EMERGING TREND IN ADRESSING THE CHALLENGES TO ORAL NANOCURCUMIN DELIVERY TO IMPROVE QUALITY OF LIFE OF PATIENTS SUFFERING FROM CANCER

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ABSTRACT

Present work was investigated for enhancement of solubility, bioavailability and anti-cancer activity of curcumin, a potent natural anticancer agent. Solid microdispersion of curcumin was prepared by melt granulation technique using *Gelucire® 50/13* as a hydrophilic carrier followed by adsorbtion on *Aeroperl® 300 Pharma.* Compatibility among curcumin and excipients were checked using FT-IR, DSC and XRD analysis. The prepared microdispersion was characterized for percent drug content, entrapment efficiency, surface morphology, particle size, solubility, bio-availability and anticancer activity. DSC analysis study had shown complete encapsulation of raw curcumin which was further supported by XRD study showing flat peaks. A 3^2 full factorial response surface quadratic model demonstrated the positive effect upon change in the concentration of independent variables *viz.* Gelucire® 50/13 and Aeroperl® 300 Pharma on dependent variables particle size; % entrapment efficiency. TEM analysis confirmed the nano particulate dispersion upon dilution in distilled water with many fold increased in solubility. Maximum solubility was observed in acidic buffer pH 1.2. *In vivo* study revealed increased in bio-availability in Wistar rats as compared to raw curcumin by HPLC analysis. In MTT assay study, curcumin microdispersion has shown more prominent anti-cancer activity than raw curcumin on breast cancer cell line culture, *MCF-7.* From above study, it may be concluded that implementation of this innovative approach could be a better alternative for oral nanocurcumin delivery to improve the quality of life patients suffering from cancer.

INTRODUCTION

According to the latest WHO statistics, global cancer deaths will increase by about 45% by 2030, with developing countries such as India contributing 70% to the total. Along with the continuous development of science and technology, the need for addressing practical problems associated with drug therapies has increased proportionately. To date, cancer therapy has been based on parenteral administration of drugs. An interesting study of patients' preferences found that almost 78.7% of the subjects wanted to be treated by the oral route for recurring breast cancer, whereas only 2.7% preferred the parenteral route and 18.6% had no preference[1].

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Chemosensitivity is the susceptibility of tumor cells to the cell-killing effects of synthetic anticancer drugs. It has been shown that natural anticancer drugs augment the cytotoxic effects of chemotherapeutic drugs, which are optimally active when cancer cells proliferate rapidly. More than 65% of the anticancer drugs used are available in oral dosage form, but very few of them are used in this form. This could be because of their limited bioavailability owing to their poor physiological properties and efflux mechanisms[2].

Many researchers have found that turmeric, which is used as a dietary spice, has high efficacy as an anticancer drug at multiple target sites. The most active component of turmeric is curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione), which makes up 2-5% of the spice. Curcumin is practically insoluble in water. It is a yellow pigment obtained from the rhizomes of turmeric (*Curcuma longa*)[3]. Curcumin is being studied in clinical trials for its use in the treatment of a wide variety of human ailments, including cancer, cardiovascular disorders and neurological diseases[4]. In a Phase I clinical trial, it was confirmed that the curcumin doses of up to 12 g daily are safe, with no significant toxicity[5].

The powerful therapeutic effects of crystalline curcumin are partly restricted by its poor solubility in water, which in turn limits its oral bioavailability[6]. The maximum solubility of curcumin in an aqueous buffer (pH 5.0) is reported to be 11ng/mL. At neutral pH, the curcumin concentration is reported to be too low to quantify[7].

Curcumin undergoes degradation extremely slowly at pH values of 1-6. It is essentially stable at acidic pH values. But it is unstable at neutral and basic pH values, under which conditions it is degraded to ferulic acid and feruloyl methane[8].

The use of nanocarrier-based formulations has been shown to increase the solubilisation potential, improved encapsulation and prevent metabolic degradation within the gastrointestinal tract. It has been reported that drug-drug interactions are prevalent in patients being treated with oral anticancer drugs. This is alarming in the sense that widely used formulations are dangerous to patients. About 46% of cancer patients receiving oral preparations reported major drug-drug interactions that can be overcome by using the carrier-based drug-release approach[9].

The apparent solubility and dissolution rate of curcumin in its amorphous form are high, as a result of which the oral bioavailability of this drug, which is poorly soluble in water, is better in this form[10].

In the present study, an attempt had made to developed highly efficient, stable nano-curcumin (amorphous) formulation, based on novel functional excipients[11]. It was found that this curcumin-loaded nanodispersion has enhanced solubility and bioavailability and very good anticancer activity[12].

MATERIALS AND METHODS

Materials

Crystalline curcumin (>98% w/w purity) was procured from Yarrowchem Products Pvt. Ltd., Mumbai, India. Gelucire® 50/13 (Stearoyl Macrogolglycerides EP, solid pastilles, nominal melting point 47–50°C, HLB 13) was generously provided as a gift by Gattefosse India Ltd., India. A sample of Aeroperl® 300 Pharma was obtained as a gift from Evonik Pharma. Mumbai, India. All other chemicals and solvents were of analytical grade.

Methods

Preparation of physical mixtures and solid microdispersionss (SMD)

Physical mixtures containing curcumin, Gelucire® 50/13(a hydrophilic carrier) and Aeroperl® 300 Pharma (an adsorbent) were prepared by trituration for 10 minutes, followed by sieving. The resulting mixtures were stored in desiccators at room temperature until use.

Solid microdispersions were prepared by first melting the Gelucire® 50/13 in a china dish at 50°C above a water bath. Crystalline curcumin was then added to this molten mass followed by adsorbtion on Aeroperl® 300 Pharma. The mixture was allowed to cool at room temperature and triturated to obtain a free-flowing powdered dispersion[13].

Selection of suitable experimental design

Response surface methodology is an effective approach for optimizing formulations. In a CCD, all the factors are studied at all possible combinations, as it is considered to be most efficient in estimating the influence of the individual variable (main effects) and their interactions, using minimum experimentation (14a, 15a) again, the added advantage of this design is to determine the quadratic response surface which is not estimable with factorial design at two levels[16]. In this study, fitting a cubic model is considered to be a better as the values of the response surface are not known from the previous findings. Hence, a CCD for two factors (Gelucire 50/13 and Aeroperl 300 Pharma) at three levels with alpha equal to 1, which in turn is equivalent to 3^2 factorial designs, were proposed for the current mucoadhesive formulation optimization study, Table I.

Table I: A 3^2 Full factorial experimental design layout translation of coded levels in actual units

Trial No.	*Formulation code	Coded factor level	
		Factor 1 (X1:mg)	Factor 2 (X2:mg)
1.	SMD1	0 (250)	0 (150)
2.	SMD2	1 (400)	0 (150)
3.	SMD3	-1 (100)	1 (200)
4.	SMD4	0 (250)	1 (200)
5.	SMD5	1 (400)	-1 (100)
6.	SMD6	1 (400)	1 (200)
7.	SMD7	0 (250)	-1 (100)
8.	SMD8	-1(100)	0 (150)
9.	SMD9	-1 (100)	-1 (100)

*SMD= Solid Microdispersions

Optimization of data analysis

The response variables considered for optimization were including the particle size, percentage entrapment efficiency. Finally, using Design-Expert (version 9.0.1, Stat-Ease, Inc., USA) to fit full second order polynomial equation with added interaction terms to correlate

the studied responses with examined variables. The polynomial regression results were demonstrated using 3-D graphs and contour plots.

Validation of Response Surface Methodology

Total of eight formulations were selected as check points to validate RSM. Solid microdispersions were formulated (as described in section above) using chosen optimal composition and evaluated for particle size, zeta potential, percentage entrapment efficiency and dissolution study. Plots between predicted and observed responses were critically compared, the residual graphs plotted and the percent error was calculated with respect to the observed responses. Correlation plots were also constructed separately for eight formulations.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR analysis was carried out using the potassium bromide (KBr) disk method. The samples were ground gently with FT-IR grade KBr in the ratio 1:15 by weight. The blends were compressed in a hydraulic pellet presser by applying a pressure of 10 tons. Thin, flat pellets of diameter 10 mm were obtained. These were analyzed for confirming the compatibility between curcumin and carrier excipients by scanning their spectra. The scanning range was 450–4000 cm^{-1}, and the spectra were recorded on an FT-IR spectrophotometer (Jasco FT-IR Plus, Japan).

Thermal analysis using differential scanning calorimetry (DSC)

The possibility of any interactions between the curcumin, the hydrophilic carrier and other excipients used was assessed through thermal analysis using DSC. Samples were accurately weighed and placed on aluminum pans, which were sealed with aluminum lids. The thermograms of the samples were obtained at a scanning rate of 10°C/minute.

X-ray diffraction (XRD)

The thermal transformation of crystalline curcumin and its physical blending and solid microdispersions were investigated using a Philips PW3710 analytical X-ray diffractometer with Cu K 2α rays, a voltage of 40 kV and a current of 25 mA. The samples were scanned for 2θ values from 5° to 50°. Diffraction patterns of curcumin and its solid microdispersions using Gelucire® 50/13 were obtained.

Scanning electron microscopy (SEM)

The surface morphology and shapes of the raw crystalline curcumin and its solid microdispersions were observed and images obtained using scanning electron microscopy. The samples were mounted on a double-faced adhesive tape sputtered with platinum prior to analysis.

Transmission electron microscopy (TEM)

Solid microdispersions were diluted 250 times with simulated gastric fluid and filtered through whatman filter paper of 0.45 µm. The physical morphology of diluted sample was then observed using transmission electron microscope (TEM) (Hitachi H-7500, Japan). Small drop was deposited on a carbon-coated copper grid and observed[17].

Particle size analysis using Zetasizer

Nanoparticle dispersions were obained by diluting 10 mg of the solid microdispersions with demineralised water to ensure that the intensity of the signal was suitable for the instrument. The zeta potential of the sample was measured using laser Doppler velocimetry, Zetasizer 3000, Malvern Instruments, Malvern, UK (14b).

Solubilization kinetics

The solubility values of raw crystalline curcumin and solid microdispersions were determined by adding excess amount to water and buffer solutions (pH values 1.2, 4, 6.8 and 8). The resultant solutions were agitated at 37.0 ± 0.5°C using shaking flask apparatus (shake flask method). After 48 hours the samples were filtered through a 0.45µm membrane filter, which yielded clear solutions. These solutions were analyzed for absorbance on a double-beam UV-visible spectrophotometer at 426 nm, and the absorbance were recorded in triplicate (15b)

Quantification of curcumin

Drug content study

The quantity of curcumin in solid microdispersions was determined by weighing a sufficient quantity of optimized solid microdispersions which was transferred to a 25 mL volumetric flask and diluted with 20 mL of methanol. The resultant mixture was sonicated for 15 minutes and diluted with methanol to make 25 mL. The mixture was filtered using a 0.45 µm filter to remove any particles. The filtrate was analyzed using a UV–visible spectrophotometer to determine the drug (curcumin) content.

The percent drug loading and percent encapsulation efficiency of the solid microdispersions were calculated using equation (1) and equation (2), respectively (18a)

$$\text{Drug loading (\%)} = \frac{\text{Weight of drug in microdispersion}}{\text{Weight of drug in micodispersion}} \times 100$$

$$\text{Encapsulation efficiency (\%)} = \frac{\% \text{ drug loading}}{\% \text{ theoratical loading}} \times 100$$

In vitro dissolution study

The average percent release of curcumin in 900 mL of 0.1 N HCl was determined through *in vitro* dissolution testing. USP type II dissolution test apparatus was used, and the sample was maintained at 37.0 ± 0.5°C, with continuous stirring at a constant speed of 100 rpm. Samples were withdrawn at 1 hour intervals (with replacement of the same quantity of fresh dissolution medium) and filtered through Whatman paper No. 41 (0.45 µm). The filtrates were also analyzed in triplicate at 426.0 nm using a UV–visible spectrophotometer (PC 1600 Shimatzu, Japan). The percentage of curcumin released was calculated using equation (3).

$$\text{Drug release (\%)} = \frac{\text{Released curcumin}}{\text{Total curcumin used}} \times 100$$

Mathematical modelling of in vitro release data

The *in vitro* drug release data was subjected to analysis with various release kinetic models using PCP disso software. Various models were fitted using the following equations (19):

Zero order kinetic models

$$M_o - M_t = k_o t$$

First order kinetic model

$$In\ (M_o - M_t = k_1 t$$

Higuchi model
$$M_t = K\sqrt{t}$$

Hixon-Crowell cube root model

$$(Wo)^{\frac{1}{3}} - (Wt)^{\frac{1}{3}} = k_{1/3}\ t$$

Korsemeyer-Peppas model
$$\frac{M_1}{M_\infty} = kt^n$$

Where, M_o, M_t and M_∞ correspond to the amount of drug taken at time t equal to zero, dissolved at a particular time, t and at infinite time, respectively. The terms M_o and, M_t are refer to the weight of the drug taken initially and at time t, respectively. Various other terms k, and K are refer to the release kinetic constants obtained from the linear curves of Korsemeyer-Peppas, zero-order, first-order, Hixon-crowell cube root law and Higuchi model, respectively (18b).

Oral bioavailability study

All the experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), 1410/c/11/CPCSEA, at Deshpande Laboratories, Bhopal, (M.P.), India.

Male Wistar rats were housed individually in ventilated cages. They were provided sterile bedding, water and food and maintained at 24°C. The animals were starved overnight, with free access to water.

The calculated doses of raw curcumin,100mg/kg (control) and solid microdispersions (equivalent to 100 mg/kg raw curcumin) were administered by oral gavages. Blood samples were collected at the 1, 4, 8, 12 and 24 hour time points in heparinised micro-centrifuge tubes, and plasma was separated. Plasma was extracted in methanol and evaluated on an HPLC C-18 column. The stationary phase used was acetonitrile: 5% acetic acid (75:25 V/V), with a flow rate of 1 mL/minute. Detection was carried out at 426 nm at room temperature for 5.8 minutes using an HPLC system (Shimadzu, Japan) (6b).

Various pharmacokinetic parameters were calculated from the plasma sample concentration–time profiles using the Thermo Kinetica software package, version 5.0 (Thermo Fischer Scientific, USA).

Cell viability assay of cancer cell lines

MTT [(3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue product, formazan. The process requires active mitochondria, and even freshly dead cells do not cleave significant amounts of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Deshpande Laboratories, Bhopal, India, using the standard operating procedures. In brief, the compounds were dissolved in DMSO and serially diluted with complete medium (MEM) to get a range of test concentrations. The DMSO concentration was maintained below 0.1% in all the samples. Cell lines maintained in appropriate conditions (MEM medium, 37°C, 98% humidity, 5% CO_2) were seeded in 96-well plates and treated with test samples of different concentrations. They were incubated at 37°C and 5% CO_2 for 96 hours. MTT was added to the wells and incubation was continued for a further 96 hours. The dark blue formazan produced by the cells was dissolved in DMSO in a safety cabinet and read at 550 nm. Percentage inhibitions were calculated and plotted against the concentrations used to calculate the IC_{50} values.
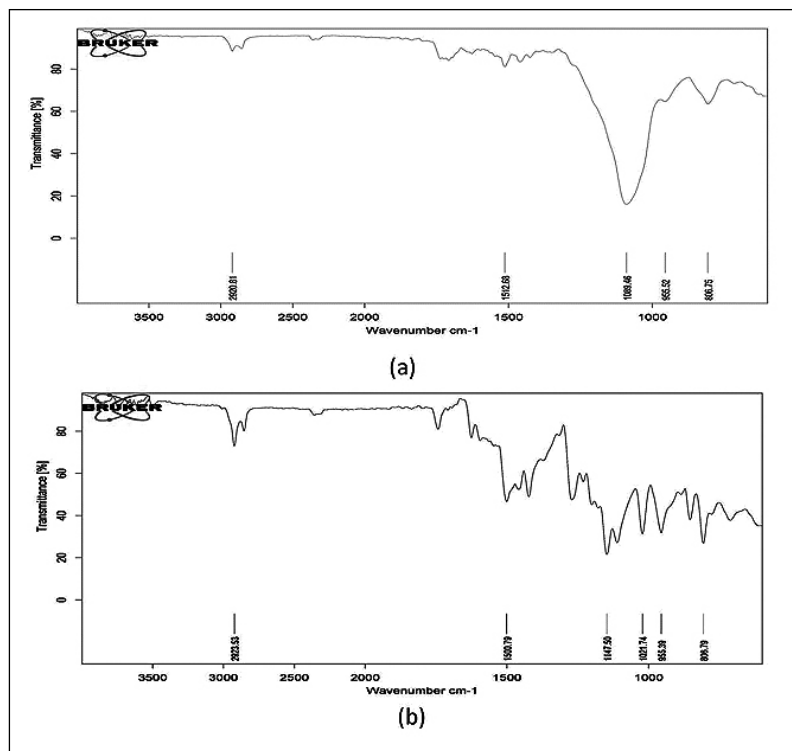
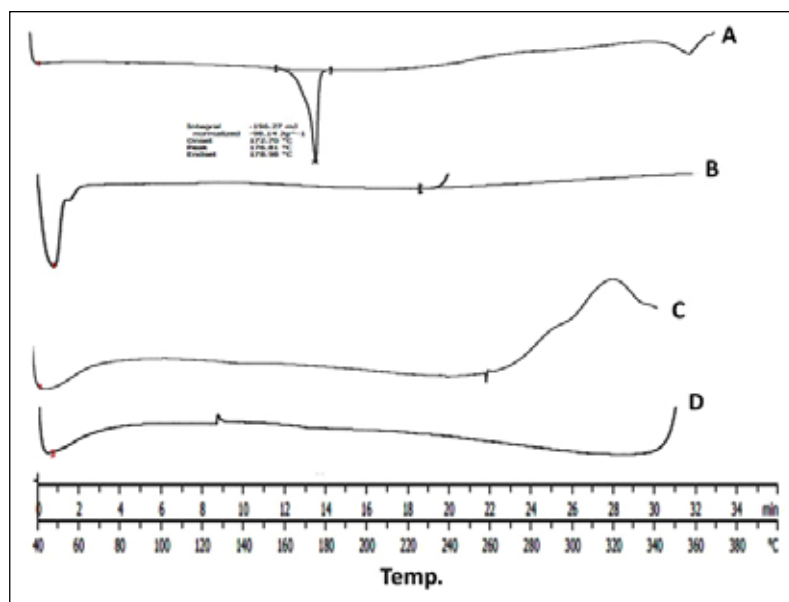**Fig. 1: FT-IR study: (a) Raw curcumin (b) Physical blend of curcumin, gelucire 50/13 and aeroperl 300 pharma**

Fig. 2: Differential Scanning Calorimetry study: (A) Raw vcurcumin (B) Gelucire 50/13 (C) Aeroperl 300 Pharma

Stability study

A physical stability study was conducted according to the guidelines of the International Conference on Harmonization (ICH). The optimized solid microdispersions were stored in a photo-stability chamber (Electrolab, India) at 40°C and 75% RH in the presence of light for 6 months. The particle sizes and curcumin content were investigated at specified time intervals over the 6 months.

RESULTS

Characterization of solid microdispersions

In the FT-IR study, no interaction among the curcumin and gelucire 50/13 was found, retaining the basic functional groups of the curcumin after analysing the physical blend, (Fig.1)

DSC study gives the information about the melting, crystallization decomposition or a change in the heat capacity and also useful to detect the status of encapsulated drug as well as interaction among the different compounds used in the formulation. Pure curcumin showed the sharp endothermic peak at 178.98°C corresponding to its melting point (15c). Gelucire 50/13 showed a broad endothermic peak at 49.5°C corresponding to its melting point (20). In case of physical mixture of curcumin and gelucire 50/13, it exhibited same broad peak as of gelucire 50/13 alone, 49.5°C. The peak corresponding to the curcumin was shifted to 278°C and become broad and very weak due to the complete dilution of the curcumin. In contrast, the characteristic peak of the curcumin was disappeared in all thermograms of the solid microdispersion (Fig. 2). This was supported by the XRD analysis, raw curcumin has shown intense and sharp °2Theta peaks at 17.35, 27.38, 18.17, 24.53 and 23.38 wherein no such distinctive peaks were found in solid microdispersion (Fig.3).

SEM analysis revealed that the solid microdispersions had a smooth and spherical encapsulated architecture because of adsorption on the surface of Aeroperl® 300 Pharma. They had a mesoporous granular structure, as a result of which they had a very large surface area of 300m^2/g. The average diameter of the dry particles was estimated from the SEM images to be 30-40 µm, which may be attributed to the highly adsorptive nature of Aeroperl® 300 Pharma, imparting the free flowing propersteis of the resultant solid microdispersion, (Fig.4.)
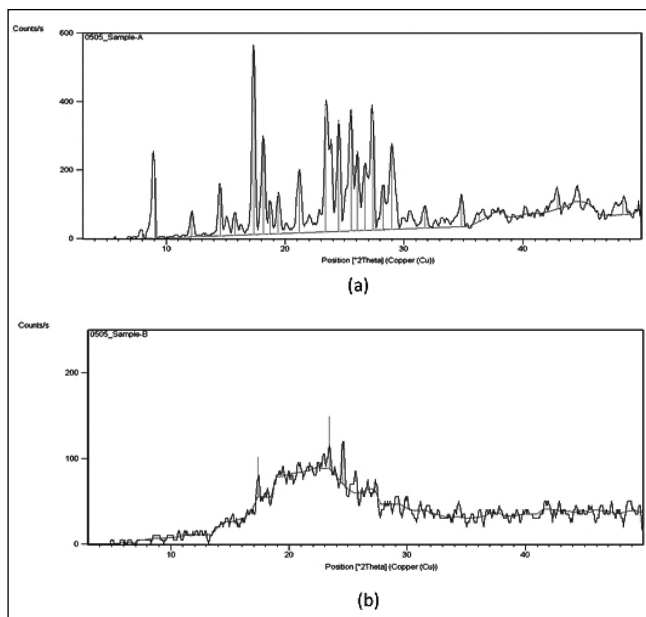
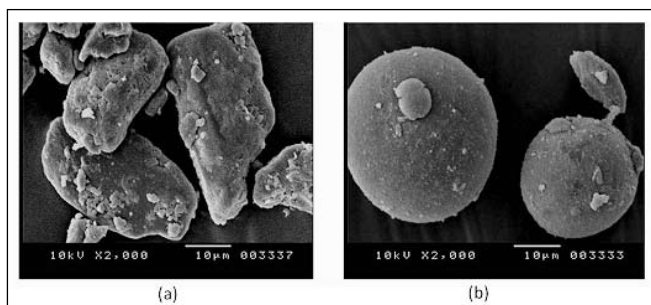**Fig. 3: X-ray Diffraction study: (a) Raw curcumin (b) Encapsulated curcumin in Gelucire 50/13**

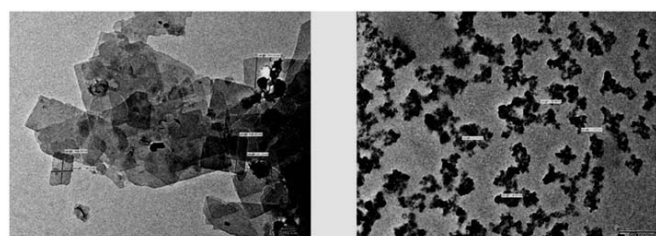**Fig. 4: Scanning electron microscopy study: (a) Raw curcumin (b) Solid microdispersion**

Fig. 5: Transmission electron microscopy study: (a) Raw curcumin (b) Solid microdispersion

TEM analysis indicated that the diameters of the prepared solid microdispersions and raw curcumin after dispersion in distilled water were 11.02-35.0 nm and 101.56-202.86 nm, respectively, (Fig.5) These dispersed particles had zeta potential values in the range of -19.56±0.65 to -32.12±0.56 mV in an aqueous buffer pH value 1.2, Table II. Average particle size of 17nm was observed using Zetasizer 3000 (Malvern Instruments, Malvern, UK), Fig.6
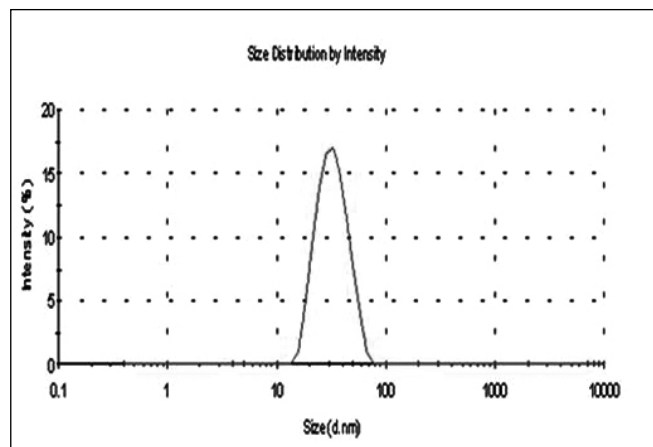

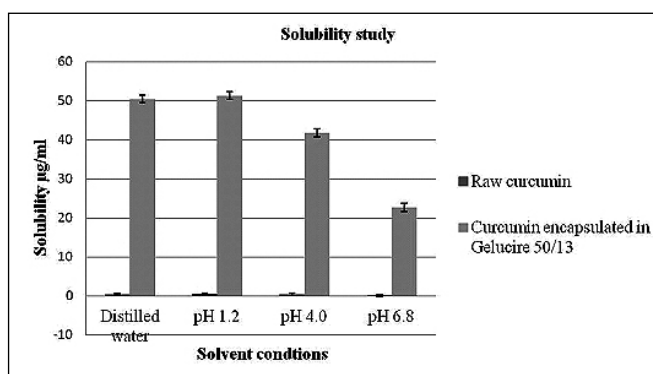**Fig. 6: Particle size analysis of solid microdispersion by Malvern zetasizer 3000**

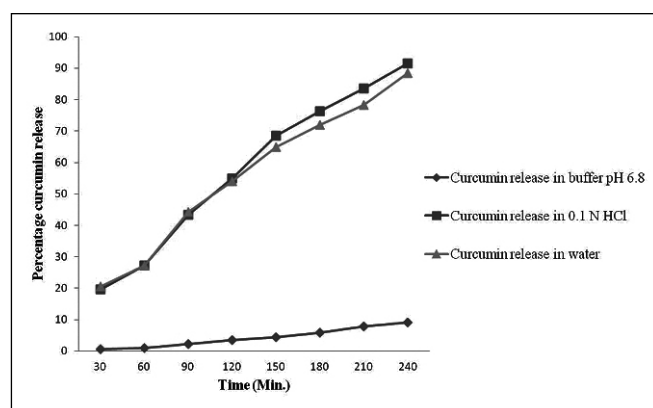**Fig. 7: Solubility analysis of raw curcumin and solid microdispersion**

Fig. 8: Comparative *in vitro* dissolution study of optimized solid microdispersion at various pH conditions

Solubilisation study

The solubility data relating to raw curcumin and its solid microdispersions in water and in buffer solutions of different pH values indicated that the Gelucire and curcumin molecules interacted mainly by electrostatic forces and occasionally through forces such as those
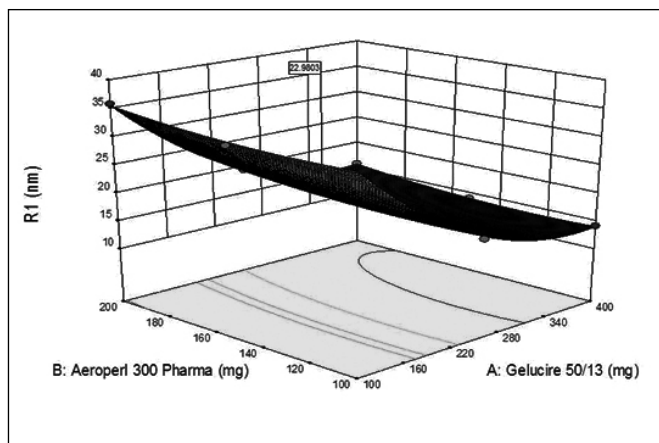
Fig. 9: Surface response plot showing effect of concentration of gelucire 50/13 and aeroperl 300 Pharma on particle size (R1, nm)
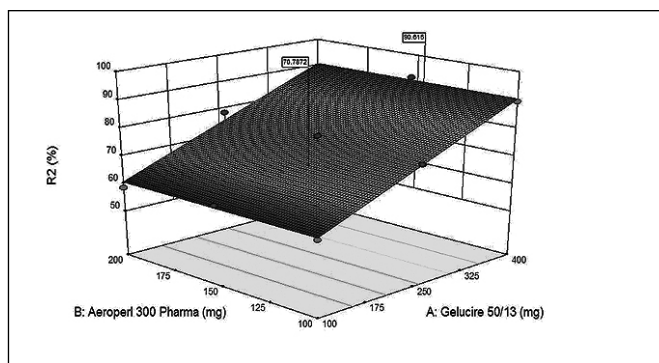

Fig. 10: Response surface plot showing effect of concentration of gelucire 50/13 and aeroperl 300 Pharma on percent entrapment efficiency (R2, %)
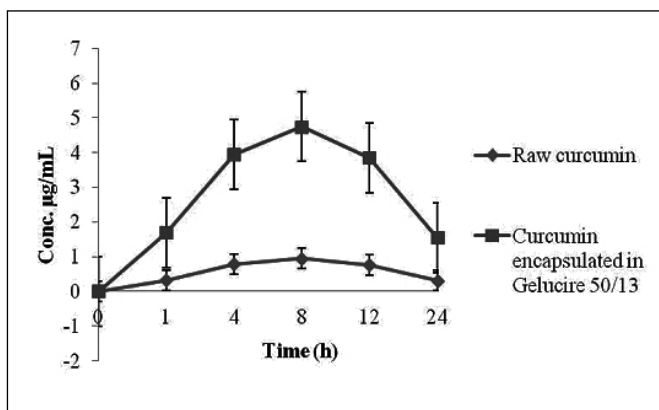

Fig. 11: Bioavailability study of optimized batch of factorial design in wistar rats

of weak hydrogen bonds. Solid microdispersion has shown many fold increase in solubility in 0.1 N HCl (51.30µg/mL) as compare to raw curcumin (0.58µg/mL). In distilled water solid dispersion has shown almost same solubility i.e. 50.42µg/mL as in 0.1 N HCl. It

has shown less solubility in phosphate buffer pH 6.8 (22.68µg/mL), (Fig 7)

In vitro release study

A faster and greater release was observed in the case of 0.1N HCl, compared with phosphate buffer of pH 6.8. Percentage curcumin release of 19.55 was observed in first 30 minutes, in acidic media and 0.56 in phosphate buffer (pH 6.8). The maximum release of 91.58% was observed at the end of 4th hour, whereas only 9.17% was observed in phosphate buffer, pH 6.8 where as in distilled water, 88.48%. The *in vitro* release profiles all the batches are shown in (Fig.8) Observed dissolution data was kinetically treated for best model fitting using PCP Disso software (v 2.08). Dissolution data was found to be best fitted to matrix system model.

Experimental design

A two-factor, three-level response surface quadratic model was used to identify the effects of the independent variables; Gelucire® 50/13 and Aeroperl® 300 Pharma (X1 and X2, respectively) on the dependent variables viz. particle size, percent entrapment efficiency (R1 and R2, respectively). ANOVA was performed using the latest version (9.0.3.1) of the Design-Expert® software package (Stat-Ease, Inc., USA). Multiple regression analysis was performed on the results. An equation in terms of coded factors can be used to make predictions about the response for given levels of each independent factor. The coded equation may be used to identify the relative impacts of the factors by comparing the coefficients of the factors. The final data relating to the equation are provided in Table III.

$$Y = \beta 0 + \beta 1 \times 1 + \beta 2 \times 2 + \beta 11 \times 1 \times 1 + \beta 22 \times 2 \times 2 + \beta 12 \times 1 \times 2 \tag{4}$$

Effect on particle size

Changes in the concentrations of the factors had a positive effect on the curcumin particle size. Formulation 3 had the maximum size (35.86 nm), with a lower concentration of Gelucire and a higher concentration of Aeroperl® 300 Pharma. Formulation 2 had the minimum particle size (13.97 nm), with a higher concentration of Gelucirre and middle concentration of Aeroperl® 300 Pharma, response surface plot. (Fig. 9)

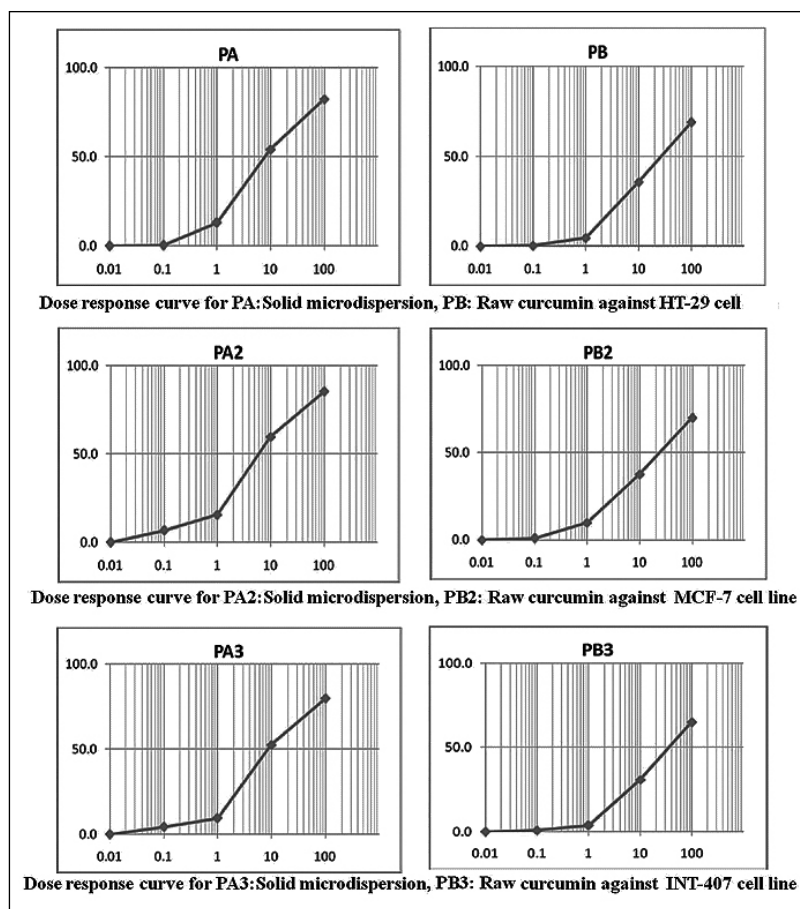The sequential model sum-of-square study suggested a quadratic model with a *p* value of 0.0026 and

Dose response curve for PA: Solid microdispersion, PB: Raw curcumin against HT-29 cell

Dose response curve for PA2: Solid microdispersion, PB2: Raw curcumin against MCF-7 cell line

Dose response curve for PA3: Solid microdispersion, PB3: Raw curcumin against INT-407 cell line

Fig. 12: MTT assay study for anticancer activity against Hep-G2, HT-29 and MCF-7 cancer cell line

a standard deviation of 0.69. The "Predicted R-Squared" value of 0.9823 is in reasonable agreement with the "Adjusted R-Squared" value of 0.9943—the difference is less than 0.2, Table IV.

Effects on percentage entrapment efficiency

Changes in the concentrations of Gelucire® 50/13 and Aeroperl® 300 Pharma had a positive effect on the percentage entrapment efficiency. Formulation 2 had the highest percentage entrapment efficiency, 91.58%, with a higher concentration of Gelucire. As designed, there was a linear increase in the percentage entrapment of curcumin. Formulation 3 had minimum entrapment efficiency (58.59%), with a lower concentration of Gelucire and a higher concentration of Aeroperl® 300 Pharma., response surface plot. Fig. 10

The Predicted R-Squared value obtained from the sequential model sum-of-square study with a p value of 0.0001 and a standard deviation of 1.97 is reasonably close to the Predicted R-Squared value of 0.9578, with the

difference being less than 0.2. The ANOVA results indicate that all the predicted levels of Gelucire and Aeroperl are close to the planned levels, Table IV.

Oral bioavailability study

The mean plasma concentration–time profiles were obtained after oral administration of raw curcumin and optimized solid microdispersion in male Wistar rats, (Fig.11) The concentration of raw curcumin in the blood was analysed using the HPLC technique and was found to be significantly low (0.32 µg/mL) compared with the corresponding value for the curcumin solid microdispersions (1.69 µg/mL) in the first hour. Higher concentration values of the curcumin had been observed due to the greater wetting and micellar solubilisation of the curcumin encapsulated in Gelucire. Conversion of the microdisperion into a nanosized form in the gastric media may also have been responsible for the quicker and greater release in the stomach. This study indicates a 5.28-fold increase in the curcumin bioavailability at the end of the first hour and a 6.53-fold bioavailability at the end of 12 hours. However, a decline in the concentration was observed after 12 hours for both raw curcumin and the solid microdispersions, the concentrations of which were 0.31µg/mL and 1.77µg/mL, respectively in 24 hour period of study.

Cell viability assay on cancer cell lines

Very low amount of solid microdispersion was required to inhibit viable human cancer cells like, liver (HT-29):8 µg/mL, human intestinal cancer cells (INT-407):10µg/mL, Table V. The prepared solid micro-dispersion had shown better IC_{50} values of 6µg/mL (IC_{50}: concentration required to inhibit 50 % of viable cancer cell count) for human breast cancer cells (MCF-7) as compared to raw curcumin which was 30µg/mL, 35µg/mL and 25µg/mL respectively, (Fig.12)

Stability study

No significant changes in the size of particle, drug content and dissolution parameters were observed in prepared solid microdispersion formulation after six month of stability study, Table VI

Table II: Particle size, zeta potential, polydisperrsity index and percent drug entrapment obtained for the experimental batches

Batch	Particle size (nm)	Zeta potential (mV)	Polydispersity index (PdI)	Percent drug entrapment (%)
SMD1	18.65±0.45	-19.56±0.65	0.109±0.63	77.45±0.95
SMD2	13.97±0.26	-23.25±0.23	0.302±0.26	91.58±0.93
SMD3	35.86±0.45	-24.15±0.89	0.184±0.12	58.59±0.53
SMD4	19.48±0.89	-22.56±0.99	0.114±0.52	78.45±0.19
SMD5	14.15±0.79	-32.12±0.56	0.248±0.35	89.56±0.83
SMD6	15.89±0.73	-30.26±0.49	0.214±0.86	88.48±0.35
SMD7	17.45±0.75	-28.25±0.62	0.264±0.47	75.63±0.54
SMD8	33.16±0.31	-23.55±0.22	0.252±0.68	61.45±0.85
SMD9	34.15±0.94	-24.58±0.35	0.202±0.64	59.89±0.45

All the batches were analysed for every parameter as mean ± S. D, n=3

Table III: Results of multiple regression analysis for measured responses

Response	Model	Coefficients					
Particle size (Y1)	Quadratic	$\beta 0$ 17.92	$\beta 1$ -9.86	$\beta 2$ 0.91	r^2 0.993	P value 0.0026	F value 77.53
% Entrapment Efficiency (Y2)	Linear	75.68	14.95	0.073	0.9773	0.0001	173.0

Table IV: ANOVA for Response Surface model

Model Summary Statistics, Particle size (nm),(Y1)						
	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Source						
Linear	3.54	0.8867	0.8490	0.7483	167.00	
2FI	3.88	0.8867	0.8188	0.4696	351.92	
Quadratic	0.69	0.9979	0.9943	0.9823	11.76	Suggested
Cubic	1.09	0.9982	0.9857	0.6746	215.87	Aliased
Model Summary Statistics , % Entrapment efficiency (Y2)						
Linear	1.97	0.9830	0.9773	0.9578	57.56	Suggested
2FI	2.16	0.9830	0.9727	0.8956	142.35	
Quadratic	1.54	0.9948	0.9861	0.9423	78.74	
Cubic	1.32	0.9987	0.9898	0.7684	315.95	

Table V: Anticancer activity of raw curcumin and its solid microdispersions

IC_{50} values					
HT-29		MCF-7		INT-407	
PA	PB	PA2	PB2	PA3	PB3
8 µg/mL	30 µg/mL	6 µg/mL	25 µg/mL	10 µg/mL	35 µg/ml

*PA: Solid microdispersion; PB: Raw curcumin

HT-29: Human liver cell line;

MCF-7: Human breast cancer cell line;

INT-407: Human intestinal cancer cell line

Table VI: Results after accelerated stability study

Batch	Particle size (nm)	Zeta potential (mV)	Polydispersity index (PdI)	Percent drug entrapment (%)
SMD1	16.85±0.82	21.86±0.59	0.119±0.89	74.88±0.98
SMD2	10.54±0.55	20.55±0.52	0.312±0.56	89.48±0.56
SMD3	31.28±0.29	21.58±0.93	0.154±0.25	54.77±0.52
SMD4	19.75±0.53	22.84±0.66	0.104±0.68	76.48±0.59
SMD5	13.48±0.69	33.48±0.55	0.248±0.69	88.88±0.25
SMD6	15.78±0.76	31.88±0.49	0.224±0.99	86.78±0.26
SMD7	16.71±0.56	25.78±0.66	0.254±0.75	73.48±0.86
SMD8	32.87±0.96	23.55±0.68	0.254±0.23	59.63±0.56
SMD9	36.44±0.93	22.57±0.95	0.209±0.66	57.82±0.35

*All the batches were analysed for every parameter as mean ± S. D, n=3

DISCUSSION

DSC study indicates that the crystalline curcumin was completely converted into amorphous form, this may be due to the complete encapsulation of the curcumin inside the gelucire matrix and the adsorption on aeroperl® 300 Pharma. This was supported by XRD analysis where the findings indicated that the amorphous curcumin was totally encapsulated and there were intermolecular interactions within the Gelucire matrix. Similar findings have been reported by other researchers, who have provided evidence that crystalline drugs were converted to an amorphous form (21, 22).

Aggregation of particles and re-crystallization of curcumin were prevented because, Aeroperl® 300 Pharma is an efficient desiccant. This helps to stabilize the amorphous curcumin inside the melted mass of gelucire making it free flowing fine powder with large surface area. The conversion of solid microdispersion into nano-size form after dilution in water and good zeta poetical values has indicated that they are very stable, with no aggregation in aqueous buffer pH 1.2.

Maximum enhancement of solubility of solid microdispersions was observed in 0.1 N HCl and distilled water as compare to phosphate buffer, pH 6.8 (in order of pH 1.2 > distilled water > 4 > 6.8), (Fig. 6) This behaviour may be due to rapid degradation of curcumin to ferulic acid and feruloylmethane at higher basic pH values.

The effect of change in concentration of gelucire has positive impact on the particle size and entrapment efficiency. Increased in concentration of gelucire had shown decreased in particle size whereas increased in entrapment efficiency of curcumin. The effect of wetting and micellar solubilization and/or deflocculation of the hydrophilic carrier, Gelucire® 50/13 resulted in an increase in the dissolution of the solid microdispersions with higher gelucire amounts as compared with raw curcumin (23).

Two different media were used in the *in vitro* dissolution study to observe the effect of the pH value on the curcumin release as this can provide an idea of the drug release behaviour in physiological systems and in intracellular regions, which are acidic. In all the formulation batches, an initial burst release was observed, which lasted 30 minutes. This may be due to the fact that Gelucire has a greater affinity for water molecules, producing rapid wetting and quick solubilization in water. After analyzing the dissolution data using PCP Disso, it was found that the releases of all the formulations followed first-order kinetics and the Korsemeyer-Peppas model (18).

Higher bioavailability values were observed for solid microdispersion as compare to raw curcumin after same time period are the indications of lowering of required dose in the patients with minimum or no toxicity (C_{max}:raw curcumin is 4.75µg/mL; C_{max}:solid microdispersion is 0.95 µg/mL at t_{max} of 8 hour).

In MTT assay study, lower IC_{50} values were obtained for solid microdispersion which has proved better anticancer activity against MCF-7 human breast cancer cell line than the raw curcumin. This could be because of immediate and high wetting property of gelucire (HLB 13) and conversion of curcumin into nano size form.

CONCLUSION

From the present study it may be concluded that solid microdispersion is a better option for improving the solubility and bioavailability of curcumin using novel carrier, gelucire 50/13. Further, it can also be concluded that oral curcumin microdispersion formulation is better alternative to the widely used anticnacer agents given by parenteral route with enhanced solubility and stability. Finally, it can be suggested that prepared oral formulation may have a very good contribution to improve the quality of life of patients suffering from cancer.

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