

Formulation and optimization of fenofibrate lipospheres using Taguchi's experimental design

CHOPPARAPU SAROJA
PALGHAT KRISHNAMANI LAKSHMI

*Department of Pharmaceutics
G. Pulla Reddy College of Pharmacy
Mehdipatnam, Hyderabad, India*

Fenofibrate lipospheres were prepared by the melt dispersion technique. Critical parameters influencing particle size and entrapment efficiency were optimized by applying the L9 Taguchi experimental design. Entrapment efficiency of up to 87 % was obtained for the optimized formulation on increasing olive oil up to 30 % in the lipid carrier. Particle size analysis by microscopy and SEM revealed narrow particle size distribution and formation of discrete lipospheres of superior morphology. *In vitro* dissolution data best fitted the Higuchi model, indicating diffusion controlled release from porous lipid matrices. Prolonged release was obtained from stearic acid-olive oil lipospheres compared to cetyl alcohol-olive oil lipospheres due to the relatively hydrophobic matrix formed by stearic acid. Lipid lowering studies in Triton induced hyperlipidemia rat model demonstrated higher lipid lowering ability for fenofibrate lipospheres compared to the commercial product and plain drug.

Keywords: fenofibrate, lipospheres, olive oil, phospholipids, sustained release

Accepted November 5, 2012

Due to their inherent biodegradability and biocompatibility, lipids are now being extensively investigated as carriers (1) for drugs and proteins. Most research is focused on the development of lipid based delivery systems over polymeric carriers. Several workers have reported the cellular toxicity (2, 3) of monomers after intracellular processing of polymeric carriers.

Lipospheres comprise a lipid core with surface coated emulsifier molecules, with the drug dissolved or dispersed in it. Lipospheres have been exploited for the delivery of anesthetics (4, 5), antibiotics (6) and antifungal agents (7), for parenteral delivery of vaccines and adjuvants, for transdermal (8) and oral delivery (9). Lipospheres can be prepared by melt dispersion, solvent evaporation, microemulsion and multiple emulsion methods (10).

* Correspondence; e-mail: drlakshmisuresh@gmail.com

Fenofibrate, a BCS class II compound has been chosen as a model drug molecule to investigate the potential of lipospheres in enhancing its oral bioavailability. Oils used in the present work have been selected so as to provide an adjuvant effect on the drug due their ability to enhance high density lipids and lower the plasma triglyceride and cholesterol levels apart from formulation considerations. Fenofibrate is commercially available as micronized powder in the form of oral tablets and capsules (Trilipix®, Lipofen®, Antara®, Lipicard®). However, micronization is a time and energy consuming process and comicronization of fenofibrate with surfactants such as sodium lauryl sulfate (Lipidil micro®) causes mucosal irritation. Hence the current research work investigates lipospheres formulated with safer excipients as carriers for fenofibrate to overcome the limitations of marketed products.

EXPERIMENTAL

Materials

Fenofibrate (unmicronized) was a gift from Alembic Limited, India. Phospholipon 90H and Phospholipin 80H were kindly provided by Lipoid GmbH (Germany). Stearic acid, cetyl alcohol, soy lecithin, sodium lauryl sulphate were purchased from S.D. Fine Chemicals Limited (India). Olive oil was purchased from Sos Cuetara (Spain). Sunflower oil was purchased from Sarawwalaa Agro Refineries Limited (India). Triton WR 1339 (Tyloxapol) was purchased from Sigma Chemical Co (USA). Marketed formulation Lipicard 200 mg capsules containing micronized fenofibrate from USV Ltd. (India) and were used to compare the performance of liposphere formulations.

Preparation of fenofibrate lipospheres by the melt dispersion technique

Melted lipid mixture containing the lipophilic model drug was emulsified into an external aqueous phase containing a suitable surfactant kept at the same temperature as the lipid phase. The emulsion was mechanically stirred with a stirrer equipped with alternative impellers. The milky formulation was then rapidly cooled by immersing the formulation flask in a cool water bath without stopping the agitation to yield a uniform dispersion of lipospheres. The obtained lipospheres were then washed with water and isolated by filtration through a Whatman filter paper. Blank lipospheres (without drug) were prepared by the same method.

Determination of entrapment efficiency

Lipospheres (50 mg) were accurately weighed into centrifuge tubes to which sodium lauryl sulphate (SLS) medium (0.1 mol L^{-1} , 10 mL) was added. The preparations were centrifuged at $10,000 \text{ g}$ at 4°C for 30 min. The pellets obtained upon filtering the centrifuged preparation were washed again with 10 mL of 0.1 mol L^{-1} SLS and re-centrifuged under the same conditions for 30 min. The filtrates from two centrifugation cycles were analyzed for un-entrapped drug.

Decanted lipospheres from the above step were dissolved in 10 mL of methanol and the preparation was sonicated for 15 min to obtain a clear solution. The resulting solution, after appropriate dilution, was analyzed spectrophotometrically at 291 nm using a UV 2600 spectrophotometer (Chemito Instruments Pvt. Ltd., India) for the amount of fenofibrate entrapped in the lipospheres.

Determination of partition coefficient by the shake-flask method

The solvent system comprising octanol (100 mL) and 100 mL of water was shaken vigorously in a separating funnel for half an hour to saturate solvent layers with one another. Accurately weighed amount of 100 mg of the drug or formulation containing an equivalent amount was added and the mixtures were shaken vigorously for 30 min to allow drug distribution between the two immiscible phases and then both organic and aqueous layers were separated. Concentration of fenofibrate present in aqueous and organic layers was determined spectrophotometrically. The partition coefficient was calculated.

Particle size and surface morphology

Approximately 1 mg of liposphere formulation was mounted on a glass slide and the sample was covered with a coverslip. The mounted sample was observed under an optical microscope (Digi 3 Labomed, The New York Microscope Company, USA) using a calibrated eye piece under 45× magnification.

Morphological examination of the surface of the optimized fenofibrate formulation was carried out with a scanning electron microscope (JEOL JSM-6100, Japan). The particles were vacuum dried, coated with a thin gold-palladium layer with a sputter coater unit and observed microscopically at an accelerating voltage of 5.0 kV.

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of pure fenofibrate and the optimized liposphere formulation were recorded with a FTIR spectrophotometer (FTIR-Shimadzu 8400 S, Japan) from 4000–400 cm^{-1} using KBr pellets. The pellets were made by applying a pressure of 100 kg cm^{-2} to a mixture of lipids and KBr (1:20) for 10 min in a hydraulic press (KP, Kimaya Engineers, India).

Differential scanning calorimetry

DSC thermograms of fenofibrate, placebo lipospheres and optimized drug loaded lipospheres were recorded using a Shimadzu instrument (model TA-50 WSI, Japan) calibrated with indium. The analysis was carried out on 3.5-mg samples sealed in standard aluminum pans. Thermograms were obtained under dry nitrogen flow (25 mL min^{-1}) between zero and 400 $^{\circ}\text{C}$ at a scanning rate of 10 $^{\circ}\text{C min}^{-1}$.

In vitro release of fenofibrate from lipospheres

Lipospheres or a marketed product equivalent to 50 mg of fenofibrate were accurately weighed and filled into gelatin capsules. Degassed 0.1 mol L^{-1} SLS medium (900 mL)

was placed into the dissolution tester jars and the temperature was maintained at $37 \pm 2^\circ\text{C}$. A USP II dissolution apparatus at 75 rpm (11) was used. Samples of 5 mL were drawn at time points of 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min and an equal amount of fresh dissolution medium was replaced each time. Samples were analyzed spectrophotometrically at 291 nm (12).

The release data was subjected to kinetic treatment by zero-order (cumulative percentage drug release *vs.* time), first-order (log cumulative percentage drug released *vs.* time), Higuchi model (cumulative % drug release *vs.* square root of time) (13) to determine the release mechanism. Determination coefficients (R^2) were calculated for all the formulations. Release exponent n was calculated from the Korsmeyer-Peppas equation (14) (log cumulative % drug release *vs.* log time).

Lipid lowering study

Approval to carry out these studies was obtained from the Institutional Animal Ethics Committee (IAEC) (320/CPCSEA) of the G. Pulla Reddy College of Pharmacy, Hyderabad, India.

Male Wistar rats (250 ± 30 g) were kept in air-conditioned rooms ($24\text{--}25^\circ\text{C}$) with constant humidity. Rats starved for 18 hours were given intraperitoneal injection of Triton WR 1339 dissolved in 0.9 percent saline at a dose of 250 mg kg^{-1} to induce hyperlipidemia within 24 h. Control groups of rats were given the vehicle (plain saline) and experimental groups were given plain fenofibrate (9 mg kg^{-1} body mass) or the liposphere formulation (equivalent to 9 mg kg^{-1} fenofibrate) or the marketed product Lipicard (fenofibrate, 200 mg) capsules (equivalent to 9 mg kg^{-1} fenofibrate). Without anesthesia and restraining rats by hand, the oral dosing was performed by intubation using an 18-gauge feeding needle (the volume to be fed was 1.0 mL in all cases). To study the effect of formulation components on lipid lowering, one more group was included; this group was fed a placebo formulation. Blood samples were drawn at 0, 24 and 48 hours. Serum was separated by centrifugation at 10000 g and used for biochemical analysis. Serum cholesterol was estimated by the modified Roeschlau's method (15) and triglycerides were estimated by McGowan *et al.* and Fossati *et al.* (16) method using a standard kit obtained from ERBA diagnostics (Germany). Statistical analysis of the collected data was performed using *t*-test (unpaired, one-tailed) to test whether there was a significant difference between treated and control groups.

Table I. Variables and levels used for the Taguchi L9 orthogonal array

	Variable	Low level (1)	Medium level (2)	High level (3)
A	Lipid composition	Stearic acid/olive oil (SA+OO)	Cetyl alcohol/olive oil (CA+OO)	Stearic acid/sun flower oil (SA+SO)
B	Lipid ratio	90:10	80:20	70:30
C	Type of emulsifier	PL90H	PL80H	Soy lecithin (SL)
D	Content of emulsifier (%)	5	10	15

Orthogonal array and Taguchi experimental design

Taguchi method (17) is a combination of mathematical and statistical techniques used in an empirical study. It determines the experimental conditions having least variability as the optimum condition. The variability of a property is due to the »noise factor«. On the other hand, a factor easy to control is called the »control factor«. Variability can be expressed by the signal-to-noise ratio. The experimental condition having the maximum S/N ratio is considered the optimum condition, as the variability of characteristics is in inverse proportion to the S/N ratio.

Variables and levels were taken as denoted in Table I. The signal-to-noise (S/N) ratio was calculated for each factor level combination. S/N response for »larger is the better option« was considered. The formula for the larger-is-better S/N ratio is

$$S/N = -10 \log(\Sigma(1/Y^2)/n)$$

where Y is the response for the given factor level combination and n is the number of responses in the factor level combination. L9 orthogonal array used in the present study is represented in Table II.

RESULTS AND DISCUSSION

Optimization of entrapment efficiency and yield from Taguchi experimental runs

The objective of the present experiment is to apply the Taguchi L9 orthogonal design for optimizing liposphere properties and thereby to arrive at an optimum formula, giving lipospheres with high yield, narrow particle size distribution and higher entrapment efficiency.

It was observed in preliminary trials that a lipid/drug ratio of a minimum of 10:1 was required to obtain high entrapment efficiency. Hence, Taguchi experimental runs

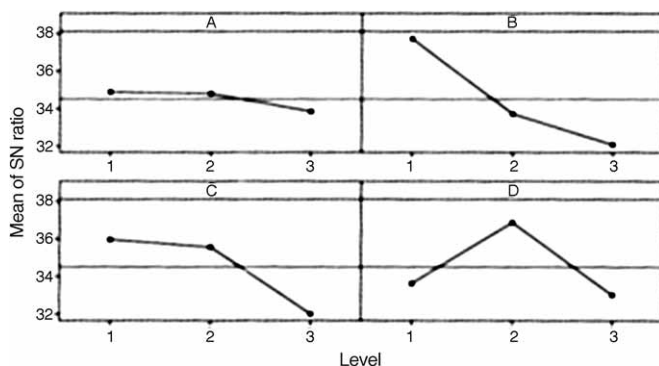


Fig. 1. Main effect plot of the S/N ratio for entrapment efficiency.

Table II. Results from Taguchi experimental runs

A	B	C	D	EE (%) ^a	S/N ratio	Yield (%)	S/N ratio	Particle size (µm)	<i>t</i> ₈₀ (min) ^b	log <i>P</i>
T1	1 (SA+OO)	1 (PL90H)	1 (5 %)	85.98	38.7	86.54	38.8	105 ± 10	210	1.36
T2	1 (SA+OO)	2 (PL80H)	2 (10 %)	73.13	37.5	80.85	38.2	90 ± 5	300	0.76
T3	1 (SA+OO)	3 (70:30)	3 (15 %)	28.11	28.5	42.05	32.0	30 ± 15	240	0.74
T4	2 (CA+OO)	2 (PL80H)	3 (15 %)	77.21	37.6	81.71	38.3	45 ± 5	30	0.74
T5	2 (CA+OO)	3 (SL)	1 (5 %)	32.50	30.6	71.60	37.2	100 ± 5	270	1.09
T6	2 (CA+OO)	3 (70:30)	2 (10 %)	65.31	36.2	61.21	35.9	150 ± 5	60	0.75
T7	3 (SA+SO)	1 (90:10)	2 (10 %)	70.01	36.9	72.72	37.3	60 ± 10	270	0.76
T8	3 (SA+SO)	2 (80:20)	1 (15 %)	45.22	33.0	61.94	35.9	75 ± 10	240	0.83
T9	3 (SA+SO)	3 (70:30)	2 (PL80H)	38.39	31.6	44.34	33.2	90 ± 5	150	1.02

SA – stearic acid, OO – olive oil, CA – cetyl alcohol, SO – sunflower oil, SL – soy lecithin, PL80H – Phospholipin 80H, PL90H – Phospholipin 90H.

^a Entrapment efficiency.

^b *t*₈₀ (min) for Lipicard® was found to be 30 min.

were done at this ratio. S/N ratios for output parameters, namely, entrapment efficiency and yield, were calculated. Results are shown in Table II.

The formula showing highest entrapment efficiency (OF1) of 89.9 % was found to be A1, B1, C1, D2, corresponding to stearic acid/olive oil, 90:10, phospholipon 90H, 10 %, respectively (Fig. 1).

The optimum formula for yield (OF2) was found to be A2, B1, C1, D2, corresponding to cetyl alcohol/olive oil, 90:10, phospholipon 90H, 10 %, respectively.

Factors influencing entrapment efficiency

Lipid/oil ratio. – At a constant phospholipid amount, the lipid core formulated with stearic acid alone (without vegetable oils) gave lipospheres of highest entrapment efficiency. As the amount of oil in the lipid core increased, the entrapment efficiency decreased. This observation is contrary to the concept of nanolipid carriers (18), which in theory should increase loadability of the drug due to the formation of an asymmetric lipid structure providing more room for drug loading. The decrease in entrapment efficiency of the fenofibrate liposphere formulation containing oils was probably due to the limiting and competing solubility of oil and lipophilic drug (fenofibrate) in the solid lipid. Olive oil (rich in monounsaturated fatty acids, MUFA) based lipospheres showed higher drug entrapment compared to sunflower oil (rich in polyunsaturated fatty acids, PUFA) based lipospheres due to higher solubility of MUFA in saturated fat (stearic acid) which provides more room for drug loading compared to PUFA (19).

Type of phospholipid. – Entrapment efficiency of fenofibrate was found to increase in lipospheres formulated with synthetic emulsifiers like Phospholipon 90H and 80H compared to the natural emulsifier used (soy lecithin). This was probably due to the reported leaky nature of phospholipid membranes formed by natural phospholipids compared to synthetic lipids.

Partition coefficient

A drastic reduction in the partition coefficient of the drug entrapped in lipospheres was observed ($\log P$ ranging from 0.7–1.3) compared to the drug itself ($\log P$ of fenofibrate is 5.3). This effective reduction of the $\log P$ value indicates improved partitioning of the drug from the formulation into aqueous phase, which is attributed to an increase in the amount of emulsifier. Results are presented in Table II.

Particle size

From Table II it is evident that the combination of stearic acid and olive oil with the highest amount (15 %) of emulsifier gave the smallest particles, as demonstrated by trial T3. Among all the emulsifiers used, soy lecithin resulted in the smallest particle size but the lipospheres were found to be aggregated when observed under microscope. As the lipid/oil ratio increased, the particle size of lipospheres in Taguchi trials (T4, T7) decreased. This decrease in particle size was probably due to the availability of higher amounts of carrier material and emulsifier for the formation of discrete spherical particles.



Fig 2. SEM image of fenofibrate lipospheres.

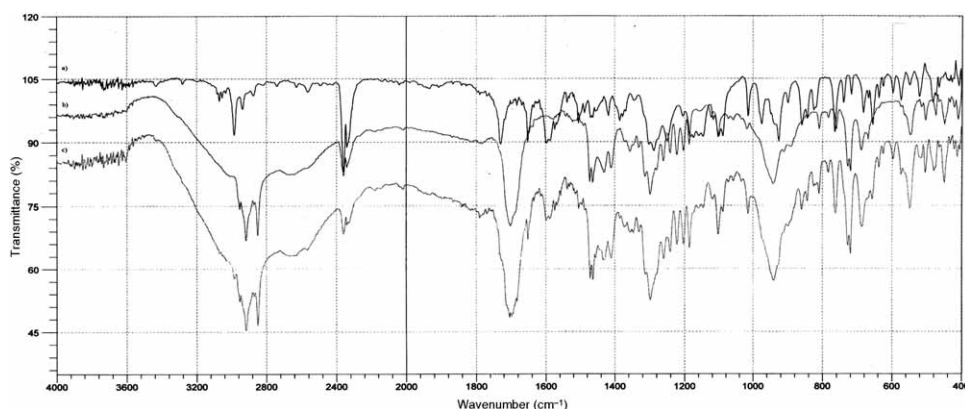


Fig. 3. FTIR spectrum of a) pure drug, b) excipients, c) physical mixture of drug and excipients of OF1 formulation.

Fig. 2 shows the scanning electron microscopic image of fenofibrate lipospheres, which substantiates the formation of discrete particles of 100 μm with smooth surfaces.

Optimized fenofibrate formulation was prepared at three different stirring speeds, namely 750, 1500 and 2500 rpm (data not shown). A drastic reduction in particle size was observed when the stirring speed of 1500 rpm was used, resulting in a particle size of 60–75 μm when compared to the stirring speed of 750 rpm (180 μm). Decrease in particle size was only minimal when the stirring speed was changed from 1500 rpm (60–75 μm) to 2500 rpm (30–45 μm). This result is in accord with the fact that the particle size decreases linearly with an increase in stirring speed up to a particular value, after which the relationship between particle size and stirring speed becomes nonlinear.

Drug-lipid compatibility

Drug-lipid compatibility in optimized lipospheres was evaluated by FTIR and DSC analysis. FTIR spectra are displayed in Fig. 3. The principal peaks of fenofibrate were observed between 600–800 cm^{-1} ; they resulted from C–Cl stretching. The peaks at 1729.4,

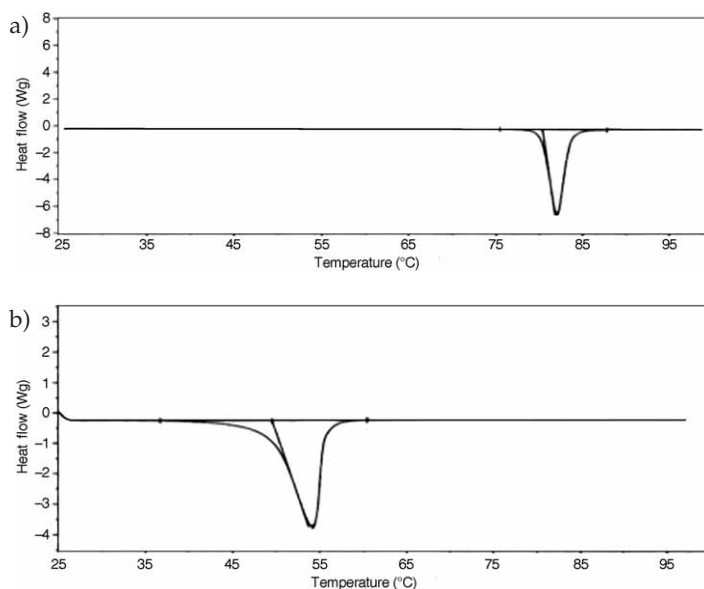


Fig. 4. DSC thermogram of: a) fenofibrate (pure drug), b) formulation (OF1).

1651.0, 1598.9 and 925.1 cm^{-1} correspond to C=O, C=C stretching of the carboxylate group and C-H bending, respectively. These characteristic peaks were identified in a physical mixture of the drug and excipients with no appreciable changes in frequency. Thus, it can be inferred that there was no chemical interaction between the drug and lipids used in the formulation.

DSC thermogram of fenofibrate showed an endothermic peak at 82 °C, which is within the melting range of the drug (Fig. 4a). DSC thermogram of pure stearic acid showed an endothermic peak at 54.13 °C, which is the reported melting point of stearic acid. Plain lipospheres prepared by the melt dispersion method showed an endothermic peak at 51.38 °C and a small exotherm at 64.46 °C. Drug loaded lipospheres prepared by the same method showed a large endothermic peak at 49.53 °C and disappearance of the drug peak (Fig. 4b), suggesting that the drug is present in form of molecular dispersion in the loaded lipospheres and that fenofibrate exists in amorphous state rather than in crystalline state. Also, the melting point for stearic acid decreased from 54.13 to 51.38 °C and 49.53 °C for plain and drug loaded lipospheres, respectively, which could be attributed to the smaller size of prepared lipospheres. Also, the inclusion of drug molecules in the lipid is normally accompanied by a depression in the melting point of the lipid.

In vitro release

It was observed in the *in vitro* release study that OF1 (stearic acid and olive oil lipospheres) extended the release of the drug for up to 5 h whereas OF2 (cetyl alcohol and olive oil lipospheres) gave immediate release of the drug similar to the marketed formulation containing fenofibrate in micronized form (Fig. 5). This is probably due to the

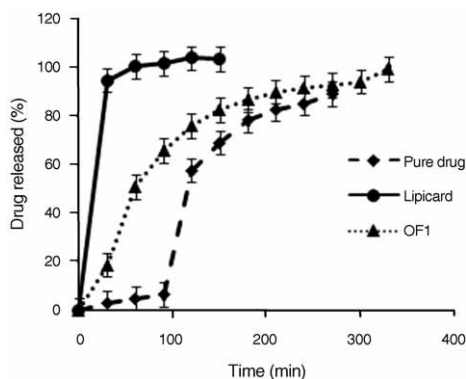


Fig. 5. Comparative *in vitro* release profiles of: pure drug, Lipicard and OF1 (stearic acid:olive oil, 90:10, phospholipon 90H, 10 % showing sustained release). Mean \pm SD, $n = 3$.

higher affinity of alcohol groups towards the dissolution medium promoting matrix hydration, which provides a hydrophilic pathway for water molecules to solubilize the drug, thus leading to an increase in dissolution rate. Owing to the crystalline nature of the lipid matrix formed due to interaction of stearic acid with sodium ions in SLS medium, drug solubility and hence release are assumed to be retarded (20).

Release profiles of Taguchi experimental runs were fitted into zero-order, first-order and Higuchi models (13) to determine release kinetics and the mechanism of drug release. Regression coefficient values from Table III clearly indicate that the data best fitted the Higuchi model, indicating diffusion controlled release from porous lipid matrices. To characterize the diffusional release, the Korsmeyer-Peppas model (14) was applied. Since the release component value n for the majority of Taguchi runs is between 0.5 and 1.0 indicating anomalous release, both diffusion and dissolution from polymer matrix appear to govern the drug release (21).

Lipid lowering studies

Table IV shows the serum cholesterol and triglyceride levels at 0, 24 and 48 h. A drastic increase in serum cholesterol and triglyceride levels after 24 h indicates induction of hyperlipidemia in male Wistar rats due to Triton treatment. However, after treatment with the plain drug, marketed product (Lipicard®) and fenofibrate lipospheres (OF1) to respective groups at 9 mg kg⁻¹ orally, the liposphere formulation demonstrated the highest lipid (cholesterol and triglycerides) lowering. *In vivo* studies indicated that the lowering of serum cholesterol and triglycerides by the liposphere formulation was comparable to that of the marketed product. Significant difference between Triton control *vs.* fenofibrate lipospheres (OF1)/marketed formulation was obtained at 24 and 48 h, especially for triglyceride levels compared to cholesterol levels. This is in accord with the fact that the fibrate class of drugs mainly act by lowering the triglyceride levels. No significant difference between the control and plain drug was observed due to the drug poor bioavailability.

Table III. Release kinetics of fenofibrate lipospheres

Formulation	Zero-order		First-order		Higuchi		Kors meyer-Peppas	Release mechanism
	R^2	k_0 (% min ⁻¹) ^a	R^2	k_1 (min ⁻¹) ^a	R^2	k_H (% min ^{-1/2}) ^a	n^b	
T1	0.911	0.256	0.819	0.001	0.971	6.338	0.603	Anomalous
T2	0.979	0.224	0.885	0.001	0.986	5.389	0.595	Anomalous
T3	0.974	0.270	0.882	0.002	0.999	6.049	0.599	Anomalous
T4	0.991	0.333	0.980	0.002	0.999	6.674	0.592	Anomalous
T5	0.960	0.261	0.820	0.003	0.987	5.855	0.993	Anomalous
T6	0.982	0.325	0.936	0.002	0.986	7.630	0.761	Anomalous
T7	0.975	0.280	0.879	0.002	0.998	6.266	0.694	Anomalous
T8	0.926	0.100	0.890	0.032	0.970	2.469	0.166	Fickian diffusion
T9	0.948	0.193	0.924	0.001	0.977	3.494	0.203	Fickian diffusion

^a k_0 , k_1 and k_H – release rate constants for zero-order, first-order and Higuchi model, respectively; ^b Release exponent.

Table IV. Treatment of serum cholesterol and triglyceride levels in vivo

Treatment group	Cholesterol (mg per 100 mL)			Triglycerides (mg per 100 mL)		
	Initial	24 h	48 h	Initial	24 h	48 h
Normal control	236.7 ± 5.6	238.9 ± 4.5	235.7 ± 6.4	180.5 ± 7.3	188.4 ± 8.9	184.9 ± 10.3
Triton control	236.7 ± 6.8	540.4 ± 5.2	549.8 ± 5.9	178.9 ± 11.8	332.0 ± 9.2	322.0 ± 5.4
Placebo	235.8 ± 5.8	580.0 ± 7.2	575.9 ± 4.1	165.6 ± 10.8	350.2 ± 8.7	360.5 ± 5.4
Plain drug	230.8 ± 10.4	642.8 ± 5.9	486.1 ± 12.3	200.2 ± 8.2	379.8 ± 7.1	288.4 ± 4.8
Marketed product (Lipicard)	230.6 ± 8.6	683.4 ± 9.3	53.5 ± 6.2	188.7 ± 9.9	414.0 ± 4.8 ^a	139.0 ± 10.6 ^a
Fenofibrate lipospheres	222.1 ± 4.3	663.0 ± 5.9 ^a	33.7 ± 7.3 ^a	163.5 ± 3.7	375.0 ± 6.9 ^a	130.9 ± 2.5 ^a

Mean ± SD, $n = 6$. ^a Indicates statistically significant difference between treated group and Triton control at $p < 0.002$.

CONCLUSIONS

Fenofibrate containing lipospheres were prepared by hot emulsification and cold re-solidification of a mixture of lipids and oils. Lipid/oil ratio was found to play an important role in the formation of discrete lipospheres and in drug entrapment. About 85 % of the drug was found to be entrapped at 10 % of oil in lipid in the optimized for-

mulation, OF1. However, entrapment efficiency was found to decrease with an increase in the amount of oil in the lipid carrier. Drug release from lipospheres varied from prolonged release shown by OF1 (stearic acid and olive oil lipospheres) to immediate release shown by OF2 (cetyl alcohol and olive oil lipospheres), indicating the role of the lipid type in altering drug release. *In vivo* studies in male Wistar rats indicated that OF1 demonstrated a lowering of serum cholesterol levels in comparison with the marketed product. It can be concluded from these results that the liposphere formulation can be exploited as a novel drug delivery system for poorly soluble drugs like fenofibrate as these lipospheres can be prepared by using easily available, inexpensive, GRAS listed excipients and simple preparation techniques resulting in bioavailable fenofibrate formulations.

REFERENCES

1. G. Fricker, T. Kromp, A. Wendel, A. Blume, J. Zirkel, H. Rebmann, C. Setzer, R. O. Quinkert, F. Martin and C. M. Goymann, Phospholipids and lipid-based formulations in oral drug delivery, *Pharm. Res.* **27** (2010) 1469–1486; DOI: 10.1007/s11095-010-0130-x.
2. Y. C. Tseng, Y. Tabata, S. H. Hyon and Y. Ikada, In vitro toxicity test of 2 cyanoacrylate polymers by cell culture method, *J. Biomed. Mater. Res. A* **24** (1990) 1355–1367; DOI: 10.1002/jbm.820241007.
3. K. Lam, J. Schakenraad, H. Esselbrugge, J. Feijen and P. Nieuwenhuis, The effect of phagocytosis of poly(L-lactic acid) fragments on cellular morphology and viability, *J. Biomed. Mater. Res. A* **27** (1993) 1569–1577; DOI: 10.1002/jbm.820271214.
4. D. B. Masters and A. J. Domb, Liposphere local anesthetic timed-release for perineural site application, *Pharm. Res.* **15** (1998) 1038–1045; DOI: 10.1023/A:1011978010724.
5. S. Toongsuwan, L. C. Li, B. K. Erickson and H. C. Chang, Formulation and characterization of bupivacaine lipospheres, *Int. J. Pharm.* **280** (2004) 57–65; DOI: 10.1016/j.bbr.2011.03.031.
6. A. J. Domb, Long acting injectable oxytetracycline-liposphere formulations, *Int. J. Pharm.* **124** (1995) 271–278; DOI: 10.1016/0378-5173(95)00098-4.
7. B. Amarji, D. Raghuwanshi, S. Vyas and P. Kanaujia, Lipid nano spheres (LNSs) for enhanced oral bioavailability of amphotericin B: development and characterization, *J. Biomed. Nanotechnol.* **3** (2007) 264–269; DOI: 10.1166/jbn.2007.029.
8. A. Bhatia, B. Singh, V. Rani and O. Katare, Formulation, characterization, and evaluation of benzocaine phospholipid-tagged lipospheres for topical application, *J. Biomed. Nanotechnol.* **3** (2007) 81–89; DOI: 10.1166/jbn.2007.009.
9. T. Bekerman, J. Golenser and A. Domb, Cyclosporin nanoparticulate lipospheres for oral administration, *J. Pharm. Sci.* **93** (2004) 1264–1270; DOI: 10.1002/jps.20057.
10. N. Sateeshbabu and K. Gowthamarajan, Manufacturing techniques of lipospheres: overview, *Int. J. Pharm. Pharm. Sci.* **3** (2011) 17–21.
11. *United States Pharmacopoeia XXXI, National Formulary XXVI*, USP Convention, Rockville (MD) 2008, pp. 2145–2146.
12. J. J. Sheng, P. J. Sirois, J. B. Dressman and G. L. Amidon, Particle diffusional layer thickness in a USP dissolution apparatus II: a combined function of particle size and paddle speed, *J. Pharm. Sci.* **97** (2008) 4815–4829; DOI: 10.1002/jps.21345.
13. T. Higuchi, Mechanisms of sustained action medications: theoretical analysis of rate of release of solid drugs dispersed in solid matrices, *J. Pharm. Sci.* **52** (1963) 1145–1149; DOI: 10.1002/jps.2600521210.

14. R. W. Korsmeyer, R. Gurny, E. Doelker, P. Buri and N. A. Peppas, Mechanisms of solute release from porous hydrophilic polymers, *Int. J. Pharm.* **15** (1983) 25–35; DOI: 10.1016/0378-5173(83)90064-9.
15. P. Röeschlau, E. Bernt and W. Gruber, Enzymatic determination of total cholesterol in serum, *Z. Klin. Chem. Klin. Biochem.* **12** (1974) 403–407.
16. P. Fossati and L. Prencipe, Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide, *Clin. Chem.* **28** (1982) 2077–2080.
17. K. D. Kim, D. N. Han and H. T. Kim, Optimization of experimental conditions based on the Taguchi robust design for the formation of nano-sized silver particles by chemical reduction method, *Chem. Eng. J.* **104** (2004) 55–61; DOI: 10.1016/j.cej.2004.08.003.
18. L. Mu and S. S. Feng, Fabrication, characterization and in vitro release of paclitaxel (Taxol®) loaded poly(lactic-co-glycolic acid) microspheres prepared by spray drying technique with lipid/cholesterol emulsifiers, *J. Control. Release* **76** (2001) 239–254; DOI: 10.1016/S0168-3659(01)00440-0.
19. A. Dubey, P. Prabhu and J. V. Kamath, Nano structured lipid carriers: A novel topical drug delivery system, *Int. J. Pharm. Tech. Res.* **4** (2012) 705–714.
20. M. Nasr, S. Mansour, N. D. Mortada and A. El. Shamy, Lipospheres as carriers for topical delivery of aceclofenac: Preparation, characterization and in vivo evaluation, *AAPS PharmSciTech.* **9** (2008) 154–162; DOI: 10.1208/s12249-007-9028-2.
21. M. Ozyazici, E. H. Gökçe and G. Ertan, Release and diffusional modeling of metronidazole lipid matrices, *Eur. J. Pharm. Biopharm.* **63** (2006) 331–339; DOI: 10.1016/j.ejpb.2006.02.005.