Formulation and evaluation of semisolid dosage forms of an anti-inflammatory drug

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Abstract

Curcumin is one of the commonly used dietary supplements with wide pharmacological activities but the main hindrance in the commercial exploitation of curcumin is the issue with its solubility and stability. Hence, the aim of the present study was to formulate curcumin silver nanoparticles (CSNP) by chemical reduction method and to evaluate its solubility, stability as well as diffusion properties. The CSNP was combined with potent anti-inflammatory drug Diclofenac sodium (DS) to prepare gels (F1–F7), cream (F8) and ointment (F9). The DS–CSNP was subjected to in vitro and in vivo anti-inflammatory activity by albumin denaturation method and carrageenan-induced paw oedema method using albino rats, respectively. Results of the present study revealed that CSNP possess good solution stability in different pH solutions compared to pure curcumin, and the particle size as well as zeta potential were found to be 115 nm and -5.69 mV, respectively for CSNP. Based on the results of in vitro release study and in vitro anti-inflammatory activity, formulation F4, F5, F9 and marketed product were subjected for in vivo anti-inflammatory activity. Among the three formulations, formulation F9 showed the maximum inhibition of the oedema (82.25%) at the end of 90 min followed by F5 and F4. In addition, formulations F4, F5, and F9 exhibited better in vivo anti-inflammatory activity in comparison with the marketed product which might be due to synergistic effect of the combination of curcumin silver nanoparticles and DS. In conclusion, CSNP-incorporated DS semisolid preparations were stable and can yield promising anti-inflammatory activity compared to marketed formulation; hence, these formulation can be exploited commercially in the preparation of anti-inflammatory dosage forms.

Keywords Curcumin silver nanoparticles · Diclofenac sodium · Semisolids · Anti-inflammatory activity

Introduction

Inflammation is a normal, protective response to tissue injury by the body which involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair. Drugs used in the treatment of such condition are known as anti-inflammatory drugs. Inflammation related with rheumatoid arthritis is traditionally treated using non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, ibuprofen, ketoprofen

and celecoxib for pain relief to reduce other symptoms of the disease (Khan et al. 2014). In addition to these synthetic compounds even several herbs such as turmeric, aloe vera, clove, cinnamon, ginger, etc. were reported to possess anti-inflammatory activity. The development of NSAIDs, with reduced potential to cause gastric ulcers, was finally realized with the demonstration that clinically useful NSAIDs inhibited the enzyme cyclo-oxygenase, which was also present in the gastric mucosa. The finding that cyclo-oxygenase present in inflammatory lesions (COX2) was distinct from that found in the stomach (COX1) led to the development of selective COX2 inhibitors (Nagalakshmi et al. 2015).

Curcumin, a yellow pigment obtained from *Curcuma longa* rhizome has been used from the ancient times as a spice, dietary supplement, coloring agent and also for curing wide types of diseases. Previous research revealed that consumption of curcumin up to 8 g/day is safe and it has a wide spectrum of therapeutic effects such as anti-inflammatory, antifungal, anticancer, antispasmodic, antibacterial,

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antioxidant, antiamebic, anti-HIV, antidiabetic, antifertility and many more (Kumar et al. 2011). The important issue associated with curcumin is the solubility in water and it is highly susceptible for degradation with the pH change (Gupta et al. 2010). It is also reported that curcumin undergoes degradation up to 90% in serum-free 0.1-M phosphate buffer of pH 7.2 at 37 °C. The decomposition of curcumin was pH dependent and occurs faster under neutral and basic pH condition (Priyadarsini 2014).

To overcome its solubility- and bioavailability-related issues, curcumin-loaded nanoparticles (liposome, niosomes, solid lipid micro-particles, such as bovine serum albumin, and chitosan) have been reported by many researchers (Kaur and Kaur 2014). Nanoparticles are the clusters of atoms ranging 1–100 nm in size. These nano-size increases the penetration potential of the particles, hence aiding in better utilization of metal properties (Koliyote and Misal 2013).

Based on the above background, the present study was designed with a primary objective of preparation of curcumin nanoparticles and its semisolid dosage form and their evaluation. The secondary objective of the study is to evaluate anti-inflammatory activity of the herbo-synthetic (CSNP and DS) semisolids dosage form as a topical delivery. In this project, DS was chosen as a model drug from synthetic source and curcumin was a model drug from herbal source. The outcome of the study may provide certain advantages in the current therapy of inflammation like better patient acceptance with minimum synthetic pharmaceutical compound and better safety aspects of herbo-synthetic dosage.

Materials and methods

Materials

Diclofenac sodium (DS) and curcumin were procured from Yarrow Chem. products, Mumbai, India. Carbopol 934, white petroleum, liquid paraffin, PEG 6000, PEG 400, lutrol F-127, polyvinyl pyrrolidone (PVP) and sodium alginate were purchased from S.D. Fine Chem. Ltd, Mumbai, India. Millipore water was obtained from Millipore System (Aqualex-5). All other reagents were of the purest grade commercially available.

Methods

Preparation of curcumin nanoparticles

To a 50 ml of freshly prepared 0.001-M silver nitrate solution, 5 ml of 0.002-M sodium borohydride solution was added with continuous stirring and kept it aside for 15 min in a clean 250-ml beaker till a clear and slightly dark solution is obtained. Further, this clear solution was heated and

maintained in water bath at 45 °C for 30 min (solution A). Curcumin (50 mg) was dissolved in 4 ml of DMSO to get orange–yellow-colored solution. This solution was added drop-wise into solution A with continuous stirring using glass rod for 30–45 min till clear yellow–orange solution is obtained. The obtained solution was allowed to reach room temperature and 0.5 ml of 0.5 mcg/ml PVP solution was added as a stabilizer and filtered to get clear golden yellow-colored solution of silver nanoparticles (Sreedhar and Srikanth 2014). This solution with curcumin nanoparticles was stored in a dark cool place in a well-closed glass container until further use.

Characterization of curcumin silver nanoparticles (CSNP)

Solution stability of curcumin and silver nanoparticles Curcumin is reported to be unstable in alkaline pH and found to be more stable in acidic pH. To evaluate the degradation pattern of curcumin, the below-given degradation kinetic study was conducted.

Accurately, 100 mg of the curcumin was dissolved in 100 ml of 9:1 ratio of DMSO and water. This solution was diluted suitably to get the final concentration of 5 mcg/ml with same solution. One ml of the resultant solution was transferred to 9 ml of the phosphate buffer of pH 1.2. Absorbance was recorded at 421 nm at 5-, 10-, 15-, 30-, 45- and 60-min time interval (Bhagath and Singh 2016). Similar experiment was repeated using other buffers and the same study was conducted even for CSNP at different buffers of pH 1.2, 6.8 and 7.4.

Zeta potential and particle size determination The prepared CSNP was subjected to zeta potential analysis (Malvern Zetasizer) to determine the surface charge of the nanoparticles and also to find out their aggregation behavior. For particle size determination, 1 ml of CSNP was diluted with 9 ml of water and the sample was taken for analysis. The analysis was carried out in computer controlled Malvern particle size analyzer to find out the particle size distribution (Bhagath and Singh 2016).

Preparation of nano-gel

Seven batches of nano-gel formulations were prepared (Table 1). Polymers such as carbopol 934P, sodium alginate, lutrol F-127 were accurately weighed and soaked in sufficient quantity of water for 15 min and triturated using clean mortar and pestle. In another 250-ml beaker, DS was dissolved in propylene glycol and other ingredients were dissolved in water completely. While preparing lutrol F 127-based gel, cold method was used. The polymer was soaked in water and refrigerated at 4 °C overnight to obtain transparent gel base; refrigeration was adopted to avoid froth.

Table 1 Formulation design of nano-gels, nano-cream and nano-ointment

Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9
DS	1	1	1	1	1	1	1	1	1
CSNP	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol 934P	0.5	0.75	1	–	–	–	–	0.4	–
Sodium alginate	–	–	–	4	6	8	–	–	–
Lutrol F 127	–	–	–	–	–	–	20	–	–
Cetostearyl alcohol	–	–	–	–	–	–	–	8	–
White petroleum	–	–	–	–	–	–	–	4	–
Liquid paraffin	–	–	–	–	–	–	–	5	–
Tween 80	–	–	–	–	–	–	–	8	–
Glycerin	–	–	–	–	–	–	–	6	–
PEG 400	–	–	–	–	–	–	–	–	32
PEG 6000	–	–	–	–	–	–	–	–	32
Propylene glycol	15	15	15	15	15	15	15	–	7.5
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ethanol	10	10	10	10	10	10	10	–	–
Triethanolamine	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	–
Water up to	100	100	100	100	100	100	100	100	100

CSNP curcumin silver nanoparticles, Qs quantity sufficient, formulations F1, F2, F3, F4, F5, F6 and F7 are nano-gels, F8 and F9 are nano-cream and nano-ointment, respectively

DS solution was transferred to the gel base with triturating. Measured quantity (10 ml of 0.83% CSNP) of CSNP was also transferred to the gel base. Further, remaining quantities of the formulae were added to make up the required quantities of gel. Finally, pH was adjusted to 6.8 using triethanolamine. All the gels were allowed to equilibrate for at least 24 h at room temperature (Vats et al. 2014; Koliyote and Misal 2013; Lakshmi et al. 2011; Baviskar et al. 2013).

Preparation of nano-cream

Diclofenac sodium nano-cream was prepared (F8) as per Table 1. Cetostearyl alcohol, white petroleum, and liquid paraffin constitute oil phase, and rest of the ingredients except flavor constitute aqueous phase. Both aqueous and oil phases were heated in water bath to 75 °C separately in 200-ml beaker. When both phases attain 75 °C, aqueous phase was transferred to oil phase with continuous constant stirring at 75 rpm. Heating was removed slowly and allowed the mixture to attain room temperature. Flavor (Rose oil) was added at 35 °C to get smooth cream (Khan et al. 2014; Rao and Kharat 2010).

Preparation of nano-ointment Diclofenac sodium nano-ointment was prepared (F9) as per Table 1. PEG 6000 and PEG 400 were taken in a china dish and melted at 75 °C. Diclofenac sodium and CSNP were dissolved in propylene glycol and transferred to the molten liquid. Paraben was dissolved in water at 75 °C and transferred to the molten mass.

This mixture was triturated using glass rod constantly and allowed for cooling to room temperature. Flavor was added to the above ointment at 35 °C (Akanksha et al. 2009).

Evaluation parameters

Physical appearance and pH determination The prepared semisolid formulations were evaluated visually for their color, appearance and phase separation (Koliyote and Misal 2013). The pH of gel formulations was determined using digital pH meter (Techno Scientific products). 1 g of gel was dissolved in 100 ml of distilled water and stored for 2 h. The measurement of pH of each formulation was done in triplicate and average values were calculated (Chauhan et al. 2013).

Spreadability A sample of 1 g of semisolid was placed in the center of the glass slide of (10 × 7 cm) dimensions above which another slide of the same dimension was placed carefully. One kilogram weight was placed on slide assembly carefully for 1 min. The diameter of the spreaded semisolid was measured in centimeters using scale. This experiment was repeated thrice for each semisolid formulation (Chauhan et al. 2013).

Extrudability The extrudability of formulations from aluminum collapsible tubes was determined using universal tube filling machine. Aluminum collapsible tube filled with 10-g gels was held between two clamps. A tube was com-

pressed and extrudability of formulation was determined in terms of weight in grams.

Rheological studies Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle (no. 6), angular velocity (5–25 rpm) and temperature (25 °C) (Lakshmi et al. 2011).

Drug content One gram of the formulations was dissolved in pH 6.8 buffer and the volume was made up to 100 ml with the same buffer. The resultant solution was suitably diluted with buffer and the absorbance was measured at 276 nm using the Shimadzu-1800 UV–visible spectrophotometer.

$$\% \text{ inhibition of paw oedema} = \left\{ \frac{\text{Paw volume after Carrageenan (ml)} - \text{Paw volume (ml) after particular time}}{\text{Paw volume after Carrageenan}} \right\} \times 100.$$

The drug content was determined from the calibration curve of DS. This test was performed in triplicate and the average drug content for each formulation was calculated (Lakshmi et al. 2011).

In vitro diffusion studies Diffusion study of the semisolid dosage forms was carried out in diffusion cell at 37 ± 1 °C using 100 ml of phosphate buffer (pH 6.8) as the diffusion medium. Sample of 1 g was applied on cellophane membrane and 5 ml of each sample diffused in diffusion medium was withdrawn at constant interval of time 1, 2, 3, 4, 5, and 6 h, and the equal volume of diffusion medium was replaced with fresh diffusion medium. The obtained sample was analyzed at 276 nm using phosphate buffer as blank (Lakshmi Pet al. 2011; Leelaprakash and Dass 2011).

Inhibition of albumin denaturation for anti-inflammatory activity In inhibition of albumin denaturation technique, the reaction mixture consists of test extracts (50 and 100 µg/ml) and 1% aqueous solution of bovine albumin fraction; pH of the reaction mixture was adjusted using small amount of 1-N HCl. Samples were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min followed by cooling the samples to room temperature. Finally, the turbidity of the sample was measured at 660 nm and the percentage inhibition of protein denaturation was determined (Hafeez et al. 2013) using the below-given formula

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100.$$

In vivo studies The male adult albino rats weighing about 180–250 g were taken and markings were made on right hind paw, behind tibia tarsal junction. Each rat was placed in an observation chamber for 10 min to minimize stress-

related behaviors. The animals were divided into 5 groups (Control, Standard, F4, F5 and F9 formulations) and each group contains 6 animals. Pleurisy was induced by injecting 0.1 ml of 1% w/v Carrageenan solution subcutaneously into the sub-plantar surface of the right hind paw of the rat and the inflammation was measured by plethysmometer. Then, 0.5 g of the formulations was gently rubbed onto the plantar surface of the right hind paw 50 times with the index finger. All rats were subsequently returned to the observation chamber. The inflammatory response was assessed by measuring the volume of the paw at 30, 60, 90 and 120 min after applying the formulations using plethysmometer. The percentage inhibition of paw oedema was calculated using following formula (Koliyote and Misal 2013).

Compatibility study using FT-IR

Infrared spectroscopy was conducted using a Tensor 27 Bruker FT-IR spectrophotometer and the spectrum was recorded in the region of 4000–400/cm. The interaction between drug and excipients was observed for any shift in peaks of drug in the spectrum of physical mixture of drug (Baviskar et al. 2013).

Accelerated stability studies

The selected formulations were subjected to a stability testing for three months as per the ICH guidelines at temperature 40 ± 2 °C. The formulations were analyzed for the change in physical appearance, pH, viscosity, drug content and percentage drug release by the earlier procedures mentioned above (ICH Q1A [R2]).

Statistical analysis

The results obtained from both in vitro and in vivo studies were analyzed statistically by one-way ANOVA and multiple comparison tests using Graphpad Prism software.

Result

Solution stability of curcumin and silver nanoparticles

Solution stability studies indicated that curcumin was unstable in all aqueous solutions but both curcumin and CSNP were found quite stable in the pH 1.2 buffer (Table 2). However, solution stability of curcumin was found to be in the

Table 2 Degradation of curcumin

Time	Curcumin degradation in (mcg/ml)					CSNP degradation studies in (mcg/ml)		
	pH 1.2	pH 4.5	pH 6.8	pH 7.0	pH 8.0	CSNP pH 1.2	CSNP pH 6.8	CSNP pH 7
0.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000
1.000	3.141	0.606	1.090	0.345	0.704	4.007	0.578	4.236
5.000	3.134	0.599	1.085	0.289	0.690	4.007	0.570	4.120
10.000	3.127	0.577	1.049	0.239	0.669	4.007	0.563	4.017
15.000	3.127	0.563	1.035	0.225	0.648	4.000	0.563	4.050
30.000	3.113	0.514	0.915	0.183	0.599	4.000	0.556	4.043
45.000	3.106	0.486	0.894	0.134	0.592	4.000	0.549	4.020
60.000	3.092	0.451	0.880	0.120	0.577	3.993	0.549	3.980
Slope ^a	0	0.002	0.004	0.003	0.002	0	0	0
R ² value	0.979	0.99	0.915	0.883	0.906	0.829	0.911	0.65

^aSlope is obtained by taking linear portion of the concentration Vs time plot

following order of pH 1.2 > pH 4.5 > pH 6.8 > pH 7.0 > pH 8.0 and followed zero-order kinetics (Table 2).

Characterization of silver nanoparticle-based formulation

Surface charge (Fig. 1) particle size (Fig. 2) and polydispersity index (Fig. 2) of the CSNP was found to be 5.98 mV, 114.9 nm and 0.382, respectively. CSNP were then dispersed into semisolid dosage forms like topical gel (F1–F7), ointments (F9) and cream (F8). All semisolid dosage forms showed good visual appearance, homogeneity, extrudability, drug content, spreadability and showed no phase separation (Table 3). In vitro drug release profile of prepared formulations was compared with marketed product (see Fig. 3). Since drug release decides

the amount of drug available for absorption. The in vitro drug release studies at 6 h of various formulations was in the order of F4 > F5 > F9 > F6 > F7 > marketed product > F10 > F1 > F2 > F3 > F8 (Fig. 4). Among all formulations, formulation F4, F5 and F9 showed the highest percentage of drug release, i.e., 66.22, 54.71 and 44.85%, respectively, over a period of 6 h. Drug release patterns showed the first-order kinetics.

In vitro and in vivo anti-inflammatory studies

The in vitro anti-inflammatory results by albumin denaturation method showed significant % inhibition in all the formulations ranging from 61 to 79% (Fig. 5). However, formulations F4, F5 and F9 showed the highest amount of drug diffusion along with good in vitro anti-inflammatory

Fig. 1 Zeta potential plot for CSNP

Fig. 2 Graph of particle size distribution of CSNP **Table 3** Physical parameters of different formulation batches ($n=6$)

Formulation code	Color	Homogeneity	Phase separation	pH	Spreadability (gm cm/s)	Extrudability	% Drug content
F1	Pale yellow	Good	No	6.5	180.43 ± 0.547	Good	93.45 ± 0.442
F2	Pale yellow	Good	No	6.5	146.15 ± 0.651	Good	93.82 ± 0.570
F3	Pale yellow	Good	No	6.8	103.57 ± 0.887	Good	94.33 ± 0.905
F4	Creamy white	Better	No	7.2	145.29 ± 0.945	Good	96.55 ± 0.663
F5	Creamy white	Good	No	7.3	310.00 ± 0.673	Good	91.22 ± 0.781
F6	Creamy white	Good	No	7.3	261.54 ± 0.546	Good	94.75 ± 0.983
F7	Creamy white	Good	No	7.5	218.42 ± 0.559	Good	93.22 ± 0.944
F8	Pale yellow	Good	No	6.8	159.26 ± 0.994	Good	94.26 ± 0.976
F9	Pale yellow	Excellent	No	6.8	177.27 ± 0.932	Good	95.55 ± 0.985

Fig. 3 Rheological pattern of CSNP based semisolid formulations

activity. Hence, formulations F4, F5 and F9 were subjected to in vivo anti-inflammatory activity in rats. The results obtained were analyzed statistically by one-way ANOVA and multiple comparison tests using Graphpad Prism software. The statistical data indicated that all three selected

Fig. 4 Drug release profile of the formulations

Fig. 5 Percentage inhibition profile of in vitro anti-inflammatory studies

Table 4 In vivo anti-inflammatory activity

Formulation code	% inhibition of paw oedema			
	At 30 min	At 60 min	At 90 min	At 120 min
Control	5.101	10.55	13.79	17.96
Standard	5.343	17.02	34.66	52.77
F4	11.31	31.98	59.63	82.25
F5	12.35	32.19	48.33	64.87
F9	20.19	41.06	54.92	70.89

formulations and marketed one were highly significant (P value < 0.001) against the control. Results of in vivo anti-inflammatory activity are presented in Table 4 and Fig. 6. In vivo anti-inflammatory activity by Carrageenan-induced paw oedema method on albino rats showed that F4, F5 and F9 formulations are highly significant (P value < 0.001) against the control, and F4 formulation was found more significant vs marketed preparation. F9 and F5 formulations vs marketed preparation were also significant with P value 0.0013 and 0.0449, respectively (data showed in Table 5 and Fig. 6a–d).

Compatibility study using FTIR

FTIR spectrum of DS pure drug, CSNP and semisolid dosage forms was recorded. Pure drug showed a N–H stretching of secondary amine at 3373.980/cm, bands at 1570.851/cm were due to C=O stretching of carboxyl ion, bands at 1552.77/cm were due to C=C ring stretching, and band at 745.42/cm was due to C–Cl stretching. CSNP and semisolid dosage forms also showed all the characteristics peaks of DS; hence, it indicated the absence of any interaction between the drug and excipients. All the characteristic peaks exhibited by DS are shown in Figs. 7, 8, 9 and 10.

Stability study

Short-term stability studies was carried out for 3 months and the results showed no significant ($P > 0.05$, student t test) change in physical appearance, pH, viscosity, drug content and in vitro release profile. Results of accelerated stability studies of optimized formulations are shown in Table 6.

Discussion

Skin is one of the vital organs covering around two square meter area of the body and it is an ideal route for topical drug administration. Skin selectively absorbs the vital components of the formulation and transfers to the blood stream through various hair follicles, sweat glands and pores available on the skin because of which it is widely exploited for drug delivery. Various physicochemical properties of the drug such as the dose, solubility, molecular size, partition coefficient, polarity, etc. influence the absorption of the drug. Considering the above factors, semisolids are promising dosage forms for local as well as systemic therapies as they possess exclusive advantages over liquids and solid dosage forms. Most of the anti-inflammatory, anti-bacterial, anti-fungal, anti-infective and anti-psoriatic drugs are usually administered through skin for local application. Recently, few attempts were also been made to deliver drug systemically through this route.

Curcuminoids are the major potent constituents of Turmeric (*Curcuma longa*) with the absorption maxima (λ_{max}) of 421 nm. Curcumin in hydro-alcoholic/aqueous solution was found to get degrading instantaneously indicating its instability in respective solutions. Hence, an attempt was made to quantify the degradation of the curcumin in various pH buffer solutions such as pH 1.2, 4.5, 6.8, 7.0 and 8.0. The amount of curcumin was determined using the calibration curve of curcumin which was linear with a regression coefficient of 0.996 and a slope of 0.142. The results indicated that curcumin was unstable in all aqueous solutions as the absorbance was decreasing with time and solution stability of curcumin was found in the following order of pH 1.2 $>$ pH 4.5 $>$ pH 6.8 $>$ pH 7.0 $>$ pH 8.0. Hence, in the present study, curcumin nanoparticles was prepared and evaluated for various parameters and incorporated into semisolid dosage form.

Preparation of nanoparticles was found to be sensitive and following observations were made during the experiment without following which proper silver nanoparticles could not be observed. Silver nanoparticles did not form when heated using heating mantle but when heated in an electrical water bath, nanoparticles were formed properly indicating that maintaining uniform temperature is critical in nanoparticles formation. Constant stirring and drop-wise addition of curcumin solution are important

Fig. 6 In vivo anti-inflammatory activity studied at different time intervals

to get clear nanoparticles; otherwise, a precipitation can be seen immediately or soon after the preparation. During the study, it has been observed that addition of more curcumin ($> 1.0\%$ w/v) to the silver nitrate solution yields curcumin precipitate immediately, and the ideal concentration of curcumin was of 0.83% w/v to be incorporated to yield proper silver nanoparticles with more stability.

In addition, a slight change in color was observed after preparation of silver nanoparticles after 15 days but no significant variation was seen in the assay of curcumin indicating further on the stability of silver nanoparticles of curcumin. The nanoparticles solution formed was clear, golden yellow in color with a pH of 6.7. Nanoparticles

Table 5 Data of statistical analysis

Comparison formulations	At 30 min		At 60 min		At 90 min		At 120 min	
	P value	Significance	P value	Significance	P value	Significance	P value	Significance
control Vs. standard	> 0.9999	No	0.7617	No	0.0057	Yes	< 0.0001	Yes
control Vs. F9	0.0096	Yes	< 0.0001	Yes	< 0.0001	Yes	< 0.0001	Yes
control Vs. F5	0.4277	No	0.0047	Yes	< 0.0001	Yes	< 0.0001	Yes
control Vs. F4	0.4483	No	0.0052	Yes	< 0.0001	Yes	< 0.0001	Yes
standard Vs. F9	0.0110	Yes	0.0016	Yes	0.0075	Yes	0.0013	Yes
standard Vs. F5	0.4611	No	0.0718	No	0.1146	No	0.0449	Yes
standard Vs. F4	0.4823	No	0.0777	No	0.0008	Yes	< 0.0001	Yes
F9 Vs. F5	0.3366	No	0.5050	No	0.7391	No	0.5794	No
F9 Vs. F4	0.3189	No	0.4822	No	0.9039	No	0.0666	No
F5 Vs. F4	> 0.9999	No	> 0.9999	No	0.2533	No	0.0020	Yes

Fig. 7 FT-IR Spectra of DS drug

size was found to be 115 nm and zeta potential was -5.98 mV.

Degradation studies for these silver nanoparticles indicated that silver nanoparticles of curcumin are more stable in solution than curcumin alone. Further acidic pH favors the curcumin silver nanoparticles. Half-life of CSNP was higher in comparison with curcumin alone in solution. These results suggested that CSNP was more stable in vitro than curcumin. Hence, CSNP were incorporated into the semi-solid dosage form (gel, ointment and cream) and was evaluated for physicochemical, in vitro release and both in vitro and in vivo anti-inflammatory studies. Viscosity of semi-solid dosage forms was decreased with increase in shear rate,

which indicates the characteristics of pseudo plastic flow and follow non-Newtonian flow (shear thinning). For the topical formulations, the consistency of the sample is an important feature, due to the fact that it must be applied to outer layer of skin. So, it is preferable to formulate Non-Newtonian formulations because of their low resistance to flow when they are applied under the high friction.

Drug content present in the prepared semisolid dosage formulations was in the range of 93–113% suggesting higher availability of drug to exhibit the desired pharmacological activity. The values of spreadability indicate that prepared formulations spread by small amount of shear. The pH of the CSNP-based semisolids was between 6.8 and 7.5 at 25 °C,

Fig. 8 FT-IR Spectra of CSNP

Fig. 9 FT-IR Spectra of DS + Carbopol combination formulation (F3)

and this is the acceptable range for topical preparations. The in vitro release studies were conducted to explore drug release patterns from which in vivo drug behavior can be analyzed. The amount of drug released from different formulations was in the order of F4 > F5 > F9 > F6 > F7 > marketed product > F10 > F1 > F2 > F3 > F8 and all the formulations showed first-order release kinetics.

In the in vitro anti-inflammatory activity by albumin denaturation method, all the formulations exhibited 61–79% percentage of inhibition. Since all the formulations including the marketed one showed greater than 60% inhibition,

three formulations such as F4, F5 and F9 were selected for in vivo anti-inflammatory activity in animals based on in vitro release studies and anti-inflammatory studies.

Results of the in vivo anti-inflammatory study evaluated by Carrageenan-induced paw oedema method on albino rats revealed that at 60-min F9 formulation shows significant ($P < 0.001$) anti-inflammatory activity among the tested samples followed by F5, F4 and standard. However at 120-min time interval, F4 formulation (CSNP and DS gel) exhibited significantly higher anti-inflammatory activity followed by F9 and F5 and marketed standard drug (DS). Hence, it

Fig. 10 FT-IR Spectra of F4 formulation

Table 6 Accelerated stability study of the optimized formulations ($n=6$)

Formulation	Month	Color	pH	Viscosity (cps)	Drug content (%)	% CDR	<i>P</i> value	significance
F4	0	Creamy white	7.2	514,400	96.55±0.14	66.22±0.66	> 0.05	No
	1	Creamy white	7.2	514,400	96.55±0.26	66.18±0.70		
	2	Creamy white	7.2	514,400	96.23±0.43	66.12±0.33		
	3	Creamy white	7.2	514,000	96.20±0.73	66.10±0.41		
F5	0	Creamy white	7.3	23,800	91.22±0.45	54.71±0.78	> 0.05	No
	1	Creamy white	7.3	23,800	91.20±0.24	54.70±0.83		
	2	Creamy white	7.3	23,750	91.20±0.73	54.56±0.52		
	3	Creamy white	7.3	23,700	91.20±0.66	54.20±0.44		
F9	0	Pale yellow	6.8	106,200	95.55±0.98	44.85±0.74	> 0.05	No
	1	Pale yellow	6.8	106,200	95.55±0.33	44.82±0.55		
	2	Pale yellow	6.8	106,195	95.48±0.55	44.79±0.70		
	3	Pale yellow	6.8	106,195	95.45±0.57	44.70±0.58		

can be concluded that all F4, F5 and F9 formulations were better than the marketed formulation in depicting anti-inflammatory activity in animal model because of the obvious reason of combining curcumin silver nanoparticles and

DS. In addition, the results of percentage inhibition of the paw oedema revealed the existence of correlation between in vitro drug release studies and anti-inflammatory activity.

FTIR studies confirmed that there was no interaction between DS and excipients, as all characteristic peaks of DS were evidenced in the spectrum of formulations indicating compatibility between drug and excipients.

Three optimized formulations were subjected for 3-month stability studies at specific temperature and relative humidity. There was no significant change in physical appearance, pH, viscosity, drug content and in vitro release profile. This indicates that there is no significant ($P > 0.05$, student t test) drug degradation during study period and prepared formulations were stable.

Conclusion

Results obtained from the present study revealed that the prepared CSNP is highly stable in comparison with the normal curcumin. The herbo-synthetic semisolid dosage forms of CSNP and DS possessed good physicochemical properties with the highest amount of drug release with good stability. Anti-inflammatory activity of these formulations confirmed that the topical delivery of herbo-synthetic semisolids of DS and CSNP are better than the marketed preparation. Further, no interaction was also observed among the drugs/excipients. Hence, we conclude that herbo-synthetic semisolids of DS and CSNP show synergistic effect and promising therapeutic effect and can be commercially exploited and released into the market after clinical trials in human beings.

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