

PREPARATION, CHARACTERIZATION AND EVALUATION OF CELECOXIB LOADED NANOSPONGES FOR THE TREATMENT OF PSORIATIC ARTHRITIS

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Abstract

Psoriatic arthritis is a chronic inflammatory joint disease which is one of the types of psoriasis. 25% of all psoriasis patients develop psoriatic arthritis. It is characterised with innate and adaptive immune responses. The main objective of the present work was to prepare characterization and evaluate the Celecoxib nanosponges for the treatment of Psoriatic Arthritis. It is a non-steroidal anti-inflammatory drug (NSAID) having low solubility and low bioavailability. In order to increase the solubility, this drug was incorporate in nanosponges by melting technique. The prepared formulation was evaluated for different parameters. SEM images confirm that the prepared formulation was spherical and porous in nature. Particle size analysis shows that as the cross-linker ratio increases, there is increase in the particle size of nanosponges. Particle size was in the size range of 201.69 nm. The *in vitro* studies were carried out for prepared nanosponges which showed drug release of 89.69% in 24 h.

Keywords: Nanosponges, Psoriatic arthritis, NSAID, Drug release

Introduction

Psoriasis is the most common chronic autoimmune disease in the United States. The immune system releases proinflammatory cytokines and growth factors that accelerate the growth of skin cells which accumulate and form thick red patches of skin on various parts of the body¹. About 25% of psoriasis patients develop inflammatory arthritis in which inflammation progresses to joints and entheses. Psoriatic Arthritis (PsA) patients exhibit joint pain, stiffness, and swelling which can affect any part of the body². PsA occurs when the immune cells release cytokines that act on healthy cells and tissues to induce skin and joint inflammation. Genetic and environmental factors interact to trigger the cellular pathways that promote skin and joint disease³.

Celecoxib is used as anti-inflammatory agent used for the treatment of psoriatic arthritis but this celecoxib have poor solubility in water and belongs to BCS Class II. Celecoxib is used to treat arthritis, pain, menstrual cramps, and colonic polyps. Prostaglandins are chemicals that are important contributors to the inflammation of arthritis that causes pain, fever, swelling and tenderness^{4,5}.

Nanosponges is a novel approach which offers controlled drug delivery for topical use and is an emerging technology for topical drug delivery, as well as is employed for the improvement of performance of topically applied drugs. Nanosponges are tiny sponges with a size of about a virus, which can be filled with a wide variety of drugs. These tiny sponges can circulate around the body until they encounter the specific target site and stick on the surface and begin to release the drug in a controlled and predictable manner⁶. Nanosponges have emerged as one of the most promising fields of life science because of their application in controlled drug delivery. Nanosponge technology offers entrapment of ingredients and is believed to contribute towards reduced side effects, improved stability, increased elegance and enhanced formulation flexibility. Nanosponges are non-irritating, non-mutagenic, non-allergenic and non-toxic⁷.

In nanosponges preparation cyclodextrins are most preferable polymer because these are having more capacity to increases solubility of poorly soluble drugs when compared to other polymers. Cyclodextrins are nanometric biomaterials with a close relationship between molecular status and supramolecular

properties⁸. They are a class of cyclic glucopyranose oligomers and are synthesised by enzymatic action on hydrolysed starch. The main common native cyclodextrins are α , β and γ , which comprise six, seven and eight glucopyranose units, respectively. They have a characteristic toroidal shape, which forms a well-defined truncated cone-shaped lipophilic cavity⁹.

Materials and Methods:

Celecoxib was procured from Aarti Drugs Ltd., India. Beta cyclodextrin was procured from Himedia, India. Dimethyl carbonate and Carbopol were procured from Loba Chemie, India. Dimethyl formamide was procured from Merk, India.

Experimental Method:

Preparation of Nanosponges by melt Technique:

A series of three types of β -CD NS were prepared using Dimethyl carbonate for the crosslinking agent. Briefly an amount of anhydrous β -CD was put to react in melted DMC at 90°C for at least 5 h. Then solid was ground in a mortar and extracted with ethanol to remove either impurities or unreacted DMC. The reaction was carried out using a cross-linker excess, at three different molar ratios, 1:1, 1:2, 1:4, 1:6 and 1:8 (β -CD: Cross-linker). After purification, NS were stored at 25 °C until further use¹⁰.

Procedure for drug loading into nanosponges:

Nanosponges for drug delivery were pre-treated to obtain a mean particle size below 500 nm. The nanosponges were suspended in water and sonicated to avoid the presence of aggregates and the sample was dried by freeze drying. The aqueous suspension of NS was prepared and the excess amount of the drug was dispersed. The suspension was maintained under constant stirring for specific time required for complexation. After complexation, the uncomplexed (undissolved) drug was separated from complexed drug by centrifugation. Then solid crystals of nanosponges were obtained by solvent evaporation or by freeze drying. Crystal structure of nanosponges played a very important role in complexation with drug. A study revealed that paracrystalline nanosponges showed different loading capacities when compared to crystalline nanosponges. The drug loading was found to be greater in crystalline nanosponges than paracrystalline one. In poorly crystalline nanosponges, the drug loading occurred as

a mechanical mixture rather than inclusion complex¹⁰.

Table 1: Preparation of nanosponge in different ratio.

Formulation Code	Polymer and cross linker Ratio	
	β -cyclodextrin	DMC
CNS1	1	1
CNS2	1	2
CNS3	1	4
CNS4	1	6
CNS5	1	8

Characterisation and Evaluation of prepared Celicoxib Nanosponges:

The prepared formulations were characterized by Fourier transform infrared spectroscopy analysis (FTIR), Differential scanning calorimetric analysis (DSC), Scanning electron microscopy analysis (SEM) and X-ray diffraction analysis (XRD), solubility studies and entrapment efficiency¹¹.

Solubility studies

Drug solubility was determined by adding excess amount of pure celecoxib and nanosponge formulations in pH 7.4 phosphate buffer at 37 ± 0.5 °C in vials respectively. These vials were kept in orbital shaker for 24 h at 100 rpm. Final solution was filtered through membrane filter of 0.45 μ m. The concentration of the samples was measured using UV Visible spectrophotometer (UV 1800, Shimadzu, Japan). Each sample was analyzed triplicate¹².

Fourier transformed infrared (FT-IR) spectroscopic analysis

Fourier transform infrared analysis was conducted to verify the interaction between drug and polymer. The sample powder was dispersed in KBr powder and pellets were made by applying 4 Kg/cm² pressure¹³. FT-IR spectra were obtained by powder diffuse reflectance on a FT-IR spectrophotometer type 8400S Shimadzu.

Scanning electron microscopy (SEM)

The surface morphology of formulations was determined using a scanning electron microscope (Zeiss EVO LS 15, Smart SEM 5.05 Germany). Samples were mounted on aluminium mount, using double-sided adhesive tape and sputtered by gold under

vacuum and were scanned at an accelerating voltage of 15 KV before observation¹⁴.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on pure drug and its formulations using DSC-60 instrument. Calorimetric measurements were made with empty cell (high purity alpha alumina discs) as the reference¹³. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10 °C min⁻¹. The energy was measured as J/Kcal.

Particle size & Zeta potential analysis

The average particle size distribution and charge of the resulting nanosponge was determined by dynamic light scattering using C:\Microtrac\FLEX 11.0.0.2 Instruments, United Kingdom. The experiment was performed using clear disposable zeta cell, water as a dispersant which has refractive index (RI) - 1.330 and viscosity (cP) - 0.898 and the temperature was kept constant at 25 °C. The sample was analyzed for three times to minimize the error¹⁵.

Entrapment efficiency

To calculate the entrapment efficiency, accurately weighed quantity of 100 mg dissolved in 7.4 pH buffer stir up to 10 min to break the complex then the solution was filtered and in that 2 ml was taken from above solution and diluted up to 10 ml with 7.4 pH buffer. It was kept aside for few minutes and absorbance was measured by UV-spectrophotometer at 252 nm¹⁶.

In vitro drug diffusion studies

In-vitro drug release studies were carried out for all the nanosponges with different polymer and cross linker ratio (1:1,1:2, 1:4, 1:6 and 1:8). This were performed using dialysis membrane method. The membrane was soaked for 24 hrs in 7.4 pH buffer and receptor compartment filled with buffer and kept for stirring on a magnetic stirrer. Nanosponges powder equivalent to 100 mg was loaded in membrane temperature was maintained at 37± 0.5 °C and the speed of stirring was kept constant (600rpm) for 12 hrs. Aliquots of drug sample 5 ml was taken at 1 hr interval and replaced with equal amount of freshly prepared buffer. Each experiment was performed in triplicate. The drug analysis was done using UV-Spectrophotometer at 252 nm. The amount of drug released was calculated and the percentage drug released was plotted against time¹⁷.

Results and Discussion:

Scanning electron microscopy (SEM):

Scanning electron microscopy for the prepared formulation was done to check the morphology for the nanosponges. The SEM photographs are shown in Figure 1. The prepared optimized nanosponge (CNS3) formulation, were roughly irregular in shape and porous in nature.

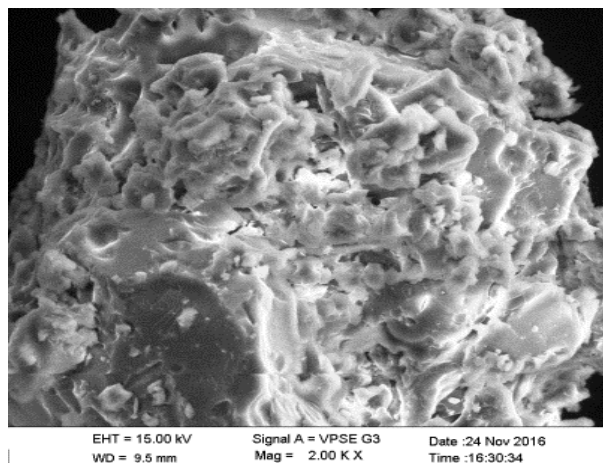


Figure 1: Surface morphology of optimized formulation (CNS3)

FT-IR studies:

The FTIR characteristic peaks of pure celecoxib and optimized formulation were observed in the Figure 2. From the data it was observed that no major interactions between celecoxib and nanosponges formulation. Hence it can be inferred that there is no chemical interaction between drug and prepared formulation and it can be concluded that the characteristic bands of pure drug were not affected by β - cyclodextrin and method used for preparation. Observed peaks in pure drug and optimized formulation were depicted in Table 2.

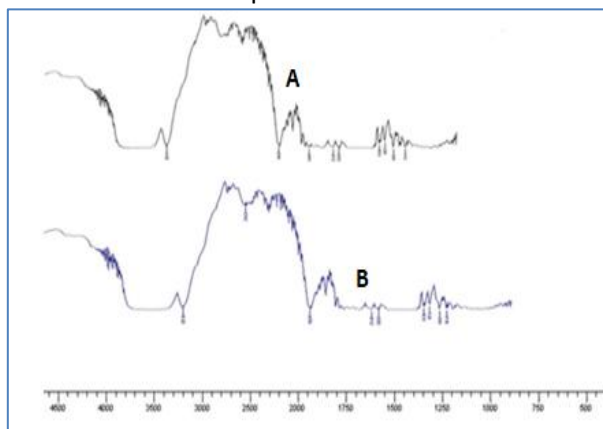


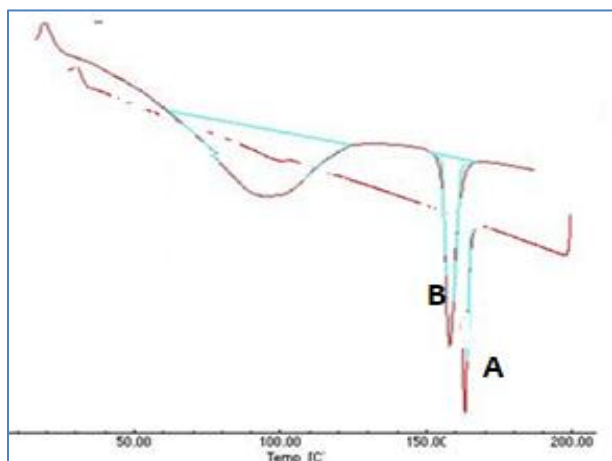
Figure 2: FTIR of A. Pure drug and B. Optimized formulation

Table 2: Observed peaks in pure drug and optimized formulation

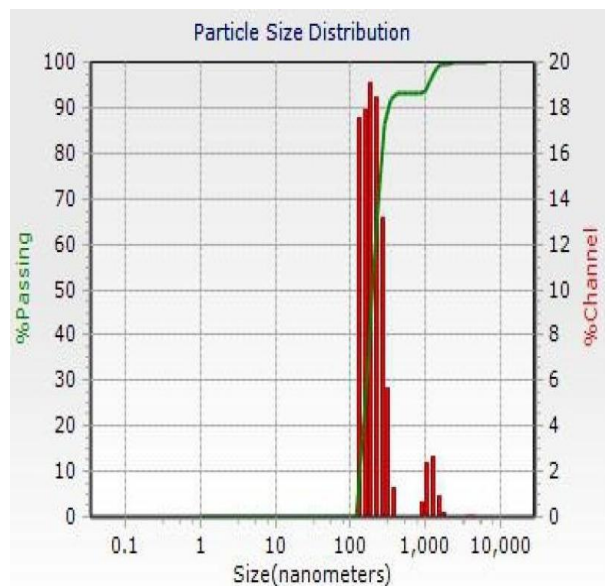
Sl. No.	Bond nature and attribute	Range	Peak observed in pure drug	Peak observed in optimized formulation
1	O-H (Alcohol)	3600-3200	3246	3401
2	C-H(Aliphatic)	~3030	3099	
3	Alkyl C-H Stretch	2950-2850	2916	2931
4	C=C	1400-1600	1487	1453
5	C-F Stretch	1000-1400	1351	1283

Differential scanning calorimetry:

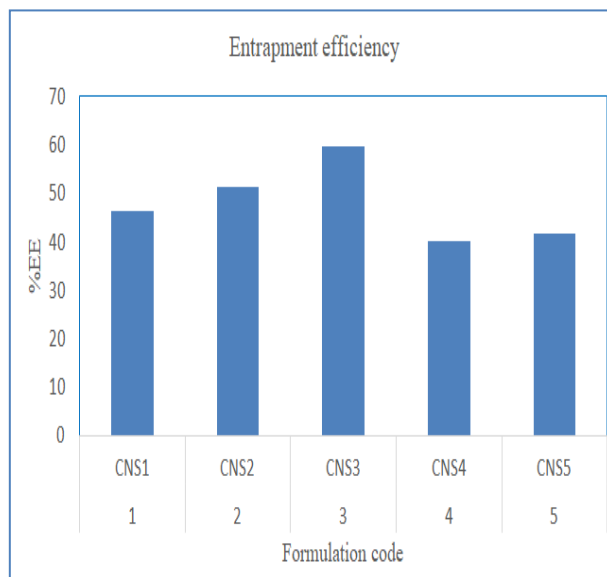
Pure drug thermogram has shown a sharp endothermic peak at 163.10 °C, which corresponds to its melting point and the prepared formulation CNS3 was showed peak at 160.06 °C represented in Figure 3. The DSC thermograms revealed that there was no significant difference between the drug and the optimized formulation. From the thermograms it was evident that melting point of celecoxib was changed when it was formulated as nanosponges.

**Figure 3:** DSC thermogram of A. Pure drug and B. Optimized formulation**Particle size and Zetapotential analysis**

The particle size analysis of optimized CNS3 formulation revealed that particle size measured by light scattering method using Zeta sizer. As seen in Figure 4, the mean diameter of formulation CNS2 is around 201.69 nm and Zeta potential gives the type of charge present on the surface of the nanosponges. This will give the stability of the prepared formulation. The dividing line between the stable and unstable suspensions is generally taken at +30 mV or -30 mV are the limits for the zeta potential. The zeta potential for the prepared formulations was found to be -17.6 mv.

**Figure 4:** Particle size of CNS3 formulation**Entrapment efficiency:**

The entrapment efficiency of prepared celecoxib nanosponge formulations were depicted in Figure 5. The entrapment efficiency gives the amount of drug entrapped in the polymer. The encapsulation efficiency was calculated for prepared nanosponges ranged from 40.28% to 59.64%.

**Figure 5:** % EE of all formulations**In vitro drug diffusion studies**

The *in vitro* test was conducted for all prepared nanosponges formulations and the obtained results were plotted in % cumulative drug release Vs time. The release data obtained for formulations CNS1 to CNS5 were showed in Figure 6.

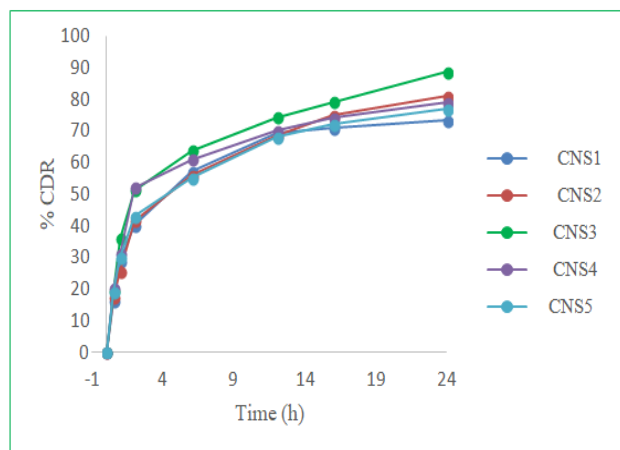


Figure 6: *In vitro* drug diffusion of prepared formulations

Conclusion:

The objective of the present work was to prepare and evaluate the celecoxib nanosponges. The prepared nanosponges were freeze dried and evaluated for particle size, zeta potential and entrapment efficiency and the results were found in the in the range. The SEM images of optimized nanosponge (CNS3) formulation showed irregular in shape and porous in nature. *In vitro* study was conducted for all prepared formulations and CNS3 formulation showed maximum drug release at 24 hrs. finally the study was concluded that the sustained release of celecoxib drug till 24 hrs was achieved by delivering it in the form of nanosponges.

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