Gelatin Adsorbed Solid Lipid Nanoparticles (SLN) For Targeted Drug Delivery Of Anti -Inflammatory Drug

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Abstract

The purpose of this research was to study the effects of surface modified solid lipid nanoparticle of diclofenac as targeted and controlled drug delivery system. Diclofenac SLN were developed using glyceryl monostearate by solvent emulsification diffusion technique followed by sonication and then characterized by particle size analysis, zeta potential, TEM, drug entrapment efficiency. The in vitro dissolution profile showed that the GSLN were able to sustain the release of the Diclofenac for considerable period of time (89.04% within 24 hr.). The in vitro data fits to zero order, first order, Higuchi model, r^2 value showed the drug release characteristics and mechanism. The paw edema test after i.p administration showed that GSLN had extended anti-inflammatory effects compared with Diclofenac. The stability study showed the no alteration in physical appearance, size, shape, drug content and in-vitro drug release after storage at 4^0C and 25^0C during the 60 day (7, 15, 30, 45, and 60 days). These results suggest that GSLN could be promising target drug delivery for Diclofenac with an extended pharmacological effect owing to delayed released of parent drug and were stable at room temperature.

Keywords: Diclofenac: solid lipid nanoparticle: sustain release system: anti-inflammatory: targeted drug delivery.

Abbreviations: SLN, solid lipid nanoparticle: GSLN, gelatin adsorbed solid lipid nanoparticle: PCS, photon correlation spectroscopy: NSAID, non-steroidal anti-inflammatory drug: GMS, glyceryl monosterate: TEM, transmission electron micro spectroscopy.

INTRODUCTION

SLN are sub-micron colloidal carriers (50-1000nm) which are composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. [1] Solid lipid nanoparticles (SLNs) have recently gained significant attention as potential alternate colloidal drug delivery systems for liposomes and lipid emulsions. The use of solid lipid is an attractive innovation that is advantageous because the solid matrix of the lipid provides more flexibility in controlling the drug release and protects the encapsulated ingredients from chemical degradation. [2] The efforts to improve drug effectiveness have led to developments in drug delivery technology. Targeted drug delivery implies selective and effective localization of pharmacologically active ingredient at preselected target in therapeutic concentration, while restricting its access to non-target area, thus maximizing the effectiveness of the drug. [3] SLNs have attracted increasing attention as a potential

drug delivery carrier owing to their numerous advantages such as the possibility of controlled drug release and drug targeting, the increased drug stability, the high drug payload, the possibility of incorporation of lipophilic and hydrophilic drugs, the biocompatibility of the lipid carrier, the avoidance of organic solvents, as well as the feasibility of large-scale production. [4] Diclofenac a Benzenacetic acid, 2, [(2, 6 - dichloro phenyl) amino] benzoic acid is an active NSAID indicated for the symptomatic treatment of pain and inflammation. It is a cyclooxygenase inhibitor and also antagonizes the actions of bradykinin. Recommended dose 150 mg daily in divided doses, mean plasma elimination half-life is 1 to 2 hour. To reduce the dosing frequency and adverse effects during prolong treatment it is needed to formulate in long acting dosage from.

Surface modification of solid lipid nanoparticles:

Surface modification of SLN with specific ligands have been attempted to use SLNs as targeted drug delivery system. Gelatin has a specific interaction with fibronectin; excess fibronectin in the local tissue is associated with a number of disease such as inflammatory disorders, cardiovascular disease, rheumatoid arthritis, and cancer. [5] In the group of Non-steroidal anti-inflammatory class of drug Diclofenac is a very useful candidate in the case of inflammation and rheumatic disorder. SLNs are used as targeted drug delivery system due to; Small size and relatively narrow size distribution which provide biological opportunities for site – specific drug delivery, controlled release of active drug over a long period can be achieved and surface modification can easily be accomplished and hence can be used for site specific drug delivery system.

MATERIALS AND METHODS

Materials:

Diclofenac is a gift sample from Modi mundi pharma pvt. Ltd., Glyceryl monosterate (GMS), Poloxamer 188, tween 80 from Hi Media Laboratories pvt. Ltd. Carrageenan sodium salt was purchased from (SD fine-CHEM. Limited, Mumbai, India) and other chemicals are analytical grades.

Preparation of diclofenac solid lipid nanoparticles:

SLN were prepared by a modified solvent emulsification diffusion followed by sonication method. Triglycerides (75mg) were each dissolved in 2 ml mixture of methanol and chloroform (1:1) respectively (internal oil phase). Powdered diclofenac (50 mg) was dispersed in the above solution and sonicate for two minute.(Sartorius,labsonic P).The resulting dispersion was poured into a homogenizer tube containing 8 ml aqueous solution of mixture of surfactant and co-surfactant (external aqueous phase) while homogenizing for 30 minutes to form o\w emulsion. After homogenization the above emulsion was poured into ice-cold water up to 50 ml and stirred for 2 hour to diffuse the organic solvent into water. The above dispersion was centrifuged at 10000 rpm for 10 minute and the solid material was re-dispersed with distilled water and sonicate for 5 minute. [6, 10]

Measurement of Size and Zeta potential of SLN:

Average particle size, surface charge (zeta potential) and polydispersity index of solid lipid nanoparticles were measured by photon correlation spectroscopy using a zetasizer (Malvern, UK). Samples were diluted appropriately with the aqueous phase of the formulation for the measurements. Zeta potential measurements were done at 25^0C, and electric field strength was around 23.2V/cm

Entrapment Efficiency:

The entrapment efficiency of the drug was determined by measuring the concentration of free drug in the dispersion medium by UV-Visible spectroscopy, as mentioned below. A quantity of 1ml solid lipid nanoparticles dispersion was measured and transfer into a volumetric flask containing 10 ml methanol, mix them and kept for overnight at mechanical shaker, after vertexing the sample was filtered through whatman filter paper and absorbance was determined spectroscopic ally using UV-VIS Spectrophotometer (Shimazdu) at 268 nm. The concentration of drug was determined from the calibration curve.

Stability studies;

Diclofenac SLNs of triglyceride were stored at 4 ± 1^0C and 25 ± 1^0C for 2 months and Sample were analyzed for average size, entrapment efficiency and physical appearance after a period of 7, 15, 30, 45 and 60 days. The % drug content vs time and log % drug content vs time graph was plotted in order to evaluate shelf-life of the formulation.

In vitro release of diclofenac from SLN:

In vitro release studies were performed using the Franz diffusion cell. Dialysis membrane having pore diameter 2.4 nm, molecular weight cutoff between 12-14 kD was used. The membrane was activated by incubating in 5% EDTA solution for 0.5 h and then in boiling water for 1h before mounting in a Franz diffusion cell. Diclofenac formulation (1ml) was placed in the donor compartment and the receptor compartment was filled with dialysis medium (phosphate buffer pH 7.4). At fixed time intervals, 1ml of the sample was withdrawn from receiver compartment through side tube. Fresh phosphate buffer solution was placed to maintain constant volume. Samples were analyzed by UV-Visible spectroscopy.

Characterization by transmission electron microscopy (TEM):

The morphology of SLNs was examined using an electronic transmission microscope (Morgagni 268 D; Japan). After diluting 20-fold with the original dispersion medium of the preparation, the samples were negatively stained with 1.5% (w/v) phosphotungustic acid (PT) acid for observation.

Pharmacokinetic study:

In the present study, the drug release data were plotted using various kinetic equations (Zero order, first order and Higuchi's kinetics, Kerseymere's equation,) to evaluate the drug release mechanism and kinetics. To study the release kinetics and mechanism of drug release, data obtained from in vitro drug release studies were plotted in various kinetic models: Zero order as cumulative amount of drug released vs time, first order as log cumulative percentage of drug remaining vs time, and Higuchi's model as cumulative percentage of drug released vs. square root of time and as log cumulative percentage of drug released vs log time. Biopharmaceutical evaluation, and in-vivo/in-vitro correlations were beyond the scope of this study and will be considered in future work.

In vivo study using carrageenan-induced paw edema model:

Paw edema was induced by injecting 0.1 ml of 1% carrageenan in phosphate buffer saline (pH 7.4) into the sub plantar tissues of the left hind paw of each rat. [7] Each groups of rats (n=6) was treated with as control (vehicle), standard (diclofenac) and test (solid lipid nanoparticles and gelatin adsorbed solid lipid nanoparticles) by i.p. injection 1 hour before the carrageenan injection. The swelling volume of the paw measured before (time o) and at 1, 2, 3, 4, 5, 6, 12 and 24 hours after the carrageenan injection, the degree of paw edema was determined by measuring the hind paw volume with pleythysmometer. The percent inhibition of the edema was calculated by the formula [1-T/Cl x 100, where T and C are the mean paw volumes in the drug/ SLNs treated and control groups respectively].

RESULTS AND DISCUSSION

Preparation of SLN:

SLN have been prepared by various researchers using different methods. In the present study, we have developed an economical, simple, and reproducible method for the preparation of SLN, i.e. solvent diffusion followed by sonication. To disperse the diclofenac homogeneously in the lipid, solvent system chloroform\ methanol (1:1) was used, concentration of surfactant was optimized to 2.5% and stirring speed to 4000rpm. There was no decrease in particle size with further increase in surfactant concentration. To reduce the particle size probe ultrasonicator was used. An eight minutes sonication was necessary to obtained solid lipid nanoparticles in the range of 80-360 nm with narrow size distribution.

Measurement of Size and Zeta potential of SLN:

An adequate characterization of the solid lipid nanoparticles is a necessity for the controle of the quality of the product. PCS the most powerful techniques for the routine measurement of particle size. [8] The average diameter of SLN and GSLN measured by PCS Zetasizer was 94.08 nm and 101.45 nm respectively (n=3) was showed in fig.1.

The measurement of zeta potential allows prediction about the storage stability of colloidal dispersion. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. The zeta potential distribution of SLN was showed in table 1. The mean zeta potential was –20.5mV (n=3) Therefore, this method had gained a relative good stability and dispersion quality. [9]

Table 1 The mean diameter, zeta potential and entrapment efficiency of different SLN formulations

Formulation	Mean diameter (nm)	Zeta potential (mV)	Entrapment efficiency (%)
SLN 1	94.08±1.06	−20.5±1.5	49.61
SLN 2	98.89±1.01	−19.6±0.6	48.92
SLN 3	106.5±0.98	−26.3±1.2	46.54
SLN 4	118.5±0.67	−15.5.±1.1	45.06
SLN 5	313.0±1.12	−20.9±0.7	41.98
SLN 6	336.5±0.93	−20.4±0.5	38.75
SLN 7	422.0±0.74	−16.4 ±1.8	37.08

SLN 8	672.0±0.69	−18.4±0.8	34.32
SLN 9	812.1±0.42	− 22.6±0.3	42.62
SLN 10	873.1±0.26	−23.8±0.1	31.25

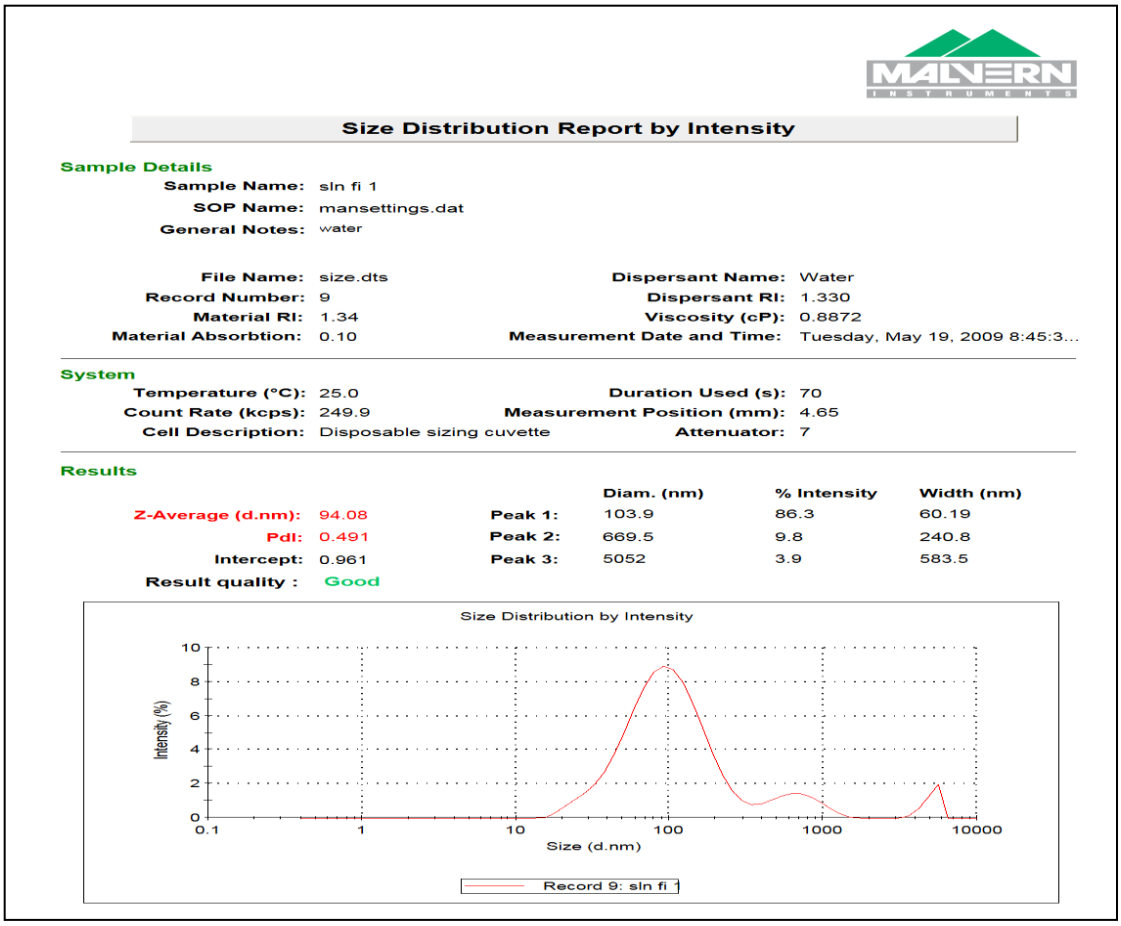

Figure 1 Size distribution report of solid lipid nanoparticles

Transmission electron microscopy (TEM)

TEM shows that the particles had round and uniform shapes. The mean diameters of SLN and GSLN were found approximately 94 nm and 101 nm, respectively (plate. 2a and 2b) was showed in fig. 2.

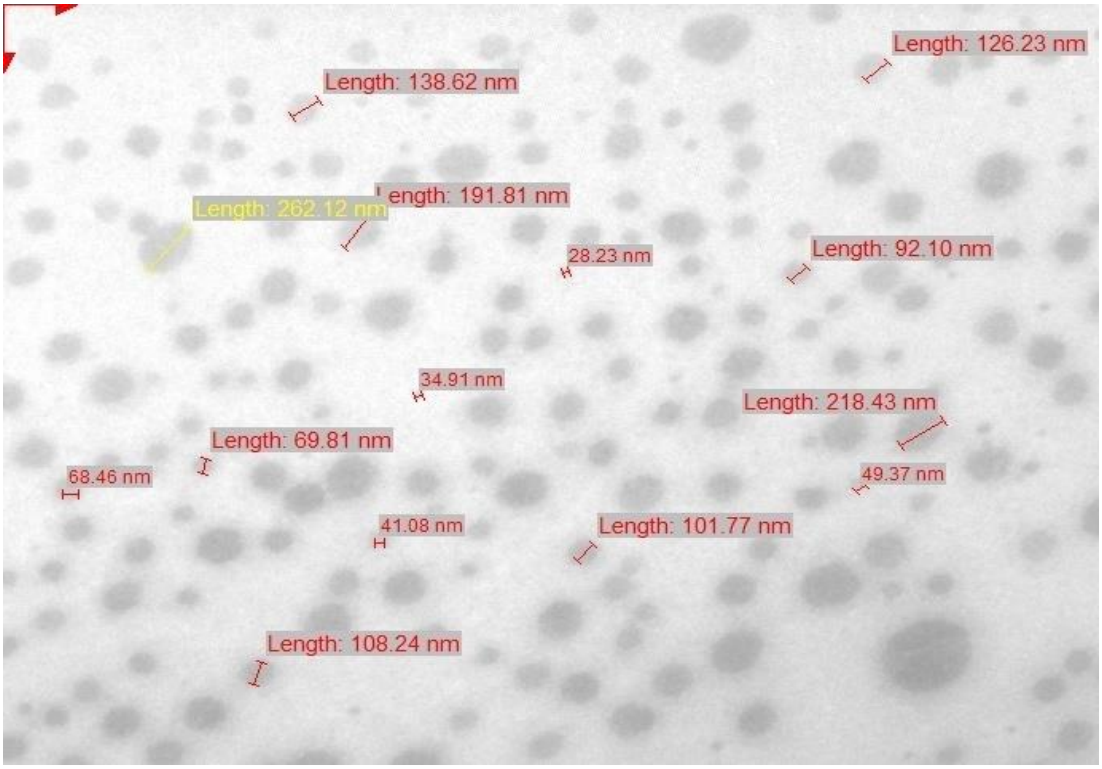

Figure 2 Plate 2 a Solid lipid nanoparticles - 94.80 nm

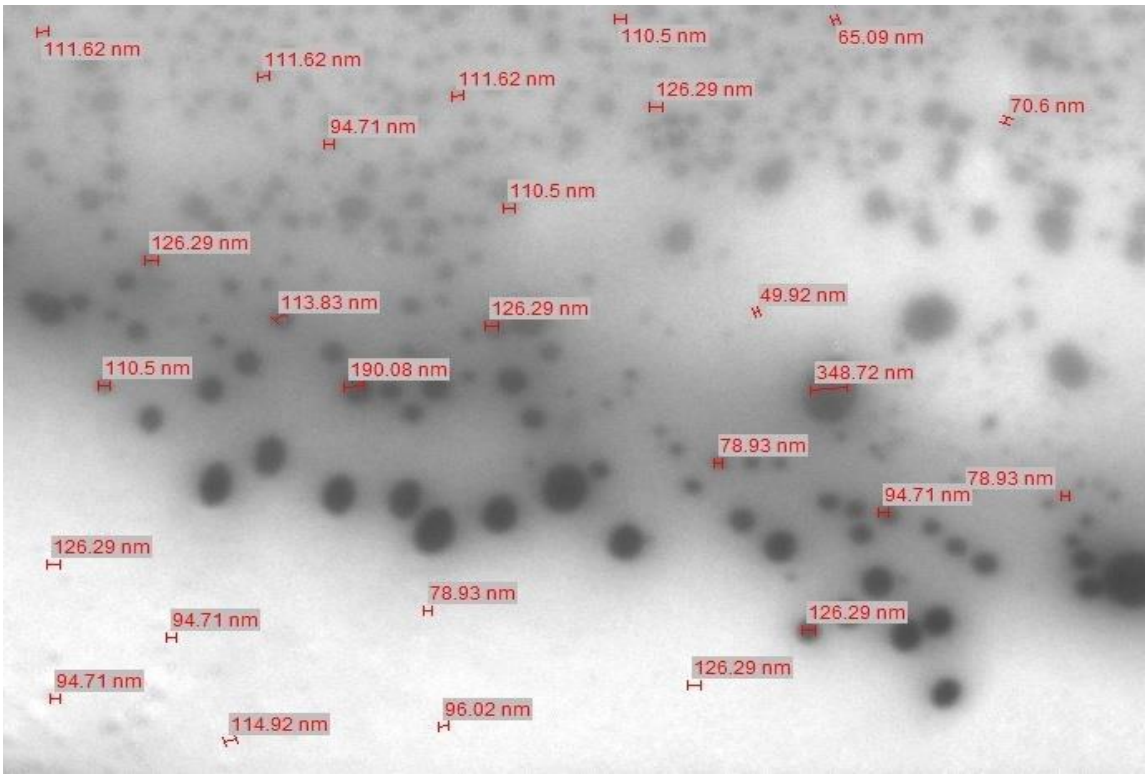

Figure 2 Plate 2 b Gelatin adsorbed solid lipid nanoparticles-101.45 nm

Entrapment Efficiency:

As shown in table 2 and Fig. 3 as the percent of surfactant increases (from 0.5 to 3.5%) the entrapment efficiency was first increase (up to 2.5%) and then becomes almost constant. This can be attributed to the fact that 2.5% surfactant led to a saturation of the higher loading level.

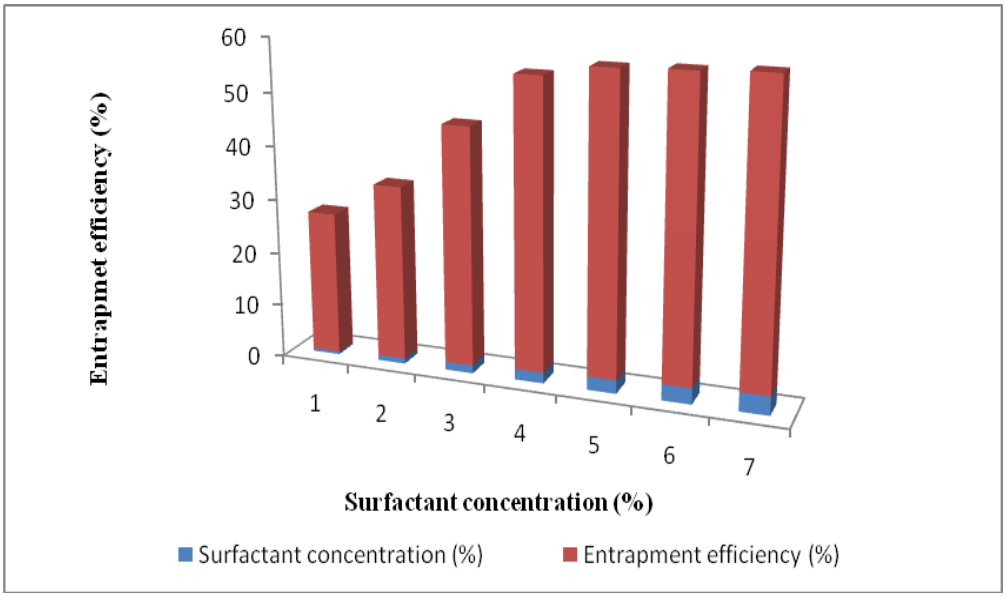

Figure 3 Effect of surfactant concentration on the drug entrapment efficiency

Table 2 Effect of surfactant concentration on the drug entrapment efficiency

S. No.	concentration of surfactant (%)	Entrapment efficiency (%)
1	0.5	26.64
2	1.0	32.52
3	1.5	44.19
4	2.0	53.51
5	2.5	55.12
6	3.0	55.03
7	3.5	54.98

Stability data:

Gelatin Adsorbed solid lipid nanoparticle of Diclofenac (GSLN-1) were stored in glass container at $4^0 \pm 1^0$C, and room temperature (25^0C) and Relative Humidity 65% (RH) for period of 60 days and evaluated for physical appearance and drug content (Table 3).

Table 3 Effect of aging on residual drug content at 4 ± 1^0C and room temperature

S. No.	parameters	storage condition	
		4 ± 1^0C	room temperature
1	K (days)	3.8×10^{-4}	5.6×10^{-4}
2	$T_{10\%}$ (days)	273	185
3	$t_{1/2}$ (days)	1796	1217

K= degradation rate constant, $T_{10\%}$= shelf life of drug, $t_{1/2}$ = half-life of drug

GSLN formulation stored at 4 ± 1^0C showed k value as 3.8×10^{-4} and $t_{10\%}$ value as 273 days, while those stored at room temperature showed k value as 5.6×10^{-4} and $t_{10\%}$ value as185 days. The $t_{10\%}$ obtained in case of formulation stored at room temperature was found lower in comparison with the formulation stored at lower temperature (4 ± 1^0C). The results of stability studies suggest that for adequate shelf life of GSLN formulation the ideal storage temperature is 4 ± 1^0C.

In vitro release of diclofenac from SLN:

It was found that the optimum drug release observed from the formulation (SLN and GSLN) was up to 24 h about 89.98% and 89.02% respectively. In vitro release gets decreases marginally after gelatin adsorption Fig. 4a and 4b.

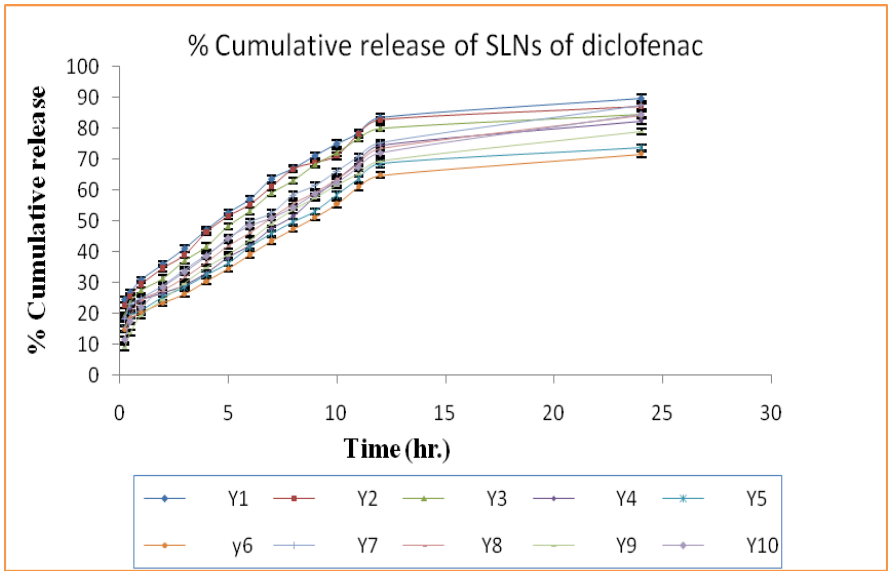

Figure 4a Percent Cumulative release of SLNs of Diclofenac in PBS (pH 7.4)

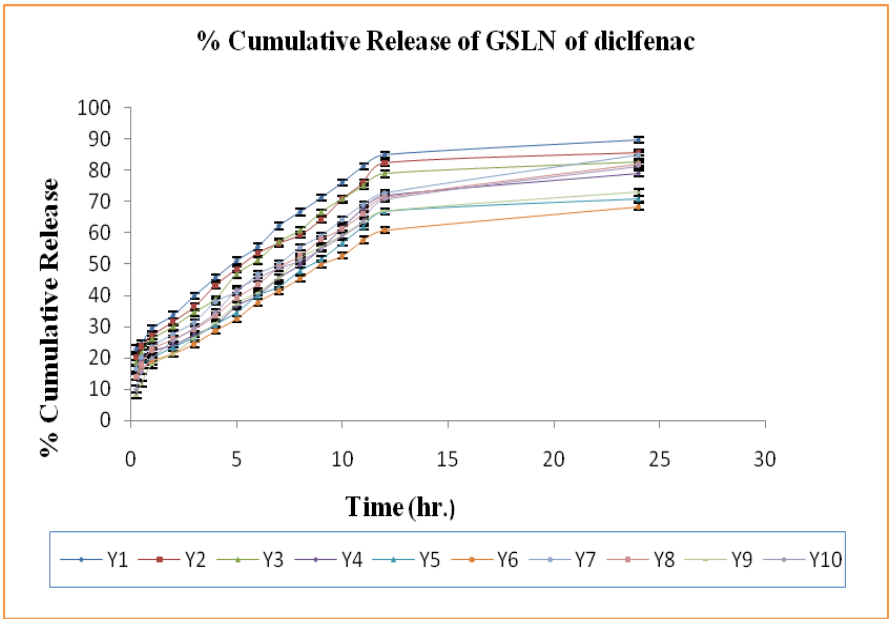

Figure 4.b Percent Cumulative release of GSLNs of Diclofenac in PBS (pH 7.4)

Pharmacokinetic study (In vitro release kinetics):

The obtained release, data was fitted into zero order, first order, Higuchi and Koresmeyer pappas plot. Release of drug from almost all the SLNs followed Higuchi equation better than zero and first order equation (Table 4).

Table 4 Nonlinear fits of Diclofenac released from SLNs

Formulation	Zero order,		First order,		Higchi,		Koresmeyer pappas
	R	R^2	R	R^2	R	R^2	N
SLN 1	0.910	0.829	0.885	0.784	0.990	0.981	3.202
SLN 2	0.874	0.765	0.878	0.771	0.989	0.980	3.12 7

SLN 3	0.892	0.796	0.892	0.796	0.968	0.938	3.159
SLN 4	0.931	0.867	0.931	0.867	0.963	0.929	3.122
SLN 5	0.918	0.843	0.918	0.843	0.971	0.943	2.810
SLN 6	0.925	0.857	0.925	0.857	0.973	0.947	2.745
SLN 7	0.936	0.877	0.936	0.877	0.984	0.969	3.202
SLN 8	0.932	0.869	0.932	0.869	0.983	0.968	3.190
SLN 9	0.913	0.835	0.919	0.845	0.982	0.965	3.193

In vivo study using carrageenan-induced paw edema model:

The anti-inflammatory activity of solid lipid nanoparticles and Gelatin adsorbed solid lipid nanoparticles of diclofenac (SLN and GSLN-1) was studied by using the Carrageenan model with the help of Pleythysmometer. Time dependent effects was shown in Table 5. A significant (P< 0.05) difference in the % inhibition of the edema at the different time intervals (1, 2,3,4,5,6,12 and 24 hours) was observed and when the graph was plotted between % inhibition of edema and time intervals (hours), the test (GSLN-1) displayed almost similar % inhibition from 1 to 6 hours and a higher % inhibition from 12 to 24 hour with respect to SLN and standard (diclofenac) (Fig. 5).

Table 5 Percent Inhibition of edema during Intra peritoneal treatment with the Solid lipid nanoparticle (SLN, GSLN-1) and pure drug (Diclofenac)

Sr. No.	Treatment	No. of animals	Dose	InitialPaw volume(ml	[Mean paw volume ± SD] Duration (hr)								[Percentage Inhibition ± SD] Duration (hr)							
					1	2	3	4	5	6	12	24	1	2	3	4	5	6	12	24
1	Control	6	1 ml	1.91± 0.204	4.33 ± 0.60	5.0 ± 0.31	5.25 ± 0.68	6.83 ± 0.98	7.00 ± 1.14	4.75 ± 0.61	4.16 ± 0.40	3.66 ± 0.51	-	-	-	-	-	-	-	-
2	Standard (Diclofenac)	6	0.075 mg/g	1.25 ± 0.41	3.33 ± 0.81	4.58 ± 1.56	3.83 ± 0.75	3.58 ± 0.37	3.08 ± 0.20	2.83 ± 0.40	2.83 ± 0.40	2.41 ± 0.49	21.37 ± 0.87	15.76 ± 1.04	14± 0.73	42 ± 1.63	57.14 ± 2.01	46.18 ± 1.17	42.0 ± 1.23	25.92± 1.52
3	Test (SLN)-1	6	0.015 mg/g	1.25 ± 0.41	3.08 ± 0.20	3.583 ± 0.80	4.16 ± 0.25	4.08 ± 0.49	3.08 ± 0.49	2.66 ± 0.25	2.41 ± 0.37	2.167 ± 0.25	18.8 ± 0.72	24 ± 0.164	26 ± 1.06	52.85 ± 1.26	53.8 ± 2.46	50.37 ± 1.23	42.1 ± 0.94	40.11 ± 0.68
4	Test (GSLN)-1	6	0.015 mg/g	1.25 ± 0.41	3.41± 1.02	3.86± 0.86	4.92± 1.01	5.34±0 .98	4.12± 1.17	3.2± 1.24	2.86± 1.56	2.32± 0.96	19.5± 1.12	26.37 ±1.04	29.0 ±1.17	56.8 ±0.86	58.92 ±0.74	54.14 ±0.92	44.37 ±1.01	42.18± 0.88

(Figures are Mean ± SD)

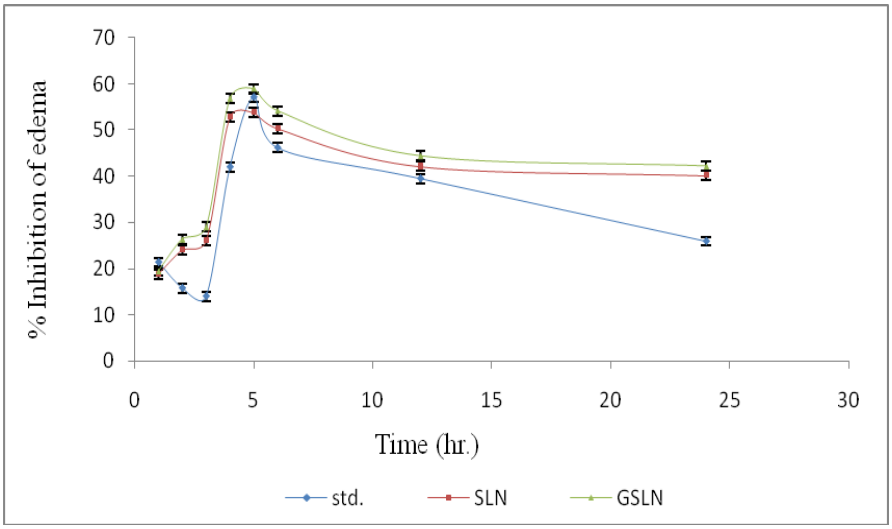

Figure 5 Comparative curves between standard and test groups (% inhibition of edema vs. time.

CONCLUSION

The results showed that gelatin surface modified solid lipid nanoparticles may be considered as a promising carrier system for controlled release and targeted delivery of diclofenac with possible clinical application for arthritis treatment.

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REFERENCES

1. G. Huang, Na Zhang, X. Bi, M.Dou, Solid lipid nanoparticles of temozolamide: potential reduction of cardial and nephric toxicity. Int. J.Pharm., 355, 1-2: (2008) 314-320.
2. K.Vivek, H. Reddy, RS. Murthy, Investigation of the effect of the lipid matrix on drug entrapment, in vitro release, and physical stsbility of Olanzapine-loaded solid lipid nanoparticles. AAPS Pharm Sci Tech. 8(4): (2007).
3. P.V. Pople, K. K. Singh, Development and evaluation of topical formulation containing solid lipid nanoparticles of vitamin A, AAPS Pharm Sci Tech.;7 (4) (2006).
4. A. Ghada, H. F. Rania., Diazepam-Loaded Solid Lipid Nanoparticles: Design and Characterization AAPS PharmSciTech, 10 (1), (2009) 211-220.
5. T.M.Jamie, B. Diane J., Preparation and characterization of gelatin surface modified PLGA microspheres, AAPS Pharm Sci Tech; 3(1) 11; (2001)1-8.
6. M.Trotta, D. Francesca, C. Otto, Preparation of solid lipid nanoparticle by a solvent emulsification diffusion technique, Int. J .Pharm 257 (2003) 153-160.
7. R. Ilavarasan, M. Mallika , S. Venkataraman, Anti-inflammatory and free radical scavenging activity of Ricinus communis root extract, J. Eth.pharmacol, 103 (2006) 478–480
8. K. Jores, W.Mehnert, M. Drechsler, H. Bunjes, C.Johann, K. Mader, Investigation on the structure of solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. J. Control. Release 95 (2004) 217-227.
9. N. AL-Haj, A. Rasedee, Solid lipid nanoparticle preparation, and characterization. Int. J. Pharmacol, , 5, 1: (2009)90-93
10. F.Q. Hu, H. Yuan, H.H. Zhang, M. Fang, Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. Int. J. Pharm., 239 (2002) 121–128