VNS Institute of Pharmacy[1], Bhopal; Department of Pharmaceutics[2], Shivdan Singh Institute of Technology and Management, Aligarh; Department of Pharmacy[3], S.G.S.I.T.S. Indore College of Pharmacy[4], IPS Acadmey, Indore, India

Formulation and evaluation of verapamil hydrochloride loaded solid lipid microparticles

U. Pilaniya[1], K. Pilaniya[2], H. K. Chandrawanshi[1], N. Gupta[3], M. S. Rajput[4]

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Urmila Pilanya, VNS Institute of Pharmacy, Bhopal 11007 Delhi, India
Pilaniya.urmila@yahoo.com

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The present study aimed to produce verapamil hydrochloride-loaded solid lipid microparticles (SLM) by the w/o/w emulsion solvent evaporation technique, using diethyl ether as solvent phase, glyceryl monostearate as biodegradable polymer and Span 60 as surfactant. SLM of spherical shape were prepared by simple dilution of the emulsion with water. To increase the lipid load the process was conducted at 50 °C, and in order to reach sub-micron size, a high-shear homogenizer was used. The encapsulation efficiency of prepared SLM reached 74.29 ± 0.76%. Particle size (98.55 ± 1.42 μm), surface morphology (spherical) and drug loading efficiency (18.57 ± 1.25% w/w) were investigated. And optimization of drug polymer ratio (3:1), nature and concentration of emulsion stabilizer in the external aqueous (0.1%), phase viscosity of external aqueous phase (0.5%), volume of external aqueous phase and stirring rate (1000 rpm) were detected. Analysis of microsphere content after processing showed that verapamil did not undergo any chemical modification within the micro-particles. The *in-vitro* release of verapamil from the microparticles was very low and an initial burst effect of 17% of the dose was observed. The slow release may help to avoid a high frequency of administration. The prepared solid lipid microparticles appear to have interesting perspectives as delivery systems for the oral administration of verapamil hydrochloride with improved half-life, improved bioavailability, and minimized local and systemic gastrointestinal disturbances of the drug.

1. Introduction

Solid lipid microparticles (SLM) with a particle size range of 1 – 1000 μm were developed in early 1990 s and have since been considered to be promising drug carrier systems, especially in view of a sustained release profile (Reithmeier et al. 2001). They are monolithic spherical structures with the drug distributed throughout the microsphere matrix either as a molecular dispersion or as particle dispersion. In recent years, biocompatible lipid micro- and nanoparticles have been reported as alternative materials to polymers (Morel et al. 1996; Erni et al. 2002). Solid lipid particles combine several advantages and avoid the disadvantages of other colloidal carriers. Various positive aspects of the potential use of SLM as drug carrier systems are that they offer the possibility of controlled drug release and drug targeting (Schwarz et al. 1994), they provide protection of incorporated active compounds against degradation and they allow an incorporation of hydrophilic and/or hydrophobic drugs (Hu et al. 2002; Lippacher et al. 2001). Solid lipids are advantageous pharmaceutical excipients being relatively cheap, natural and biodegradable products with physiological, non-toxic properties. The drug solubility and miscibility in melted lipid, chemical and physical structure of lipid materials, and their polymorphic state determine the loading capacity of drug in the lipid particles (Muller et al. 2000). The amount of drug encapsulated can vary from 1 to 5% for hydrophilic compounds (Cavalli et al. 2003; Reithmeier et al. 2001) and reach up to 80% for lipophilic compounds (Zur et al. 1998).

Solid microparticles in dispersions are usually obtained using a melt dispersion method or a solvent evaporation method (Savolainen et al. 2003). Solid lipid particles have been proposed as a colloidal drug carrier therapeutic system for different administration routes such as oral, topical (Wissing et al. 2001; Kunisawa et al. 200), ophthalmic, subcutaneous and intramuscular injection (Yang et al. 1999; Cavalli et al. 1997). New technological advances have brought many innovative drug delivery systems into the commercial circulation. A variety of approaches have been investigated for the controlled release of drugs and targeting to selective sites including polymeric prodrugs, liposome, monoclonal antibodies and microspheres. Solid lipids are commonly used as lipid matrices with a variety of functions (Severine et al. 2005). Here we prepared solid lipid microparticles containing the calcium ion influx inhibitor verapamil hydrochloride.

Given orally, 90–100% of verapamil is absorbed, but due to high first-pass metabolism, bioavailability is much lower (10–35%). It is bound to 90% to plasma proteins and has a volume of distribution of 3–5 L/kg^{-1}. It is metabolized in the liver to at least 12 inactive metabolites (though one metabolite, norverapamil, retains 20% of the vasodilating activity of the parent drug). Of its metabolites, 70% are excreted in the urine and 16% in the feces; 3–4% are excreted unchanged in urine. There is a non-linear dependence between plasma concentration and dosage. Onset of action is 1–2 h after oral dosage. Half-life is 5–12 h (with chronic dosages). It is not cleared by hemodialysis (Indian Pharmacopoeia 1996; Krishnan et al. 2003).

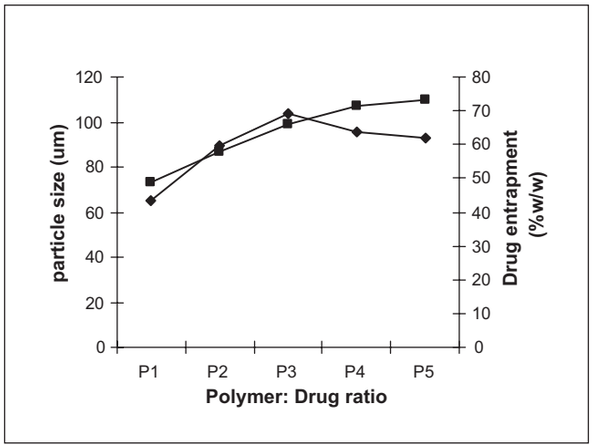

Fig. 1: Effect of polymer:drug ratio on average particle size and percent drug entrapment – ■–mean diameter–♦– drug entrapment

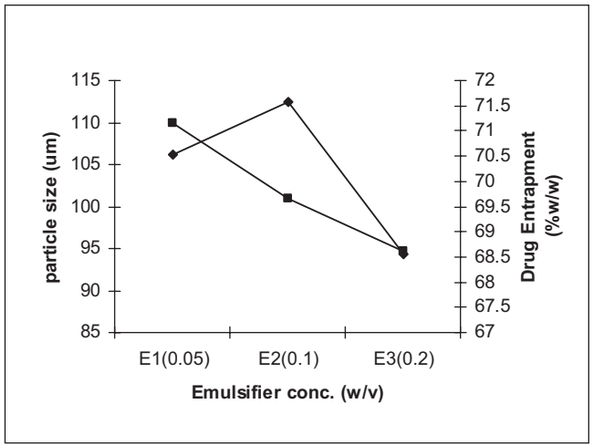

Fig. 2: Effect of emulsifier concentration on average particle size and percent drug entrapment. –■– Mean diametera (μm) –♦– Entrapment efficiency a, c (%, w/w)

Biodegradable polymers retain their properties for a limited period of time *in vivo* and then gradually degrade into materials that can become soluble or are metabolized and excreted from the body. In order to be used for *in vivo* applications the polymers used for such systems must have favorable properties for biocompatibility, processability, sterilization capability, and shelf life. Here glycerol monostearate is used to prepare the solid lipid microparticle of verapamil hydrochloride (Martindale 2005).

2. Investigations, results and discussion

2.1. Polymer:drug ratio

Verapamil hydrochloride loaded microparticles were prepared using different polymer:drug ratios (1:1, 2:1, 3:1, 4:1, 5:1, w/w). Increasing the weight of polymer in a fixed volume of organic solvent resulted in an increase in mean particle size (Fig. 1). Table 1 summarizes the results.

2.2. Nature and concentration of emulsion stabilizer in the external aqueous phase

Of the stabilizers studied (Tween 80, Span 20, Span 60), Span 60 (0.1% w/v) was selected as the stabilizer of choice, since it allowed the preparation of particles in the size range of 101.04 ± 1.56 μm with an interesting drug loading of 18.57 ± 1.25% (w/w) (Fig. 2). It could be shown that changes in Span 60 concentrations were devoid of the effect on drug entrapment, particle size and particle size distribution (Rafati et al. 1997). Table 2 summarizes the results.

2.3. Aqueous phase:oil phase ratio

As external dispersing phase different volumes of polyvinyl alcohol (PVA) aqueous solution (150, 200, 300 ml) were used, resulting in different ratios between aqueous external and oil internal phases (w/o ratio), namely 15:1, 20:1, 30:1. Particles produced by a 20:1 w/o ratio (200 ml) enabled the production of spherical microparticles with a mean diameter of 101.37 ± 2.14 μm, a process yield of 84.24 ± 1.67% (w/w), an encapsulation efficiency of 74.13 ± 1.85% (w/w) drug loading of 18.23 ± 1.38% (w/w). Table 3 summarizes the results obtained.

2.4. Viscosity of the external aqueous phase

Increasing viscosity of the external phase achieved by addition of the increasing concentrations of PVA (0.1, 0.5, 1.0% w/v) led to a slight increase in particle size (92.33 ± 1.62 μm) with

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)
01	1:1	73.19 ± 2.97	13.54 ± 1.64	43.57 ± 2.62	59.89 ± 2.67
02	2:1	87.00 ± 2.65	15.75 ± 1.27	59.86 ± 2.78	62.36 ± 2.52
03	**3:1**	$\mathbf{99.21 \pm 1.75}$	$\mathbf{17.42 \pm 2.17}$	$\mathbf{69.28 \pm 1.74}$	$\mathbf{85.74 \pm 2.32}$
04	4:1	107.34 ± 1.26	14.86 ± 1.19	63.92 ± 2.42	69.73 ± 1.28
05	5:1	109.57 ± 2.15	12.48 ± 2.43	61.97 ± 1.73	62.87 ± 1.77

Mean ± S.D. (Standard deviation), n = 3

Table 2: Effect of emulsifier concentration on microparticles characteristics

Batch code	Emulsifier conc. (%w/w)	Mean diameter (μm)	Drug loading (%w/w)	Entrapment efficiency (%, w/w)	Process yield (%w/w)
01	0.05	109.87 ± 1.61	16.78 ± 2.15	70.54 ± 1.62	62.20 ± 1.63
02	**0.1**	$\mathbf{101.04 \pm 1.56}$	$\mathbf{18.57 \pm 1.25}$	$\mathbf{71.58 \pm 1.31}$	$\mathbf{82.83 \pm 2.41}$
03	0.2	94.65 ± 1.23	17.06 ± 2.73	68.56 ± 1.96	65.80 ± 1.48

Mean ± S.D. (Standard deviation), n = 3

Table 3: Formulation parameters of the microparticles

Batch code	Polymer:drug ratio (w/w)	Emulsifier concentration (%, w/v)	Aqueous:oil phase ratio (v/v)	Viscosity of aqueous phase (%, w/v)	Stirring speed (rpm)	Stirring time (h)
01	1:1	0.1	20:1	0.5	1000	2
02	2:1	0.1	20:1	0.5	1000	2
03	**3:1**	**0.1**	**20:1**	**0.5**	**1000**	**2**
04	4:1	0.1	20:1	0.5	1000	2
05	5:1	0.1	20:1	0.5	1000	2

Mean ± S.D. (Standard deviation), n = 3

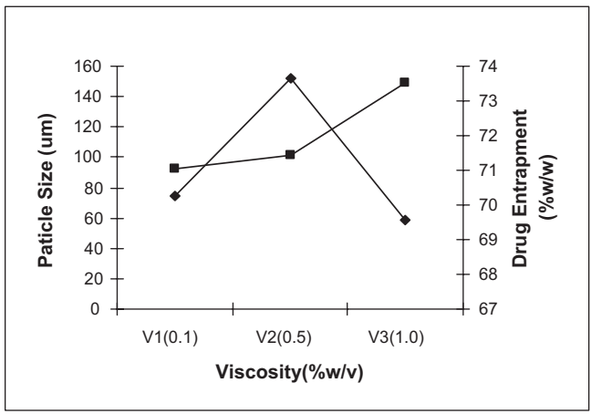

Fig. 3: Effect of viscosity of aqueous phase on average particle size and percent drug entrapment. –■– Particle size –♦– Drug entrapment

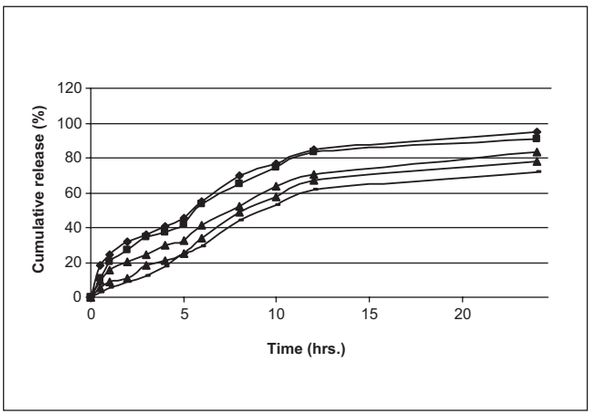

Fig. 4: In-Vitro release curves of microparticles. –♦– Drug entrapment –■– Drug entrapment –▲– Drug entrapment –▲– Drug entrapment –■– Drug entrapment

0.1% PVA to 148.72 ± 1.73 μm with 1.0% PVA with out alteration on the unimodal size distribution (Arshady et al. 1990). The use of 0.5% PVA led to the formation of spherical particles with a mean diameter of 101.49 ± 1.63 μm, a process yield of 84.74 ± 1.52% (w/w), drug loading of 18.63 ± 1.35% (w/w) and an encapsulation efficiency of 73.64 ± 1.68% (w/w) (Fig. 3). Table 3 summarizes the comparison of obtained results.

2.5. Stirring speed

Stirring speed plays an important role in the microparticles size distribution and drug loading. In fact using 3:1 polymer:drug ratio, 20:1 w/o ratio and 0.5% viscosity of aqueous phase, it was found that a 500 rpm stirring speed produced particles with rough and irregular surface. On the contrary, a stirring speed of 1500 rpm led to spherical microparticles, characterized by 78.52 ± 1.32 μm mean diameter, 63.54 ± 2.17% (w/w) process yield, drug loading of 14.31 ± 0.16% (w/w) and 60.94 ± 3.43% (w/w) encapsulation efficiency (Arshady et al. 1990; Esposito et al. 1996). The best results in term of process yield were obtained under 1000 rpm stirring speed (84.33 ± 1.22%, w/w), microparticles produced this way were spherical, with a 96.26 ± 1.53 μm mean diameter, drug loading of 18.53 ± 2.04% (w/w) and 72.61 ± 1.34% (w/w) encapsulation efficiency. Table 3 summarizes results obtained.

2.6. Duration of agitation during emulsification

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 of microparticle 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 (73.08 ± 1.62% w/w) than after 2 h (67.92 ± 1.23%, w/w, Table 3)

2.7. In vitro release studies

In vitro verapamil hydrochloride release studies from glycerol monostearate microparticles were performed in pH 1.2 buffer (first 2 h) and phosphate buffer, pH 7.2 (after 2 h) at 37 ± 0.5 °C. Verapamil release from the microparticles was found to be slow and spread over extended period of time (24 h) (Rafati et al. 1997). The amount of verapamil released from the microparticles was decreased with an increase in coat material in the microparticles ($p < 0.05$) (Fig. 4). The increased density of the polymer matrix at higher concentrations resulted in an increased diffusional pathlength (Bonferoni et al. 2000). 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 release (Muller et al. 1995; Palmieri et al. 2002). The dissolution profiles of the microparticles could be divided into three stages.

2.7.1. 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 dissolve quickly and probably account for the rapid initial release (Palmieri et al. 2002).

2.7.2. Release in acid after initial stage

Most of the drug release during this time period is due mainly of the initial release and could be removed with an acid wash

Table 4: ***In vitro*** **release profile of microparticles in pH 1.2 buffer and pH 7.2 buffer**

Time (h)	01	02	03	04	05
0.5	18.31(±0.47)	11.15 ± 0.38	**9.28 ± 0.028**	5.58(±1.05)	2.43(±0.64)
1	24.73(±0.76)	20.39(±0.48)	**15.675 ± 0.014**	8.78(±0.71)	5.38(±0.34)
2	31.63(±0.72)	26.81(±0.14)	**20.102 ± 0.076**	11. 04(±0.37)	8.65(±1.22)
3	35.84(±1.64)	34.49(±0.78)	**24.465 ± 0.042**	18.19(±1.41)	12.13(±0.76)
4	40.78(±0.53)	37.33(±1.06)	**29.770 ± 0.056**	21.35(±1.54)	17.87(±0.98)
5	45.33(±1.21)	42.37(±1.52)	**32.697 ± 0.074**	25.34(±0.33)	24.84(±0.67)
6	54.82(±1.75)	53.88(±0.28)	**41.323 ± 0.042**	33.94(±1.95)	29.17(±1.19)
8	69.52(±1.40)	65.08(±1.21)	**52.256 ± 0.027**	48.96(±1.87)	44.36(±0.88)
10	76.28(±1.41)	74.24(±0.91)	**63.452 ± 0.067**	57.73(±1.34)	53.13(±1.32)
12	85.01(±0.71)	83.18(±1.20)	**70.211 ± 0.038**	66.93(±1.36)	61.94(±0.63)
24	95.24 ± 0.44	91.17 ± 1.78	**83.351 ± 0.082**	77.67 ± 0.67	71.56 ± 1.12

Mean ± S.D. (Standard deviation), n = 3

prior to the dissolution analysis (Palmieri et al. 2002). Verapamil release from microparticles was negligible during this stage.

2.7.3. Release at pH 7.2

In the slightly neutral buffered medium pH 7.2, higher release rates were noticed for the microparticles due to the creation of pores and channels and probably to swelling of polymers to some extent. It is evident that slopes are larger in the slightly neutral medium than in acidic one.

2.8. Kinetics of drug release

In order to investigate the release mechanism of the present drug delivery system, the data obtained from *in vitro* release of the final optimized batch (03) were fitted into equations for the zero-order, first-order, and Higuchi release model and Peppas equation. 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 ($R^2 = 0.9636$) and Peppas model ($R^2 = 0.9827$) and a value of n = 0.587 (Fig. 5) indicating anomalous transport.

2.9. 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 and polydispersed with a diameter of 101.75 ± 0.82 μm (Fig. 6). The surface of these microparticles was found to be smooth with quite a few pock marks.

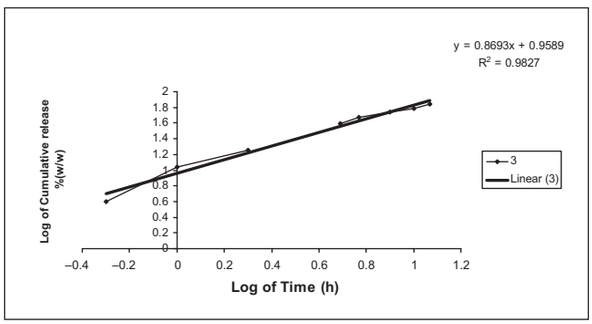

Fig. 5: Korsmeyer Peppas plot of the optimized batch (03)

2.10. Differential scanning calorimetric studies

Differential Scanning Calorimetry (DSC) studies of verapamil and glycerol monostearate was carried out by heating the samples from 40 to 240 °C at a rate of 20 °C/min, in order to carry out drug polymer compatibility studies (Fig. 7) using an UNIVERSAL Q 200 V 23.5 instrument. In the drug loaded microparticles of glycerol tripalmitate, the thermogram showed a peak at 63.75 °C corresponding to the melting point of glycerol monostearate. This shift may be due to a physicochemical interaction of drug and polymer. No drug peak was observed in drug loaded microparticles suggesting that drug was molecularly dispersed throughout the polymer matrix.

2.11. Stability studies

Verapamil hydrochloride microparticles in the form of lyophilized powder were stored in glass bottles at 4 ± 1 °C, room temperature and 40 ± 1 °C, 75% 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 40 ± 1 °C, agglomerates of microparticles were found after storage for three months, which may be attributed to polymer softening and fusion. Optimal storage condition of the formulation assessed by analyzing the residual drug content after the time intervals of 15, 30, 45, 60 and 90 days. The percent residual drug content was determined and found to be 98.15 ± 0.13 at 4 ± 1 °C (Table 5) and 97.17 ± 0.21 at room temperature (Table 6) respectively after storage for 90 days (Figs. 8 and 9). Microparticle formulations stored at 4 ± 1 °C showed the *k* value as 2.35×10^{-4} and $t_{10\%}$ value of nearly 448.734 days, while those stored at room temperature showed the *k* value as 3.18×10^{-4} and $t_{10\%}$ value of nearly 331.245 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.

3. Experimental

3.1. Material

Verapamil HCl as an almost white, crystalline powder, practically free of odor, with a bitter taste was received as gift sample (50 g) from Piramal Healthcare Ltd, Baddi, in a press sealed polyvinyl chloride envelope pack. Glycerol monosteaeate as a polymer, Span 60 as an emulsifier, PVA as the stabliser and diethyl ether as solvent were used. Studies have shown that simply mixing the ingredient is not sufficient to ensure controlled-release SLM formulations. Drug release cannot be prolonged, based on a solid matrix where drug and lipids are just physically mixed. The production method must allows the drug to dissolve or to disperse into lipids. Preformed lipid polymers can be used for the preparation of microparticles by the following methods.

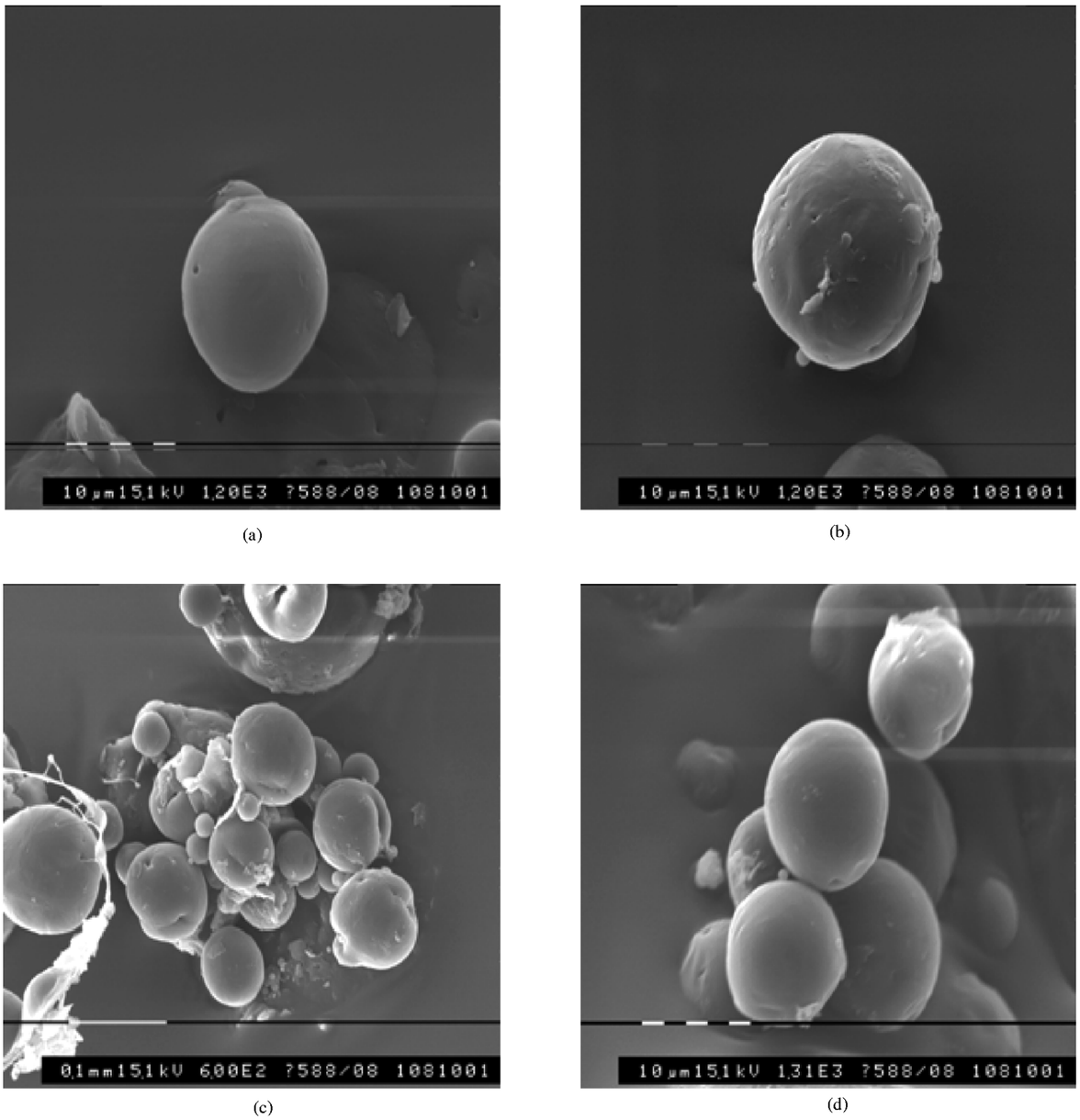

Fig. 6: SEM photographs of microparticles: (a) verapamil loaded single particle, (b) drug free single microparticle, (c) placebo group of particles, (c) verapamil loaded group of particles

3.2. Preparation of SLM

Verapamil hydrochloride SLM were prepared by w/o/w emulsion solvent evaporation. A solution of verapamil hydrochloride (5 ml containing 120 mg) in distilled water (internal aqueous phase) was emulsified with a (360 mg) solution of glycerol monostearate (10 ml) in diethyl ether (oil phase) at high speed on a magnetic stirrer. The resulting water-in-oil (w/o) emulsion was then emulsified at room temperature into a small volume (45 ml) of 0.5%, w/v PVA solution using 0.1% w/v Span 60 by vortex-mixing (Eldem et al. 1991; Esposito et al. 2005). The emulsion was poured into a larger volume (155 ml) of an ice-cooled (5 °C) aqueous phase (PVA 0.5%, w/v) and stirred with a mechanical stirrer (4 × 4 cm) (United Electrical Industries, Varanasi, India) at a stirring rate of 1000 rpm for 2 h to allow the evaporation of the organic solvent. The hardened microparticles were separated from the aqueous phase by filtration, rinsed with 40 ml of acetone. The washings were checked for the absence of organic solvents spectrophotometrically (Shimadzu UV 1700, Japan) (Brakland et al. 2007).

Table 5: Effect of aging on residual drug content at 4 ± 1 °C

	% Residual drug content (at 4 ± 1 °C)					
Formulation	0 Days	15 Days	30 Days	45 Days	60 Days	90 Days
03	100	99.53 ± 0.05	99.12 ± 0.04	98.66 ± 0.06	98.35 ± 0.14	98.15 ± 0.13

Mean ± S.D. (Standard deviation), n = 3

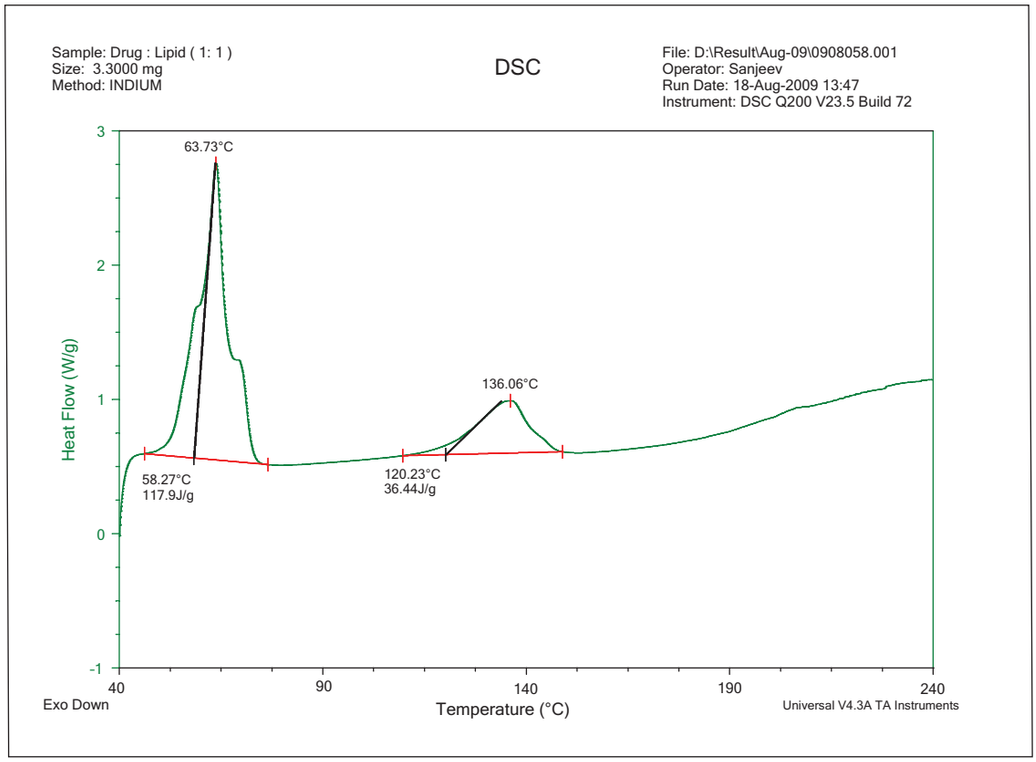

Fig. 7: DSC thermogram of pure drug (verapamil hydrochloride) and lipid

Table 6: Effect of aging on residual drug content at room temperature

	% Residual drug content (room temperature)					
Formulation	0 Days	15 Days	30 Days	45 Days	60 Days	90 Days
03	100	99.37 ± 0.18	98.52 ± 0.15	98.12 ± 0.12	97.73 ± 0.16	97.17 ± 0.21

Mean ± S.D. (Standard deviation), n = 3

3.3. Surface morphology and particle size distribution

The morphology and appearance of microparticles were examined by scanning electron microscopy (SEM). The prepared microparticles were coated with gold palladium under an air atmosphere for 150 s to achieve a 20 nm film (Coater Polaron,18 mA current at 1.4 kV). The coated samples were then examined using SEM (Philips 505, Philips, Holland). The particle size and size distribution of the prepared microparticles were measured by laser diffraction in a particle size analyzer (Mastersizer, Malvern Instruments, UK). The dried powder samples were suspended in deionised water and sonicated for 1 min with an ultra-sound probe before measurement.

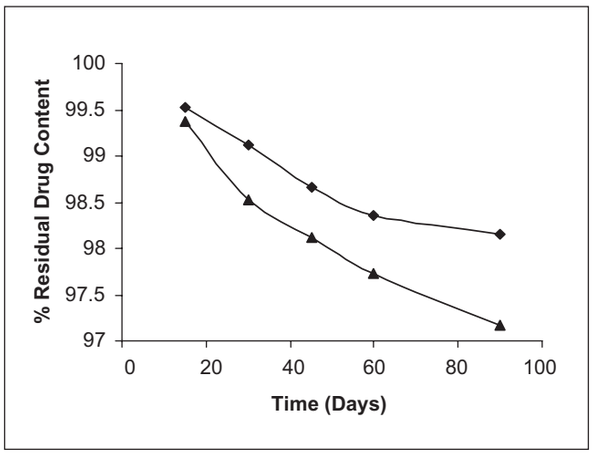

Fig. 8: Effect of aging on %residual drug content at 4 ± 1 °C and room temperature. –♦– % residual Drug content at 4 °C –▲– %Residual at room temp.

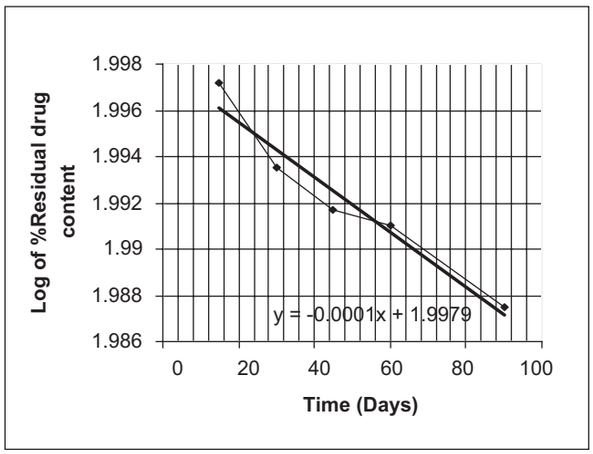

Fig. 9: Effect of aging on log % residual drug content at 4 ± 1 °C and room temperature –♦– log % residual drug content at Room temperature ◆ Linear (log %residual drug content at Room temperature)

3.4. Encapsulation efficiency

Microparticles were crushed and powdered in a mortar. Accurately weighed 100 mg of this powder were extracted in 100 ml pH 1.2 buffer for 24 h. The solution was then filtered; a sample of 5 ml was withdrawn from this solution, diluted to 50 ml with water and assayed spectrophotometrically (Shimadzu UV 1700, Japan) at 236 nm to determine the verapamil content of the microparticles.

3.5. Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) studies of verapamil and glycerol monostearate was carried out by heating the samples from 40 to 240 °C at a rate of 20 °C/min, using UNIVERSAL Q 200 V 23.5 instrument. In the drug loaded microparticles of glycerol tripalmitate, the thermogram showed a peak at 63.75 °C corresponding to the melting point of glycerol monostearate.

3.6. In-vitro release study

In vitro dissolution studies were carried out on the microparticles at 37 ± 0.5 °C at 100 rpm with USP Dissolution Apparatus II (Type II, Veego DA, 6DR Japan). For the acid stage, an accurately weighed sample of microparticles was suspended in the dissolution media consisting of 500 ml of 0.1 N (pH 1.2) hydrochloric acid without enzymes and dissolution was done for 2 h. At the end of the 2 h, 400 ml of 0.1 M tribasic sodium phosphate was added to all dissolution vessels, the pH was adjusted to 7.2 (± 0.2) and the dissolution was continued until the microparticles were depleted of drug or for 24 h (Bonferoni et al. 2000; Palmieri et al 2000).

3.7. Stability study

Formulations were stored in screw capped small glass bottles at 4 ± 1 °C, room temperature and 40 ± 1 °C and 75 ± 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) (Figs. 8 and 9), which reflected an almost linear relationship.

3.8. Statistical analysis

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

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

$\sum_{i=1}^{n-1} C_{uc}$ = 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.

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