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Cubosomes Dispersions as Enhanced Indomethacin Oral Delivery Systems: *In vitro* and Stability Evaluation

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IMA and BAQ contributed equally to this work. They designed the study, performed the experiments and statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Authors BA, AA, EE and KAA performed the experiments and statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Author MMB provided the required chemicals and instruments to complete this research. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To improve the dissolution of indomethacin through developing liquid indomethacin loaded cubosomes dispersion for oral delivery.
Methodology: Glyceryl monooleate based indomethacin loaded cubosomes dispersion were prepared using Taguchi design to study the effect of indomethacin to the disperse phase ratio and poloxamer 407 (PLX%) concentrations on the particle size and entrapment efficiency (%EE). Furthermore, in vitro release in phosphate buffer (pH 6.8), and morphology were investigated. Also, the stability of indomethacin loaded cubosomes dispersions was examined after 6 months storage at 25°C in the dark.
Results: The prepared indomethacin cubosomes dispersions were in the nanoscale (184.53±0.7 to 261.33±0.8 nm) with reasonable %EE (49.30±2.6 to 95.55±3.4 %). Moreover, a biphasic release

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profile was predominant for all formulations, up to 50% of payload released after 2h followed by a second continuous sustained release phase over 24h. The kinetics of indomethacin release was best explained by Higuchi model and the mechanism of drug release from these cubosomes dispersions was by fickian diffusion mechanism. In general, the indomethacin loaded cubosomes dispersions were stable after 6 months storage at 25°C in the dark.
Conclusion: Indomethacin loaded cubosomes dispersions proved to be a successful platform to encapsulate and enhance the release of indomethacin with a good stability profile over 6 months.

Keywords: Cubosomes; dispersions; poorly water-soluble drugs; indomethacin; lipids; oral delivery.

1. INTRODUCTION

One of the major challenges of the pharmaceutical industry is the delivery of biopharmaceutics classification system (BCS) Class II drugs. BCS Class II drugs are characterized by high permeability and poor water solubility, thus the dissolution of the drug from the dosage form is the rate-limiting step, leading to low bioavailability [1]. Indomethacin (IND) is a non-steroidal anti-inflammatory drug (NSAID). IND demonstrates better oral bioavailability and blood-brain barrier penetration than other NSAIDs. Thus, IND has been widely used as an analgesic, anti-pyretic and anti-inflammatory. However, the use of IND was limited, because it is one of BCS Class II drugs [2,3]. Many approaches have been described to increase the solubility and dissolution rate of IND by different formulation strategies in order to reduce adverse reactions, administration frequency, and/or the daily dose [4–6].

To date, drug delivery systems based on lipid carriers remain apparently one of the most important delivery systems to improve the oral absorption of poor-water soluble drugs. Recently, among these carriers is the cubosome (CUB) which have attracted much attention as promising versatile delivery system to encapsulate both hydrophilic and hydrophobic drugs, improve drug absorption and offer protection for drugs against degradation [7]. CUB is nanostructured liquid-crystalline particles, in a liquid-crystalline phase with cubic crystallographic symmetry formed by amphiphilic lipids which self-assemble into the complex three-dimensional cubic phase structure through the self-organization into bilayers around bicontinuous non-intersecting water channels. In the gastrointestinal tract, CUB can maintain the drug in a solubilized state by entrapping drugs into the mixed micelles made by the digestion of CUB, therefore facilitate drug dissolution and absorption leading to enhanced oral bioavailability. Furthermore, CUB is highly biocompatible with low-cost production and easily scaled-up manufacturing technology [8–10].

This research proposes an original oral delivery system for the dissolution enhancement of IND, which can address the multiple demands of reproducible performance, easily scaled-up manufacturing technology, non-toxic and low-cost formulation and prolonged stability. Furthermore, the prepared liquid CUB dispersion will improve the ease of administration of the IND oral delivery for geriatric and pediatric patients.

2. MATERIALS AND METHODS

2.1 Materials

Glyceryl monooleate (GMO) (Peceol®) was purchased from Gattefosse, Saint-Priest Cedex, France. Indomethacin (IND), poloxamer 407 (PLX) and polyvinyl alcohol (PVA) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Spectra/ Pore dialysis membrane (12000–14000 molecular weight cut- off) was purchased from Spectrum Laboratories Inc. (U.S.A.). All other chemicals used were of analytical grade and were obtained from standard commercial suppliers.

2.2 Preparation of Indomethacin Loaded Cubosome Dispersions

IND loaded CUB dispersions were prepared by emulsification of the lipid phase (GMO) and surfactant (PLX) in water containing stabilizing agent (PVA) for the CUB dispersion [11,12]. Briefly, GMO and PLX were melted on a hot plate at temperature 70°C. Then, IND was dissolved in the molten mixture. After that, 2.5%w/w PVA was dissolved in 2 mL distilled water at 70±2°C then was added dropwise to the molten mixture and the dispersions were subjected to sonication using probe sonicator. To form the CUB dispersions, water containing 2.5% PVA was added dropwise to the cubic gel at

70±2°C under mechanical stirring so that the disperse phase (molten mixture) would constitute 5% from the CUB dispersion. CUB dispersions were maintained under stirring for 2h and were cooled to room temperature and then stored in glass vials at room temperature for further investigations.

2.3 Statistical Design of the Study

Taguchi design L9 orthogonal array was constructed using Minitab 19.2 Statistical Software (Minitab Inc., Pennsylvania, USA). It was composed of two variables set at three levels (Table 1). This design was used to investigate the influence of the formulation variables that would influence the CUB size and the drug entrapment efficiency (EE%). A high signal-to-noise (S/N) ratio indicated the optimum conditions. The signal factor (S) was the outcome, that is, particle size or EE% and noise factors (N) included humidity, room temperature, experience of researcher and so on. Optimization of the particle size and EE% was performed using the Taguchi's 'smaller-is-better' and 'larger-is- better' criterion, respectively [13].

2.4 Morphological Analysis

Morphological analysis of CUB dispersions was performed by transmission electron microscopy (TEM). One drop of the dispersion was deposited on carbon-coated copper grid (200 mesh) and negatively stained using phosphotungstic acid (1% w/v) with the excess stain removed using a filter paper. The grid was completely dried at room temperature and the measurements were performed with a TEM microscope (JEM-1010, Jeol, Tokyo, Japan).

2.5 Particle Size Analysis

The particle size of the dispersions was determined by using photon correlation spectroscopy. Samples were diluted (100-fold) with de-ionized water and placed in a scattering chamber where the light scattering was monitored at 90° scattering angle using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, U.K.). All measurements were performed at 25±0.5°C in triplicate and the values were expressed as mean ± S.D.

2.6 Drug Encapsulation Efficiency

IND loaded cubosomes were separated from unentrapped IND by centrifuging 0.5 mL of IND cubosomes dispersion in an eppendorf tube at 30,000 rpm for 45 min at 4°C (Sigma 3–30k; SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). The supernatant was removed, and the precipitates dispersion was diluted with ethanol and analyzed for entrapped IND content using spectrophotometry at a wavelength of 320 nm (Biochrom Libra S22 UV/Vis spectrophotometer, Biochrom Ltd., Cambridge, United Kingdom). Each formulation was analysed in triplicate and the mean ± SD was reported. The entrapment efficiency (EE%) was thus determined as the percentage ratio between the amount of IND encapsulated in the CUB and the initial amount of IND included in the formulation. It was calculated using the following equation:

$$EE\% = \frac{IND\ ENTRAPPED}{INITIAL\ IND\ USED}$$

2.7 *In vitro* Drug Release Studies

The release profiles of IND from CUB dispersions were evaluated by means of the dialysis method using cellulose acetate dialysis tubing (Spectra/Por with molecular cutoff 12,000–14,000 by Spectrum Laboratories Inc., Eindhoven, The Netherlands) sealed at both ends with clips [7,14–18]. A phosphate buffer solution pH 6.8 containing 1% SLS to maintain sink conditions constantly shaken at 100 rpm and warmed to 37 ± 0.1◦C was used as the release medium. The membrane was washed with distilled water several times to remove preservative and soaked in release medium overnight before use. CUB dispersion containing the equivalent of 20 mg IND were placed into dialysis bags, which were then transferred into beakers containing 100 mL of the release medium. At predetermined time intervals 0.5, 1, 2, 4, 6, 8 and 24 hours, 3 mL of the release medium was withdrawn and instantly replaced by equal amount of fresh release medium to maintain a sink condition. The amount of released IND was determined by measuring the absorbance at 320 nm. The release profile of IND was obtained by plotting the % release of IND as a percentage versus time. All release experiments were done in triplicates and the values were expressed as mean ± S.D.

2.8 Release Kinetics Studies

The % IND release was assessed using various kinetic models: zero-order, first- order and Higuchi. The criteria employed to select the ''appropriate model'' was the one with the highest coefficient of determination (r^2) [19,20].

Table 1. The composition and experimentally measured values of particle size, polydispersity index and encapsulation efficiency of indomethacin loaded cubosomes dispersions

Formulation code	Parameters		Particle size (nm)	Polydispersity index	Encapsulation efficiency (%)
	A	B			
CUB1	1	1	261.33±0.8	0.35±0.002	85.26±5.9
CUB2	1	2	191.40±0.4	0.35±0.011	76.22±9.7
CUB3	1	3	186.30±1.3	0.35±0.004	67.70±4.2
CUB4	2	1	196.63±0.9	0.20±0.007	91.32±1.3
CUB5	2	2	237.63±0.8	0.20±0.003	87.00±0.2
CUB6	2	3	187.30±0.9	0.20±0.011	77.15±1.5
CUB7	3	1	184.53±0.7	0.19±0.007	95.55±3.4
CUB8	3	2	192.20±0.7	0.20±0.002	66.93±4.7
CUB9	3	3	235.07±0.6	0.19±0.009	49.30±2.6

A: Poloxamer% (numerical value indicates parameter levels1=2.5; 2=5; 3=10); B: indomethacin: disperse phase ratio (numerical value indicates parameter levels1=1:75; 2=1:37.5; 3=1:18.75). Mean ± S.D. = Mean values ± Standard deviation

$$Q_t = Q_0 + K_0 t$$

Where, Q_t is the amount of drug released at time t; Q_0 the amount of drug in the medium at t = 0 and K_0 the zero order release constant. Accordingly, a plot of the amount of drug-released versus time will be linear. Drug delivery systems following this model release the same amount of drug by unit of time [20].

$$\log Q_t = \log Q_0 + (K_1 /2.303)\, t$$

where, Q_t is the amount of drug released at time t; Q_0 the amount of drug in the medium at t = 0 and K_1 the first order kinetic constant. Accordingly, a plot of the decimal logarithm of the released amount of drug versus time will be linear. In this model the amount of drug released is proportional to the amount of drug remaining, in such way, that the amount of drug released by unit of time diminish [20].

$$Q_t = K_H t^{1/2}$$

where, Q_t is the amount of drug released at time t and K_H is the Higuchi rate constant. Accordingly, a plot of the amount of drug-released versus the square root of time will be linear. In this model the amount of drug released is proportional to the square root of time.

Further, to better characterise the mechanism of drug release from CUB dispersions, release data were analyzed using the equation proposed by Korsmeyer and Peppas [19,20].

$$Q_t /Q_\infty = K_k t^n$$

where, Q_t corresponds to the amount of drug released at time t, Q_∞ is the amount released at time ∞, Q_t /Q_∞ is the fraction of drug released at time t, K_K a constant, and n is the release exponent, a measure of the primary mechanism of drug release. If n < 0.5, Fick diffusion is the dominant release mechanism. The release mechanism mainly driven by erosion when n approaches to 1. When 0.5<n<1, non-Fickian (anomalous) transport could be attributed to the drug release by diffusion and erosion of the delivery system [19,20].

2.9 Stability Studies

The IND loaded CUB dispersions were freshly prepared and stored in dark for six months at 25°C and 30% relative humidity. Particle size and in vitro drug release were evaluated after the storage period. The methodology here were the same as described in Sections 2.5 and 2.7.

2.10 Statistical Analysis

Standard curves were constructed and assessed using regression analysis. A one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparisons was used to assess statistical significances where required. A *P*-value <.05 is considered statistically significant. All statistical analysis was performed using Minitab Statistical Soft- ware Release 19.1 (Minitab Inc., State College, Pennsylvania).

3. RESULTS AND DISCUSSION

3.1 Preparation of Cubosomes Dispersions

The Taguchi design was applied in this study to identify the important factors that would influence the size and %EE of IND loaded CUB

dispersions. Considering PLX concentration and IND: disperse phase ratio to be investigated. Table 1 illustrates the structure of the L9 orthogonal array and the corresponding results. GMO (Fig. 1) was used in the preparation of CUB dispersions in this study due to its ability to spontaneously form cubic phases upon mixing it with water, in addition to being a safe, non-toxic, biocompatible and biodegradable ester. PLX composed of polypropylene oxide (PPO) AND polyethylene oxide (PEO) (PEO_{99}–PPO_{67}–PEO_{99}) and PVA (CH_2CHOH) were used as a surfactant and stabilizer, respectively [9].

3.2 Characterization of Cubosomes Dispersions

Table 1 shows the L9 orthogonal array and the measured particle size and EE%. Analysis of the results indicated particle sizes ranging from 184.53±0.7 to 261.33±0.8 nm and EE% ranging from 49.30±2.6 to 95.55±3.4 % were obtained (Table 1). Figs. 2 and 3 show the mean SN graph of the particle size and EE% of IND loaded CUB, respectively, for each parameter level. The factor with the largest range and corresponding rank (indicating the relative importance compared with other factors) was considered as the significant factor influencing the size or EE%.

All the formulations were in the nanoscale range, with sizes between 184.53 ± 0.71 and 261.33 ± 0.81 nm (Table 1). Analysis of results following the Taguchi design indicated that the particle size of CUB dispersions was influenced mainly by IND to the disperse phase ratio followed by PLX concentration. As evident from the main effects plot for SN ratio (Fig. 2), the particle size was affected by the IND to the disperse phase ratio where the particle size increased as the ratio of the IND to the disperse phase was decreased and can be seen comparing CUB 1 (261.33 ± 0.8 nm) and CUB 3 (186.30±1.3 nm). This could be attributed to high level of GMO used. Similar finding reported by Hosny [21]. Indeed, GMO does not form a stable CUB dispersion in water per se necessitating the addition of a stabilizer. It was demonstrated that PLX dramatically stabilized CUB dispersion. Particularly, PLX has been shown to effectively enhance the stability of bicontinuous cubic phases dispersions. As reported elsewhere, the

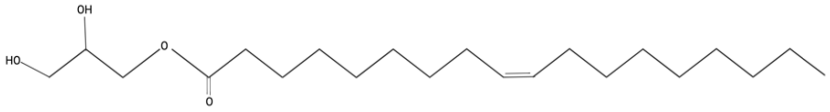
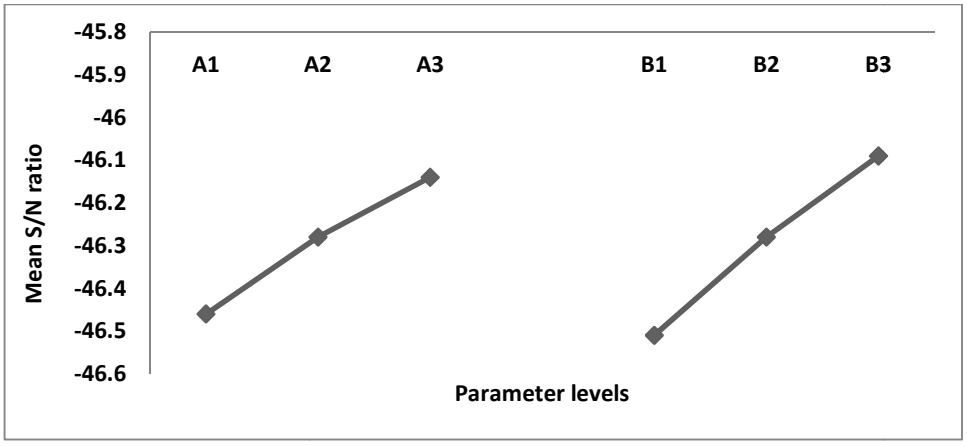


Fig. 1. Chemical structure of glyceryl monooleate (GMO); Created with BioRender.com



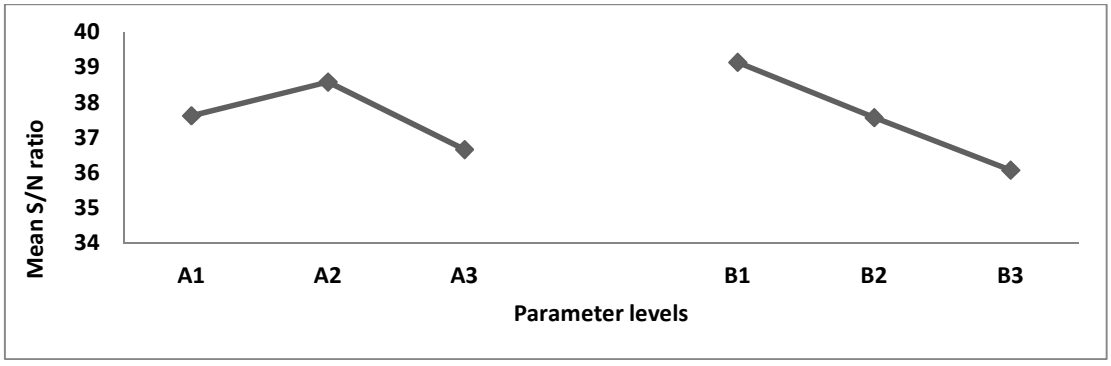
Range	0.32	0.42
Rank	2	1

Fig. 2. Mean signal-to-noise (S/N) graph for particle size response. Letters (A and B) indicate the experimental parameters and the numeric value indicates the parameter levels

phase diagram of GMO/PLX evidence that PLX is not merely absorbed at the particle surface [22]. It is suggested that the PPO blocks of PLX are moored in the non-polar zone or at the surface of the GMO-based bilayers, while the PEO tails are dissolved in the water. This order should stabilize the vesicles toward coalescence into bigger ones by a strong steric repulsion among bilayers. Also, the resultant decrease in particle size with increasing PLX concentration (up to 10%) might be attributed to the ability of PLX as a surfactant to decrease the surface tension and consequently decrease the surface energy of the CUB, thereby preventing particle aggregation and decreasing particle size. This can be seen comparing CUB 1 (261.33 ± 0.8 nm) and CUB 7 (184.53 ± 0.7 nm) (Table 1). This finding comes in line with previous reports [23,24].

All the prepared CUB dispersion achieved successfully entrapped IND with %EE ranging from 49.30±2.61 to 95.55± 3.4% (Table 1). This success can be attributed to the lipophilic nature of IND [25] which causes it to possess high affinity to the hydrophobic region of the cubic phase (GMO). From a structural perspective, GMO (Fig. 1) consists of a long aliphatic chain (hydrophobic) and glycerol moieties (hydrophilic). The hydrophobic moiety would form a lipid bilayer with a cubic phase, with the hydrophilic moieties forming a water channel. Poorly water-soluble drugs such as IND would therefore be most likely incorporated into the hydrophobic region of the cubic phase of CUB, leading to high EE% [16]. This pattern could be explained by the saturation of the bulk cubic phase with the IND due to its lipophilic nature, thereby causing a disturbance of the bulk cubic phase making the IND escape to the aqueous medium [26].

The concentration of PLX and amphiphilic nonionic triblock copolymer (PEO_{99}–PPO_{67}–PEO_{99}) is critical for the formation of stable CUB. It is suggested that the polypropylene oxide blocks (PPO) (hydrophobic part) of PLX are adsorbed onto or incorporated at the surface of the CUB, while the polyethylene oxide tails (PEO) (hydrophilic part) are solubilized in the water. This disposition should stabilize the CUB toward fusion by a strong steric repulsion between bilayers [9]. In the present work, different concentrations of PLX were investigated (2.5%, 5%, and 10% w/w, Table 1). As evident from the main effects plot for SN ratio (Fig. 3), the %EE was affected by PLX concentrations with 5% PLX resulting in higher EE% as seen comparing CUB 1 (85.26±5.9%) and CUB 4 (91.31 ± 1.3 %). These results suggest that the effect of PLX as a surfactant on the partitioning and the solubilization of IND in the hydrophobic phase of the CUB thus affecting its %EE inside CUB. Decrease in %EE of IND from 91.32±1.3 to 49.3 ± 2.6% was seen with increasing PLX concentration from 5% to 10%. IND being a hydrophobic molecule will tend to stay in the hydrophobic phase of CUB. But with an increase in PLX concentration in the preparation, IND may diffuse out from the hydrophobic phase and solubilize in the hydrophilic phase of the CUB. More solubilization of the IND in the hydrophilic phase will result in the decreased amount of IND encapsulated [21,27]. Such high drug EE% is desirable as they can reduce the volume of dosage form required to achieve the desired therapeutic effect.



Range	1.92	3.07
Rank	2	1

Fig. 3. Mean signal-to-noise (S/N) graph for % entrapment efficiency. Letters (A and B) indicate the experimental parameters and numeric value indicates the parameter levels.

3.3 Morphological Analysis

The morphology of the CUB dispersion was examined using TEM. Fig. 4 clearly confirms the formation of CUB. Also, it is evident that the formed CUB are in nano range sizes, non-aggregated and well separated from each other.

3.4 *In vitro* Drug Release Studies

IND in vitro dissolution from differently prepared CUB dispersions was compared. Fig. 5 showed a general biphasic IND dissolution feature from all the CUB dispersions, with relatively fast drug release in the first 2 hours (%Q2 ca. up to 49±8.33 %) and slower release in the following 22 hours (%Q24 ca. between 68.29±1.95 to 96.37±8.37 %). Recently, Paolino et al. cubosomes containing fluorescein were successfully developed with a high entrapment ratio and showed a rapid release during the first hour followed by a continuous release up to 6 hours [28].

The initial fast dissolution could be explained by IND fraction adsorbed or weakly bonded to the large surface area of the CUB [29]. Also, it can be attributed to the ability of the CUB to keep the insoluble IND in a solubilized nano-size state with the formation of a concomitant large surface area for the diffusion of IND from the CUB upon exposure to the release medium in appropriate sink conditions [30]. While in the second slow phase, IND snakes its way through the tortuous inner, narrow pore size aqueous nanochannels of the CUB (the principal route of drug release for both lipophilic and hydrophilic drugs) which were responsible for the slowing down of IND release [31]. Another factor that could contribute to slow release of IND is the presence of GMO as one of their main components, which might lead to slower partitioning of IND (lipophilic drug) from the oily medium to the aqueous one [32]. This pattern is advantageous as the initial rapid drug dissolution phase can achieve high IND concentration in a short time, while the slow steady state dissolution of the remaining product would provide successful drug delivery along the time [33].

Comparing the release profile of IND from different CUB dispersions at 2 hours revealed that increasing the ratio of IND to disperse phase ratio significantly (P <.001) increased IND percent released. This could be attributed to decrease in %EE of IND at higher ration of IND to disperse phase ratio leading to more amount of the IND remained as a free drug [34].

3.5 Release Kinetic Studies

The IND release data were plotted in various kinetic models, including zero order, first order and Higuchi to describe the IND release mechanism. From Table 2, it was demonstrated that the in vitro drug release of IND was best fitted into Higuchi's equation (diffusion-controlled release) with the highest coefficient of determination (r^2) [12,35]. This illustrates the IND diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred to as Higuchi's kinetics. This is attributed to the unique lipid bilayer structure of CUB where IND was distributed both in the lipid bilayer and

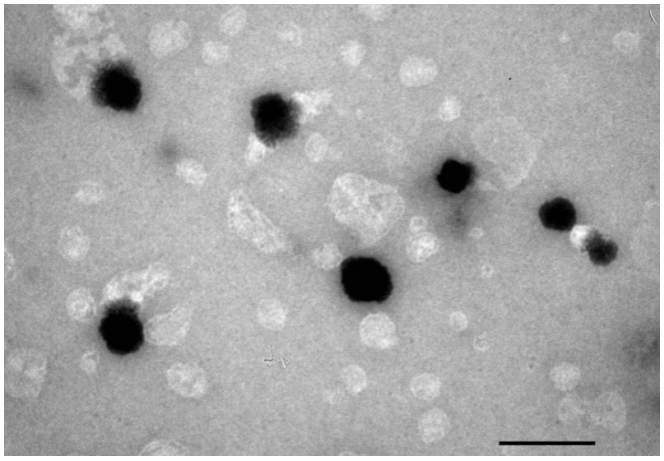


Fig. 4. Transmission electron microscopy photograph of the IND loaded cubosome dispersion (CUB 5). Scale: 200 nm

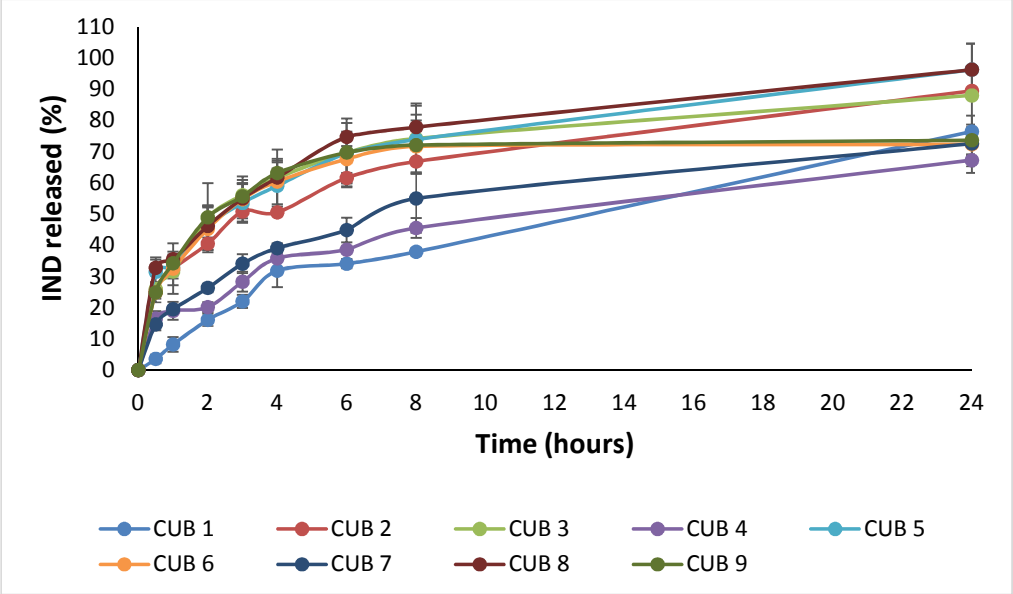


Fig. 5. *In vitro* Release Profiles of IND from IND loaded cubosome dispersions in phosphate buffer (pH 6.8) containing 1% SLS at 37°C
Mean ± S.D. = Mean values ± Standard deviation

Table 2. Correlation coefficient (r^2) values and (n) values in the analysis of release data.

Formulation code	CUB 1	CUB 2	CUB 3	CUB 4	CUB 5	CUB 6	CUB 7	CUB 8	CUB 9
Higuchi	0.990	0.960	0.900	0.976	0.948	0.700	0.958	0.920	0.700
First-order	0.988	0.950	0.843	0.928	0.980	0.521	0.903	0.969	0.511
Zero-order	0.915	0.688	0.561	0.806	0.660	0.414	0.754	0.620	0.398
Korsmeyer-Peppas model	0.96	0.990	0.904	0.960	0.980	0.900	0.985	0.970	0.864
n	0.775	0.331	0.337	0.389	0.316	0.303	0.435	0.309	0.299

inner water channel due to the addition of PLX. The initial release was attributed to the IND diffused from the inner water channel of CUB to the release medium. The slow and continuous release was explained by the IND in lipid bilayer diffusing to the inner water channel first, and then diffusing from the inner water channel to the release medium [15]. Moreover, it was reported that cubosomes should be categorized as a burst release drug carrier, whereby the drug is released by diffusion from the cubic phase matrix and the detrimental factor is the hydrophobicity of surfactants [19].

A more in-depth analysis revealed that the IND release was fitted to Korsmeyer- Peppas model with a good linearity (Table 2). For the majority of the prepared CUB dispersions, the calculated values of n ranged from 0.303 to 0.435, indicating the Fickian diffusion of drug ($n < 0.5$) was the usual molecular diffusion of the drug due to a chemical potential gradient. The values of n for CUB 1 were found to be 0.775, indicating that the release is shifted from Fickian diffusion to non-Fickian (anomalous) diffusion-controlled release where the diffusion may be combined with swelling of GMO bilayers [9,19].

3.6 Stability Studies

The CUB dispersions are free from phase separation phenomena for almost 6 months from production. The CUB dispersions appear opalescent, whitish, and odorless. In order to evaluate the physical stability of the CUB dispersions, release behavior and particle size were evaluated after storage for a period of 6 months at 25°C in the dark. There results revealed a slight increase in particle size of some CUB dispersions which do not exceed 400 nm

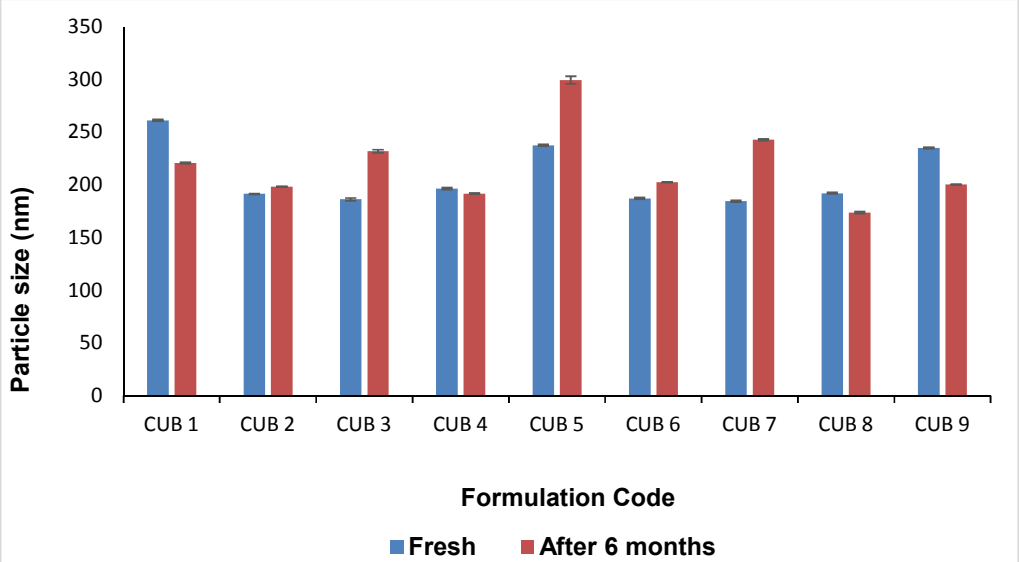


Fig. 6. Changes in the mean particle size of CUB dispersions after 6 months storage at 25°c.
Mean ± S.D. = Mean values ± Standard deviation

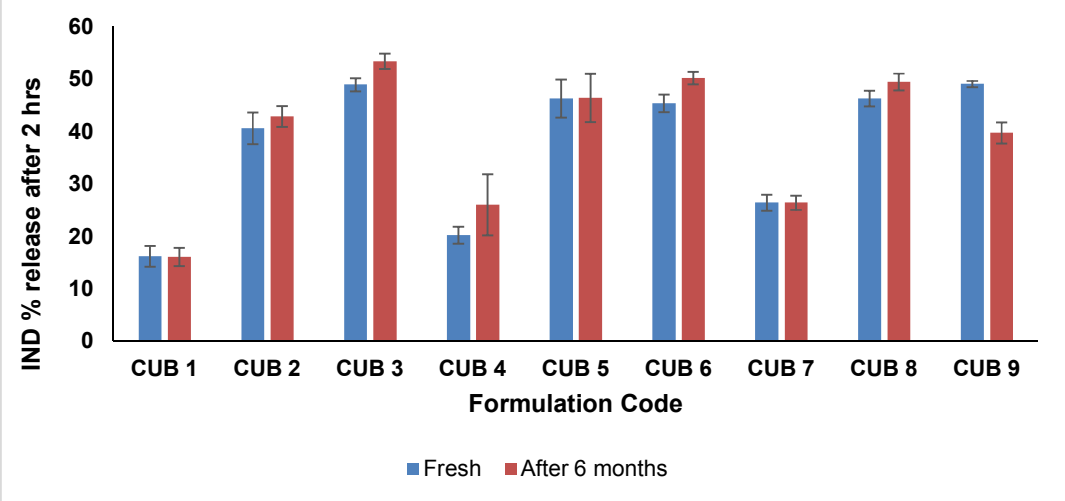


Fig. 7. Indomethacin (IND) % released within 2 hours after 6 months storage at 25°C
Mean ± S.D. = mean values ± standard deviation

after 6 months from their production (Fig. 6) [36]. Moreover, the in vitro release study showed a slight change in % release of IND after 2 hours (Fig. 7), indicating good stability of the selected CUB dispersion [15].

4. CONCLUSION

The present study report that CUB dispersions offer a promising technique for increasing IND solubility with high entrapment efficiency and nanoscale particle size. The Taguchi design applied in this study revealed that the ratio of the drug to the disperse is the most important and contributing factor affecting the attributes of the prepared CUB dispersions followed by PLX% making these factors a critical decision in the formulations of CUB dispersions. The in vitro release profiles of the IND loaded CUB dispersions showed that they released up to 50% of their encapsulated IND after 2 hours. Moreover, the results of a stability study suggested that the particle size values of the IND loaded CUB dispersions and in vitro release

profile changed slightly after 6 months of storage. These findings pave the road for CUB dispersions to offer a new platform for encapsulating and enhancing the release properties of BCS class II drugs. Further research in this area is recommended on different drugs to explore the great potential of CUB dispersion to improve oral bioavailability of BCS class II drugs *in vitro* and *in vivo*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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