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PREFORMULATION AND DEVELOPMENT OF CURCUMIN MAGNETIC NANOSUSPENSION USING MAGNETITE (Fe_3O_4) AND METHYL CELLULOSE

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ABSTRACT

This work aims at the preformulation and development of intra-articular drug delivery system by enhancing the release of curcumin from curcumin magnetic nanosuspension for the management of rheumatoid arthritis. The preformulation studies such as solubility, incompatible studies of drug, magnetite and polymer with various stress conditions, LOD, particle size, hygroscopicity and thermal properties were carried out. The results give evidence that curcumin was absolutely suitable for the development of magnetic nanosuspension. Magnetite (Fe_3O_4) and methyl cellulose has been used for the preparation of magnetic nanosuspension using the solvent displacement method. Physiochemical characterization of CMNS was carried out to obtain information about particle size distribution, drug loading efficiency, morphology, pH, viscosity, density, sedimentation rate, magnetic susceptibility test, assay and release was found from *in vitro* dissolution studies using 0.01N hydrochloric acid with 0.8% SLS at $37^0C \pm 0.5^0C$. Curcumin release from magnetic nanosuspension of selected formulations (F6) show better release with external magnetic fields among other formulations (F1 – F5).

KEY WORDS: Preformulation studies, Curcumin, Magnetic nanosuspension, *In vitro* release studies

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INTRODUCTION

It is necessary to improve the bioavailability of curcumin in order to fully utilize the potential of this agent, and therefore a growing number of research groups are working on this aim. There are studies designed to investigate new approaches that could overcome these limitations seen with free curcumin. Number of studies has evaluated the nanosuspension formulation *in-vitro* and their effectiveness [1].

In pharmaceutical product development, magnetic microspheres / magnetic nanosphere based on polymer have been widely investigated as a carrier for IA drug delivery [2]. The use of magnetic nanosuspension composed of curcumin on methylparaben, span 60 and methylcellulose an elicited various levels of inflammation following their IA injection and these polymers might not be suitable drug carriers for IA treatment of RA.

Biodegradable polyester biomaterial methylcellulose (MC) has been very widely used because of their biodegradation characteristics and they have been approved by the Food and Drug Administration (FDA). MC degrades slowly when compared to PLGA and is more suitable for long-term drug delivery [3].

The magnetic component of the CMNS in general is magnetite Fe3O4, a proven bio compatible iron oxide, which is FDA- approved and used clinically as MRI contrast agent in products [4]. The most common synthesis of magnetite micro particles is based on Elmore's co precipitation of ferrous (Fe2+) and ferric(Fe3+) ions under basic overall reaction is written as follows:

$$Fe2^{+} + 2Fe3^{+} + 8OH^{-} \longrightarrow Fe_3O_4 + 4H_2O$$

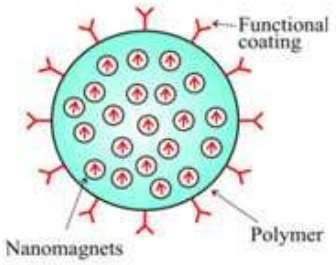


Figure 1
concept of magnetic particle

The objective of this study is to develop a methyl cellulose curcumin magnetic nanosuspension in order to improve its solubility and stability.

Rheumatoid arthritis (RA) is a slow, degenerative disease whereby the white blood cells interact with antigen-derived immune complexes causing a moderate influx of monocytes and neutrophils due to increased vascularization [5]. Curcumin has shown to be able to block inflammation, stops cancer, kill infectious microbes, and improve heart health. Curcuminoids are found in the root of turmeric, a spice used in cooking and in ancient Asian herbal remedies [6].

Scientifically researches prove that curcumin has effective therapeutic outcome acting as an antioxidant, anti-inflammatory, antispasmodic, anticoagulant, anticarcinogenic, immune modulatory activities and even in wound healing [7]. There is not enough research to know if it helps with rheumatoid arthritis natural and alternative therapy.

MATERIALS AND METHODS

Curcumin were received from Sigma Aldrich, India. Methyl cellulose was purchased from Dow chemicals, Mumbai. Magnetite (Fe_3O_4) was purchased from Samsung Fine Chemicals, Mumbai. Polyvinyl Pyrolidine K30 and Methyl paraben were obtained from Zhejang-Al Pharma Chemicals, China. Methanol and formaldehyde were purchased from Jain Enterprises, Chennai. All the other used chemicals are analytical grade reagents appropriately.

(i) Preformulation studies[8,9]:

Background information such as chemical name, structure, solvent of recrystallization, purity, therapeutic category, appearance, color and odour studied according to literature review.

Physical Properties

i. Hygroscopicity

100mg of samples were spread into Petridis and weighed then stored in desiccators, elevated room temperature and 40^0C / 75% RH; then observed the weight of each Petridis containing powder the result were calculated.

$$\text{Hygroscopicity} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where,

W1 = Weight of the empty Petridis in grams.

W2 = Weight of the Petridis with sample in gram (Before experiment).

W3 = Weight of the Petridis with sample in grams (After experiment) – As time specified.

ii. Loss on drying

Transfer about 100mg of the samples to the weighing bottle and place the loaded bottle in the drying chamber (drying oven) remove the stopper and leave it also in the chamber. Dry the sample at 105° for 3 hours. After drying has completed, allow it to cool to room temperature weigh the bottle contents and calculate LOD according to the formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in Weight}}{\text{Sample Weight}} \times 100$$

iii. Solubility studies

Curcumin is completely soluble in ethanol, slightly soluble in chloroform, dichloromethane, acetone, and methanol was studied according to the literature.

iv. Drug-Excipient Compatibility Study

Incompatibility studies were done by inducing a stress at different temperature such as Freezer (20^0C- 10^0C), Cold (2^0C- 8^0C), Room temperature, 25^0C /60% RH and 40^0C / 75% RH by means of fridge and stability chamber with the ratios of 1:1:1:1 in polymer and Curcumin: MC: Magnetite: PVP K30 : Span 60 : Me. Paraben [10]. The study was done for one month (1^{st}. 2^{nd}, 3rd, 21^{st} and 30^{th} day) and observation for the physical changes such as color, liquefaction etc., and chemical changes were observed by FT-IR (Model - 4100) these results are given in the table1.

Table 1
Incompatibility study of curcumin with excipients

S.No	*Curcumin: MC : Magnetite: PVP k30 : Span 60 : Me. Paraben (1:1:1:1:1)*	*Sample interval*				
		1st day	7thday	15thday	21st day	30th day
	Conditions	**Physical / chemical changes**				
1	Freezer (10^0C- 20^0C)	Nd	Nd	Nd	Nd	Nd
2	Cold (2^0C- 8^0C)	Nd	Nd	Nd	Nd	Nd
3	Room temperature	Nd	Nd	Nd	Nd	Nd
4	25^0C /60% RH	Nd	Nd	Nd	Nd	Nd
5	30^0C /65% RH	Nd	Nd	Nd	Nd	Nd
6	40^0C / 75% RH	Nd	Nd	Nd	Nd	Nd

Nd --- Not detectable
MC --- Methyl Cellulose

v. Differential Scanning Calorimetrical (DSC) Analysis

A differential scanning calorimetry was used to study the thermal analysis of drug-excipients compatibility. Thermal analysis of the curcumin, methyl cellulose, empty nanosuspension and curcumin-loaded nanosuspension (optimized) was performed with a differential scanning calorimeter (JADE DSC, Perkin Elmer. USA) [11]. Samples of 2.5 – 12mg placed into aluminum containers and heated, at a constant rate (108^0C for 1min), from 20 to 40^0C under nitrogen atmosphere.

vi. Particle size

About 200mg of sample was weighed and placed on top of the sieve and mechanically shaken by means of mechanical sieve shaker. The sieves were then removed and the curcumin retained on each sieve was weighed [12]. The percentage weight of powder retained on each sieve was calculated and the results are tabulated.

Weight Size = Mean size of sieve opening X % weight retained on smaller sieve

Particle size = Weight size / 100

(ii) Formulation of CMNS

The CMNS was prepared by solvent displacement method coupled with ultrasonication. The polyvinylpyrolidine, span60, methylparaben and methylcellulose were dissolved separately in 5ml of water under continuous stirring using a magnetic stirrer. Curcumin was dissolved in 2ml of methanol and magnetite (Fe3O4) with particle size less than 50nm was added and dispersed into the above solution [13]. The solutions of suspending agents (polyvinyl pyrolidine), surfactant (span60) and preservative (Methylparaben) were added simultaneously to a beaker containing 12ml of water under stirring, followed by adding the drug solution.

To the homogenous solution, the dispersion containing the polymer (methylcellulose) and magnetite were added, stirred at 2500rpm for 15min in an ice bath under nitrogen atmosphere using a magnetic stirrer and then sonicated at 25% amplitude for 20min in pulse mode at 6:4s under nitrogen atmosphere to produce CMNS. The drug–polymer–Excipient compatibility and the functional integrity of the drug in CMNS were confirmed through FT-IR spectral study as a preliminary confirmation.

Table 2
Working formula CMNS F1-F6

S.No	Ingredients	Quantity in milligram					
		F1	F2	F3	F4	F5	F6
1	Curcumin	100	100	100	100	100	100
2	Methyl cellulose	45	47.5	52.5	55.7	58	58.5
3	Magnetite (Fe_3O_4)	90	82.7	77.6	72.5	70.7	70
4	PVP K30	15	14	13	12	12	12
5	Span 60	18	20	22	22	22	22
6	Methyl paraben	0.25	0.25	0.25	0.25	0.25	0.25
7	Ethanol	4ml	3ml	3ml	2ml	2ml	2ml
8	Purified water	10ml	12ml	15ml	15ml	15ml	15ml

Evaluation of CMNS

(i) Drug–Excipients Interaction and Polymorphism Studies

FTIR spectra were taken on to investigate the possible chemical interactions between the drug and the excipients in the magnetic nanosuspension formulation[14]. Sample will be centrifuged under 2500rpm for 30min. Decantation followed by washing with water and dried under hot air oven at 1000C for 1hr. Dried granules crushed with KBr to get the pellets. The spectrum of curcumin, methyl cellulose, PVP K30, Span60, magnetite and curcumin -loaded magnetic nanosuspension (optimized) was recorded.

(ii) Particle Size, Shape

Magnetic nanosuspension particle size and particle size distribution would measure by a laser light scattering analyzer (zetasizer Nano ZS90). A suitable amount of dried magnetic microspheres from each formulation is suspended in water and sonicated for 1min before measurement[15]. The resulting homogenized suspension will then analyzed for the volume mean diameter and particle size distribution.

(iii) Morphology

Morphological analysis of CMNPs in suspension was performed using transmission electron microscopy (Hitachi, H-7500, Japan). Samples of the nanosuspension (5 to10 µL) were dropped onto Formvar-coated copper grids and dried [16]. Digital Micrograph and Soft Imaging Viewer software were used to perform the image capture and analysis, including particle sizing.

(iv) Drug Loading (DL) and Encapsulation Efficiency

The entrapment efficiency measurements were performed on UV-spectrophotometer (Tesco, UK).In order to quantify the content of curcumin in supernatant and suspension in samples; series of standard solutions were prepared. The known amounts of curcumin were dissolved in ethanol and diluted to obtain a stock solution of 1000ng/mL. The standard solutions were then prepared using the stock solution the respective concentrations (100, 200, 400, 600, 800 and 1000 ng/mL). The absorbance was measured at 425 nm based on the spectral analysis. A calibration curve of curcumin was developed by plotting absorbance versus concentration of standard solutions [17]. The entrapment efficiency was calculated using the following equation:

$$\%\,\text{Drug loading (DL)} = \frac{\text{Weight of curcumin microspheres}}{\text{Weight of microspheres}} \text{X}100$$

$$\% \text{ Theoretical loading (DL)} = \frac{\text{Weight of curcumin added}}{(\text{Weight of curcumin added} + \text{Weight of polymer adde}} \text{ X } 100$$

$$\% \text{ Entrapment efficiency (EE)} = \frac{\% \text{ drug loading}}{\%\text{theoretical loading}} \text{ X } 100$$

(v) Magnetic susceptibility test

Magnetic susceptibility reflects a material's degree of sensitivity to magnetic fields. Standardized equipment (Magnometer / Model-7014) is available to hold the sample, generate a magnetic field, and recorded the results[18].

(vi) Sedimentation test

The sedimentation of suspended particles of the prepared nanosuspension was determined by measuring the changes in nepheloturbidimetric units using a digital nepheloturbidity meter (Model-132, Systronics, India) at regular time intervals for a period of 12h [19].

(vii) pH

Immerse the electrode(s) of the pH meter (Trans Instruments, Malaysia) into the magnetic nanosuspension and turn the beaker slightly to obtain good contact between the suspension and the electrode then the pH was measured[20].

(viii) Density

The nanosuspension was measured on a high quality balance and entered into the pycnometer-290 software to give the sample density.

(ix) Viscosity

This procedure determines the viscosity of a fluid by the use of a Brookfield Viscometer (Model- DV-E).

Viscosity is a measure of the ratio of shearing stress to rate of shear.

$$\text{Poises} = \frac{\text{Shear Stress (dynes)}}{\text{Rate of Shear (cm/sec)}}$$

(x) Content uniformity

50mg of curcumin equivalent of CMNS was dissolved in small amount of ethanol and sonicated 30mts. To this add 50ml of double distilled water and filtered through 0.22µ filter paper [10]. 1ml from this stock solution dilute with the same solvent to make 50ml. The absorbance was measured at 425 nm spectrophotometrically.

Standard dilution

50 mg of curcumin was dissolved in small amount of ethanol and diluted to 50ml of double distilled water. From that 1ml dilute with the same solvent to make 50ml.The absorbance was measured at 425nm based on the spectral analysis.

Formula

$$\text{Amount of drug} = \frac{\text{Sample Abs.}}{\text{Std. Abs.}} \text{ X } \frac{\text{Std. dilution}}{\text{Sample dilution}} \text{ X Avg.volume of mag.susp.}$$

(xi) In-vitro drug release studies

Dissolution tests were performed in a USP type II (paddle method - TDT-08 L, Electro lab, Mumbai, India) at 37^0C ± 0.5^0C and paddle was rotated at a speed of 100 rpm. Open ended tube was tightened with semipermeable

membrane at one end and 8.5ml of CMNS was added into it. This setup was immersed on the dissolution medium (see fig.1). Samples of 5ml were collected at intervals 10, 30, 60, 90, 120, 150, 180, 210, 240, 300, 330 and 360minsand required dilution with dissolution medium then detected at 425nm using UV-spectrophotometer [13]. The peak area of standard solution and sample collected at intervals 10, 30, 60, 90, 120, 150, 180, 210, 240, 300, 330 and 360mins was recorded and the percentage drug release was calculated according to the following formula.

Dissolution apparatus used for evaluating Curcumin release from CMNS

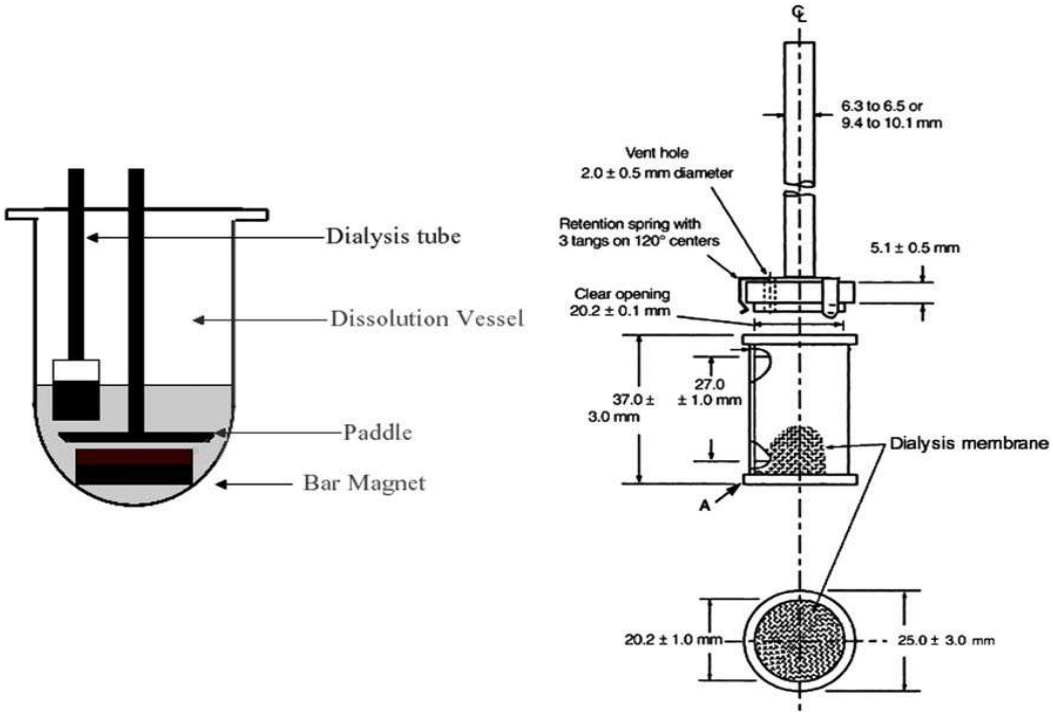


Figure 1
Schematic diagram of the apparatus used for evaluating Curcumin release from CMNS.

$$\text{Amount of drug} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \text{Average volume of CMNS}$$

RESULTS

(i) Preformulation studies

The physical and chemical mix of curcumin with different excipients such as methyl cellulose, magnetite, methyl cellulose, PVPK30,span60 and magnetite at various conditions such as freezer, cold, 25^0C/60%RH, 30^0C/65%RH, 40^0C/75%RH and room temperature. Result shows that no physical and chemical changes are observed by FTIR spectroscopy (Fig.2) in the value of curcumin up to 1st, 7th, 21st and 30th day, which indicates that curcumin is stable in various stress condition. Loss on drying (0.52%) and hygroscopicity (0.19%) was calculated with curcumin alone, which is not hygroscopic substance so it can dry at 100^0C.

Spectrum curve from FT-IR

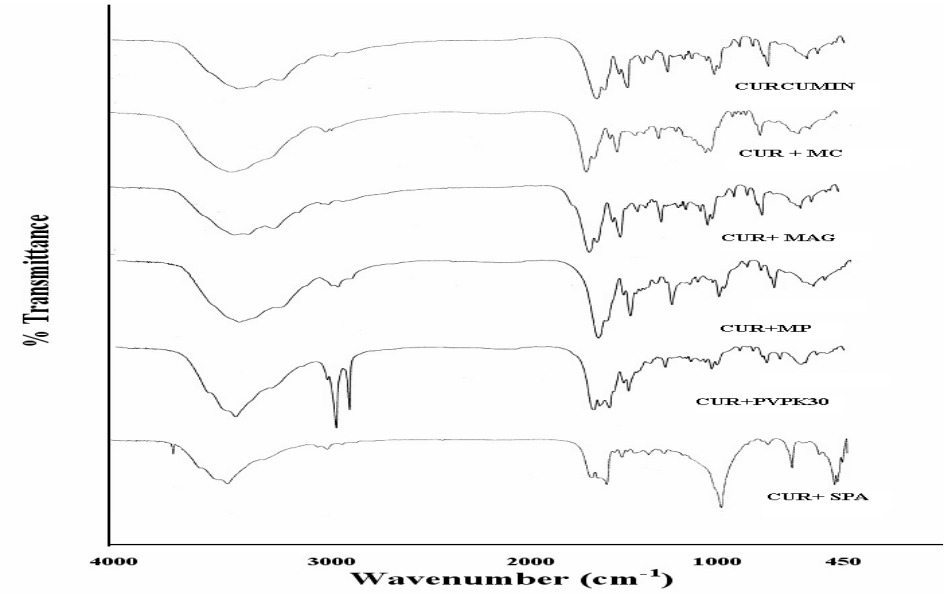


Figure 2
FTIR spectra of Curcumin, MC, Magnetite, MP, PVP and Span60

DSC thermograms of drug and drug-excipients mixtures and corresponding peak temperatures and enthalpy values (ΔH) of curcumin with various excipients mixtures are shown in figure 3. DSC curve of curcumin showed a sharp endothermic peak at 189.23^{0}C corresponding to its melting point. The endothermic peak of the drug was well retained in majority of cases. However, in some combinations there were slight changes in peak temperature and peak shape, which might be due to mixing of excipients with the drug as this reduces the purity of component in mixtures.

Thermogram curves from differential scanning colorimetry

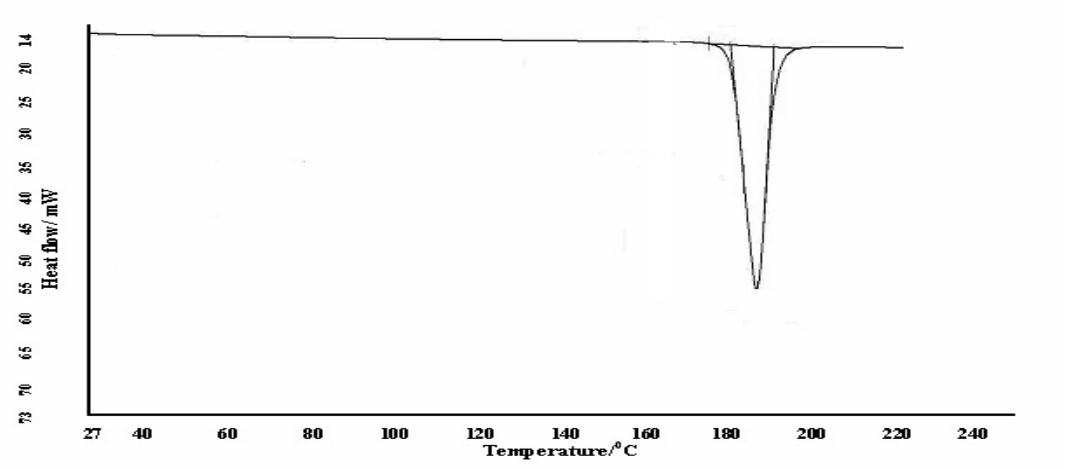


Figure 3
DSC Thermogram of Curcumin drug

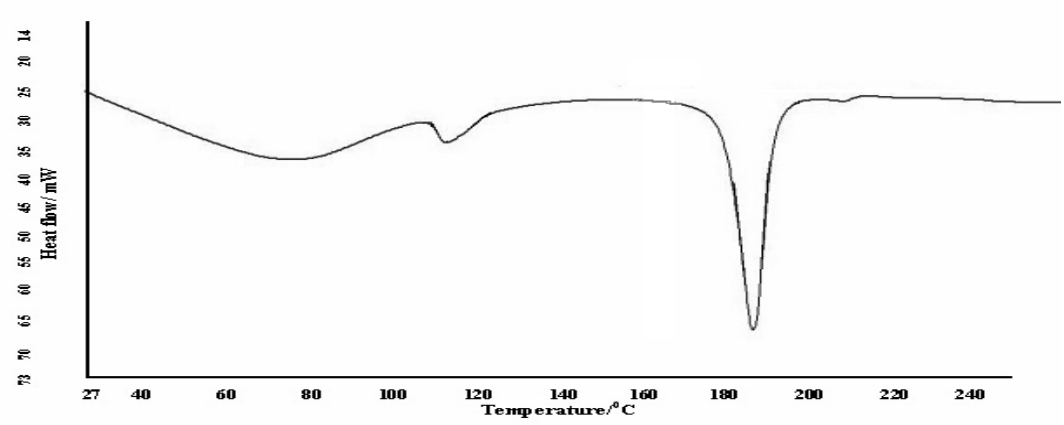


Figure 4
DSC Thermogram of Curcumin with PVP K30 (1:1)

In the DSC thermogram of curcumin and methyl cellulose, the endothermic peak of curcumin was well retained in the mixture (fig. not shown), with a slight change in the enthalpy value. The DSC curve of PVP K-30 show that the melting endotherm of curcumin was well preserved in the mixture and heat of enthalpy is nearly same to the parent drug, concluding its suitability with curcumin. One extra peak was observed at 82^0C (Fig.4), which is of the adsorbed water present on PVP K-30.

Particle size distributed was calculated and the result has shown in table 3

Table 3
Particle size determinations of curcumin

Sieve No	*Weight Retained in mg*	*% Retained*	*Mean size*	% Relative mean size
#20	0.33	33.8	850	28730
#20/#40	0.31	31.4	425	13345
#40/#60	0.12	12.3	250	3075
#60/#100	0.13	13.3	150	1995
#100/pan	0.09	9.2	125	1150
Total		**48295**		
=48295/100				**=482.9µm**

(ii) Formulation consideration

A simple technique using high-frequency ultrasonication was developed for the formulation of CMNS and the process was optimized. From formulation F-F4, phase separation occurs due to methyl cellulose concentration around 16%, which was improved by increasing the polymer concentration and decreasing binder concentration of formulation (F2). Formulation (F3) shows that the above problem has successfully overcome due to sustain increased in polymer concentration, even though under magnetic field (with magnet) excessive release was observed with in150mins. More over the same problem occurred at formulation (F4). Above problems successfully overcome at formulation (F5) and also need to optimize the formula from F5, formulation F6 was developed and drug release and content uniformity shows within the limit.

(iii) Evaluations

a. Morphology

The particle size distribution in the CMNS was found to be biphasic in nature, ranging from 112 to1380nm and the average particle size (hydrodynamic diameter) was found to be 655nm (Fig.5).

TEM images of magnetic nanosuspension

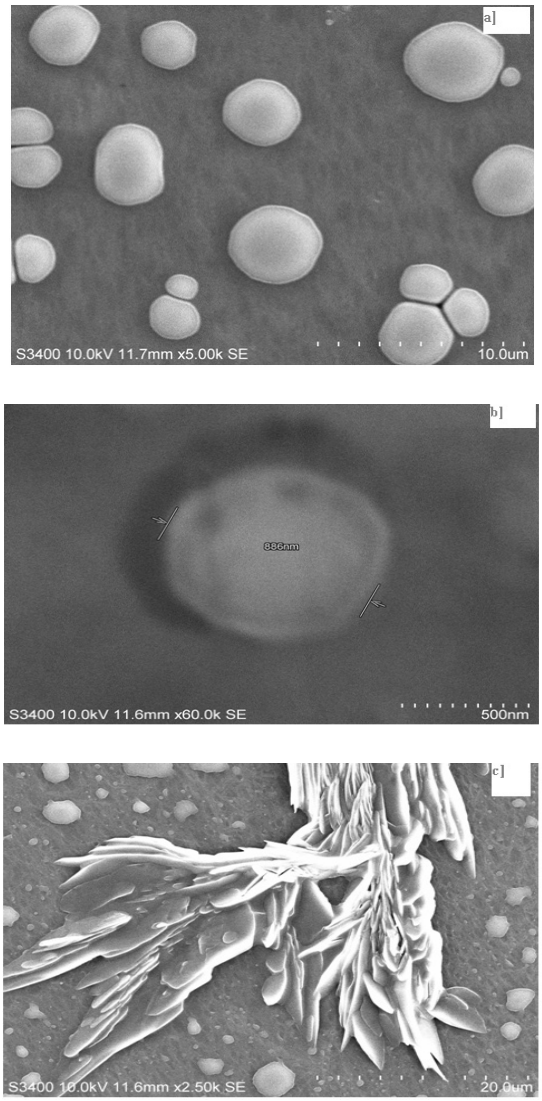


Figure 5

TEM images of magnetic nanosphere [a] Nanosphere at 10.0um [b] nanosphere size 886nm [c] Nanosphere at 20.0 um

The zeta potential and polydispersity index of the formulation (F-6) was found to be -15.2 and 0.680mV, respectively shown in figure-6.

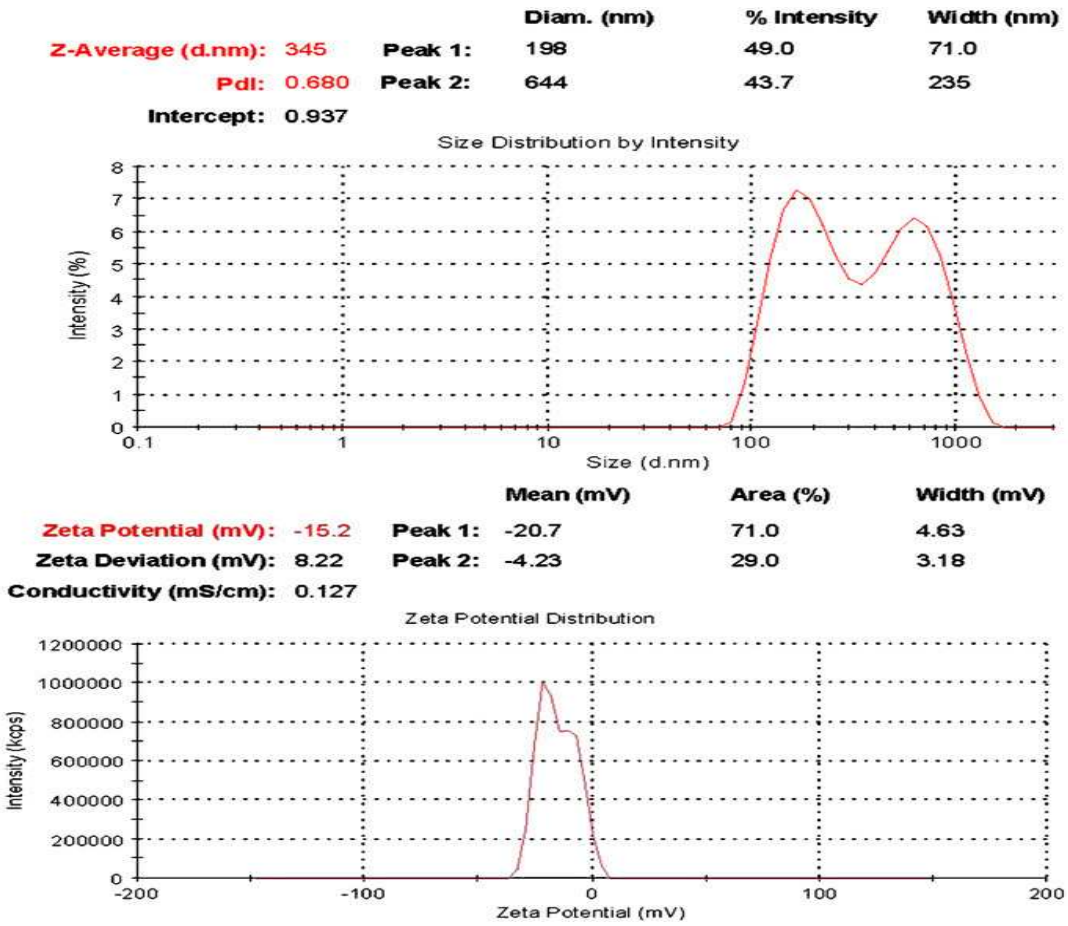


Figure 6
Size and zeta potential distribution for CMNS

Magnetic susceptibility of the formulated CMNS was found to be 5 X 105. The hysteresis curve (Figure not shown) clearly indicates that the formulated CMNS is ferromagnetic in nature with a saturation magnetization (Ms) of 0.4emu/g and coercivity of 37Oe.

The formulation was found to be stable and no sedimentation of suspended particles could be observed up to 60 days. Sedimentation was found after a period of one month, but could be easily re-dispersed by shaking. The drug content was determined from the standard calibration curve and was found to contain 97.18% of curcumin in the formulated magnetic nanosuspension. The density, pH, viscosity and conductivity of the CMNS was found to be 0.762 g/ml, 6.8, 0.877cP and 0.138 mS/cm, respectively.

All the suspended Curcumin magnetic nanoparticles were found to get attracted towards the applied external magnetic field within 10mins.

b. In-vitro drug release study:

The *in-vitro* curcumin release was not steady, stable (F1-F4) and in an enhanced fashion of release at dissolution medium without magnetic fields and was found to have a burst and enhanced release shown in table 4.

Table 4
Comparative dissolution study of F1-F6 without magnetic fields

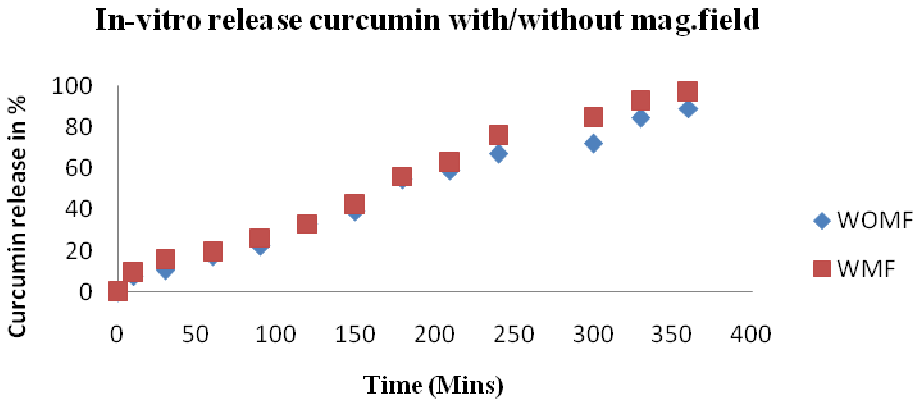
Time (Minutes)	*Percentage drug release*					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
10	26.26	22.19	20.66	14.41	10.60	07.88
30	31.84	27.23	22.19	16.70	12.25	10.66
60	40.22	30.11	26.77	19.68	18.15	17.54
90	-	33.10	34.19	30.01	25.67	22.44
120	-	44.09	40.91	38.45	34.36	33.23
150	-	55.70	52.10	48.11	39.87	39.19
180	-	-	-	60.19	57.20	54.56
210	-	-	-	72.56	59.45	58.87
240	-	-	-	88.12	67.66	66.98
300	-	-	-	-	75.00	72.12
330	-	-	-	-	81.01	84.33
360	-	-	-	-	98.47	89.18

The *in-vitro* curcumin release from CMNS (F5-F6) was steady, stable and an enhanced fashion of release at dissolution medium with magnetic fields and was found to have a burst and enhanced release as shown in table 5.

Table 5
Comparative dissolution study of F1-F6 with magnetic fields

Time (Minutes)	*Percentage drug release*					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
10	28.75	24.72	22.12	15.16	12.62	09.23
30	34.09	28.42	25.25	18.15	16.15	15.65
60	42.28	32.18	29.19	22.18	20.22	19.22
90	-	36.18	37.16	32.10	28.12	26.34
120	-	49.09	46.98	48.19	34.76	33.16
150	-	62.09	58.18	54.18	44.09	42.09
180	-	-	-	66.12	55.20	56.20
210	-	-	-	75.42	63.41	62.67
240	-	-	-	88.14	71.14	75.87
300	-	-	-	-	79.87	84.32
330	-	-	-	-	84.10	92.54
360	-	-	-	-	91.14	96.84

The in-vitro curcumin release from CMNS (F6) was steady, stable and a controlled fashion with or without magnetic field were found to have a burst and enhanced release shown in figure 7.



WMF – with magnetic field; WMF – without magnetic field

Figure 7
In-vitro releases with/without magnetic field

CONCLUSION

A simple technique using high-frequency ultrasonication for the formulation of CMNS was developed and optimized, which produced a stable nanosuspension. The cavitation and shear force produced during the exposure to ultrasound waves breaks the particles in to distinct nanoparticles in suspension.

The application of an external magnetic field either influences or alters the curcumin release pattern in this *in-vitro* dissolution study. For the amount of drug release, only the pH, viscosity of suspension is important. The magnetic nanoparticles were, however, curcumin magnetic nanosuspension which released the drug in an identical fashion to the non-magnetic suspension, can be magnetically directed and seem more biologically active to the arthritis than then nonmagnetic preparation.

More works are needed to confirm the *in-vivo* activity and mechanism. Furthermore, optimized applications for targeted therapy of curcumin magnetic nanosuspension will have to be investigated in different animal models under the influence of an external magnetic field. Hence, it may be suggested that curcumin as nanoparticles may have had better intra-arterial diffusion and thereby may reduce the time taken for healing of arthritic symptoms.

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