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Bioavailability Enhancement of Curcuminoids using Natural Polymer

Harsha Kharkwal[1], Kumud Bala[2], DD Joshi[3] and Deepshikha Pande Katare[2]*

[1]*Amity Center for Carbohydrate Research, Amity University Uttar Pradesh, Noida-201303; India*
[2]*Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida-201303; India.*
[3] *Amity Institute of Phytomedicine and Phytochemistry, Amity University Uttar Pradesh, Noida-201303, India*

ABSTRACT

Curcuminoids plays a vital role in treating various ailment in traditional as well as allopathic system of medicine pertaining to its immense utility it acts as a Dietary supplement, an anti-cancer agent; reduces β-amyloid plaques in Alzheimer's etc. This paper is an attempt to enhance its bioavailability/ aqueous solubility of Curcuminoids by imparting hydrophilic character of water-soluble carriers such as guar-gum. Solid Dispersion Technique has been used to increase the dissolution and absorption of poorly soluble drugs by dispersing the drug in a highly water soluble carrier in a solid state. Formulations with 1:1, 1:2, 1:3, 1:4, 1:5 drug: polymer ratio were prepared and comparatively evaluated for their better release profile. Pure Curcuminoids exhibited the slowest dissolution rate pertaining to hydrophobicity. The study done here reveals Solid Dispersions of Curcuminoids were remarkably enhanced compared to the pure Curcuminoids.

Keywords: Curcuminoids, Solid Dispersions, Bioavailability, Guar gum

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INTRODUCTION

Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa* has a wide spectrum of biological and pharmacological activities. Chemically, Curcumin is a bis-R,-unsaturated _-diketone (commonly called diferuloylmethane,), which exhibits keto–enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium. Commercial it contains approximately 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin. Traditionally, turmeric has been used for many ailments, particularly as an anti-inflammatory agent, and Curcumin has been identified as the active principle of turmeric. Curcumin is known for its antitumor, [1][2] antioxidant, antiarthritic, anti-amyloid, antiischemic [3] and anti-inflammatory properties.[4] Anti-inflammatory properties may be due to inhibition of eicosanoid biosynthesis. In addition, it may be effective in treating malaria, prevention of cervical cancer, and may interefere with the replication of the HIV virus.

Inspite of its efficacy and safety, impressive array of beneficial bioactivities, the bioavailability of Curcumin in animals and man remains low. Research carried out on Curcumin demonstrates poor systemic bioavailability after p.o. dosing [5] which relates to its inadequate absorption and fast metabolism. Curcumin bioavailability obtained from Standardized Curcumin extract was shown poor on Colorectal cancer patients by work carried out by Li and his co-workers. [6] Indirect evidence suggests that Curcumin is metabolized in the intestinal tract. Curcumin

undergoes metabolic O-conjugation to Curcumin glucuronide and Curcumin sulfate and bioreduction to tetrahydrocurcumin, hexahydrocurcumin and hexahydrocurcuminol in rats and mice in vivo [7], in suspensions of human and rat hepatocytes and in human and rat intestine. [8] Metabolic conjugation and reduction of Curcumin was more in human than in rat intestinal tissue. Experiments carried out with [3H] labeled Curcumin and incubated with inverted rat gut sacs suggested intestinal tract plays an important role in the metabolic disposition of Curcumin. [9] Analysis of Curcumin present before and after treatment of tissue extracts. Intestinal mucosa, as well as liver and kidney tissue from the rat, can glucurodinate and sulfate Curcumin, as judged by the analysis of differential amounts of Curcumin present before and after treatment of tissue extracts with conjugate-hydrolyzing enzyme. Thus, gut metabolism contributes substantially to the overall metabolic yield generated from Curcumin in vivo. In human intestinal fractions, conjugation with activated sulfuric or glucuronic acids was much more abundant, whereas conjugation in human hepatic tissues was less extensive, than in the rat tissues. [10]

Although p.o. administered Curcumin inhibits chemically induced liver and skin carcinogenesis [11] with poor bioavailability and non-measurable blood levels [12] this route of administration inhibits chemically induced skin and liver carcinogenesis. Curcumin, when administered orally inhibits the initiation of radiation-induced mammary and pituitary tumors. [13]

Improved solubility for enhanced bioavailability of Curcumin has been researched in length. .In the past, several classical techniques based on physical parameters such as heat, pH, and complexations with metal ions, serum have been applied to prepare more soluble Curcumin formulations. Curcumin analogues were also prepared by chemical modifications. Kurien et al. in his work carried out claimed enhancement of both turmeric and curcuma by 3-fold and 12-foldusing heat without heat-mediated disintegration of Curcumin. [14] In another study carried out by Zebib et al.[15] wherein he and his co-workers prepared complexes of Curcumin with met al ions (Zn^{2+}, Cu^{2+}, Mg^{2+} and Se^{2+}) being readily soluble in water-glycerol (1:1; w/w) and stable towards light and heat serum albumin complexes of Curcumin significantly increased its solubility, delayed the erythrocyte membrane damage by reducing the toxic effect of amphoterecin. Chemically modified 4-arylidene Curcumin derivatives prepared by Qiu et al. t were found to be more soluble and more potent anti-cancer targeted analogues. [16] In addition, Differently modified Curcumin by chemical process were also prepared to enhance its activity against cancer and NF-κB. [17-22] Water-soluble Curcumin conjugates with two differently sized poly (ethyleneglycol) molecules exhibited solubility in water and enhanced cytotoxicity for anti-cancer treatment. [23]

The present research work is focused on hydrophilic polymers in the design of oral controlled drug delivery systems because of their flexibility to obtain a desirable drug release profile, cost effectiveness. Hydrogels are polymeric networks with three-dimensional configuration capable of imbibing high amounts of water or biological fluids from about twenty to thousand times their dry weight. Their affinity to absorb water is attributed to the presence of hydrophilic groups such as-OH,-CONH-,CONH2-,and-SO3H in polymers forming hydrogel structure The crosslinks present in the hydrogel structure which show swelling characteristics instead of being dissolved in the aqueous surrounding environment. Among the hydrophilic polymers, cellulose derivatives such as methyl cellulose, hydroxyl propyl methyl cellulose and sodium carboxy- methyl cellulose are generally considered to be stable and safe as release retardant excipients in the development of oral controlled release dosage forms. These semi-synthetic polymers are quite expensive when compared with natural polymers such as guar gum, Xanthan gum and so forth. The use of natural polymers for pharmaceutical applications is attractive because they are economical, readily available, non-toxic, and capable of chemical modifications, potentially biodegradable and biocompatible.

MATERIALS AND METHODS

Preparation and evaluation of Solid Dispersions of Curcuminoids

Curcuminoids (>95% Curcumin**)** were purchased from Sigma**,** Guar gum (Merck Chemicals), Dichloromethane (Merck Chemicals), Acetone (Merck Chemicals), Tween 80 (Sigma) and all other reagents were of analytical-reagent grade.

Solid Dispersion Preparations

Solid dispersions of Curcuminoids : guar gum in the weight ratio of 1:1 1:2, 1:3, 1:4 and 1:5 were prepared by solvent method. . The required quantity of guar gum was dissolved in 10 ml of water and stirred well until a homogenous solution was formed. And this was added to the solution of curcumin (1 g) in acetone (50 ml). The solvents were removed under reduced pressure at 40°C and dried under vacuum at room temperature for 5 hours.

The samples were pulverized using mortar and pestle and the 0.125mm size was obtained by passing through sieve no.120 in a sieve shaker. All solid dispersion of different composition 1:1, 1:2, 1:3, 1:4 and 1:5 were prepared in same manner.

Fourier Transform Infrared Spectroscopy Studies
Fourier transform infrared spectra were obtained on a Perkin-Elmer spectrometer equipped with a deuterated triglycine sulfate detector. Samples were prepared in KBr discs. Five milligrams of each sample were mixed with 30 mg of KBr and compressed at 10 ton by a hydraulic press.

Method for Preparation of standard graph for the estimation of Curcuminoids in acetone.
10mg of accurately weighed Curcuminoids was transferred into 10ml beaker. To this a little quantity of acetone was added and made to dissolve and made up to 10ml (1000mcg/ml).This is stock solution. From this 1ml was pipette out into a 10ml and made up to 10ml (100mcg/ml). From this 0.5 to 9 mcg/ml solution was prepared and absorbances were determined at 427nm using Elico make UV-VISIBLE spectrophotometer.

Method for Preparation of standard graph for the estimation of Curcuminoids in Distilled Water.
10mg of accurately weighed Curcuminoids was transferred into 10ml beaker. To this a little quantity of acetone was added and made to dissolve and made up to 10ml.This is stock solution. From this 1ml was pipetted out into a 10ml and made up to 10ml with water (100mcg/ml). From this 0.5 to 9 mcg/ml solution was prepared by diluted with water and absorbances were determined at 427nm using Elico make UV-VISIBLE spectrophotometer.

Method for Preparation of standard graph for the estimation of Curcuminoids in DMSO
10mg of accurately weighed Curcuminoids was transferred into 10ml beaker (1000mcg/ml). To this a little quantity of DMSO was added and made to dissolve and made up to 10ml (1000mcg/ml).This is stock solution. From this 1ml was pipetted out into a 10ml and made up to 10ml with DMSO (100mcg/ml). From this 0.5 to 9 mcg/ml solution was prepared by diluted with DMSO and absorbances were determined at 427nm using Elico make UV-VISIBLE spectrophotometer.

Method for Preparation of standard graph for the estimation of Curcuminoids in Dichloromethane
10mg of accurately weighed Curcuminoids was transferred into 10ml beaker. To this a little quantity of Dichloromethane was added and made to dissolve and made up to 10ml (1000mcg/ml).This is stock solution. From this 1ml was pipetted out into a 10ml and made up to 10ml with Dichloromethane (100mcg/ml). From this 0.5 to 9 mcg/ml solution was prepared by diluted with Dichloromethane and absorbances were determined at 427nm using Elico make UV-VISIBLE Spectrophotometer.

Solubility of solid dispersions
An excess amount of pure Curcuminoids and solid dispersion of different composition was added in the 15 ml of distilled water in conical flask to get the super saturated solution and placed in incubator shaker with constant shaking for 24 hrs at 37 ºC until equilibrium was attained. At the end of the 24 h shaken, the solutions were immediately and rapidly filtered through a 0.45 µm Millipore filter and the filtrate was diluted into 10 times volume with distilled water. The amount of Curcuminoids in each diluted sample was analyzed by UV-VISIBLE Spectrophotometer and the absorbance was read at 427 nm against blank.

In vitro drug release
The drug release rate from Solid dispersions and pure Curcuminoids was determined using Electrolab-USP Dissolution test apparatus with the basket method .A volume of 900-ml 0.02%w/v tween 80 solution equilibrated at 37^0C, at a rotation speed of 100 rpm, was utilized as dissolution fluid. Perfect sink conditions prevailed during the drug release studies. 5 ml sample was withdrawn at each interval, filtered through Whatman filter paper No 1 and analyzed by UV method to determine the concentration of drug present in the dissolution medium at 427nm. The initial volume of the dissolution medium was maintained by adding 5ml of fresh dissolution medium after each withdrawal. Dissolution studies were carried out over the period of 48hr.

Drug release kinetics
Several mathematical models have been published, to elucidate the water and drug transport processes and to predict the resulting drug release kinetics. To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics.

The following plots were made:

1. cumulative % drug release vs. time (zero order kinetic model);
2. log cumulative of % drug remaining vs. time (first order kinetic model);
3. cumulative % drug release vs. square root of time (higuchi model)
4. log cumulative % drug release vs. log time (korsmeyer model)
5. cube root of drug % remaining in matrix vs. time (hixson-crowell cube root law).

Mechanism of drug release

To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model:

$$M_t / M_\infty = k\, t^n$$

Where Mt / M_∞ is fraction of drug released at time t, k is the rate constant and n is the release exponent The 'n' values used for the elucidation of the drug release mechanism from the solid dispersions were determined from log cumulative percentage of drug released versus log time plots **(**i.e., log M_t / M_∞ x 100) versus log t). A value of n = 0.5, indicates Case- I (Fickian) diffusion or square root of time kinetics, 0.5 < n < 1 anomalous (non – fickian) diffusion, n=1, Case-II transport and n > 1 super Case-II transport.

Statistical Analysis

All the data of solubility studies and in vitro dissolution rate studies were analyzed statistically by ANOVA (analysis of variance) test.

Drug content uniformity

All the solid dispersions were tested for drug content uniformity .Accurately weighed amount of SD was dissolved in Dichloromethane (50ml)and then centrifuged for 5 min at 10,000rpm.The solution was then filtered and then suitably diluted with dichloromethane and assayed for drug content by measuring the Curcuminoids content at an absorbance of 427nm.

RESULTS AND DISCUSSION

The sustained release solid dispersions of Curcuminoids were formulated by using guar gum as polymer. The formulations were coded as shown in table-1. . These sustained release solid dispersion system may be useful for enhancing bioavailability and sustained action.

Table-1 Formulation codes of Solid dispersion of Curcuminoids

Formulation	Formulation Code	Ratio of Drug and Biopolymer
1	SD1	1:1(Guargum)
2	SD2	1:2(Guargum)
3	SD3	1:3(Guargum)
4	SD4	1:4(Guargum)
5	SD5	1:5(Guargum)

The standard graph for the estimation of Curcuminoids in distilled water, dichloromethane, DMSO, acetone was plotted. The drug content and in vitro release of Curcuminoids was studied using UV, Dissolution Apparatus respectively.

Standard curve of Curcuminoids in DMSO

The graph was plotted to determine the Curcuminoids in DMSO by determining the absorbance at 427nm by using different concentrations, such as 2,3,4,5,6,7,8,9 mcg /ml, and the absorbance was found to be 0.212, 0.2839, 0.3522, 0.4317 and 0.515,0.615,0.716,0.7934 respectively .(Figure-1)

Standard curve of Curcuminoids in distilled water

The graph was plotted to determine the Curcuminoids in distilled water by determining the absorbance at 427nm by using different concentrations, such as 1,2,4,5,6 ,7mcg/ml, and the absorbance was found to be 0.0899, 0.1811, 0.2788, 0.3353 and 0.3924, 0.4689 respectively.(Figure-2)

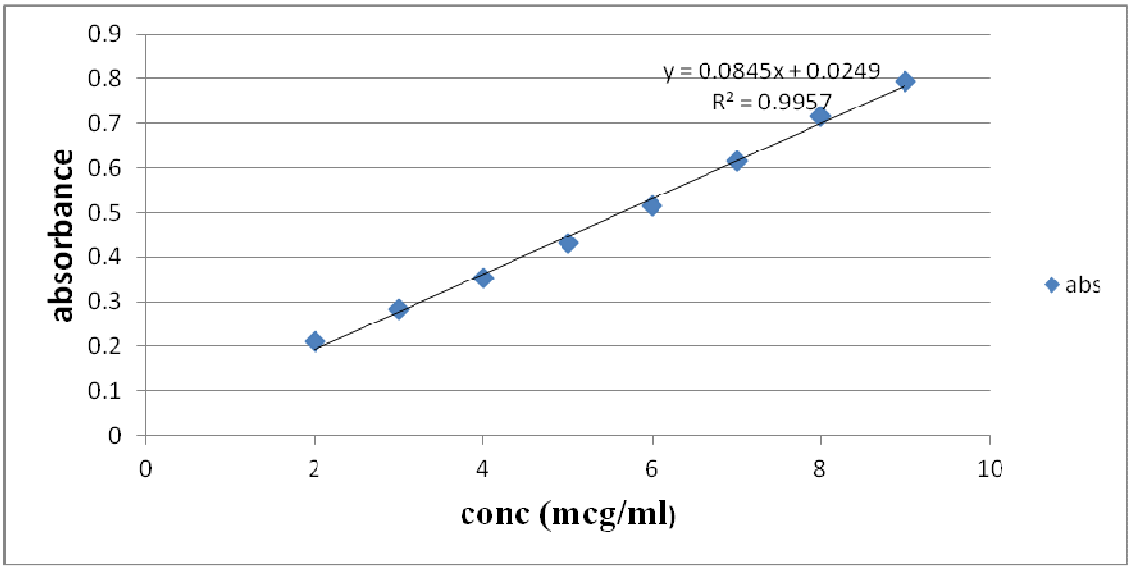

Figure 1. Standard curve of curcuminoids in DMSO

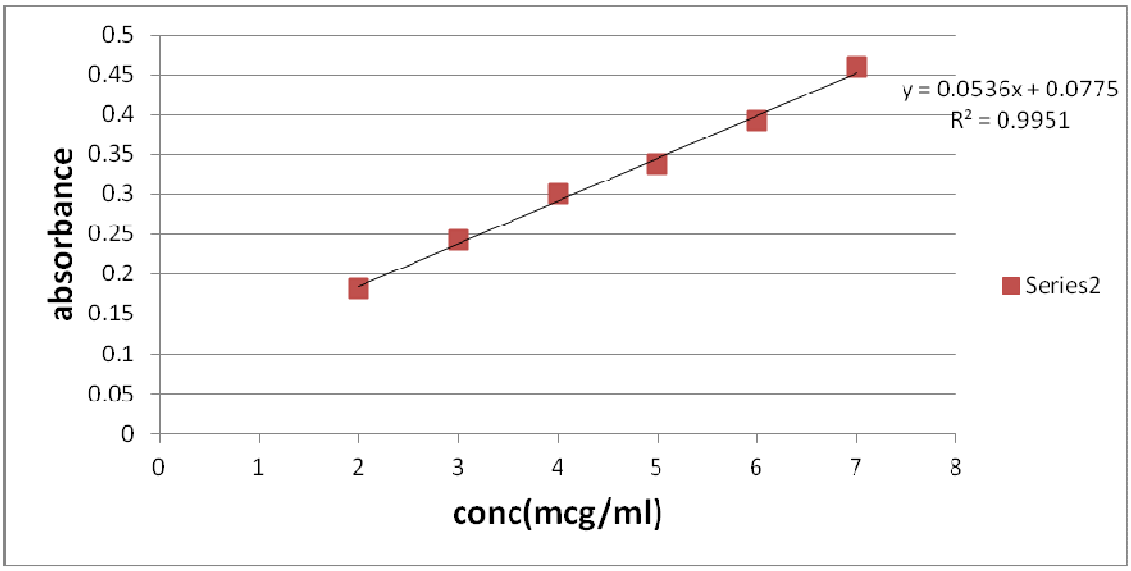

Figure 2. Standard curve of Curcuminoids in distilled water

Standard Curve Of Curcuminoids in Acetone

The graph was plotted to determine the Curcuminoids in acetone by determining the absorbance at 427nm by using different concentrations, such as 1,2,3,4,5mcg/ml, and the absorbance was found to be 0.2621, 0.3549, 0.4819, 0.6178 and 0.7418, respectively.(Figure-3)

Standard Curve Of Curcuminoids in Dichloromethane

The graph was plotted to determine the Curcuminoids in dichloromethane by determining the absorbance at 427nm by using different concentrations, such as 3,4,5,6,8 and 9mcg/ml, and the absorbance was found to be 0.1218, 0.2987, 0.4013, 0.4997 ,0.8038 and 0.9206 respectively.(Figure-4)

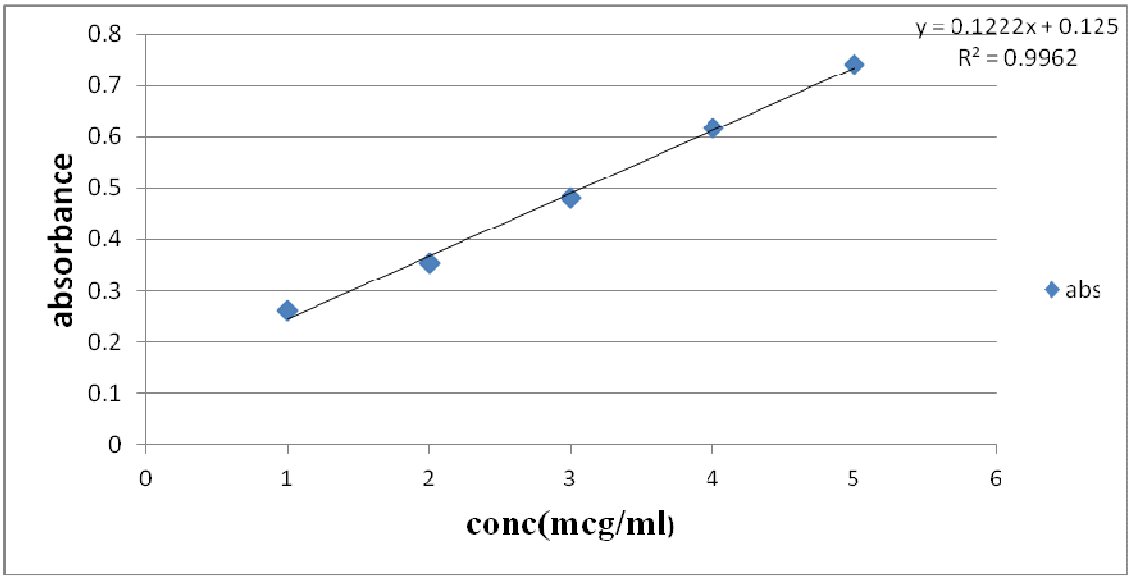

Figure 3. Standard curve of curcuminoids in acetone

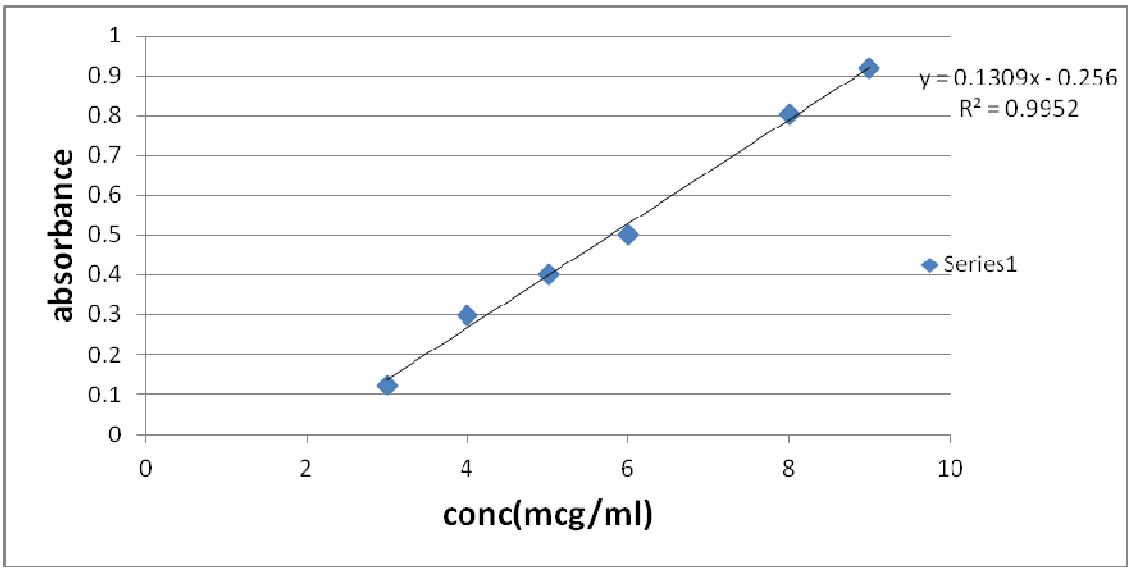

Figure 4. Standard curve of Curcuminoids in Dichloromethane

Drug content uniformity

The weighed quantities of SD formulations were evaluated for drug content uniformity by UV-Spectrophotometer. The results of the drug content uniformity in each of SD are presented in Table 2.It was found that SD4 Formulation showed maximum drug content, 80.9% whereas SD5 showed minimum drug content, 75.09%.

Table-2: Drug content uniformity of solid dispersion(SD) of curcuminoids

S. No	SD Formulation	% Drug Content
1	SD1	78.79%
2	SD2	77.09%
3	SD3	79.92%
4	SD4	80.9%
5	SD5	75.90%

Solubility Profile

The solubility data for Curcuminoids and solid dispersions (SD1,SD2,SD3,SD4, SD5) are given in table-3 .It was found that by imparting the hydrophilic character of guar-gum the solubility of Curcuminoids increases and solid dispersion SD2 show maximum solubility in comparison with others .The order of solubility is in following manner SD2> SD1>SD4>SD5>SD3>Pure Drug. ANOVA ($P<0.05$) performed on the solubility parameter demonstrated that there was a statistically significant differences between the solubility of Curcuminoids and formulation. **(**Figure-5)

Table-3 Solubility profile of various solid dispersions and pure drug

Formulation	Solubility (mcg/ml)
SD1	7.5
SD2	10.2
SD3	4.3
SD4	6.4
SD5	4.8
Pure Drug	1

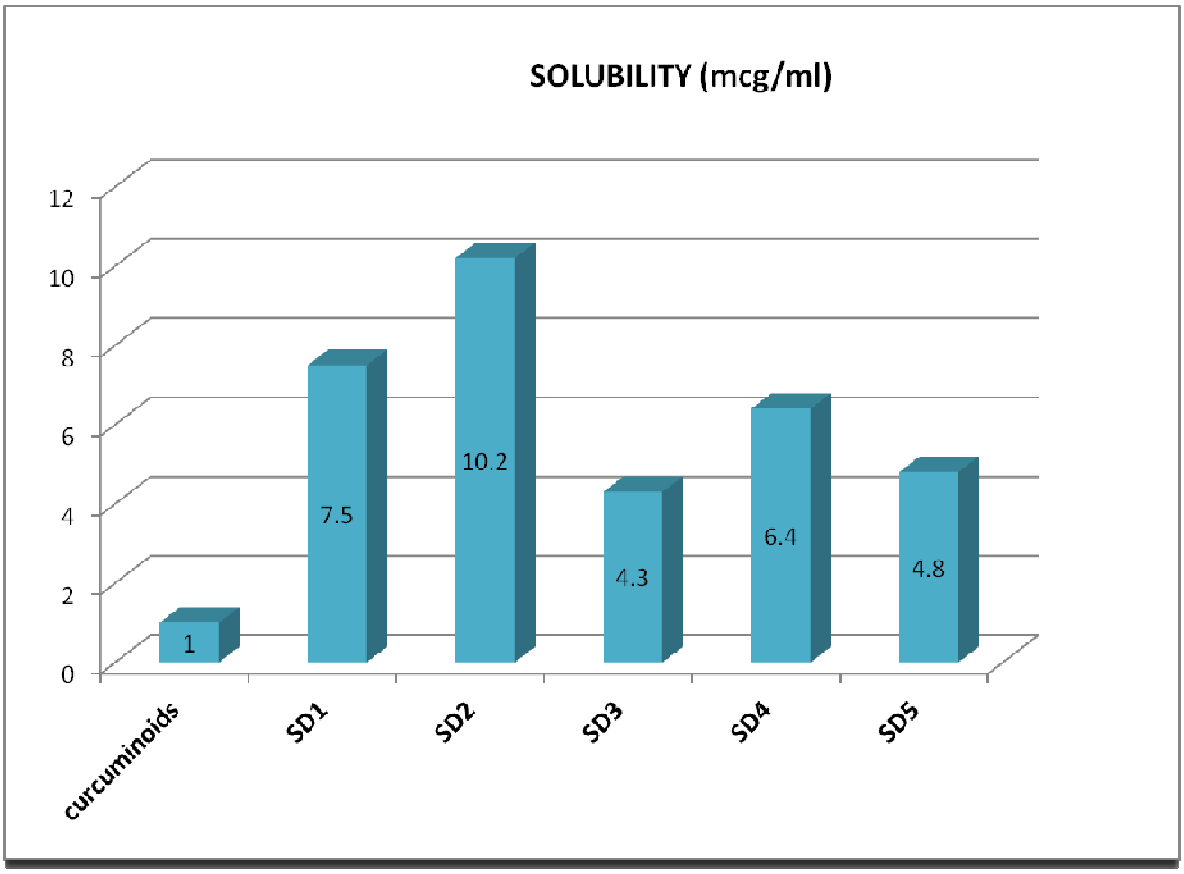

Figure 5. Solubility profile of solid dispersions and Curcuminoids

In vitro Dissolution Rate Studies

Figure -6 show the in vitro dissolution profiles of the solid dispersions in comparison with pure Curcuminoids. It is found that the rate of dissolution of Curcuminoids is very low as compared to solid dispersions .Solid Dispersion SD1 showed the higher % of drug release (70%) in 24 hours. The rank order of release of drug is SD1>SD2>SD3>SD4>SD5>Curcuminoids. The pure Curcuminoids exhibited the slowest dissolution rate because of its hydrophobicity that caused the powder to float on the surface of dissolution medium and prevented its surface contacting the medium. In all cases, the dissolution rates of Curcuminoids SD were remarkably enhanced compared to the pure Curcuminoids. Several mechanisms have been proposed to account for the increase in the dissolution kinetic of drug from solid dispersions. These mechanism include the reduction of drug crystallite size, a

solubilization effect of carrier, an absence of drug aggregation and agglomeration, an improved in drug wettability, the conversion of drug to the amorphous state. The increased in Curcuminoids dissolution from these solid dispersions can thus be contributed by several factors such as an excellent wettability, which could be observed clearly from the solid dispersion since it rapidly left the surface and was dispersed in the bulk of the dissolution medium, a markedly increase in Curcuminoids solubility, the solubilizing effect of the carrier, an absence of aggregation and agglomeration and the formation of high energy amorphous state as confirmed by FTIR data.

Release Kinetics

The pattern of drug release is in sustained manner, the release should follow three steps.

First step is the penetration of the dissolution medium in the polymer matrix (hydration). Second step is the swelling with concomitant or subsequent dissolution or erosion of the matrix and third step is the transport of the dissolved drug, either through the hydrated matrix or from the parts of the eroded surface to the surrounding dissolution medium. Drug release from hydrophilic matrices is known to be a complex interaction between dissolution, diffusion and erosion mechanisms. Water penetration, polymer swelling, drug dissolution, drug diffusion and matrix erosion from dosage form is controlled by the hydration of guar gum, which forms the gel barrier through which the drug diffuses. Drug release is also influenced by drug polymer ration with increase in polymer ratio from 1 to 5, the drug release decreases from 70.2% to 49.1.% (SD1 to SD5) shown in Fig.6 . This release retardant property may be due the matrix formation of the polymer and enclosing the drug in matrix commencing slow release of drug either through diffusion or erosion of polymer.

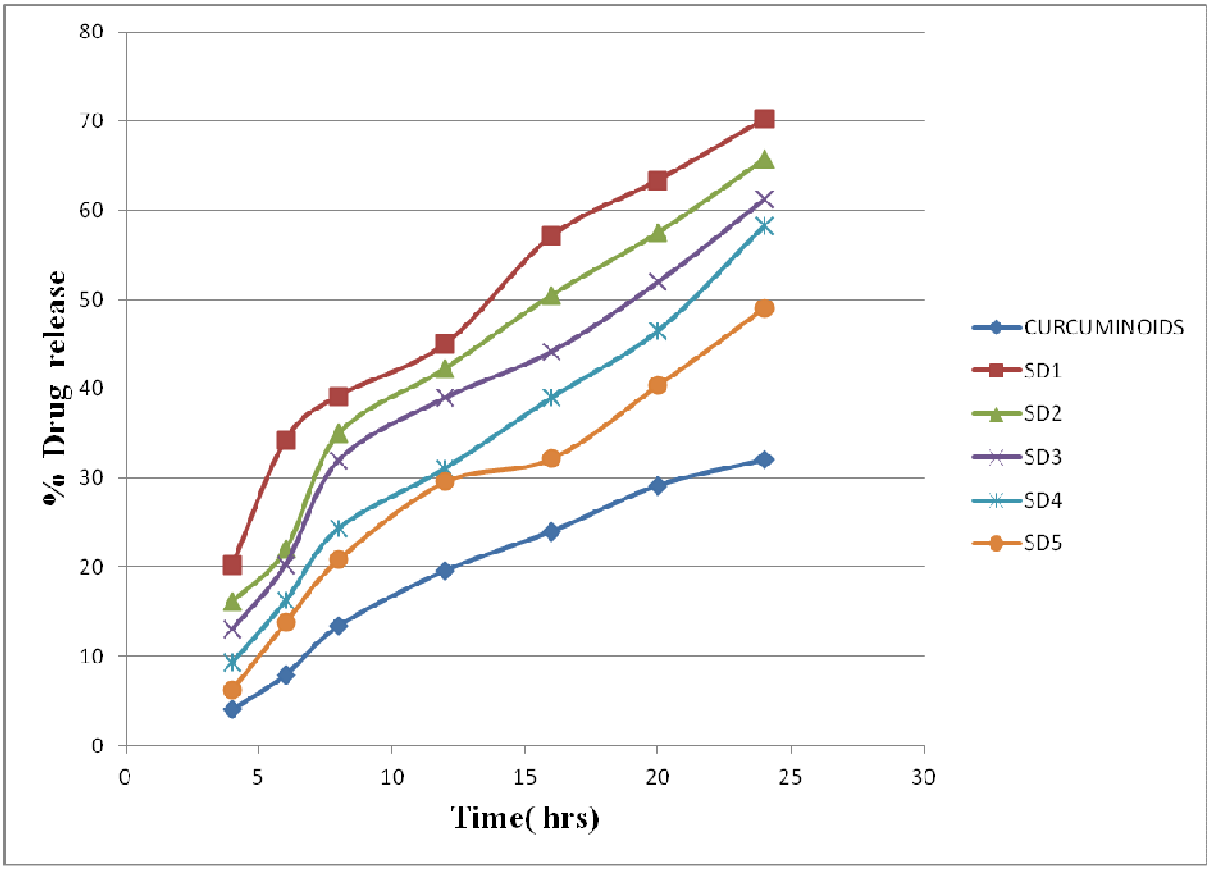

Figure 6: Comparative dissolution study of solid dispersions and pure drug

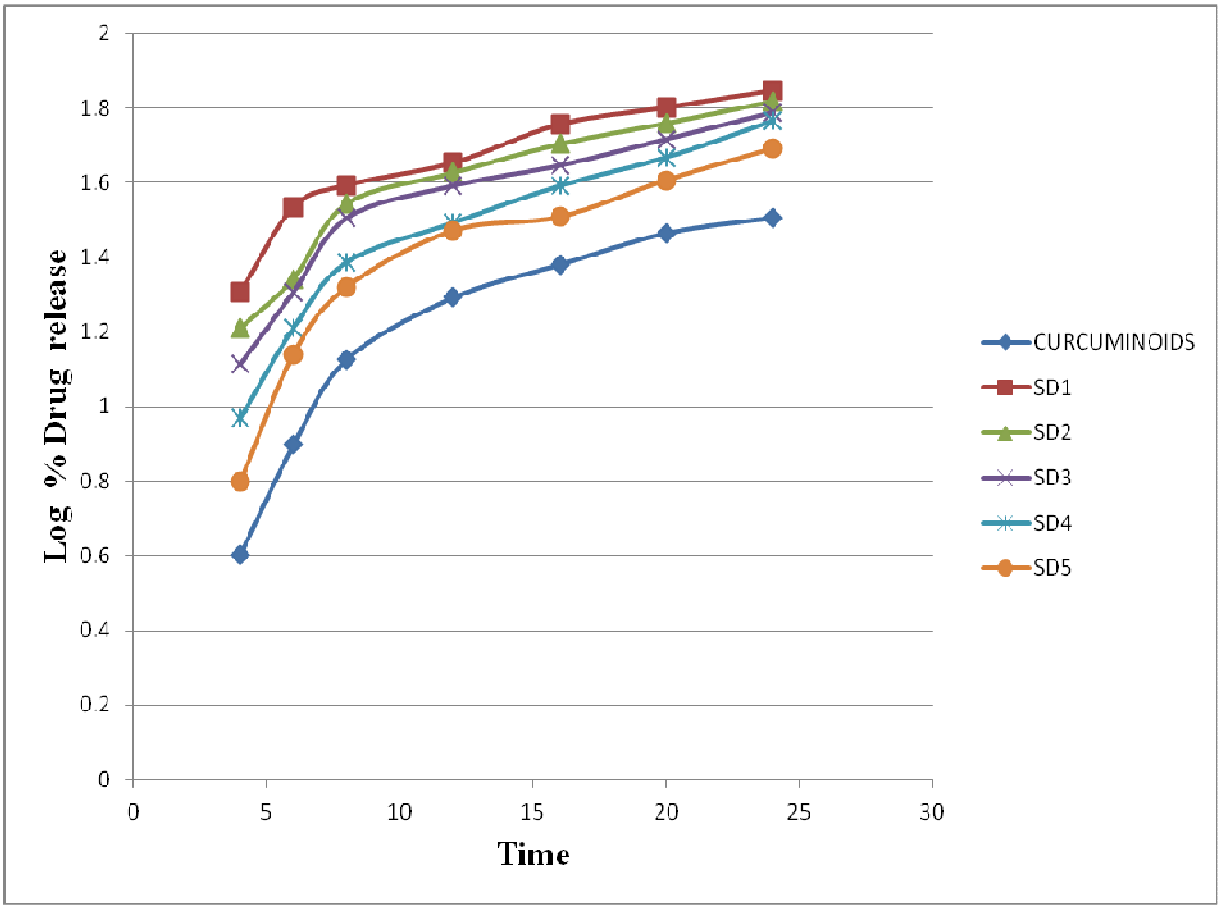

Figure 7. First order release model of Curcuminoids from various solid dispersions

Table-4

Formulations	Zero order kinetics ($R^{2)}$	First order kinetics (R^2)	Higuchi model (R^2)	Hixson-crowell model (R^2)	Korsmeyer-Peppas (R^2)
SD1	0.959	0.857	0.981	0.982	0.958
SD2	0.964	0.870	0.987	0.984	0.972
SD3	0.987	0.848	0.985	0.973	0.960
SD4	0.960	0.885	0.981	0.978	0.976
SD5	0.969	0.818	0.984	0.976	0.990
Pure drug	0.971	0.831	0.995	0.958	0.961

The regression coefficients **(**table 4**)** obtained for zero order kinetics were found to be higher (R_2= 0.95 to 0.987) when compared with those of first order kinetics (R_2= 0.81 to 0.88), indicating that drug release independent of concentration from all formulation. All the formulation showed good linearity with Hixson Crowell cube root law (R_2 = 0.99 to0.981) signifying that the drug release from the SD was erosion based means decrease in surface area and diameter of particles with polymer erosion. To categorize the mechanism of drug release, in vitro information was en suite in korsmeyer-pepas model (table 4). All the formulation shows good linearity (R_2= 0.95 to 0.996), with the slope (*n*) values 0.6367 to 0.9589, indicating that release mechanism was anomalous non-Fickian or anomalous release ($0.5 < n < 1$) anomalous (non – fickian) diffusion, n=1.The n values increased as the drug polymer ratio increases. Based on swelling and erosion studies, it was concluded that SD undergo swelling based diffusion as well as erosion during the dissolution study, which indicates that polymer relaxation had a role in drug release mechanism. However, it can be over and done with that release kinetics was found to be diffusion coupled with erosion as shown from figures 7 to 10.We can say that the drug is dispersed within the three-dimensional structure of the hydrogel and drug release is controlled by drug diffusion through the gel or erosion of the polymer.

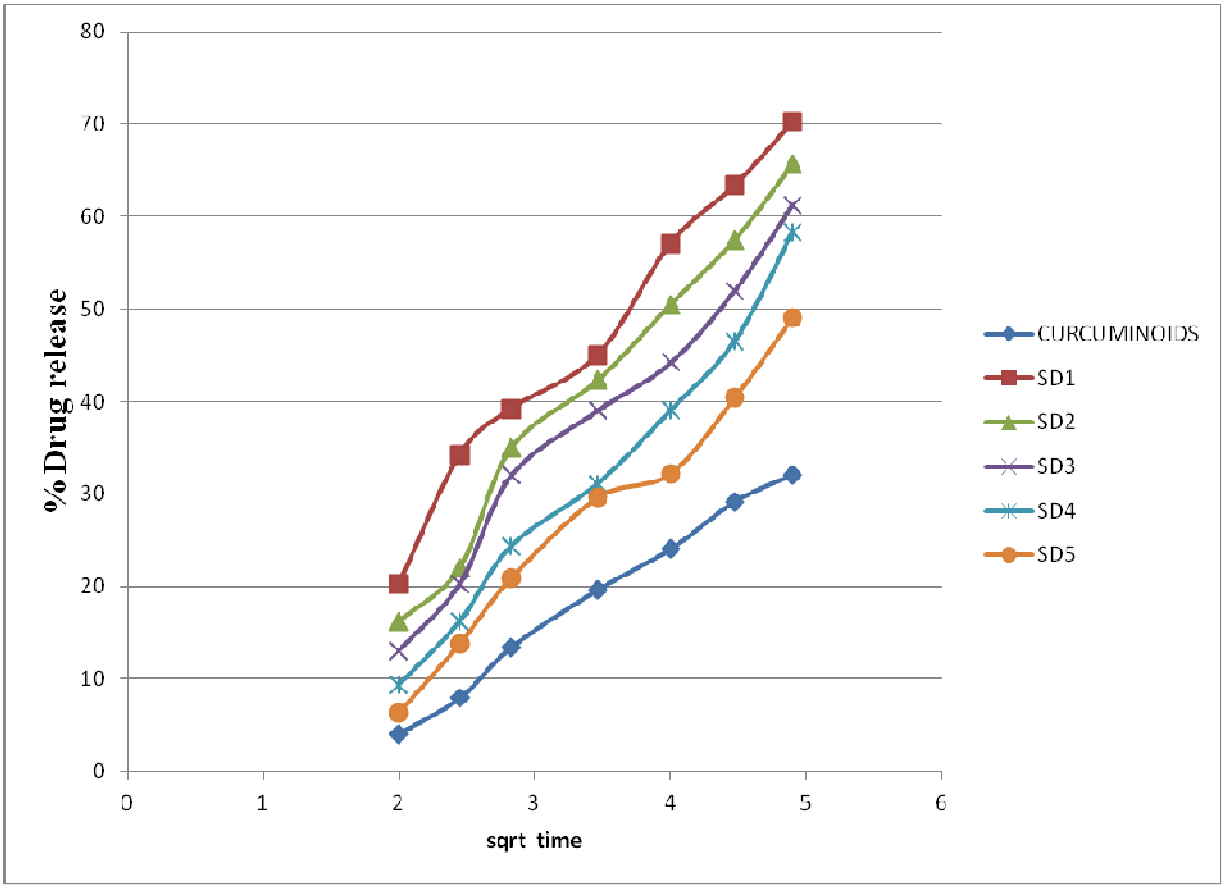

Figure 8. Higuchi model of Curcuminoids release from various solid dispersions

Fourier Transform Infrared Spectroscopy

FT-IR spectroscopy was carried out to elucidate the interaction between Curcuminoids and guar-gum in the solid state. Figs. 11-13 show the FTIR spectra of Curcuminoids, guar gum and solid dispersions (SD1).

In FTIR spectrum of pure Curcuminoids ,the C=O stretching and O-H stretching were appeared at 1637.9 and 3518.2 cm^{-1} respectively .The characteristic IR Frequencies of guar-gum is shown in table 5 .The chemical structure of Curcuminoids shows the functional groups of phenolic OH, C=O and aromatic C=C which shows the peaks at 3500-3300 cm-1, 1625 –1640 cm-1 and 1520 – 1400 cm-1 in the IR spectrum. These peaks were observed in both pure drug as well as in solid dispersion indicating no interaction between drug and polymer.

Table-5 Characteristic IR Frequencies of Guar Gum

Wavenumber (cm-1)	Characteristic group
3384	O–H stretching vibration
2924	C–H stretching of CH_2 group
1657	ring stretching
1350,1414,1450	symmetrical deformations of CH_2 group
1155,1078	C–OH and primary alcoholic –CH_2OH stretching mode
1021	–CH_2 twisting vibration
871	galactose and mannose
930,770	(1–4), (1–6) linkage of galactose and mannose respectively

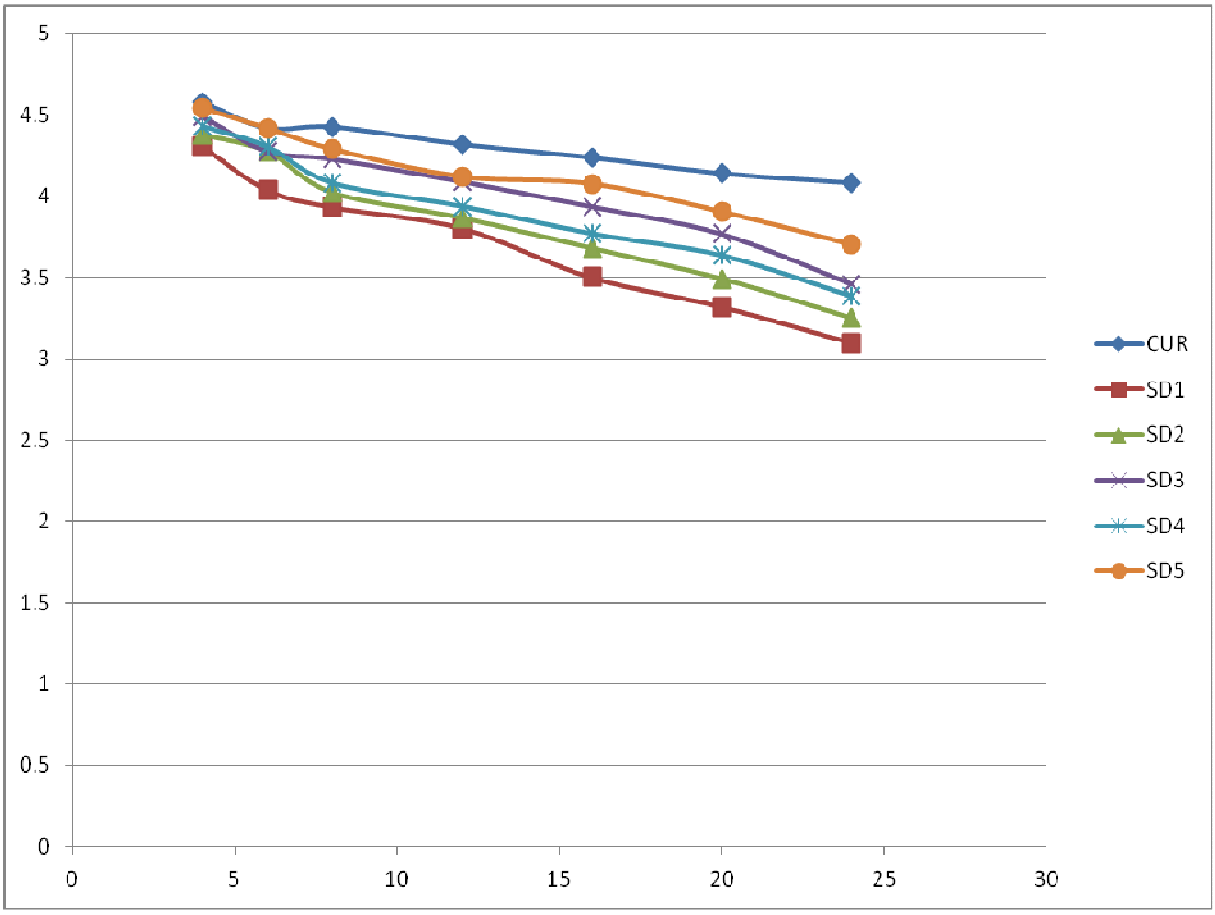

Figure 9. Hixson-crowell release model of Curcuminoids from various solid dispersions

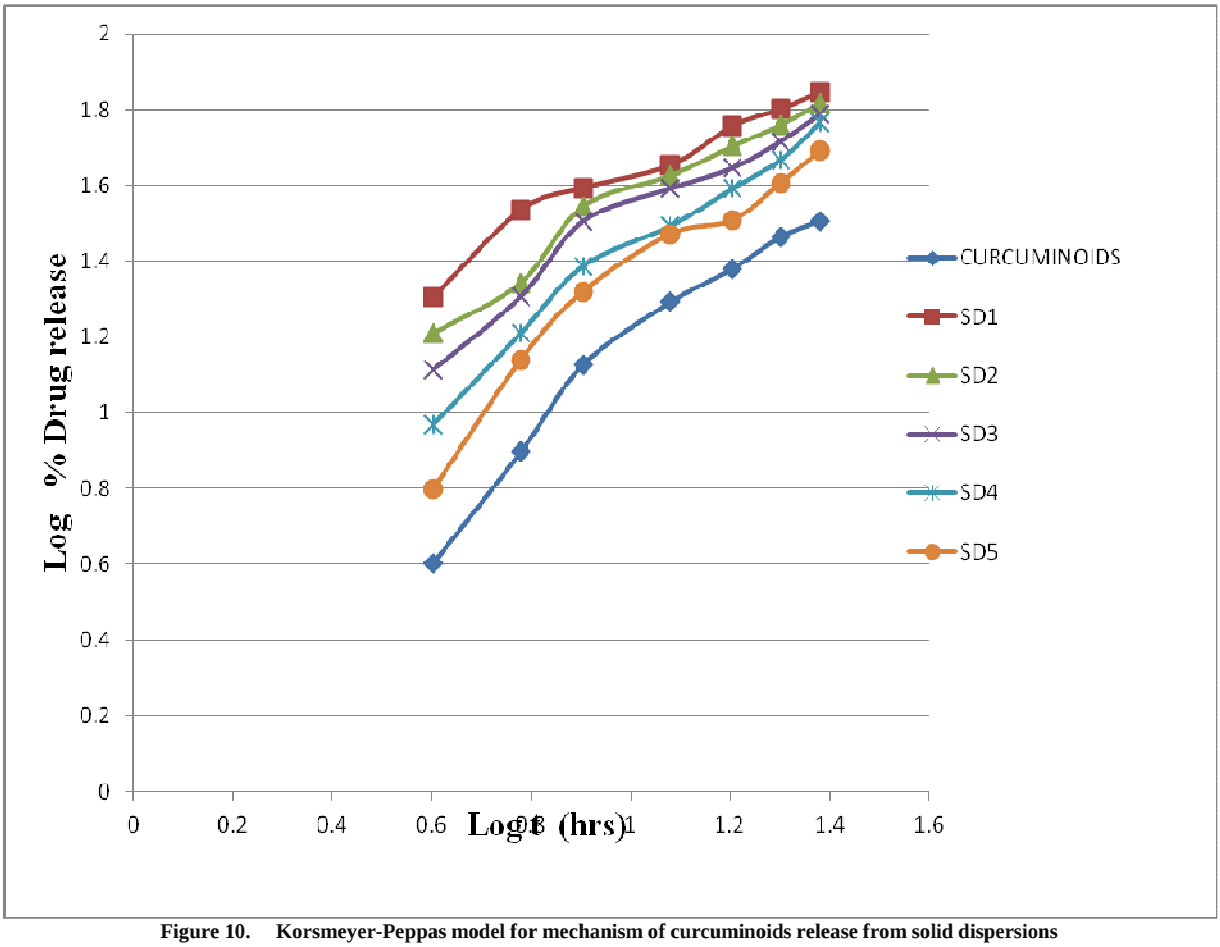

Figure 10. Korsmeyer-Peppas model for mechanism of curcuminoids release from solid dispersions

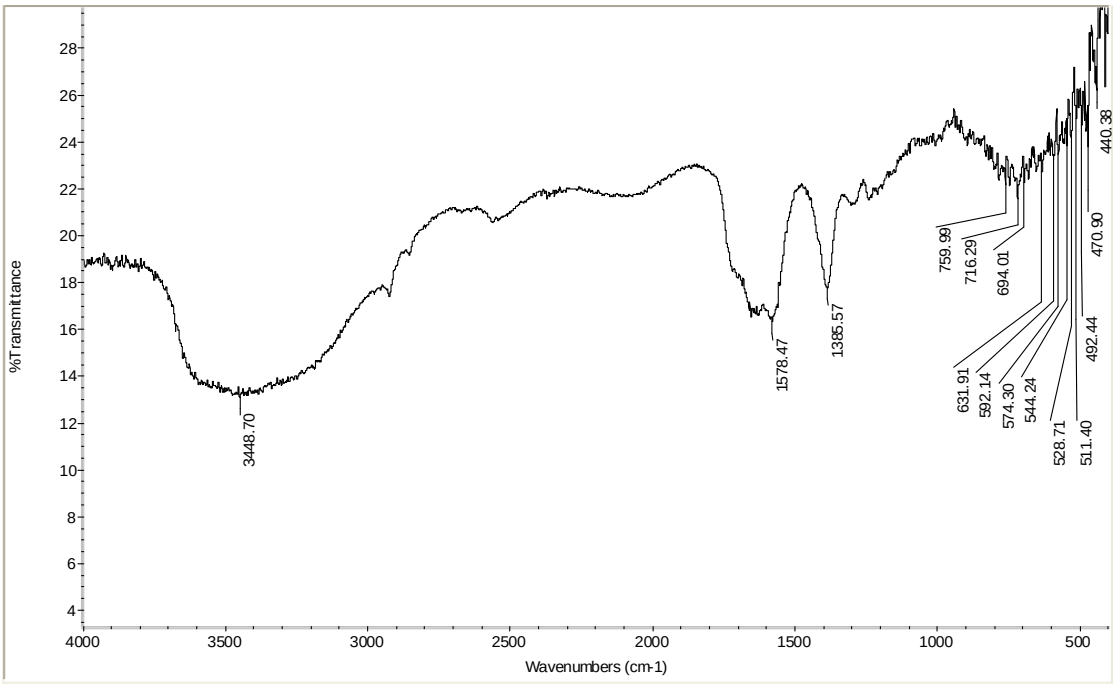

Figure 11. FT-IR Spectra of Guar Gum

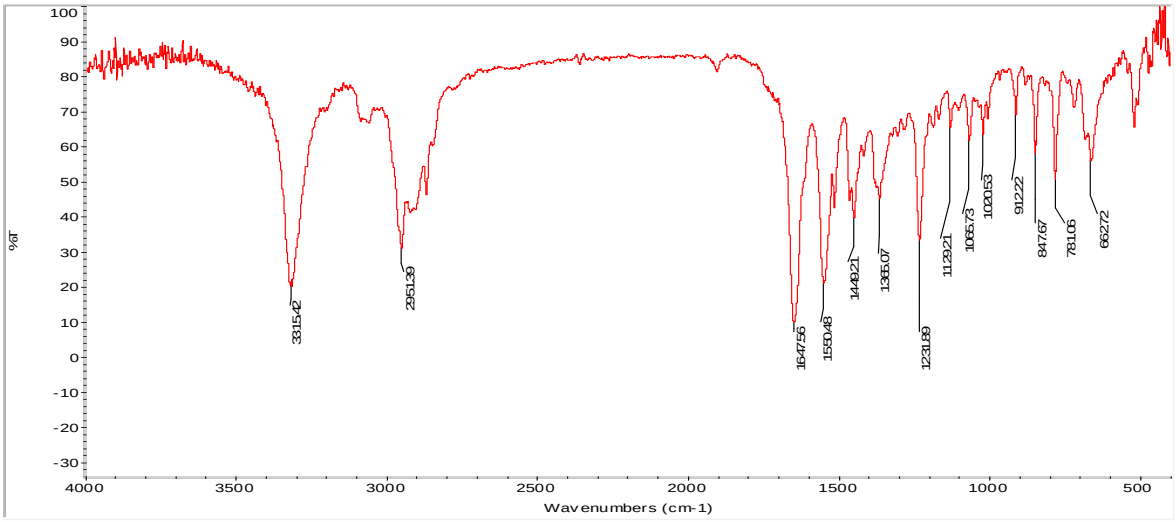

Figure 12. FT-IR Spectra of Curcuminoids

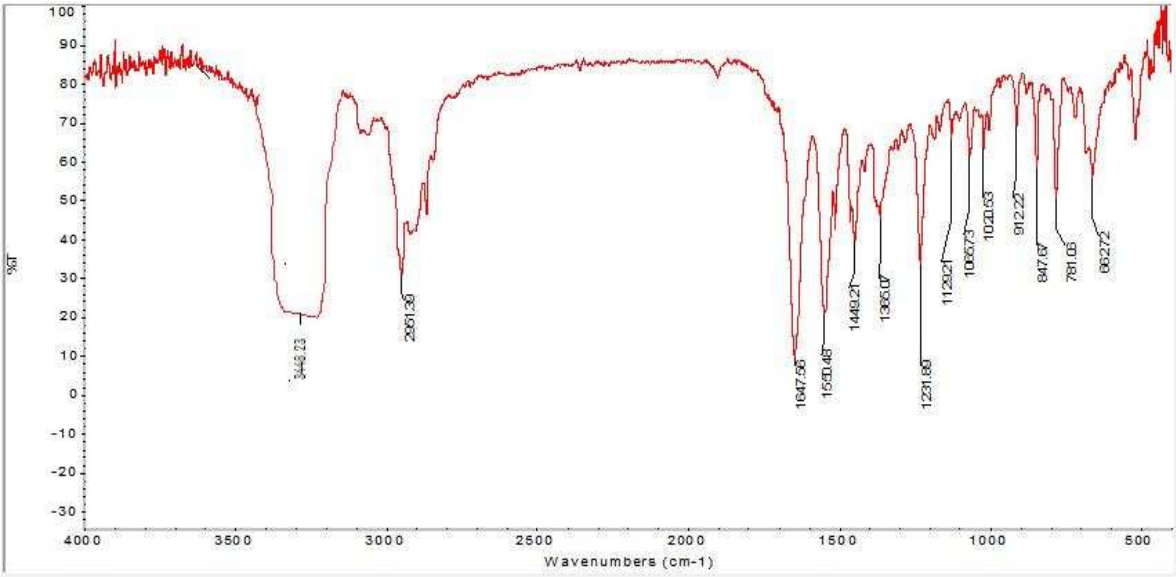

Figure 13. FT-IR Spectra Of Solid Dispersions (SD1).

CONCLUSION

Solid dispersion of Curcuminoids using guar-gum as a release retarding polymers was successfully prepared. Drug content uniformity was made for all prepared sustained release solid dispersion. IR study was performed and confirmed absence of any possible solid state drug and polymer interactions. Solubility of curcuminoids solid dispersions increased upto 10 times by imparting hydrophilic character of guar gum. In vitro release profile studies suggested that the drug release has been extended up to 24 hrs when guar gum as a polymers is used. Exhibit zero order kinetics and drug release is controlled by drug diffusion through the gel or erosion of the polymer.

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