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EXTRACTION, NANOFORMULATION AND EVALUATION OF CURCUMIN

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ABSTRACT: Nanosuspensions have versatile potential for efficient exploitation of different drug delivery formulations and routes because of the properties provided by their small size. In present study, Curcumin extraction was carried out from *Curcuma Longa* (crude curcuminoids) using soxhlet extraction method with ethanol as solvent which gives high yield. Formulations of nanosuspensions with nanoprecipitation technique are made using polycaprolactone (PCL) as polymer, with extracted natural curcumin and with synthetic curcumin. A comparison is made for parameters such as product yield, drug content, drug entrapment efficiency and in-vitro studies between formulation of nanosuspensions with naturally extracted curcumin and synthetic curcumin. Average particle size obtained ranging from 297.4nm to 973.1nm for different formulations with natural curcumin and 253.8nm to 850.1nm for different formulations with synthetic curcumin. Entrapment efficiency of nanosuspension ranged between 66.7% to 80.78% for natural curcumin and 68.78% to 80.86% for synthetic curcumin. Drug content ranged from 67.41% to 85.70% for formulation with natural drug and 68.27% to 83.27% for formulation with synthetic drug. The zeta potential values shows good stability and negative surface charge in the range of -72.95mv to -8.59mv. The prepared nanosuspension showed enhanced dissolution which may lead to enhanced oral bioavailability.

INTRODUCTION: Extraction, as the term used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components. The techniques to extraction process are maceration, percolation, soxhlet extraction, decoction, continuous counter current extraction etc. Curcumin from rhizome curcuma longa (raw turmeric) can be extracted using maceration, percolation and soxhlet extraction techniques [1].

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Curcumin is an orange yellow crystalline powder with three colouring components in various portions which are all dicinnamoyimethane derivatives. (i) 1, 7-Bis-(4-hydroxy- 3-methoxyphenyl)-hepta-1, 6-diene- 3, 5 dione = diferuloyl methane (Chemical formula: C21H20O6, Formula weight: 368). (ii) 1-(4-hydroxyphenyl)-7-(4-hydroxy 3- methoxyphenyl)-hepta-1,6-diene-3, 5 dione = phydroxycinnamoyl ferulo methane (Chemical formula: C20H18O5, Formula weight: 338). (iii) 1, 7-Bis-(4-hydroxyphenyl) - hepta-1, 6-diene-3, 5dione=p, pdihydroxy dicinnamoyl methane (Chemical formula: C19H16O4, Formula weight: 308).

Nanopharmaceuticals are the pharmaceuticals which are designed by using nanotechnology techniques. The applications of Nanopharmaceuticals range from smart materials

for tissue engineering tools to drug delivery systems, to the production of nanomaterials. Nanopharmaceutics is the future of healthcare and has great promise. Nanosuspensions are one of the preferred dosage forms of oral bioavailability for poor water soluble drugs and to increase therapeutic performance of these drugs in any route of administration [2]. Nanosuspension [3] is fine dispersion of uniform-sized solid particles in an aqueous vehicle.

A nanosuspension not only solves the problems of poor solubility and bioavailability, but also alters the pharmacokinetics of drug and thus improves drug safety and efficacy. The techniques to prepare nanosuspension [4] are supercritical fluid technique, melt emulsification technique, solvent evaporation technique etc. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster. This approach is useful for molecules with poor solubility, poor permeability, or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without any blockade of the blood capillaries.

Advantages of nanosuspension are suitable for hydrophilic drugs, higher drug loading can be achieved and dose reduction is possible. Nanosuspension increases dissolution rate and absorption of drug due to smaller particle size and larger surface area. For large-scale production of nanosuspension, media milling and high-pressure homogenization technology have been successfully used.

MATERIALS AND METHODS:

Materials:

Model drug synthetic curcumin from sigma laborites, Hyderabad. Polymer polycaprolactone and polyvinyl alcohol from sigma Aldrich, Bangalore. Sodium tri-polyphosphate from finer chemicals, Hyderabad. Ethanol from srinivasa scientific, Hyderabad. Hexane, acetone, di-chloro methane from qualigans fine chemicals, Mumbai.

Methods:

Extraction of curcumin [5 6]**:** The soxhlet extractor is filled with 150gm of turmeric powder and the round bottom flask is filled with 175 ml of ethyl alcohol. The extraction is started by the heating mantel and temperature is maintained at the set point of 60^0c. The extraction is continued for 3 to 4 hours until the solvent which fills siphon cycle unit is filled with almost colorless solvent. Then, remove the round bottom flask from soxhlet extractor and transfer it to a beaker.

Concentrated the extract with continuous stirring at room temperature, until ethyl alcohol is evaporated off from the extract. Then, 50ml of hexane is added to the concentrated extract and stir the solution using a magnetic stirrer at 500 rpm. Water is added slowly to the solution until curcumin precipitate is observed. Filter the precipitate of curcumin using filtration and recrystallize from ethanol. The obtained precipitate is dried and characterization is done by using UV-spectrophotometer and HPLC [7] [8]. The extraction results are shown in **Table 1**. The increasing range of extraction of curcumin and characterization results of UV-spectrophotometer and HPLC are showed in the **Figures 1 & 2**.

TABLE 1. OBSERVED RESULTS FOR VALIDATING THE EXTRACTION PROCEDURE

S. No	Time for extraction	Raw Turmeric powder (gm)	Solvent vol.(ml)	Product yield (gm)	% yield	% extracted
1.	1 hrs	50	150	1.2	2.4%	41.49%
2.	2hrs	50	150	1.9	3.8%	64.83%
3.	3hrs	50	150	3.25	6.5%	76.11%
4.	4hrs	50	150	4.13	8.6%	87.3%

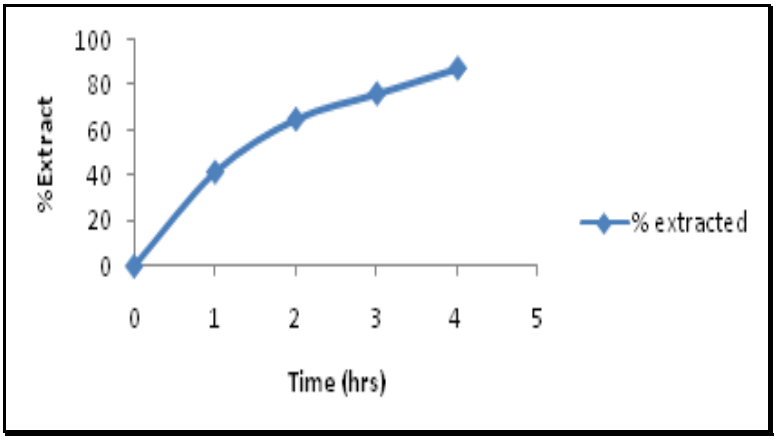

FIG 1. RESULTS OF EXTRACTION OF CURCUMIN

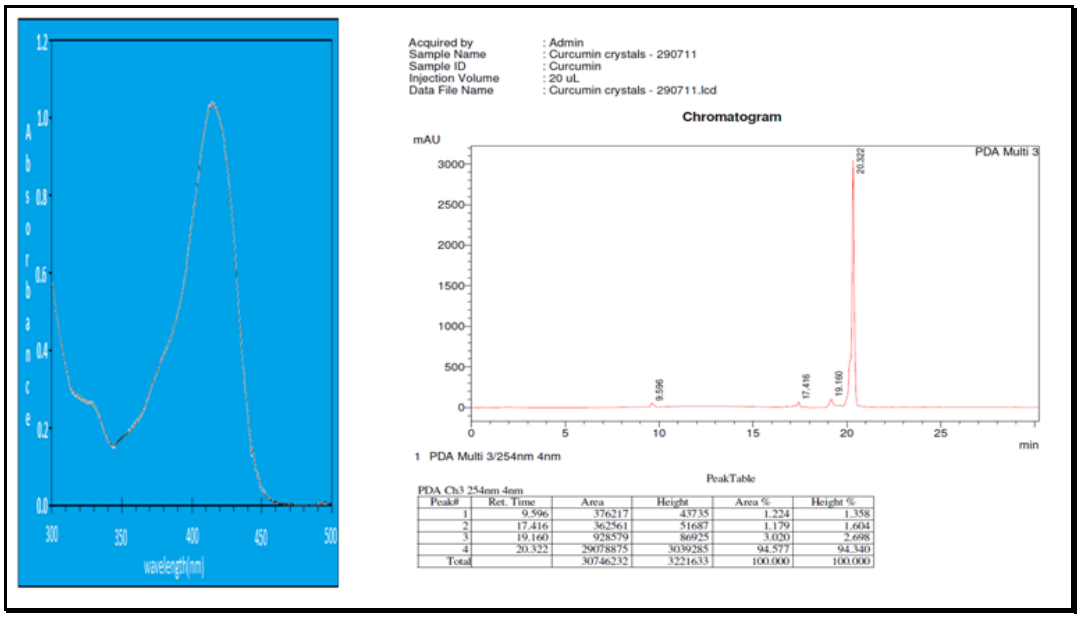

FIG 2. UV-VISIBLE WAVELENGTH AND HPLC RESULTS OF CURCUMIN AT MAXIMUM (λ_{max}) ABSORBANCE

Preparation of nanosuspension using nanoprecipitation technique:

Nanosuspension of curcumin was prepared by using curcumin drug and different polymer concentration (0.8%, 1.6%, 3.2% &4.8%) by nanoprecipitation technique as shown in. Required quantities of drug, polymer, cross **Table 2** linking agent and stabilizer are added under continuous stirring for 8hrs and at last water is added as anti solvent agent. The prepared nanosuspension is placed in an air tight container and stored in cool place.

TABLE 2. FORMULATIONS WITH CURCUMIN DRUG AND ITS PRODUCT YIELD

S.No	Polymer Concentration	Synthetic curcumin Concentration	PVA Concentration	STPP Concentration	Stirring Speed	Time of Stirring	Product yield on natural drug	Product yield of synthetic drug
1.	0.8% w/v	100mg	4% w/v	1% w/v	1200 rpm	8 hrs	69.5%	72.9%
2.	1.6% w/v	100mg	4% w/v	1% w/v	1200 rpm	8 hrs	76.3%	82.2%
3.	3.2% w/v	100mg	4% w/v	1% w/v	1200 rpm	8 hrs	78.4%	83.3%
4.	4.8% w/v	100mg	4% w/v	1% w/v	1200 rpm	8 hrs	83.3%	86.1%

Estimation of drug content and entrapment efficiency: 100mg of Drug loaded nanosuspension is taken and dissolved in 100ml of suitable buffer solution of P^H 7.4. Now these samples are analyzed against a blank spectro-photometrically at 421nm using Schimadzu UV spectrophotometer. Dilute the stock solution until the minimum absorbance reaches to 0.899Abs or below. Then the last addition of solvent is taken as a dilution factor. This procedure is repeated for the evaluation of entrapment efficiency with the stock solution samples are centrifuged in centrifuge tube for 1 hr at room temperature and at 5000rpm. . The calculations of drug content and entrapment efficiency values are shown in **Table 3**.

***In vitro* release studies** [9 10]**:** In vitro drug release was carried out using dissolution test apparatus USP XXII using a dialysis membrane to hold nanosuspension. The dissolution consisted of phosphate buffer (P^H 7.4) for 9 hours. An amount of 900ml of the dissolution fluids were used at 37±1^{0}C with a stirring speed 70±2 rpm. Aliquots of 5ml were withdrawn at predetermined time intervals and equivalent amount of free dissolution media maintained at the same temperature was replaced. The samples were analyzed by measuring the absorbance at 421nm by UV spectrophotometer. The drug release profile is shown in the **Figure 4**.

RESULTS AND DISCUSSION:

The present investigation was undertaken to formulate and evaluate the nanosuspension with natural drug and synthetic drug for sustained release dosage form. Extraction studies indicated that 8.6% of curcumin were extracted, which has normally 6-9% in a natural curcuma longa. The entrapment efficiency indicates good compatibility between drug, polymer and stabilizer. The results of drug content, entrapment efficiency are shown in the table 3. The results drug content was between 67±0.5% to 83±0.5% and the results of entrapment efficiency was between 66±0.5% to 80±0.5% which indicates the good to excellent drug content and entrapment between polymer and drug.

TABLE 3. DRUG CONTENT AND ENTRAPMENT EFFICIENCY FOR FORMULATIONS OF NANOSUSPENSION WITH CURCUMIN

Sample name	Samples	% Drug content		% Entrapment efficiency	
		Formulation with natural curcumin	Formulation with synthetic curcumin	Formulation with natural curcumin	Formulation with synthetic curcumin
Cur-S1	0.8%PCL	67.41%	68.27%	66.75%	67.75%
Cur-S2	1.6%PCL	70.34%	71.72%	71.25%	70.89%
Cur-S3	3.2%PCL	80.10%	81.10%	77.57%	78.06%
Cur-S4	4.8%PCL	85.75%	83.75%	80.78%	80.86%

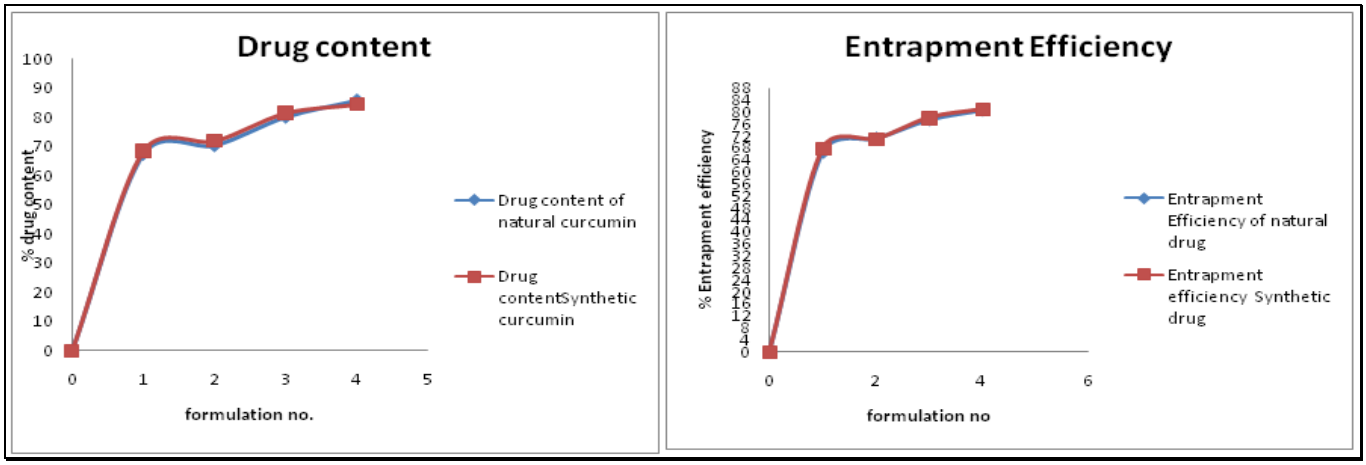

FIG 3. COMPARISON OF DRUG CONTENT AND ENTRAPMENT EFFICIENCY OF NANOSUSPENSION BETWEEN SYNTHETIC DRUG AND NATURAL DRUG FORMULATIONS

The selected formulations based on near entrapment efficiency between natural curcumin and synthetic curcumin were subjected to in vitro studies and subjected various evaluation parameters. The results obtained for in vitro studies were shown in **Table 4** and kinetic models evaluation parameters are shown in **Table 5**.

TABLE 4. DRUG RELEASE PROFILE OF FORMULATED NANOSUSPENSION

Time (hrs)	%Drug Release of Cur-N2	%Drug Release of Cur-S2	%Drug Release of Cur-N3	%Drug Release of Cur-S3
0	0	0	0	0
1	5.1	5.7	5.2	5.7
2	7.4	9.6	11.7	11.3
3	12.23	16.41	22.3	18.4
4	21.59	23.6	27.9	26.5
5	37.23	38.4	42.9	34.6
6	57.5	51.2	59.4	49.04
7	68.79	68.1	68.8	64.8
8	78.6	73.3	77.3	81.3
9	80.4	81.1	80.7	83.4

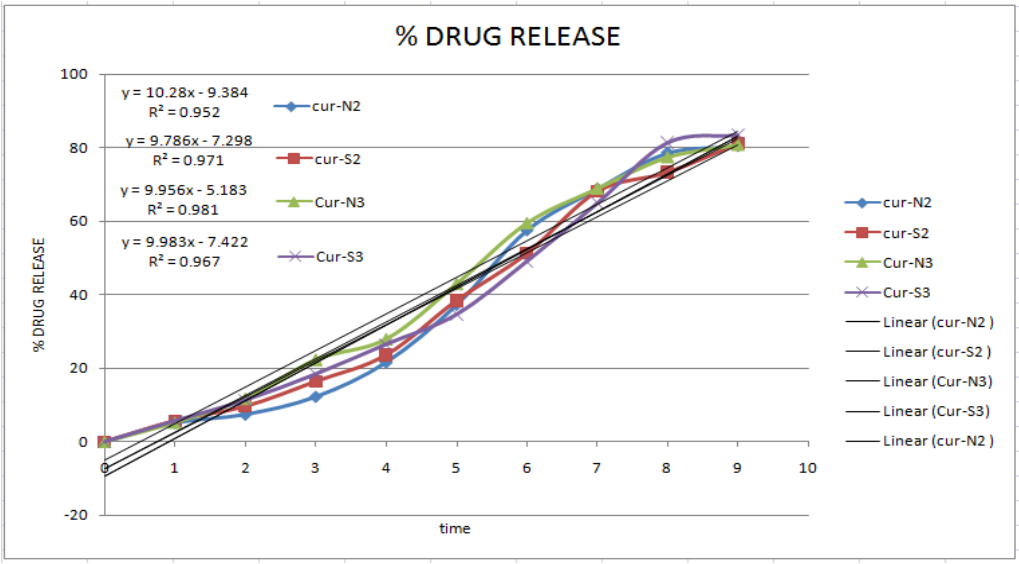

FIG 4. DRUG RELEASE PROFILE FOR DIFFERENT SAMPLES

TABLE 5: KINETIC DATA FOR *IN -VITRO* STUDIES

Model and Parameter		CUR N2	CUR N3	Average of R^2	CUR S2	CUR S3	Average of R^2
Zero order kinetics	R^2	0.952	0.981	0.966	0.971	0.967	0.969
	K_0 (h^{-1})	10.28	9.956		9.786	9.983	
First order kinetics	R^2	0.930	0.958	0.944	0.937	0.892	0.914
	K_1(h^{-1})	-0.096	-0.092		-0.090	-0.098	
Higuchis kinetics	R^2	0.796	0.861	0.828	0.824	0.816	0.82
	K_H ($h^{-1/2}$)	30.73	30.47		29.46	29.97	
Korsmeyer peppas kinetics	R^2	0.951	0.993	0.972	0.977	0.988	0.982
	n	0.843	0.831		0.831	0.879	

Upon model fitting analysis of nanosuspension, korsmeyer peppa's model are with 'n' values 0.831 to 0.880 and average correlation coefficient 'R^2' 0.970 to 0.980. The obtained in vitro results of selected formulations are evaluated with various kinetics models like zero order kinetics, first order kinetics, Higuchi's model and korsmeyer peppa's model **(Figures 4 to 6)** [11, 12]. This indicates that the release of curcumin loaded polycaprolactone nanosuspension follow zero order kinetics with sustained release pattern. The results particle size distribution according to the Beckman coulter nanosize analyzer [13] showed 297±0.5nm to 973±0.5nm which are in the range of 1nm to 1000nm **(Table 6)**. The charge of particle was determined by zeta potential analyzer as shown in **Table 6.**

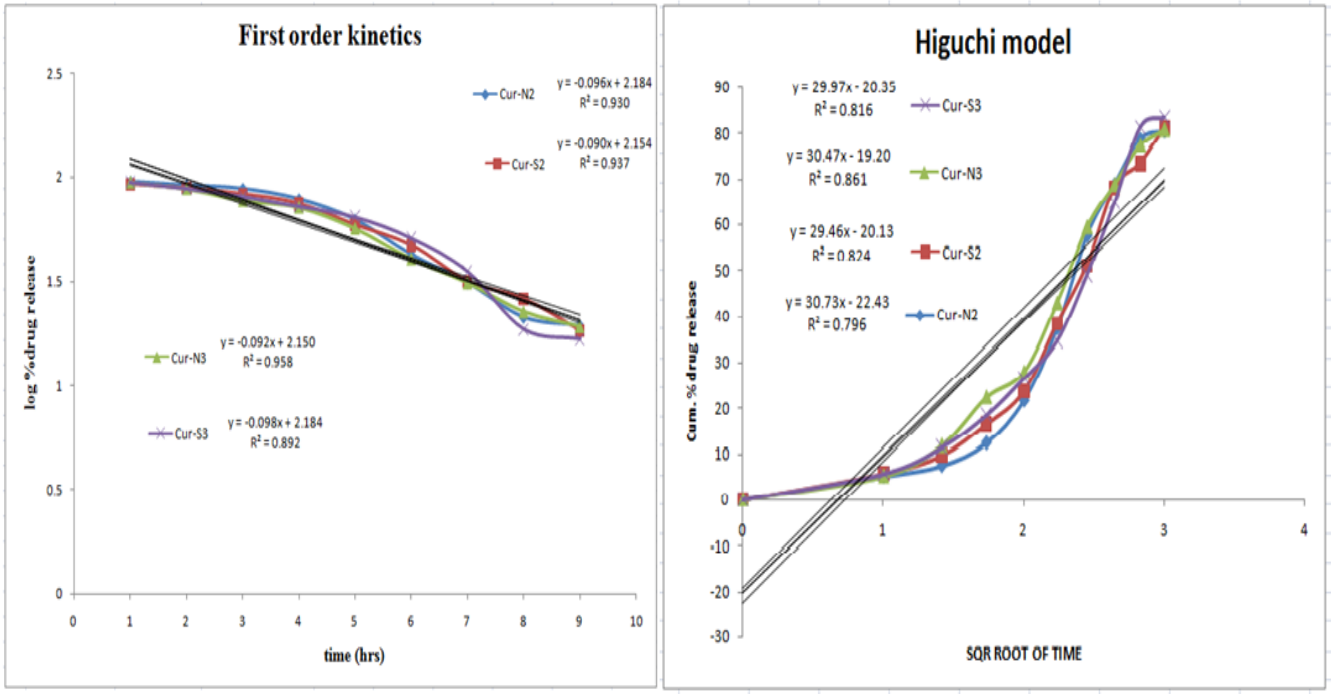

FIG 5. FIRST ORDER KINETICS AND HIGUCHI'S MODEL FOR SELECTED FORMULATIONS

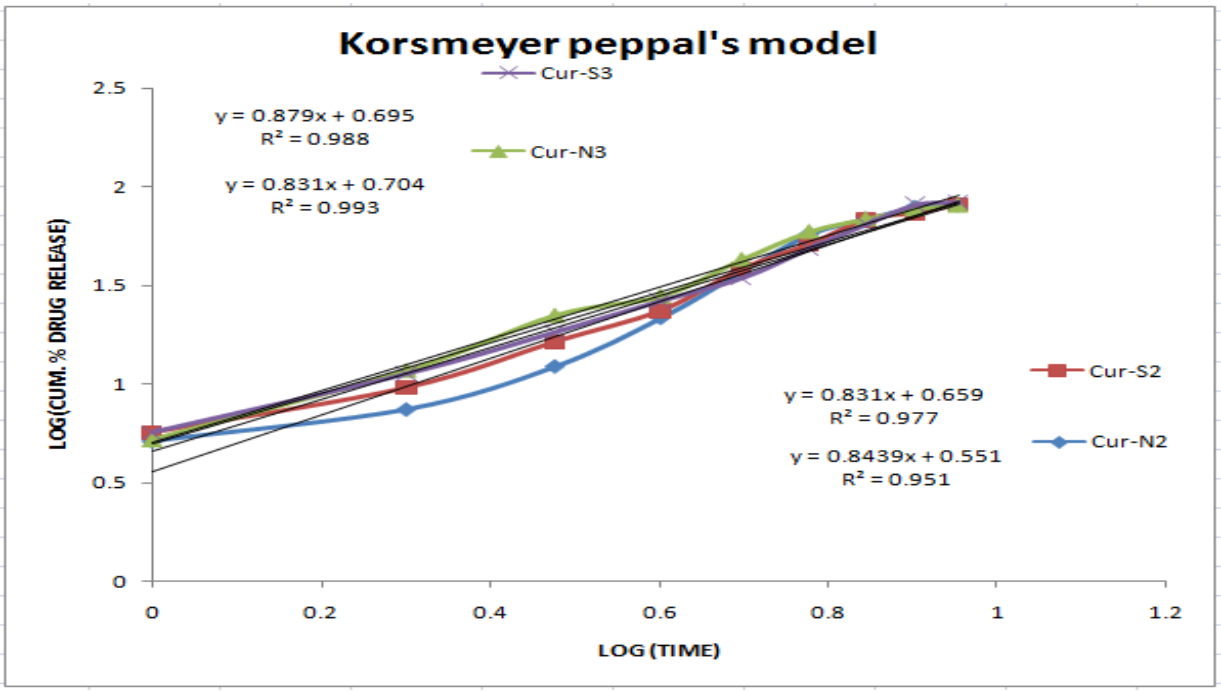

FIG 6. KORSMEYER PEPPAL MODEL FOR SELECTED FORMULATIONS

TABLE 6. AVERAGE PARTICLE SIZE FOR NANOSUSPENSIONS FORMULATIONS

Sample name	AVERAGE PARTICLE SIZE		ZETA POTENTAL	
	NATURAL DRUG, nm	SYNTHETIC DRUG, nm	Natural Curcumin, mv	Syntheyic curcumin, mv
0.8% w/v	297.4	253.8	-8.41	-9.19
1.6% w/v	410.6	413.2	-18.95	-17.96
3.2% w/v	668.5	667.5	-26.42	-28.36
4.8% w/v	973.1	850.1	-70.59	-72.59

Comparison of all evaluation studies of particle size analysis and zeta potential analysis are shown in the **Figures 7**.

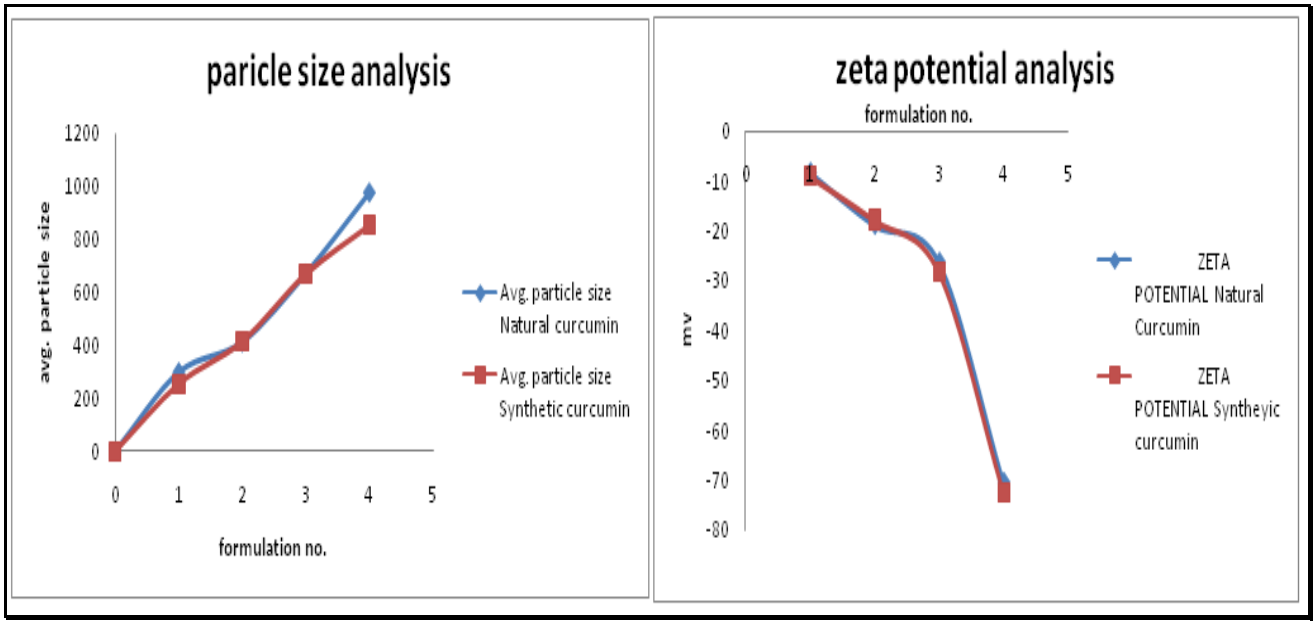

FIG 7. RESULTS OF AVERAGE PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS FOR NANO-SUSPENSIONS FORMULATIONS

CONCLUSIONS: Extraction, formulation and evaluation of nanosuspension containing curcumin were found to be potential, cost effective and satisfactory in vitro release studies. It may enable to release the drug in a sustained pattern for prolonged time and thereby accompanying some of the benefits like target delivery of drug, reduction of dose related side effects and better patient compliance.

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