Buoyant *in situ* Gels of Meloxicam- β -Cyclodextrin-Triethanolamine Ternary Complex for Oral Delivery; From a Box-Behnken Experimental Design to *in vivo* Activity Detail

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The aim of the present study was to ameliorate the aqueous solubility and dissolution rate of meloxicam by preparing its ternary complex with β -cyclodextrin and triethanolamine and to develop its gastric buoyant *in situ* gels to improve *in vivo* anti-inflammatory activity of meloxicam. Meloxicam- β -cyclodextrin-triethanolamine ternary complexes were characterized by FTIR, XRD and SEM techniques. A total of fifteen meloxicam- β -cyclodextrin-triethanolamine ternary complex incorporated buoyant *in situ* gels were prepared and optimized using a Box-Behnken design. Independent variables (concentrations of gellan gum, calcium carbonate and meloxicam respectively) were optimized in order to achieve the desired responses. The response surface plots and the possible interactions between the independent variables were analyzed using the Design Expert software 10.0.3. (Stat-Ease, Inc, USA). The results showed that the optimized buoyant *in situ* gels with short floating lag time (0.7 min), low viscosity (210 cps) and high *in vitro* drug release at 6th hour (92 %) was obtained using an optimized combination of calcium carbonate (0.75 % w/v), gellan gum (0.25 % w/v) and MLX- β -CD-TEA ternary complex (equivalent to 11 mg of meloxicam), respectively. The optimized formulation exhibited significantly high anti-inflammatory activity compared to *in situ* gel containing pure meloxicam. It was stable for over 3 months. Hence it may be used for the effective oral delivery of meloxicam.

Keywords: Meloxicam, Inclusion complex, Solubility, *in situ* gel, *in vivo* activity.

INTRODUCTION

Meloxicam, a quite new cyclo-oxygenase inhibitor, belongs to the enolic acids class of NSAIDs. It is approved by FDA for the long-term treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and so forth. It has a poor aqueous solubility [1] and therefore, on oral administration, it remains as unionized lipophilic form in the highly acidic stomach region. These conditions support the migration into the surface epithelial cells where it is dissociated into an ionized form that traps hydrogen ions, consequently producing highly critical local concentration, which lead to irritation in the stomach wall, stomach pain, rupture of gastric mucosa, gastric bleeding and ulceration [2,3], this risk may become much higher for people who are older in age [4], have poor health, or consume a large quantity of alcohol [5]. Moreover, the poor solubility and slow dissolution rate of the lipophilic drugs in the gastrointestinal fluid, besides posing difficulties in the manufacture of pharmaceutical preparations such as liquid orals, may also give rise to fluctuating oral bio-availability and poor therapeutic response of the drug [6].

Cyclodextrins (CDs) a structurally interrelated oligosaccharides with six (α -CD), seven (β -CD) and eight (γ -CD) α -1,4-glucopyranose units, play a vital role in improving therapeutic efficacy of drug molecules with poor solubility [7]. They are capable of assuaging the undesirable characteristics of drugs through the formation of inclusion complexes. Among the various cyclodextrins, β -cyclodextrin has been the most commonly used inclusion compound owing to its vast availability, low cost, absolute biocompatibility and also wide regulative acceptance [8]. Moreover, recent report evidenced that pre-associating NSAIDs with β -CD could save rats against the damaging GI adverse effects of NSAIDs while enhancing their bioactivity [9]. Nevertheless, the poor water solubility of β -CD is a major hurdle in its vast application.

A surge of scientific studies in this area revealed that the incorporation of a small amount of suitable auxiliary materials like hydrophilic polymers, organic acids, amino acids and hydroxyl organic amines, to a drug- β -CD complex could ameliorate both the complexation and solubilizing potentialities of the β -CD and ultimately reduce its amount in pharmaceutical

formulations. Such outcomes are ascribed to the complementary effect of added substance and β -CD on the formation of ternary complexes. Triethanolamine (TEA) evidently has been successfully employed with the aim to enhance the effectiveness of drug delivery and boost the cyclodextrin solubilizing potential towards acidic drugs such as methotrexate [10], gemfibrozil [11], flurbiprofen [12], ketoprofen and chlorthalidate [13]. Regarding its oral application, TEA could favourably improve in rats the per-oral absorption/bioavailability of piroxicam [14] and meloxicam [15] *via* salt formation.

Oral solutions offer several advantages over other oral pharmaceutical formulations such as emulsions, suspensions, tablets and capsules, but they often suffer from abrupt gastrointestinal transit. This could be a serious concern for the majority of drugs, particularly weakly acidic drugs as they are absorbed from the stomach and/or upper part of the small intestine [16,17]. The gastric retention of oral solutions containing these drugs could be favourably achieved through a radical approach of liquid *in situ* gelling system. These systems are polymeric gel formulations respond to physical or chemical signals, including pH, ionic factor, metabolite or temperature [18-20]. Numerous polymers such as pectin, chitosan, gellan gum, xyloglucan and xanthan gum have been investigated for this purpose. Gellan gum is a bacterial anionic deacetylated polysaccharide produced by *Pseudomonas elodea*. It undergoes gel formation by virtue of temperature change or because of the presence of cations (*e.g.* Na^+ , K^+ , Ca^{2+}). It is a water soluble polysaccharide. It produces a gel *via* formation of double helices, followed by their ionic cross linking. Incorporation of suitable amounts of gas forming substance like calcium carbonate, to the aqueous solutions of the above polymers could make them float on the surface of the gastric fluid [21]. This floating property of the gels could help in minimizing the gastric irritant effect of weakly acidic drugs by preventing direct contact with the stomach mucosa [22].

Over the years, substantial work mainly related to the solubility enhancement of meloxicam with the use of hydrophilic excipients alone [23-25] or in combination with cyclodextrins [26,27] has been reported and also there some reports appeared in the literature about the meloxicam-cyclodextrin and/or hydrophilic excipient complex/dispersion incorporated formulations such as tablets [28], suspension [29], suppositories [30] and buccal patches [31]. However, there is a lack of evidence in the literature about the meloxicam ternary complex (prepared using cyclodextrins and alkali substance combination) incorporated gastric buoyant *in situ* gels, even though this has been considered to be very beneficial from the pharmaceutical and therapeutic point of view.

Therefore the present study was planned to achieve the following objectives: 1) To improve the aqueous solubility and *in vitro* dissolution rate of poorly soluble meloxicam by preparing its ternary complexes with β -cyclodextrin and triethanolamine and characterize them by spectral, diffractometric and microscopic techniques and 2) To develop gastric buoyant *in situ* gels by incorporating selected ternary complex of meloxicam to improve its *in vivo* anti-inflammatory activity. Box-Behnken design [32], which gives small number of experimental runs and takes less time and thus offers a far more

effective approach than the traditional approaches concerning statistical optimization of a pharmaceutical formulation [33] is used in the design of buoyant *in situ* gels of meloxicam- β -cyclodextrin-triethanolamine ternary complex.

EXPERIMENTAL

Meloxicam is purchased from the UFC Biotechnology New York (USA), β -Cyclodextrin, triethanolamine, gellan gum and calcium carbonate were purchased from the Research lab fine chemicals Mumbai (India), while other ingredients used were of analytical research grade.

Phase solubility studies: Solubility determinations of meloxicam were conducted as per the reported method [34]. An excess amount of meloxicam (50 mg) was added to 20 mL aqueous β -cyclodextrin solutions of increasing concentration (3 to 15 mM) in 50 mL stoppered conical flasks. After shaking the contents of the flask at 37 °C for 72 h on a mechanical shaker, the undissolved meloxicam was filtered through a 0.45 mm filter paper (Whatman Grade 2589a) and the solutions after appropriate dilutions were assayed for meloxicam content at 362 nm spectrophotometrically. Phase solubility studies of meloxicam were also performed with the incorporation of triethanolamine at a concentration of 0.5 % w/v to the solutions containing β -cyclodextrin. The blank experiments were run simultaneously in the same concentrations of β -cyclodextrin in distilled water in order to cancel out any absorbance if showed by β -cyclodextrin molecules. The above solubility experiments were repeated for two more times to get accuracy in the results. The apparent stability constants ($K_{1:1}$) were computed from phase solubility diagrams using the below equation:

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})}$$

where S_0 is the intrinsic solubility of pure meloxicam.

Preparation of solid complexes: Meloxicam- β -cyclodextrin-triethanolamine (MLX- β -CD-TEA) ternary complexes at 1:1:0.5, 1:1:1 and 1:1:1.5 molar ratios respectively, were prepared by the solvent evaporation method [35] as follows: Calculated amounts of meloxicam, β -cyclodextrin and triethanolamine were dissolved in a small quantity of dimethyl formamide in a beaker and stirred at 600 rpm for 1 h to attain equilibrium. The solvent was removed using a rotary evaporator (IKA, Germany) under reduced pressure at room temperature. MLX- β -CD-TEA ternary complexes thus obtained were dried in vacuum at room temperature for 12 h. The dried mass was pulverized and sieved through a 60-mesh screen and stored in desiccators until used for further investigation. A batch of meloxicam- β -cyclodextrin (1:1 molar ratio) binary complex and also physical mixtures in equal ratios to the ternary complexes were prepared as the references for the characterization.

Characterization of raw materials and solid complexes of meloxicam

Fourier transformed infrared spectroscopy: FTIR spectra of MLX, β -CD, TEA, MLX- β -CD binary complex, MLX- β -CD-TEA ternary complexes and physical mixtures of MLX- β -CD-TEA were measured as potassium bromide

discs on Nicolet iS 50 FTIR (Thermo Fisher Scientific) spectrophotometer.

X-ray diffractometry: XRD pattern of MLX, β -CD, TEA, MLX- β -CD binary complex, MLX- β -CD-TEA ternary complexes and physical mixtures of MLX- β -CD-TEA were determined using Rigaku Ultima IV X-ray diffractometer. $\text{CuK}\alpha$ radiation was used in the wavelength 1.54060 Å. The samples were step scanned between 0 and 70° at 2 θ scale while measuring the intensities of the diffraction peaks.

Scanning electron microscopy: The surface morphology of the MLX, β -CD, physical mixture and selected inclusion complexes were examined by scanning electron microscopy (SEM). Samples were fixed on the brass stub using double-sided tape and made electrically conductive by coating with a thin layer of gold by sputter coater (ion sputter). ePhotographs were taken at an electric voltage of 20 kV.

***in vitro* Dissolution study:** Dissolution studies were carried out in a USP XXIV rotating paddle apparatus (8 basket Dissolution Test Station, Electrolab, India) at 37 °C using the paddle method at 50 rpm for meloxicam alone and for MLX- β -CD binary complex, MLX- β -CD-TEA ternary complexes and physical mixtures of MLX- β -CD-TEA by powder dispersion method [36]. Quantities sufficient of each powder which were equivalent to 15 mg of meloxicam were placed in the 500 mL of dissolution medium (pH 1.2), which was prepared and degassed using a media preparator (EMP-21 DO, Electrolab, India). 5 mL samples were withdrawn at preset time intervals and analyzed spectrophotometrically at 362 nm. After each sampling the fresh dissolution medium was added to maintain a sink condition.

Preparation of buoyant *in situ* gels: Buoyant *in situ* gels of MLX- β -CD-TEA ternary complex were prepared as per the reported method [37]. Weighed quantity of gellan gum was transferred to a beaker containing half of the total volume of double distilled water, which contains 0.25 % w/v of sodium citrate. The contents of the beaker were heated to 90 °C with continuous stirring on a magnetic stirrer until a clear solution is obtained and then the solution was allowed to cool below 40 °C. Calculated amounts of calcium carbonate and MLX- β -CD-TEA ternary complex were dissolved in the second half of the double distilled water in a separate beaker and this solution is slowly with continuous stirring added to the cooled gellan gum solution. Finally the *in situ* gelling solution obtained was stored in an amber coloured glass bottle in a cool place until further investigation.

Optimization: Based on phase solubility and *in vitro* dissolution studies MLX- β -CD-TEA ternary complex (1:1:1 molar ratio) was selected as one of the factors influencing the performance of buoyant *in situ* gels of meloxicam. The other two independent variables or factors, *i.e.* calcium carbonate and gellan gum were selected based on factor screening study. The Box-Behnken experimental design was employed to optimize the floating *in situ* gelling solutions wherein the concentrations of calcium carbonate (A), gellan gum (B) and MLX- β -CD-TEA ternary complex (C) were selected as independent variables or factors. Each factor was kept as low, medium and high levels. Floating lag time, Viscosity and percent cumulative drug release at 6th h were taken as dependent variables or responses (Table-1). The effect of factors on the observed responses was analyzed employing Design expert version 10.0.3. (Stat-Ease, Inc, USA) software. Fifteen experimental runs obtained from the design with 3 middle points with their observed and predicted responses are depicted in Table-2. The responses were statistically analyzed by ANOVA test method. The optimum formulation was chosen by the numerical optimization process using the desirability function. In order to assess the impact of each factor on the observed responses, the polynomial coefficients for *in situ* gelling solutions were ascertained. The polynomial equation generated by this experimental design is as follows:

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

where Y is the response; b_0 is the intercept; b_1 - b_{33} are the regression coefficients calculated from the observed experimental values; and A, B and C are the coded levels of the factors. The terms A, B and C_i^2 ($i = 1, 2$ or 3) constitute the interaction and quadratic terms respectively.

***in vitro* evaluation of buoyant *in situ* gels of MLX- β -CD-TEA ternary complex**

Appearance: The colour and the clarity of the buoyant *in situ* gels of MLX- β -CD-TEA ternary complex was evaluated by the visual inspection of the solutions against a dark illuminating background.

pH measurement: The pH of buoyant *in situ* gels of MLX- β -CD-TEA ternary complex was measured by a calibrated digital pH meter (HI-2214 logging pH bench meter, UK) at room temperature using 30 mL of the sample. pH determination for each sample was performed in triplicate.

TABLE-1
VARIABLES IN A BOX-BEHNKEN DESIGN FOR THE FORMULATION OF
BUOYANT *in situ* GELS OF MLX- β -CD-TEA TERNARY COMPLEX

Factors	Level used, actual coded		
	Low (-1)	Medium (0)	High (+1)
Independent variables			
A = Calcium carbonate (%)	0.25	0.5	0.75
B = Gellan gum (%)	0.25	0.5	0.75
C = MLX- β -CD-TEA equivalent to meloxicam (mg)	7	11	15
Dependent variables			
Y ₁ = Floating lag time (sec)	Goals		
Y ₂ = Viscosity (cps)	Shorten		
Y ₃ = Cumulative drug release (%)	Decrease		
	Enhance and prolong		

TABLE-2
OBSERVED RESPONSE IN BOX-BEHNKEN DESIGN FOR BUOYANT *in situ* GELS OF MLX- β -CD-TEA TERNARY COMPLEX

Run No.	Variables*			Floating lag time (min)		Viscosity (cps)		Cumulative drug release (%)	
	A	B	C	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	0.75	0.75	11	0.9	0.88	1250	1251.00	67	66.88
2	0.25	0.50	7	6.1	6.10	501	501.88	86	85.75
3	0.25	0.25	11	6.1	6.13	200	199.00	98	98.13
4	0.50	0.50	11	3.0	3.00	520	520.00	81	81.00
5	0.50	0.25	15	3.0	2.98	224	225.13	95	94.88
6	0.50	0.75	15	3.0	3.03	1245	1244.87	70	69.88
7	0.75	0.25	11	0.7	0.72	210	209.75	92	91.88
8	0.75	0.50	15	0.8	0.80	551	550.13	79	79.25
9	0.75	0.50	7	0.8	0.80	525	525.13	78	78.00
10	0.25	0.75	11	6.1	6.08	1200	1200.25	73	73.13
11	0.50	0.50	11	3.0	3.00	520	520.00	81	81.00
12	0.50	0.75	7	3.0	3.03	1230	1228.87	70	70.13
13	0.25	0.50	15	6.1	6.10	512	511.88	84	84.00
14	0.50	0.25	7	3.0	2.98	206	206.13	95	95.13
15	0.50	0.50	11	3.0	3.00	520	520.00	81	81.00

*A, Calcium carbonate; *B, Gellan gum; *C, MLX- β -CD-TEA ternary complex

Viscosity measurement: The viscosity of buoyant *in situ* gels of MLX- β -CD-TEA ternary complex was determined by a Viscometer (SV-10 Japan) at room temperature using 30 mL of the sample. Viscosity determination for each sample was done in triplicate.

in vitro Gelation study

in vitro Gelation study was conducted on buoyant *in situ* gels of MLX- β -CD-TEA ternary complex as described in the literature [38]. 10 mL of the gelling solution was transferred to 500 mL of 0.1 N HCl (pH 1.2) in a beaker without much turbulence to prevent shattering of the formed gel. Gelling was observed in the beaker by visual inspection and the formulations based on their gelling consistency were given with different grades.

***in vitro* Buoyancy study:** *in vitro* buoyancy study of *in situ* gels of MLX- β -CD-TEA ternary complex was conducted as per the reported method [39]. 500 mL of the dissolution medium (pH 1.2) is taken in a dissolution flask (USP Type-II) and the temperature of the dissolution medium was maintained at 37 ± 0.5 °C. 10 mL of the solution is transferred to a Petri dish (4.5mm internal diameter) and the Petri dish is carefully placed at the bottom of a dissolution flask without much disturbance in the flask. The time taken by the *in situ* gel to come up to the surface of the medium (floating lag time) and also about how much time the gel continuously floated on the surface of the medium (duration of floating) was recorded.

***in vitro* Drug release study:** *in vitro* Release of meloxicam from the buoyant *in situ* gels of MLX- β -CD-TEA ternary complex was determined in a USP XXIV rotating paddle apparatus (8 basket Dissolution Test Station, Electrolab, India) at 37 °C using the paddle method at 50 rpm. The dissolution medium used was 500 mL of 0.1 N hydrochloric acid (pH 1.2) which was prepared and degassed using a media preparator (EMP-21 DO, Electrolab, India). The *in situ* gel is added to a Petri dish (4.5mm internal diameter) and the Petri dish is then transferred to the bottom of a dissolution basket without much disturbance. 5 mL samples were withdrawn at fixed time intervals and analyzed at 362 nm using UV-visible spectro-

photometer (BT-600 UK). After each withdrawal an immediate replacement of 5 mL fresh dissolution medium was done to maintain a sink condition. Each determination was performed in triplicate till 6 h.

***in vivo* Anti-inflammatory activity:** Carrageenan induced rat hind paw edema method [40] was used to assess the anti-inflammatory activity of optimized buoyant *in situ* gels of MLX- β -CD-TEA ternary complex. The experimental protocol was approved by the Institutional Review Board and Animal Ethical Committee of the University of Dammam, Kingdom of Saudi Arabia (Approval number: IRB-2014-3-199).

Animals: Wistar rats of either sex weighing 200-250 g were used in the present study. The selected animals were housed in polypropylene cages under standard laboratory conditions (temperature 25 ± 2 °C) with a cycle of 12 h of darkness and light. The animals were fed with standard diet and water *ad libitum* and were fasted for at least 12 h prior to the experiment.

Carrageenan induced rat hind paw edema method: Rats were divided into three groups, each group has six rats. The animals of group I received 1 mL of control [calcium carbonate/gellan gum (0.75 %w/v/0.25 %w/v)] p.o. and groups II and III received p.o. standard [calcium carbonate/gellan gum/pure meloxicam (0.75 %w/v/0.25 %w/v/1 mg)] and optimized buoyant *in situ* gel [calcium carbonate/gellan gum/(0.75 %w/v/0.25 %w/v/MLX- β -CD-TEA ternary complex equivalent to 11 mg of meloxicam)] respectively (dose of meloxicam: 1 mg/kg body weight). After 1 h of the test drug and the control treatment animals of group I, II and III were injected with subcutaneous injection of 0.1 mL of 1 % w/v solution of carrageenan (Sigma Chemical Co, St. Louis MO, USA) in normal saline into the plantar side of the left hind paw. Plethysmograph (UGO Basile, Italy) was used to measure the rat paw volume. The paw volume was measured before (0) h and 1, 2, 3, 4 and 6 h after the injection of carrageenan.

The edema inhibitory activity was computed using the following formula:

$$\text{Edema inhibition (\%)} = \left(1 - \frac{D}{C}\right) \times 100$$

where, D represents the percentage difference in increased paw volume after the administration of test drugs to the rats. C represents the percentage difference of increased volume in the control groups.

Stability study: Optimized *in situ* gel of MLX- β -CD-TEA ternary complex was packed in glass bottles and stored at accelerated temperature and humidity ($40 \pm 2^\circ\text{C}/75\% \pm 5\text{ RH}$) in the stability chamber for 3 months. Small amounts of samples from each bottle were removed after 0, 1, 2 and 3 months and evaluated for appearance, pH, floating lag time, viscosity and *in vitro* drug release.

RESULTS AND DISCUSSION

Phase solubility studies: Phase solubility graphs of MLX- β -CD and MLX- β -CD-TEA complexes are illustrated in Fig. 1. According to Higuchi and Connors classification, the graphs were classified as A_L type, which revealed the formation of 1:1 soluble complexes in the presence or absence of TEA. The apparent stability constant (K_c) values obtained were 166.2 M^{-1} for MLX- β -CD binary complex and 235.6 M^{-1} for MLX- β -CD-TEA ternary complex. The K_c values specify that the stability of the MLX- β -CD-TEA ternary complex is greater than that of MLX- β -CD binary complex. The incorporation of TEA greatly increased the solubilizing potential of β -CD. The mechanism involved in enhancing the solubility of meloxicam could be ascribed to the synergistic effect of salt formation and inclusion in β -cyclodextrin.

Fig. 1. Phase solubility graphs of MLX- β -CD (a) and MLX- β -CD-TEA complexes (b)

Characterization of raw materials and solid complexes of meloxicam

Fourier transformed infrared spectroscopy: FTIR spectrum of meloxicam (Fig. 2a) showed distinct peaks at 3291.86 cm^{-1} (Secondary-NH or -OH), 1621.02 cm^{-1} (C=O

stretching), 1347.09 cm^{-1} (S=O stretching) and 855.75 cm^{-1} to 565.18 cm^{-1} (-CH aromatic ring bending and heteroaromatics). FTIR spectrum of β -cyclodextrin (Fig. 2b) showed distinct peaks at 3315.43 cm^{-1} (O-H stretching), 1154.25 cm^{-1} and 1078.81 cm^{-1} , 1026.48 cm^{-1} (-OH and C-O respectively). In the FTIR spectrum of MLX- β -CD-TEA physical mixture (Fig. 2c) the characteristic -NH or -OH absorption was shifted and broadened, whereas this absorption is disappeared in binary (Fig. 2d) and ternary (Fig. 2e) complexes of meloxicam. Moreover, appearance of new peaks in the FTIR spectra of physical mixture and in solid binary and ternary complexes indicates inclusion complex formation. These results also indicate that an intermolecular interaction has occurred between meloxicam and triethanolamine resulting in salt formation. The salt formation could be due to an electrostatic interaction between negative charge of the oxygen atom of meloxicam and positive charge of the triethanolamine nitrogen.

Fig. 2. FTIR spectrum of meloxicam (MLX) (a), β -cyclodextrin (β -CD) (b), MLX- β -CD-TEA physical mixture (c), MLX- β -CD binary complex (d) and MLX- β -CD-TEA ternary complex (e)

X-ray diffractometry: The diffractogram of pure meloxicam (Fig. 3a) showed characteristic peaks at 13° , 14.8° , 18.5° and 25.8° due to its crystalline nature. Series of intense peaks at 4.46° , 8.9° , 12.4° , 17.1° , 22.7° , 25.6° and 39.2° observed in the diffractogram of β -cyclodextrin (Fig. 3b) are indicative of its crystalline nature. Most of the principal peaks of both pure meloxicam and β -cyclodextrin are present in the MLX- β -CD-TEA physical mixture (Fig. 3c) indicates no major interaction between meloxicam, β -cyclodextrin and triethanolamine.

In contrast to these results, the X-ray diffractograms of both MLX- β -CD binary complex (Fig. 3d) and MLX- β -CD-TEA ternary complex (Fig. 3e) showed significant changes in the crystalline nature of meloxicam. Reduction of peak intensity with slightly shifting of peaks of pure meloxicam at 18.5° and 25.8° and forming new peaks such as that at 19.3° and 29.5° in MLX- β -CD-TEA ternary complex is an example of these changes. These results are indicative of a reduction in crystallinity of meloxicam in MLX- β -CD-TEA ternary complex, which might be because of combined effect of salt formation and inclusion complex formation between meloxicam, triethanolamine and β -cyclodextrin.

Fig. 3. XRD spectra of meloxicam (MLX) (a), β -cyclodextrin (β -CD) (b), MLX- β -CD-TEA physical mixture (c), MLX- β -CD binary complex (d) and MLX- β -CD-TEA ternary complex (e)

Scanning electron microscopy: From the SEM images (Fig. 4a), pure MLX particles appeared as crystalline, β -CD particles (Fig. 4b) appeared like cluster structure. Microscopic observation of ternary physical mixture (MLX- β -CD-TEA) (Fig. 4c) showed the presence of MLX crystals adhered to the surface of β -CD particle revealing no apparent interaction between both powders in the solid state. Binary and ternary inclusion complexes (Fig. 4d and 4e) showed a small and irregular piece and like inclusion of material in the cavity. Pandya *et al.* [41] have reported that a modification in the shape of drug particles was indicative of a new solid state. Thus, changes in the morphology of complex as compared to drug showed interaction between MLX and a complexing agent. These results are consistent with the XRD results.

***in vitro* Dissolution study:** Dissolution profiles of pure meloxicam, MLX- β -CD binary complex, MLX- β -CD-TEA physical mixtures and ternary complexes are depicted in Fig. 5. Both binary and ternary complexes of meloxicam exhibited improved dissolution as compared to pure meloxicam and physical mixtures. The trend observed for percent dissolution of meloxicam from physical mixtures and ternary complexes was an increase in the dissolution rate by an increase in triethanolamine concentration. However the physical mixture

Fig. 4. SEM images of meloxicam (MLX) (a), β -cyclodextrin (β -CD) (b), MLX- β -CD-TEA physical mixture (c), MLX- β -CD binary complex (d) and MLX- β -CD-TEA ternary complex (e)

Fig. 5. *in vitro* dissolution profiles of pure meloxicam (MLX), MLX- β -CD binary complex (A1), MLX- β -CD-TEA physical mixtures (A2 to A4) and MLX- β -CD-TEA ternary complexes (A5 to A7)

and the ternary complex containing 1:1:1.5 molar ratio of MLX- β -CD-TEA respectively showed decreased rate and extent of drug dissolution as compared to the physical mixtures and the ternary complexes containing 1:1:0.5 and 1:1:1 molar ratios of MLX- β -CD-TEA respectively. It may be imputed to salting out effect due to the presence of higher concentration

of TEA in the complexes. MLX- β -CD-TEA ternary complex (1:1:1 molar ratio) showed comparatively highest drug dissolution. The mechanisms of dissolution of meloxicam from the physical mixtures and complexes were studied. The data was used to study the best linear fit for the following equations [42]: (1) Zero order, (2), First order, (3) Matrix (Higuchi matrix), (4) Peppas-Korsmeyer equation, (5) Hixson-Crowell equation:

$$R(\%) = K_t$$

$$\log \% \text{unreleased} = K_t/2.303$$

$$R(\%) = Kt^{0.5}$$

$$\frac{\text{Amount of drug released at time } t}{\text{Amount of drug released at time } \infty} = K_t n$$

$$(\% \text{unreleased})^{1/3} = K_t$$

where 'n' is the diffusion coefficient, which is suggestive of transport mechanism.

The dissolution mechanism of the complex with highest rate and extent of drug dissolution was found to be the first order type ($r = 0.99870$, $a = 0.00820$ and $b = 1.97610$). The r , a and b are correlation coefficient, slope and constant, respectively, for the best fit kinetic model.

Post-characterization and *in vitro* evaluation, MLX- β -CD-TEA ternary complex (1:1:1 molar ratio) was selected to prepare and evaluate its floating *in situ* gelling solutions.

Optimization: Fifteen buoyant *in situ* gels of MLX- β -CD-TEA ternary complex were developed according to the experimental design and characterized by different responses such as floating lag time, viscosity and drug release. The mathematical correlations were set up and coefficients of the second order polynomial equations were derived using multiple linear regression analysis for a floating lag time, viscosity and drug release were found to be quadratic in nature with interaction terms. The coefficients of the polynomials fit well to the data, with the values of R^2 ranging between 0.9995 and 0.9999 ($p < 0.0009$ in all cases). The three dependent values ranged from 0.7 to 6.1 min, 200 to 1250 cps and 67 to 98 % floating lag time, viscosity and drug release, respectively. A positive value in polynomial equations corresponds to an effect that favours the optimization, whereas a negative value represents an inverse relationship between the factor and the response. The polynomial equations derived by the statistical analysis of the results are given in Table-3. Where A, B and C

corresponds to the coded values of the calcium carbonate, gellan gum and meloxicam, respectively. The effect of calcium carbonate on floating lag time is comparatively more significant than the effect of gellan gum. Other independent variable meloxicam has not shown any significant change in the floating lag time of *in situ* gelling solutions. All three independent variables, namely calcium carbonate, gellan gum and meloxicam individually and also in combinations (calcium carbonate + gellan gum, calcium carbonate + meloxicam) have the positive effect on viscosity. But the effect of the said variables is negative on drug release. This could be due to sol to gel transformation of the formulations in an acidic medium.

All the responses studied for fifteen ternary complexes *in situ* gel formulations were collectively fitted to various models using Design Expert version 10.0.3. (Stat-Ease, Inc, USA). The best-fitted model of the three factors and their comparative values of R^2 predicted R^2 , adjusted R^2 , SD and % CV are given in Table-3. The "predicted R^2 " was more or less in accordance with the "adjusted R^2 " values (Fig. 6). The 3D response surface

Fig. 6. Actual vs. predicted values

TABLE-3
MODEL SUMMARY STATISTICS GIVEN BY A BOX-BEHNKEN DESIGN

Model	R ²	Adjusted R ²	Predicted R ²	SD	CV (%)
Floating lag time = +3.00-2.65* A+0.025* B+0.000* C+0.050* AB+0.000* AC+0.000* BC+0.45* A ² +0.000* B ² +0.000* C ²					
Linear	0.9865	0.9828	0.9740	0.26	0.98
2FI	0.9866	0.9766	0.9415	0.31	
Quadratic	0.9999	0.9998	0.9986	0.032	
Viscosity = +520.00+15.38* A+510.62* B+8.75* C+10.00* AB+3.75* AC-0.75* BC-4.50* A ² +199.50* B ² +6.75* C ²					
Linear	0.9334	0.9152	0.8727	116.41	0.18
2FI	0.9336	0.8838	0.7111	136.30	
Quadratic	1.0000	1.0000	1.0000	1.12	
Cumulative % Drug Release (6 th h) = +81.00-3.13* A-12.50* B-0.13* C+0.000* AB+0.75* AC+0.000* BC+0.38* A ² +1.13* B ² +0.37 * C ²					
Linear	0.9942	0.9926	0.9898	0.84	0.27
2FI	0.9959	0.9928	0.9875	0.83	
Quadratic	0.9998	0.9995	0.9970	0.22	

graphs presenting the interaction effects of the factors on the responses are illustrated in Fig. 7. The fitting results showed that the optimized *in situ* gels of MLX- β -CD-TEA ternary complex with short floating lag time (0.7 min), low viscosity (210 cps) and high drug release at the 6th hour (92 %) was obtained using an optimized combination of calcium carbonate (0.75 % w/v), gellan gum (0.25 %w/v) and meloxicam content (11 mg), respectively. All the response surfaces were best fitted with quadratic polynomial models and are capable of predicting the interaction effects as well. Finally, the model was analyzed for ANOVA ($p < 0.0001$), which disclosed that the model terms for main effects and interaction effects were highly significant.

***in vitro* Evaluation of buoyant *in situ* gels:** All the 15 ternary complexes *in situ* gels of MLX- β -CD-TEA ternary complex were clear, viscous and pale yellow in colour. The pH of the solutions was in the range of 6.8 to 7.5. The *in situ* gelling solutions containing 0.5 % and above amount of calcium carbonate exhibited desired *in vitro* gelation property and also showed a long duration of floating on the surface of an acidic medium (pH 1.2). This could be due to the free availability of a sufficient number of calcium ions and carbon dioxide content from the calcium carbonate in an acidic medium to boost the gelling potential of

gellan gum and also induce floating characters in it (Table-4). These findings are consistent with the reported results [21].

The *in situ* gels demonstrated a considerable increase in viscosity with increasing amount of gellan gum. It was attributed to an increasing chain interaction with gellan gum concentration. Similarly, an increase in the amount of calcium carbonate also increases the viscosity of the *in situ* gels at all three gellan gum percentage. It could be due to the high concentration of finely dispersed particles of calcium carbonate in the gelling solution. On the other hand, meloxicam has not contributed much in increasing the viscosity of *in situ* gels as it was in its freely soluble ternary complex form with β -cyclodextrin and triethanolamine.

A marked decline in the rate and extent of *in vitro* drug release was noted with the increase in gellan gum concentration in *in situ* gels (Fig. 8). It is attributed to the high density of the system and also to the increase in the drug's diffusional path length. In order to study the drug release mechanism, the *in vitro* release data obtained were fitted to various kinetic equations. The release model of optimized *in situ* gel formulation followed Matrix (Higuchi matrix) kinetics with a best-fit r value 0.9698, a value 12.3273 and b value 8.0428.

Fig. 7. 3D graphs of independent variables

TABLE-4
in vitro EVALUATION OF BUOYANT *in situ* GELS OF MLX- β -CD-TEA TERNARY COMPLEX

Formulation Code/Run No.	Clarity	Colour	pH	Gelation (pH 1.2)	Duration of floating (h)
F1	Clear	Pale yellow	7.4	Very good	> 24
F2	Clear	Pale yellow	7.2	Poor	< 1
F3	Clear	Pale yellow	6.8	Poor	< 1 h
F4	Clear	Pale yellow	7.3	Good	12
F5	Clear	Pale yellow	7.0	Good	12
F6	Clear	Pale yellow	7.5	Good	12
F7	Clear	Pale yellow	6.9	Very good	> 24
F8	Clear	Pale yellow	7.2	Very good	> 24
F9	Clear	Pale yellow	7.3	Very good	> 24
F10	Clear	Pale yellow	7.2	Poor	< 1
F11	Clear	Pale yellow	7.3	Good	12
F12	Clear	Pale yellow	7.2	Good	12
F13	Clear	Pale yellow	7.1	Poor	< 1
F14	Clear	Pale yellow	6.8	Good	12
F15	Clear	Pale yellow	7.2	Good	12

Fig. 8. *in vitro* drug release profile of buoyant *in situ* gels MLX- β -CD-TEA ternary complex

***in vivo* Anti-inflammatory activity:** The optimized floating *in situ* gel of MLX- β -CD-TEA ternary complex has showed significant inhibition of Carrageenan-induced rat paw edema from 2 to 6 h in rats following oral administration, as compared to the control and standard group of animals. The highest percentage of inhibition of standard drug pure meloxicam was found to be 59.37 % ($p < 0.05$), whereas the optimized floating *in situ* gel of MLX- β -CD-TEA ternary complex exhibited the maximum percentage of inhibition of paw edema at 6 h is 84.38 % ($p < 0.05$) (Table-5), even though the standard drug has exhibited the significant inhibition of paw edema, but the optimized buoyant *in situ* gel has been found to be more significant ($p < 0.05$) paw edema inhibition from 2 h to till 6 h as compared to the control and standard group of animals.

Stability study: Optimized buoyant *in situ* gel of MLX- β -CD-TEA has not shown any significant difference in the appearance, pH, floating lag time, viscosity and *in vitro* drug release characteristics after 3 months of storage (Table-6), which clearly indicates the stability of the optimized buoyant *in situ* gel.

Conclusion

Solubility and dissolution rate of meloxicam were successfully increased by preparing its ternary complex with β -cyclodextrin and triethanolamine and was formulated as buoyant *in situ* gel. The optimized buoyant *in situ* gel of meloxicam- β -cyclodextrin-triethanolamine ternary complex demonstrated a desired gelling and floating property with prolonged and almost complete drug release (92 %) in an acidic medium (pH 1.2). Improved anti-inflammatory activity of the

TABLE-5
ANTI-INFLAMMATORY ACTIVITY OF OPTIMIZED BUOYANT *in situ* GELS OF MLX- β -CD-TEA TERNARY COMPLEX

Group	Treatment	Initial paw volume	Paw volume (mL)						Edema inhibition at 6 th h (%)
			1 h	2 h	3 h	4 h	5 h	6 h	
I	Control	1.17±0.06	1.22±0.02	1.47±0.07	1.65±0.07	1.67±0.02	1.92±0.04	2.18±0.06	—
II	Standard	1.07±0.09	1.18±0.04	1.30±0.03*	1.39±0.05*	1.42±0.01*	1.48±0.05*	1.57±0.01*	59.37
III	Optimized formulation	1.04±0.02	1.17±0.05	1.20±0.01*	1.19±0.02*	1.32±0.06*	1.35±0.03*	1.32±0.04*	84.38

Values are presented as the mean $\pm$ SEM, $n = 6$ in each group; One-way ANOVA followed by multiple Tukey's comparison test. * $p < 0.05$, as compared to the control group. ANOVA = analysis of variance; SEM= standard error of the mean

TABLE-6
STABILITY STUDY FOR THE OPTIMIZED BUOYANT *in situ* GELS OF MLX- β -CD-TEA TERNARY COMPLEX

Months	Clarity	Colour	pH	Floating lag time (min)	Viscosity (cps)	Cumulative drug release at 6 th h (%)
0	Clear	Pale yellow	6.93 $\pm$ 0.057	0.70 $\pm$ 0.10	200 $\pm$ 1.00	92.30 $\pm$ 0.22
1	Clear	Pale yellow	7.00 $\pm$ 0.100	0.83 $\pm$ 0.15	205 $\pm$ 1.73	91.67 $\pm$ 0.39
2	Clear	Pale yellow	7.06 $\pm$ 0.152	0.93 $\pm$ 0.25	206 $\pm$ 2.08	92.76 $\pm$ 0.27
3	Clear	Pale yellow	7.10 $\pm$ 0.100	1.06 $\pm$ 0.37	204 $\pm$ 3.46	91.83 $\pm$ 0.57

optimized formulation as compared to the standard could be due to the ameliorated solubility and dissolution of meloxicam in gastric fluid. The formulation was remained stable for over 3 months. Thus, it can be concluded that buoyant *in situ* gels of meloxicam- β -cyclodextrin-triethanolamine ternary complex satisfied the pharmaceutical and pharmacodynamic requirements as a novel drug delivery system and hence may be used for the effective oral delivery of meloxicam.

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