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Preparation, Characterization and Stabilization of Curcumin Nanosuspension

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Abstract: Curcumin is a natural product found in the rhizome of *Curcuma longa* being responsible for its biological actions. It exhibits anti-inflammatory, anti-viral, anti-bacterial, antioxidant and nematocidal activities. Due to its poor solubility in water there is a need to formulate this curcumin for better bioavailability. Curcumin was extracted by acetone using soxhlet apparatus and evaporated by vacuum rotary evaporator. Nano suspension was prepared by bottom up method using acetone and water as solvent system in which curcumin was dissolved in acetone and added to this solution drop wise into a beaker containing water with constant stirring. Different amount of SDS was used as stabilizer and the stability was optimized. Nanosuspension was characterized using particle size analyzer, zeta potential analyzer and SEM. The stability of this formulation was characterized by the measurement of zeta potential. Result showed that prepared nanosuspension has good stability (Zeta potential -33.3 ± 2.45 mV) up to 4 weeks from the day of preparation. The method adopted for the preparation of nanosuspension was found to be good. The use of SDS for the stability was well demonstrated.

Key words: Curcumin, *Curcuma longa*, nanosuspension, natural products, formulation, Solubility.

Introduction

Turmeric or *Curcuma longa* is a tropical plant native to southern and southeastern tropical Asia. Turmeric is one of the most essential spices used all over the world with a long and distinguished human use[1]. Curcumin is a natural product found in the rhizome of *Curcuma longa* being responsible for its biological actions[2]. It has been shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, anticancer activities and has a potential against various malignant diseases, diabetes, allergies, arthritis, alzheimer's disease etc[3]. Indian system of medicines claims the use of its powder against biliary disorders, anorexia, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis[4]. Curcumin also binds with heavy metals such as cadmium and lead, thereby reducing the toxicity of these heavy metals. This property of curcumin explains its protective action to the brain[5]. Components of turmeric are named curcuminoids, which

include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin[6]. All of these compounds are poorly soluble in water.

It is well established in the literature that poor bioavailability is one of the leading causes of compound failure in preclinical and clinical development[7]. Bioavailability of a compound depends on its solubility or dissolution rate[8]. Dissolution may be the rate determining step for bioavailability and medicinal value therefore, efforts to increase dissolution rate for water insoluble drug is often needed[9].

Nanosuspensions found to be promising methodology that can be used for enhancing the dissolution of poorly water soluble compounds[10]. Nano-suspensions play an important role in drug delivery system associated with water-insoluble and both water and lipid-insoluble drugs[11]. These suspensions keep pharmaceutical active ingredient at the submicron levels in a liquid phase stabilized by added stabilizers[12].

Preparation of nanosuspensions was reported to be a more cost effective and technically simple alternative and yield physically more stable product than liposomes[13]. An alternative and most promising strategy to exploit science and technology at the nanometer scale is offered by the bottom-up approach, which starts from nano to subnano structures[14]. The bottom-up approach is widely accepted in the area of nanoscience and nanotechnology .The basic challenge of this technique is that during the precipitation procedure the growth of the crystals needs to be controlled by the addition of stabilizers to avoid Agglomeration or Aggregation that develop the formation of microcrystals. The zeta potential (ZP) is a function of the surface charge which develops when a particulate matter is placed in a liquid[15]. It is a good index of the magnitude of the electrostatic repulsive interaction between particles. This ZP is commonly used to predict stability of the formulation[16].

Materials and methods

Preparation of extract

The commercially available food graded rhizomes of *C. longa* (100 g) was purchased from local market. It was broken into small pieces and covered by the filter paper. This material was placed inside the soxhlet extractor. About 500 ml of Acetone was used for this process. The extract obtained from soxhlet apparatus was transferred to rotary vacuum evaporator. At 40°C solvent was completely removed. The remaining residue was collected aseptically and kept at the airtight container. It was stored at 4°C after the mass ratio and yield was measured. This residue was recrystallized by saturation solubility method using different solvent system. The crystals obtained from evaporation was subjected for characteristic analysis such as physical parameter like melting point and solubility then by instrumentations like TLC and UV-VIS spectro photometer to find out the presence and purity of curcumin.

Solubility: Curcumin is soluble in acetone, methanol, DMSO and dimethyl formamide. The solubility was measured by taking known amount of saturated solution and the solvent was evaporated to dry.

TLC : A glass plate (20x10cm) layered with 2mm thickness of silica gel and activated at 105°C for 15 minutes after drying. Methanolic solution of curcumin extract was spotted on this plate. Then the plate was kept in a chromatographic tank containing chloroform: hexane: methanol (1:1:0.5) as mobile phase.

Analysis of curcumin by UV-Vis spectrophotometer

A double beam scanning UV-VIS spectrophotometer (Model No.2310 made in China) was used to measure the absorbance of the solution. A 10 mg of residue was dissolved in acetone and placed in a UV-VIS spectrophotometer for the identification of curcumin and other impurities by wavelength scanning. The wavelength of the respective absorbance plotted by the instrument is given (Figure-2).

Preparation of Nanosuspension

Bottom up method

In bottom up technology the poor water soluble drug is dissolved in a non polar solvent then added to polar solvent to form precipitate. This experiment prepared this nano suspension by bottom up method using saturation solubility with acetone as non polar solvent and water as polar solvent. About 200 mg of curcumin

crystal was dissolved in 10 ml of acetone. Then the solution was added dropwise to 100 ml of distilled water which was kept on a magnetic stirrer for continuous stirring.

Stability optimization

At the above process water alone was taken without any added stabilizer that served as blank. Different quantities (100, 200, 300, 400 and 500 mg) of SDS were added to this 100 ml distilled water to prepare the stabilizer with different concentration having 100, 200, 300, 400 and 500 mg/dl) of SDS. The stability of each suspension was observed by measuring zeta potential immediately and thereafter at the interval of a week and continued up to 4 weeks.

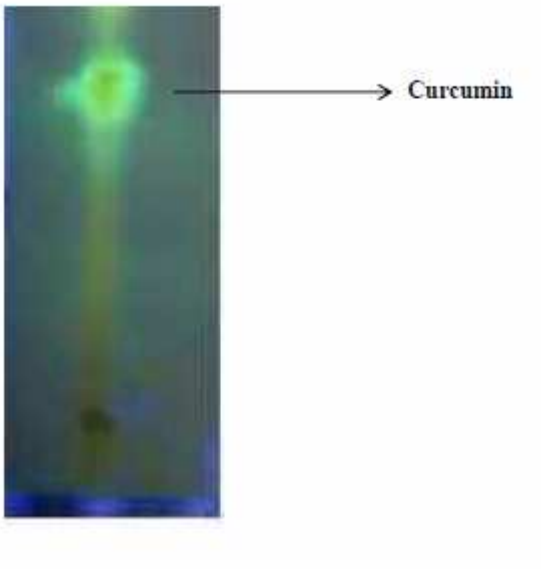
Characterization of Nanoparticles

The particle size and distribution of particles of prepared curcumin nanosuspension was measured by Dynamic Light Scattering principle using a particle size analyzer Nanotrac NPA151 - Nanotrac ULTRA version 10.5.2 at CSIR – CECRI Government of India. The surface morphology was observed using scanning electron microscope (SEM) and zeta potential by Zeta potential analyzer.

Result and Discussion

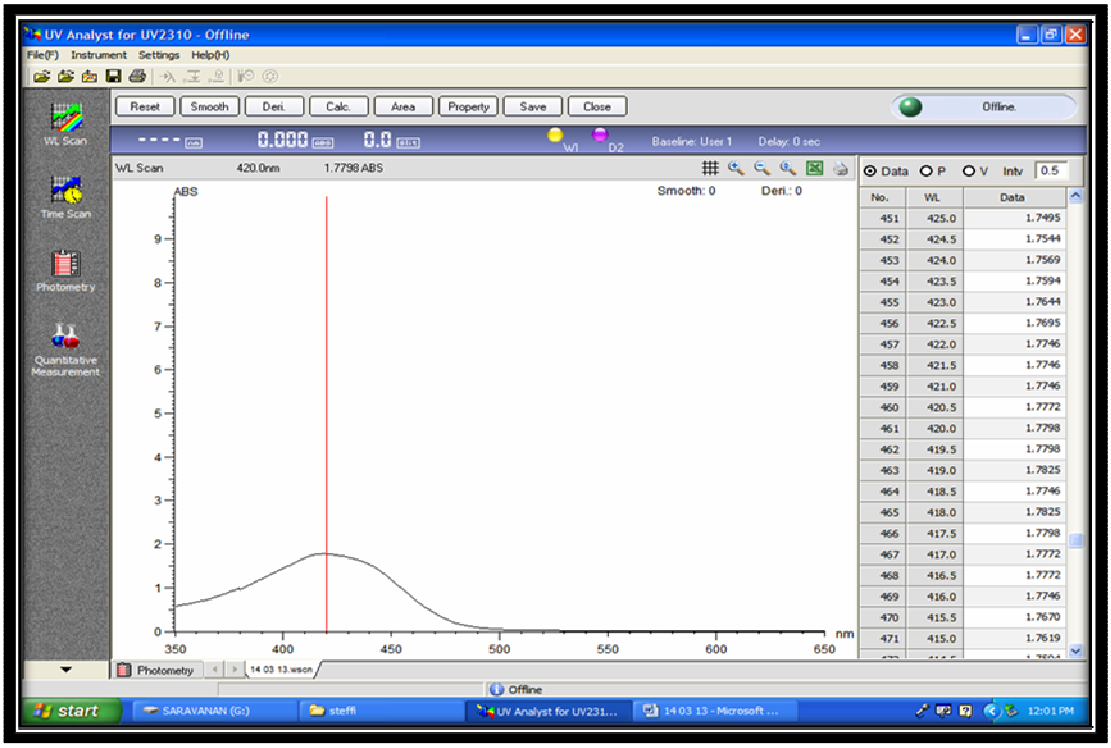
Physical parameters: Melting point was found to be 183°C. The residue obtained was weighed to find out the dissolved quantity of curcumin. **Solubility**: curcumin was found to be highly soluble in acetone (about 20 mg/ml) and poor in other solvents (less than 1 mg/ml).**TLC**: After the development of chromatogram, it was illuminated by UV light. The presence of curcumin was confirmed by the appearance of fluorescence light found around the developed spot on illumination by UV (Figure -1).

Fig. 1: Thin layer chromatography



UV-VIS spectrophotometer: Wavelength scanning by UV-VIS spectrophotometer showed absorption maximum at 420 nm. That confirmed the presence of curcumin (Figure -2).

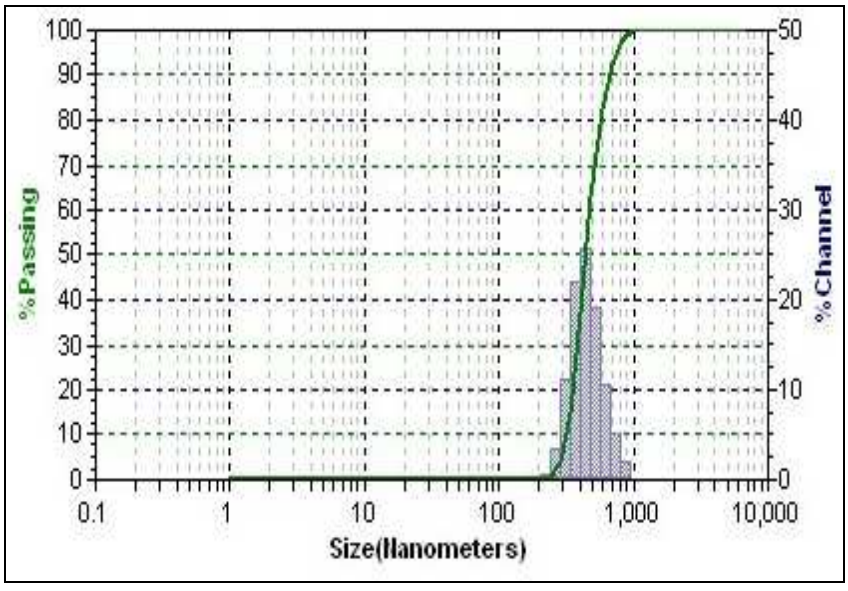
Fig. 2: UV spec interference - 420nm curcumin



Particle size Analysis

The particle size of the prepared suspension showed the diameter of 436 ± 122.8 (1SD) nm. The entire suspension had the particle size range 323 - 733 nm (Figure -3). The density of the suspension was 3.2g/cc at 27.6°C.

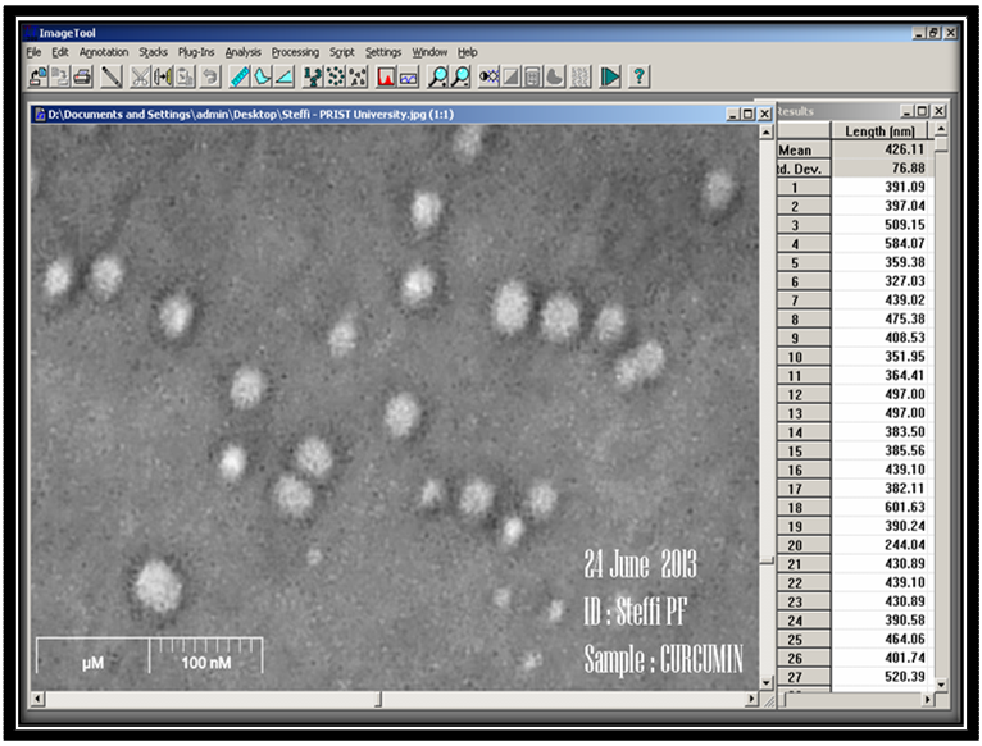
Fig. 3: Particle size analysis



Scanning electron microscope (SEM)

Analysis of SEM image using image analysis software UTHSCSA showed the formation of spherical and smooth nanoparticles. The image also proved the particles are almost uniform in size and distribution (Figure - 4). The measured particle size 426.11 ± 76.8 (1SD).

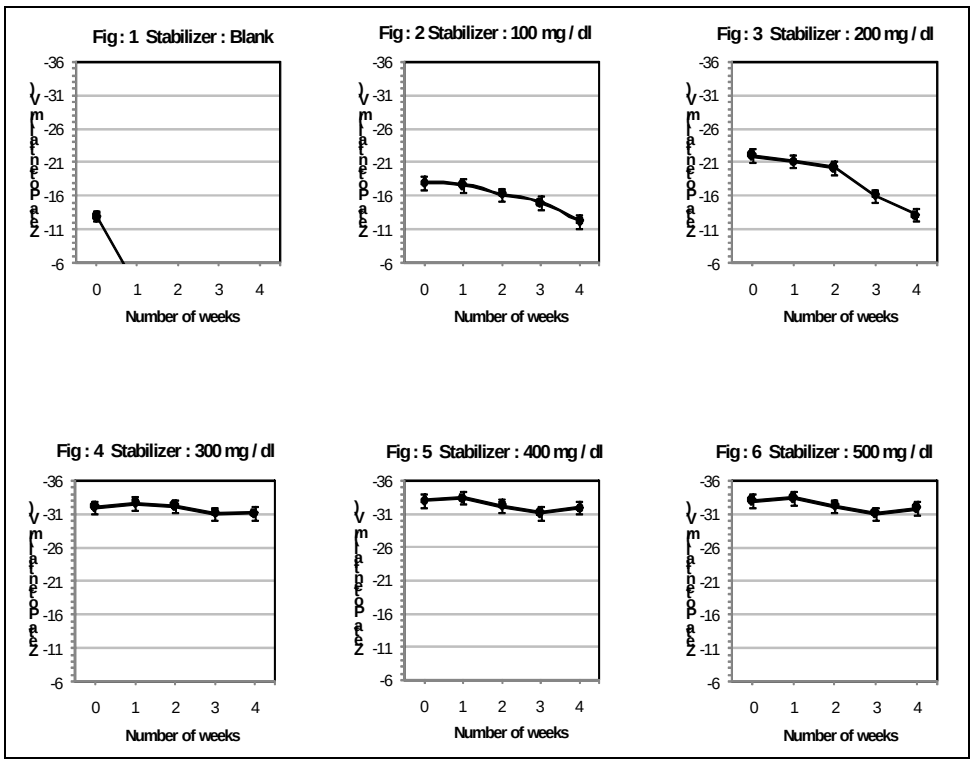
Fig. 4: SEM image analysis by UTHSCSA software



Stability Optimization:

Among the five different concentration of stabilizes subjected for experiment, the blank showed the stability only for a week where as the concentration 100mg / dl and 200mg / dl showed a gradually decreased zeta potential values from first and second week onwards respectively. The concentration 300mg / dl and more did not show any change in its stability up to four weeks. Suggesting the minimum concentration of 300mg / dl is required to achieve the maximum stability. Result showed that prepared nanosuspension has good stability (-33.3 ± 2.43 mV) up to 4 weeks from the day of preparation (Figure -5).

Fig. 5: Stability assay - Zeta potential



The aim of this study was to prepare and evaluate curcumin nanosuspension for therapeutic application. The fast growing research on curcumin clearly confirms the versatility and flexibility of curcumin for structural modifications. This study describes various approaches that have been undertaken to solve the problems associated with curcumin and to improve its bioavailability[17].

Nanosuspension is widely prepared by bottom up technology. The bottom-up technology is an assembling method from which molecules to nano-sized particles are formed[13]. Ali et al[18] discussed that on comparison of methodologies, milling, high pressure homogenization, bottom up techniques, antisolvent precipitation are quite simple, cost effective, and easy for scaling-up. Baglioni et al[19] adopted bottom-up technique with different mode such as microprecipitation, microemulsion and melt emulsification due to their simplicity and economy. Kwangjae Cho et al[24] studied the suitable particle diameter (400 to 600 nm) used in a drug delivery system. It should be large enough to prevent their rapid leakage into blood capillaries but small enough to escape capture by fixed macrophages that are lodged in the reticuloendothelial system, such as the liver and spleen. The current study showed an average particle diameter falling within this range suggesting this suspension as a better formulation in cancer treatment[20,21].

Heni et al (Rachmawati 2013)[22] used five different stabilizers like polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), d-α-tocopherol polyethylene glycol 1000 succinate (TPGS), sodium dodecyl sulfate (SDS), carboxymethylcellulose sodium salt for better stability of nanosuspension. In the present study SDS was used as stabilizer to improve the stability of the nanosuspension. According to Mora-Huertas et al[23] the zeta potential is an indication of the stability of the suspension. A suspension can be stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is required for good stability.

Conclusion

Study concludes that addition of stabilizer SDS may be the better choice for the stabilization of curcumin nanosuspension when the suspension is required to be kept for a period of about a month.

Contributions

All authors of this manuscript have materially participated in the research and article preparation and have approved the final article.

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