APPLICATION OF 2^3 FULL-FACTORIAL DESIGN FOR DEVELOPMENT AND OPTIMIZATION OF BIOCOMPATIBLE, BIODEGRADABLE SOLID LIPID NANOPARTICLES CONTAINING CURCUMIN

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ABSTRACT

Curcumin (C), a natural anticancer agent suffers extremely low aqueous solubility and rapid systemic elimination, therefore, the aim from the present study was to develop and optimize biocompatible, biodegradable solid lipid nanoparticles containing curcumin (CSLNs) using Solvent Injection Method. The components selected to develop the SLNs were Glycerol MonoStearate (GMS; the lipidic carrier), Poloxamer 407 (P407; Surfactant) and Ethanol (solvent for the drug and the lipidic carrier). The combination and ratios for the optimization process were carried out using 2^3 full-factorial designs. The experimental design runs (8 formulations H1–H8) were prepared and the design dependent responses (assessment of particle size, Entrapment Efficiency % and the in vitro release study) were characterized. The developed formulae showed a nanometric particle size range (203.0–345.0nm), high Entrapment Efficiency % (62.77–87.42 %) and prolonged release over 24 hr periods (55.97–89.64 %). These results were analyzed using JMP® 10 software and its analytical tools were used to draw Pareto charts and the interactions plots. On the basis of the software analysis, formulation H9 with a desirability factor of 0.582 was selected as the optimized formulation and was evaluated for the dependent responses. Formulation H9 showed a particle size of 249.1 nm, 74.51 % Entrapment efficiency, 85.72 % in vitro release over 24 hr and these results suggested that the solid lipid nanoparticles formulations could be a promising method to sustain the release of curcumin.

INTRODUCTION

Traditional medicine is known to be fertile ground for the source of modern medicines **(Corson and Crews, 2007; Aggarwal and Sung, 2008; Wu *et al.*, 2011)** as Nature's chemical diversity and complexity form a unique source of molecules for a wide range of therapeutic targets **(Balunas and Kinghorn, 2005; Coimbra *et al.*, 2011)**.

Several natural compounds at present are being appreciated because of their broad spectrum of activities making them more appropriate to interfere in multifactorial diseases, such as cancer **(Nobili *et al.*, 2009; Pan *et al.*, 2009; Harvey *et al.*, 2010)**.

In addition, compared to synthetic compounds, natural bioactive compounds generally have better safety profiles, are well accepted by the public (opinion) and are usually relatively cheap **(Pan *et al.*, 2009; Gupta *et al.*, 2010)**. However, their physicochemical properties are generally not drug-like and pose a number of challenges that need to be overcome for their establishment as effective therapeutics **(Shoji and Nakashima, 2004; Nobili *et al.*, 2009; Gupta *et al.*, 2010; Coimbra *et al.*, 2011)**.

A great example of these natural compounds is curcumin which has considerable potentials against various human diseases like cancer **(Anand *et al.*, 2007; Tsai *et al.*, 2011; Yallapu *et al.*, 2012)**. Though curcumin has demonstrated safety and efficacy profiles (as a chemotherapeutic agent) but it has restrictive pharmaceutical role because of its extremely low aqueous solubility and rapid systemic elimination which severely curtails its bioavailability profile **(Anand *et al.*, 2008; Anand *et al.*, 2010; Mohanty and Sahoo, 2010)**.

Therefore, design and development of biodegradable controlled drug delivery system for curcumin was the main research aspect on which extensive work has been done in the past few decades **(Mulik *et al.*, 2009)**.

Solid Lipid Nanoparticles (SLNs) were developed as the first generation of Lipid based Nanoparticless and are being extensively studied as promising approaches for poorly soluble drugs **(Almeida and Souto, 2007; Martins *et al.*, 2007; Nayak *et al.*, 2010)**.

SLNs as drug delivery systems have a well known advantages such as good tolerability, lower cytotoxicity, higher bioavailability by oral administration, increase in the drug stability as well as other advantages provided by lipid excipients, such as biodegradability and cost effectiveness that promote their use as novel drug carriers **(Mehnert and Mader, 2001; Mukherjee *et al.*, 2007)**. Also since they are consisting of physiological and biodegradable lipids, lipid nanoparticles are suitable for the incorporation of lipophilic, hydrophilic, and poorly water-soluble drugs within the lipid matrix in considerable amounts **(Bunjes *et al.*, 2001).**

Lipidic carriers used to prepare SLNs can be highly purified lipids such as tristearin or tripalmitin, hard fats such as stearic acid or behenic acid, waxes such as cetyl palmitate, and acylglycerol mixtures such as compritol or GMS **(Souto and Müller, 2006).**

The objectives behind the selection of GMS (a non-polar lipid, $C_{21}H_{42}O_4$) as the lipid phase to formulate SLNs formulations were its high drug entrapment efficiency as the presence of high amounts of Mono-, Di-glycerides in GMS help the drug to solubilize in the lipid fraction as well as the less defined mixture of acylglycerol provides additional space for drug molecules to get entrapped **(Manjunath *et al.*, 2005; Shah and Pathak, 2010).**

In addition to that GMS depicts some surface tension activity due to the fact that it is composed of ~ 50% of Monoglycerides which was held responsible for the small particle size of GMS-based lipid nanoparticles in comparsion to other lipidic carriers such as Trimyristin and Tristerin which are triglycerides of higher molecular weight and lacking the surface tension activity **(Jenning *et al.*, 2000; Nayak *et al.*, 2010).**

Poloxamers or Pluronic (marketed by BASF Corporation) are the series of commercially available difunctional triblock copolymers of non-ionic nature. They comprise of a central block of relatively hydrophobic polypropylene oxide surrounded on both sides by the blocks of relatively hydrophilic poly ethylene oxide and they are regarded as PEO-PPO-PEO copolymers **(Kabanov *et al.*, 2002).** Among number of natural and synthetic polymers, Poloxamer 407 (P407), a US Food and Drug Administration-approved polymer, is the most attractive due to its biocompatibility, biodegradability as well as its low toxicity profile **(Hussain and Saxena, 2012).** Also P407 had been used to produce micellar formulations of different drugs to increase their solubility, their metabolic stability and their circulation time **(Oh *et al.*, 2004; Zhang *et al.*, 2011; Hussain and Sexana, 2012).**

Furthermore, P407 had been used either alone or with other polymers like Carbopol 934 and HPMC to provide controlled release delivery systems of different **drugs (Kabanov *et al.*, 2002).**

The objective of the present study was to develop SLNs of curcumin and to optimize it for the independent variables selected (amount of glycerol monostearate, concentration of

poloxamer, and volume of ethyl alcohol) in order to achieve desired particle size with maximum entrapment efficiency (EE %) and percent cumulative drug release (CDR %). Factorial design enables all the factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. Many researchers have optimized nanoparticulate formulations using 2^3 full-factorial design **(Bozkir and Saka, 2005; Bhavsar *et al.*, 2006; Derakhshandeh *et al.*, 2007; Shah and Pathak, 2010).**

MATERIALS AND METHODS

Materials

Curcumin (C; 98% pure powder and molecular weight = 368.38 D) was purchased from Acros Organics, NJ, USA; Poloxamer 407 (P407) powder from Spectrum Chemicals MFG. CORP., CA, USA; Purified Glycerol MonoStearate (GMS) from VWR, West Chester, PA, USA; Phosphate Bufferd Saline (PBS) pH 7.4 powder packets from Sigma-Aldrich Co, St. Louis, MO, USA. Absolute Ethyl alcohol (Ethanol) from Avantor Performance Materials INC., NJ, USA. Methanol HPLC Grade from EMD chemicals In., NJ, USA. All other chemicals were standard pharmaceutical grade and used without further purification.

Methods

Experimental Design of SLNs

In this study, a 2^3 full-factorial experimental design was used to optimize SLNs. In order to optimize, the amount of GMS (X1), concentration % of P407 (X2) and volume of Ethanol (X3) were selected as the independent variables while the particle size in nm (Y1), Entrapment Efficiency percent (EE %, Y2) and Cumulative Drug Released percent (CDR %, Y3) were selected as the responses parameters. Eight formulations of SLNs (H1–H8) were prepared according to the experimental design where the actual values of the independent variables as well as the observed values for the responses parameters are illustrated in Table I.

Table (1): 2^3 full factorial design of C-SLNs formulations:

Formula Code	Independent Parameters			Dependent Parameters		
	GMS amount (mg)	P407 concentration (%)	Ethanol Volume (ml)	Particle Size (nm ± SD)	EE% ± SD	CDR % over 24 hr
H1	-1	-1	-1	294.9 ± 2.8	79.22 ± 0.89	87.64
H2	-1	-1	+1	262.9 ± 0.7	71.21 ± 0.74	88.3
H3	-1	+1	-1	214.1 ± 1.6	66.78 ± 0.89	89.64
H4	-1	+1	+1	203.0 ± 4	62.77 ± 0.83	55.97
H5	+1	-1	-1	345.0 ± 4.7	87.42 ± 0.96	56.5
H6	+1	-1	+1	320.2 ± 3.4	82.53 ± 0.88	74.55
H7	+1	+1	-1	268.2 ± 3.3	76.58 ± 0.81	77.5
H8	+1	+1	+1	249.3 ± 0.7	68.1 ± 0.93	69.34

Actual and coded values of the independent parameters:

Independent Parameter	Low Level	Actual Value	High Level	Actual Value
GMS amount (mg) X1	-1	100	+1	200
P407 concentration % X2	-1	1	+1	2
Ethanol Volume (ml) X3	-1	1	+1	2

Preparation of Solid Lipid Nanoparticles containing Curcumin (CSLNs)

Solid lipid nanoparticles containing curcumin (CSLNs) were prepared according to solvent injection technique reported early by **(Schubert and Muller-Goymann, 2003; Shah and Pathak, 2010)** with slight modification where curcumin (2% of the lipid phase) and specified amount of GMS was dissolved in the specified volume of ethanol with gentle heating. The resulting solution was rapidly injected into the 10 ml of aqueous phase containing specified amount of P407 that was continuously stirred at 400 rpm for 30 min on a magnetic stirrer; 0.1NHCl (4ml) was added to the dispersion and the dispersion then was centrifuged to 10,000 rpm for 30 min at 10°C in Allegra® 64R Benchtop centrifuge (Beckman Coulter Inc, Palo Alto, CA, USA), and aggregates were resuspended using 10 ml distilled water containing 4% poloxamer 407 (by weight) as stabilizer with stirring at 1,000 rpm for 10 min and lyophilized in FreeZone® 2.5 tabletop Freeze dryer (Labconco Corporation, Kansas city, Missouri, USA).

HPLC analysis method

Curcumin analysis was preformed according to **Cui *et al.* (2009)** with gentle modification where all the samples were analyzed by the *WATERS*® HPLC system using Perkin Elmer HPLC column (SPHERI–5 RP – 18, Perkin Elmer LLC, Norwalk city, CT, USA) with the following specifications 5µm, 4.6mm×250mm Preceded by a guard column (SecurityGurad™, Phenomenex Inc., Torrance, CA, USA) filled with C18 cartridges (4 x 2.00 mm ID) at room temperature. The mobile phase was Methanol: H_2O (containing 3.6% glacial acetic acid) (73:27, v/v), freshly prepared on the day of use, filtered through a 0.45 µm filter and was degassed by sonication for 15 min **(Dandekar and Patravale, 2009).** The flow rate 1ml/min, the retention time for curcumin was 6.3 min and curcumin was detected at 428 nm with a sample run time of 10 min.

Physical Characterization of C-SLNs formulations
Determination of particle size

The particle size of C-SLNs formulations was determined by Photon correlation spectroscopy (PCS) employing the ZetaPALs® particle size analyzer (Brookhaven Instruments Incorporation, New York, USA) that was fitted with a 4mW He–Ne diode laser operating at 633 nm. An aliquot of lyophilized SLNs formulations was resuspended in deionized water (DIW) prior to measurements to yield a suitable scattering intensity. Analysis was performed at a fixed angle of 90° to the incident light and data were collected over a period of 9 min. The particle size analysis data were evaluated using the hydrodynamic diameter **(Nayak *et al.*, 2010)**.

Determination of Encapsulation Efficiency Percent (EE %)

The Encapsulation Efficiency Percent (EE %) of the developed C-SLNs formulations was determined as described earlier by **(Tiyaboonchai *et al.*, 2007; Nayak *et al.*, 2010)** with a suitable modification. Ten milligrams of lyophilized C-SLNs formulae were accurately weighed and dispersed in 10 ml of methanol. The samples were then

centrifuged at 13,000 rpm/30 min. The amount of curcumin in the supernatant was determined using HPLC analysis method and the EE % was determined as follows:

$EE\ \% = [(T_{CU} - S_{CU})/T_{CU}] \times 100$

Where T_{CU} stands for the total amount of curcumin added to the formulation and S_{CU} stands for the amount of drug measured in the supernatant.

In-Vitro Release Profile of curcumin

The cumulative curcumin percent released (CDR %) was determined as described earlier by **(Nayak *et al.*, 2010; Bisht *et al.*, 2007)** with a suitable modification where known amount of lyophilized C-SLNs formulations (100 mg) were dispersed in PBS pH 7.4 and the dispersion was divided into 8 aliquots (1 ml each) in microfuge tubes which were kept in a thermo stable water bath at 37°C ± 0.5°C. At predetermined time intervals, the dispersion was centrifuged at 200×*g* for 5min and the resulted pellets were then redissolved in 1ml of methanol. After that, the drug content was determined using HPLC analysis method and the concentration of the released curcumin was calculated using a standard curve of curcumin in methanol.

RESULTS AND DISCUSSION

Preparation of Curcumin Solid Lipid Nanoparticles (C-SLNs) formulations

C-SLNs formulations were successfully prepared by Solvent Injection Method which gave a yellowish sponge product after the freeze-drying process. The lyophilized formulations were easily redispersed in water and the resultant suspensions were subjected to physical characterization tests.

Physical Characterization of C-SLNs formulations

Evaluation of the physical characteristics of the developed C-SLNs formulations is a crucial step in determining the efficiency of the delivery system as it influences the physical stability, cellular uptake, biodistribution and release of the encapsulated drug **(Acharya *et al.*, 2009; Mohanty and Sahoo, 2010)**.

Determination of particle size

Particle size is a critical determinant for the fate of administered nanoparticles as it governs the distribution of nanoparticles in the body and the small size of particles is considered advantageous for passive targeting to tumor tissue by enhanced permeability and retention effect **(Panyam *et al.*, 2002; Sahu *et al.*, 2008; Das *et al.*, 2009; Mohanty and Sahoo, 2010; Akhtar *et al.*, 2012).** It is clear that the particle size measurements obtained for all the eight formulations were in the submicron range (203.00 – 345.00 nm) where formulation H4 showed the smallest value (203.0 nm) and H5 showed the highest value (345.00 nm). The best explanation for the small particle size range obtained using GMS as the lipid phase comes from the surface tension activity provided by the high percent of Monoglycerides (main composition in GMS) which facilitate emulsification process and improve the surfactant film around the nanoparticles leading to prevention of particle aggregation and crystal growth **(Westesen *et al.*, 1993; Ahlin *et al.*, 1998; Jenning *et al.*, 2000; Nayak *et al.*, 2010)**.

Determination of Encapsulation Efficiency (EE)

Determination of the EE % for the developed SLNs formulations is an important factor for determining the therapeutic efficacy of drug delivery system **(Mohanty and Sahoo, 2010).** The encapsulation efficiency (EE %) of curcumin was determined by

measuring the concentration of free drug in the dispersion medium (methanol) after centrifugation of SLN suspension using HPLC analysis method. From table (1) the EE % values showed a range between 62.77 % and 87.42 % with the highest EE % obtained was in case of H5 and the lowest EE% in case of H4 indicating relatively good entrapment efficiency of curcumin in the developed SLNs formulations, probably because of its lipophilic character. It is known that three drug incorporation models can be deduced to describe how drug molecules are incorporated in the SLNs formulations:

(1) Solid Solution Model.

(2) Core–Shell Model, Drug Enriched Shell.

(3) Core–Shell Model, Drug-Enriched Core **(Müller *et al.*, 2000; Lv *et al.*, 2009).**

The drug incorporation model relates to specific nature of the solid lipid matrix as well as the lipophilicity of drug **(Schäfer-Korting *et al.*, 2007; Lv *et al.*, 2009)**. Depending on the previous reports and literatures we believe that C-SLNs formulations were comprised of a high melting point GMS core with a protective hydrophilic coating of P407 **(García-Fuentes *et al.*, 2002; Heiati *et al.*, 1996)**. From the obtained results in table (1) it was evident that the EE % increased with increasing GMS content as the increase in GMS content can afford more space to encapsulate drug under the condition of given surfactant concentration in outer phase **(Lv *et al.*, 2009)**. Also, another factor should be considered for the relatively high EE % of curcumin in GMS-based SLNs formulations which is the effect of Poloxamer 407 (P407). Based on the reported studies by **Fang *et al.* (2008); Hsu *et al.* (2003); Liu *et al.* (2007a); Singh et al. (2008) and Lv *et al.* (2009),** the SLNs formulations developed using P407 in the outer phase had the highest EE% over other known surfactants such as Brij 78, Tween 80 and Tween 20. These results might be related to various effects such as the hydrophile–lipophile balance (HLB) value, structure of the surfactant and its concentration in the outer phase for example high concentration of Poloxamer in the outer phase results in increasing the thickness of the hydrophilic coating and then more drugs can disperse and dissolve in it **(Lv *et al.*, 2009).**

In-Vitro Release Profile

The release profile of curcumin from the developed SLNs was investigated over a period of 24 hours and the final release results after 24 hr are shown in table (1) while the complete release over the whole period is represented in figure (1). In order to mimic the in vivo condition, PBS pH 7.4 was chosen as the release medium and since the solubility of curcumin in aqueous solutions is extremely low, it will remain in the form of crystals in the releasing medium **(Bisht *et al.*, 2007; Nayak *et al.*, 2010)**. At the predetermined time intervals, the dispersion was centrifuged (200×*g*/5min) in order to separate the released curcumin crystals and it did not affect the lipid nanoparticles because they do not sediment at this speed **(Bisht *et al.*, 2007; Nayak *et al.*, 2010)**. The curcumin amount present in the precipitated pellets was then determined using HPLC analysis system after being dissolved in methanol and the concentration of the released curcumin was calculated using the standard curve of curcumin in methanol. From table (1) it was clear that the in-vitro release of curcumin from the investigated formulations was markedly different from one formulation to another and the highest release % was 89.64 (H4) while the lowest % was 55.97 (H5) which could be explained based on the particle size measurements obtained as the increase in particle size causes a decrease in the total surface area and as a result, the CDR % after 24 hr.

Statistical analysis of experimental data

The results of the experimental design were analyzed using JMP ®10 software that was very helpful in elucidating the relationship between the dependent and independent variables through providing the main effects, interactions profile, response surface and contour plots. Additionally it was used to express the mathematical relationships between the independent variables and the dependent responses in the form of the polynomial equations listed in table (2). The confidence that the regression equations would predict the observed values better than the mean for Y_1, Y_2 and Y_3 was 99.83 %, 98.58 % and 97.96 %, respectively. These polynomial equations represented the quantitative effect of process variables (X_1, X_2 and X_3) and their interaction on the designated responses and they were used to generate the response surface plots which represented the simultaneous effect of any two variables on the response parameter taking one variable at constant level. The values of the coefficient X_1, X_2 and X_3 are related to the effect of these variables on the designated response. Coefficients with more than one factor term represent interaction terms and coefficient positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect.

Table (2): Regression equations for the responses:

Response	Regression equation
Particle size (nm) Y_1	354.075 + 0.5765X1 - 86.95X2 - 41.35X3 - 0.035X1X2 - 0.003X1X3 + 13.4X2X3
Entrapment Efficiency (EE %) Y_2	82.1637 + 0.129675X1 - 8.5525X2 - 5.6425X3 - 0.02195X1X2 - 0.00675X1X3 + 0.205X2X3
Cumulative Drug Released (CDR %) Y_3	45.7025 - 0.14225X1 + 15.705X2 + 35.915X3 + 0.0931X1X2 - 0.1156X1X3 - 9.69X2X3

Effect of X_1, X_2 and X_3 on Y_1, Y_2 and Y_3

The selected independent variables (the amount of GMS, concentration of P407 and volume of ethanol) significantly influenced the dependant parameters (particle size, EE % and CDR %) that is very much evident from the results in Table I. Figure (2) represent the pareto chart while figures (3 and 4) represent the main effects plot and the interaction effects plot of GMS amount in mg (X_1), P407 concentration % (X_2) and ethanol volume in ml (X_3) on the dependable responses for the developed C-SLNs formulations. From these figures it was obvious that X_1 and X_2 had the main effects on the dependant parameters while X_3 had the same effect as X_2 but to a lesser extent. X_1 had a positive effect on both Y1 and Y2 while it had a negative effect on Y3 while X2 and X3 had negative effects on Y1 and Y2 and positive effects on Y3. Table (3) show the ANOVA analysis for the dependable parameters where the statistical significance of each effect was tested by comparing the mean square against an estimate of the experimental error.

From the previous results, the following conclusions could be drawn:

1. Increased amount of GMS caused an increase in both the particle size and the EE % and a decrease in the CDR % for the developed C-SLNs formulations and this effect regarding the particle size could be interpreted to the fact that the size of lipid nanoparticles is highly dependent on lipid concentration as lipid tend to coalesce at high lipid concentration and according to Stoke's law, this behavior can be explained by difference in density between internal and external phase **(Leroux *et al.*, 1994)**. Additionally, **Schubert and Müller-Goymann, (2003)** have reported that an increase in particle size of SLNs is due to reduction in the diffusion rate of the solute molecules in the outer phase as a result of viscosity increase in the lipid–solvent phase.

Table (3): Analysis of variance for the dependent responses:

	Particle Size (nm, Y1)			**EE % (Y2)**			**CDR % (Y3)**		
Source	***Sum of Squares***	***Mean Square***	***P-Value***	***Sum of Squares***	***Mean Square***	***P-Value***	***Sum of Squares***	***Mean Square***	***P-Value***
X1	5397.61	5397.61	5397.61	150.078	150.078	0.1373	619.52	619.52	0.1297
X2	10396.8	10396.8	10396.8	266.228	266.228	0.1038	458.136	458.136	0.1501
X3	941.78	941.78	941.78	80.5815	80.5815	0.1849	32.6432	32.6432	0.4664
X1X2	6.125	6.125	6.125	2.40901	2.40901	0.6662	43.3381	43.3381	0.4221
X1X3	0.045	0.045	0.045	0.227812	0.227812	0.8879	66.8168	66.8168	0.3574
X2X3	89.78	89.78	89.78	0.0210125	0.0210125	0.9656	46.948	46.948	0.4098
Total error	28.125	28.125	28.125	7.20101	7.20101		26.4265	26.4265	
Total (corr.)	16860.3	16860.3	16860.3	506.746			1293.83		
R^2	99.8332			98.579			97.9575		
R^2 (adjusted for d.f.)	98.8323			90.0528			85.7025		
Standard Error of Est.	5.3033			2.68347			5.14067		
Mean absolute error	1.875			0.94875			1.8175		

Concerning the EE %, the behavior of GMS could be explained by the fact that on increasing the amount of GMS, EE % is bound to increase because of the increased concentration of mono-, di-, and triglycerides that act as solubilizing agents for highly lipophilic drug **(Mukherjee *et al.*, 2007)**. Moreover, the behavior of GMS concerning the CDR % could be related to its effect on the particle size where the increase in particle size might be due to the increased amount of lipid provides additional space for drug molecules to entrap, thus decreasing the total surface area and as a result, the CDR % after 24 hr was least for the formulation H5 that had the maximum particle size of 345.0 nm.

2. The behavior of P407 on the developed C-SLNs formulation is opposite to GMS behavior where it was found that on increasing the concentration % of P407, both the particle size and EE % were decreased but the CDR % was found to be increased. Concerning the particle size, this behavior is attributed to the reduction in the surface tension between the aqueous phase and the organic phase caused by the increase of the surfactant concentration, also the surfactant helps to stabilize the newly generated surfaces and prevents particle aggregation **(Schubert and Müller-Goymann, 2003).**

Concerning the EE %, this behavior may be attributed to the well-known fact that the aqueous solubility of drug increases with increasing concentration of surfactant in aqueous phase **(Shah and Pathak, 2010)**. Concerning the CDR %, this behavior is attributed to the fact that the corresponding decrease in the particle size causes in turn an increase in the surface area available for dissolution **(Shah and Pathak, 2010)**.

3. The behavior of Ethanol on the developed C-SLNs formulation was similar to that of concentration % of P407 but to a lesser extent as could be observed in Figures (2, 3 and 4).

Concerning the particle size, the behavior of ethanol could be attributed to the resultant decrease in the viscosity of organic phase that in turn led to increase in the diffusion rate. The behavior of ethanol concerning the EE % is related to the fact that curcumin has higher solubility in ethanol as compared to water. The behavior of GMS concerning the CDR % could be attributable to the corresponding decrease in particle size, which in turn increased the surface area and hence dissolution.

4. The possible interactions between X1X2, X2X3, and X1X3 for each response were also investigated in figures (2, 3 and 4) Graphically, the interactions are visualized by lack of parallelism in the lines, but in this case, parallel lines obtained for each interaction term(s) for each response parameter(s) indicated lack of interactions, which in turn indicated that the experimental design has maximum efficiency in estimating main effects **(Bolton and Bon, 2004)**.

Optimization of the formulation ingredients

According to our criteria of lower particle size, higher EE % and higher CDR % after 24hr, H9 was developed as the optimized formulation (desirability value of 0.582) using 148 mg of GMS, % of P407 and ml of ethanol and evaluated for responses. The predicted and experimental values for the responses were recorded in table (4) while the complete release profile was represented in figure 5. From figures (1 and 5) all the investigated C-SLNs formulations as well as H9 showed a biphasic drug release pattern (i.e. a burst release at the initial state followed by a sustained release state).The first stage of the release (the burst release) was usually attributed to the dissociation of the fraction of drug which is adsorbed onto or nearby the surface of nanoparticles or the polymeric matrix **(Shaikh *et al.*, 2009; Misra *et al.*, 2009; Mohanty and Sahoo, 2010; Nayak *et al.*, 2010).** Upon placing the nanoparticles within the release medium, the curcumin located onto the SLNs surface was released following a burst profile due to the short diffusion distance for

the drug adhered on the surface region of the nanoparticles to go in the dissolution medium **(Souto *et al.*, 2004).** The second stage of release (sustained release), the release profile will slow down and become sustained due to the time required for the entrapped drug to diffuse from the lipid core into the release medium **(Nayak *et al.*, 2010)** or might be due to the partition of drug in the hydrophobic core of GMS and subsequently diffusion/erosion of polymeric matrix made the drug released from C-SLNs formulations to the aqueous medium **(Mohanty and Sahoo, 2010)**. Our results came in agreement with the studies conducted by **Bisht *et al.* (2007); Tiyaboonchai *et al.* (2007); Mulik *et al.* (2009); Shaikh *et al.* (2009); Mohanty and Sahoo, (2010); Mulik *et al.* (2010; Nayak *et al.* (2010) and Wang *et al.* (2012)** who observed the similar trends of in vitro release (i.e. showing an initial burst release followed by a slower and constant release thereafter).

Table (4): Evaluation of the optimized C-SLN formula (H9):

Dependant Response (Y)	Predicted Value	Experimental Value
Particle size (nm,Y1)	240.54	249.1 ± 2.9
EE % (Y2)	71.59	74.51 ± 0.75
CDR % (Y3)	82.97	85.72

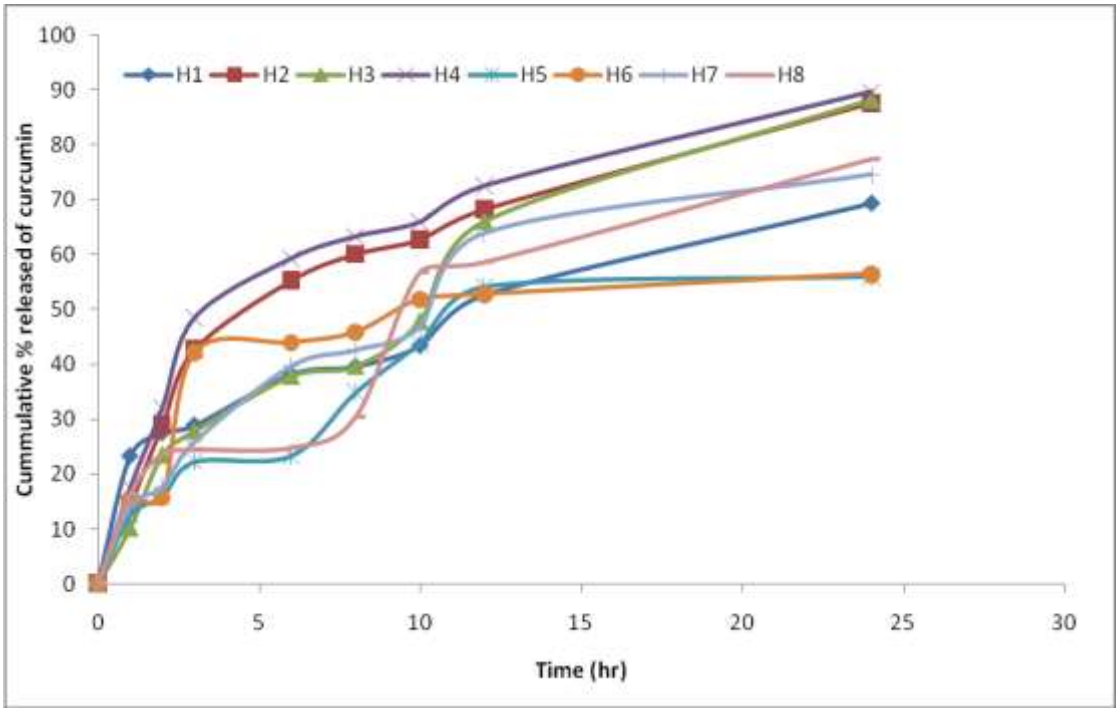
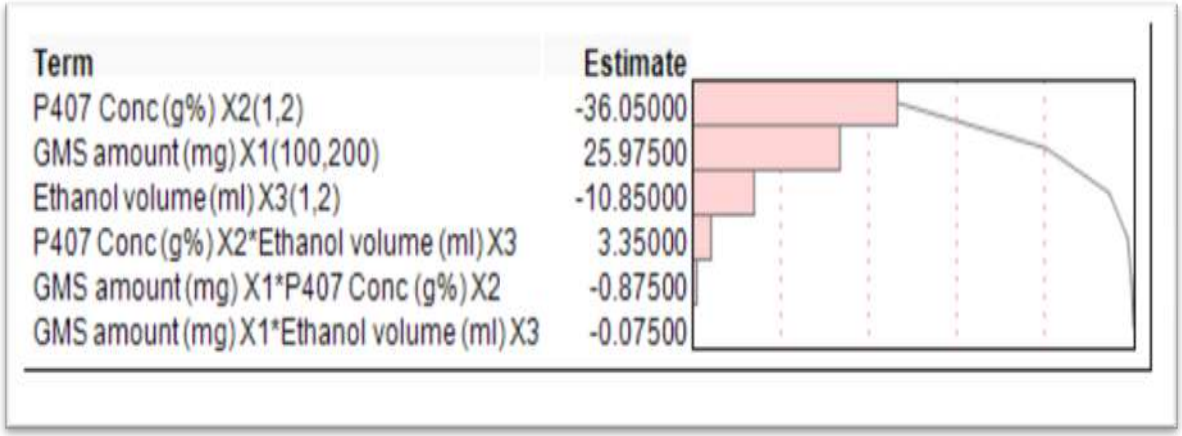


Fig (1): Cumulative % released of curcumin over 24 hr from the experimental design Formulations (H1–H8).

A)



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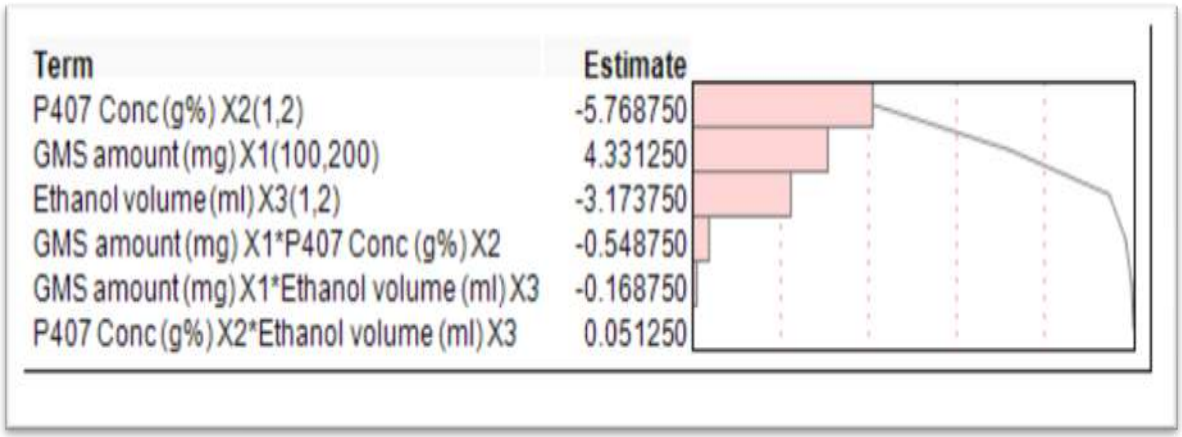


C)

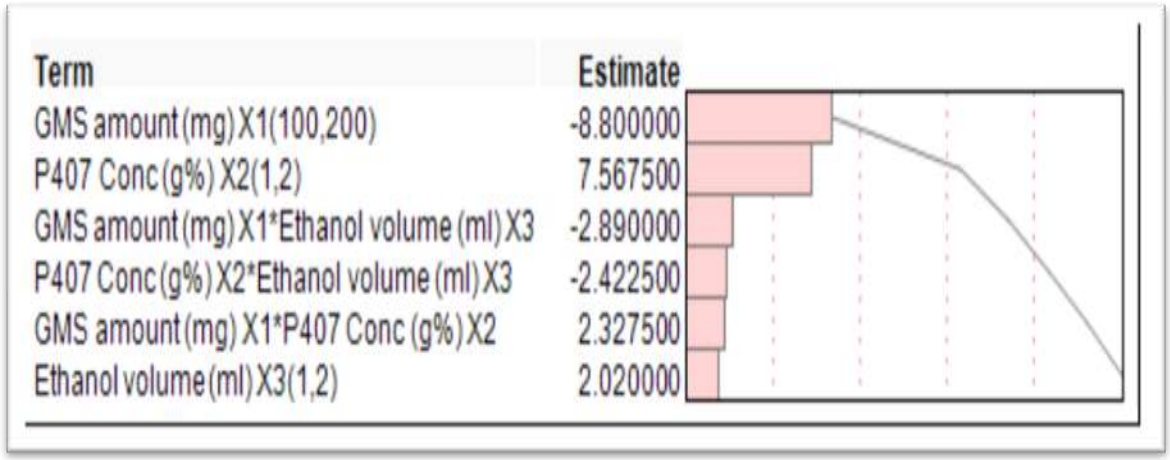
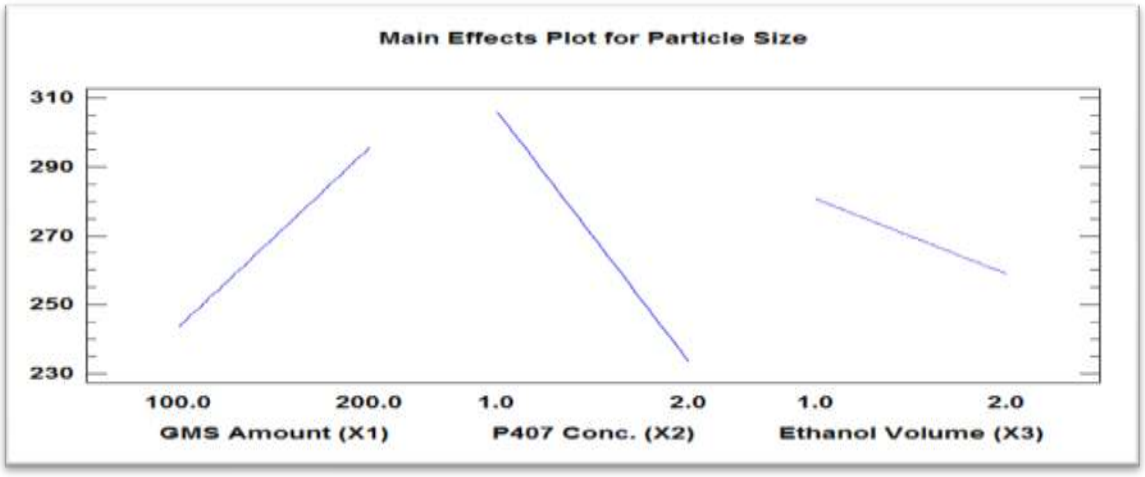
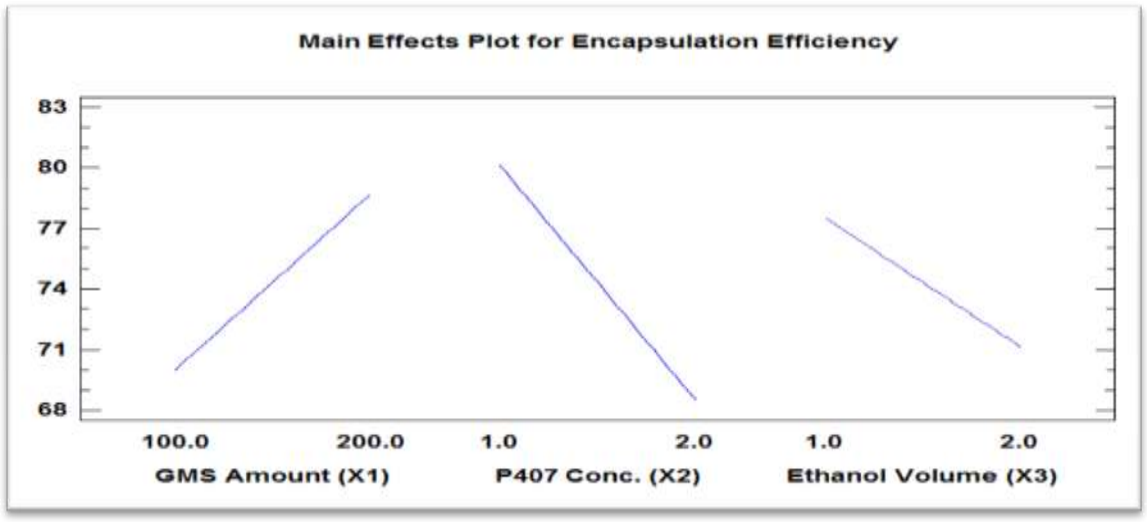


Fig (2): Patero charts showing the effects of X1, X2 and X3 on A) particle size, B) EE % and C) CDR %.

A)



B)



C)

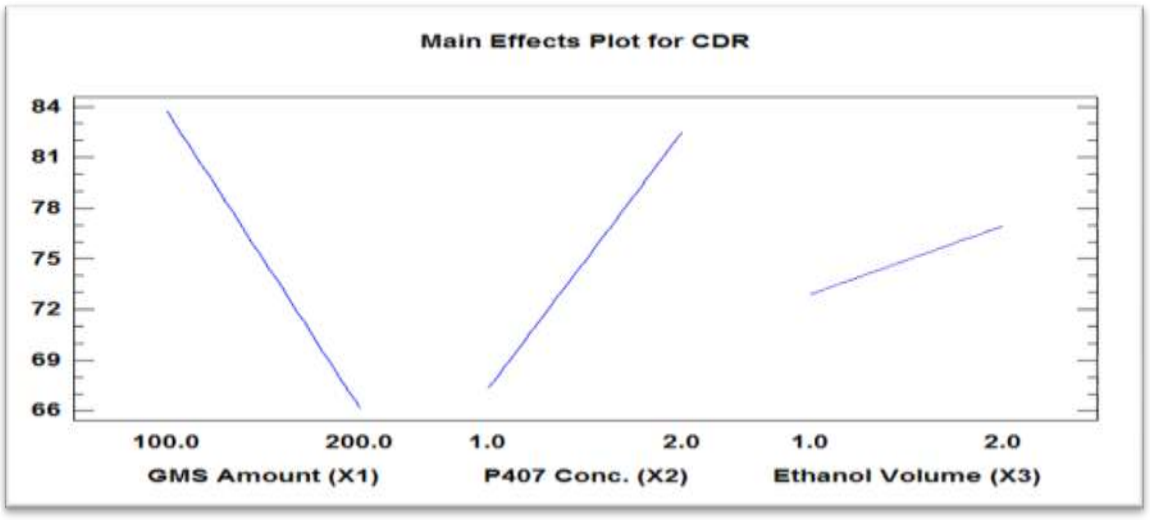
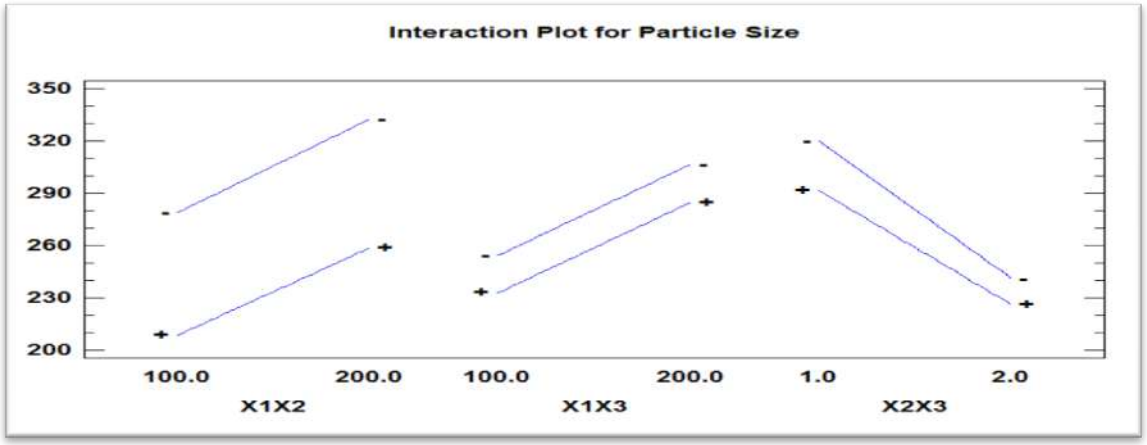
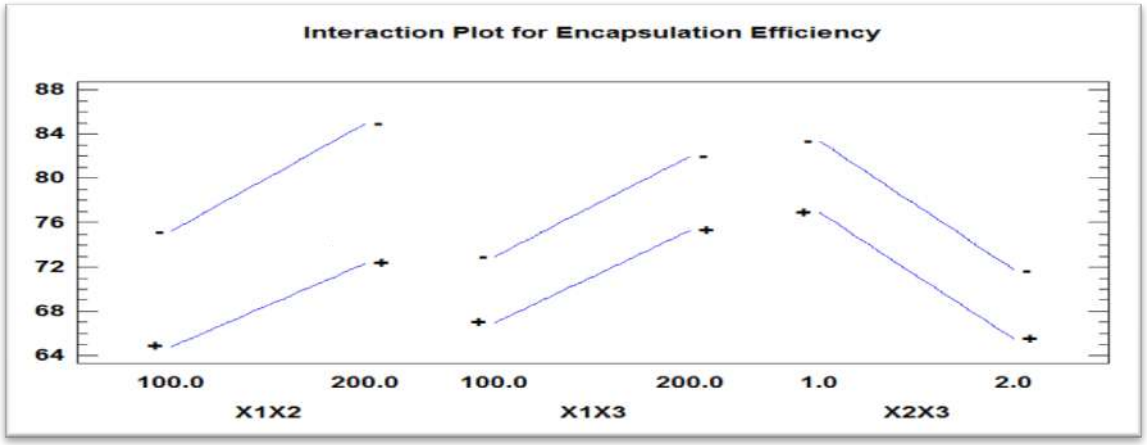


Fig (3): Main effects plot of X1, X2 and X3 on A) particle size, B) EE % and C) CDR %.

A)



B)



C)

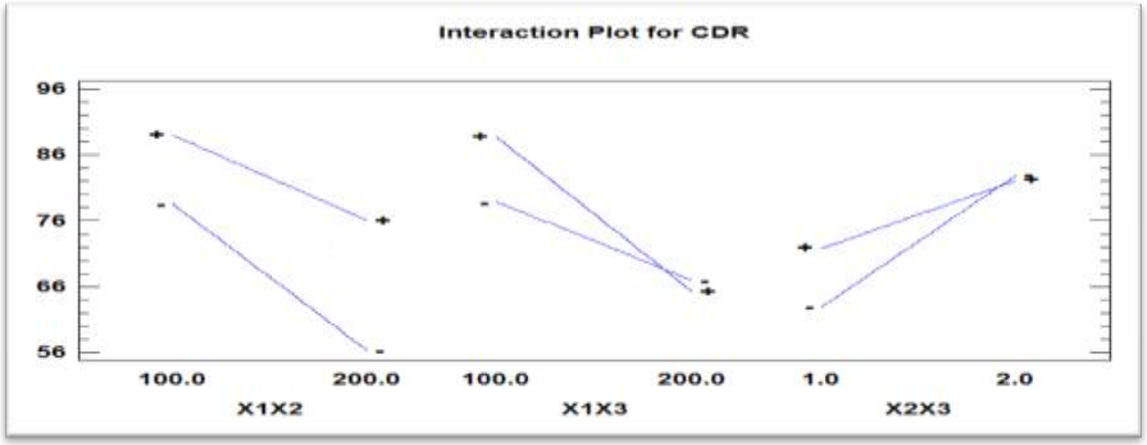


Fig (4): interaction plot of X1, X2 and X3 on A) particle size, B) EE % and C) CDR %.

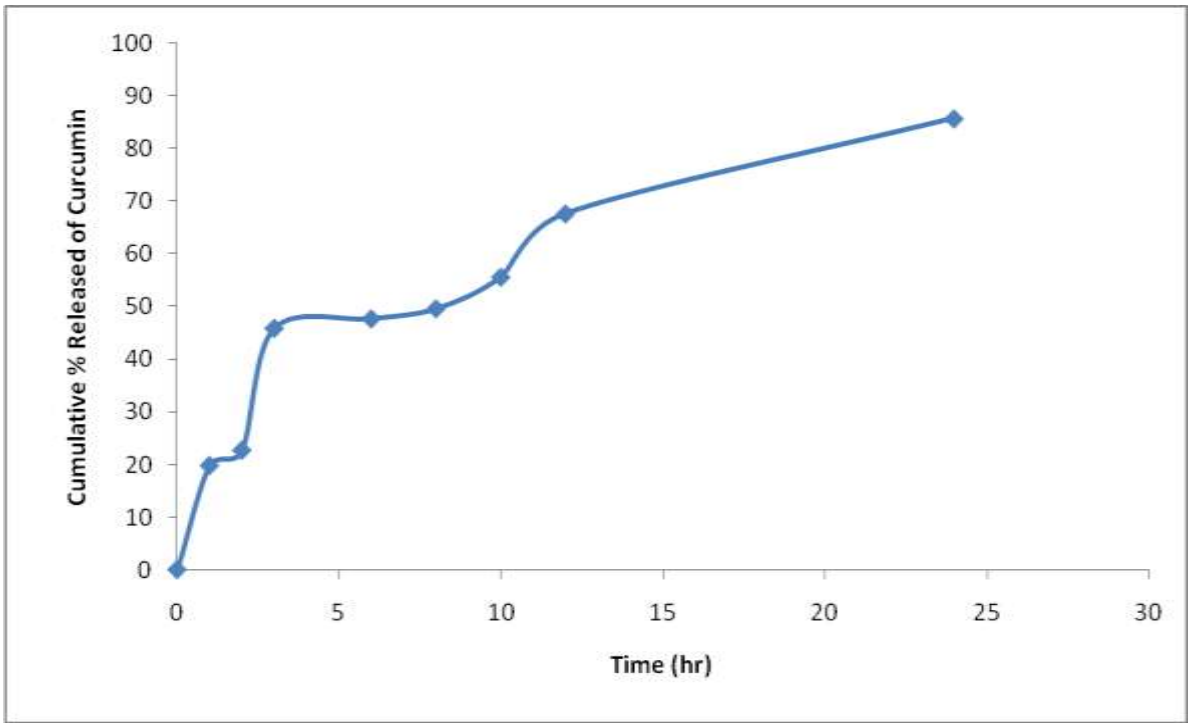


Fig (5): Cumulative % released of curcumin over 24 hr from Formula H9.

CONCLUSION

Solid Lipid Nanoparticles of curcumin were successfully developed to yield an optimized formulation with least nanometeric particle size and highest possible entrapment efficiency that could sustain the release of curcumin over 24 hr. The use of 2^3 full-factorial design enabled to develop an acceptable formulation using minimum raw materials and in minimum time.

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استخدام نظام 2^3 المعامل الكامل لتطوير وتعظيم الجسيمات في حجم النانو المحتوية علي عقار الكوركيومين في صورة جسيمات مع الدهن قابلة للتكسير ومتوافقة حيويا

حمدي محمد دوابة، أحمد محمود سامي، ايهاب أمين فتوح، فتحي ابراهيم عبد الله، محمد نوتان

الكوركومين هو عقار طبيعي لعلاج السرطان وهو شحيح الذوبان في الماء وله فترة نصف حياة قصيرة جدا.
وعلي ذلك، فأن الهدف من هذه الدراسة هو تطوير وتعظيم الجسيمات في حجم النانو في صورة جسيمات مع الدهن محتوية علي عقار الكوركيومين قابل للتكسير حيويا وفي نفس الوقت متوافق حيويا باستخدام طريقة الحقن بالمذيب.
والمحتويات المختارة لتطوير الجسيمات الدهنية في حجم النانو هي أحادي شمعات الجليسرول (الحامل الدهني)، بوليكسيمار ٤٠٧ (مادة ذات نشاط سطحي)، الجليسرين (مذيب للعقار والحامل الدهني).
وقد استخدم طريقة ٣٢ نظام المعامل الكامل لتحديد النسب المناسبة للمواد المستخدمة لتعظيم المنتج من الجسيمات. وقد تم اختيار ثمانية صيغ وتحديد الاستجابة كالاتي: حجم الجسيمات، والنسبة المئوية للعقار الموجود في الجسيمات، ومعدل انطلاق العقار من الجسيمات.
وقد اعطت الصيغ المطورة حجم جسيمات من ٢٠٣ الي ٣٤٥ نانومتر، والنسب المئوية الموجودة في الجسيمات من ٦٢,٧٧ الي ٨٧,٤٢%، واعطت معدل انطلاق للعقار من الجسيمات في مدة ٢٤ ساعة من ٩٧,٥٥ الي ٩٨,٩٤ %.
وباستخدام برنامج مناسب لتحليل النتائج، وجد أن الصيغة H9 (الصيغة المثلي) قد أعطت حجم جسيمات ٢٤٩,١ نانومتر، ٧٤,٥١% محتوي عقار، ٨٥,٧٢% انطلاق للعقار علي مدي ٢٤ ساعة. مما يعطي صيغة ناجحة طويلة المفعول لعقار الكوركيومين.