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Research Article

Curcumin as a model Drug: Conformation, Solubility Estimation, Morphological, *in vitro* and *in vivo* Biodistribution Study

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Abstract

Curcumin (CRM) is a golden pigment from turmeric, has been linked with anticancer, antioxidant, anti-inflammatory and antidiabetic properties. This article provides a brief about the conformation, solubility estimation, morphology, in vitro and in vivo bioavailability curcumin. In vitro release was conducted pH 1.2 phosphate buffers (94.02 ± 0.25), pH 6.4 phosphate buffer saline (99.74 ± 0.55), pH 6.8 phosphate buffer (90.74 ± 0.87) and pH 7.4 phosphate buffer (92.48 ± 0.12). The highest concentration was observed in the plasma after IV administered CRM saline solution (9854.65 ± 445.74 ng/mL) compared to oral administered CRM saline solution (5428.25 ± 479.20 ng/mL).

Keywords: Curcumin, anticancer, CRM, in vitro, SEM, in vivo pharmacokinetics.

Introduction

Curcumin (CRM) is a polyphenolic compound it belongs to class of flavonoids glycosides . CRM, a polyphenol known as diferuloylmethane, has been extensively studied for its therapeutic efficacy for many disorders including several inflammatory diseases, Alzheimer"s, and brain cancer. The extensive research on CRM has revealed several of its important functions. It interacts with various proteins, inhibits the activity of various kinases, and controls the activation of transcription factors that are involved in cell proliferation and survival. CRM-induced apoptosis through down-regulation of the NF-κB and Akt pathways [1-3]. Curcumin ((1E, 6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione), a polyphenol known as diferuloylmethane. Molecular formula of CRM C21 H20 O6 and Molecular weight of curcumin (CRM) is 368.39 g/mol. CRM is a highly lipophilic drug having a log P value is 1.82 respectively. Dissociation constant of CRM was 8.3±0.04. CRM is a bright yellow-orange powder material. CRM has maximum solubility in methanol and acetonitrile. Melting point of CRM 175ºc respectively. Reported λmax of CRM is 423 nm. CRM was identified by Melting point, infrared vibrational spectrophotometry (IR), Differential scanning Calorimetry (DSC), UV spectroscopy, thin layer chromatography (TLC). CRM interacts with various proteins, inhibits the activity of various kinases, and controls the activation of transcription factors that are involved in cell proliferation and survival. This study was aimed at conformation of CRM, Solubility estimation of CRM in different solvent, morphological examination of CRM, in vitro release in different solvent media and in vivo kinetics study was determined in pure CRM [4-5].

Materials and Methods

Materials

Curcumin (CRM) was obtained from Sunpure Extracts Ltd. (Delhi, India) and All other reagents used were of analytical grade.

Methods

Melting point

Melting point determination is prime confirmation of drug. In this method, drug whose analysis to be carried out in glass capillary method and melting point apparatus.

Infrared Spectroscopy (IR)

FT-IR spectra of CRM (pure drug), CRM with methanol, CRM with ethanol and CRM with chloroform. CRM was mixed with potassium bromide (KBr) of IR grade in the ratio of 1:100 and compressed using motorized pellet press (Kimaya Engineers, India). The pellets were then scanned using FT-IR spectrophotometer (Shimadzu 8400S, Japan). In case of CRM with methanol, CRM with ethanol and CRM with chloroform, they were placed inside the liquid sample holder.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) of CRM (pure drug), mixtures of CRM with methanol, CRM with ethanol and CRM with water were conducted using differential scanning calorimeter (Mettler Toledo®, Switzerland) at a heating rate of 10 °C /min. The measurements were performed at a heating range of 35 to 400 °C under nitrogen atmospheres.

Thin Layer Chromatography (TLC)

Aluminium backed Silica Gel 60 F254 HPTLC plates (10 cm × 20 cm, layer thickness 0.2 mm, E-Merck, Darmstad, Germany) was used for the study. These plates were prewashed with methanol and a mixture of Hexane: Ethyl Acetate (6:4) was employed as the mobile phase. The principal spot of were applied on plates and kept in chromatographic chamber. After removing the plate from the chromatographic chamber, allowed to dry exhaustively in air or in a current of cool air. The chromatogram examined in ultraviolet light (254 nm). The retention factor of CRM was determined.

UV Spectroscopy

According to European pharmacopoeia, 10 mg of CRM was dissolved in 100 ml of methanol (100 µg/mL). Final concentration was 10 µg/mL and it examined between 200-800 nm. The maximum absorbance was determined using UV-Vis Specrophotometer (UV 1700, Shimadzu, Japan) to confirm the λmax of the drug.

Standard calibration curve of Curcumin (CRM) in different solvent

Standard calibration curve of Curcumin (CRM) was conducted in Methanol (10-80 µg/mL), Water (5-35 µg/mL), pH 1.2 phosphate buffer (10-60 µg/mL), pH 6.8 phosphate buffer (10-80 µg/mL), pH 7.4 phosphate buffer (3-27 µg/mL) and ethanol (2-20 µg/mL). Absorbance of the above solution were taken at 423 nm using UV-Vis spectrophotometer (UV 1700, Shimadzu, Japan).

Solubility of Curcumin (CRM) in different solvent

Solubility of CRM was conducted in water, methanol, pH 1.2 phosphate, pH 6.4 phosphate, pH 6.8 phosphate, pH 7.4 phosphate

buffer and DMSO. An excess amount of CRM was added to each of above solvent and the samples were sonicated for 30 minutes. The mixture were kept at ambient temperature 37°C ± 0.5°C for 24 h for continually shaken in to orbital shaker (Remi mechanical shaking incubator, Mumbai) to get equilibrium. The equilibrated samples were centrifuged at 3000 rpm for 5 min. Aliquot portions of the supernatants were taken and properly diluted with above solvent for quantification of CRM spectrophotometrically at 423 nm (UV 1700, Shimadzu, Japan).

Assay estimation by using HPLC

10 mg of CRM was dissolved in 10 mL of methanol (1000 µg/mL), take 0.1 mL of solution and diluted with 10 mL of mobile phase up to mark. Centrifuged for 15 min at 3,500 rpm (REMI Instruments, Mumbai, India) and analyzed by HPLC. An Agilent HPLC system with UV detector was used which is composed of quaternary pump and photo diode array detector (PDA). Chromatography was performed on a reverse-phase C18 column (Eclipsed XDB 5 µm, 4.6 mm x 150 mm, Singapore) using acetonitrile: water with 0.1 % ammonia (30:70) mixture as mobile phase. Elution was performed isocratically at 25 °C at a flow rate of 0.2 mL/min at 423 nm with retention time of 9 min. for CRM. All data were acquired and processed using EZ chrome elite software version 3.3.2 and % drug content was calculated.

Particle size analysis

Droplet size of CRM was determined by photon correlation spectrophotometer, which analyses the fluctuations in light scattering due to the Brownian motion of the particles using a Zetasizer ZS 90, (Malvern Instruments Ltd., UK). CRM was diluted with double distilled water and light scattering was monitored at 25˚C at a 90˚ angle [6].

Zeta Potential

The CRM was diluted 100 times using double distilled water and analyzed using Zetasizer ZS 90, (Malvern Instruments Ltd. UK).

In vitro release of CRM in different media

In vitro diffusion study of CRM was carried out pH 1.2, 6.4 saline, 6.8 and 7.4 phosphate buffer by using diffusion cell apparatus, Franz diffusion cell having 2.0 cm diameter and 25 ml capacity. Dialysis membrane (Himedia) used as diffusion membrane. The temperature was maintained at 37 ˚C. CRM was dispersed in the donor chamber. Samples were periodically withdrawn from the receptor compartment for next 4 hours and replaced with the same amount of fresh pH 1.2, 6.4 saline, 6.8 and 7.4 phosphate buffer solution and assayed by a HPLC at 423nm[7].

Morphological estimation

The CRM was examined morphologically by Scanning Electron Microscope (JSM-6390LV, JEOL, Japan) with 20 kV accelerating voltage. Samples were prepared by placing onto an aluminium specimen stub, dried overnight and sputter coated with gold prior to imaging [8].

In vivo biodistribution study

The in vivobiodistribution studies were performed according to the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The animal protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur, registration number 651/PO/ReBi/S/02/CPCSEA & 29th July 2014.

In vivopharmacokinetic study was carried out using male Wistar albino rats. This in vivo bio-distribution studies can be divided in two groups each group consist of six animals. Group I, 100 µl of the CRM in saline solution administered in intravenously (IV) with the help of tuberculin syringe (1 mL), at the delivery site. For second group 100 µl of CRM in saline solution are administered oral delivery site. The rats are anaesthetizeand blood sample was collected at different time intervals by retro-orbital puncture. Blood samples were anticoagulated with heparin and centrifuged at 5000 rpm for 10 min to obtain plasma. At each time point, 6 rats are taken for measurements. All plasma samples were stored for up to 48 h in a deep freezer (−70˚C) until HPLC analysis [9-10].

Sample Processing

To 100 µl of plasma sample, 100 µl IS Hydrochlorothiazide (30 µg/ml) and add extraction solvent 2 mL of acetonitrile was spiked and vortex mixture for 20 min. This sample was ultracentrifuge at 10,000 rpm for 10 min. The supernatant layer was collected and 20 µl was injected in HPLC system and the whole procedure was carried out at room temperature.

Chromatographic conditions

The chromatographic separation was performed at ambient temperature with reversed-phase, 150 X 4 mm base specific column packed with 5µm C18 column (Eclipsed XDB 5 µm, 4.6 mm x 150 mm, Singapore). The mobile phase was a mixture of acetonitrile: water with 0.1% ammonia (30:70 v/v) pumped at a flow-rate of 0.2 mL/min. Detection was performed at a wavelength of 423 nm.

Data Analysis

The non-compartmental model was considered as a best suited model for calculation of the different pharmacokinetic parameters. The Cmax and Tmax were directly computed from the concentration vs. time plot. The trapezoidal method was used to calculate the concentration time curve (AUC0→ t). The Kinetica 5® (Thermo Fisher Scientific Demo version) software was employed for study.

Result and Discussion

Melting point

Melting point of CRM by glass capillary method and melting point apparatus was found to 173-175°c, the observed melting point of CRM was confirmed with the standard melting point of CRM (175 °C) reported in literature.

Infrared Spectroscopy (IR)

Observed peaks of CRM, CRM with methanol, CRM with ethanol and CRM with chloroform was similar to the standard IR spectra of drug reported in the literature (Figure 1).

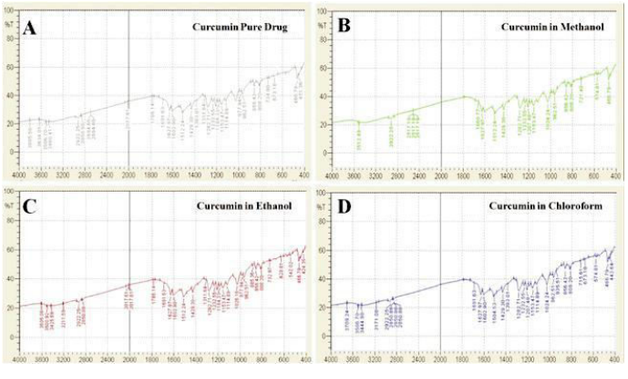

Figure 1: IR spectra: Curcumin (A), Curcumin in Methanol (B), Curcumin in Ethanol (C), Curcumin in Chloroform (D)

Differential scanning calorimetry (DSC)

Melting point of CRM was measured by using DSC (Metler Toledo), Showed melting point of 174.25˚c which is similar as reported in literature, Melting point of mixtures of CRM with methanol, CRM with ethanol and CRM with water measured by using DSC (Metler Toledo), Showed melting point of 175.58˚C which is similar as reported in literature (Figure 2).

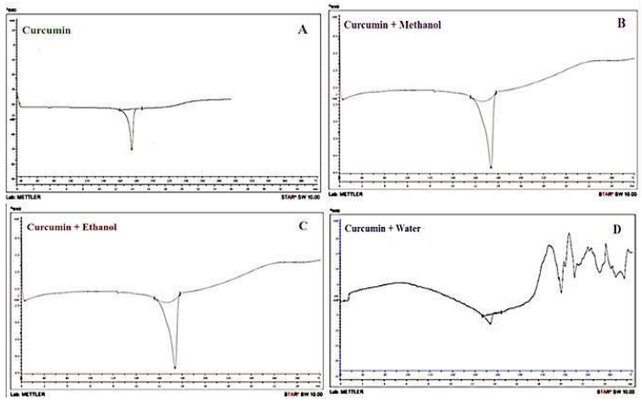

Figure 2: DSC: Curcumin (A), Curcumin + Methanol (B), Curcumin + Ethanol (C), Curcumin + Water (D)

Thin Layer Chromatography (TLC)

TLC is important test for conformation of CRM in which retention factor of CRM was found to be 0.51. The experimental retention factor was correspondence to reported retention factor (0.52) (Figure 3)

Figure 3: TLC of CRM

UV Spectroscopy

The solution of CRM in methanol was found to exhibit maximum absorption at 423 nm after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature and thus the procured drug sample of CRM complies with the reference spectra (Figure 4).

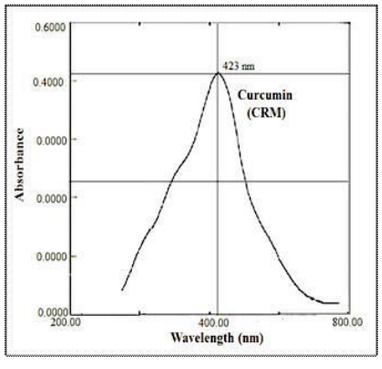

Figure 4: UV spectra of CRM

Standard calibration curve of Curcumin (CRM) in different solvent

Graph of absorbance Vs concentration was plotted and all concentration of all solvent was linear over the range indicating its compliance with Beer's and Lambert's law. Correlation coefficient of (R_2) CRM in methanol was found to be 0.9994. Correlation coefficient of (R_2) CRM in water was found to be 0.9982. Correlation coefficient of (R_2) CRM in pH 1.2 phosphate, pH 6.8 phosphate and pH 7.4 phosphate buffer was found to be 0.9986, 0.9994 and 0.999 respectively. Correlation coefficient of (R_2) CRM in ethanol was found to be 0.9994 (Figure 5).

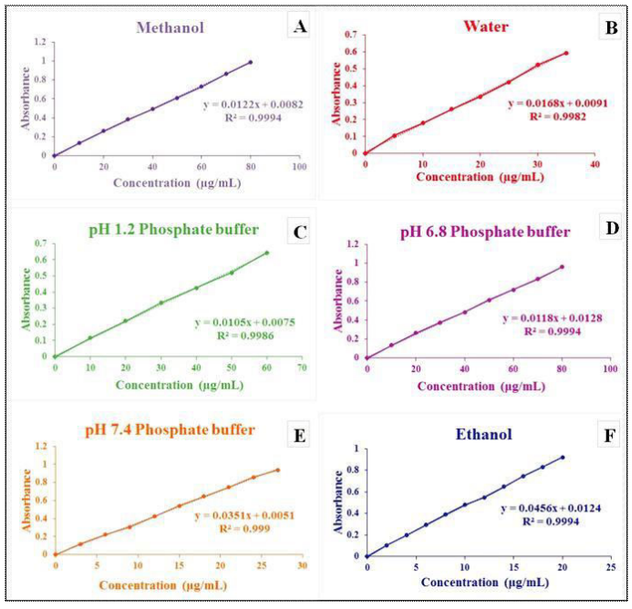

Figure 5: Standard calibration curve of Curcumin (CRM): Methanol (A), Water (B), pH 1.2 phosphate (C), pH 6.8 phosphate(D), pH 7.4 phosphate(E),Ethanol (F)

Solubility of Curcumin (CRM) in different solvent

The solubility of Curcumin (CRM) was assessed in different solvent system such as water, methanol, pH 1.2 phosphate, pH 6.4 phosphate, pH 6.8 phosphate, pH 7.4 phosphate buffer and DMSO (solubility in mg/mL). CRM is a BCS class II drug having minimum solubility in aqueous media. Solubility of CRM in Water (0.15 ± 0.22), methanol (10.0 ± 0.33), pH 1.2 Phosphate buffer (2.0 ± 0.52), pH 6.4 phosphate buffer (5.0 ± 0.11), pH 6.8 phosphate buffer (4.0 ± 0.26), pH 7.4 phosphate buffer (3.0 ± 0.56) and DMSO (12.0 ± 0.99) was optimum to reported limit. The solubility of CRM in different solvent was shown in Figure 6.

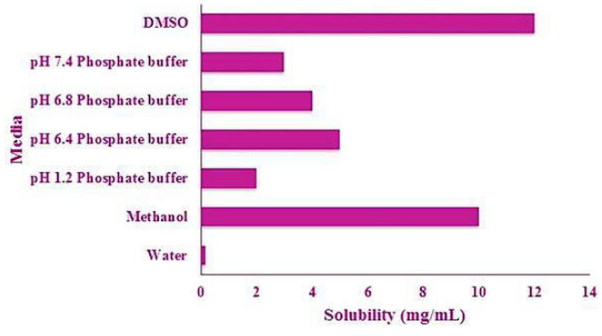

Figure 6: Solubility of Curcumin (CRM) in different solvent

Assay estimation by using HPLC

Drug content or assay estimation of CRM was found to 99.22 % for CRM under HPLC analysis and chromatogram was shown in Figure 7.

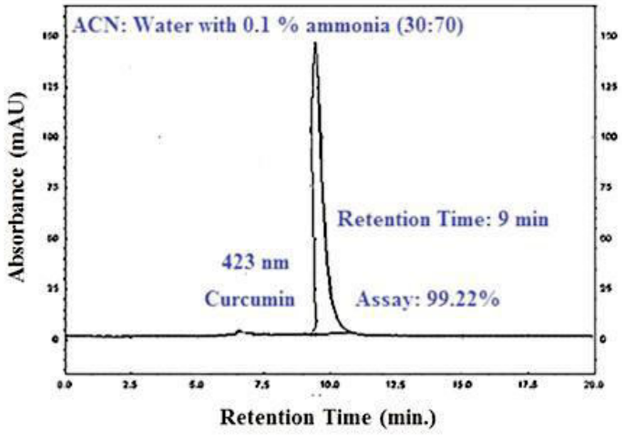

Figure 7: Assay estimation by using HPLC

Particle size analysis

The Particle size of CRM was found to be 568.6 ± 0.52 nm (mean ± SD, n=3) was minimum to acceptable limit. The polydispersity index of CRM was lowest having a value of 0.363 ± 0.05 (mean ± SD, n=3). Since the diameter of the dispersed particle of the CRM was much smaller than the 1000 nm. The particle size of the CRMwas important factor as this determines the rate and extent of drug release as well as absorption (Fig. 8).

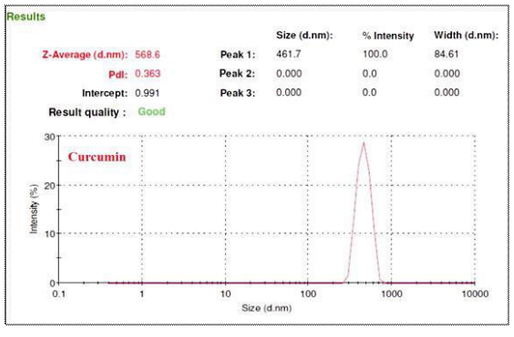

Figure 8: Particle size of CRM

Zeta Potential

Zeta potential of CRM was found to be -9.11 ± 0.13 mV (mean ± SD, n=3). The ZP values, either positively or negatively charged, mean that dispersion will have greater to shows long-term stability because the charged particles repel one another and thus overcome the natural tendency to aggregate (Figure 9).

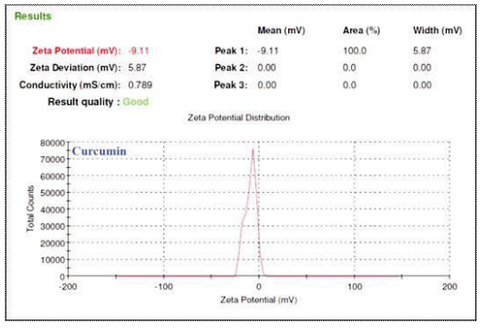

Figure 9: Zeta Potential of CRM

In vitro release of CRM in different media

The release profile of CRM in pH 1.2 phosphate buffer (94.02 ± 0.25), pH 6.4 phosphate buffer saline (99.74 ± 0.55), pH 6.8 phosphate buffer (90.74 ± 0.87) and pH 7.4 phosphate buffer (92.48 ± 0.12). The release profile of CRM in pH 6.4 phosphate buffer saline shows maximum drug release as compared to other Media (Figure 10).

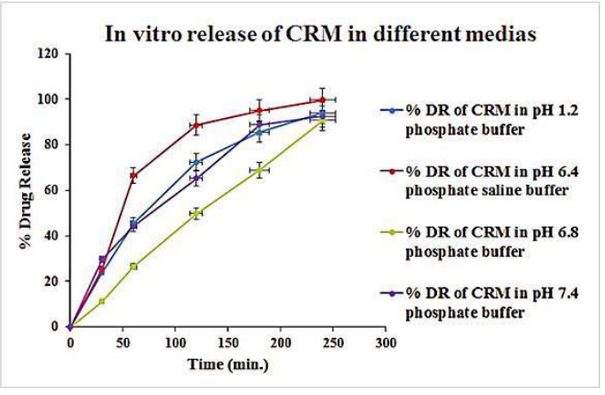

Figure 10: *In vitro* Release of CRM in different media

Morphological estimation

Morphological study of CRM was done by taking SEM pictures. Studies showed that the predicted particle size and measured size with a Zetasizer was comparable with size of particles that were observed by SEM (Figure 11).

Figure 11: Morphological estimation

In vivo biodistribution study

The results of bio-distribution studies showed the time profile of CRM concentration in plasma higher after intravenous (IV) administration of CRM in saline solution as compared to oral administration of CRM in saline solution respectively. The profiles of CRM level in Plasma displayed an initial absorption phase and maximum concentration achieved after 15 min in IV administration. After the initial 15 min, the drug concentration in the plasma was found higher for IV delivered CRM saline solution (9854.65 ± 445.74 ng/mL) at Tmax 15 ± 0.00 than the oral administered CRM saline solution (5428.25 ± 479.20 ng/mL) at Tmax 15 ± 0.00. The highest concentration was observed in the plasma after IV administered CRM saline solution compared to oral administered CRM saline solution (Fig. 12). Thus, the results of the present investigation prove that CRM could be transported directly IVroute of administration, thereby enhancing drug concentration in blood and also enhancing bioavailability of CRM.

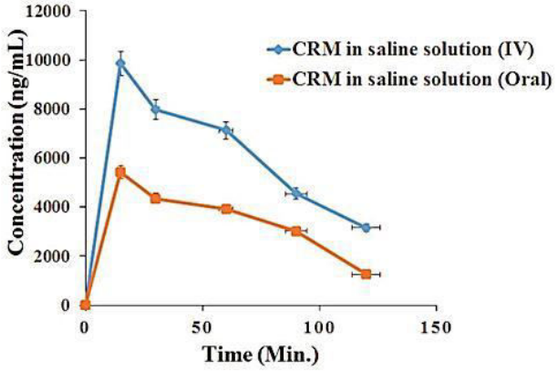

Figure 12: *In vivo* biodistribution study

Conclusion

CRM was conformed melting, IR, DSC, TLC and UV examination. Solubility of CRM was conducted in different solvents and chromatographic assay by HPLC was found 99.22%. Morphological examination of CRM was conducted in SEM imaging. In vitro release of CRM conducted in different solvents that indicated that CRM shows maximum solubility saline buffer. In vivo biodistribution study of CRM was concluded that IV administered CRM was maximum release as compared to oral administered CRM.

Conflicts of interest

The authors confirm that this article content has no conflict of interest.

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