

**Research Article****Chondroitin sulphate mediated targeted delivery of methotrexate and aceclofenac to the joints for effective management of rheumatoid arthritis****Satish Shilpi\*, Shikha Upadhaye, Roshni Shivvedi, Ekta Gurnany, Pranali Chimaniya, Amrita Singh, Megha Chouhan, Shikha Jain, Sunil K. Yagnik, Kapil Khatri***Pharmaceutics Research Laboratory, Department of Pharmaceutics, Ravishankar College of Pharmacy, Bhopal, MP, India*

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**Abstract**

**Objective:** Rheumatoid arthritis (RA) is an autoimmune disease in which immune system of body mistakenly attacks the joints. This creates inflammation and severe pain around the joints due to thickening or degeneration of synovial fluid. Frequent high dosing of drug may precipitate side effect. Purpose of this work was site specific and simultaneously delivery of methotrexate and aceclofenac by chondroitin sulphate conjugated lipid nanocarrier i.e. solid lipid nanoparticles (SLNs) for the effective management of RA. **Material and Methods:** The SLNs were prepared by solvent injection method and further they were surface engineered with Chondroitin sulphate. They were further characterized for size and its size distribution, shape and surface morphology, zeta potential, % entrapment efficiency and in vitro drug release profile. Anti-inflammatory activity and in vivo performance was also predicted. **Results:** The particle size of the unconjugated SLNs and chondroitin sulphate conjugated-SLNs (CS-SLNs) were found to be  $127.9 \pm 5.3$  nm and  $131.2 \pm 4.6$  nm, respectively. SLNs showed matrix diffusion sustained drug release pattern. In vitro aceclofenac release was found  $61.35 \pm 1.08\%$  and  $58.97 \pm 2.30\%$  and release of methotrexate was found to be  $58.08 \pm 0.93\%$  and  $56.18 \pm 1.27$  in 24 h for chondroitin uncoupled and coupled SLNs. **Conclusion:** Highest uptake of SLNs by the knee joint due to highly expressed receptors associated with chondroitin sulphate. Targeting efficiency of prepared chondroitin sulphate conjugated lipidic nanoparticles were potential nanocarrier for the effective management of RA.

**Keywords:** Rheumatoid arthritis, SLNs, Chondroitin sulphate, immunosuppressant

**Introduction**

Rheumatoid arthritis (RA) is an autoimmune disease in which immune system mistakenly attacks the joints. This creates inflammation that causes the synovium tissue to thicken, resulting in swelling and pain around the joints. The synovium responsible to makes synovium fluid that lubricates joints and helps them move smoothly. Over the time, cartilage, the elastic tissue and bones may irreversible damage in this autoimmune disease and the joint spacing between bones can become smaller which may leads to severe pain and lose their mobility (Arrar et al., 1995; Calleja et al., 2004; Janusz et al., 2002). It effect to joints of the hands, feet, wrists, elbows, knees and ankles in

symmetrical manner i.e. if one knee is affected then the other one will also be affected. If disease will not be treated or managed then it will be affect the other body part like cardiovascular and respiratory system or may be whole systemic body (Zhou et al., 2010; Sandhu et al., 2017).

In current available approaches various oral and topical NSAIDs like ibuprofen, ketoprofen and naproxen sodium, COX-2 inhibitor but on frequently administering high dose, these drugs may precipitate severe toxic affect to GIT and other body organ (Bishnoi et al., 2014). Corticosteroid medications, including prednisone, prednisolone and methyprednisolone, are potent and quick-acting anti-inflammatory medications but these drugs could not adminsterd for long period of time due to their side effect (Mahor et al., 2010). In case of permanent damage, joint replacement surgery is an option which can relieve from pain and restore function in joints which severely damaged. But their higher cost and discomfort for patient make these approaches avoidable. In some other approaches in which

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use the JAK inhibitors like Tofacitinib that block the Janus kinase or JAK pathways which are responsible for immune response of the body (Janusz et al., 2011; Hombach et al., 2009). Some immunomodulators like methotrexate, hydroxychloroquine, sulfasalazine, leflunomide, cyclophosphamide and azathioprine can also use for the modification or minimise the immune response (Onishi et al., 2013; Muller et al., 2010; Sandhu et al., 2017).

These all drugs or available therapy approaches have some own limitation like high cost and severe side effect and some other complication like patient discomfort. One of them a major limitation is that the potent drug molecules easily and rapidly eliminates from the synovial cavity via lymphatic system and need to administer another dose of drug frequently. Due to these limitations of current drug therapy, it is need to develop such a strategy which easily target and deliver therapeutic molecules in high concentration with sustained manner to the joint area. Solid lipid nanoparticles are a nontoxic lipid nature drug carrier that cover all these criteria and have ability to cross all biological barriers very efficiently (Garg et al., 2016; Gupta et al., 2007). Lipid nanoparticles (SLNs) can carry high payload and release drug in sustained manner for longer period of time. They are small enough to circulate in the microvascular system, and their hydrophilic nature, prevent macrophage uptake (Wissing et al., 2002; Venkateswarlu et al., 2004; Shilpi et al., 2015).

Chondroitin sulphate is a molecule that is occurs in the body; normally it is a major component cartilage or synovial fluid around joints in the body (Jeremy et al., 2011; Kumar et al., 2017). It provides toughness to connective tissue that provide smoothness to the joints and it help to produce new cartilage. Lack of Chondroitin sulphate in the body may cause RA. In case of RA, connective tissue, overexpress the receptor (CD44, annexin and leptin receptors) for more uptake of chondroitin sulphate (Hombach et al., 2009; Zhou et al., 2010; Jeremy et al., 2011; Onishi et al., 2013). According to this phenomenon, chondroitin sulphate can be used as targeting ligand which can target drug loaded nanocarrier to the joints (Li et al., 2017). It can avoid the unwanted drug distribution and minimise side effect and dose of drug. Aceclofenac and methotrexate were chosen for this study. Aceclofenac is a selective inhibitor of PGE<sub>2</sub> via cyclooxygenase (COX-1 and COX-2); interleukin (IL)-1b, IL-6 and tumor necrosis factor in human which are responsible for pain. It is safe and not causes gastric irritation or ulcer that may cause with other NSAIDs. It was reported that the encapsulation is high and easy with SLNs. Methotrexate is a is used as a disease-modifying treatment for some autoimmune diseases, including rheumatoid arthritis, Generally it is used as a chemotherapy drug but in low doses, methotrexate safely use for treatment of certain autoimmune diseases and today, it becomes first-line therapy for the treatment of rheumatoid arthritis. Due

to its target specific steps in the inflammatory process, they not affect the entire immune response of body.

In this study, aceclofenac and methotrexate loaded SLNs were prepared and further they were surface engineered with chondroitin sulphate as a targeting ligand (for annexin 6 and CD44 receptor) to achieved targeted drug delivery of both drug in high concentration to the arthritic joint and minimize the unwanted drug distribution which reduce side effect and also minimize the dose of drugs. This novel targeted approach designed to effectively and efficiently management of RA.

## Method and Materials

### Materials

Methotrexate and aceclofenac was purchased from AvansureLifesciences Pvt. Ltd. (Gurgaon, India). Chondroitin sulphate, Hydrogenated soya phosphatidylcholine (HSPC) was received as a gift from Lipoid (Ludwigshafen Am Rhein, Germany). Monosodium iodoacetate, Stearylamine, Sephadex G-50, dialysis membrane (MWCO, 15 KDa), 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDC), Triton X100, N-hydroxysulfosuccinimide (Sulfo-NHS), were purchased from Sigma (St Louis, MO). Tween 80, Span 80, and Tristearin purchased from Himedia (Mumbai, India). All other reagents and chemicals used were of analytical grade.

### Preparation of SLNs

SLNs were prepared according to the solvent injection method previously developed by Shilpi et al., (2015). In this method, tristearin (100 mg), HSPC (100 mg) and stearylamine (10 mg) were dissolved in a minimum quantity of absolute ethyl alcohol and heated to about 60° C. In another container, phosphate buffer (pH 7.4) with Tween 80 (0.5 % v/v) used as aqueous phase was heated at the same temperature. Then the organic lipid phase added drop wise using a preheated syringe to aqueous phase at the same temperature under continuous mechanical stirring (Remi Instruments, Mumbai, India) at 4000 rpm for a definite time period. At the same temperature, The mixture was sonicated for 120 s using probe sonicator (Probe sonicator, Lark Innovative Technology, Chennai, India) of an 10/-amplitude lever at a 20-s pulse rate (power output 2 KW) and a 20-kHz frequency, to obtain uniformity in the size of the SLNs. Then SLNs suspension was continuously stirred for 60 min at room temperature. Drug-loaded SLNs were prepared by the same procedure in which the aceclofenac (50 mg) and methotrexate were (10 mg) was dissolved in lipid phase.

### Conjugation of Chontroitin Sulphate with SLNs

Chondroitin sulphate was conjugated on the SLNs surface using carbodiimide chemistry. In this method carboxylic

group of Chondroitin sulphate had been conjugated with the amine group of stearylamine with the help of N-ethyl-N-(dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS). In this method, the drug-loaded SLNs (100 mg) were suspended in a PBS (pH 7.4; 10 ml) containing Chondroitin sulphate (10 mg) in the presence of EDC and NHS (10.0 % W/W) and then left for conjugation for 2 h at room temperature. Unconjugated Chondroitin sulphate was removed by passing the formulation through a mini column of Sephadex G-50 gel (Figure 1). The conjugation was confirmed by IR spectroscopy.

### Characterization of SLNs

#### Particle size, polydispersity index and zeta potential

Average particle size, polydispersity index (PDI) and surface charge of SLNs was determined using Zetasizer (DTS ver.4.10, Malvern Instruments, England, UK). The SLNs formulation was diluted with deionized water (1:9 v/v) and analysed for average size and PDI and it was performed at the Department of Pharmaceutical sciences, Rajiv Gandhi Pradyogiki Viswavidyalaya, Bhopal, India.

Measurement of surface charge was based on the zeta potential ( $\zeta$ ) that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of surface charge, zetasizer with a field strength of 20 V/cm on a large bore measures cell was used and samples were analysed after diluted with 0.9 % NaCl to adjust a conductivity of 50 IS/cm.

#### Particle shape and surface morphology

Scanning Electron Microscopy (SEM) was used to examine surface morphology of SLNs. Samples were prepared by sprinkling lyophilized SLNs on double adhesive tape adhere on aluminum stub. Then gold coating (thickness about 300Å) was carried out using a sputter coater. Samples were examined and photomicrographs were taken under scanning electron microscope (LEO 435 VP, Eindhoven, Netherlands) at an acceleration voltage of 30 kV.

Transmission electron microscopy (TEM; Philips CM12 Electron Microscope, Eindhoven, Netherlands) used to analyse the shape of SLNs. Samples were negatively stained with 2 % aqueous solution of phosphotungstic acid and examined at an acceleration voltage of 20 kV to visualize nanoparticles. SEM and TEM were performed at the Indian Institute of Science Education and Research (IISER), Bhopal, MP, India. A TEM and SEM image of coupled chondroitin sulphate SLNs are presented in figure 2a and 2b respectively.

#### Entrapment efficiency

Entrapment efficiency of SLNs for Aceclofenac and Methotrexate was determined according to the method described by Shilpi et al. (2015) and Fry (1978) using Sephadex G-50 mini

column. Sephadex G-50 minicolumns were prepared by taking weighed amount of Sephadex G-50 and mixed with sufficient amount of double distilled water and kept for 24 h for complete swelling. Then it was placed in a 1-ml PVC syringe (Dispovan) previously packed with a plug of glass wool and a small piece of Whatman filter paper at the bottom end. These columns were used to determine entrapment efficiency of SLNs.

The amount of un-entrapped drug was removed by passing 2.0 mL of SLNs formulation from Sephadex column and then it was centrifuged at 3,000 rpm to complete removal of SLNs. Un-entrapped drug was remain in sephadex column and SLNs eluted from it. Eluted SLNs was treated with 1% triton X-100 to lyse the SLNs. Solution was centrifuged at 10000 rpm and supernatant was analysed on UV spectrophotometer (Shimadzu 1700) at  $\lambda_{max}$  of 278nm and 255nm for aceclofenac and methotrexate respectively. Same procedure was repeated for determining the drug entrapment efficiency of chondroitin sulphate conjugated SLNs. The % drug entrapment efficiency was calculated according following formula.

$$\% \text{ Drug Entrapment} = \frac{\text{Amount of drug entrapped in SLNs formulation}}{\text{Initial amount of drug taken for loading}} \times 100$$

#### In vitro drug release

The drug release was performed in PBS (pH 7.4) for chondroitin sulphate conjugated SLNs and unconjugated SLNs separately using dialysis bag technique. In this study 2.5 mL of SLNs formulation (suspension of lyophilized 100 mg SLNs in PBS pH 7.4) equivalent to 20 mg of drug was taken in dialysis tubing (MWCO, 15 KDa, Himedia) and placed in a beaker contain 50 mL of PBS pH 7.4. The dialysis bag retains SLNs and allows passing the free drug into the dissolution media. Temperature was maintained at  $37 \pm 1^\circ \text{C}$  throughout the study. The samples were withdrawn after specified time intervals i.e. 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24 and 48 h and replaced with the same volume of fresh PBS pH 7.4 and analysed for drug concentration by using UV spectrophotometer (Figure 3).

#### Coupling efficiency

The chondroitin sulphate concentration in the conjugated SLNs was measured by the Bradford method of protein estimation (Calleja et al., 2004). Briefly, 1 ml of chondroitin sulphate conjugated SLNs containing about 100 mg of SLNs was taken in a volumetric flask with 1 ml (10 %) Coomassie blue G dye solution, and then volume was attuned to 10 ml with distilled water and measured spectrophotometrically at 530nm to determine the chondroitin sulphate concentration and compared with their

blank containing the same amount of dye. Conjugation was further confirmed by IR spectroscopy (Figure 1).

### In vivo study

#### Selection of animal

Albino rats (Wistar strain) of male sex and approximately 120–150 g of weight were selected and maintained on standard diet and water. All studies were carried out according to the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India and approved by the Institutional Animal Ethical Committee, Ravishakar College of Pharmacy, Bhopal MP, India.

#### Estimation of drug in serum and various organs

Selected albino rats were divided into four groups and each group consist of three animals. Before dose administration they were fasted overnight and in first in which the first group was taken as control. Plain drug, unconjugated SLNs and conjugated SLNs were administered via IV route through tail vein to second, third and fourth group respectively. Drug solution and formulation administered is in equivalent to 3.0 mg/kg and 1.0 mg/kg body weight for amount of aceclofenac and methotrexate respectively. A comparative study was performed for drug plasma concentration profile and tissue distribution time profile. After administration of plain drug solution and SLNs preparations, one rat from each group was sacrificed after 2, 4, 6 and 12 h (Fig 4).

Blood samples were collected through cardiac puncture in a centrifuge tube containing heparin and centrifuged at 2000 rpm for 15 min. In collected supernatant, 2mL of 0.4% ortho-phosphoric acid was mixed and deproteinized with 2 mL of acetonitrile. Mixture was centrifuged at 3000 rpm for 10 min to separate precipitated protein and supernatant was filtered using 0.22 mm membrane filter. The filtrate, analyse for drug concentration using HPLC having. In drug distribution study, one gram from each organ (kidney, liver, spleen and knee joint) was homogenized with PBS pH 7.4 using tissue homogenizer. If organs weighing less than 1 g, then complete organ was used. Homogenise mixture was centrifuged and deproteinized with acetonitrile and supernatant was analyse by using the high performance liquid chromatography (HPLC) having reverse phase C18 HPLC column (4.6 × 250 mm, 5µm) with a UV

detector. Water and methanol in 4:6 ratios was used as mobile phase and flow-rate was kept 1 ml/min. The chromatography analysis was carried out at 25°C and monitored at 382 and 307nm for aceclofenac and methotrexate.

#### Anti-inflammatory activity

Selected albino rats for this study were divided into four groups and each group have three animals including one control group. They were fasted overnight with water ad libitum prior to the day of experiment. Right paw of all the animal was marked with ink at the level of tibiotarsic articulation, so that every time the paw was dipped into the plethysmometer (UGO, BASILE 7140, Italy) to measure paw volume.

Arthritis was induced by intra articular (IA) administering single dose of Monosodium iodoacetate (2 mg), in the infra-patellar ligament of the right knee of all animal till three day and measured the paw volume. The plain drug and drug loaded formulations i.e. chondroitin sulphate conjugated SLNs, and Un-conjugated SLNs (equivalent to 3.0mg/kg for aceclofenac and 2.5 mg/kg for methotrexate) suspension or solution in PBS (pH 7.4) were administered intravenously to second, third and fourth group of albino rats. Paw volume was measured at suitable time interval i.e. 1, 2, 4, 6, 8, 12, 24, 36 and 48 h and calculated according to following formula.

$$\% \text{ Edema Volume} = \frac{\text{Edema volume of control group} - \text{Edema volume of treated group}}{\text{Edema volume of treated group}} \times 100$$

#### Statistical analysis

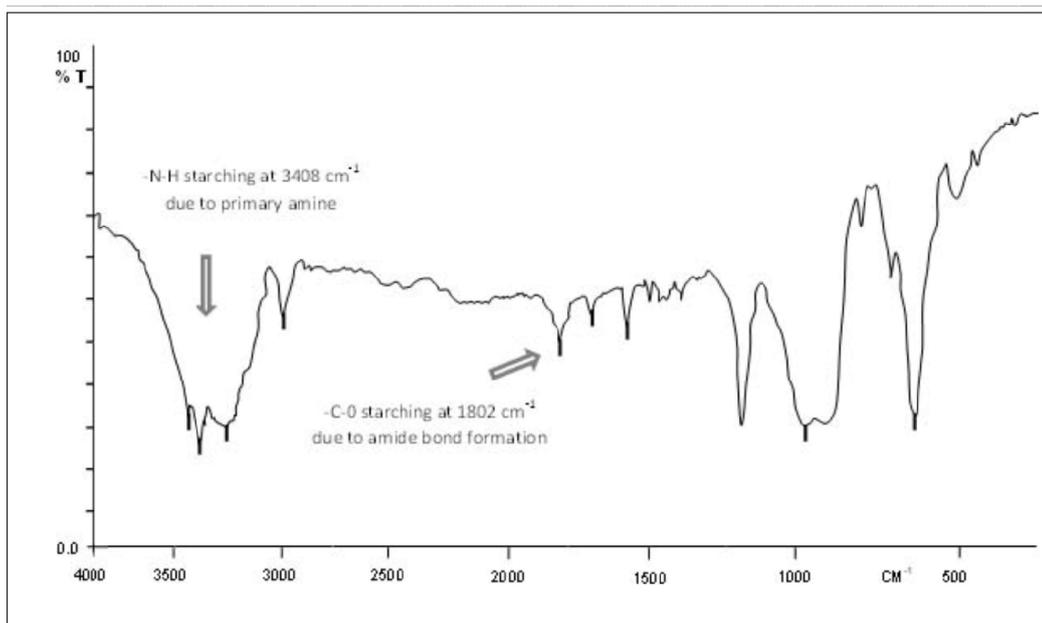
Data are expressed as the mean ± standard deviation (SD) and statistical analysis was carried out employing the oneway analysis of variance (ANOVA) test using the PRISM software (Graph Pad). A value of P\0.005 was considered statistically significant.

#### Results and Discussion

SLNs were prepared by the solvent injection method using HSPC, tristearin, and stearylamine and optimized for various process and formulation variables. The average diameter of CS-conjugated and unconjugated SLNs was found to be 131.2 ± 4.6 nm and 127.9 ± 5.3 nm respectively

**Table 1.** Various characteristic of Chondroitin sulphate conjugated and unconjugated SLNs bearing Aceclofenac and Methotrexate.

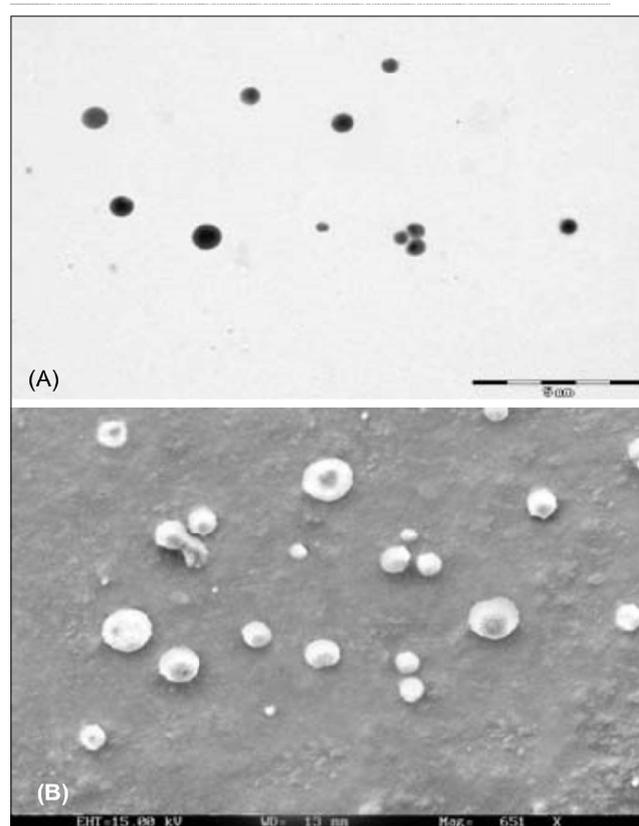
Formulation Code	Average Size (nm)	PDI	Zeta Potential (+mV)	% EE	
				Aceclofenac	Methotrexate
SLNs	127.9 ± 5.3	0.239	24.3 ± 1.6	73.08 ± 2.4	78.6 ± 2.7
CS-SLNs	131.2 ± 4.6	0.247	17.3 ± 0.9	68.95 ± 3.2	75.12 ± 3.9



**Figure 1.** FT-IR spectra of coupling efficiency of chondroitin sulphate on conjugated SLNs (CS-SLNs)

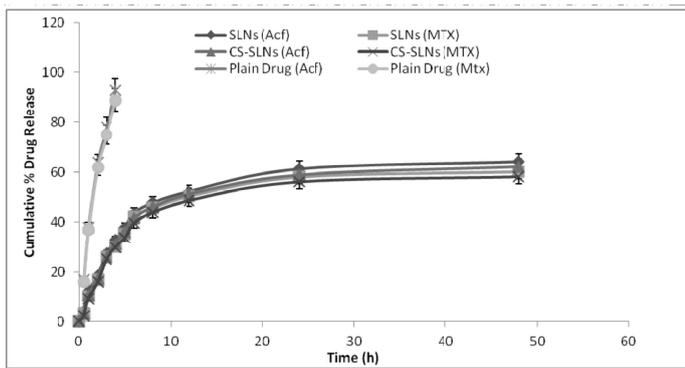
(Table 1). The size of the CS-conjugated SLNs was found to be higher when compared to the unconjugated formulation which could be due to the surface conjugation of CS. The PDI was found to be  $< 0.3$  indicating uniformity in size of both the SLNs formulation. TEM and SEM photomicrograph were shown that both CS-conjugated and unconjugated SLNs were spherical in shape and smooth in surface (Figure 2a-b). The zeta potential was found to be  $24.3 \pm 1.6$  and  $17.3 \pm 0.9$  respectively for uncoupled and coupled optimized formulation of SLNs. The positive, high zeta potential value represents good stability of SLNs formulation. The entrapment efficiency of CS-conjugated SLNs for aceclofenac was  $68.95 \pm 3.2\%$ , while  $73.08 \pm 2.4\%$  for unconjugated SLNs and % EE of MTX in CS-conjugated was  $78.6 \pm 2.7\%$ , while  $75.12 \pm 3.9\%$  for unconjugated SLNs. The drug was leached out during the incubation for the CS conjugation process and it was the reason behind decrease in % EE in case of CS-conjugated SLNs.

Chondroitin sulphate coupling of optimized SLNs formulations was carried out using carbodiimide chemistry. The amide linkage ( $-\text{CONH}-$ ) forms between the  $-\text{NH}_2$  groups stearylamine (SLNs surface) and  $-\text{COOH}$  group of chondroitin sulphate with help of EDC and NHS as a coupling agent (Figure 1). The coupling efficiency was determined by the Bradford method of protein estimation and found to be  $57.45 \pm 1.4\%$ . It was further confirmed by IR spectroscopy, the FT-IR spectra shown characteristic peak at  $3,289 \text{ cm}^{-1}$  represents  $-\text{N}-\text{H}$  stretching of the primary amine, and the peak at  $1,628 \text{ cm}^{-1}$  represents  $-\text{C}-\text{O}$  stretching. The disappearance of the secondary amine peak in the spectra was additional confirmation of chondroitin sulphate conjugation Using UV spectroscopy, CS coupling efficiency was found to be  $57.45 \pm 1.4\%$ .



**Figure 2.** (A) TEM and (B) SEM photomicrograph of Chondroitin sulphate conjugated SLNs

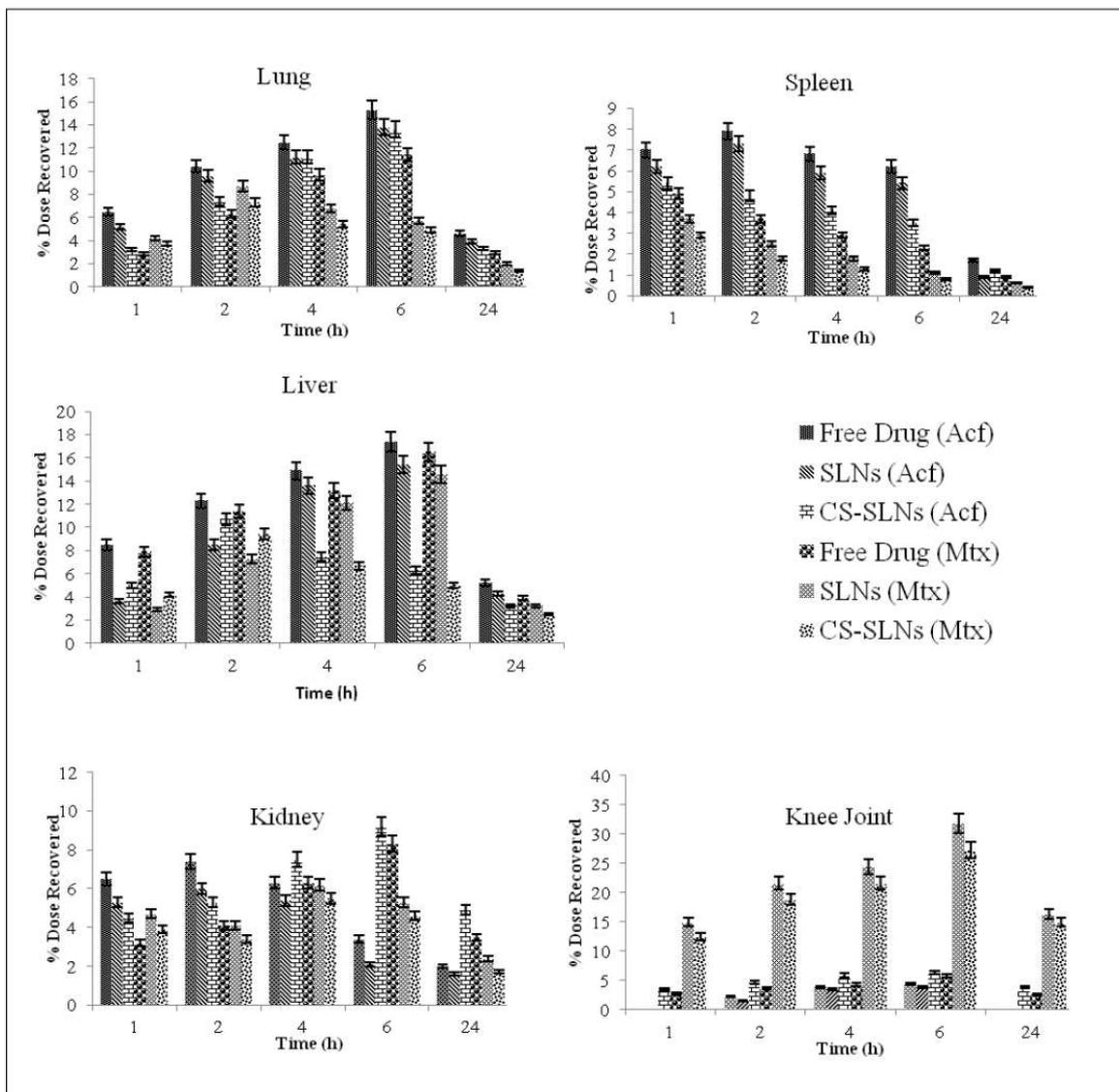
In vitro drug release of CS-conjugated and unconjugated SLNs were studied using the dialysis bag technique in PBS (pH 7.4). It was found that, In vitro aceclofenac release was  $61.35 \pm 1.08\%$  and  $58.97 \pm 2.30\%$  and release of MTX was found to be  $58.08 \pm 0.93\%$  and  $56.18 \pm 1.27$  in 24 h for



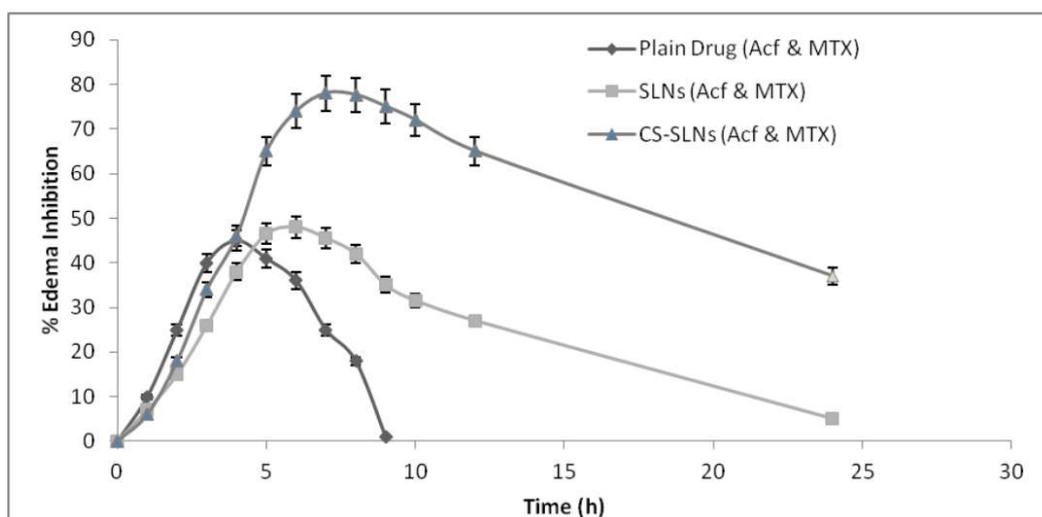
**Figure 3.** *In-vitro* drug release of Acf and MTX from chondroitin sulphate conjugated SLNs (CS-SLNs) and unconjugated SLNs.

chondroitin uncoupled and coupled SLNs (Figure 3). The delayed drug release pattern of CS-conjugated SLNs was due to due to the structural integrity of Chondroitin sulphate which creates a barrier to release drug from SLNs to dissolution media.

In the serum drug concentration study, both SLNs formulations exhibited greater performance in comparison of free drug. While, in case about chondroitin sulphate conjugated SLNs (CS-SLNs) 13.9% of drug were found after 24 h. In case of plain drug solution administration, the percent aceclofenac recovered after 6 h in different organs was  $17.4 \pm 1.1$ ,  $3.4 \pm 0.2$ ,  $6.2 \pm 0.3$ ,  $15.3 \pm 1.3$  and  $4.4 \pm 0.09$  % while MTX was  $16.5 \pm 1.5$ ,  $2.1 \pm 0.1$ ,  $5.4 \pm 0.2$ ,  $13.8 \pm 1.2$  and  $3.9 \pm 0.3$  % respectively for liver, kidney, spleen, lungs and knee joint. Chondroitin sulphate conjugated SLNs and unconjugated SLNs shown sustained drug release behaviour. Dose recovered of aceclofenac from unconjugated SLNs formulation was  $15.4 \pm 1.3$ ,  $9.2 \pm 1.1$ ,  $3.5 \pm 0.1$ ,  $13.6 \pm 1.2$ , and  $6.4 \pm 0.7$ , while for MTX it was  $14.6 \pm 2.1$ ,  $8.3 \pm 0.8$ ,  $2.3 \pm 0.3$ ,  $11.4 \pm 0.6$ , and  $5.8 \pm 0.1$  respectively for liver, kidney, spleen, lungs and knee joint. In case of CS-SLNs formulation, it was found that  $31.8 \pm 1.9$ % aceclofenac and  $27.2 \pm 1.4$ % MTX was



**Figure 4.** Organ distribution profile of CS-SLNs, SLNs and plain drug solution after IV administration



**Figure 5.** Anti-edema effect of Acf and MTX loaded SLNs formulations.

recovered after 6 h. After 24 h aceclofenac was recovered in concentration of  $16.3 \pm 1.4\%$  and MTX was  $14.9 \pm 2.2\%$  in knee joint which was 6 times more in comparison of unconjugated SLNs and 80-140 times more in comparison to plain drug solution (Figure 4). Concentration of both drugs in other organ was negligible and can be shown in figure. It is only due to the chondroitin sulphate which was conjugated with SLNs and it was responsible for targeting and high uptake in knee joint.

In anti-inflammatory study, it was observed that edema inhibition start after 1.5 h and it was maintained upto 3 h while unconjugated SLNs start edema inhibition after 3h and maintained upto 8 h. In case of CS-SLNs, it was given significant effect and starts inhibition of edema after 2 h and maintained it up to 24 hr. Both the formulation was given significant effect and due to presence of MTX, it helps to reduce immunity at the joint area. CS-SLNs formulations were given more significant result than unconjugated SLNs formulation due to site specific targeting of chondroitin sulphate. In case of CS-SLNs, the concentration of both drug higher in knee joints due to its targeting affinity to bind receptor which has overexpressed (CD44, Chondroitin sulphate, annexin receptor) on RA joints (Figure 5). Nano-matrix size and lipoidal nature of SLNs was helped to cross the biological barrier and beneficial to reach in joints.

### Conclusion

As significant result obtained from different studies, it can be concluded that on the basis of the result that the chondroitin sulphate have targeting efficiency to the RA joints and can be used as targeting ligand for the delivery of different potential therapeutic agent via a SLNs type of nanocarrier. MTX and aceclofenac was simultaneously successfully delivered by SLNs in this research work and hence, it is concluded that SLNs is a potential multi drug delivery system for effective management of RA.

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### Declaration of interest

Authors have no financial interest, direct or indirect, in the subject matter or materials discussed in the manuscript.

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