

Scanning electron microscopic

The surface topography of microparticles prepared by different methods was analyzed by scanning electron microscopy. The prepared microparticles were coated with gold palladium under an air atmosphere for 150 seconds to achieve a 20nm film (Coater Polaron, 18mA current at 1.4 kV). The coated sample were then examined using SEM (Philips 505, Philips, Holland).

Particle size distribution

A laser light scattering technique (Mastersizer, Malvern Instruments, UK) was employed to confirm the particle size distribution of solid lipid microparticles. The dried powder samples were suspended in deionised water and sonicated for 1 min with an ultrasound probe before measurement. The compressed air system was utilized to inhibit the aggregation of dried microparticles²³.

Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry studies of Diltiazem and glycerol monostearate was carried out by heating the samples from 40°C to 240°C at the rate of 20°C/min, using UNIVERSAL Q 200 V 23.5 instrument²⁴.

In-vitro release study

In-vitro dissolution studies were carried out on the microparticles at the temperature of 37°C±0.5°C and at 100 rpm using USP Dissolution Apparatus II. Initially the dissolution studies were performed in simulated gastric fluid consisting of 0.1N (pH 1.2) hydrochloric acid without enzyme. An accurately weighed sample of microparticles was suspended in the media and dissolution study was carried out for 2 hr. At the end of the 2 hr, 400 ml of 0.1M tribasic sodium phosphate, pH was adjusted to 7.2±0.2 and added to all 6 dissolution vessels. The dissolution was continued until the microparticles were depleted of drug²⁵⁻²⁶.

In-vivo release study

The oral pharmacokinetic study of diltiazem hydrochloride SLM intended for the sustained release delivery, was carried out in albino rats, which were divided in two groups each with six animals of weight 250-300 grams. The animals were fasted for 24 hr following the oral administration of 0.5 ml suspension of diltiazem hydrochloride SLM in distilled water. The plasma samples were withdrawn from retro orbital plexus region of rats at time intervals of 0, 1, 2, 4, 8, 12 and 24 hr of post administration, in the animals lightly anesthetized with ether.

Analysis of diltiazem hydrochloride from rat plasma

The plasma samples withdrawn at 0, 1, 2, 4, 8, 12 and 24 h time interval were deproteinised using acetonitrile, subjected to centrifuge at 10,000 rpm for 10 minutes at the temp of -4°C. The clear supernatant was separated through micropipette and the drug content was analyzed using HPLC.

Pharmacokinetic parameters of diltiazem hydrochloride SLM

Oral Pharmacokinetic parameters such as maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), half life (T_{1/2}), mean residence time (MRT), were calculated using graph pad Version 5.0 software.

Stability study

Prepared formulation was stored in screw capped small glass bottles at 4±1°C, 60±5% RH and 25±1°C, 65±5% RH. Samples were analyzed for residual drug content after a period of 15, 30, 45, 60 and 90 days. Initial drug content was taken as 100 % for each formulation. The log percent residual drug content was plotted against time (t), which reflected an almost linear relationship.

Statistical analysis

Determination of corrected drug concentration: the corrected drug concentration at each time interval was calculated using the formula given below:

$$C_c = C_{uc} + V_s/V_t \sum_{i=1}^{n-1} C_{uc}$$

$$\sum_{i=1}^{n-1} C_{uc} = \text{Sum of previous uncorrected drug concentration.}$$

C_c = Corrected drug concentration.

C_{uc} = Uncorrected drug concentration.

V_s = Volume of sample withdrawn.

V_t = Total volume of dissolution medium.

RESULTS AND DISCUSSION

The method employed for the preparation of drug loaded biodegradable microparticles is the solvent evaporation technique. The present study investigated that diltiazem containing lipid microparticles can be produced in the preferred size range as a substitute to polymeric microparticles.

Selection of optimum Polymer: drug ratio

Diltiazem hydrochloride loaded microparticles were prepared using different polymer: drug ratio. Increasing the weight of polymer in a fixed volume of organic solvent resulted in an increase in mean particle size (from 71.43 ± 2.38µm to 113.52 ± 2.85µm for 1:1 to 5:1) as shown in Fig. 1 and Table 1.

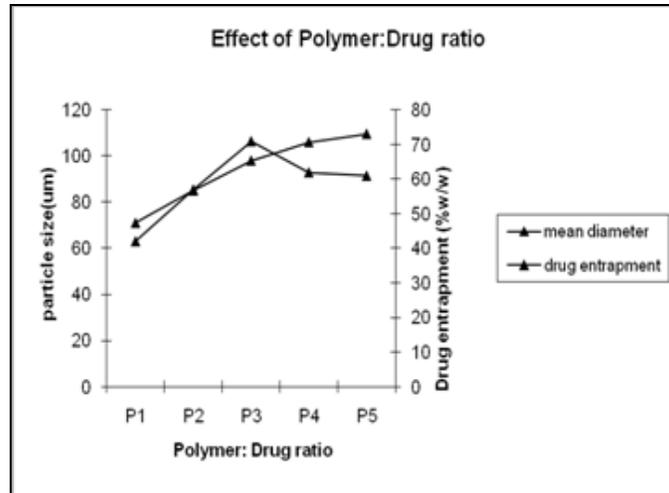


Figure 1: Effect of polymer: drug ratio on average particle size and percent drug entrapment

Table 1 Effect of polymer: drug ratio on microparticles characteristics

Batch Code	Polymer: drug ratio (w/w)	Mean diameter (µm)	Drug Loading (% w/w)	Entrapment efficiency (% w/w)	Process yield (% w/w)
DT-1	1:1	71.43 ± 2.38	22.74 ± 1.46	42.48 ± 2.46	62.08 ± 1.34
DT -2	2:1	85.83 ± 1.47	19.75 ± 1.46	57.26 ± 1.45	64.46 ± 2.47
DT -3	3:1	98.52 ± 1.37	16.62 ± 2.56	71.48 ± 1.66	87.43 ± 1.45
DT -4	4:1	106.44 ± 1.47	13.98 ± 1.89	60.92 ± 2.56	63.53 ± 1.54
DT -5	5:1	113.52 ± 2.85	11.48 ± 2.45	61.87 ± 1.64	62.67 ± 1.65

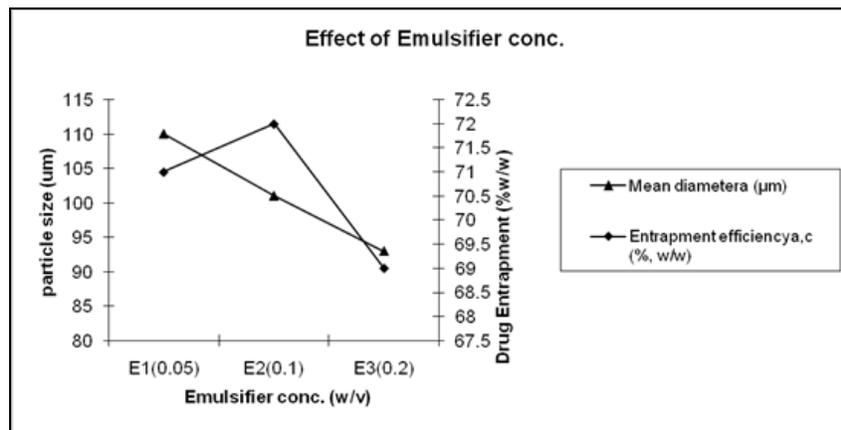


Figure 2: Effect of emulsifier concentration on average particle size and percent drug entrapment

Table 2 Effect of emulsifier concentration on microparticles characteristics

Batch Code	Emulsifier conc. (% w/v)	Mean diameter (µm)	Drug Loading (% w/w)	Entrapment efficiency (% w/w)	Process yield (% w/w)
DT-3	0.05	110.98±1.34	18.98±2.37	71.94±1.82	62.30±1.83
DT -3	0.1	101.64±1.56	19.37±1.04	72.48±1.01	84.30±2.11
DT -3	0.2	93.54±1.84	18.16±1.98	69.66±0.96	65.80±1.58

Nature and concentration of emulsion stabilizer in the external aqueous phase

Among the entire stabilizer studied, span 60 resulted in successful preparation of microparticles. Finally, 0.1% w/v span 60 was selected as a stabilizer of choice, since it allowed the preparation of particles in the size range of $101.64 \pm 1.56 \mu\text{m}$ with a considerable higher drug loading about 19.37 ± 1.04 (w/w) in Fig. 2 and table 2.

Selection of optimum aqueous: oil phase ratio

As external dispersing phase different volumes of PVA aqueous solution were employed, resulting in different ratios between aqueous as external and oil as internal phases. Particles produced by 20:1 w/o ratio (200 ml) enabled the production of spherical microparticles with a mean diameter of $101.37 \pm 2.14 \mu\text{m}$, having the process yield of $83.24 \pm 1.47\%$ (w/w), the encapsulation efficiency of $75.13 \pm 1.98\%$ (w/w) and drug loading of $17.23 \pm 1.38\%$ (w/w). Table 3 and Fig. 3 summarizes the obtained results.

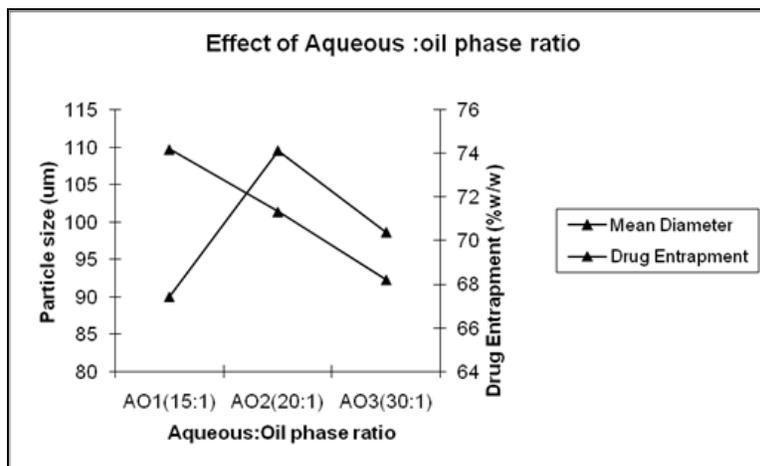


Figure 3: Effect of aqueous: oil phase ratio on average particle size and percent drug entrapment.

Table 3 Effect of aqueous:oil phase ratio on microparticles characteristics

Batch Code	Aqueous:oil phase ratio	Mean diameter (µm)	Drug Loading (% w/w)	Entrapment efficiency (% w/w)	Process yield (% w/w)
DT-3	15:1	109.74 ± 2.67	16.58 ± 1.54	67.42 ± 2.45	65.24 ± 2.15
DT -3	20:1	101.37 ± 2.14	18.23 ± 1.38	74.13 ± 1.85	84.24 ± 1.67
DT -3	30:1	92.28 ± 1.18	17.56 ± 2.13	70.39 ± 2.37	67.54 ± 1.88

Optimum viscosity of the external aqueous phase

Increasing viscosity of the external phase by addition of the increasing concentration of PVA led to a slight increase in the particle size $93.53 \pm 1.4 \mu\text{m}$ with 0.1% PVA to $138.85 \pm 0.89 \mu\text{m}$ with 1.0% PVA. The use of 0.5% PVA led to the formation of spherical particles with a mean diameter

of $101.47 \pm 1.73 \mu\text{m}$ with the process yield of $84.34 \pm 0.64\%$ (w/w), drug loading of $18.46 \pm 1.25\%$ (w/w) and an encapsulation efficiency of $72.82 \pm 1.34\%$ (w/w) which was highest among all the three concentrations of PVA (Fig. 4 and Table 4).

Table 4 Effect of viscosity of aqueous phase on microparticles characteristics

Batch Code	Viscosity (% w/v)	Mean diameter (µm)	Drug Loading (% w/w)	Entrapment efficiency (% w/w)	Process yield (% w/w)
DT-3	0.1	93.53 ± 1.4	17.83 ± 2.18	71.47 ± 1.45	64.34 ± 2.55
DT -3	0.5	101.47 ± 1.73	18.46 ± 1.25	72.82 ± 1.34	84.34 ± 0.64
DT -3	1.0	138.85 ± 0.89	17.19 ± 1.98	70.75 ± 2.09	65.34 ± 2.56

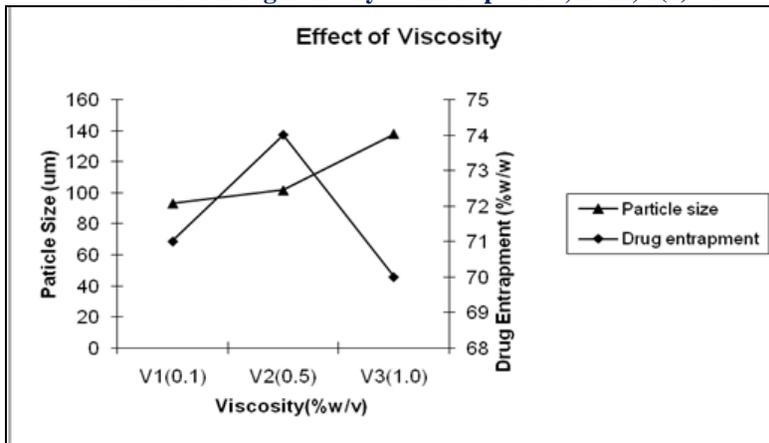


Figure 4: Effect of viscosity of aqueous phase on average particle size and percent drug entrapment

Optimum stirring speed

Stirring speed plays an important role in the microparticles size distribution and drug loading. On increasing the stirring speed up to 1500 rpm spherical microspheres were obtained. The microspheres at such high shear rate possessed 79.52 ± 1.32 µm mean diameter, 64.54 ± 2.17% (w/w) process yield, drug loading 16.31 ± 0.16% (w/w) and 62.94 ± 3.43% (w/w)

encapsulation efficiency [28]. The best results in term of process yield were obtained by the use of 1000 rpm stirring speed (84.33 ± 1.22%, w/w), microparticles in this condition were spherical, with 98.26 ± 1.53 µm mean diameter, drug loading 19.53 ± 2.04% (w/w) and 74.61 ± 1.34% (w/w) encapsulation efficiency. Table 5 summarizes the comparison of obtained results (Fig. 5)

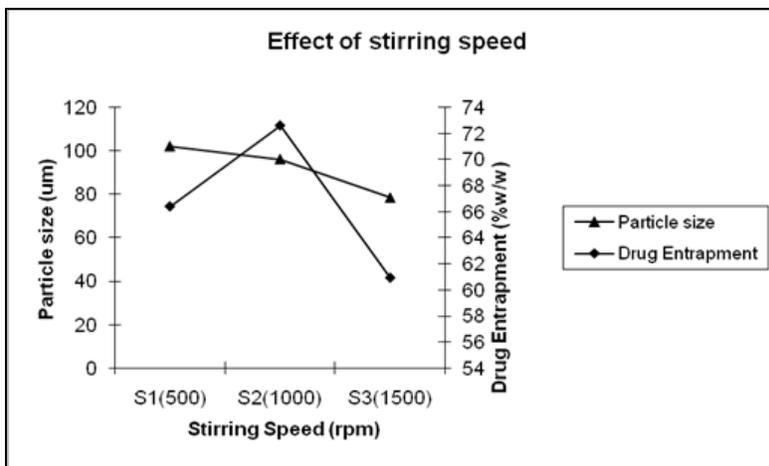


Figure 5: Effect of stirring speed on average particle size and percent drug entrapment

Table 5 Effect of stirring speed on microparticles characteristics

Batch Code	Stirring speed (rpm)	Mean diameter (µm)	Drug Loading (%w/w)	Entrapment efficiency (% w/w)	Process yield (%w/w)
DT-3	500	109.43±1.34	17.18±1.45	65.71±1.85	63.34±1.08
DT -3	1000	98.26 ± 1.53	19.53 ± 2.56	74.61 ± 1.34	84.33 ± 1.22%
DT -3	1500	83.45±1.92	14.61±1.54	57.44±2.13	62.34±2.37

Optimum stirring time

For a constant speed of 1000 rpm, a polymer:drug ratio of 3:1, a w/o ratio of 20:1 and a 0.5% viscosity of aqueous phase, an increase of the stirring time from 1 to 3 h

resulted in reduction in microparticles size (from 115.68 ± 2.43 to 81.66 ± 2.68 µm). A 2 h stirring time was chosen because the entrapment efficiency was higher (76.08 ± 1.62%, w/w) than after 2 h (69.92 ± 1.23%, w/w) (Fig. 6).

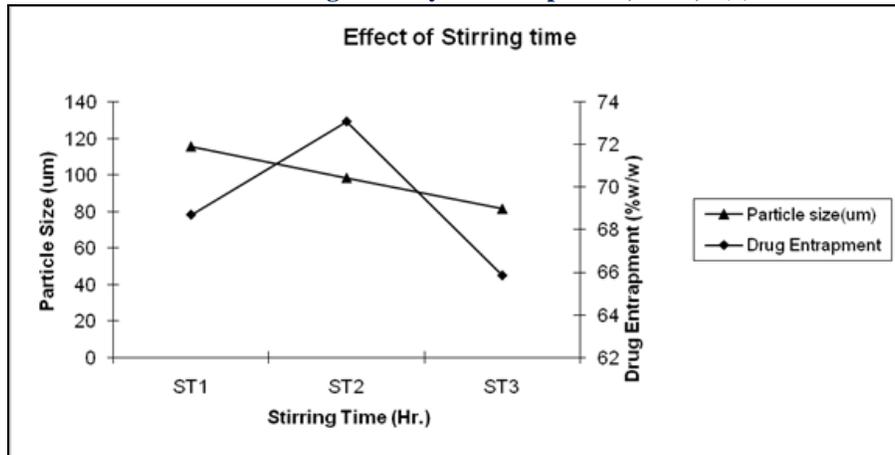


Figure 6: Effect of stirring time on average particle size and percent drug entrapment

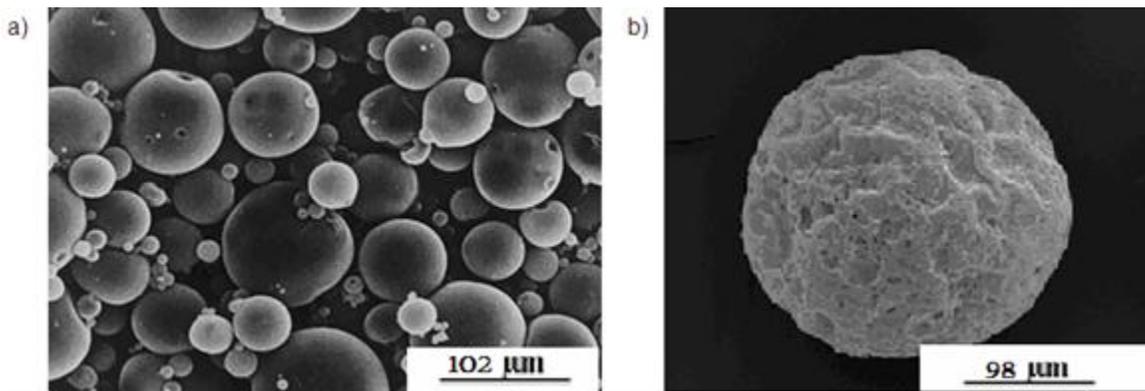


Figure 7: SEM photographs of microparticles: a) Diltiazem loaded group of particles and b) Diltiazem loaded single particle

Scanning electron microscopic studies

The spherical shape of microparticles was established by SEM. The surface analysis of empty and of drug loaded microparticles prepared by the w/o/w emulsion solvent evaporation method revealed that the microparticles were spherical with a diameter of $101.75 \pm 0.82 \mu\text{m}$ and polydispersity index of as shown in Fig. 7

Differential scanning calorimetric studies

In the drug loaded microparticles of glycerol monostearate, thermogram showed a peak at 63.75°C corresponding to the melting point of glycerol monostearate. This shift may be due to physicochemical interaction of drug and polymer as shown in fig. 8.

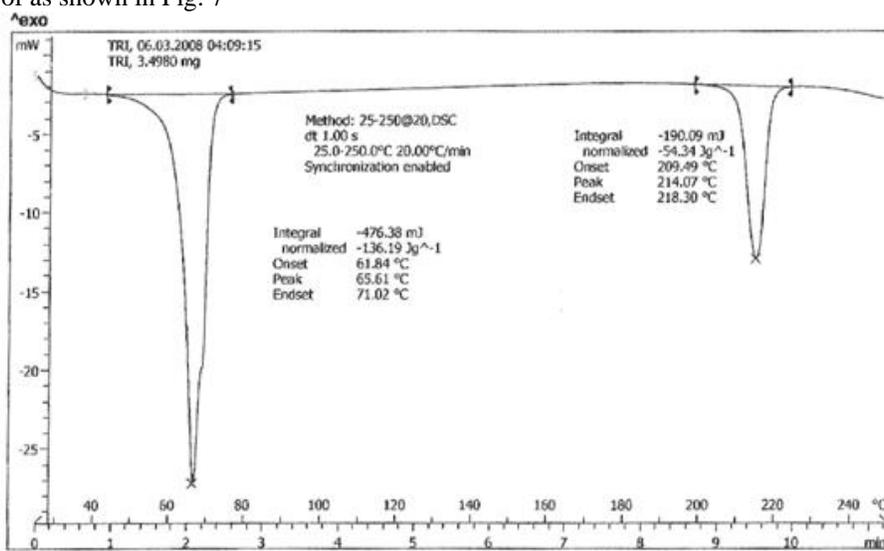


Figure 8: DSC thermogram of pure drug (Diltiazem Hydrochloride) and lipid

Size distribution and Size statistics

The measurement achieved by laser light scattering in order to estimate the average diameter of solid lipid microparticle of diltiazem hydrochloride evidenced the presence of some aggregates with average diameter 105.71 μm , and particle size ranged between 95.76 to 115.38 μm thus presenting unimodal and narrow particle size distribution as shown in Fig. 9.

In-vitro release studies

In-vitro Diltiazem Hydrochloride release studies from glycerol monostearate microparticles were performed for 2 h in pH 1.2 buffer (Simulated gastric fluid) and after 2 h in phosphate buffer, pH 7.2 (Simulated intestinal fluid) at temperature of $37 \pm 0.5^\circ\text{C}$. Diltiazem release from the microparticles was found to be slow and spread over extended period of 24 h²⁸ as shown in Fig. 10. Percent of

Diltiazem released from the microparticles was decreased with an increase in amount of coat material in the microparticle formulation ($p < 0.05$). The increased density of the polymer matrix at higher concentrations results in an increased diffusional pathlength²⁶. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microparticles are formed at a lower polymer concentration and have a large surface area exposed to dissolution medium, giving rise to faster drug release¹⁰⁻²⁷.

Initial release stage

About 17% rapid drug release was noticed just a few minutes after suspending the microparticles in the acid solution medium and higher release rates were associated with smaller size fraction. Under the microscope, drug crystal could be observed at or near the surface of the microparticles. These surface crystals would dissolve quickly and probably account for the rapid initial release²⁷.

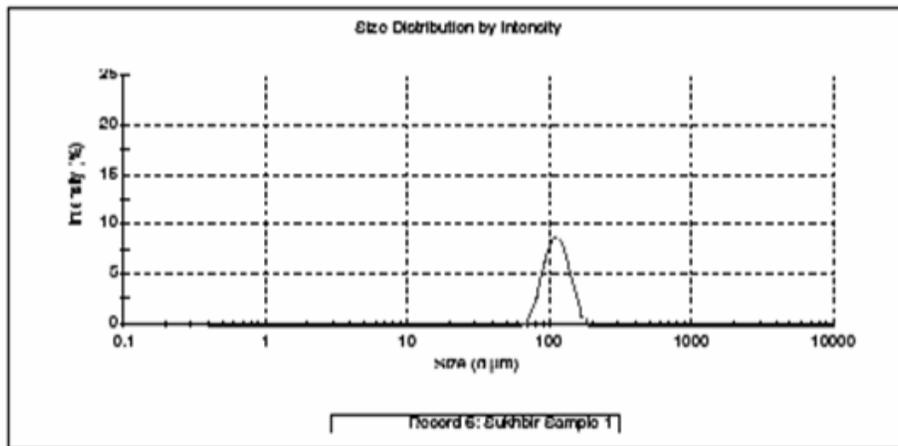


Figure 9: Size distribution and Size statistics report by intensity

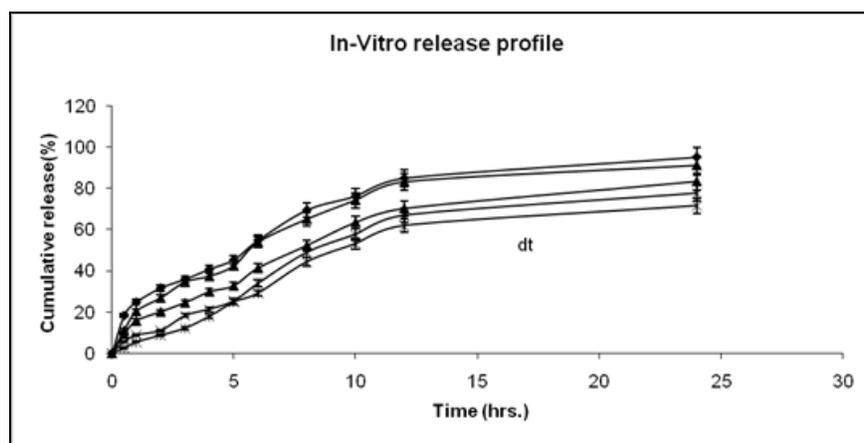


Figure 10: In-Vitro release curves of microparticles

Release in acid after initial stage

Most of the drug release during this time period is mainly due to the initial release and could be removed with an acid wash prior to the dissolution analysis²⁷. Diltiazem release from microparticles was negligible during this stage.

Release at pH 7.2

During the dissolution in the slightly neutral buffered medium pH 7.2, higher release rates were noticed for the microparticles as a result of the creation of pores and channels and probably due to swelling of polymers to some

extent. It is evident that slopes are larger in the slightly neutral medium than in acidic one.

Kinetics of drug release

In order to investigate the release mechanism of present drug delivery system, the data obtained from *in-vitro* release of final optimized batch (DT-03) were fitted into equations for the zero-order, first-order, Higuchi release model and

Krosmeyer Peppas model. The interpretation of data was based on the values of the resulting regression coefficients. The *in-vitro* drug release showed the regression coefficient values for Higuchi's model ($r^2 = 0.9759$) and Krosmeyer Peppas model ($r^2 = 0.986$) and a value of $n = 0.576$ represented in Fig. 11 and 12 respectively, indicating anomalous transport²⁹

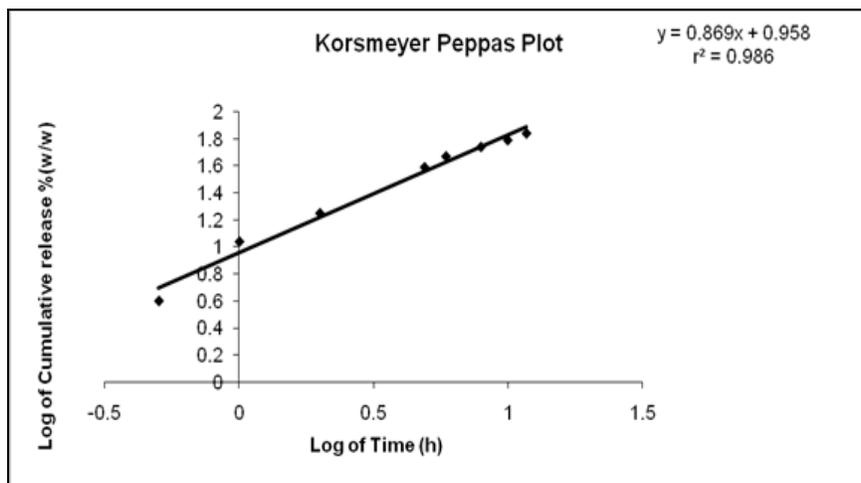


Figure 11: Korsmeyer Peppas plot of the optimized batch (DT-03)

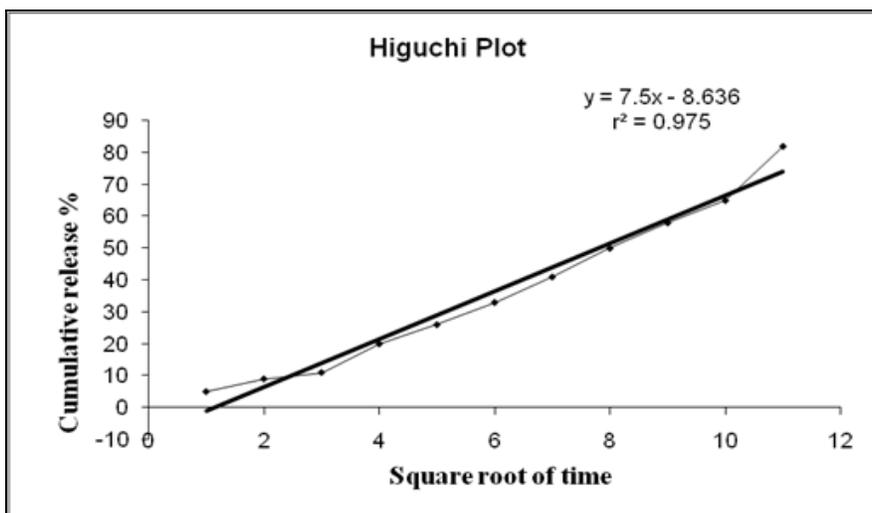


Figure 12: Higuchi's plot of the optimized batch (DT-03)

In-vivo studies

In-vivo studies were carried out in albino rats as per the established protocol and the pharmacokinetic parameters were determined.

Pharmacokinetic parameter of SLM

Pharmacokinetic analysis was carried out to determine oral parameters using comparative results from drug aqueous suspension and SLM of the diltiazem hydrochloride administered to II and III group respectively. Significantly higher C_{max} values were observed for SLM formulation compared to aqueous oral suspension of same drug as shown in Fig. 13. The highest concentration value achieved in case

of drug suspension was 218.59 ± 1.47 and the C_{max} value for SLM formulation was observed as 235.85 ± 1.29 . T_{max} value remained for 8 h (i.e., between 4h to 12 h time interval) in the SLM formulation indicating sustained release of drug whereas in pure drug without polymer, the highest concentration (C_{max}) was observed at first time point, i.e., 2 h, this was considered as T_{max} . MRT of diltiazem SLM formulation was considerably higher than the drug suspension. Half life of drug increased many fold and was found to be 12 h in SLM formulation and 2 h in drug suspension of diltiazem hydrochloride as shown in Table 8.

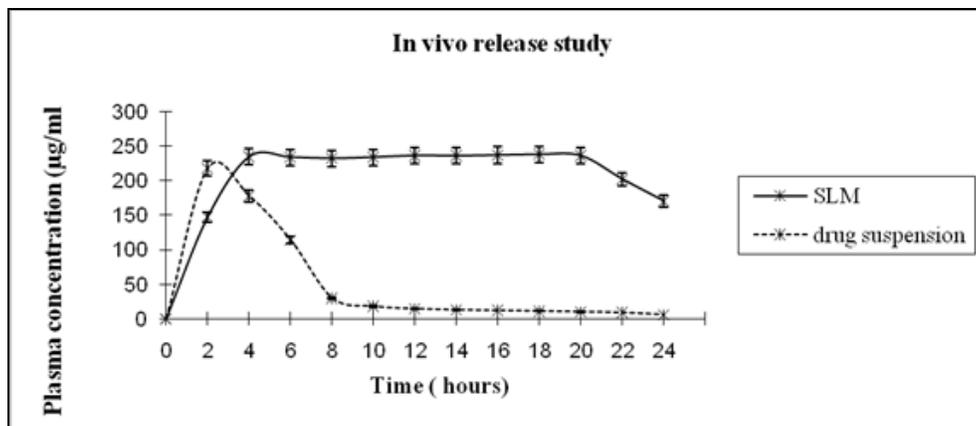


Figure 13: In-vivo release study of SLM and pure drug without polymer

Table 8 Pharmacokinetic parameters following the oral administration of diltiazem-loaded SLMs to albino rats, as compared to oral free drugs

Parameters	Control	SLM	Drug suspension
Cmax	0	235.85±1.29	218.59±1.47
Tmax	0	4±2.10	2±3.29

Cmax, maximum concentration; Tmax, maximum time to reach Cmax concentration

Mean ± S.D. (Standard deviation), n=3

Stability studies

Diltiazem Hydrochloride microparticles in the form of lyophilized powder were stored in glass bottles at 4 ± 1°C, 60 ± 5 % RH and 25 ± 1°C, 75 ± 5% RH for period of 3 months and evaluated for any change in the shape and structural integrity by microscopic examination and residual drug content. At 25 ± 1°C, agglomerates of microparticles were found after storage for three months, which may be attributed to polymer softening and fusion. Optimal storage conditions for the formulation assessed by analyzing the residual drug content after the time interval of 15, 30, 45, 60 and 90 days. The percent residual drug content was

determined and found to be 99.11±0.59 at 4 ± 1°C (Table 9) and 98.28±0.98 at 25 ± 1°C (Table 10) respectively after storage for 90 days. (Fig. 14 and 15) Microparticles formulation stored at 4 ± 1°C showed the k value as 11.51 × 10⁻⁴, t_{10%} value of nearly 94.45 days, t_{1/2} value was 602.61 days, while those stored at 25 ± 1°C showed the k value as × 10⁻⁴ and t_{10%} value of nearly 45.1 days, t_{1/2} value of nearly 301.30 days.

The log % residual drug content vs. time graph was plotted for the optimized formulation in order to evaluate k (specific rate constant or degradation rate constant), t_{1/2} and t_{10%} of the formulation.

Table 9 Effect of aging on residual drug content at 4 ± 1°C

Formulation	% Residual Drug Content (at 4 ± 1°C.)					
	Mean ± S.D., n=3					
	0 Days	15 Days	30 Days	45 Days	60 days	90 Days
DT-3	100	99.57±0.36	99.41±0.20	99.28±0.38	99.19±0.64	99.11±0.59
Log % of drug remaining	2	1.998	1.997	1.996	1.996	1.996

Table 10 Effect of aging on residual drug content at room temperature

Formulation	% Residual Drug Content (at 25 ± 1°C) Mean ± S.D., n=3					
	0 Days	15 Days	30 Days	45 Days	60 days	90 Days
DT-3	100	99.12±0.48	98.98±0.29	98.67±1.29	98.48±1.38	98.28±0.98
Log % of drug remaining	2	1.996	1.995	1.994	1.993	1.992

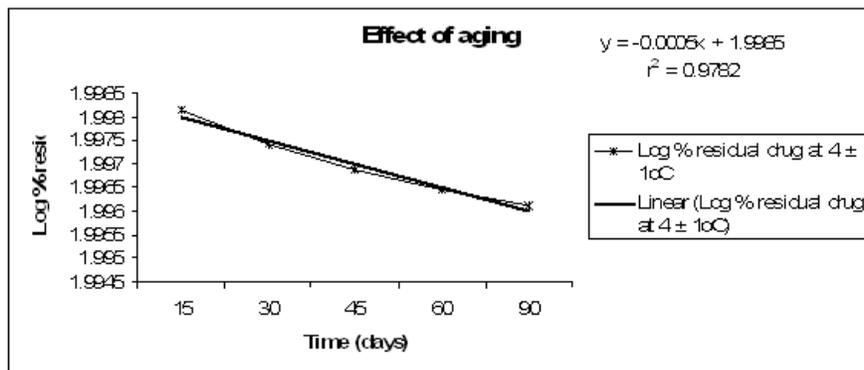


Figure 14: Effect of aging on log % residual drug content at 4 ± 1°C

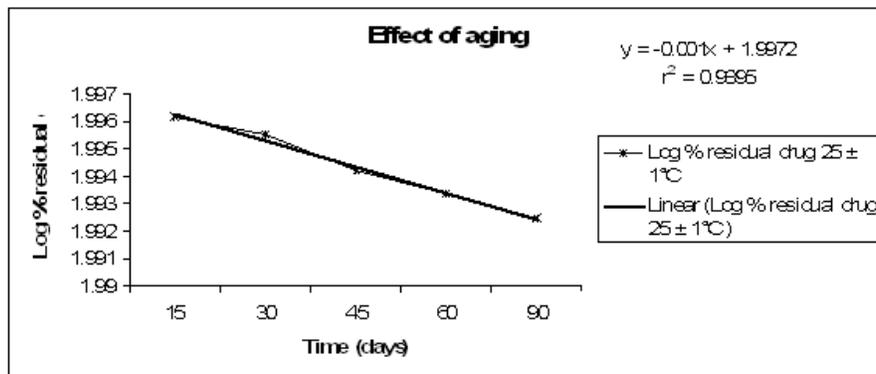


Figure 15: Effect of aging on % residual drug content at 25 ± 1°C

CONCLUSIONS

The present study deals with formulation and evaluation of solid lipid microparticles in the size range suitable for oral delivery by solvent evaporation technique melt dispersion technique and w/o/w emulsion solvent evaporation techniques using organic solvents. A W/O/W emulsion solvent evaporation technique was finally considered best method to prepare the SLM since it generated microspheres with spherical shape. The encapsulation efficiency of the drug into glyceryl monostearate microparticles was substantially influenced by the preparation method and the physical state of the drug to be incorporated. The w/o/w emulsion solvent evaporation techniques and the incorporation of the drug as an aqueous solution gave the best results with actual drug loadings up to 16% and an

encapsulation efficiency of approximately 72%. The *in-vitro* release study of diltiazem hydrochloride was carried out for 24 which resulted in initial burst release of 17 % followed by slow release of 84.51 %. The release data were fitted into equations for the zero-order, first-order, Higuchi release model and Krosmeier Peppas model. The interpretation of data was based on the values of the resulting regression coefficients. The *in-vitro* drug release showed the regression coefficient values for Higuchi's model ($r^2 = 0.9759$) and Krosmeier Peppas model ($r^2 = 0.986$) and a value of $n = 0.576$. *In-vivo* studies were carried out in albino rats as per the established protocol and the pharmacokinetic parameters were determined. C_{max} value for SLM formulation was observed was 235.85 ± 1.29 where C_{max} value achieved in case of drug suspension was 218.59 ± 1.47 T_{max} value

remained for 8 h (i.e., between 4h to 12 h time interval) in the SLM formulation indicating sustained release of drug whereas in pure drug without polymer, the highest concentration (C_{max}) was observed at first time point, i.e., 2 h, this was considered as T_{max}. Microparticles formulation stored at 4 ± 1°C showed the k value as 11.51 × 10⁻⁴, t_{10%} value of nearly 94.45 days, t_{1/2} value was 602.61 days, while those stored at 25 ± 1°C showed the k value as × 10⁻⁴ and

t_{10%} value of nearly 45.1 days, t_{1/2} value of nearly 301.30 days.

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REFERENCE

- Hardman JG, Limbird LE, Gilman's G. The Pharmacological basis of Therapeutics. McGraw-Hill Publications 10th edition; 2001. p. 712-13.
- Indian Pharmacopoeia. Government of India, Ministry of Health & Family welfare, published by controller of publications, Delhi Vol.II; 1996. p. 797.
- Krishnan P, Venkataman K. Current Index of Medical Specialities; 2003. p. 235.
- Reithmeier H, Hermann J, Gopferich A, Development and characterization of lipid microparticles as a drug carrier for somatostatin, International journal of pharmaceuticals, 2001, 218, 133-143.
- Morel S, Ugazio E, Cavalli R, Gasco MR, Thymopentin in solid lipid nanoparticles, International journal of pharmaceuticals, 1996, 132, 259-261.
- Schwarz C, Mehnert W, Lucks JS, Muller RH, Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization, Journal of Controlled Release, 1994, 30, 83-96.
- Hu FQ, Yuan H, Zhang HH, Fang M, Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization, International journal of pharmaceuticals, 2002, 239, 121-128.
- A, Muller RH, Mader K, Preparation of semisolid drug carriers for topical application based on solid lipid nanoparticles, International journal of pharmaceuticals, 2001, 214, 9.
- Muller RH, Mehnert W, Lucks, JS, Solid lipid nanoparticles: an alternative colloidal carrier system for controlled drug delivery, European Journal of Pharmaceutics and Biopharmacokinetics, 1995, 41, 62-69.
- Cavalli R, Bargoni A, Podio V, Muntoni E, Zara GP, Gasco MR, Duodenal administration of solid lipid nanoparticles loaded with different percentages of tobramycin, Journal of pharmaceutical Science, 2003, 92, 1085-1094.
- Reithmeier H, Herrmann J, Gopferich A, Lipid microparticles as a parenteral controlled release device for peptides, journal of Controlled Release, 2001, 73, 339-350.
- Savolainen M, Herder J, Khoo C, Evaluation of polar lipid-hydrophilic polymer microparticles, International journal of pharmaceuticals, 2003, 262, 47-62.
- Wissing SA, Muller RH, Solid lipid nanoparticles (SLN): a novel carrier for UV blockers, Pharmazie, 2001, 56, 783-786.
- Kunisawa J, Okudaira A, Tsutsumi Y, Characterization of mucoadhesive microspheres for the induction of mucosal and systemic immune responses, Vaccine, 2000 19, 589-594.
- Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ, Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain, Journal of Controlled Release, 1999, 59, 299-307.
- Cavalli R, Caputo O, Parlotti ME, Trotta M, Scarnecchia C, Gasco MR, Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles, International journal of pharmaceuticals, 1997, 148, 47-54.
- Severine J, Geraldine P, Luc D, Brigitte E, Solid lipid microparticles: formulation, preparation, characterization, drug release and applications, Expert Opinion Drug Delivery, 2005, 2(1), 1-12.
- Schwendeman SP, Brandon MR, Cardamone M, Klivanov A, Langer R. Stability of proteins and their delivery from biodegradable polymer microparticles. In: Smadar, C., (Ed.), Microparticulate Systems for the Delivery of Proteins and Vaccines. Drugs and the pharmaceutical Sciences, vol. 77, Decker, New York; 1996. p. 1-49.
- Muller RH, Mader K, Gohla S, Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art, European Journal of Pharmaceutics and Biopharmacokinetics, 2000, 50, 161-177.
- Wang PY. Implant preparations containing bioactive macromolecule for sustained delivery. US Patent 5110595; 1992, 5 May.
- Cady SM, Steber WD, Fishbein R, Development of a sustained release delivery system for bovine somatotropin, Proceeding International Symposium on Controlled Release of Bioactive Material, 1989, 16, 22-23.
- Esposito E, Menegatti E, Cortesi R, Hyaluronan-based microparticles as tools for drug delivery: a comparative study, International journal of pharmaceuticals, 2005, 288, 35-49.
- Eldem T, Speiser P, Hincal A, Optimization of spray-dried and congealed lipid micropellets and characterization of their surface morphology by scanning electron microscopy, Pharmaceutical Research, 1991, 8, 47-54.
- Suahada N, Alhnan MA, McConnell EL, Basit AW, Drug distribution in enteric microparticles, International journal of pharmaceuticals, 2009, 379, 1-8.
- Bonferoni MC, Rossi S, Ferrari F, Stavik E, Pena-Romero A, Caramella C, Factorial Analysis of the Influence of Dissolution Medium on Drug Release from Carrageenan-Diltiazem Complexes, AAPS Pharmaceutical Science and Technology, 2000, 1(2), 15-30.
- Palmieri GF, Bonacucina G, Martino PD, Martelli S, Gastro-resistant microspheres containing ketoprofen, Journal of Microencapsulation, 2002, 19, 111-119.

27. Rafati H, Coombes AGA, Adler J, Holland J, Davis SS, Protein – loaded poly (DLlactide- co-glycolide) microparticles for oral administration: formulation, structural and release characteristics, *Journal of Controlled Release*, 1997, 43, 89-102.
28. Arshady R, Albumin microparticles and microcapsules: methodology of manufacturing techniques, *Journal of Controlled Release*, 1990, 14, 111-114.
29. Muhlen AZ, Schwarz C, Mehnert W, Solid lipid nanoparticles (SLN) for controlled drug delivery-drug release and release mechanism, *European Journal of Pharmaceutics and Biopharmacokinetics*, 1998, 45, 149-155.