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Research Article

Development and Evaluation of Solid-Lipid Nanoparticles of Sulfasalazine for Anti-rheumatic Activity

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ABSTRACT

Chronic rheumatoid arthritis (RA) can cause irreversible joint deterioration over time. Solvent-based lipid nanoparticles (SLNs) are widely used as an efficient method to increase the oral bioavailability of poorly soluble medicines like sulfasalazine. The present study aimed to formulate and evaluate the anti-rheumatic potential of sulfasalazine's solid lipid nanoparticles (SLNs). Drug-loaded SLNs were formulated and coated with chitosan (CS) for sustained delivery and characterized for particle size, polydispersity index and *in-vitro* drug release. The safety and efficacy profile of the optimized batch was analyzed in an animal model. The particle size of the optimized formulation was 269 ± 2.45 nm with a PDI of 0.217 ± 0.008 and entrapment efficiency of about 79.9 ± 2.21. The zeta potential of particles was 35.7 mV. Particles had spherical shape with sizes ranging 100 nm, which was determined by TEM analysis. The created formulation showed that the medication was released from the lipid matrix under regulated conditions, with 83.2 ± 1.5% of the drug released in 24 hours. C_{max} for the drug was higher (337 ± 24) when administered as an SLNs drug. Similarly, T_{max} was longer when administered as lipid nanoparticles (6 hours), indicating a sustained drug release from SLNs. Complete Freund's adjuvant (CFA) activity in rats administered with CS-SSZ-SLN (300 mg/kg) equivalent to doses of 300 mg/kg SSZ showed a reduction in paw edema by day 9 (53.1 ± 1.75% ($p < 0.005$), day 18 (68.68 ± 2.08%) ($p < 0.001$) and 78.24 ± 2.36% ($p < 0.001$) on day 21, respectively. A significant increase in the Tmax and the $T_{1/2}$ values for the nanoparticles indicates sustained release of the drugs by the SLNs. Sulfasalazine decreases inflammation, which is likely responsible for lessening the signs and symptoms of inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.

INTRODUCTION

The chronic illness known as rheumatoid arthritis (RA) can cause permanent joint damage and destruction, which can cause chronic pain, loss of function, and disability. The immune system reacts to its own antigen because the body is unable to discriminate between foreign and self-antigens in this autoimmune disease. [1,2]

As our understanding of the pathophysiology of RA has advanced, tumor necrosis factor-α (TNF-α) has been identified as a key cytokine that damages joints.[3] Available methods of treatment of RA help to treat only the symptoms by decreasing joint discomfort, inflammation, and articular structural destruction, thus delaying the progression of the disease. Patients with this disease have traditionally been cured by non-steroidal anti-inflammatory medications (NSAIDs). Many therapy modalities for rheumatoid arthritis (RA) can control symptoms by lowering inflammation, joint discomfort, and articular structural damage, which also delays the disease's progression.[4,5] The therapy of RA has advanced significantly over the last ten years, with the major utilization of disease-modifying anti-rheumatic drugs (DMARDs) & immunological agents that specifically target cells involved in RA immuno-pathogenesis.[6] Anti-rheumatic drugs, including gold, methotrexate, penicillamine, and sulfasalazine, can be used to treat arthritis. These are the choice of drugs prescribed for the treatment of RA.[7,8]

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Nonetheless, a sizable portion of patients show resistance to several DMARDs. Patients with "difficult-to-treat RA" (D2T RA) are specifically defined as those whose disease activity remains uncontrolled even after using two or more bDMARDs or tsDMARDs (b/tsDMARDs) with distinct mechanisms of action (MOA).[9,10] There are numerous problems with the typical dosage formulations that are used to treat RA. Short half-lives, low bioavailability, poor solubility, and low patient compliance are the main issues with drugs. A number of RA treatment classes, including as steroids, DMARDs, and NSAIDs, have also been linked to issues with drug-associated toxicity.[11,12]

Therefore, the development of affordable and minimally harmful RA treatments is imperative. Newer techniques, such as solid lipid nanoparticles (SLNs), are being explored to create innovative dosage forms in order to ensure prolonged and sustained pharmaceutical delivery.[13] It could help reduce drug toxicity as well as issues with the short half-lives, low bioavailability, and poorly soluble nature of medications.[14]

By stabilizing and encapsulating the drug and improving its solubility and half-life, medication delivery systems utilizing nanoparticles are a favorable means of delivering therapeutics. Medication delivery using nanomaterials is superior to conventional therapy because of its regulated drug release, solubility of hydrophobic compounds, targeted specificity through active and passive targeting, and good drug transport capabilities. Therefore, it's imperative to investigate novel and more potent therapeutic medications for the therapy of RA that precisely target ill joints without harming healthy tissues.[15-17]

The ability of solid lipid nanoparticles to load drugs is high and easy permeability, making them an important tool in the area of nanotechnology for arthritis treatments.[18,19] SLNs are innovative drug delivery methods that outperform traditional colloidal and polymeric nanocarriers in a number of ways. Sulfasalazine nanoparticles (NPs) overcome the drawbacks of traditional techniques by providing lipid carriers' biological compatibility and the stability of solid matrices. It also facilitates scalability, enhances biodegradability, and allows for regulated and adjusted release patterns.[20-22] Therefore, an attempt has been made in this work to develop and evaluate SLNs for the successful delivery of sulfasalazine in addition to evaluating for drug release characteristics, particle size, and entrapment effectiveness.

Materials and Methods

Study Design

This work was performed to develop, optimize and evaluate SSZ solid-lipid nanoparticles for anti-RA potential. Before beginning any treatments, a thorough review of the literature on drugs and innovative drug delivery systems for RA was conducted. Additionally, several papers from reputable journals were searched for information on all aspects of current research. The study's goal and objectives were defined based on tests that were done in the lab using different parameters connected to the aforementioned activity.

Materials

Sulfasalazine was purchased from Healthy Life Pharma Pvt. Ltd. Mumbai, stearic acid, tween 80 was received as gift sample from Molychem, Mumbai, India.

Methods

Selection of suitable lipids and surfactants

Lipids (stearic acid) were chosen based on drug solubility and compatibility. Surfactants were chosen based on the literature analysis and their safety profile. The surfactants had chosen included sodium taurocholate and tween 80.

Formulation of sulfasalazine SLNs by micro-emulsion-based method

To make SLNs, stearic acid was first melted at a temperature that was 70°C above its melting point (65–70°C). Next, 300 mg of a separate medication, sulfasalazine, which had previously dissolved in ethanol, was added, and the mixture was agitated for 5 minutes before being sonicated for 60 seconds using a 120 W power source. The mixture was agitated for two minutes after the addition of tween 80 and soy lecithin, which act as surfactants. The chitosan was already combined using an in situ approach. After heating an aqueous phase to 80°C, 50 mg of sodium taurodeoxycholate, a co-surfactant, was added to the melted lipid phase. Using a mechanical stirrer, this liquid was swirled for 20 minutes at various rpm. After that, the emulsion was thrice cleaned with distilled water.[23]

Optimization of formulation variables of CS-SSZ-SLNs

The RSM was employed to optimize the formulations of the SLNs. Formulation variables such lipid concentration,

Table 1: Formulation variables in RSM design for SSZ-CS NP

Independent variables	*Symbol*	*Unit*	*Coded levels*			*Response (Y1)*	*Response (Y2)*	*Response (Y3)*
			-1	*0*	*+1*			
Surfactant concentration	X_1	mg/mL	1	1.5	2	Particle size (nm)	PDI	%EE
Homogenization speed	X_2	rpm	12k	15k	18k			
Lipid content	X_3	mg	150	300	450			

surfactants, and homogenization speed were the main determinants of the particle size, PDI, drug entrapment efficacy, and percentage of drug release of the SSZ-loaded chitosan nanoparticle (CS-SSZ-NP) preparations (Tables 1, and 2).

Characterization, Evaluation and Optimization of Sulfasalazine SLNs

Measurement of particle size and distribution

The prepared SLNs' particle sizes (z-average) and particle size distributions (PDI) were ascertained using photon correlation spectroscopy (PCS). Zetasizer ZS 90 from Malvern Instruments Ltd., UK, was used for the measurements.

Particle shape and morphology

Transmission electron microscopy (TEM; Hitachi H7500, Tokyo, Japan) was used to examine the form and morphology of produced SLNs. The TEM method of microscopic analysis focuses the structure's image using magnetic lenses after sending electrons through nanoparticles.

Measurement of zeta potential of SLNs

Zeta potential can be utilized to predict long-term stability and improve formulation. About 1-mL of SLN dispersion (Millipore, India) was diluted with 10 mL HPLC grade water for zeta potential determination, and measurements were taken using Zetasizer Ver. 7.01 (Malvern Ltd., UK).

Determination of drug content

To determine the total drug content in the prepared SLNs, 0.1 mL of SLN dispersion was extracted in chloroform:ethanol mixture (1:9) volumetrically. Required dilutions were further carried out in ethanol. Drug content was estimated using UV spectrophotometric method at a maximum wavelength of 359 nm. The experiment was carried out in triplicate.

Determination of entrapment efficiency

By evaluating the amount of free drug (un-entrapped) in the supernatant obtained after centrifuging SLN dispersion, entrapment efficiency (EE) was measured. The SLN dispersion was centrifuged using an ultra-centrifuge at a speed of 6000 to 12000 rpm at a temperature of 4°C. The amount of un-entrapped medication was then calculated by analyzing the supernatant, & the entrapment efficiency was estimated using standard formula.

In-vitro release studies of drugs from SLNs

Studies on *in-vitro* release were carried out using a water bath incubator shaker. The molecular weight cutoff for the 12,000 to 14000 Da pore size dialysis membrane was employed. At intervals of 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hours, an aliquot of 5 mL was taken out. The volume was taken at regular intervals to maintain constant volume and replaced with fresh medium kept at the same temperature. The %cumulative release was determined by using UV.

Table 2: Design matrix with recorded responses

		Factor 1	*Factor 2*	*Factor 3*	*Response 1*	*Response 2*	*Response 3*
Std	*Run*	*A: Surfactant (X1) Mg/ml*	*B:Speed (X2) rpm*	*C: Lipid content (X3) Mg*	*Particle size (Y1) nm*	*PDI*	*%EE*
7	1	1	15000	450	359	0.224	80.54
5	2	1	15000	150	152	0.185	72.6
11	3	1.5	12000	450	312	0.248	77
14	4	1.5	15000	300	287	0.214	72.75
6	5	2	15000	150	210	0.198	74.5
1	6	1	12000	300	254	0.195	80.8
3	7	1	18000	300	198	0.21	76.2
12	8	1.5	18000	450	286	0.208	82.5
4	9	2	18000	300	210	0.205	79.8
8	10	2	15000	450	324	0.232	84.2
13	11	1.5	15000	300	225	0.198	78.6
17	12	1.5	15000	300	196	0.195	82
2	13	2	12000	300	235	0.192	83.6
16	14	1.5	15000	300	254	0.2	77.9
10	15	1.5	18000	150	162	0.178	72.1
9	16	1.5	12000	150	189	0.186	74.5
15	17	1.5	15000	300	218	0.198	81.45

Table 3: Experimentally observed responses of optimized formulations

Process	X_1	X_2	X_3	*Predicted*	*Experimental (n = 3)*	*Error (%)*
Particle size (Y1)	336.32	2.00	12000	275.4	269 ± 2.45	2.18
PDI (Y2)	336.32	2.00	12000	0.213	0.217 ± 0.008	1.87
EE % (Y3)	336.32	2.00	12000	81.37	79.9 ± 2.21	1.78

Table 4: The numerical optimization criteria -CS-SSZ-NPs

Parameter	*Goal*	*Lower limit*	*Upper limit*	*Lower weight*	*Upper weight*	*Importance*
A: Surfactant	is in range	1	2	1	1	3
B: Speed	is in range	12000	18000	1	1	3
C: Lipid	is in range	150	450	1	1	3
Particle size	is in range	152	359	1	1	3
PDI	is target = 0.213	0.178	0.248	1	1	3
EE	maximize	72.1	84.2	1	1	3

Solution

Lipid	Surfactant	Speed	Size	PDI	%EE	Desirability	
336.32	2.00	12000.2	275.4	0.213	81.37	0.876	Selected

In-vivo pharmacokinetic and PK studies

After a specified oral dose of optimized formulation, the concentrations of metabolites in plasma were assayed at different time points. Calibration curves in rat plasma were plotted against the concentration of the corresponding standard solutions in the range of 5 to 30 µg/mL for SSZ. The pharmacokinetic parameters were reported as mean ± SD values and the C_{max} and T_{max} values of the drug were estimated.

CFA-induced arthritic model in rats

The efficacy studies were performed using the CFA-induced arthritis model in rats. For each study, the animals were divided into different groups of six, and the animals' right paws were injected with CFA to cause inflammation. Following the induction of inflammation, the treatment schedules were followed for each study, and the effect of the treatment protocols on various parameters was assessed.

Storage stability study

In accordance with ICH recommendations, the sulfasalazine nanoparticle formulation was kept at 4°C in the refrigerator, 25°C in a stability chamber with a humidity of 60%, and 40°C with a temperature of 75% for the duration of 6 months. Particle size and EE of the samples removed at 0, 2, 4 and 6 months intervals were evaluated.

Results and Discussion

Optimization of Formulation Variables of CS-SSZ-SLNs

The extended effect of formulation variables X1 (surfactant concentration), X2 (homogenization speed), and X3 (amount of lipid) on the responses, Y1 (particle size), Y2 (poly-dispersity index–PDI), and Y3 (%EE) were estimated through a systematic optimization process using the Box–Behnken design of RSM.

The range for particle size is 152 to 359 nm. For PDI it was obtained in the range of 0.178 to 0.248 and for entrapment efficiency (%EE) in the range of 72.1 to 84.2%. A linear model for particle size, PDI and %EE was found to be suitable as no effect of interactions between the factors was observed.

Data Optimization and Validation of the Experimental Model

The optimized formulation has a particle size 269 ± 2.45 nm, PDI 0.217 ± 0.008 and entrapment efficiency of about 79.9 ± 2.21, It showed a strong correlation with the expected results. The response parameters have prediction errors of 2.18, 1.87, and 1.78%, with an absolute error of 1.08 percent ± 0.5%. The low error numbers indicate the response surface methodology's great predictive ability (Tables 3, 4 and Figs 1 and 2).

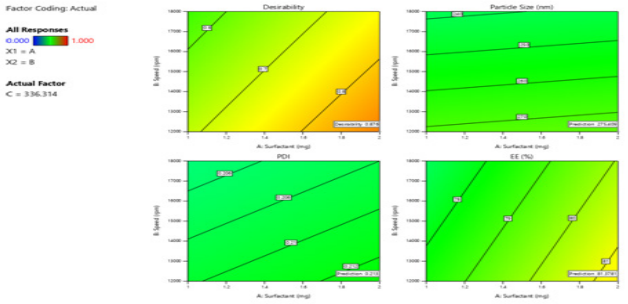

Fig. 1: Desirability plot

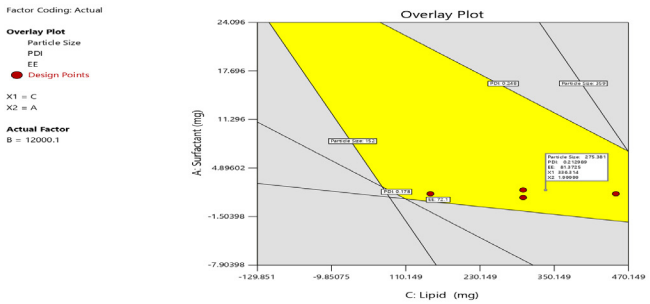

Fig. 2: Overlay plot for optimization of CS-SSZ-SLNs

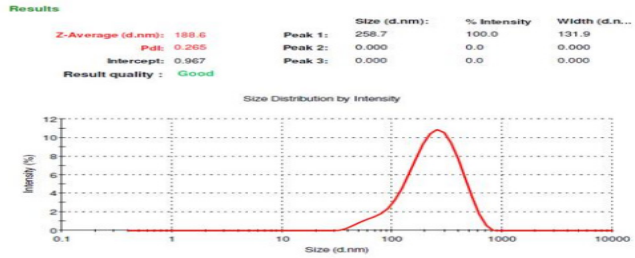

Fig. 3: Particle size distribution of optimized batch

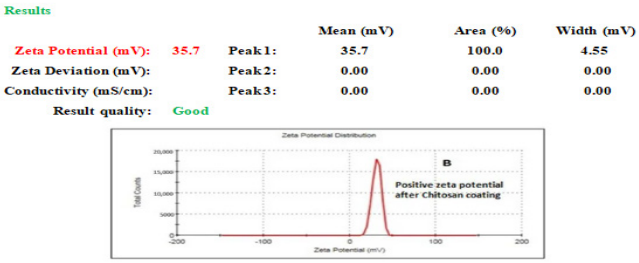

Fig. 4: Zeta potential of optimized batch

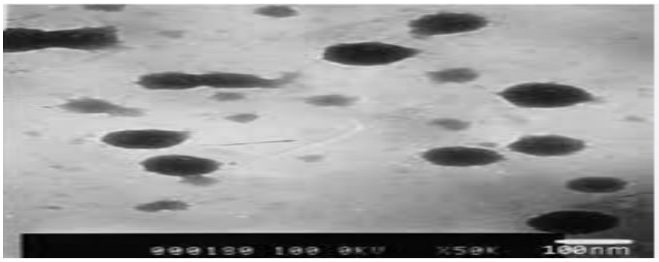

Fig. 5: TEM image of optimized batch

Characterization of the Optimized Formulations (CS-SSZ -SLNs)

Measurement of particle size and PDI

When compared to uncoupled SLNs, drug loading and coupling with chitosan ligand appear to alter the size and PDI of the SLNs. The change in concentration of the lipid and homogenization speed alters the size of the particle. The improved formulation measured 254.2 nm in size, and an overlay plot created with design expert software revealed a polydispersity index of 0.265. Uniformity in size in a specific range indicates optimum polymer incorporation with surfactant and homogenization speed. It also results in the desired entrapment and drug release efficiency of SLNs (Fig. 3).

The results also show that particle size reduces as surfactant concentration rises. This might be because a greater surfactant covers new surfaces more quickly, lowering surface tension and facilitating particle partitioning during emulsification.[24] The drug's particle size has an impact on the drug's loading capacity, formulation stability, and release characteristics. They also determine how nanoparticle delivery systems behave *in-vivo*, their biological fate, and their targeting capacity. [25,26]

Analysis of zeta potential of SLNs

Zeta potential is a widely used metric to estimate the stability of colloidal suspensions. It represents the degree of repulsion between similarly charged particles in dispersion. In the dispersion medium, nanoparticles with a ZP greater than +30 mV or lower than -30 mV are extremely stable. The zeta potentials of roughly 35.7 mV displayed in Fig. 4 suggest that the formulation is stable. Lipid and tween 80, which reduce electrostatic repulsion between the particles and sterically stabilize the nanoparticles by producing a coat around their surface, may be responsible for this.

Entrapment efficiency

In CS-SSZ-SLNs, the entrapment efficiency was found to be 79.12 ± 2.05%. The findings suggested that SSZ in the SLNs had a decent EE. SSZ has poor lipid solubility, which enhances the likelihood that it will partition out, particularly after the lipid solidifies into the crystalline matrix. Cationic SLN were developed to improve the EE of particular medications that have low water and lipid solubility.[18] Therefore, the high EE of the medication in the SLNs has been made possible by the usage of stearic acid to boost SSZ affinity towards the lipid.

Particle shape and morphology

The spherical CS-SSZ-SLNs ranged in size from 100 nm. This was accomplished by modifying the ideal parameters for CS-SSZ-SLN preparation. CS, tween, and stearic acid combinations in varying concentrations generate chemically polyelectrolytic complexes that promote the creation of perfectly spherical polymeric nanoparticles (Fig. 5).

X-ray diffraction analysis

The crystalline nature of the drug was shown by the strong peaks in the XRD pattern of SSZ powder at diffraction angles of 2 θ = 14.08°, 18.74°, 25.12°, and 29.54°. (Fig. 6) When encapsulated in nanoparticles, SSZ mostly exists and is distributed in a non-crystalline state, as demonstrated by the sharp characteristic peaks of the drug being suppressed in the CS-loaded nanoparticle. This conclusion

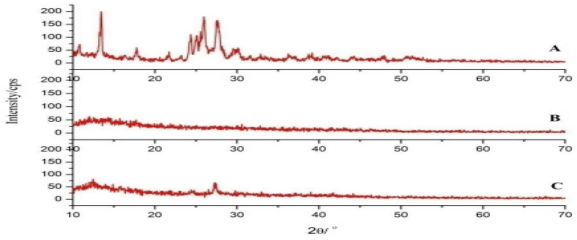

Fig. 6: XRD pattern of (A) SSZ, (B) Blank NP and (C) CS-SSZ-SLNs

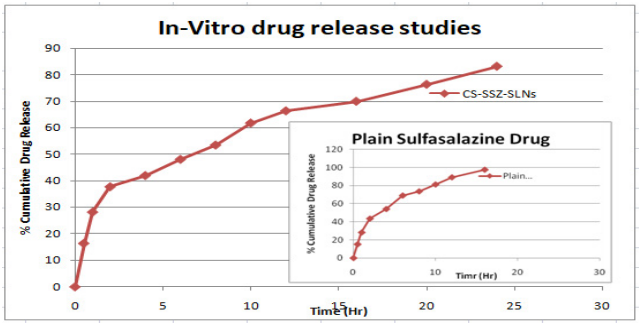

Fig. 7: *In-vitro* drug release of pure SSZ and optimized batch

Table 5: Dissolution studies in phosphate buffer

Time (Hours)	*%Cumulative drug release (%CDR) in phosphate buffer pH 7.4)*	
	CS-SSZ-SLNs	Plain sulfasalazine
0.5	16.4 ± 1.15	15.2 ± 1.2
1	28.2 ± 1.8	28.25 ± 1.35
2	37.76 ± 1.35	43.5 ± ± 0.8
4	42 ± 2.1	54.1 ± 0.72
6	48.1 ± 0.8	69 ± 1.21
8	53.5 ± 1.3	73.6 ± 1.25
10	61.8 ± 1.6	81.2 ± 1.12
12	66.4 ± 1.5	89.1 ± *0.85*
16	70 ± 1.24	97.5 ± 1.45
20	76.4 ± 2.25	
24	83.2 ± 1.6	

Table 6: Concentration of SSZ and peak area ratio

Concentration (µg/mL)	*Peak ratio of analyte/Int. standard*
5	0.189
10	0.342
15	0.602
20	0.77
25	0.954
30	1.08

is consistent with the findings from the DSC study.

In-vitro release studies of drugs from SLNs

Table 7: Plasma concentration of plain SSZ and CS-SSZ-SLNs

Time (hours)	*Concentration (µg/mL)*	
	Plain SSZ	*CS-SSZ-SLNs*
0.25	210	355
0.5	274	369
1	282	384
2	296	398
4	378	406
8	324	470
12	312	428
24	286	383

Table 8: Comparative pharmacokinetic profile of SSZ and CS-SSZ-SLNs

Formulation/ Parameters	AUC_{0-t} *(µg.h\ml)*	$AUC_{0-\infty}$ *(µg.h\ml)*	$T_{1/2}$ *(Hr)*	C_{max} *(µg/ml)*	T_{max} *(Hr)*
Plain SSZ	4685 ± 126	232045 ± 458	5.26 ± 0.09	225 ± 14	4
CS-SSZ-SLNs	6742 ± 189	32568 ± 624	11.14 ± 0.36	337 ± 24	6

Table 9: Animal groups in the CFA rat model

Rat Group	*Applied formulation for treatment*
I	NC – Saline (no CFA injection)
II	Arthritic control – Saline (no treatment-only vehicle)
III	Standard Sulfasalazine (300 mg/kg)
IV	CS-SSZ-SLN (150 mg/kg of SSZ)
V	CS-SSZ-SLN (300 mg/kg of SSZ)

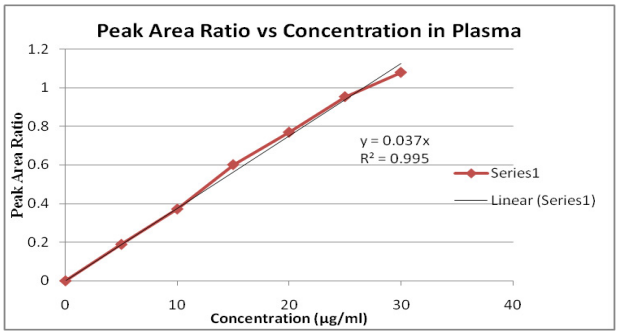

Fig. 8: Standard curve of SSZ in plasma

The results shown in Table 5 and Fig. 7 optimized CS-SSZ-SLNs released 83.2 ± 1.5, in 7.4 pH phosphate buffer. The result indicates that the release of drug from pure SSZ (89.1 ± 2.2) was better but it doesn't show a controlled release profile as complete drug release occurred within 12 hours, while in case of nanoparticles the drug release was good as well as it shows controlled release more than 24 hours. The delayed diffusion of the lipophilic drug from the polyelectrolyte complex matrix allowed for controlled

Table 10: %increase in rat paws thickness on treatment

Rat group	*%increase in paw thickness at different time intervals*						
	Day 3	*Day 6*	*Day 9*	*Day 12*	*Day 15*	*Day 18*	*Day 21*
I	0	5 ± 0.24	6 ± 1.02	4 ± 0.4	3 ± 0.72	2 ± 0.38	3 ± 0.6
II	96 ± 3.14	85 ± 4.12	78 ± 3.4	72 ± 3.56	72 ± 3.1	68 ± 2.42	65 ± 1.24[a]
III	65 ± 1.24	46 ± 2.75	54 ± 2.48	42 ± 2.2	36 ± 1.7[a]	30 ± 0.8[b]	30 ± 1.2
IV	80 ± 2.46	65 ± 3.2	46 ± 1.8	54 ± 3.1	45 ± 2.05[a]	40 ± 1.2[a]	36 ± 0.68[b]
V	64 ± 2.65	52 ± 1.7	48 ± 2.62[a]	42 ± 1.86	35 ± 1.1	30 ± 1.78[b]	21 ± 0.8 [b]

[a] Significant at $p < 0.05$, [b] Significant at $p < 0.001$

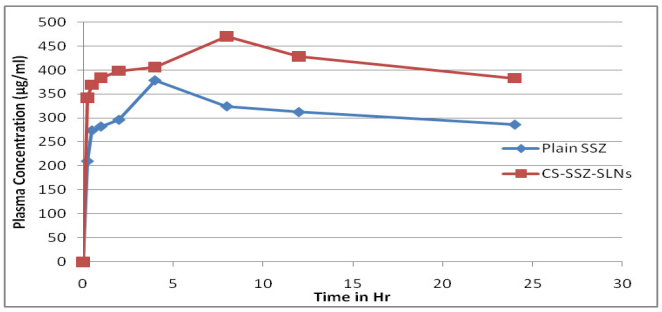

Fig. 9: Curve B/W plasma concentrations and time of SSZ, CS-SSZ-SLNs

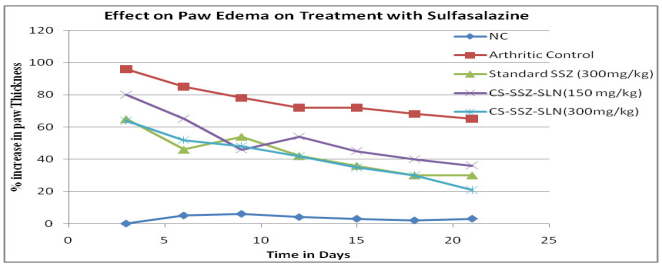

Fig. 10: Percent increase in paw edema in CFA-induced arthritis in rats of normal control (no CFA), arthritic control (No treatment), standard SSZ 1-mg/kg, CS-SSZ-SLNs 150 and 300 mg/kg/day, respectively

release. The polyelectrolyte complex is formed by the ionically interacting chitosan amino groups and lipid carboxyl residues. Chitosan and lipid complexation lowers the porosity of colloidal particles and lessens medication leaking from encapsulated particles.

In-vivo pharmacokinetic and PK studies

Firstly, acute oral toxicity studies are conducted to understand the adverse effects in an appropriate animal model to create a safety database of the formulation.[27] All the studies in this work were performed on healthy female wistar albino rats (150–200 g), aged 8 to 12 weeks. The LD_{50} value calculated according to the guidelines of OECD was found to be more than 2000 mg/kg by oral route. The regression equation and the coefficient of determination (R^2) obtained from the standard curve was y = 0.037x and 0.995 for SSZ.

PK Studies of Pure Drug Sulfasalazine and Nanoparticles

The $AUC_{0-\infty}$ value of nanoparticles was found to be 1.42 folds higher than the plain drug solution, suggesting the relative bioavailability of SSZ-SLNs to be 142% of the standard plain drug solution, thus indicating an increase in bioavailability. It was observed that the plasma drug concentration of SSZ was higher at all time points when administered in the form of solid lipid nanoparticles, with the C_{max} being higher and T_{max} being significantly longer than when compared to standard plain SSZ solution, indicating a sustained drug release from nanoparticles. The $T_{1/2}$ of drug also significantly increased when administered in form of nanoparticles compared to that of standard drug solution, confirming the sustained release of SSZ from CS-SSZ-SLNs (Tables 6-8 and Figs 8 and 9).

Effect on Paw Edema on Treatment with Sulfasalazine

On day 1, every rat given a CFA injection experienced inflammation in the paw area. Paw inflammation significantly decreased in all treatment groups in a dose-dependent manner from day 1 to 21.

From day 1 to 21, the rats in group 2, the arthritic control group, had paw sizes that were reduced by 32.21 ± 1.05%. Paw edema significantly decreased in rats given conventional plain SSZ at a dose of 300 mg/kg (Group III) by days 15 (61.52 ± 2.74%) ($p < 0.05$) and 18 (68.75 ± 2.62% $p < 0.001$). Action on day 21 was similar to that of day 18. The rats treated with CS-SSZ-SLN 150 mg/kg (Group IV) produced a remarkable decrease in paw edema by day 18 (58.33 ± 1.42%) ($p < 0.001$), and day 21 there is a slight decrease in paw edema (60.94 ± 2.12%)) ($p < 0.001$), whereas rats administered with CS-SSZ-SLN (300 mg/kg) equivalent to doses of 300 mg/kg SSZ (Group V) showed reduction in paw edema by day 9 (53.1 ± 1.75% ($p < 0.005$) day 18 (68.68 ± 2.08%) ($p < 0.001$) and 78.24 ± 2.36 % ($p < 0.001$) on day 21 reductions in paw inflammation, respectively (Tables 9, 10 and Fig. 10).

Conclusion

As per the design approach, the micro-emulsion-based technology was successfully utilized to synthesize SSZ

and CS-coupled SLNs. *In-situ* method was used to coat SLNs with chitosan ligand in order to increase their oral bioavailability and maybe the targeted delivery of the drugs at the site of action.[28] Stearic acid, a cationic lipid, was used to improve the negatively charged drug's affinity for the lipid phase.[29] The particle size analysis and TEM examination confirmed that the optimized CS-SSZ-SLNs had a PDI of less than 0.3 and were roughly 300 nm in diameter. The EE for SSZ varied from 80 to 90%. The drug release experiments showed that CS-SSZ-SLNs exhibited prolonged drug release when contrasted with pure drug solutions.

The HPLC approach allowed for the detection of the SSZ in plasma using a drug-standard solution. When SSZ was delivered as SLNs, it was found that the C_{max} was higher (337 ± 24), and when CS-SSZ-SLNs was administered (6 hours later), the T_{max} was longer, suggesting a prolonged drug release from SLNs. Confirming the prolonged release of SSZ from SLNs, the $T_{1/2}$ of SSZ increased from 5.26 to 11.14 hours when given as solid lipid nanoparticles as opposed to a typical plain drug solution. The results show that, at the same dose levels, the anti-arthritic activity of nanoparticles was greater than that of plain SSZ, suggesting that SLNs were able to increase the bioactivity of SSZ. Because chitosan may attach itself to the intestinal mucosa, it improves the muco-adhesive quality of the carrier. [30] This results in an extended residence period at the intestinal absorption sites, which increases the drug's bioavailability.

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