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Article

Anionic Surfactant Based Topical Curcumin Nanosuspension: Fabrication, Characterization and Evaluation

Moorthi Chidambaram[1*], Senthilkumar Chinnamaruthu[2], Mohan Sellappan[2], Kathiresan Krishnasamy[1]

[1]Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India
[2]Department of Pharmacy, Shri JJT University, Vidyanagari, District-Jhunjhunu, Rajasthan, India

* Corresponding author: cmoorthitgodu@gmail.com

Abstract

Curcumin, a hydrophobic polyphenol isolated from dried rhizomes of turmeric exhibits diverse pharmacological activities including anti-bacterial. However, the clinical usefulness of curcumin was limited mainly due to low aqueous solubility and stability. The primary aim of the study was to prepare anionic surfactant based curcumin nanosuspension and to assess its *in-vitro* anti-bacterial efficacy on *Escherichia coli* in comparison with ethanolic solution of curcumin. Curcumin nanosuspension was prepared by nanoprecipitation method, characterized for the average particle size, span, uniformity, surface area, and surface morphology and assessed for its anti-bacterial activity against gram-negative bacteria *Escherichia coli* using agar well diffusion method. Prepared curcumin nanosuspension showed an average particle size of about 175 nm, span of about 1.5, uniformity of about 0.8, surface area of about 57 m^2/g and nanoparticles were spherical in shape. Curcumin nanosuspension have shown significant ($P<0.05$) anti-bacterial activity against *Escherichia coli* at various concentrations in comparison with ethanolic solution of curcumin. We conclude that the size reduction of curcumin in nano range has increased the surface area resulting in increased aqueous solubility and reactability of curcumin, which in turn have increased the potency of curcumin nanosuspension.

Keywords: Anionic Surfactant, Curcumin, Nanoprecipitation Method, Nanosuspension, Sodium Lauryl Sulfate

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1. Introduction

Curcumin (diferuloylmethane), a hydrophobic polyphenol isolated from dried rhizomes of turmeric (*Curcuma longa* Linn.) exhibits diverse pharmacological activities including anti-inflammatory, anti-oxidant, and anti-bacterial due to wide spectrum of molecular targets and useful in the treatment of various diseases including acquired immune deficiency syndrome, allergy, alzheimer's disease, arthritis, atherosclerosis, cancer, cataract, cystic fibrosis, diabetes mellitus, epilepsy, fever, gall stones, gastric ulcer, hypothyroidism, inflammatory bowel disease, leishmaniasis, lung diseases, malaria, multiple sclerosis, myocardial infarction, osteoporosis, pancreatitis, parkinson's disease, psoriasis, scleroderma, and wound [1-5].

However, the clinical usefulness of curcumin was limited mainly due to low aqueous solubility and stability. Many approaches have been tried to enhance curcumin aqueous solubility, which includes albumin nanoparticles, co-solvent, cyclodextrin inclusion, liposome, magnetic nanoparticles, microcapsule, microsphere, nanosponges, phospholipids complexes, polymer micelles, polymeric nanoparticles, solid dispersion, solid lipid nanoparticles and surfactant free nanoparticles [6,7]. However, anti-bacterial activity of anionic surfactant based curcumin nanosuspension was not yet reported. Hence, the primary aim of the study was to prepare anionic surfactant based curcumin nanosuspension and to assess its *in-vitro* anti-bacterial efficacy on *Escherichia coli* in comparison with ethanolic solution of curcumin.

2. Experimental section

2.1 Materials

The chemicals used in this study were obtained from commercial sources and used as received without any further purification. Curcumin, β-cyclodextrin, nutrient broth and nutrient agar were obtained from Himedia Laboratories (Mumbai, India). Analytical grade ethanol was obtained from Brampton (Ontario, Canada). Sodium Lauryl Sulfate was obtained from S.D Fine Chemicals (Mumbai, India).

2.2 Fabrication of anionic surfactant based topical curcumin nanosuspension

Anionic surfactant based topical curcumin nanosuspension was prepared based on nanoprecipitation method

[2,6]. Briefly, 100 mg of curcumin was dissolved in 20 ml of ethanol and sonicated (Lark, India) for 5 minutes. The prepared organic phase was then added to 50 ml of aqueous phase containing 50 mg of sodium lauryl sulphate and 50 mg of β-cyclodextrin under the influence of sonication (40 kHz; Lark, India) for 60 minutes. Prepared curcumin nanosuspension was subjected to ultra centrifugation (Remi, India) at 19,000 rpm for about 45 minutes at −20°C to separate sodium lauryl sulphate coated curcumin nanoparticles, which was washed and re-suspended in distilled water and used for further characterization.

2.3 Characterization of anionic surfactant based topical curcumin nanosuspension

The average particle size, span, uniformity and surface area of the prepared anionic surfactant based topical curcumin nanosuspension was measured based on laser light scattering principle using Mastersizer (Malvern, UK) [6]. Briefly, prepared curcumin nanosuspension was added drop-wise in to the water maintained in the sample dispersion unit of particle size analyser, where the nanoparticles scattered using single shaft pump and stirrer and re-circulated continuously around the measurement zone of the particle size analyser. The surface morphology of the prepared curcumin nanosuspension was determined by transmission electron microscopy (Hitachi H7500) at 20,000 magnifications.

2.4 *In-vitro* anti-bacterial assay of anionic surfactant based topical curcumin nanosuspension

Gram-negative bacteria *Escherichia coli* were used to study the anti-bacterial activity of anionic surfactant based topical curcumin nanosuspension using agar well diffusion method [8]. Briefly, sterilized nutrient agar media was poured into standard petri plates (60×15 mm) with 4 mm depth and allowed to solidify at room temperature. *Escherichia coli* were then inoculated on the solidified agar plate. A 3 mm wide well/cavity was made using a sterilized steel borer in each petri plates. Various concentrations of prepared anionic surfactant based topical curcumin nanosuspension and ethanolic solution of curcumin were added to corresponding well/cavity using micropipette. The petri plates were maintained undisturbed at room temperature for 30 minutes to allow the diffusion of samples and then incubated for 24 hours at 37°C. Zone of inhibition diameter was measured after incubation period. The entire assay was carried out under aseptic condition in triplicate. The anti-bacterial potential of the samples were assessed based on the mean diameter of zone of inhibition around the wells.

2.5 Statistical analysis

The *in-vitro* study results were expressed as mean ± standard deviation (SD) and student's t-test was used to assess the difference between groups using GraphPad Prism software (version 5.04). The differences were considered significant if P value < 0.05 and nonsignificant if P value > 0.05.

3. Results and Discussion

3.1 Fabrication of anionic surfactant based topical curcumin nanosuspension

Anionic surfactant based topical curcumin nanosuspension was prepared based on nanoprecipitation method. During fabrication, addition of organic phase in to aqueous phase leads to rapid miscibility of ethanol with water resulting in initiation of curcumin nucleation. Simultaneously, sonication process produces cavitation (bubbles), which oscillates nonlinearly and ultimately collapse resulting in production of high temperature, high pressure, and shocking wave, which inhibits curcumin nucleation at the initial stage. However, nucleated curcumin forms complex with β-cyclodextrin and increases the solubility of curcumin in the aqueous phase. Subsequently, anionic surfactant sodium lauryl sulfate get adsorbed on the formed complex. Anionic nature of the surfactant provides higher zeta potential to the formed nanoparticles and develops an electrostatic force, which maintains the nanoparticles in Brownian motion and overcomes the Van der Waals force of attraction

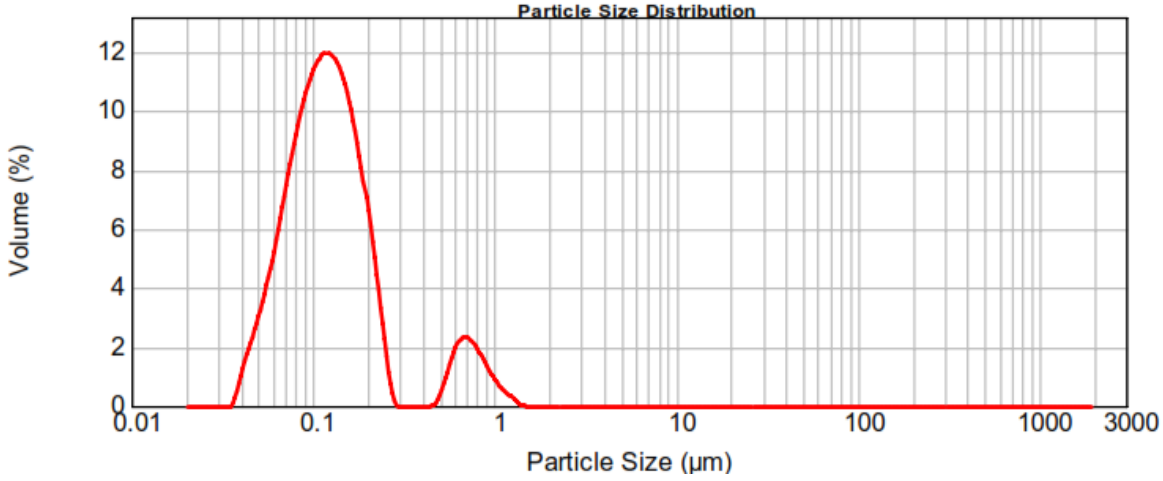

Fig. 1 Particle size distribution of prepared anionic surfactant based topical curcumin nanosuspension .

and gravitational force resulting in the prevention of nanoparticle aggregation and sedimentation. Though sodium lauryl sulphate coated curcumin nanoparticles were formed instantaneously, sonication process was continued up to 60 minutes to remove the residual ethanol present in the curcumin nanosuspension. Prepared curcumin nanosuspension was subjected to ultra centrifugation at 19,000 rpm for about 45 minutes at −20°C to separate sodium lauryl sulphate coated curcumin nanoparticles and washed with distilled water to remove the free curcumin, sodium lauryl sulphate, and β-cyclodextrin.

3.2 Characterization of anionic surfactant based topical curcumin nanosuspension

Prepared curcumin nanosuspension was characterized for average particle size, surface area, span, uniformity and morphology as these parameters determines the solubility, cellular uptake and consistency of performance. Prepared curcumin nanosuspension showed an average particle size of about 175 nm, span of about 1.5, uniformity of about 0.8 and surface area of about 57 m^2/g (Fig. 1). Transmission electron microscopy (TEM) image of prepared curcumin nanosuspension has shown spherical morphology of sodium lauryl sulphate coated curcumin nanoparticles at 20,000 magnifications (Fig. 2).

3.3 *In-vitro* anti-bacterial assay of anionic surfactant based topical curcumin nanosuspension

Prepared anionic surfactant based topical curcumin nanosuspension was studied for its anti-bacterial activity against gram-negative bacteria *Escherichia coli* at various concentrations in comparison with ethanolic solution of curcumin and the results are summarized in table 1. At 50 µL concentration, ethanolic solution of curcumin has produced about 14 mm zone of inhibition and further increase in concentration to 100 µL and 150 µL has produced only 16 mm zone of inhibition, which shows the non-linearity in response when increase in concentration. However, 50 µL concentration of curcumin nanosuspension has produced 21 mm zone of inhibition

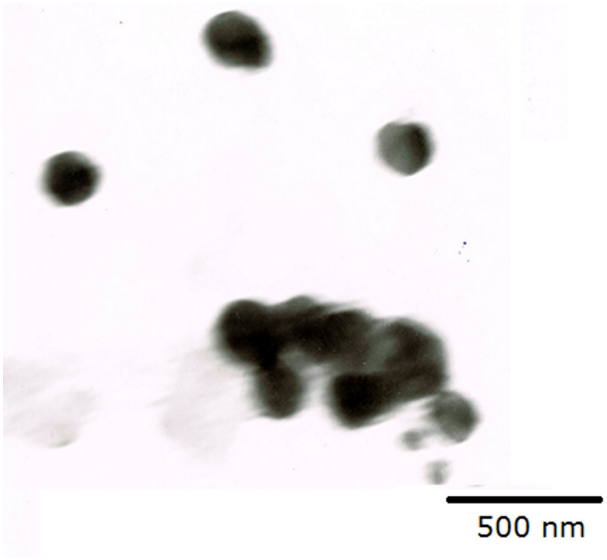

Fig. 2 TEM image of anionic surfactant based topical curcumin nanosuspension.

and further increase in concentration to 100 µL and 150 µL has produced 25 mm and 29 mm zone of inhibition, which shows the linearity in response when increase in concentration. However, curcumin nanosuspension have shown significant ($P<0.05$) anti-bacterial activity against *Escherichia coli* at various concentrations in comparison with ethanolic solution of curcumin (Fig. 3).

Table 1 Anti-bacterial activity of anionic surfactant based topical curcumin nanosuspension and ethanolic solution of curcumin against *Escherichia coli*

Samples	Concentrations of samples		
	50 µL	100 µL	150 µL
	Mean inhibition zone diameters (in mm)		
Ethanolic solution of curcumin (2 mg/ml)	14±0.20	16±0.23	16±0.19
Curcumin nanosuspension (2 mg/ml)	21±0.12*	25±0.05*	29±0.08*

*P < 0.05, as compared to ethanolic solution of curcumin.

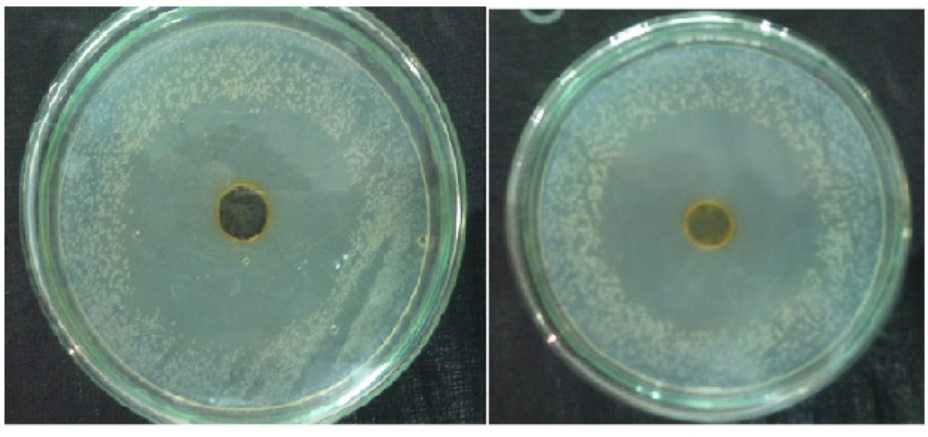

Fig. 3 Anti-bacterial assay of curcumin nanosuspension against *Escherichia coli* in comparison with ethanolic solution of curcumin.

4. Conclusion

We conclude that the prepared curcumin nanosuspension has produced average particle size less than 200 nm with narrow distribution range. Moreover, size reduction of curcumin in nano range have increased the surface area resulted in increased aqueous solubility and reactability of curcumin, which in turn have increased the potency of anionic surfactant based topical curcumin nanosuspension.

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