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Design and Development of *Cuminum cyminum* L. Seed Extract Microemulsion for Anaemia

D. A. Chougule*¹, D. T. Gaikwad², R. J. Jarag³

^{*1,2}Departments of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur-416013, Maharashtra, India; ³Department of Pharmacology, Bharati Vidyapeeth College of Pharmacy, Kolhapur-416013, Maharashtra, India

ABSTRACT

The aim of study was to develop and evaluate microemulsion for permeation enhancement of Cumin in treatment of anaemia. Successful attempt was made to carry out formulation of Cumin loaded microemulsion. Oleic acid selected as oil phase, tween 80 and propylene glycol selected as surfactant and co-surfactant, from pseudoternary phase diagram different concentration of oil, surfactant, co-surfactant and distilled water were optimized and water dilution method was used for microemulsion preparation. Cumin extract loaded microemulsion were formulated and characterized for particle size, PDI, zeta potential, viscosity, percent transmittance, RI and drug content. The ex vivo permeation study was performed on goat small intestine. It was observed that 55.50% Cumin extract and 87.19% Cumin loaded microemulsion was permeated. This data indicates that Cumin loaded microemulsion has greater permeation as compared to extract. In vivo study data observed that microemulsion formulation increases the Hb, RBC, MCV, MCH, MCHC level as compared with extract. The results clearly indicate that microemulsion increases the Hb and RBC and bring towards the normal level.

Keywords: Cuminum Cyminum, Microemulsion, Anaemia

***Correspondence to Author:**
D. A. Chougule

Departments of Pharmaceutics,
Bharati Vidyapeeth College of
Pharmacy, Kolhapur-416013,
Maharashtra, India

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1.0. Introduction

Thermodynamically microemulsions are clear, stable, transparent, isotropic dispersions. (1) Microemulsion is important for the enhancement of solubility and the bioavailability of less soluble drugs and first time introduced by Hoar and Schulman in 1943. Microemulsions are truly a quaternary (pseudoternary) system composed of oil and aqueous phases accompanied with a surfactant and a cosurfactant. (2) The structurally microemulsion is divided into three parts, water in oil (w/o), oil in water (o/w) and bicontinuous microemulsions. (1) Pharmaceutically acceptable, non-toxic and non irritating excipients were used. Oleic acid selected as oil phase, span 80 and propylene glycol selected as surfactant and co-surfactant. From pseudoternary phase diagram different concentration of oil, surfactant, co-surfactant and distilled water were optimized and further proceed for microemulsion formulation. Water titration method was used for preparation of microemulsion. *Cuminum (Cumin) cyminum* L. is a tiny low annual herb that's a member of the aromatic family Umbelliferae. It is indigenous of the Japanese Mediterranean countries and Upper Egypt, however is now cultivated in Morocco, Iran, Turkey, India, China and also the Americas. (3) This spice is contains excellent source of minerals like, iron, copper, calcium manganese, selenium, Zn and Mg. *Cuminum cyminum* L. is known as Jeera and has been used in diet since ages. Recently it has been reported to have beneficial effect on metabolic disorders. *Cuminum cyminum* L. seeds are a nutritious additive for people with anaemia Cumin is very rich in iron (above 66 mg. in each 100 grams) which is more than 5 times the daily requirement of iron for an adult. (4). Iron is the main constituent in synthesis of haemoglobin. Cumin contains non heme iron, and it is not bound to heme protein, gets absorbed less easily, there is problem with permeation also. So an approach was made to

improve the permeation of non heme iron by formulating it in microemulsion.

Anaemia is a disease in which decreases the total RBC or Hb or lowers the ability of blood to carry oxygen. The symptoms of anaemia are, feeling tired, weakness. (5, 6) Anemia is that the commonest blood disease out of different types of anaemia, iron deficiency anaemia is the most common. Its common in girls and women's than men. (7)

2.0. Material and Method

2.1. Material

2.1.1. Chemicals

Phenylhydrazine (Loba Chemie), Methanol 99.8% (Loba Chemie), iso-Propyl myristate (Loba Chemie), Ethyl oleate (Loba Chemie), Span 80 (Loba Chemie), Tween 80 (Loba Chemie), Span 20 (Loba Chemie), Tween 20 (Loba Chemie), Propylene glycol (Loba Chemie), Olive oil (Loba Chemie) PEG (Loba Chemie).

2.1.2. Collection of cumin seed

Cuminum cyminum seed were purchase from local market Kolhapur, Maharashtra, India. A voucher specimen was deposited and authenticated by Dr. Madhukar Bachulkar, Plant Taxonomist, and Principal Shri Yadav Arts & Science College Pethvadgaon. The seeds were crushed in a mechanical grinder and used for further analysis

2.1.3. Animal

Either sex albino rats were selected as experimental model having weight 200-250 gm. The study protocol was approved by CPCSEA animal ethical committee with reference number BVCPK/CPCSEA/IAEC/01/15/2017-2018 at Bharati Vidyapeeth College of Pharmacy, Kolhapur. They were kept under standard conditions as per guidelines of CPCSEA and were fed with pellet feed and sterile water.

2.2. Method

2.2.1. Preparation of Cumin seed extract

50 gm of Cumin seed powder was taken in 500 ml mixture of water and methanol in a ratio of 7:3. It was kept in orbital shaker for 3 days at 60 rpm. After 3 days of maceration, macerate was subjected to filtration by whatman filter paper (0.45 μ m) and filtrate was collected. Drying of filtrate was carried out by Rotary evaporator at pressure 10mmHg and temperature 60°C. Obtained extract was stored in vial at 2-8°C till the use. (8)

2.2.2. Phytochemical screening

Extract was subjected to various phytochemicals test to determine the nature of constituent of extract. (khandelwal book)

2.2.3. Determination of absorbance maxima (λ_{max})

Stock solution was prepared by addition of 10 mg of Cumin in 100 mL of water containing flask. Obtained solution containing 100 μ g/mL of Cumin was scanned in the range of 200-600 nm in SHIMADZU 81220 spectrophotometer. The maximum wavelength was determined.

2.2.4. ATR-FTIR

The ATR-FTIR spectra of Cumin were recorded by using Infrared spectrophotometer (JASCO FTIR-4100). About 15-20 mg of extract in a drop form was placed in a dwell to fill it by 60-80% uniformly mixed sample kept in sample holder & spectra was recorded over the wave number 1000-4000 cm^{-1} on ATR-FTIR spectrophotometer. (9)

2.2.5. Atomic absorption spectroscopy (Estimation of Iron)

Nitric-hydrochloric acid digestion (1:3)

0.1 gm of Cumin extract and 1.0 gm of Cumin powder were accurately weighed and placed in 100 mL of beaker. 9 ml of 65 % HNO_3 and 37 % HCl was added. Then, the mixture is dissolved completely over a water bath. During the digestion process add 2 ml of water to prevent the loss of sample, then sample was filtrate and add sufficient amount of water and make up volume up to 30 mL. Sample was analyzed on atomic absorption spectroscopy. (10)

2.2.6. Solubility study of surfactant, co-surfactant and oil

The solubility of Cumin in surfactants, co-surfactants and oil was determined by dissolving an excess amount of Cumin in 2 mL of each of the selected oils, surfactants and co-surfactant. The vials are kept in orbital shaker for 72 hours at 37°C. Then it centrifuged to 3000 rpm for 15 minutes and filtrated using membrane filter. Measured the absorbance and find out concentration of surfactant, co-surfactant and oil. (11)

2.2.7. Construction of ternary phase diagram

For the construction of ternary phase diagram CHEMIX SCHOOL (3.60, Arne Stendnes) used to define the extent of the microemulsion regions. For the ratio of surfactant and co-surfactant were at first selected. Here four ratio of surfactant (Span 80) over co-surfactant (propylene glycol) were selected ($K_m=1$, $K_m=2$, $K_m=3$, $K_m=4$). For each ratio, increases the oil phase (oleic acid) concentration from 10% to 90% with respect to decreasing the concentration of Smix from 90% to 10% and drop wise addition of double distilled water until the first sign of turbidity appearance in order to identify end point. Our aim was to determine microemulsion region, the stage at which turbidity occurred were recorded. (12).

2.2.8. Preparation of microemulsion

Required amounts of the drug were dissolved in water. Oleic acid, surfactant and co-surfactant were mixed together by using vortex mixer at 2000 rpm for 20 minutes. Add distilled water drop wise in to the above mixture with continuous stirring. After that it kept for 2 days at room temperature, which resulted in transparent and homogenous microemulsion. (12) composition (% w/w) of C1 formulation Smix 40, oil 58 water 02, C2 Smix 50, oil 43, water 07 and C3 Smix 60, oil 30, water 10

2.2.9. Induction of anaemia

Phenylhydrazine 30 mg/kg i.p. injection given for 2 day to induce anaemia. (17)

2.2.10. *In Vitro* drug release

In vitro drug release study was performed by using egg membrane. The microemulsion formulation (3 mL) was added to egg membrane (0.45 μ m pore size). Before the experiment egg membrane was washed with phosphate buffer pH 6.8. The syringe which content 3 ml of Cumin microemulsion was injected into the egg membrane. The egg membrane was placed in 250 mL of phosphate-buffered (pH 6.8) sample was stirred at magnetic stirrer that at a rate of 100 rpm at 37°C. 5 mL Sample was taken up to 12 hr for 1 hour interval, and replace same volume of phosphate buffer to maintain a constant volume. The concentration of Cumin in the samples was analyzed using UV spectrophotometer. The amount of cumulative drug released was calculated as a function of time and the release rate was determined.

2.2.11. *Ex vivo* Drug Permeation

To check the permeation of drug through intestine, small intestine of goat was collected from local market and used for the study. To remove any mucous and lumen contents of intestine, tissue was washed with pH 6.8 phosphate buffered saline. The syringe which content 3ml of Cumin microemulsion was injected into the lumen of the small intestine. The tissue was placed in a beaker containing 250 ml of phosphate-buffer pH 6.8 with constant stirring at 37°C. The oxygen tube was placed in beaker containing tissue. The two ends of tissues were fixed horizontally on to a beaker with the help of a thread. 4 mL sample was taken up to 12 hr for 1 hr interval and replace same volume of phosphate buffer. Measure the absorbance on UV spectrophotometer at a wavelength of 210 nm. The amount of drug permeated (%) was calculated by using PCP disso software. (18)

2.2.12. Preclinical study

A total 24 albino rats should randomly assign to four groups (6 animals in each group) and treated daily for 15 days as follows

Group I: Serve as control

Group II: Treated with Standard Elemental iron containing suspension 0.68 ml/kg

Group III: Treated with Cumin extract 400 mg/kg

Group IV: Treated with Cumin extract loaded microemulsion

Retro-orbital venous was used for the collection of blood. The blood was collected before induction of anaemia, after induction of anaemia with PHZ and 15 day of treatments. The volume of blood collected (0.5ml). The different haematological parameters like RBCs, Hb, MCV, MCH, and MCHCS were determined at 0 and 15 days. (19)

2.3. Characterization of microemulsion

2.3.1. Physical characteristics

The microemulsion has different physical characteristics such as homogeneity and optical clarity. By the visual observation under black and white background clarity of the formulations was determined.

2.3.2. pH

The pH of microemulsion was measured by using Digital pH Meter (MK-6).

2.3.3. Percentage transmittance

Percent transmittance of optimized batch of microemulsion was measured by using double beam UV Spectrophotometer (SHIMADZU 81220). Transparency of microemulsion was measured at 650 nm wavelength.

2.3.4. Viscosity

The viscosity of the microemulsion was evaluated by Brookfield viscometer (Brookfield DV-II + Pro) using LV 2 spindle, 2-5 rpm for 60 seconds at 25°C. A sample volume 50 ml was used.

2.3.5. Refractive index

For the measurement of refractive index of microemulsion Abbe's refractometer was used. The instrument was calibrated by distilled water which has refractive index 1.3325 at room temperature. A few drop of Cumin

microemulsion was placed into the prisms through capillary tube and the refractive index was observed and reported.

2.3.6. Particle size analysis and polydispersity index

Particle size and PDI was measured by Malvern Instruments (Serial Number: MAL1140144)

2.3.7. Zeta potential

Zeta potential was determined using Zetasizer Ver. 7.12 (Malvern Instrument Ltd., Serial Number: MAL1098084). The sample was diluted with oleic acid. Samples were placed in zeta cells and results were recorded.

2.3.8. Entrapment efficiency (EE %)

The 1 ml of microemulsion was given in suitable solvent. Then sample was placed in orbital shaker for 2 days at 25°C to achieve equilibrium. The samples centrifuged at 3000 rpm for 15 minutes. Take supernatant liquid and filtered through a Whatman filter paper (0.45 μm) and analyzed by UV spectrophotometer at respective wavelength. (13)

2.3.9. Drug content

1 mL of microemulsion which content 10 mg of drug was given in 4 ml of Solvent. The sample was kept in orbital shaker for 1 day to achieve equilibrium. The sample centrifuged at 3000 rpm for 15 minutes. Take supernatant liquid and filtered through a Whatman filter paper (0.45 μm) and analyzed by UV spectrophotometer at respective wavelength. (14)

2.3.10. Thermodynamic stability study (16)

a. Heating cooling cycle

The heating cooling cycle was performed on refrigerator temperature and 45°C temperature, six cycles were performed and stored at each temperature of not less than 48 hr. Those formulations, which pass this test, were selected for centrifugation test.

b. Centrifugation

Optimized microemulsion formulation was centrifuged at 4000 rpm for 15 minute at 0°C and observed any changes in homogeneity of microemulsions. Those formulations, which pass this test, were selected for Freeze-thaw cycles

c. Freeze-thaw cycles

This test was carried out between refrigerator temperature and 25°C with storage conditions of temperature for not less than 48 hours.

2.3.11. Dilution test

The continuous phase was added in microemulsions, and observed the crack or phase separation. Oleic acid was added in w/o type of microemulsions and it will be remain stable. (15)

2.3.12. Compatibility studies

Briefly 1mL formulation was taken in sample holder spectra were recorded over the wave number 500-4000 cm^{-1} . FTIR spectrum showed fundamental peaks corresponding to the structured components of the Cumin and excipients.

2.3.13. Microscopy

The globule size was confirmed by optical microscopy in 45X resolution. Microemulsion formulation was placed over a glass slide and measures the size of globule.

3.0. Result and discussion

3.1. Physical evaluation of Cumin

Extract was studied for physical evaluation by considering different parameters like colour, percentage yield, and nature of dry residue. The % yield (9.8%) was found in water and methanol solvent.

3.2. Phytochemical screening

The different tests were performed extracts showing presence of various active constituents have been shown in Table 2.

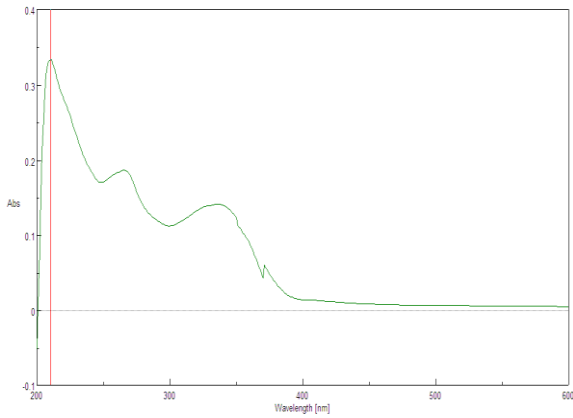


Figure 1: Maximum wavelength of Cumin

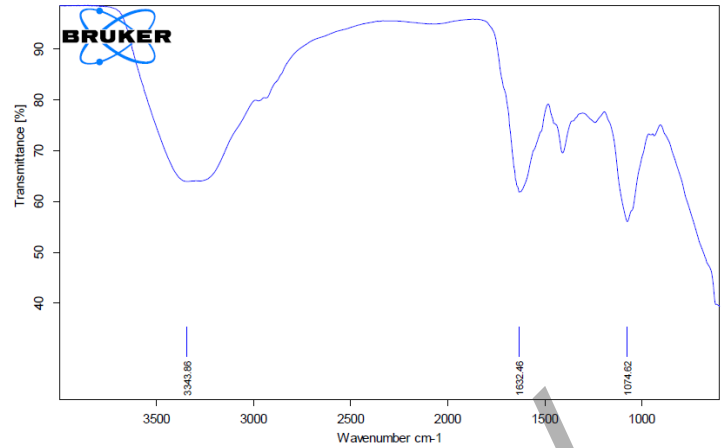


Figure 2: ATR-FT-IR spectra of Cumin



Figure 3: Optical microscopy (Motic microscopy 45X)

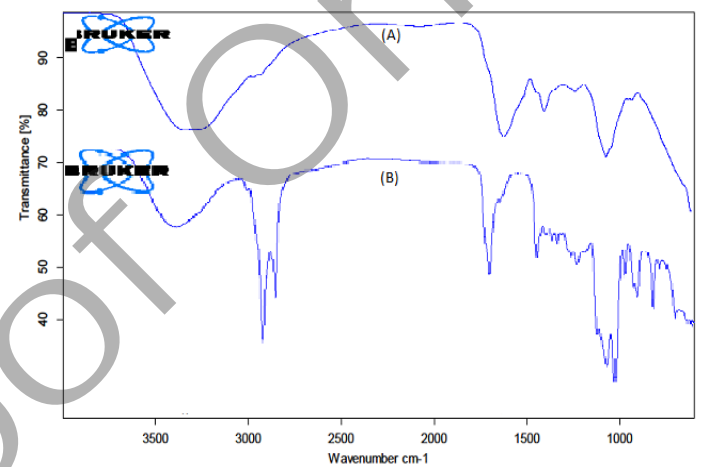


Figure 4: Compatibility of Cumin extract and Cumin loaded microemulsion

3.3. Determination of λ_{max}

For characterization of drug by UV spectroscopy, it is important to know the λ_{max} . The spectra of Cumin in water were taken and are given in figure 1 λ_{max} of Cumin in water given is 210 nm.

3.4. ATR- FT-IR Cumin extract

The FTIR spectrum of Cumin was taken for the characteristic peaks which indicate presence of different active constituents in the solvent extracts. ATR-FT-IR spectra of Cumin show presence of peaks at various wave numbers. The observed frequencies for the presence of different groups in aqueous and methanolic extract are Alcohol/Phenol O-H Stretch 3343, Amide N-H 1632.46, Alkyl halide C-F 1074.62.

3.5. Atomic absorption spectroscopy (Estimation of iron)

In 1gm of Cumin raw powder the concentration of iron was found to be 0.57 mg/g whereas in 0.100 gm extract the iron concentration was 0.48 mg/gm.

3.6. Solubility study in various oil, surfactant and co-surfactant

Solubility of drug in oil, surfactant and co-surfactant is another important factor, it helps maximum amount of drug in solubilize form. The solubility of drug in surfactant, co-surfactant and oil is given in table 3

3.7. Ternary phase diagram

Pseudoternary phase diagram were constructed separately for Km ratio so O/W

microemulsion regions could be identified and microemulsion formulations could be optimized. The ternary phase diagram was constructed by various ratios of Smix (Tween 80 & Propylene glycol), oil (Oleic acid), and water shown in figure 7.

Pseudo ternary phase diagram at $K_m=1$ shows more region of microemulsion than $K_m=2$, $K_m=3$ and $K_m=4$ as decreases the concentration of Smix less microemulsion region observed.

Phase behavior investigations of optimized system demonstrated the suitable approach in determining the water phase, oil phase and Smix concentration with which formed transparent, viscous microemulsion. The phase behavior study revealed that the maximum proportion of oil and Smix was incorporated in microemulsion formulation. From the formulation point of view more concentration of oil provides greater opportunity to permeation of Cumin.

3.8. Physical characteristics

The Cumin loaded microemulsion batches prepared by water titration method were found to be transparent microemulsions with characteristic odor and viscous in consistency. This is due to presence of surfactant and oil in the dispersions. There was no precipitation in microemulsions.

3.9. pH

The pH of microemulsion was measured by using Digital pH Meter (MK-6) which shown in table 1 as concentration of water increases pH of formulation also increases. C3 batch contains more amount of water than C1 and C2.

3.10. Percentage transmittance

Percentage transmittance of optimized batch of microemulsion was measured by using double beam UV Spectrophotometer (SHIMADZU 81220). Transmittance shows that clarity of formulation which shown in table 1.

3.11. Viscosity

The viscosity of microemulsion was determined by Brookfield Viscometer at room temperature. The formulations of various batches exhibit Newtonian Flow. Viscosity of microemulsion decreases with decrease in oil concentration and increases water concentration. C3 batch has less oil and more water content than other batches. Hence it has low viscosity than other batches.

3.12. Refractive index

For the measurement of refractive index of microemulsion Abbe's refractometer was used. The refractive index values are more than water because it contains oil and Smix. The refractive index of different batches of Cumin loaded formulation shown in table 1.

3.13. Particle size analysis and polydispersity index

Particle size distribution is one of the most important characteristics for the evaluation of the stability of oil based microemulsions. A graphical representation of particle size distribution of freshly prepared Cumin loaded micro-emulsions is given in Figure 8.

For Cumin loaded microemulsion indicated that the particle size of batches C1, C2, and C3 were 327.7, 431.1, 292.4, d. nm, respectively. Good reproducibility of the mean particle size was obtained for all microemulsion batches.

3.14. Zeta Potential

The zeta potential is the indicator of the stability of formulation. Negative zeta potential observed in all the batches this may be due to carboxylic group of oleic acid and insufficiency of counter ions for neutralization within electrically double diffuse layer. The C3 batch has zeta potential -34.5 it indicates that microemulsion is stable. Molecule and particle are small enough hence it has high zeta potential and it confirms the stability of microemulsion.

The C1 and C2 batch contains -13.6 and -21.1 it indicates that they are stable there was no aggregation and fluctuation in the microemulsion. All batches have a sufficient

charge to and mobility to inhibit aggregation of particles.

Zeta potential of C1, C2, and C3 batches contains -13.6, -21.6 and -34.5 respectively. It was observed that zeta potential values decreases as concentration of oil increases.

3.15. Entrapment efficiency (EE %)

In entrapment efficiency we measure the concentration of free compound in the dispersion medium. The different formulation batches C1, C2 and C3 were evaluated for entrapment efficiency. Entrapment efficiency of C1, C2, and C3 batches contains 74, 76.23 and 80 percentage respectively. The C3 batch has more entrapment value than C1 and C2 because it contains more amount of water hence more amount of drug is entrapped.

3.16. Drug content determination

The drug content of the microemulsions was determined by UV spectroscopy method. Drug content all batches is found shown in Table 1.

3.17. Thermodynamic stability study

The optimized formulation did not show any phase separation, creaming or cracking after heating-cooling cycles, centrifugation tests and freeze thaw tests hence it is thermodynamically stable.

3.18. Dilution test

Upon addition of continuous phase (oil), the microemulsions did not crack separate into phases which show that the formed microemulsion is w/o type and is stable.

3.19. Microscopy:

Globule size of microemulsion was determined by optical microscopy which was found to be in μm . shown in Figure 7.21

3.20. COMPATIBILITY TEST

The objective behind compatibility studies of drug and formulation by FTIR is to study the chemical interaction between them, which may affect the results positively or negatively. FTIR analysis indicated that the formulation absorption peak was closed to the Cumin extract. It indicated that there was no significant

interaction between Cumin and excipients. The IR characteristic peaks of Cumin observed at 3343, 1632.46, 1074.62. These peaks were found to be similar to the peaks that represent the O-H stretching (phenol), N-H stretching (amide), C-F stretching (alkyl halide). The IR spectrum of formulation indicated principal peaks belonging to measure functional group such as 3383 (O-H stretching phenol), 2922 (O-H stretching Acid), 1710 (C=O stretching Acid), 1042.80 (C-F stretching Alkyl halide), 1038.95 and 837.38 (=C-H stretch, Alkene).

3.21. Optimization of microemulsion

Prepared microemulsion was evaluated by particle size, PDI, zeta potential and viscosity for optimization.

The batch which lower particle size, zeta potential, PDI and higher % transmittance and drug content were selected. The selection of this batch was based on, lower the particle size results grater permeation and absorption of drug, from PDI find out the uniformity of formulation which is less than 1. More negative zeta potential is considered as more physical stability of the formulation and higher % transmittance indicate the uniform system more amount of drug content gives more therapeutic action.

From the results, batch C3 (30% Oleic acid, % 60% Smix 10% water) was selected as optimized batch having globule size of 292.4 nm, -34.5 mV zeta potential, 0.254 PDI and 98.50% transmittance and 99.30% drug content.

3.22. *In vitro* drug release

In vitro drug release studies were carried out in egg membrane. Egg membrane retained microemulsion and allowed Cumin molecules to pass through, which were released over the time into the dissolution medium. The drug release profile is shown in Figure 6. Significant variation in the drug release rate was observed. Results reveal initial burst release in first 2 hours with 23.50% of drug release. Drug

release in later stage was continuous and indicating slow drug release.

Data generated shows drug release profile of formulation shows first order model. The developed microemulsion formulation improved the solubility and in-vitro drug release of Cumin. The total 79.42% of the drug release at 12 hours.

3.23. Ex vivo drug permeation

The drug permeation profile of Cumin and Cumin loaded microemulsion shown in figure 5. The drug release data obtained in permeation study was fitted to models representing zero, first order, Higuchi's, and Korsmeyer's equation to know the release mechanisms. The 55.50% of pure Cumin was permitted and 87.19% of Cumin loaded microemulsion was permitted. We compare the Cumin loaded microemulsion with pure Cumin to check the permeation. The observed data shows that release exponent is an anomalous transport. It was concluded that

Cumin loaded microemulsion has a more drug permitted than Cumin extract.

3.24. Priclinal study

To check anti-anaemic effect of Cumin seed extract and Cumin loaded microemulsion. Either sex albino rats were selected as experimental model having weight 200-250gm. The different haematological parameters like RBCs, Hb, PCV, MCV, MCH, and MCHCS were measured

All the groups like control, standard, extract and microemulsion increases the level of all parameters. After treatment extract and Cumin loaded microemulsion has Hb 9.2 and 11.4 resp. it indicates that more amount of drug is permeated in microemulsion. In standard treatment we observe that there was a significant rise in Hb level as compared to microemulsion. We also observed that significant increases the level of other parameters like MCH, MCV, MCHC after the treatment.

Table 1: Evaluation studies of w/o microemulsion

Formulation	pH	Percentage Transmittance	Viscosity	Refractive Index	Entrapment Efficiency	Drug Content
C1	4.45 ± 0.04713	98.08 ± 0.2760	65.33 ± 0.1632	1.448 ± 0.000471	74.00	98.25
C2	4.65 ± 0.04714	98.06 ± 0.1228	64.26 ± 0.0942	1.428 ± 0.000471	76.23	98.10
C3	4.85 ± 0.04714	98.95 ± 0.1433	63.86 ± 0.0471	1.415 ± 0.000471	80.00	99.30

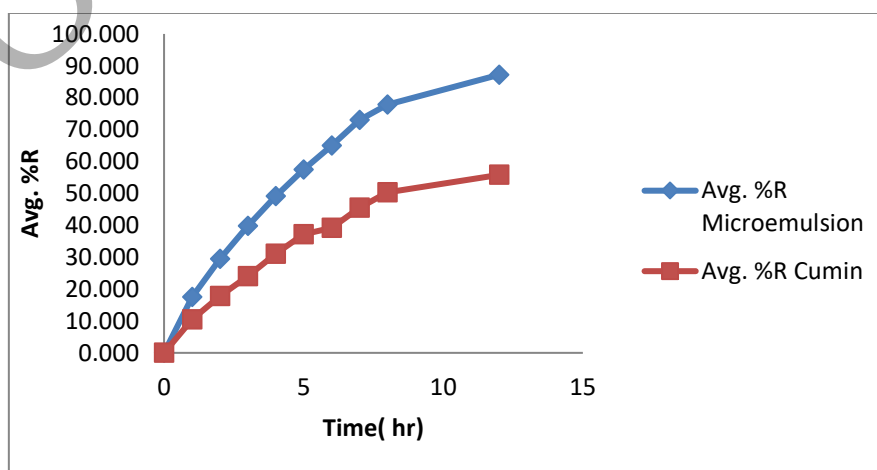


Figure 5: Ex vivo permeation of Cumin and microemulsion

Table 2: Phytochemical screening

Sr. No.	Test	Results
1	Test for Carbohydrates	+
2	Test for proteins	+
3	Test for Glycosides	
	Saponin glycosides	+
4	Flavonoids	+
5	Alkaloids	-
6	Tannins	+
7	Phenols- Dil. Iodine solution	+
8	Test for iron	
	Ammonium thiocyanate	+

Table 3: Solubility of Various components

Solubility of Cumin	Component	Solubility in mg/ml
Surfactant	Tween 20	3.96
	Tween 80	16.37
	Span 20	5.23
	Span 80	12.47
Oil	Oleic acid	3.99
	Ethyl oleate	3.70
	Iso-Propyl Myristate	3.30
	Olive oil	3.90
Co-surfactant	Ethanol	3.42
	PEG	3.50
	PG	4.14

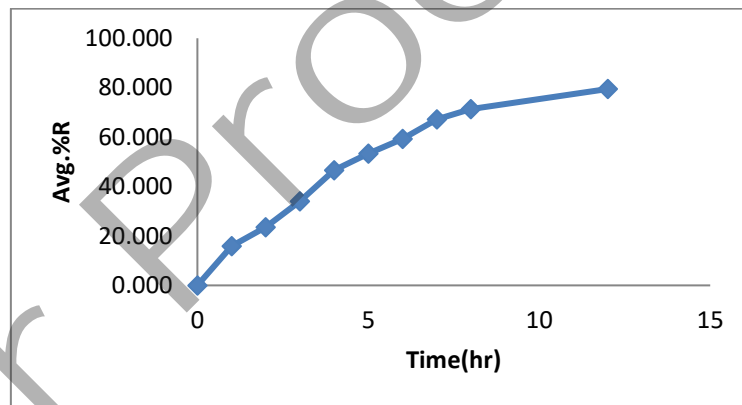
Figure 6: *In vitro* drug release profile

Table 4: Evaluation studies of w/o microemulsion

Formulation	Particle Size	PDI	Zeta Potential
C1	372.7	0.246	-13.6
C2	431.1	0.345	-21.1
C3	292.4	0.254	-34.5

Group	Hb (g/dl)		RBC($\times 10^6$ ul)		MCV (fl)		MCH (pg)		MCHC (g/dl)	
	Day									
	0	15	0	15	0	15	0	15	0	15
Extract	5.8	9.2	2.75	3.85	47.4	69.8	21.0	23.8	49.1	84.2
microemulsion	5.6	11.4	1.37	4.40	70.0	93.1	40.8	25.9	27.8	58.3
Standard	3.2	15.1	1.62	4.98	47.5	69.5	19.7	30.3	29.7	33.3
Control	13.2	14.6	7.27	7.29	51.0	51.0	18.9	20.0	37.1	39.2

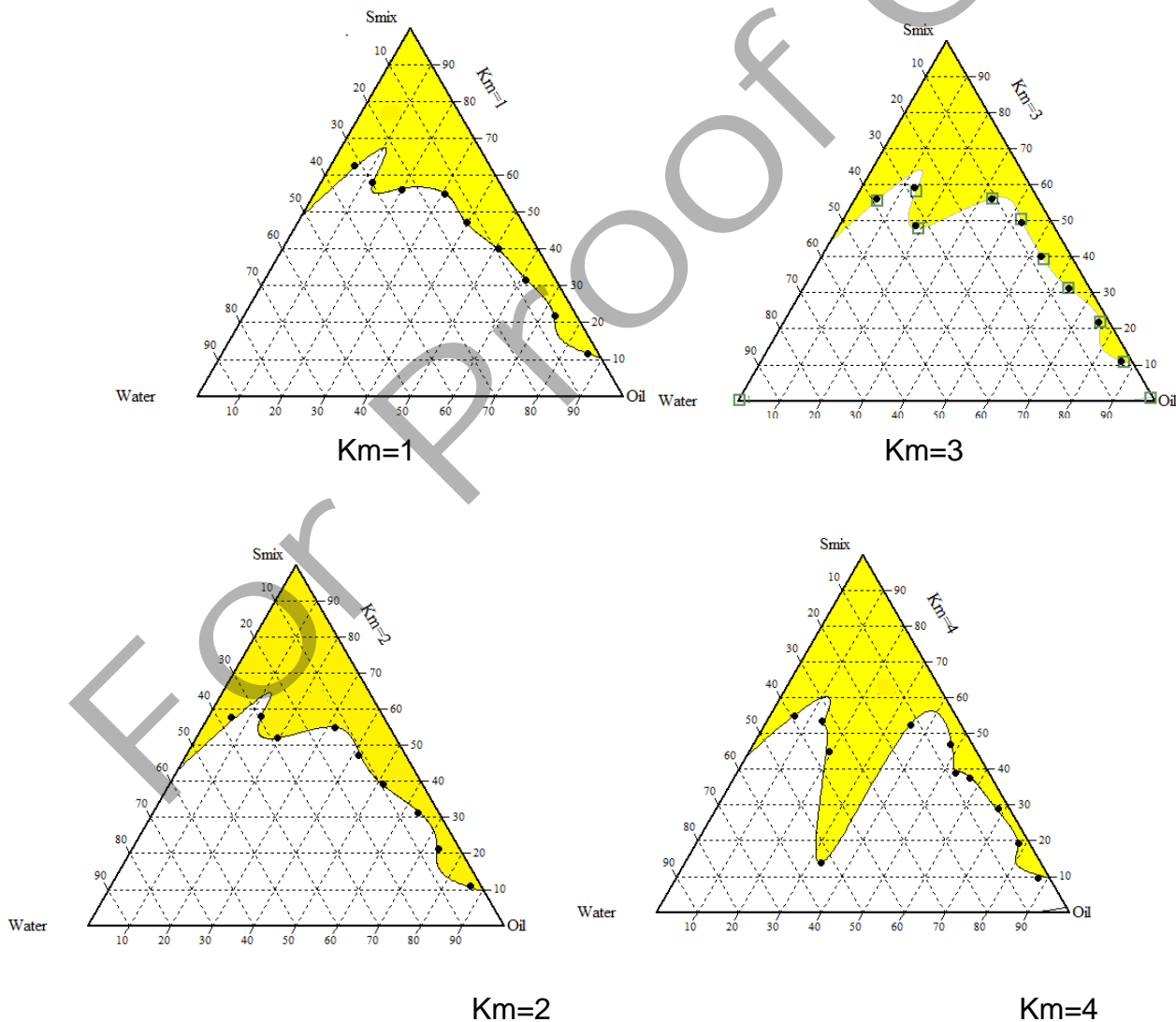


Figure 7: Ternary phase diagram

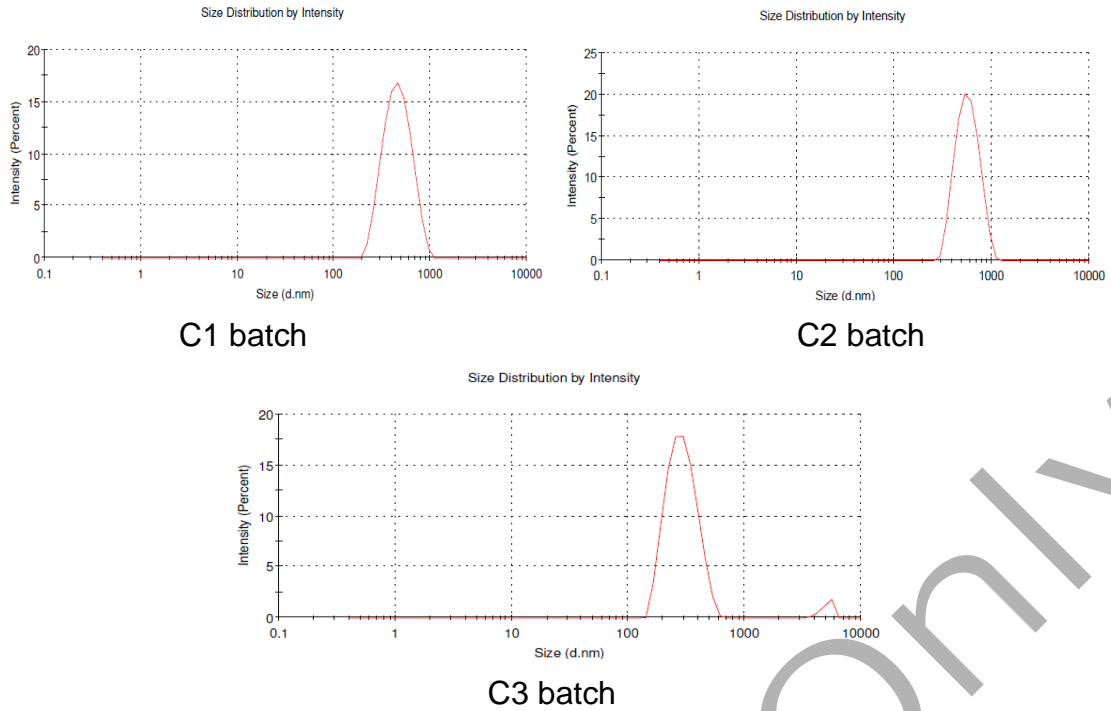


Figure 8: Particle size of microemulsion

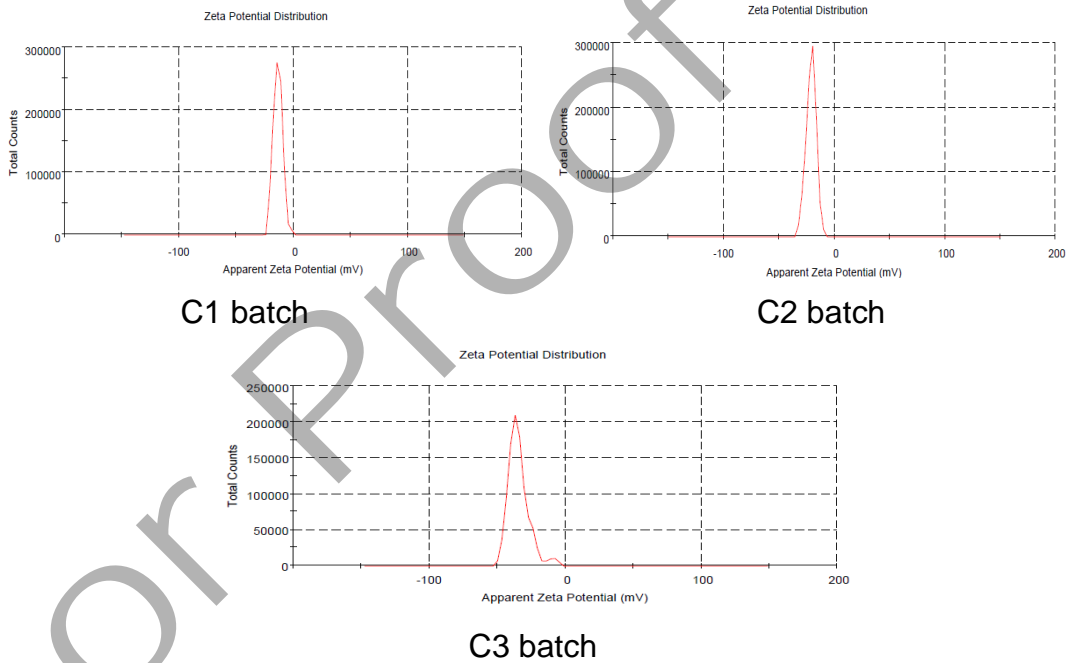


Figure 9: Zeta potential of microemulsion

Conclusion

Successful attempt was made to carry out Cumin extract microemulsion for anaemia. Prepared Cumin loaded microemulsion has low zeta potential, particle size and higher drug content and percentage transmittance, it was found to be stable. The experimental finding of

ex vivo study supports that microemulsion has a potential to improve the permeation of non heme iron and is thus a promising delivery system. *In vivo* study in albino rats confirms that significant increases in Hb, RBC, MCV, MCH, and MCHC as compared with extract group this was due to presence of oil and Smix.

Hence Cumin extract microemulsion shows promising anti-anaemic activity in albino rats.

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