Antioxidant and radical scavenging properties of curcumin solid dispersions

Biresh Sharkar[*1], Pritesh Paliwal[1]

[1]Sri Balaji College of Pharmacy, Jaipur, Rajasthan.

[2]College of Pharmacy, IPS Academy, Indore.

*Corresponding Author E-mail: biresh.sharkar@rediffmail.com

Abstract

Curcumin is a phenolic compound and a major component of *Curcuma longa* L. In the present paper curcumin solid dispersions were evaluated for their *in vitro* antioxidant activity using various assays such as 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH.) scavenging and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity. Curcumin solid dispersions showed effective DPPH. scavenging and ABTS.+ scavenging activities. Curcumin solid dispersion in the ratio of 4:1 (Curcumin: HPMCAS) showed significant *in vitro* free radical scavenging activity; in ABTS assay 81.99 ± 0.59 % inhibition and in DPPH assay 85.22 ± 0.71 % inhibition of free radical was observed. Solid dispersions were also characterized by various means which confirm no significant drug: polymer interaction. According to the present study curcumin solid dispersions can be used as potent antioxidant with enhance solubility and bioavailability.

Keywords: ***Antioxidant activity, Curcumin, Radical scavenging, DPPH***

Introduction

Curcumin is a polyphenolic compound derived from turmeric and gives the spice its yellow color [1]. The main problem in the study and application of Curcumin is low bioavailability due to poor water solubility. These polyphenols have an oral bioavailability in the range of 2-20%. Researchers have found that the molecular dispersion of drugs into polymer matrices, forming sub-nanometer scale domains of drug within the polymer matrix, effectively increases drug water solubility and often bioavailability [2]. Cellulose derivatives increase the solubility of the flavonoid by disrupting its crystal structure. The polymer does this by forming hydrogen bonds and hydrophobic interactions. Cellulose derivatives are excellent matrices because they have high glass transition temperatures (130^{o}C or higher), are extremely safe for human ingestion and are widely used in pharmaceutical and food applications [3], for example, Hydroxypropyl Methylcellulose Acetate Succinate (HPMCAS) has been of growing interest in recent years [4]. All aerobic organisms have antioxidant defenses, including antioxidant enzymes. Antioxidants can protect the human body from free radicals. They retard the progress of many chronic diseases as well as lipid peroxidation [5-7]. In present study an attempt was made to establish a successful method for increasing the solubility of Curcumin without affecting its biological effect such as antioxidant property. The focus of this study was to prepare amorphous mixtures of curcumin using a spray drying technique and evaluation of antioxidant activities of these formulations, which was expected to give more bioavailability with potent antioxidant property.

Experimental

Materials:

Pure curcumin was isolated in laboratory. Dried powder of *Curcuma longa* rhizome was macerated in hexane and ethyl acetate. The ethyl acetate extract was then dried in vacuum, subsequently separated by silica gel column chromatography using chloroform-methanol system as an eluent and LH-20 gel filtration chromatography using methanol as eluent to produce curcumin (yield; 4.65%). ABTS, DPPH**.**, were obtained from Sigma–Aldrich. All other reagents were of analytical grade.

Preparation of Spray Dried Powders and Physical Mixtures:

Different ratios (4:1, 3:1, 1:1, 1:3, 1:4) as shown in **Table 1** of HPMCAS and curcumin were prepared as 2% solutions by dissolving the two solids in a mixture of acetone/ethanol (2:4). Physical mixture was prepared to compare to the powders obtained by spray drying. The physical mixture of curcumin and HPMCAS was made by grinding weighed portion of solids together with a mortar and pestle.

Characterization:

Fourier Transform Infrared

FTIR Spectroscopy measurements were recorded on a Nicolet 8700 FT-IR Spectrometer. FTIR pellets were composed of 3 mg of the polymer matrix mixture and 100 mg of potassium bromide.

Differential Scanning Calorimetry Studies

Differential scanning calorimetry was performed on a differential scanning calorimeter. Samples were heated in hermetically sealed aluminum pans with a heating rate of 10°C/min under nitrogen atmosphere (flow rate 20 ml/min).

Solubility Studies

The solubilities of various formulations of curcumin solid dispersions were determined by adding excess amount of curcumin to glass vials containing 20 ml of aqueous solutions of polymer. These vials were shaken in a thermostatically water bath maintained at 37±0.1^{0}C until equilibrium. The supernatants were filtered through a 0.45 µm pore size millipore membrane filter at the same temperature. The filtrates were suitably diluted with phosphate buffer pH 6.5 and assayed spectrophotometrically at 427 nm for the concentration of curcumin. All experiments were determined in triplicate**.**

Antioxidant activity:

Free radical-scavenging ability by the use of a stable DPPH radical.

The DPPH radical-scavenging activity was determined using the method proposed by *Yen and Chen* [8]. DPPH (0.1 mM) was dissolved in pure ethanol (96%). The radical stock solution was prepared freshly. The DPPH solution (1 ml) was added to different sample with 3 ml of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm. The results were corrected for dilution and expressed in % inhibition. Equal volume of ethanol & DPPH was used as control. All determinations were performed in triplicate. (Results are shown in Table 1).

*Free radical-scavenging ability by the use of a stable ABTS radical cation***:**

The free radical-scavenging activity was also determined by ABTS radical cation decolorization assay [9]. The ABTS radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in the dark for 16 h. The ABTS solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.005 at 734 nm. The ABTS solution (3.9 mL; absorbance of 0.700 ± 0.005) was added to different sample and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min, and the absorbance

was immediately recorded at 734 nm. All determinations were performed in triplicate. (Results are shown in Table 1).

Statistical analysis

The results are expressed as mean values ± S.E.M. (standard error of mean). Statistical comparison was carried out by analysis of variance (ANOVA).

Results and discussion:

FTIR spectra of formulation F5 (1:4 HPMCAS: curcumin) showed all characteristic peaks of curcumin which suggest that there was no significant drug: polymer interactions took place (**Figure 1**). Solubility profile of curcumin solid dispersions was shown in **Fig. 2**. Curcumin was practically insoluble in water, significant increase in its solubility was observed in solid dispersion form. It was suggested that HPMCAS might form the soluble complex with curcumin. In all solid dispersion systems, the curcumin solubility was markedly higher than that of pure curcumin. The total antioxidant capacity was estimated by the ABTS and DPPH methods; results of *in vitro* antioxidant activity reveals that solid dispersions showed marked inhibition in the both model but in ABTS assay more inhibition of free radical was observed as compared to DPPH assay **(Figure 3)**. Results suggested that curcumin retains its antioxidant activity as solid dispersion complex with HPMCAS. All formulations showed significant *in vitro* free radical scavenging activity; curcumin solid dispersion in the ratio of 4:1 (curcumin and HPMCAS) showed 81.99 ± 0.59 % and 85.22 ± 0.71 % inhibition of free radical in ABTS and DPPH assay respectively. **(Table 2)**.

Table 1. Various formulations of curcumin solid dispersions

		Ratio	
S.No.	**Formulations**	**HPMCAS**	**curcumin**
1.	F1	4	1
2.	F2	3	1
3.	F3	1	1
4.	F4	1	3
5.	F5	1	4

Table 2. Result of free radical-scavenging activity of curcumin solid dispersion (% Inhibition by DPPH and ABTS assay).

		% Inhibition	
S.No.	**Formulations**	**DPPH assay**	**ABTS assay**
1.	F1	51.8 ± 0.69	57± 0.32
2.	F2	42.94 ± 0.67	50.45 ± 0.44
3.	F3	72.37 ± 0.49	76.29 ± 0.56
4.	F4	61.19 ± 0.77	71 ± 0.68
5.	F5	81.99 ± 0.59	85.22 ± 0.71

Note: Data are the mean ± SD of three measurements.

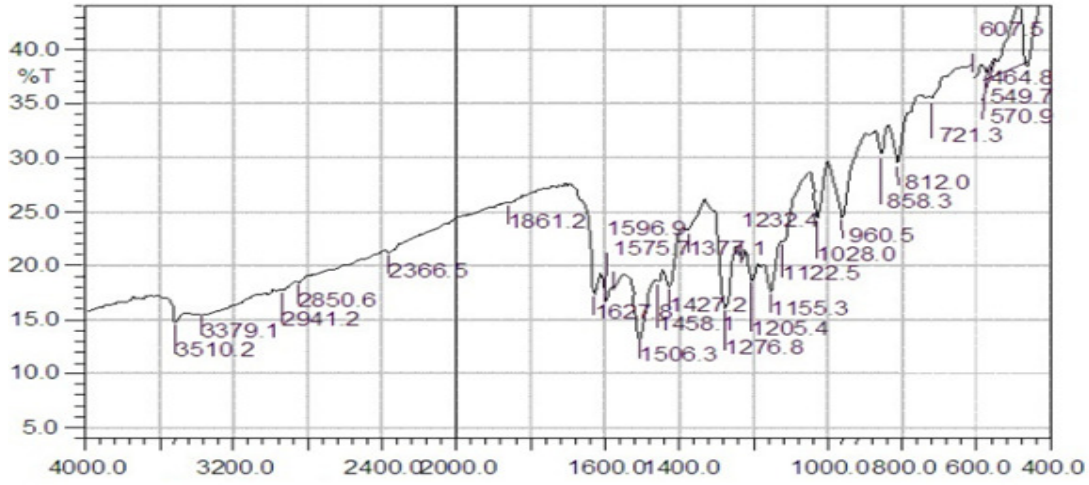

Figure 1. IR spectrograph of Formulation; F5 showing characteristic peaks of curcumin

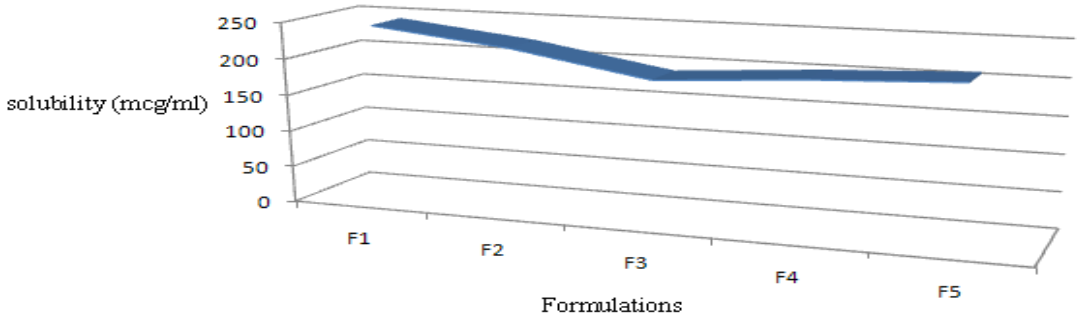

Figure 2. Solubility profile of various curcumin formulations

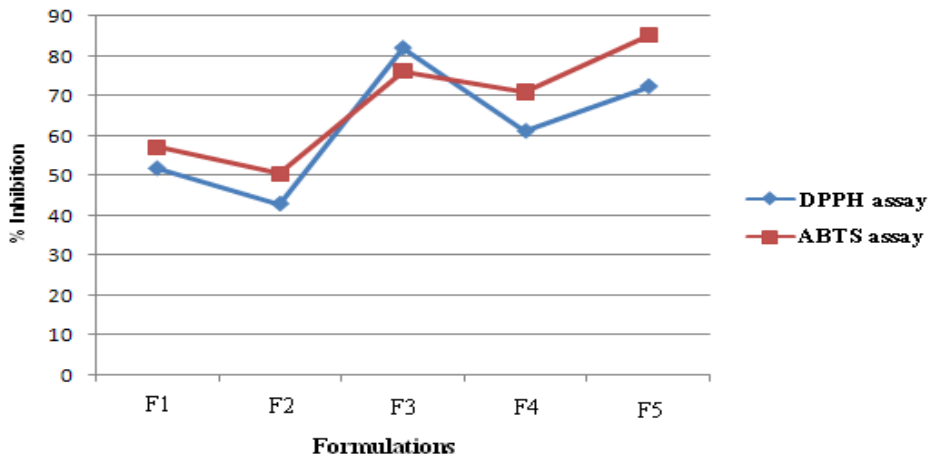

Figure 3. Comparative % Inhibition by DPPH and ABTS assay.

Conclusions

Characterization studies demonstrated that solid dispersions retain characteristic peaks of curcumin, with enhance solubility. Various curcumin solid dispersions were prepared in present study showed good biological activity and overcome the solubility problem of curcumin. Thus present study suggests that curcumin solid dispersions can be used as potent antioxidant; with enhance solubility and bioavailability.

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