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THERAPEUTIC MICROEMULSION OF CURCUMIN FOR THE MANAGEMENT OF OSTEOARTHRITIS

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ABSTRACT

Curcumin (diferuloylmethane) is a natural polyphenolic compound with potent anti-inflammatory, anticancer and antioxidant activities. However, its bioavailability is low as it is poorly absorbed in the gastrointestinal tract. Microemulsions offer the potential to improve the solubility and bioavailability of bioactive compounds; the present work investigated the topical delivery potential of microemulsion gel loaded with curcumas. Curcumin microemulsion was prepared by spontaneous emulsification method using oil (Oleic acid), surfactant:cosurfactant (S_{mix}) (Ethanol and Tween 80, Span 80 and n Butanol) and water. The optimized formulations of microemulsions were subjected to thermodynamic stability tests. After stability study, stable formulation was characterized for droplet size, pH determination, centrifugation, % drug content in microemulsion, zeta potential and vesicle size measurement and then microemulsion gel were prepared and characterized for spreadability, measurement of viscosity, drug content, *In-vitro* diffusion, *in-vitro* release data. Tween 80, Span 80 was selected as surfactant, ethanol, n Butanol as co surfactant and Oleic acid as oil component based on solubility study. The optimized formulation contained Curcumin (10 mg). The *in vitro* drug release from curcumin microemulsion gel was found to be considerably higher in comparison to that of the pure drug. The *in-vitro* diffusion of microemulsion gel was significantly good. Based on this study, it can be concluded the solubility and permeability of curcumin can be increased by formulating into microemulsion gel.

Keyword: Curcumin, Microemulsion, I*n-vitro* diffusion, Spreadability, Zeta potential, Stability, span 40

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INTRODUCTION

Curcumin is a yellow hydrophobic polyphenol derived from the rhizome of the herb Curcuma longa (family Zingiberaceae) is a diferuloylmethane [1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] and has been used for centuries as a spice in the Indian subcontinent [1]. Conventionally, it has been used for many conditions, particularly as an anti-inflammatory agent [2]. Curcumin also possesses antiviral, antibacterial, antifungal, antioxidant, antiparasitic, antitumoral, and anticarcinogenic activities. Additionally, it is considered a hepato-, nephro-, and myocardial infarction- protector 3. It has also been established that curcumin displays antirheumatic and immunomodulatory activities. These effects are mediated through the regulation of various transcription and inflammatory cytokines, protein kinases, growth factors, and other enzymes [4]. The pharmacological safety and efficacy of curcumin makes it a potential compound for treatment and prevention of a wide variety of chronic illnesses, diabetes, allergies, arthritis, and Alzheimer's disease [5]. Similarly, the efficacy of curcumin in various malignant diseases including cancer has been established [6]. Nevertheless, the poor bioavailability of curcumin has been underlined as a major drawback. The first reported study that examined the uptake of curcumin using Sprague-Dawley rats showed negligible amounts of curcumin in the rat's blood plasma after oral administration of 1 g/kg of

curcumin, suggesting that curcumin was poorly absorbed from the GIT [7]. Recently, nanotechnological delivery systems for drugs and nutraceuticals have emerged as promising solutions to improve the bioavailability of therapeutic agents and bioactive compounds [8]. Several nanoformulations of curcumin have been extensively investigated over the last decade like liposomes, nanoparticles, nanoconjugates, and microemulsions, which have been aimed at enhancing bioavailability by solubilization or encapsulation thus protecting against hydrolysis and achieving sustained release at the site of targeting [9-14]. Microemulsion as a topical carrier system is very suitable with merits like low skin irritation, excellent permeation, and high drug-loading capacity as compared to other colloidal carriers [15, 16]. However, due to low retention at the site of application by virtue of low viscosity of microemulsion system and inconvenient application, it is often converted into a gel form [17-20]. Osteoarthritis is characterized by the breakdown of cartilage, joint lining, ligaments and underlying bone.[1-3] It typically involves an entire joint, with the most commonly affected joints being the knees, hips, hands, and spine. Common manifestations of osteoarthritis are pain and stiffness. There are a variety of risk factors for osteoarthritis, including obesity, high impact Sports, and bone deformities. The prevalence of osteoarthritis increases with age [21-23].

Hence, in the present study a gel based Microemulsion system loaded with curcumin intended to be used as adjunct therapy for the treatment of Osteoarthritis was developed and evaluate the effect of varying the composition, entrapment efficiency, curcumin concentration, Zeta potential, vesicle size and stability studies.

MATERIALS AND METHOD

The sample of curcumin used in this study was supplied by Sigma Aldrich (MKE, USA). Ethanol, methanol, analytical grade Tween 80 and Span 80 were purchased from Merck (MA, USA). Oleic acid, Carbopol 934 and n Butanol was purchased from S. D. Fine Chem. Ltd., Mumbai. All other surfactant and co surfactant were purchased from Hi Media, Mumbai. Double distilled water was prepared freshly and used whenever required. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

Solubility studies

Solubility determination in the various oils, surfactants and co-surfactants for formulating microemulsion drug delivery system. The solubility of the drug in different oils is an essential step for the microemulsion formulation. So before starting the phase diagram one must have to select the oil, surfactant and co-surfactant in which the drug shows maximum solubility, to be in the desired solubility range, which is essential for the formulation of microemulsion drug delivery system.

Formulation of Curcumin -loaded microemulsion

Eight different formulations has been selected by keeping the total quantity of the formulation constant as 100% and varying all components of the system. Each formulation has been loaded with Curcumin of 10mg/ml. All eight formulations have been evaluated for different parameters such as pH, *In-vitro* release and solubility (Table 1).

Table 1: Formulation composition of prototype formulations

Formulation code	Drug	Oil (Ratio)	Surfactant: Co-surfactants (Smix ratio)
F1	10	1	1:2
F2	10	1	1:3
F3	10	1	1:4
F4	10	1	1:5
F5	10	1	1:6
F6	10	1	1:7
F7	10	1	1:8
F8	10	1	1:9

Evaluation of Formulations

pH Determination

The pH of each formulation was found before and after dilution by using pH meter Table-2.

Table 2: Results of pH of Curcumin loaded microemulsion

S. No.	Formulation code	pH*
1	F1	6.98±0.02
2	F2	7.01±0.01
3	F3	6.99±0.02
4	F4	7.01±0.02
5	F5	6.98±0.02
6	F6	6.99±0.03
7	F7	6.65±0.01
8	F8	6.66±0.02

Centrifugation

This parameter characterized to check the physical stability of formulation. The microemulsion system was centrifuged at 5000 rpm for 10 minutes to determine whether the system shows signs of creaming or phase separation. The system was observed visually for appearance Table-3.

Determination of % Drug Content in microemulsion

The mixture (Microemulsion) was centrifuged at 1000 rpm for 15 min, 0.2 ml of supernatant was taken and diluted with 0.1 N HCL. Absorbance was measured at 428nm by UV Spectrophotometer. Concentration of Curcumin was determined using standard curve equation and % drug content was calculated Table-3.

Table 3: Results of Centrifugation and % Drug Content in microemulsion

Formulation Code	Centrifugation	% Drug Content in microemulsion*
F1	Translucent	75.65±0.45
F2	Translucent	74.56±0.36
F3	Translucent	69.98±0.21
F4	Transparent	71.45±0.25
F5	Transparent	75.65±0.14
F6	Transparent	83.32±0.15
F7	Transparent	80.14±0.25
F8	Transparent	75.01±0.32

*Average of three determinations

Zeta Potential and Vesicle size

Zeta Potential of samples was measured by Zetasizer. Samples were placed in clear disposable zeta cells and results were recorded (Fig 1&2).

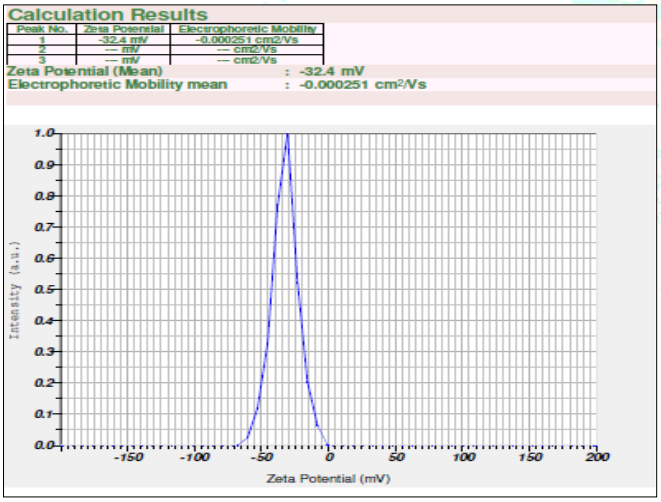

Figure 1: Result of Zeta Potential of Optimized Batch F6 (-32.4mV)

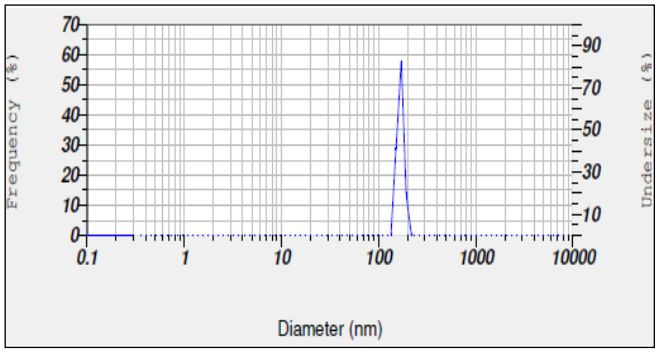

Figure 2: Result of Vesicle size of Optimized Batch

Preparation of carbopol gel base: 0.5 g Carbopol 934 was weighed and dispersed in water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 2 ml of glycerin was added to for gel consistency. Similarly 1 and 2% carbopol gels were prepared.

Preparation of microemulsion gel: Equivalent to 1g of microemulsion formulation was dissolved in 10ml of ethanol and centrifuged at 6000 rpm for 20 minutes to remove the unentrapped drug. The supernant was decanted and sediment was incorporated into the gel vehicle.The incorporation of the microemulsion into gels was achieved by slow mechanical mixing at 25 rpm for 10 minutes. The optimized formulation was incorporated into three different gel concentration 0.5, 1 and 2% w/w (Table-4).

Table 4: Composition of different gel base

Formulation	Carbopol (%)
F1	0.5
F2	1.0
F3	2.0

Evaluation of Gel

Determination of pH: Weighed 50 gm of gel formulation were transferred in 10 ml of beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 6–7 to treat the skin infections.

Spreadability: A modified apparatus suggested was used for determining spreadability. The spreadability was measured on the basis of slip and drag characteristics of the gels. The modified apparatus was fabricated and consisted of two glass slides, the lower one was fixed to a wooden plate and the upper one was attached by a hook to a balance. The spreadability was determined by using the formula: S=ml/t, where S, is spread ability, m is weight in the pan tied to upper slide and t is the time taken to travel a specific distance and l is the distance traveled. For the practical purpose the mass, length was kept constant and‘t’ was determined.

The measurement of spreadability of each formulation was in triplicate and the average values are presented.

Measurement of viscosity: The viscosity of gels was determined by using a Brook Field viscometer DV-II model. A T-Bar spindle in combination with a helipath stand was used to measure the viscosity and have accurate readings.

The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size etc. which affect the viscosity were maintained during the process. The helipath T- bar spindle was moved up and down giving viscosities at number of points along the path. The torque reading was always greater than 10%. Five readings taken over a period of 60 sec. were averaged to obtain the viscosity.

Drug content: Equvalant to 10mg (Curcumin) of the prepared gel was mixed with 100 ml. of ethyl alcohol. Aliquots of different concentrations were prepared by suitable dilutions after filtering the stock solution and the absorbance was measured at 242 nm. Drug content was calculated by linear regression analysis of the calibration curve (Table-5).

Table 5: Results of microemulsion gel formulations

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
F1	98.15± 0.021	6.98±0.021	18.32±0.075	5898±15
F2	98.05 ± 0.021	7.10±0.040	17.56±0.042	5945±23
F3	99.45 ±0.027	7.05±0.060	16.95±0.059	6123±45

***In-vitro* diffusion study:** An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. Microemulsion gel equivalent to 5mg of drug was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer (Table-6).

Table 6: Interpretation of diffusional release mechanisms

Release exponent (*n*)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5<*n*<1.0	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

Mathematical treatment of *in-vitro* release data: The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used Table-7-9.

Zero-order kinetics: The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model Fig-3:

$$Q_t = Q_o + K_o t$$

where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution (most times, Q_o=0) and K_o is the zero order release constant (Bourne, 2002).

First-order kinetics: The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution and K_1 is the zero order release constant Table-10.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish Fig-4.

Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The simplified Higuchi model is expressed as:

$$Q = K_H \cdot t^{1/2}$$

Where Q is the amount of drug released in time t and K_H is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the

Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drugs.

Korsmeyer-Peppas model: Korsmeyer *et al*. used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\, t^n$$

Where M_t/M_∞ is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log M_t/M_∞ versus log time curve. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of *n* =0.5 for fickian diffusion and higher values of *n*, between 0.5 and 1.0, or *n* =1.0, for mass transfer following a non-fickian model. In case of a cylinder *n* =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent *n* the portion of the release curve where $M_t/M_\infty < 0.6$ should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (*l*) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and b value would be zero and only at^n is used. This mathematical model, also known as *Power Law*, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms.

Table 7: *In-vitro* drug release data for formulation F1

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	13.560	1.132	86.440	1.937
1	1.000	0.000	32.560	1.513	67.440	1.829
2	1.414	0.301	65.560	1.817	34.440	1.537
4	2.000	0.602	75.580	1.878	24.420	1.388
6	2.449	0.778	76.200	1.882	23.800	1.377
8	2.828	0.903	76.210	1.882	23.790	1.376

*Average of three readings

Table 8: *In-vitro* drug release data for formulation F2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.250	1.306	79.750	1.902
1	1.000	0.000	45.580	1.659	54.420	1.736
2	1.414	0.301	68.890	1.838	31.110	1.493
4	2.000	0.602	73.250	1.865	26.750	1.427
6	2.449	0.778	73.560	1.867	26.440	1.422
8	2.828	0.903	74.150	1.870	25.850	1.412

*Average of three readings

Table 9: *In-vitro* drug release data for formulation F3

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	18.890	1.276	81.110	1.909
1	1.000	0.000	38.890	1.590	61.110	1.786
2	1.414	0.301	42.560	1.629	57.440	1.759
4	2.000	0.602	54.650	1.738	45.350	1.657
6	2.449	0.778	69.980	1.845	30.020	1.477
8	2.828	0.903	87.980	1.944	12.020	1.080

*Average of three readings

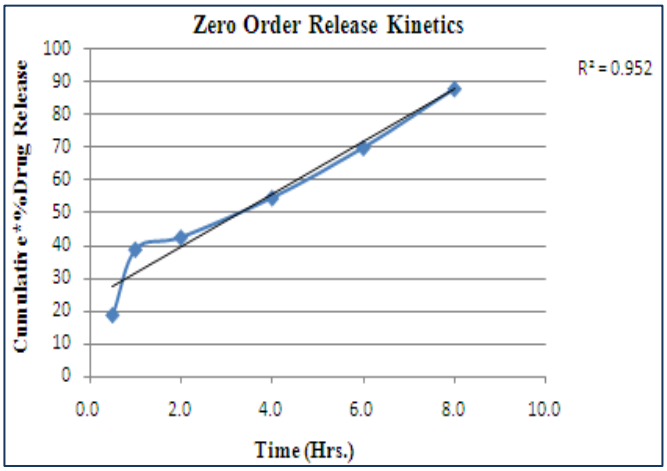

Figure 3: Cumulative % drug released Vs Time

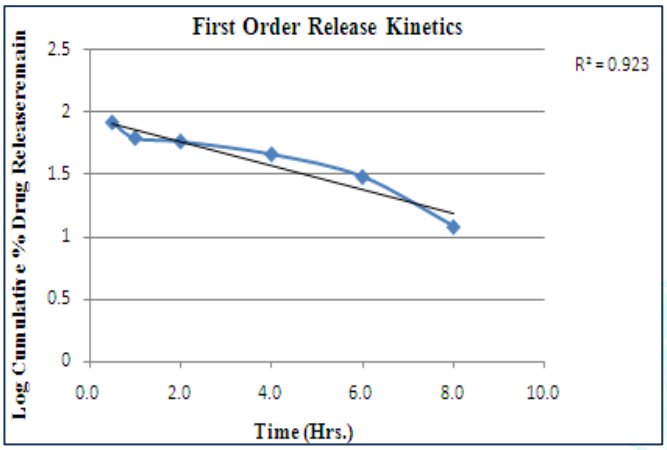

Figure 4: Log cumulative % drug remaining Vs Time

Table 10: Regression analysis data of optimized formulation

Batch	Zero Order	First Order
	R^2	R^2
F3	0.952	0.923

Stability studies

Curcumin loaded microemulsion gel was prepared and stored for 2 months first at cold condition (2ºC – 8ºC), room temperature and at elevated temperature (50º ±2ºC) and evaluated by visual inspection (phase separation).

RESULTS AND DISCUSSION

The preliminary study showed that curcumin is yellow, aromatic, bitter powder. It is freely soluble in ethanol, methanol, soluble in 0.1 N NaoH and 7.2 pH phosphate buffer and insoluble in water, chloroform and 0.1 N HCl. The melting point was in the range of 182-184^{0}C which is in compliance with the standard value of 183^{0}C as per Indian Pharmacopoeia. From the FT-IR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak. On the basis of above study it was concluded that the solubility in the oils, surfactants and co surfactants like Span 40, Span 80, Tween 20, Tween 80, Pluronic F-127, Castor Oil was found to be soluble and Sunflower Oil, Oleic acid was found to be slightly soluble for the microemulsion preparation of curcumin. Different physicochemical properties of the selected oils were studied and were found to be favourable for microemulsion drug delivery system. The Vesicle size analysis of the optimized formulation F6 was done using particle size analyzer (Horiba).The mean Vesicle size was found to be 41.6nm. Zeta potential of the optimized formulation F6 was determined using particle size analyzer (Horiba). Zeta potential of optimized formulation was found to be -32.4mV. Drug content is most important in microemulsion formulation and the data found are satisfactory. It was found to be 69.98±0.21to 83.32±0.15% which shows the good capacity of formulation to hold the drug. Three Different carbopol gel base prepared for optimization (0.5%, 1.0% and 2%) and evaluated for pH, spreadibility, viscosity measurements and *in vitro* drug release studies. The spreadability was measured on the basis of slip and drag characteristics of the gels and was in the range of 16.95±0.059– 18.32±0.075gms. cm. /sec. *In vitro* drug release study of Optimized formulation was carried out using modified franz Diffusion cell. The optimized formulation F6 showed the maximum 83.32±0.15% drug release in 8 hrs.

CONCLUSIONS

Studies on phase behavior for optimization of microemulsion properties are important when low-energy emulsification method is used, because the phases involved during emulsification are determinant in order to obtain microemulsions of small and uniform droplet size.All the prepared formulations exhibited the microemulsion properties. The optimized formulation was evaluated for zeta potential, globule size analysis and stability study. The results suggest that the optimized formulation was stable and produced microemulsion. The in-vitro diffusion study of the formulation was higher as compared to pure drug, indicating that the prepared formulation is having higher solubility and permeability. Thus it can be concluded that microemulsion formulation can be used as a one of the formulation technique to enhance the bioavailability of the poorly soluble and permeable drugs.

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