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Original Article

PREPARATION AND CHARACTERIZATION OF EDIBLE OIL NANOEMULSIONS FOR ENHANCED STABILITY AND ORAL DELIVERY OF CURCUMIN

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ABSTRACT

Objective: This work aims to improve the oral bioavailability and long-term aqueous stability of curcumin using various edible oil nanoemulsions (NEs).

Methods: NEs were optimized using the water titration method. Curcumin was loaded into optimized emulsions, and the physicochemical characteristics were determined. Long-term stability of curcumin in the edible oil NEs was analyzed by determining the droplet size, PDI and curcumin concentrations over 4 mo. Release of curcumin from the NEs was determined using a Franz diffusion apparatus and analysed using 5 mathematical models.

Results: The absorbance of curcumin was linear over the concentration range of 1 to 10 µg. ml^{-1}. The LOD and LOQ ranged from 0.57 to 1.26µg. ml^{-1} and 1.89 to 4.19µg. ml^{-1} respectively. All the NEs were monodisperse and had a droplet size less than 150 nm. Long-term emulsion stability shows no change in droplet size and PI (Dunnett's multiple comparisons test with a confidence interval of 95%). Olive oil NE showed significantly low release in gastric fluid (9.28%) with a good release (92.99%) in intestinal fluid and 48% in a body fluid by 8 h.

Conclusion: The work highlights the use of olive oil NEs as a delivery vehicle for curcumin with excellent release characteristics and the ability to protect curcumin in an aqueous environment.

Keywords: Edible oil nanoemulsions, Nanoemulsion, Olive oil emulsions, Curcumin delivery system, Oral bioavailability, Release kinetics

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INTRODUCTION

Curcumin, the potent phytochemical present in turmeric is obtained from the rhizomes of the plant *Curcuma longa* [1]. This phytochemical has a significant biological activity such as anti-inflammatory [2] anti-oxidant [3], anti-cancerous [4], antimicrobial [5], and daily intake with good bio-distribution ensures good cerebral [6] and cardiac health [7].

Various studies focussing on metabolism and distribution of curcumin have demonstrated very low or no availability of curcumin to the cells and the target tissues. The causes for this include, poor absorption, rapid metabolism and elimination with poor activity of the breakdown products of metabolism [8]. To compensate the issue of poor oral bioavailability various approaches have been identified, such as, nanosized curcumin preparation [9], lipid-based drug delivery with liposomal curcumin encapsulation [10], curcumin encapsulation in nano starch [11], complex formation of phospholipid and curcumin [12] and combinatorial metal and curcumin chelation [13].

The entrapment of the curcumin in oil phase can counteract the issue of poor aqueous solubility, as the aim of a nanoemulsion (NE) formulation of any compound is to increase the solubility, long-lasting stability, and improvement in biological activity. The release of curcumin from the emulsion has been studied through various *in vitro* models [14].

Although significant work has been done on nano-formulations of curcumin to improve the oral bioavailability, no studies, as far as we know, have demonstrated the stability and release characteristics of curcumin from edible oil NEs. This work thus focuses on utilization of edible oils such as coconut oil, castor oil, sunflower oil, gingelly oil and olive oil for the preparation of oil in water NE using bio-compatible surfactants, the determination of the long-term and accelerated stability of curcumin in these emulsions and the determination of the release characteristics of curcumin from these emulsions in simulated body fluids.

MATERIALS AND METHODS

Chemicals

Curcumin (95% purity), castor oil, olive oil, sodium chloride (NaCl), concentrated hydrochloric acid (conc. HCl), anhydrous disodium hydrogen phosphate (Na_2HPO_4), sodium hydroxide (NaOH), potassium chloride (KCl), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), potassium phosphate monobasic ($KH_2PO_4 \cdot 3H_2O$), magnesium chloride ($MgCl_2 \cdot 6H_2O$), sodium sulfate (Na_2SO_4), tris (hydroxymethyl) aminomethane ($NH_2C(CH_2OH)_3$) and the cellulose dialysis membrane (Pore size: 14 kDa) were purchased from HiMedia, India. Coconut oil (Marico Ltd. India), Fortune Sunlight refined sunflower oil (Adani Wilmar Limited, India), and Idhayam gingelly oil (V. V. V. and Sons Edible Oils Ltd., India) were procured and used without any modifications. Surfactants such as Cremophor EL and Tween 80 were obtained from Sigma–Aldrich, India. All chemicals were analytical grade and used without any modifications. Water with a resistivity of 18.2 MΩ. cm^{-1} was purified in-house using the Cascada™ Bio water purification system (Pall India Pvt. Ltd, India) and used for all experiments.

All experiments were performed in triplicate and results mentioned as mean±standard deviation (SD).

Curcumin standard graphs

The λ_{max} of curcumin (10 µg. ml^{-1}) was determined from its absorption spectrum (200 to 800 nm) in the UV and visible range using a spectrophotometer (U2910, Hitachi, Japan) with methanol as the solvent, blank and reference. A stock solution of curcumin was made in 15 ml volumetric flasks at a concentration of 1 mg. $ml^{-1}$and diluted to 1 to 10 µg. ml^{-1}in 1 µg. ml^{-1}increments in each of the oils and the absorbance of each concentration at 425 nm (λ_{max}) was used to plot the standard graphs. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following formulas respectively:

$$\text{LOD} = 3.3 \times \frac{\text{standard deviation of the regression line}}{\text{Slope (S) from regression line}}$$

$$LOQ = 10 \times \frac{\text{standard deviation of the regression line}}{\text{Slope (S) from regression line}}$$

Curcumin solubility

Excess of curcumin was added to each of the five oils and allowed to dissolve by mixing at 150 rpm in an orbital shaker for 48 h at ambient temperature. The undissolved curcumin was separated out by centrifugation at 5000 x g (SpinWin, Tarsons Products Pvt. Ltd., India) and the concentration of curcumin in the supernatant was determined by measuring the absorbance of the oils at 425 nm.

Nanoemulsion formulation

Pseudo-ternary phase diagram construction

To determine the optimum range of the components of the NE, pseudo-ternary phase diagrams were constructed based on the water titration method at ambient temperature [15]. The objective was to choose the most suitable surfactant for each oil. Briefly, to a fixed concentration of oil and surfactant, water is added in 100 µl increments with stirring at 300 rpm (SpinIt 4010, Tarsons Products Pvt. Ltd., India). The visual observations of different classes, such as transparent or clear, cloudy, turbid were tabulated, and pseudo-ternary phase diagrams were drawn for the clear emulsions.

Nanoemulsion preparation

A set of emulsions ranging from 1:1:48 to 1:9:40 (oil: surfactant: water) ratios were prepared with each oil (curcumin free or curcumin loaded) by stirring at 300 rpm for an emulsion volume of 5 ml. Cremophor EL was used as a surfactant for castor, coconut, sunflower, and gingelly oil emulsions while Tween 80 was used for olive oil emulsions. These coarse emulsions were sonicated using Ultrasonicator (Sonics, Vibra cell, USA) for 20 min at 40% intensity, for its conversion to NEs [16]. The final concentration of curcumin in the NEs was 1 mg. ml^{-1}. This was used for physicochemical characterization and stability studies.

Characterization of nanoemulsions

The series of NEs formulated were characterized by the following methods:

Hydrodynamic diameter and ζ-potential

The hydrodynamic diameter of the oil droplets was measured by the dynamic light scattering (DLS) technique with a nanoparticle analyzer (SZ-100, Horiba, Japan) using a diode pumped frequency doubled laser working at 532 nm (10mW) at 25 °C with detectors at 90° and 173° for backscatter detection. Poly-dispersity index (PDI) of the droplets was also analyzed. All the formulations were diluted 1:4 with water, before readings were taken, to reduce the multiple scattering effects. The electrophoretic mobility of the droplets was determined by laser Doppler method in the nanoparticle analyzer at a constant temperature of 25 °C. Here the particles moved through a capillary channel placed within an electric field, and the mobility of the droplets was determined by Doppler frequency shifts of scattered laser light from which ζ-potential was calculated using the provided software.

Stability of NEs

The kinetic stability of the NEs was determined by centrifuging them at 1500 × g for 30 min. The clear emulsions without phase separation with lowest surfactant concentration were selected as optimized emulsions and used for further experiments. Long-term stability was checked for a period of 4 mo, during which the droplet size, the PDI and the concentration of curcumin were determined as mentioned earlier.

Physicochemical characterization

The physicochemical characteristics of only the optimized emulsions were determined. The conductivity of the emulsions was carried out using a conductivity meter (CM 180, Elico, Hyderabad, India), apparent pH was determined using a pH meter (Mark VI, Systronics, Ahamedabad, India). The turbidity of the emulsions was determined by measuring the absorbance at 600 nm using a UV–Visible spectrophotometer (U2910, Hitachi, Japan) with water as the blank and reference.

Drug release kinetics

The kinetics of curcumin release was determined in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated body fluid (SBF) prepared according to the US Pharmacopeia as described earlier [17].

The Franz diffusion apparatus was used to determine the kinetics of drug release. The apparatus had a 500 µl donor compartment and a 5 ml recipient compartment separated by a cellulose nitrate dialysis membrane. The recipient compartment was maintained at 25 °C using a temperature controlled water circulator (Lab Companion RW-0525G, Jeio Tech Co., Ltd., South Korea) and stirred at 50 rpm. Every 15 min for the first hour followed by every hour for 8 h, samples were taken from the recipient compartment and the volume withdrawn was replaced by fresh media to maintain total volume of the release media. The concentration of curcumin was measured after suitable dilution by measuring the absorbance at 425 nm. Curcumin suspended in water at the same concentration was used as the control.

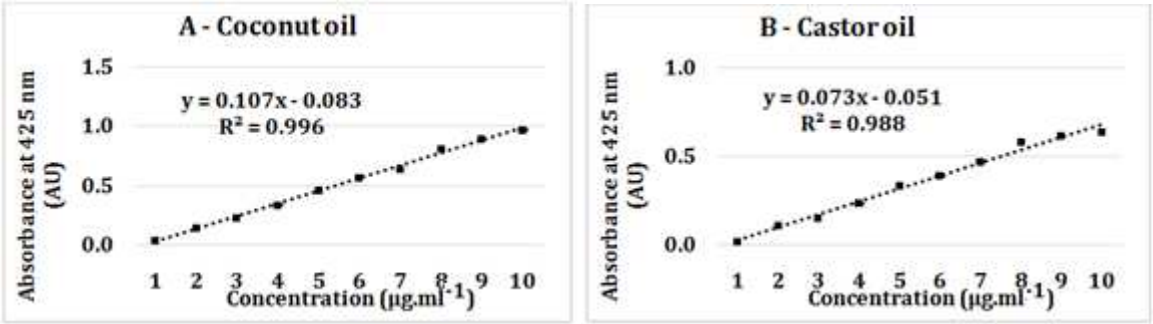
Mathematical models were then used to analyze the release profile. The models used were the Zero order model, first-order model, Higuchi model, Korsmeyer-Peppas model, and Hixson-Crowell model.

Statistical analysis

The significance of the long-term stability was determined using the Dunnett's multiple comparisons test with a confidence interval of 95%. The statistical analysis was done using GraphPad Prism v6.01 (GraphPad Software Inc., CA).

RESULTS

Curcumin standard graphs



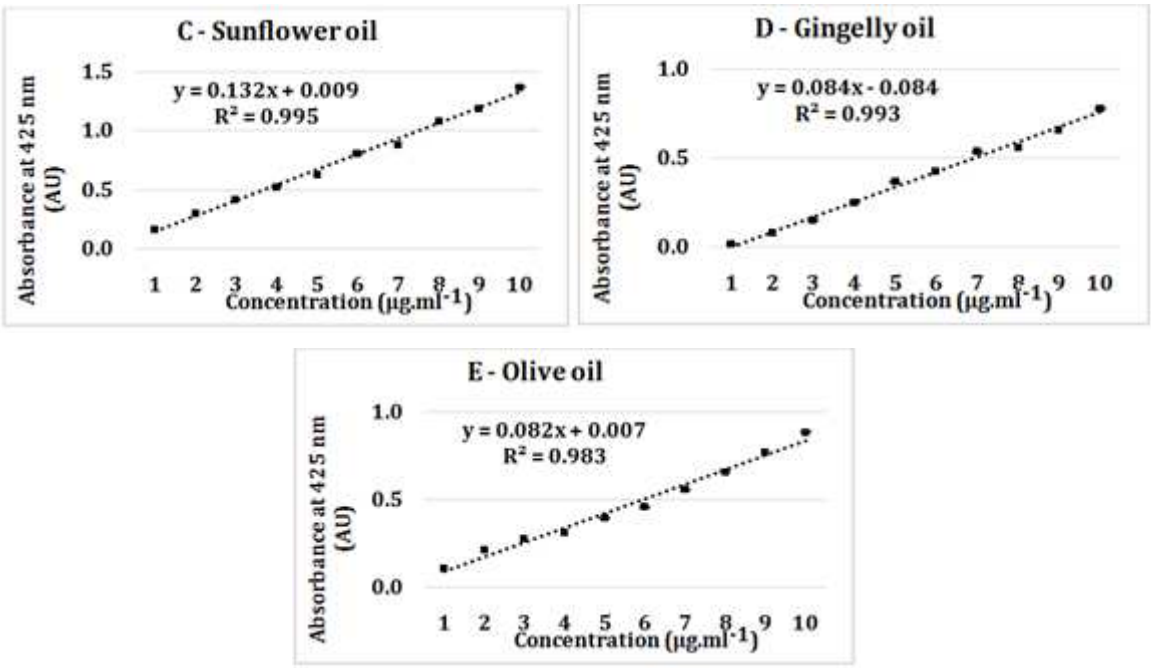


Fig. 1: Standard graphs for curcumin in various oils from 1 to 10 µg. ml^{-1}, all values presented as mean±standard deviation; n=3

Curcumin standard had good linearity from 1 to 10 µg. ml^{-1} in all the tested oils with an R^2 value ranging from 0.9832 to 0.99657 (fig. 1 A-E). The Limit of detection (LOD) and the limit of Quantification (LOQ) were calculated mathematically (table 1).

Curcumin solubility

Curcumin had a solubility in all the oils ranging from 54.36 to 94.28 mg. ml^{-1}. Olive oil had the lowest solubility while gingelly oil had the highest solubility (table 1).

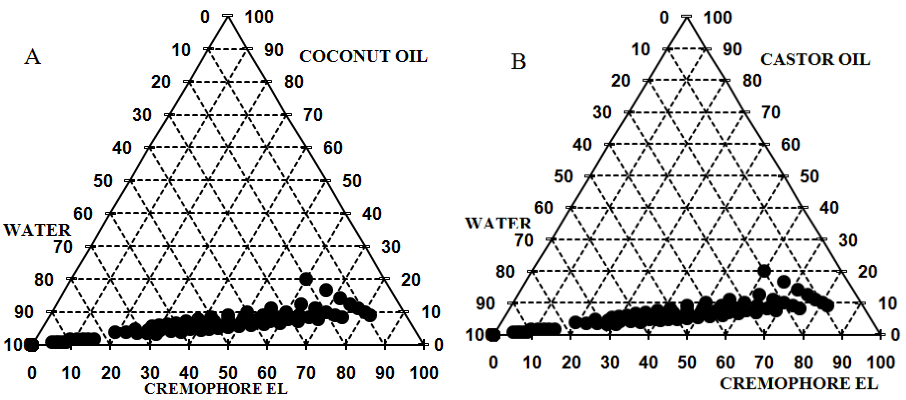
Table 1: LOD LOQ and solubility of curcumin in various oils

Oils	LOD (µg. ml^{-1}, mean±SD)	LOQ (µg. ml^{-1}, mean±SD)	Curcumin solubility (mg. ml^{-1}, mean±SD)
Olive Oil	1.26±0.013	4.19±0.044	54.36±1.32
Coconut Oil	0.57±0.043	1.89±0.143	58.62±0.76
Sunflower Oil	0.68±0.004	2.27±0.013	79.61±0.38
Castor Oil	1.03±0.019	3.43±0.063	82.35±1.05
Gingelly Oil	0.96±0.020	3.21±0.067	94.28±0.43

mean±SD (Standard deviation), n=3

Nanoemulsion formulation

Pseudo-ternary phase diagram construction



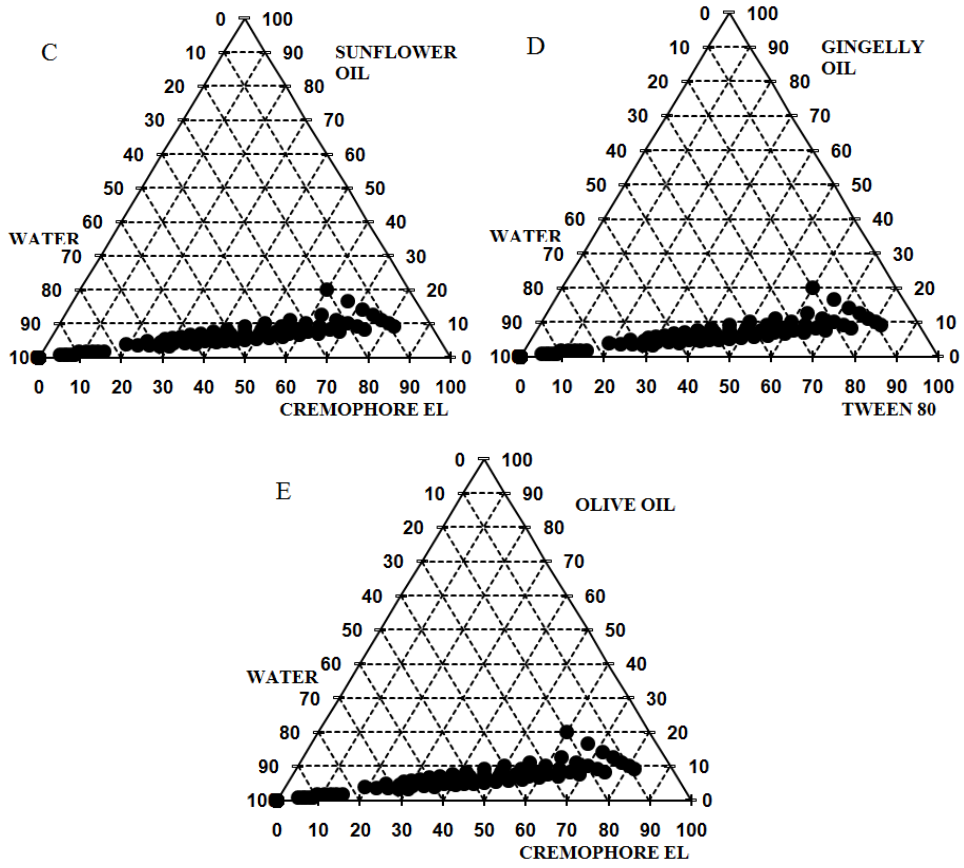


Fig. 2: Pseudo-ternary phase diagrams of the NE systems

The pseudo-ternary phase diagrams of coconut, castor, sunflower and olive oils with Cremophor El and gingelly oil with Tween 80 showed a large area of clear emulsions (fig. 2 A-E). This demonstrated that these surfactants were suitable to form NEs with the respective oils.

Nanoemulsion preparation and characterization

The coconut oil, castor oil, and gingelly oil NEs were stable above a ratio of 1:5:44, sunflower oil NE was stable above a ratio of 1:3:46 while olive oil NEs were stable above a ratio of 1:2:47. The emulsions with lower surfactant concentrations showed phase separation upon centrifugation at 1500 × g for 30 min, which indicates that they were unstable. These ratios were characterized (table 3) and used for further experiments.

The mean hydrodynamic diameter of the curcumin free NE system ranged from 19.4±3.7 nm to 142±6.5 nm while the curcumin-loaded NEs had smaller droplet size ranging between 21.2±2.1 nm to 47.3±1.8 nm. Sunflower oil NE had a 3 fold decrease in the mean hydrodynamic diameter of the curcumin-loaded NEs compared to its curcumin free NE system. The PDI of all the NEs were less than 0.5 proving that the emulsions were homogenous. The ζ-potential of all the NEs were low. The pH of the curcumin-loaded NEs was lower than the curcumin free NE systems. There was no statistically significant change in the mean hydrodynamic diameter or poly-dispersity index of the optimized curcumin loaded NEs over 4 mo (fig. 3) when maintained in dark conditions at ambient temperature (Dunnett's multiple comparisons test, alpha=0.05).

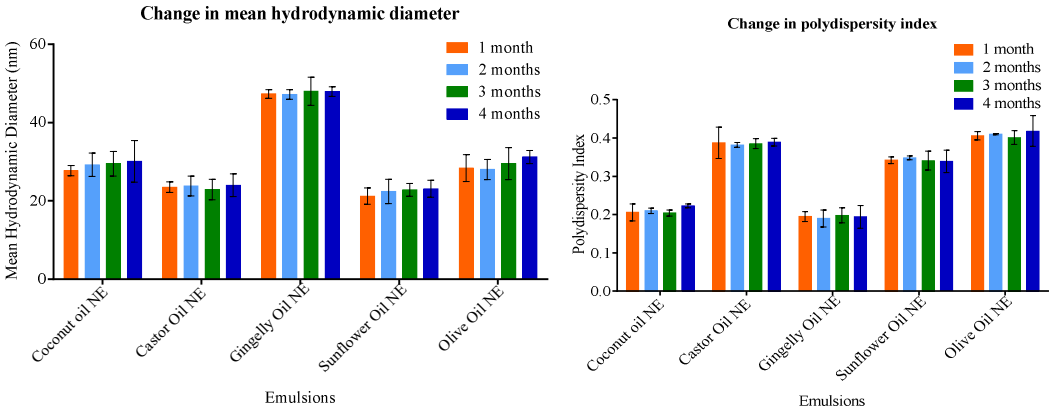


Fig. 3: Long-term stability of curcumin loaded emulsions at ambient temperature in dark conditions. All values presented as mean±standard deviation; n=3. No statistically significant difference in mean hydrodynamic diameter or polydispersity index (Dunnett's multiple comparisons test, alpha=0.05)

Table 3: Physicochemical characteristics of optimized curcumin free and curcumin loaded NEs

Emulsions	Curcumin free (mean±SD)						Curcumin-loaded (mean±SD)					
	Droplet size (nm)	PDI	ζ-potential	Conductivity (µS. cm^{-1})	Turbidity (OD600 nm) AU	pH	Droplet size (nm)	PDI	ζ-potential	Conductivity (µS. cm^{-1})	Turbidity (OD600 nm) AU	pH
Coconut oil NE (1:5:44)	75.1±10.2	0.163±0.035	-0.52±0.0	0.50±0.02	0.095±0.003	5.43±0.13	27.7±1.2	0.206±0.084	-2.27±0.02	1.33±0.05	0.106±0.08	4.52±0.17
Sunflower oil NE (1:3:46)	142±6.5	0.325±0.004	-1.24±0.01	0.35±0.01	0.105±0.005	6.66±0.11	21.2±2.1	0.387±0.021	-1.55±0.09	1.39±0.03	0.141±0.03	5.51±0.11
Castor oil NE (1:5:44)	19.4±3.7	0.207±0.015	-0.83±0.0	0.20±0.04	0.110±0.01	5.67±0.08	23.5±2.8	0.195±0.035	-2.37±0.03	1.25±0.07	0.165±0.04	4.37±0.20
Gingelly oil NE (1:5:44)	71.2±2.3	0.329±0.041	-2.57±0.02	0.56±0.04	0.112±0.007	5.34±0.12	47.3±1.8	0.342±0.047	-2.74±0.07	1.08±0.04	0.159±0.09	4.43±0.15
Olive oil NE (1:2:47)	38.8±2.5	0.387±0.029	-3.43±0.03	1.30±0.03	0.115±0.009	5.77±0.22	28.4±1.2	0.406±0.019	-3.16±0.05	1.38±0.03	0.179±0.07	4.07±0.18

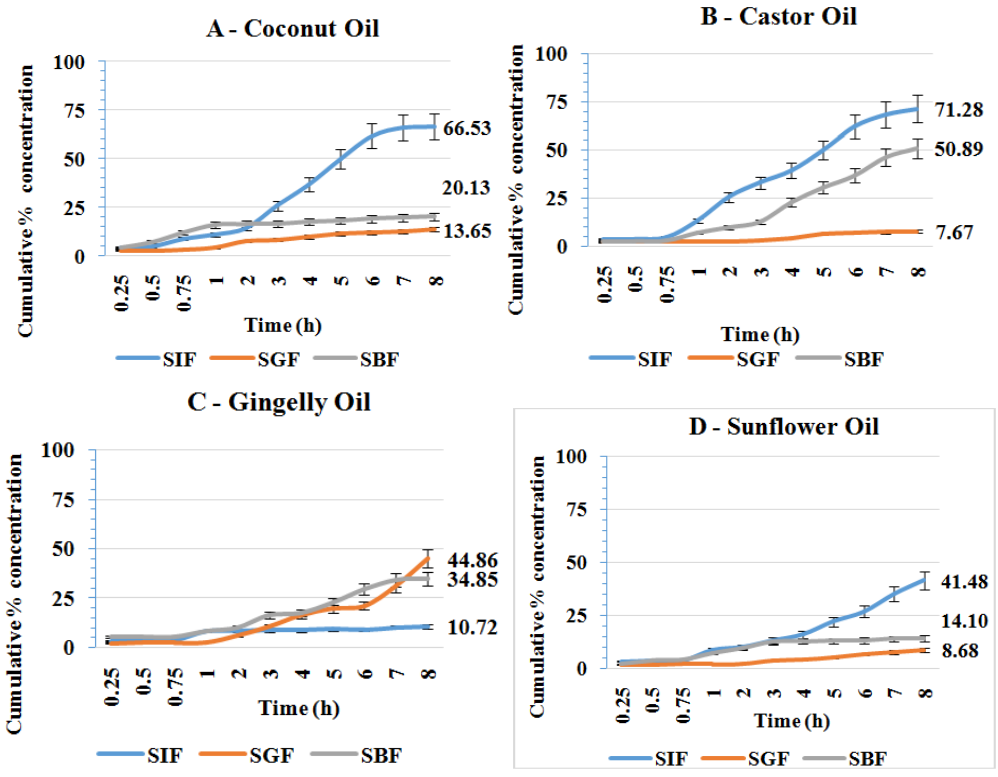
mean±SD (Standard deviation), n=3

The Dunnett's multiple comparisons test with a confidence interval of 95% showed no significant difference in the mean hydrodynamic diameter and the PDI of the curcumin-loaded emulsions. There was also no significant decrease in the concentration of curcumin in the NEs proving that the NEs were able to maintain the stability of curcumin over 4 mo (table 4).

Table 4: Changes in curcumin concentration in the NEs with long-term storage

Oils	Concentration of curcumin (mg. ml^{-1}, mean±SD)				
	Initial	1 Mo	2 Mo	3 Mo	4 Mo
Olive Oil	1.0±0.0	0.98±0.01	0.95±0.02	0.95±0.01	0.94±0.03
Coconut Oil	1.0±0.0	0.97±0.01	0.97±0.01	0.96±0.02	0.95±0.02
Sunflower Oil	1.0±0.0	0.98±0.01	0.98±0.01	0.98±0.01	0.96±0.01
Castor Oil	1.0±0.0	0.99±0.0	0.96±0.02	0.95±0.02	0.95±0.3
Gingelly Oil	1.0±0.0	0.98±0.0	0.97±0.01	0.96±0.02	0.94±0.3

mean±SD (Standard deviation), n=3. No statistically significant change in curcumin concentration over 4 mo, NEs stored at ambient temperature in dark conditions (Dunnett's multiple comparisons test, alpha=0.05)



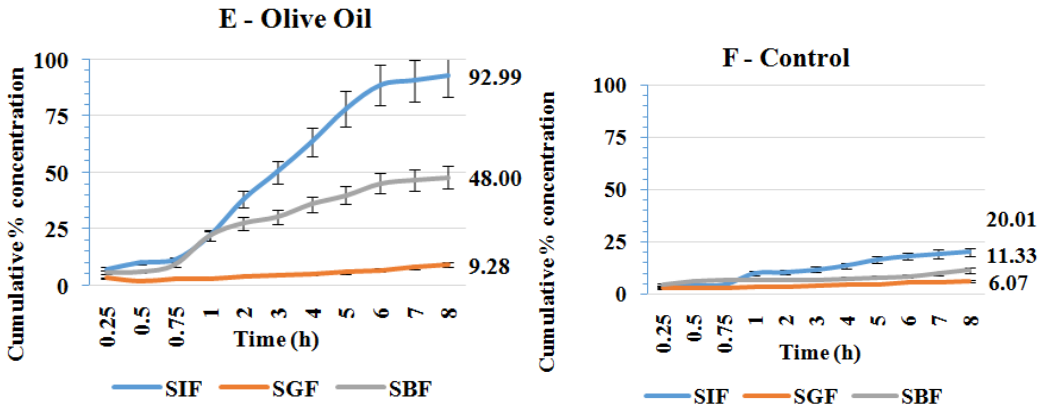


Fig. 4: Release profile of curcumin in simulated intestine, gastric and body fluids. All values presented as mean±standard deviation; n=3

Drug release kinetics

Olive oil had the highest release of curcumin (92.99%) followed by castor oil (71.28%) and coconut oil (66.53%). Gingelly oil and sunflower oil released less than 50% of the curcumin from emulsion (fig. 4 A-F). Interestingly, gingelly oil NE showed higher release in simulated gastric fluid than in the simulated intestinal fluid. There was a very low release of curcumin in control. The release of curcumin from olive oil and castor oil NEs in the simulated intestinal fluid followed the Hixson Crowell model (R^2–0.989and 0.992 respectively). Release from coconut oil NE and sunflower oil NE followed the Zero order model with $R^2$of 0.979 and 0.971 respectively (table 5-7).

Table 5: External factors evaluation matrix analysis for the release of curcumin in SIF

Models	Curcumin release from NEs in simulated intestinal fluid														
	Olive oil			Coconut oil			Sunflower oil			Castor oil			Gingelly oil		
	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept
Zero order model	0.959	12.115	9.055	0.979	9.032	0.990	0.971	4.671	0.728	0.984	9.343	2.182	0.688	0.846	4.630
First order model	0.980	-0.152	2.065	0.970	-0.065	2.024	0.949	-0.026	2.004	0.987	-0.070	2.022	0.695	-0.004	1.979
Higuchi model	0.987	0.024	0.470	0.952	0.032	0.663	0.904	0.060	0.679	0.981	0.031	0.608	0.786	0.258	-0.269
Korsmeyer-Peppas model	0.953	0.013	-0.241	0.895	0.016	-0.133	0.854	0.031	-0.126	0.947	0.016	-0.168	0.780	0.137	-0.642
Hixson-Crowell model	0.989	0.368	-0.053	0.977	0.197	-0.057	0.962	0.087	-0.016	0.992	0.209	-0.043	0.634	0.016	0.062

Table 6: External factors evaluation matrix analysis for the release of curcumin in SGF

Models	Curcumin release from NEs in simulated gastric fluid														
	Olive oil			Coconut oil			Sunflower oil			Castor oil			Gingelly oil		
	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept
Zero order model	0.960	0.821	2.192	0.944	1.477	2.926	0.977	0.918	0.982	0.952	0.800	1.576	0.933	4.851	-2.089
First order model	0.959	-0.004	1.991	0.950	-0.007	1.987	0.975	-0.004	1.996	0.951	-0.004	1.993	0.885	-0.027	2.017
Higuchi model	0.892	0.335	0.000	0.985	0.194	0.128	0.907	0.304	0.420	0.888	0.341	0.202	0.847	0.054	0.885
Korsmeyer-Peppas model	0.905	0.180	-0.509	0.972	0.103	-0.429	0.852	0.157	-0.258	0.827	0.176	-0.367	0.806	0.028	-0.020
Hixson-Crowell model	0.944	0.015	0.022	0.918	0.026	0.036	0.971	0.016	0.009	0.934	0.014	0.017	0.909	0.089	-0.057

Table 7: External factors evaluation matrix analysis for release of curcumin in SBF

Models	Curcumin release from NEs in simulated body fluid														
	Olive oil			Coconut oil			Sunflower oil			Castor oil			Gingelly oil		
	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept
Zero order model	0.899	5.518	10.063	0.656	1.515	9.978	0.793	1.455	4.718	0.984	6.462	-1.655	0.985	4.124	3.139
First order model	0.940	-0.034	1.959	0.676	-0.008	1.954	0.802	-0.007	1.979	0.966	-0.038	2.020	0.979	-0.022	1.991
Higuchi model	0.962	0.050	0.225	0.779	0.140	-0.451	0.908	0.173	-0.006	0.923	0.044	0.779	0.941	0.069	0.479
Korsmeyer-Peppas model	0.938	0.026	-0.372	0.699	0.071	-0.684	0.876	0.091	-0.490	0.870	0.023	-0.073	0.891	0.036	-0.230
Hixson-Crowell model	0.912	0.112	0.130	0.598	0.029	0.145	0.766	0.025	0.067	0.977	0.124	-0.063	0.981	0.078	0.020

DISCUSSION

NEs improve the biological activity, safety, stability and bioavailability of various water-insoluble bioactive molecules [18]. Microemulsions are inherently stable emulsions and are thermodynamically stable while NEs are inherently unstable and are only kinetically stable. NEs are increasingly used as they utilize much lower surfactant concentrations to obtain stable emulsions [19, 20].

The stable curcumin is containing emulsions which had excellent release properties lie around the center of the stability region of the pseudo-ternary phase diagrams. Al Adham has analyzed the relationship between the position of a NE in the stability region and the intensity of its biological activity. They suggested that the maximal activity is to be found at the center of the stability region [21].

Olive oil NE has the lowest solubility among the tested oil systems, but the release profile and the percentage release is the highest. This may be due to the easier partitioning of the curcumin from the NE. 50% release of curcumin from olive oil NE is seen by 3 h and there is an extended release till 8 h. This is important for absorption as curcumin levels can be maintained as curcumin is rapidly degraded by the body [8]. Olive oil also contains polyunsaturated fatty acids and is one of the best oils for human consumption [22].

Curcumin stability is decreased in aqueous environments at pH of 3 to 7 [23]. The entrapment of curcumin in NEs allows the improvement in stability, shelf life and oral bioavailability [24] of the emulsion. The extended stability of curcumin in the NEs up to 4 mo demonstrates the protective nature of the oil in the emulsion [25].

The various mathematical models to study the release kinetics operate with various assumptions and parameters. The zero order model describes a dosage form which does not dissolve and releases the drug linearly with respect to time [26]. Release kinetics follow a first-order model when the rate of release is proportional to the concentration of drug in the dosage form [27]. The Hixson and Crowell model is based on the principle that the particle size and surface area is proportional to the cube root of its volume [26]. NEs owing to their small droplet size has a very large surface area and this model best demonstrates the release profile of curcumin from olive oil NEs.

CONCLUSION

Curcumin-loaded edible oil NEs have low toxicity, exceptional biocompatibility and can help overcome the poor oral bioavailability and stability of curcumin. Olive oil is one of the best oils for human consumption and can act as an effective oral delivery system for curcumin. This study only deals with the formulation and *in vitro* release of curcumin from NEs. Further studies into the interactions between curcumin and olive oil as well as the determination of *in vivo* translation are warranted. Given the heterogeneous composition of edible oils, multiple compounds may contribute to the activity.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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