Ultrasound Assisted Curcumin Nanoformulation Imparting Enhanced Functionality

Bharti Sharma, Jaismeen Kaur, and Minni Singh

***Abstract*—**Curcumin, a bioactive with astounding nutraceutic efficacy and therapeutic properties is poorly aqueous soluble, which hampers its bioaccessibility and hence functionality. In this investigation, ultrasound assisted oil-in-water nanoemulsions were prepared with olive oil as a lipid carrier and response surface methodology (RSM) was used to optimize formulation variables using polynomial regression model. RSM revealed that curcumin concentration and viscosity had a profound impact on particle size and distribution. A nanoformulation was developed with particle size 194.8nm, PdI 0.203±0.02, and a zeta potential of -52.1±7.0 mV with an encapsulation efficiency of 95.7±1.9%. The aqueous dispersibility of the nanoformulation was observed to be an enhanced 84.7±2.3% in comparison to that of 0.3±0.01% for free curcumin. SEM and TEM micrographs revealed spherical morphology and uniform distribution of nanodroplets. These observations suggest that use of curcumin in a nanoformulation could lead to curcumin based therapeutic foods with improved functionality.

***Keywords*—** Aqueous dispersibility, Curcumin, Nanoemulsion, Ultrasound-assisted

I. Introduction

Curcumin, a polyphenolic compound with exceptional therapeutic and functional properties is derived from the Indian dietary spice turmeric (*Curcuma longa*). It is the main bioactive component that lends it a wide range of therapeutic properties and thus makes it a favourable compound to develop value added food products [1], [2]. Extensive research has revealed that majority of the orally administrated curcumin is excreted in the faeces and urine, either no or little amount is detected in blood plasma. However, pitiable aqueous solubility of curcumin attributes to its low bioavailability of less than 1%. The hydrophobic nature of curcumin is due to the presence of phenyl rings, methoxy groups and two unsaturated carbonyl groups. Its low membrane permeability, instability in gastrointestinal tract, rapid metabolism in liver and intestine and formation of inactive conjugates such as glucuronoids and sulphates limits its use as a nutraceutic [3], [4]. Various attempts have been made to increase aqueous solubility, stability and bioavailability of curcumin by either forming complexes of curcumin with polymers like gelatin and polysaccharides [5] and encapsulation in curcumin phospholipid complexes [6], [7].

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As organic solvents have been employed in these processes this renders them unsuitable for food applications, therefore it is necessary to develop alternative approaches which are safer and lead to enhancement of aqueous solubility, stability in GI tract and bioavailability of curcumin. Nanotechnology has emerged as a promising tool that holds potential to enhance the bioavailaibility of these lipophilic compounds. Nanoencapsulation enhances solubility, stability and bioactivity of various oil-soluble phytochemicals due to their small droplet size, increased surface area and high kinetic stability. In the food industries emulsion based delivery systems have emerged as an attractive alternative approach for delivery of bioactive compounds [8].

Nanoemulsions can be prepared by using various methods which are classified as either low energy or high energy. Among the high energy methods, ultrasound assisted emulsification is considered an efficient method to obtain emulsions with a reduced droplet size. However, the particle size and stability in case of nanoemulsions prepared by ultrasonication depends on various formulation and process parameters such as sonication time, amplitude and pulse [9]. In the present study ultrasound assisted oil-in-water (O/W) nanoemulsions were prepared to encapsulate curcumin and Response Surface Methodology (RSM) was used to optimize the formulation variables. Central Composite Design was used and limited numbers of experimental runs were generated The influence of ultrasonic parameters was investigated to develop the nanoformulation. The focus of current study was to enhance the aqueous solubility and stability of curcumin which will impart enhanced functionality.

II. Materials and Methods

A. Screening of oil type in the organic phase

The bulk physicochemical characteristics of the organic phase influence the formation, stability and properties of nanoemulsions [8]. This study was undertaken to examine how these properties influence the droplet size, phase behaviour and storage stability of nanoemulsions. For this, butter, clove oil, fish oil and olive oil were chosen for preparation of nanoemulsions based on their viscosity, edibility and chain length characteristics.

The solubility of curcumin in these oils was determined by adding 30mg curcumin to 5g oil. The mixture was vortexed followed by continuous shaking at 150 rpm at 37^{o}C for 48 h. The equilibrated samples were centrifuged at 1500g for 10 min to eliminate the undissolved curcumin and the supernatant was quantified for curcumin after re-extraction from oil. For this,

the supernatant (1.8 ml) was mixed with 0.1M sodium phosphate buffer (pH 6.8) containing 0.1% EDTA, 1.8 ml distilled water and 5.4 ml methanol. The mixture was vortexed for 3 min followed by addition of hexane to remove oil. Thereafter the mixture was shaken vigorously and centrifuged at 1000g for 10 min and hexane layer was discarded. Finally, ethyl acetate was added to the residual aqueous layer to partition curcumin which was quantified spectrophotometrically by observing OD at 421nm.

$$\%\text{solubility of curcumin} = \frac{\text{weight of curcumin retrieved (mg)}}{\text{initial weight of curcumin (mg)}} \times 100 \quad (1)$$

B. Preparation of coarse emulsion

For the preparation of curcumin loaded O/W nanoemulsions, olive oil and water:glycerol were used as dispersed phase and continuous phase, respectively. The non-ionic surfactant Tween-20 was used as an emulsifier and added into continuous phase at a concentration of 2.5% (v/v). Coarse emulsion was prepared by adding dispersed phase to the aqueous phase dropwise with continuous stirring for 15 min at ambient temperature. The prepared coarse emulsions were subjected to ultrasonication at 20 kHz on a 130 W sonicator – (Sonics® vibra cell VCX 130) equipped with 6 mm probe to further reduce the droplet size.

C. Optimization using Response Surface Methodology

Response surface methodology (RSM) was used to investigate the influence of five independent variables on the curcumin nanoemulsions. A central composite rotatable design (CCRD) of five levels was used to find out the best combination of these variables for responses like smaller particle size and narrower PdI. The Design Expert (Version 10 Stat-Ease Inc., Minneapolis, MN, USA) was used for experimental design, data analysis and model building. The five variables were water: glycerol ratio (A), curcumin concentration (B), sonication conditions- amplitude (C), pulse (D) and time (E) and their factorial combinations were generated as shown in Table I to examine their effects on the observed responses. The half factorial matrix of CCD design generated 26 runs. A second-order polynomial equation was used to express the response (Y) of the nanoemulsions as a function of the independent variables as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 \quad (2)$$

Where Y is the response variable, β_0 is constant, β_i, β_{ij}, and β_{ii}, represent the coefficients of the linear, interaction and quadratic parameters. Analysis of variance (ANOVA) was employed to determine the significant difference between the independent variables. The significant ($p<0.05$) independent variables were incorporated in the model and the non-significant ($p>0.05$) were excluded. Then, the three-dimensional response surface plots were constructed to see the interactive effect on the response variables- particle size (R_1) and polydispersity index (R_2). The sonication time (min.), pulse (sec.) and amplitude (%) were varied based on the runs specified in the central composite design matrix.

D. Characterization of curcumin nanoemulsion

Curcumin nanoemulsion prepared using optimized parameters were characterized by particle size, ζ-potential, aqueous solubility, scanning and transmission electron microscopy, entrapment efficiency.

TABLE I: MATRIX OF INDEPENDENT VARIABLES, THEIR ACTUAL AND CODED LEVELS FOR A ROTATABLE CENTRAL COMPOSITE DESIGN

Independent variables	**Levels**				
	-1.49 (-α)	-1	0	1	1.49 (α)
Water:Glycerol	0.25	0.5	1	1.5	1.74
Curcumin concentration	0.00186	0.2	0.6	1	1.19
Amplitude	23.7	25	27.5	30	31.23
Pulse	0.128	0.5	1.25	2	2.37
Time	0.046	5	15	25	29.9

III. RESULTS AND DISCUSSION

A. Screening of oil type

Four different oils such as olive oil, fish oil, clove oil and butter, were compared for the preparation of curcumin nanoemulsion in terms of their solubilising capacity. The solubility of curcumin was observed maximum in clove oil, followed by butter, fish oil and olive oil. Similar results were also reported by other research group where it was observed that maximum solubility of curcumin was in short chain triglycerides (clove oil), followed by the mixture of medium chain and long chain triglycerides (butter) and long chain triglycerides (fish oil, olive oil) [10]. Besides solubility, other factors such as mean particle size and polydispersity index were also considered for the comparison of above mentioned oils for the subsequent preparation of curcumin nanoemulsion as given in Table II.

TABLE II: PARTICLE SIZE AND POLYDISPERSITY INDEX OF CURCUMIN NANOEMULSION WITH DIFFERENT OIL TYPES

Oil type	**Mean Particle size (nm)**	**PdI**
Butter	743.9	0.695
Clove oil	676.5	0.756
Fish oil	563.2	0.878
Olive oil	594.7	0.415

As already reported that nanoemulsions prepared with long chain triglycerides (LCT) and medium chain triglycerides (MCT) have small mean particle diameter with narrow particle size distribution, where as nanoemulsions prepared with short chain triglycerides have large paricle diameter and broad particle size distribution respectively. Both olive oil and fish oil are long chain triglycerides but based on the observations of size and poly-dispersity Index, olive oil was chosen for subsequent studies, particularly so for the favourable size distribution that was exemplified, whereas butter is a mixture of

long chain and medium chain triglycerides and clove oil is a short chain triglyceride. In case of butter and clove oil the average droplet size is quite large with broad size distribution. Furthurmore, nanoemulsion prepared with clove oil showed clear phase separation and this accounts for the lack of physical stability of the emulsion prepared by this lipid. This can be explained on the basis of Stoke-Einstein equation which describes viscosity as an inversely proportional factor that controls the hydrodynamic diameter of the nanodroplets [8].

B. RSM optimization for nanoformulation

Response Surface Methodology was employed to determine the variation in particle size and PdI of the curcumin nanoemulsion as the function of formulation variables. CCD model was employed and 26 runs were generated with different independent variables and their corresponding responses of droplet size and particle distribution. From the experimental data the regression coefficient value for three responses were obtained. The positive values indicate an effect that favours the optimization conditions having a synergistic effect and the negative value show the antagonistic effect between the factors and the response. Then the three dimensional plots were generated to interpret the interaction effect on the variables.

By applying ANOVA, the model was found to be significant as p value is <0.0001 for particle size and 0.0324 for PdI response. R^2 value for the model with respect to each response variables is given in Table III. Also, predicted R^2 is in reasonable agreement with the adjusted R^2.

TABLE III: R^2 VALUE OF THREE RESPONSE VARIABLES

Regression coefficient	R_1 (Particle size in nm)	R_2 PdI
R^2	0.9997	0.9574
Adjusted R^2	0.9985	0.7868

The equations derived from the experimental design for two responses: particle size (R_1) and PdI(R_2):

$$Y_{\text{particle size}} = 252.00-269.74A-5.88B-5.82C+39.29D-75.78E-123.18AB-107.6AC-156.302AD-62.91AE-368.10BC-188.43BD-67.16BE+28.95CD+131.30CE+320.69DE+177.60A^2+6.92B^2+5.26C^2+8.31D^2+ 54.20E^2 \quad (3)$$

$$Y_{PDI} = 0.38-0.002675A-0.016B-0.024C+0.042D+0.070E+0.024AB+0.062AC-0.042AD-0.049AE+0.032BC+0.023BD+0.005795BE-0.015CD-0.017CE-0.053DE+0.068A^2+0.024B^2+0.005530C^2-0.045D^2+0.006201E^2 \quad (4)$$

Particle size

It is evident from Fig.1A, 1B, 1C and 1D that water:glycerol ratio which is a measure of viscosity of the aqueous phase has a substantial effect on the particle size of the nanodroplet, thereby becoming an important parameter which needs to be optimized for the preparation of nanoemulsion.

The figure IE clearly indicates that there is a decrease in particle size with an increase in sonication time thereby suggesting sonication time to be a significant parameter for obtaining nanodroplets of desired size. However the experimental design suggests that though increase in sonication time will further decrease the size of the nanodroplet but prolonged sonication will accelerate aggregation and also deteriorate encapsulated bioactive compound [10].

Fig. 1D and 1F suggest that there is an increase in particle size with an increase in curcumin concentration which is suggestive of the fact that the amount of highly lipophilic material that can be dissolved in an oil phase has considerable effect on particle size of the nanoemulsion.

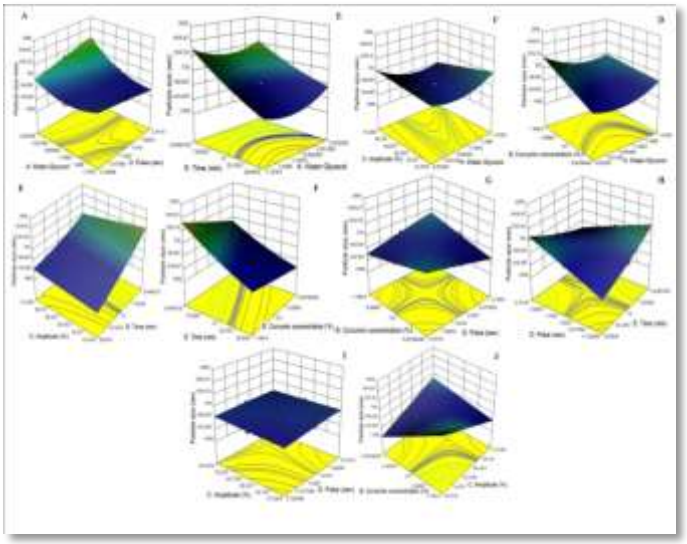


Fig.1. Three dimensional plots of ccd-rsm showing the effect of formulation variables on the particle size of curcumin nanoemulsion

PdI

The polydispersity index gives a measure of the overall particle size distribution in the system. The response variable R_2 (PdI) was significantly influenced by water:glycerol ratio (viscosity) as is evident from fig. 2A, 2B, 2C and 2D. Therefore it can be ascertained that the viscosity of the continuous phase has a substantial effect on droplet disruption mechanism during ultrasonic assisted emulsification.Other factors showed negligible effect on PdI thus making viscosity of the nanoemulsion as the predominant factor to affect particle size distribution. Also from the ANOVA analysis of the model, the equation that was generated suggests that the estimated responses are well described by the model as proved by the F-value of 5.61 (p<0.0324) as shown in Table IV.

Optimization of formulation conditions and verification of the model

By using the Design-Expert software five levels of five independent variables were optimized to achieve the best possible reponse for the formulation of nanoemulsion. Experimental and predicted values of optimized curcumin loaded nanoemulsions were compared to check the accuracy of the model and the optimized predicted and experimental conditions for the formulation of nanoemulsions are shown in Table V. The droplet size of curcumin loaded nanoemulsion after optimization of all the process parameters was 194.8nm with a PdI of 0.203±0.02 as shown in Fig.3.

TABLE IV: ANALYSIS OF VARIANCE (ANOVA) AND STATISTICAL PARAMETERS FOR QUADRATIC RESPONSE SURFACE FOR PARTICLE SIZE AND PdI

	Source	Particle size		PdI	
		F-value	p-value	F-value	p-value
	Model	836.48	< 0.0001	5.61	0.0324
Linear terms	A-Water:Glycerol	1285.12	< 0.0001	0.019	0.8966
	B-Curcumin concentration	0.61	0.4695	0.67	0.4496
	C-Amplitude	0.60	0.4743	1.55	0.2677
	D-Pulse	27.26	0.0034	4.71	0.0822
	E-Time	101.43	0.0002	12.86	0.0158
Interaction terms	AB	143.25	< 0.0001	0.80	0.4123
	AC	109.35	0.0001	5.33	0.0691
	AD	230.65	< 0.0001	2.44	0.1790
	AE	37.36	0.0017	3.36	0.1263
	BC	1279.21	< 0.0001	1.47	0.2801
	BD	335.21	< 0.0001	0.72	0.4334
	BE	42.59	0.0013	0.047	0.8372
	CD	7.91	0.0374	0.33	0.5915
	CE	162.75	< 0.0001	0.40	0.5569
	DE	970.92	< 0.0001	3.95	0.1036
Quadratic terms	A^2	1370.57	< 0.0001	30.00	0.0028
	B^2	2.08	0.2088	3.73	0.1115
	C^2	1.20	0.3224	0.20	0.6762
	D^2	3.00	0.1438	12.74	0.0160
	E^2	127.64	< 0.0001	0.25	0.6404

TABLE V: OPTIMUM PREDICTED AND EXPERIMENTAL CONDITIONS FOR THE FORMULATION OF NANOEMULSIONS

Optimal conditions		Optimal responses		
Variable factors	**Predicted**	**Responses**	**Predicted**	**Experimental**
Water:Glycerol	1	Particle size	205.3 nm	194.8nm
Curcumin concentration	0.60%	PdI	0.296	0.203±0.02
Amplitude	27.43%			
Pulse	0.50 sec.			
Time	15 min.			

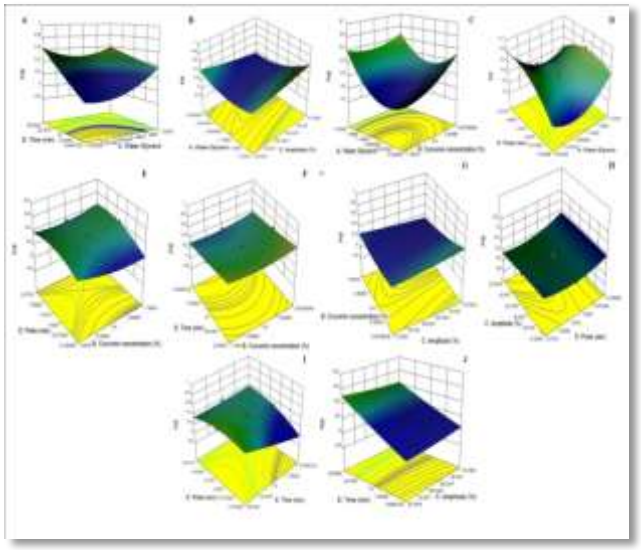


Fig.2. Three dimensional plots of CCD-RSM showing the effect of formulation variables on the PdI of curcumin nanoemulsions

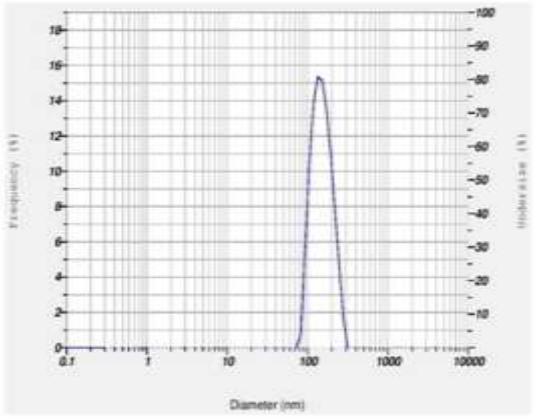


Fig.3. Droplet size of curcumin nanoemulsion

C. *Encapsulation efficiency*

The encapsulation efficiency of curcumin in the nanoformulation was determined by evaluating the concentration of curcumin encapsulated within the nanodroplets which was done by breaking the nanoemulsion and repartitioning curcumin in the organic phase. The encapsulating efficiency, as determined spectophotometrically, was found to be 95.7±1.9%.

D. *Aqueous solubility*

Free curcumin is known to have poor aqueous solubility (<1.0%) that limits its nutraceutic properties [12]. Curcumin nanoemulsion was observed to have enhanced aqueous solubility of 84.7±2.3% in comparison to free curcumin which exhibited a solubility as low as 0.3±0.01%. Fig. 4. justifies the claim.

(a) (b)

Fig.4. Aqueous solubility of free curcumin (a), curcumin nanoemulsion in water (b)

E. *Stability studies of curcumin nanoemulsion*

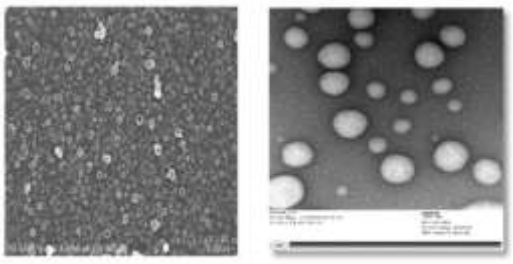
The curcumin nanoemulsion was found to be stable for more than three months based on the zeta potential values as shown in Table VI. The magnitude of zeta potential gives an indication of the stability of the colloidal system. If the values of zeta potential are large, the particles will tend to repel each other and will not coalesce.

TABLE VI: ZETA POTENTIAL VALUES OVER A PERIOD OF THREE MONTHS

Days	Zeta potential (mV)
30	-52.1
60	-46.9
90	-40.1

F. *Microscopic observations of curcumin nanoemulsions*

The morphology of curcumin nanoemulsion was observed using FE-SEM. The particle size of nanodroplet was found to be in nano range and nanodroplets showed spherical morphology as shown in Fig. 5a. The nanoemulsion droplets are randomly dispersed and distributed without any gathering throughout the field as observed under TEM shown in Fig. 5b. The nanoemulsion droplets are spherical in shape and show clear loading of curcumin in the oil phase.



(a) (b)

Fig.5. Microscopic observation by FE SEM (a) and TEM (b)

https://doi.org/10.17758/EAP.EAP0717210

IV. Conclusion

To increase the aqueous solubility and enhance the functionality of curcumin, a nanoformulation was prepared. The process parameters for the preparation of curcumin nanoemulsion were successfully optimized by RSM using the central composite design. Besides, optimization by RSM also revealed that viscosity of the continuous phase has a great influence on the droplet disruption mechanism and hence the particle size of the nanodroplet. A stable curcumin nanoemulsion was formulated with droplet size of 194.8nm using olive oil as a lipid carrier and non-ionic surfactant Tween20 by ultrasound assisted emulsification. From the stability study and morphological analysis it is ascertained that the formulation is a suitable candidate for value addition of food thereby increasing its functionality.

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