

Solid State Analysis and In-Vitro Dissolution Behavior of Meloxicam-Hydroxy Propyl Beta Cyclodextrin-Ethanolamines Ternary Complexes

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ABSTRACT

The present study was aimed to improve the aqueous solubility and dissolution rate of an NSAID meloxicam by hydroxy propyl β -cyclodextrin ternary complexes employing ethanolamines. Initially, meloxicam (MLX) binary complexes with Hydroxy propyl β -Cyclodextrin (HP β CD) were formulated by kneading and solvent evaporation techniques which was followed by ternary complex preparation of selected MLX-HP β CD binary complex employing different ethanolamines by solvent evaporation method. The solvent evaporation was used in preparing ternary complexes of MLX, because it was proved to be the best method comparatively in yielding promising binary complexes of meloxicam in the initial stage of this study. MLX formed 1:1 M stoichiometric binary and ternary inclusion complexes as demonstrated by the A_L-type of phase solubility curve. An increment in the stability constant value (K_c) of MLX- HP β CD complex in the presence of ethanolamines conceded higher complexation efficiency. Solid state analysis (FTIR, TGA, and SEM studies) of ternary compounds evidenced the perfect inclusion complex formation. Ternary complexes showed significant improvement in drug dissolution compared to pure MLX and MLX-HP β CD binary complex. The ternary complex containing 1:1:1 molar ratio of MLX-HP β CD-DEA exhibited 86.91% drug dissolution in 1 hour, which was significantly high in relation to ternary complexes containing mono and tri ethanolamines, and it was found to follow imperatively matrix order release mechanism. On aging studied complexes showed no significant change in physical appearance, drug content and drug dissolution attributes, which clearly shows high in-vitro stability of the complexes.

Keywords: Meloxicam; Ternary complex; Solubility improvement; Stability.

INTRODUCTION

Meloxicam (MLX), 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's poor solubility¹ in gastrointestinal fluid besides causing severe GI adverse effects like irritation, bleeding, perforation, and ulceration², could give rise to fluctuating oral bioavailability and thus poor clinical response due to sub therapeutic plasma drug levels³. Several strategies (Figure 1) to address low drug solubility were reported⁴ in the literature and also successfully employed in commercial production of several drug products. Nevertheless, recent studies revealed the several benefits (besides enhancing drug's solubility) of using these strategies in appropriate combination over the traditional use of single strategy⁵. One such quality approach is acidic drug-cyclodextrin-ethanolamines ternary complexation strategy⁶.

Cyclodextrin (CD) complexation of non-polar drug molecules has been well-known to render the drugs more soluble by several orders of magnitude when compared to the parent or un-complexed drug molecules. CDs are highly water-soluble polymers that can improve the solvation of dissolved drug molecules with the ability to stabilize supersaturated solutions and inhibit precipitation⁷. Hydroxypropyl- β -cyclodextrin (HPBCD) presents the highest aqueous solubility (>60% at 25°C) among the natural CDs and their derivatives and has been employed in several marketed pharmaceutical products⁸. Through the formation of ternary complexes with drug/CD/excipients it is feasible to improve the complexation efficiency (CE) of CDs and at the same time enhance the oral bioavailability of drugs from CD containing drug formulations⁹. Moreover Cyclodextrin complexation could reduce local irritation and side effects associated with some drugs¹⁰. Ethanolamines evidently have been successfully employed with the aim to enhance the effectiveness of drug delivery and boost the

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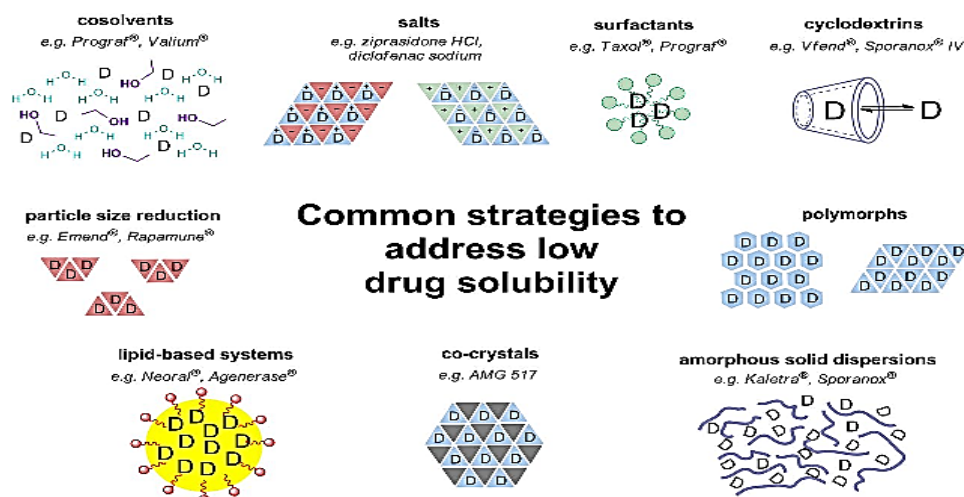


Figure 1: Common methods to address low drug solubility.

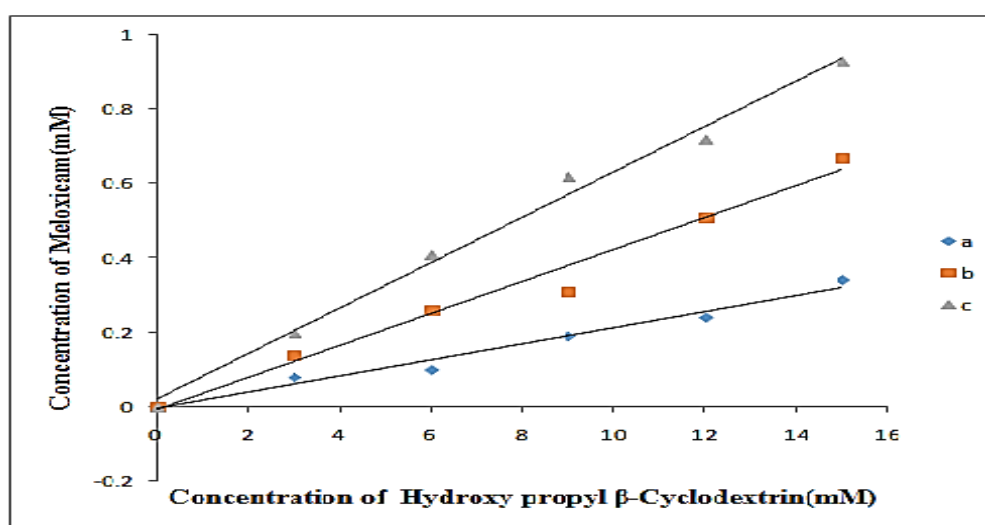


Figure 2: Phase solubility curves of a) MLX-HPβCD-MEA b) MLX-HPβCD-TEA c) MLX-HPβCD-DEA.

cyclodextrin solubilizing potential towards acidic drugs such as methotrexate¹¹, flurbiprofen¹², Ketoprofen, and chlorthiazide¹³. Furthermore, in our previous investigation we found that combined use of β -cyclodextrin and triethanolamine could be a quality approach in ameliorating solubility and dissolution potential of meloxicam¹⁴. The present investigation is the other part of the same project. Thus the specific aim of our present investigation was to combine the merits of hydroxyl propyl- β -Cyclodextrin (HP β CD) with that of alkali compounds (Monoethanolamine, Diethanolamine, and Triethanolamine) to improve the solubility and dissolution rate and thus oral bioavailability of poorly soluble weak acidic drug meloxicam.

METHODOLOGY

Materials

Meloxicam is purchased from the UFC Biotechnology New York (USA), Hydroxy propyl β -Cyclodextrin (HP β CD), Monoethanolamine (MEA), Diethanolamine (DEA), Triethanolamine (TEA), were purchased from the Research lab fine chemicals Mumbai (India), while other ingredients used were of analytical research grade.

Methods

Phase Solubility Studies

Solubility determinations of meloxicam were conducted as per the reported method¹⁵. An excess amount of meloxicam (50 mg) was added to 20 ml aqueous Hydroxy propyl β -Cyclodextrin solutions of increasing concentration (3mM to 15mM) in 50 ml Stoppard conical flasks. After shaking the contents of the flask at 37°C for 72 hours on a mechanical shaker, the undissolved meloxicam was filtered through a 0.45mm filter paper and the solutions after appropriate dilutions were assayed for meloxicam content at 362nm spectrophotometrically. Phase solubility studies of meloxicam were also performed with the incorporation of Alkali substances (Mono, di and Triethanolamines) at a concentration of 0.5% w/v to the solutions containing Hydroxy propyl β -Cyclodextrin. The blank experiments were run simultaneously in the same concentrations of Hydroxy propyl β -Cyclodextrin in distilled water in order to cancel out any absorbance if showed by Hydroxy propyl 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

Initially, binary inclusion complexes of meloxicam were prepared with Hydroxy propyl- β -cyclodextrin at 1:0.5, 1:1 and 1:1.5 molar ratios by kneading and solvent evaporation methods¹⁶ respectively. Finally, to check for potential synergistic effect of salt forming alkaline substances such as mono, di and tri ethanolamines, ternary inclusion

complexes were formulated by the solvent evaporation method, by keeping the 1:1 molar ratio of meloxicam:HP β CD constant but varying the amounts of above alkaline substances. The solvent evaporation method was used in preparing ternary complexes, because it was proved to be the best method comparatively in yielding promising binary complexes of meloxicam in the initial stage of this study. Meloxicam- Hydroxy propyl β -Cyclodextrin-Alkali substances (ethanolamines) ternary complexes at 1:1:0.5 and 1:1:1 molar ratio respectively was prepared.

Solid state analysis of raw materials and complexes of meloxicam

Fourier Transformed-Infrared Spectroscopy

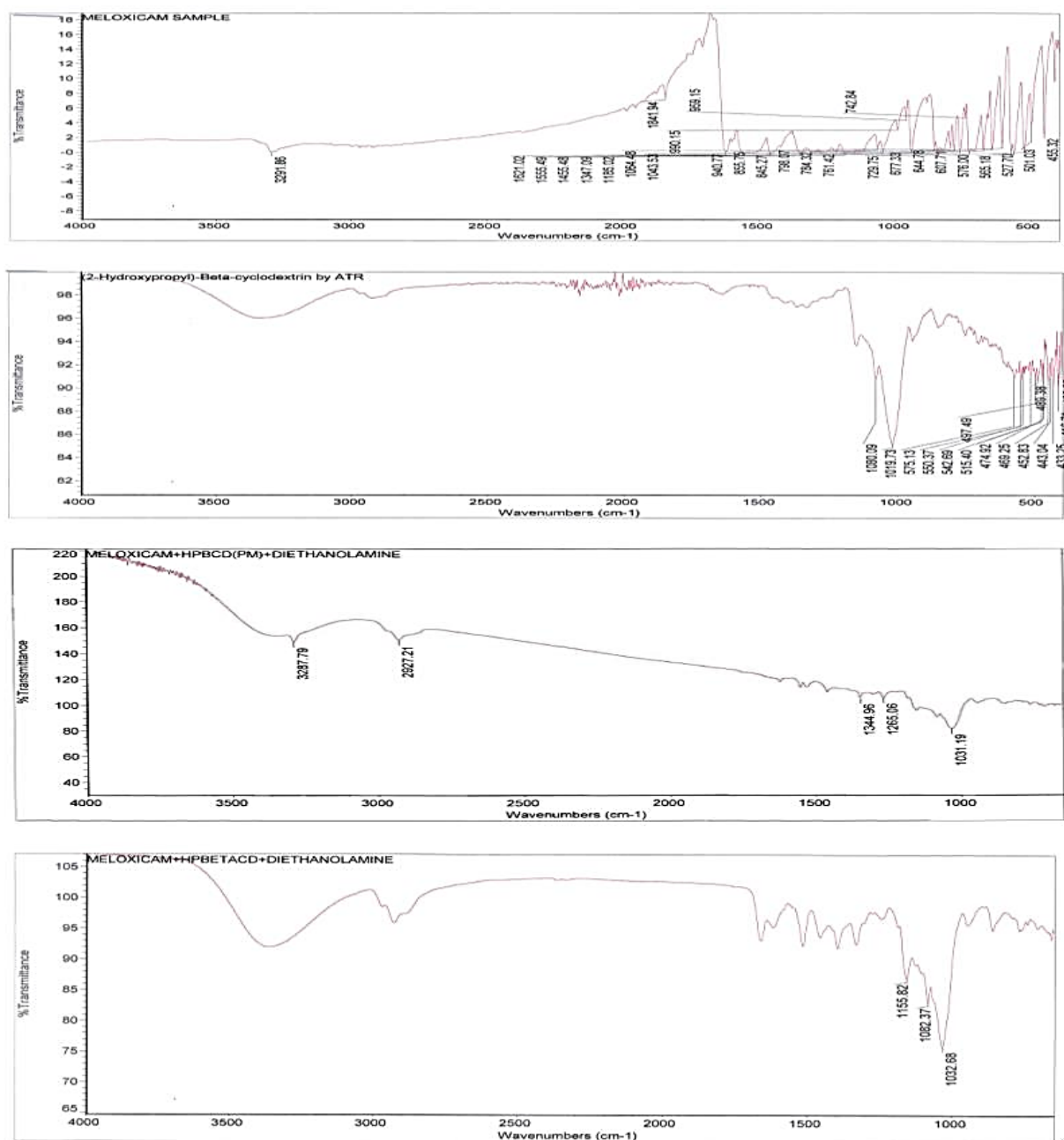


Figure 3: FTIR Spectra of a) Meloxicam b) HP β CD c) MLX-HP β CD-DEA (PM) d) MLX-HP β CD-DEA inclusion complex.

FTIR spectra of MLX, HP β CD, MLX-HP β CD-DEA (PM), MLX-HP β CD-DEA ternary complex were measured as a potassium bromide disc on Nicolet iS 50 FTIR (Thermo Fisher Scientific) Spectrophotometer.

Thermo gravimetric analysis

Thermogravimetric analysis of MLX, HP β CD, MLX-HP β CD-DEA (PM), MLX-HP β CD-DEA ternary complex were performed with a NETZSCH TG 209 (Germany)

model thermogravimetric analyzer at a temperature program of 50-400°C with a heating rate of 10°C/min.

Scanning electron microscopy

The surface morphology of MLX, HP β CD, MLX-HP β CD-DEA (PM), MLX-HP β CD-DEA ternary complex 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

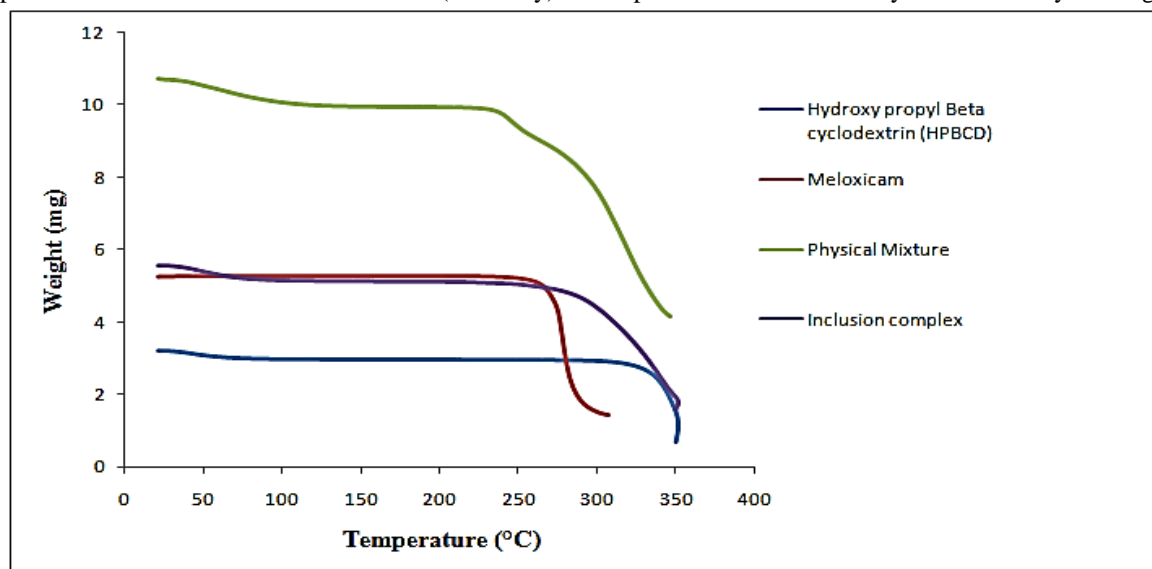


Figure 4: TGA curves of Meloxicam, HP β CD, MLX-HP β CD-DEA (PM) and MLX-HP β CD-DEA inclusion complex

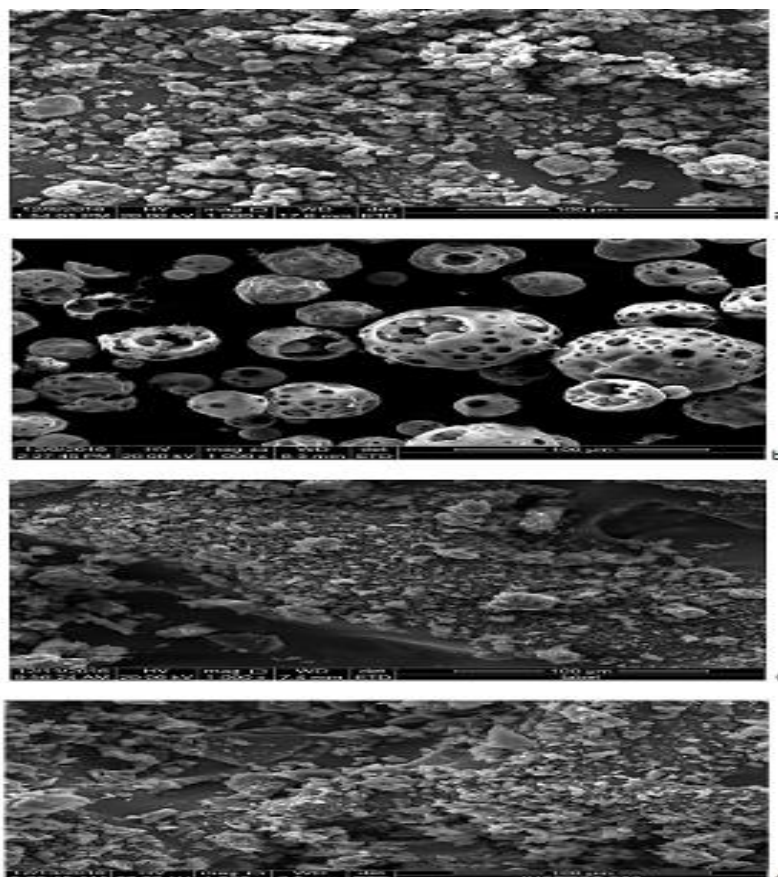


Figure 5: SEM images of a) Meloxicam b) HP β CD c) MLX-HP β CD-DEA (PM) d) MLX-HP β CD-DEA inclusion complex.

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 50rpm for meloxicam alone and for MLX- HP β CD binary complex, and MLX-HP β CD-ethanolamines ternary inclusion complexes by powder dispersion method¹⁷. 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). 5ml samples were withdrawn at preset time intervals, and analyzed spectrophotometrically at 362nm. After each sampling the fresh dissolution medium was added to maintain a sink condition.

Stability Study

Stability Study for selected solid inclusion complexes of meloxicam was carried out by storing 1gm of each selected complex in an ambered coloured screw capped glass bottles at accelerated and controlled temperatures and relative humidities for one year as per ICH guidelines. During stability study complexes were evaluated for physical appearance, drug content and in-vitro drug dissolution.

RESULTS AND DISCUSSION

Phase solubility studies

Phase solubility graphs of MLX-HP β CD-MEA, MLX-HP β CD-TEA and MLX-HP β CD-DEA complexes are illustrated in Figure 2. According to Higuchi & Connors classification, the graphs were classified as A_L type, which revealed the formation of 1:1 soluble complexes. The apparent stability constant (K_c) values obtained were 172.4M⁻¹, 199.6M⁻¹, and 253.8M⁻¹ for MLX-HP β CD-MEA, MLX-HP β CD-TEA, and MLX-HP β CD-DEA ternary inclusion complexes respectively. The K_c values specify that the stability of the MLX-HP β CD-DEA ternary complex is greater than the other two complexes. The

mechanism involved in enhancing the solubility of meloxicam could be ascribed to the synergistic effect of salt formation and inclusion in HP β -cyclodextrin.

Solid state analysis of raw materials and complexes of meloxicam

Fourier Transformed-Infrared Spectroscopy

FTIR spectrum of meloxicam (Figure 3a) 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). As observed in Figure 3b, the FTIR spectrum of HP β CD showed a prominent broad absorption band at 3395.9 cm⁻¹ due to -OH stretching and there is 1642 cm⁻¹ (O-C, 2-bands, C=O amide I band), 1410 cm⁻¹ (O-H bending, in-plane, α -CH₂ bending, CH₂ deformation), 1332 cm⁻¹ (O-H bending in plane), 1152.29 cm⁻¹ (C-C-C bending), 1080.09 & 1019.73 cm⁻¹ (O-H, H-bonded, usually broad C-O), 937 cm⁻¹ & 860 cm⁻¹ & 753.30 cm⁻¹ (=C-H & =CH₂ stretching, C-H bending & ring puckering, 703 (cis-RCH=CHR). In the FTIR spectrum of MLX-HP β CD-DEA physical mixture (Figure 3c) the characteristic -NH or -OH absorptions were present, whereas this absorption is disappeared in MLX-HP β CD-DEA (Figure 3d) complexes, indicating ternary inclusion complex formation.

Thermo gravimetric analysis

In typical TGA experiments the mass loss of a sample is precisely measured as a function of temperature at controlled temperature increase rates under inert atmosphere. The onset of thermal degradation is then determined as the temperature at which mass loss begins. Inclusion complex formation is known to improve the thermal stability of thermally labile drug molecules. Thus, thermal degradation temperatures of inclusion complexes may be observed at temperature shigher than the thermal degradation temperature of the pure drug¹⁸. This is illustrated in Figure 4. The drug and the CD each have a unique thermal degradation onset, as identified in the above said figure. In the MLX-HP β CD-DEA physical mixture, two onsets are observed representing the thermal

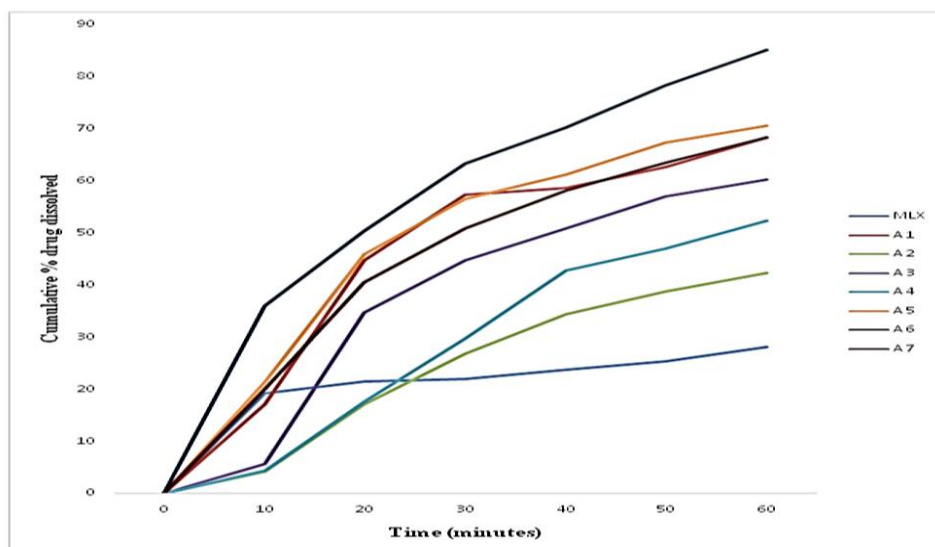


Figure 6: In-Vitro dissolution profile of pure meloxicam (MLX) and its ternary inclusion complexes.

degradation of each component. The relative mass loss in each step of the thermal degradation should be the same as the MLX-HP β CD-DEA mass ratio in the physical mixture. In a ternary inclusion complex, drug thermal degradation is shifted to a higher temperature, since the drug is protected and stabilized in the CD cavity.

Scanning electron microscopy

From the SEM images as seen in Figure 5a, pure MLX particles appeared as crystalline, HP β CD particles (Figure 5b) appeared likespherical structure. Microscopic observation of ternary physical mixture (MLX-HP β CD-DEA) (Figure 5c) showed the presence of MLX crystals adhered to the surface of HP β CDparticle revealing no apparent interaction between both powders in the solid state. Ternary inclusion complex (Figure 5d) showed a small and irregular pieces and like inclusion of material in the cavity. Pandya P, et al, has reported that a modification in the shape of drug particleswas indicative of a new solid state¹⁹. Thus, changes in themorphology of complex as compared to drug showed interaction between MLX and a complexing agent.

In-Vitro dissolution Study

Dissolution profiles of pure meloxicam, MLX-HP β CD binary complex, MLX-HP β CD-ethanolamines ternary complexes are depicted in Figure 6. Both binary and ternary complexes of meloxicam exhibited improved dissolution as compared to pure meloxicam. The trend observed for percent dissolution of meloxicam from ternary complexes was an increase in the dissolution rate by an increase in ethanolamine concentration. MLX-HP β CD-DEAternary complexes showed comparatively highest drug dissolution and these findings are in consistent with the phase solubility results. The mechanisms of dissolution of meloxicam from the complexes were studied. The data was used to study the best linear fit for the following equations²⁰;

Zero order

First order

Matrix (Higuchi matrix)

Peppas-Korsmeyer equation

Hixson-Crowell equation

%R = Kt.

Log % unreleased = Kt/ 2.303

% R = Kt^{0.5}

Amount of drug released at time t

----- = Kt n

Amount of drug released at time '∞'

(% unreleased) 1/3 = Kt

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 matrix order type (r=0. 99962, a=0. 00768, and b=1.99523). The r, a, and b are correlation coefficient, slope and constant, respectively, for the best fit kinetic model.

Stability Study

There was no significant change in the physical appearance, drug content and percent drug dissolution in the meloxicam complexes. Stability results clearly

indicated that the complexes were sufficiently stable under accelerated and controlled conditions.

CONCLUSION

From the present study it is concluded that the hydroxy propyl beta cyclodextrin ternary inclusion strategy, particularly with the alkaline substances, appears to be promising in terms of ameliorating the solubility and dissolution potential of poorly soluble weak acidic drug meloxicam in biopertinence ambience. However further pharmacokinetic and pharmacodynamic evaluation of our promising formulation containing MLX-HPBCD-DEA (1:1:1 molar ratio) would yield much rewarding outcomes.

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REFERENCES

1. Luger P, Daneck K, Engel W, Trummlitz G, Wagner K. Structure and Physiochemical properties of Meloxicam, a new NSAID. *Eur J Pharm Sci.* 1996; 4(3):175-187.
2. Wallace J L. How do NSAIDs cause ulcer disease? *Baillieres Best Pract Res Clin Gastroenterol.* Feb 2000; 14(1):147-59.
3. Sostres C, Gargello CJ, Arroyo MT, Lanás A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Practice & Research Clinical Gastroenterology.* April 2010; 24(2): 121-132.
4. Williams HD, Trevaskis NL, Charman SA, Shanker RM, Charman, Pouton CW, and Porter CJH, Strategies to Address Low Drug Solubility in Discovery and Development. *Pharmacological Reviews.* Jan 2013; 65: 315-499.
5. Dehghan MH, Jafar M, Improving dissolution of meloxicam using solid dispersions. *Iranian Journal of Pharmaceutical Research* 2006; 4:231-238.
6. Mora MJ, Longhi MR, Granero GE, Synthesis and characterization of binary and ternary complexes of diclofenac with a methyl- β -CD and monoethanolamine and *in vitro* transdermal evaluation. *Eur. J. Med. Chem.* 2010; 45: 4079–4088.
7. Srivalli KMR and Mishra B. Improved Aqueous Solubility and Antihypercholesterolemic Activity of Ezetimibe on Formulating with Hydroxypropyl- β -Cyclodextrin and Hydrophilic Auxiliary Substances. *AAPS PharmSciTech.* 2016 Apr; 17(2): 272–283.
8. Zoeller T, Dressman JB, Klein S. Application of a ternary HP- β -CD-complex approach to improve the dissolution performance of a poorly soluble weak acid under biorelevant conditions. *Int J*

- Pharm. 2012;430:176–83
9. Mohammed Jafar, Ashok Malpani, Mohammed Imran Khan. Ameliorated solubility and dissolution rate of glimepiride by cyclodextrin ternary complexes employing aminoacids. *Int Journal Pharmaceutical Sciences & Research*. June 2017; 8(6): 2443-2451.
10. Alsarra IA, Ahmed MO, Alanazi FK, Eltahir KEH, Alsheikh AM, and Neu SH. Influence of cyclodextrin complexation with NSAIDs on NSAID/Cold stress-induced gastric ulceration in rats. *Int.J.Med.Sci*. 2010; 7(4):232-239.
11. Jahamunna A. A. Barbosa, Ariana Zoppi, Mario A. Quevedo, Polyanne N. de Melo, Arthur S. A. de Medeiros, Leticia Streck, Alice R. de Oliveira, Matheus F. Fernandes-Pedrosa, Marcela R. Longhi, Arnobio A. da Silva-Junior. Triethanolamine Stabilization of Methotrexate- β -Cyclodextrin Interactions in Ternary Complexes. *Int. J. Mol. Sci*. 2014, 15, 17077-17099.
12. Maitre MM, Longhi MR, Granero GG. Ternary Complexes of Flurbiprofen with HP- β -CD and Ethanolamines Characterization and Transdermal Delivery. *Drug Dev Ind Pharm*. 2007; 33:311–326.
13. Redenti E, Szente L, Szejtli J. Cyclodextrin complexes of salts of acidic drugs. Thermodynamic properties, structural features, and pharmaceutical applications. *J Pharm Sci*. 2001; 90(8):979-986.
14. Mohammed Jafar, Mohammed Salahuddin, Tarek S Kayed, Niyaz Ahmad, Huda A Al-Eid, Abdullah H Al-Qarros. Buoyant in situ Gels of Meloxicam-[Beta]-Cyclodextrin-Triethanolamine Ternary Complex for Oral Delivery; From a Box-Behnken Experimental Design to in vivo activity detail. *Asian Journal of Chemistry*. April 2017; 29 (6): 1275-1284.
15. Higuchi T, Connors KA: Phase solubility techniques. *Advances in Analytical Chemistry and Instrumentation*. 1965; 4:117-212.
16. Jafar M, Dehghan MHG, Sharif A. Enhancement of dissolution and anti-inflammatory effect of meloxicam using solid dispersions. *International Journal of Applied Pharmaceutics* 2010; 2:22-27.
17. Jafar M, Ali S, Ghonaim H M. Improved Solubility and Dissolution Rate of Ketoprofen by Beta Cyclodextrin Ternary Complexes Incorporating Hydrophilic Polymers. *International Journal of Drug Delivery Technology*. 2017; 7(1): 45-51.
18. Pessine FBT, Calderini A. Synthesis and characterization of inclusion complex of the vasodilator drug minoxidil with beta-cyclodextrin, *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 2008; 60: 369-377.
19. Pandya P, Pandey NK, Singh SK, Kumar M. Formulation and characterization of ternary complex of poorly soluble duloxetine hydrochloride. *J. Appl. Pharm. Sci*. 2015, 5, 88–96.
20. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci*. 2001; 13(2):123-33.