Research Article

Enhancement of Meloxicam Solubility and Dissolution Using Natural Solubilizers

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ABSTRACT

A solubilizer is an ingredient that helps solubilize a drug which is insoluble in medium. The aim of this work was to improve the solubility and dissolution of a poorly water-soluble meloxicam (MX) using natural solubilizers from animal, marine and plant resources lyophilized milk (LM), shark bile salts (SBS), xanthan (XN) and β-cyclodextrin(β-CD) as synthetic solubilizer. The physical mixtures (PMs) of the drug with different solubilizers were prepared by physical mixing (PM) technique. The solubility and dissolution of MX were investigated as a function of the solubilizer concentration in the prepared formulations. In a ratio 1:3 MX:LM the solubility and dissolution of MX were improved to a great extent. Dissolution was found to be a function of the solubility. Results were confirmed by DSC, IR, XRPD and SEM which suggested solid-solid transition of MX when formulated with LM, β-CD, SBS and XN. The results suggested that natural solubilizers added promising results in enhancing the solubility and dissolution of the poorly water-soluble drugs as MX and application in the field of dosage form design without side effects.

Keywords: Meloxicam, Lyophilized milk, β-cyclodextrin, Shark bile salts, Xanthan.

INTRODUCTION

Meloxicam is chemically 4-hydroxy-2-methyl-1-N (5-methyl-2-thiazolyl)-2H-1, 2- benzothiazine-3-carboxamide-1, 1-dioxide, used as a non steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties[1]]. Prostaglandins are substances that contribute to inflammation of joints. Meloxicam inhibits prostaglandin synthetase (cylooxygenase 1 and 2) and leads to a decrease of the synthesis of prostaglandins, therefore, inflammation is reduced. Survey of literature reveals that the drug is determined by using HPLC[2,3] HPTLC[4] and few spectrophotometric method[5, 6]. This drug has low solubility in water so that it can cause problems in formulating and limiting the bioavailability[7-10]. The improvement of its solubility thereby its oral bio-availability remains one of most challenging aspects of drug development process especially for oral drug delivery system.

Many techniques were used for enhancing the solubility of drugs such as microwave induced fusion technique, nanocarrier delivery systems [6,7].

In this study natural solubilizers have been used to enhance the solubility. Thus, the study opens the chances of preparing such PMs Formulations of poorly water-soluble drugs with natural solubilizers which adds a promising technique for enhancing solubility and dissolution without side effects of synthetic solubilizers.

MATERIALS AND METHODS

Materials

Meloxicam (MX), Beta cyclodextrin (β-CD) absolute alcohol were purchased from Sigma Chemical Co. St.Louis; Xanthan (kindly supplied from pharmacognosy department); lyophilized milk (LM) and Shark bile salts (SBS). All water used was distilled and de-ionized. All other chemicals were of reagent grade and used as received.

Preparation of Lyophilized Milk

The skimmed buffalo milk was transferred to a deep freezer and kept at -21°C for 48 hours. The frozen skimmed milk was lyophilized for 72 hours at -45°C using Novalyphe-NL500 Freeze Dryer. The lyophilized powder was kept in the desiccators until use.

Preparation of Shark Bile Salts

The bile contents were evacuated from the gallbladder. The contents were dissolved in a suitable amount of distilled water, filtered, water was evaporated using hot air oven (Strok Tonic, Germany). The dried contents were pulverized and passed through sieve 60. The powder was kept in a desiccator until use.

Preparation of Physical Mixture

MX was uniformly mixed with LM, SBS, β-CD and XN using mortar and pestle in ratios illustrated in table 1 using a mortar and pestle. The prepared PMs were passed through sieve-60 and kept in the desiccators until use.

UV Analysis

Few milligrams of drug were dissolved in a small quantity of methanol and volume was made up to 100 ml with distilled water. Then 1 ml of this stock solution produced was pipette into a 10 ml volumetric flask and volume made up to the mark with distilled water. The sample was scanned in the range of 400-200 nm using UV/visible spectrophotometer to determine the λ_{max}.

Table 1: Qualitative Amounts of MX, LM and β-CD

Formulation	MX	LM	β-CD	SBS	XN
F_{MX}	1	-	-		
F1	1	1	-		
F2	1	3	-		
F3	1	5	-		
F4	1	-	1		
F5	1	-	3		
F6	1	-	5		
F7	1	-	-	1	
F8	1	-	-	3	
F9	1	-	-	5	
F10	1	1	-	1	
F11	1	-	-	-	1
F12	1	-	-	-	3
F13	1	-	-	-	5

Calibration Curve of Meloxicam in Sorensen's Phosphate Buffer (SPB) pH7.4

Meloxicam stock solution was prepared by weighing 10 mg of MX, transferred in to 1000 ml volumetric flask (previously calibrated) add 40 ml of methanol; shake for 10 min and volume was made up to 1000 ml with Sorensen's phosphate buffer pH 7.4 to get a concentration of 10 µg/ml . From this solution an aliquot of 3,4,5,6,7,8 and 9 ml were mixed with 7,6,5,4,3,2 and 1 ml SPB pH 7.4 to get concentration of 3,4,5,6,7,8,9 ug/ml. Absorbance of these solutions were measured at 362 nm using UV Spectrophotometer against blank (Methanol +SPB pH 7.4).

Drug Content

Amounts of PMs Equivalent to a theoretical content of 10 mg were accurately weighed and allowed to disintegrate completely in 100 ml of absolute alcohol. After filtration, the solution was assayed spectrophotometrically for drug content at 362 nm.

Differential Scanning Calorimetry Studies (DSC)

Samples weighing approximately 5 mg were sealed in aluminum pans and analyzed using a Shimadzu DSC-60 (Kyoto, Japan). The samples were heated in an atmosphere of nitrogen and thermograms were obtained by heating at a constant heating rate of 10°C/min in the range of 50–350°C. Thermograms for MX and its PMs were obtained.

X-ray Powder Diffraction Analysis (XRPD)

X-ray diffraction experiments were performed in a Scintag x-ray diffractometer (USA) using Cu K α radiation with a nickel filter, a voltage of 45 KV, and a current of 40 mA. Diffractions for MX and its PMs were obtained.

Infrared Spectroscopy (FTIR)

IR spectra were determined using infrared spectrophotometer (Shimadzu IR-345-U-04, Japan. An amount of 2-3 mg MX and its PMs were mixed separately with 400 mg dry potassium bromide powder, compressed into transparent discs and their IR spectra were recorded.

Scanning Electron Microscope (SEM)

Plain MX, its PMs were mounted on metal stubs with conductive silver paint and sputtered with 150 A thick layer of gold in a Bio-Rad apparatus (SEM, Jeol LTD., Japan) and scans were saved.

Meloxicam Phase Solubility Study

The experiments were carried out according to the method described by Higuchi and Connors [13]. Excess amounts of MX and different formulae of PMs were placed into stoppered conical flasks containing 20 ml distilled water. The resulting suspensions were shaken at ambient temperature until equilibrium was reached, i.e. for 48 h, on a Heidolph Vibramax 100 shaker. The solutions were filtered through a membrane filter (0.45 µm) and the dissolved drug was measured spectrophotometrically at 362 nm. This experiment was done in triplicate.

Dissolution Studies

The dissolution patterns of MX and its PMs were determined in a dissolution tester (VK 7000 Dissolution Testing Station, Vankel Industries, Inc., NJ) following the USP paddle method. All tests were conducted in 900 mL of SPB 7.4 at 37± 0.5°C with a paddle rotation speed at 100 rpm. The amount of PM used was equivalent to 10 mg MX. After specified time intervals, samples of dissolution medium were withdrawn, filtered, and assayed for drug content spectrophotometrically at 362 nm after appropriate dilution with buffer. The experiment was repeated in triplicate. The time required for 50% drug dissolved was determined ($T_{50\%}$)

Kinetic Analysis

The release data of plain drugs and its PMs were subjected to kinetic analysis according to zero order, first order kinetics Higuchi diffusion and Hixon- Crowell model models.

Statistical Analysis

The dissolution data were statistically analyzed using software to calculate probability, T_{test} using analysis of data program. Coefficient of variation (COV) was determined using excel.

RESULTS AND DISCUSSION

The different compositions of milk in different mammals are illustrated in table 2.

Table 2: Milk composition analysis, per 100 grams[11, 12]

Constituents	UNIT	Cow	Goat	Sheep	Buffalo
Water	g	87.8	88.9	83.0	81.1
Protein	g	3.2	3.1	5.4	4.5
Fat	g	3.9	3.5	6.0	8.0
Saturated fatty acids	g	2.4	2.3	3.8	4.2
Monounsaturated fatty acids	g	1.1	0.8	1.5	1.7
Polyunsaturated fatty acids	g	0.1	0.1	0.3	0.2
Carbohydrate (i.e the sugar form of lactose)	g	4.8	4.4	5.1	4.9
Cholesterol	mg	14	10	11	8
Calcium	mg	120	100	170	19
Energy	kcal	66	60	95	110

UV Analysis

The UV spectrum of MX depicted three bands at 205 nm, 270 nm and 362 nm(fig.1). The band at 362 nm was selected for spectrophotometric analysis of MX and its PMs.

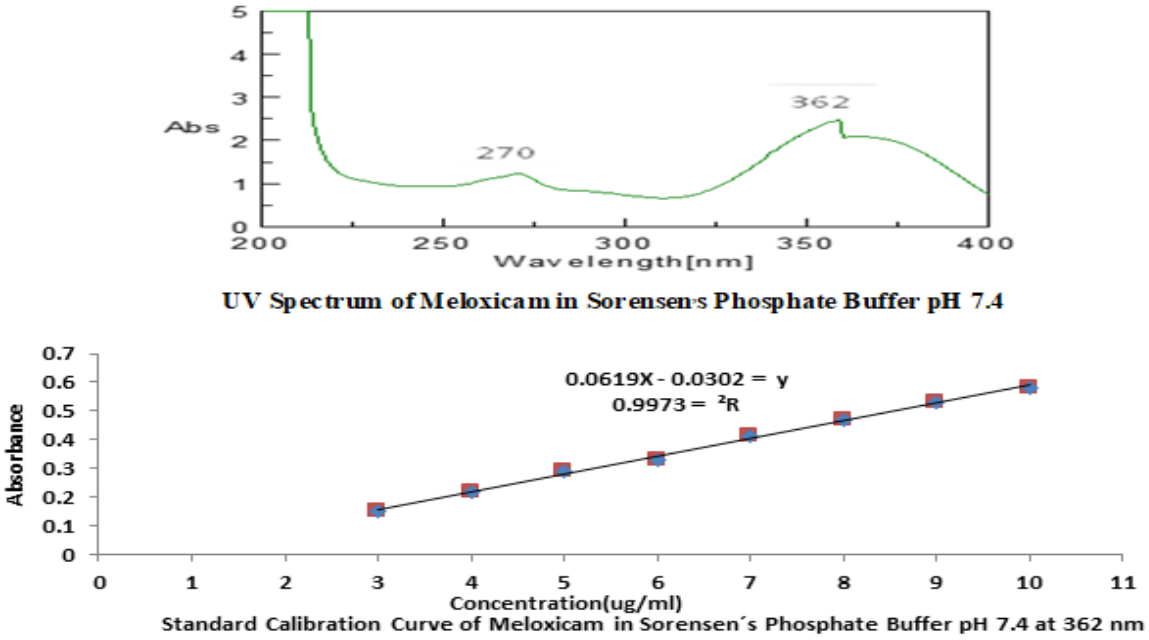

Figure 1: UV Spectrum & Standard calibration curve of MX in Sorensen's Phosphate Buffer pH 7.4

Table 3: UV Absorbance of MX at λmax 362 nm in phosphate buffer pH 7.4

Concentration of MX (ug/ml)	Absorbance
3	0.1496
4	0.2195
5	0.2891
6	0.3278
7	0.4104
8	0.4682
9	0.5298
10	0.5821

Calibration Curve of Meloxicam in Sorensen's Phosphate Buffer (pH 7.4)

Table (3) illustrates the absorbance determined at λ_{max} 362 nm in SPB PH 7.4 for serial concentrations of MX. The relation between different concentrations of MX and their corresponding absorbance was represented graphically in fig (1).

Drug Content

The value of the experimental drug content of MX was very close to the theoretical one for all prepared PMs.

Differential Scanning Calorimetry (DSC)

Representative thermograms for pure MX and its PMs with different solubilizers are presented in Figure 2. The DSC thermogram of pure MX exhibited a sharp

endothermic peak at 261.66ºC corresponding to its melting point, and reflecting crystalline nature of the drug. The thermal behavior of all formulae with the exception of F5 showed a sharp decrease in melting of drug. This reflects the prominent effect of natural solubilizers in lowering the melting point of MX This sharp decrease in melting point of drug indicates solid-solid transition from crystalline to the amorphous state.

X-ray Powder Diffraction Analysis (XRPD)

The results obtained with DSC were further confirmed by x-ray diffraction studies (Fig2). The x-ray diffraction pattern of the pure MX reflects its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. PMs of drug with LM and β-CD (1:3) depicted the absence of the majority of sharp peaks of drug and appearance of amorphous characters of both polymers especially with LM.

Infrared Spectroscopy (FTIR)

The FTIR spectra of pure MX and its PMs with different solubilizers are depicted in Figure 2.

In functional group region, FTIR Spectra of meloxicam was characterized by principal absorption bands at 3274.69 cm^{-1} due to secondary amine stretch ,2995.4 cm^{-1} due to C-H stretch, aromatic, 1538.9 cm^{-1} due to C = N stretch The IR spectrum of F2 is characterized by principal absorption band at 3245.7 cm^{-1} (secondary amine stretch). The IR spectrum of F5 depicted absorption band around 3240 cm^{-1}, IR bands of both SBS and XN were around 3200 and 3300 cm^{-1} respectively.

Based on these results, it can be concluded that PMs of MX with all solubilizers depicted no change in functional group region indicating the absence of chemical reaction between meloxicam with both natural and synthetic solubilizers. On the other hand their fingerprint regions are not superimposed the fingerprint region of meloxicam indicating physical change. This was in accordance with DSC and XRD results.

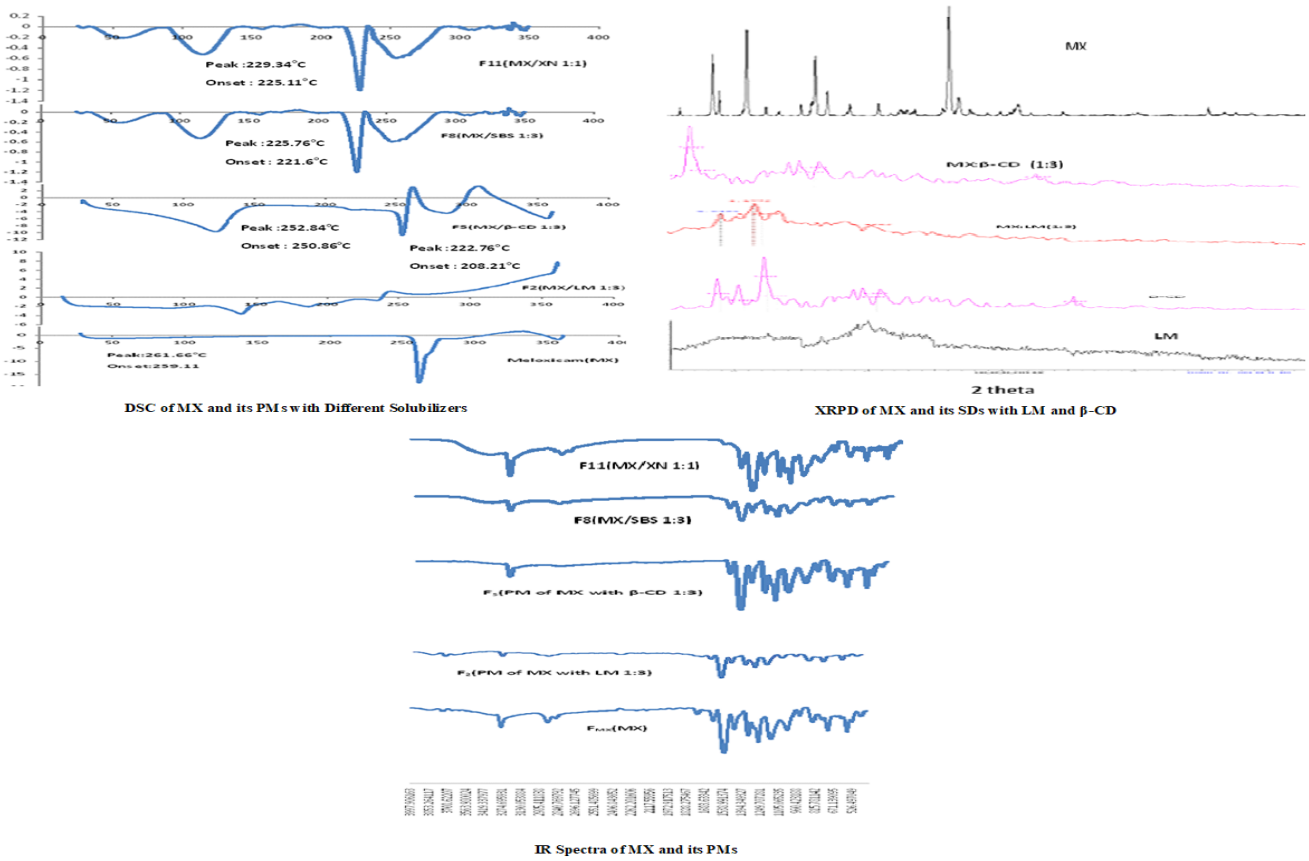

Figure 2: DSC, XRPD & IR of MX and its PMs with Different Solubilizers.

Scanning Electron Microscope

SEM micrographs of MX and with its different PMs are shown in Fig.3. The results show the particles of polymers and the drug. While, the micrographs of MX with its PMs with different solubilizers showed matrices of different homogeneity. The best homogeneity was obtained with LM and β-CD. The SEM micrographs of PM of MX with both LM and β-CD suggest that the particles of drug might have been reduced during dissolution and incubation in polymer matrix through inclusion complexes. Based on these results, it can be concluded that PM of MX with different solubilizers could be suitable form to enhance the solubility and dissolution of MX.

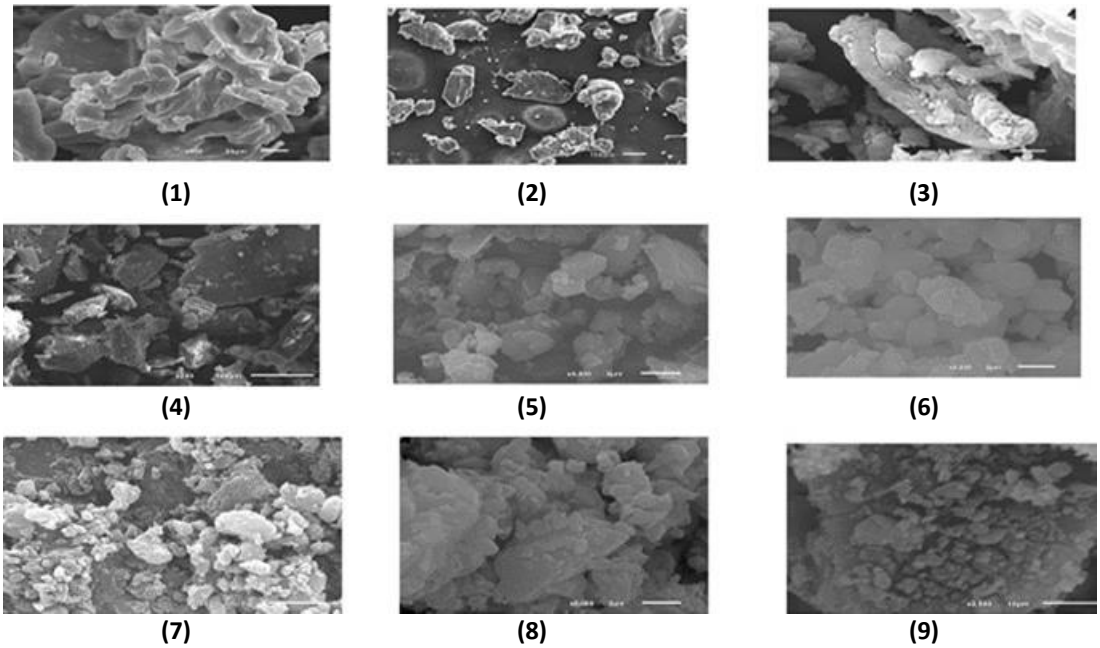

Figure 3: SEM Micrographs of LM(1),SBS(2),XN(3),β-CD,(4),MX(5), MXwith LM(1:3)(6), MX with SBS(1:3)(7), MX with XN(1:1)(8),MX with β-CD(1:3)(9)

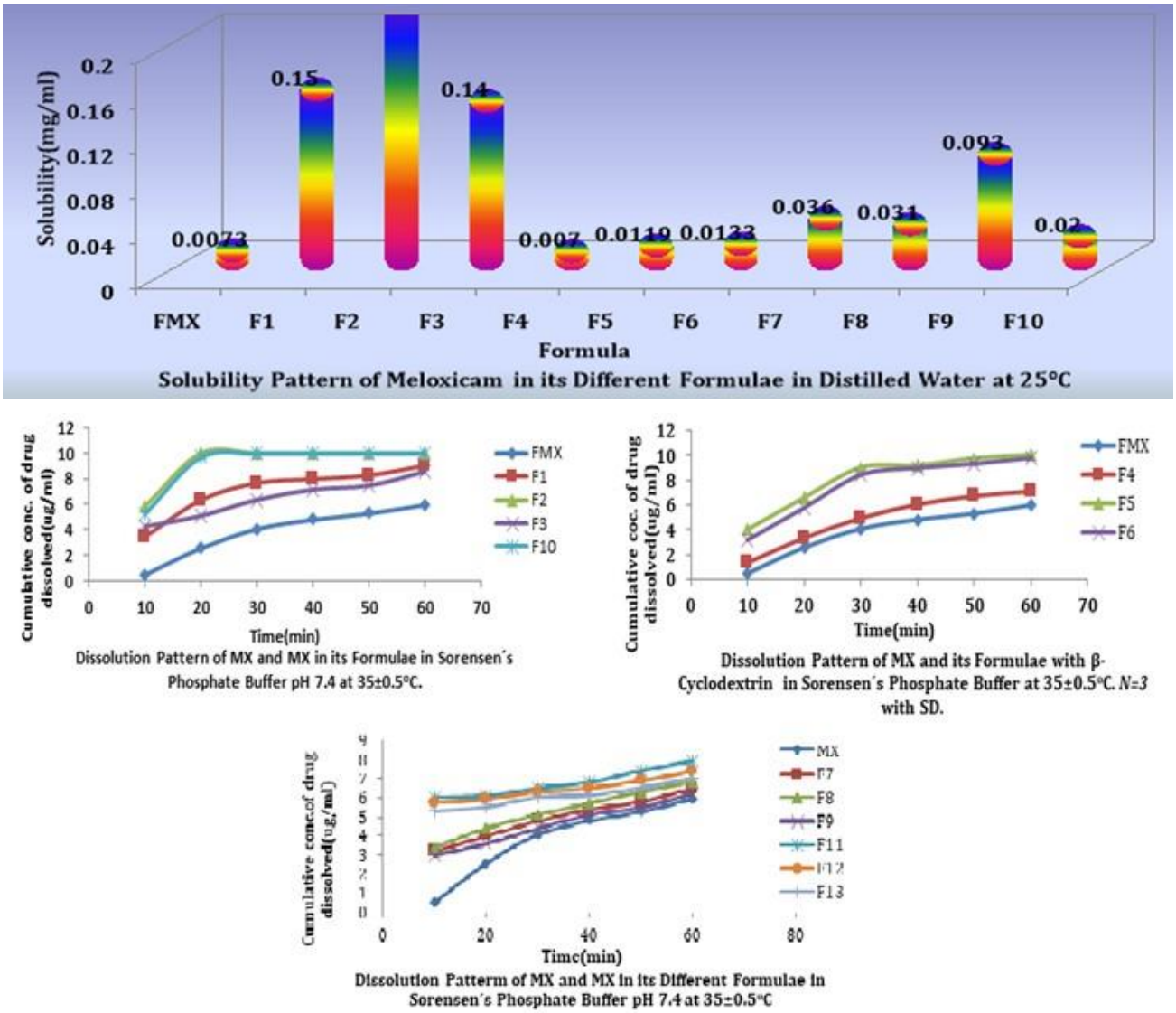

Figure 4: The dissolution pattern of MXfrom is different formulae.

Table 4 (A): Kinetic analysis of release data of Meloxicam and its Different Formulae

Formula	Model	R^2	Slope	Y-Intercept	Mechanism of drug release	$T_{50\%}$ (min)
F_{MX}	Zero	0.9190	1.031	0.26	Diffusion	44.7
	First	0.971	-0.007	1.019		
	Diffusion	0.972	1.166	-2.787		
	Hixon-Crowell	0.7930	-0.017	3.765		
F1	Zero	0.844	0.102	3.569	First	15.75
	First	0.932	-0.016	0.951		
	Diffusion	0.914	1.168	0.472		
	Hixon-Crowell	0.778	-0.010	3.088		
F2	Zero	0.696	0.078	6.206	Diffusion	2
	First	0.0701	-0.011	0. 571		
	Diffusion	0.796	0.9187	3.697		
	Hixon-Crowell	0.686	-0.006	2.800		
F3	Zero	0.980	0.0830	3.546	Diffusion	17.3
	First	0.958	-0.011	0.900		
	Diffusion	0.983	0.922	1.213		
	Hixon-Crowell	0.967	-0.008	3.075		
F4	Zero	0.931	0.115	0.904	First	35
	First	0.983	-0.009	1.014		
	Diffusion	0.978	1.296	2.471		
	Hixon-Crowell	0.852	-0.015	3.525		
F5	Zero	0.820	0.113	4.132	Diffusion	11.1
	First	0.650	-0.021	0.841		
	Diffusion	0.900	1.301	0.657		
	Hixon-Crowell	0.778	-0.010	3.013		
F6	Zero	0.840	0.126	3.191	First	15.9
	First	0.974	-0.029	1.16		
	Diffusion	0.915	1.448	-0.658		
	Hixon-Crowell	0.790	-0.012	3.13		
F7	Zero	0.989	0.064	2.698	Diffusion	31
	First	0.994	-0.005	0.888		
	Diffusion	0.995	0.710	0.897		
	Hixon-Crowell	0.972	-0.007	3.211		
F8	Zero	0.985	0.066	2.953	First	29.5
	First	0.999	-0.006	0.877		
	Diffusion	0.999	0.737	1.075		
	Hixon-Crowell	0.964	-0.007	3.172		
F9	Zero	0.993	0.064	2.393	Zero	32
	First	0.993	-0.005	0.904		
	Diffusion	0.988	0.701	0.627		
	Hixon-Crowell	0.983	-0.007	3.259		
F10	Zero	0.471	0.070	6.68	Diffusion	10.4
	First	0.065	-0.005	0.208		
	Diffusion	0.584	0.865	2.215		
	Hixon-Crowell	0.464	-0.006	2.774		
F11	Zero	0.961	0.039	5.413	Hixon-Crowell	7.3
	First	0.929	-0.005	0.693		
	Diffusion	0.902	0.417	4.402		
	Hixon-Crowell	0.968	-0.003	2.875		
F12	Zero	0.980	0.033	5.28	Hixon-Crowell	7.4
	First	0.956	-0.004	0.692		
	Diffusion	0.939	0.359	4.395		
	Hixon-Crowell	0.985	-0.003	2.892		
F13	Zero	0.974	0.033	4.906	Hixon-Crowell	7.4
	First	0.957	-0.003	0.721		
	Diffusion	0.941	0.358	4.021		
	Hixon-Crowell	0.978	-0.003	2.934		

$T_{50\%}$: Time required for 50% Drug Release

Table 4 (B): Statistical analysis of MX and its SDs Formulae

Formula	Mean	Pooled variance	$T_{stat.}$	DF	Probability (P)	COV
FMX	9.56	1.322115	6.897519166	8	FMX	9.56
F_1	9.65	0.985825	2.739041028	8	0.012743495	20.9%
F_3	9.56	1.317835	3.638918248	8	0.003299643	25.8%
F_4	9.56	1.648175	4.815541174	8	0.00066445	48.8%
F_5	9.56	1.408325	0.879351473	8	0.202424602	11.8%
F_6	9.56	1.72596	1.290177389	8	0.116514765	17.3%
F7	10	0.45626	10.99	8	2.08E-06	44%
F8	10	0.4515	10.212	8	3.63E-06	39.4
F9	10	0.5015	11.25292	8	1.75E-06	39.4
F10	10	0.009	1.00	8	0.173297	16.8
F11	10	0.2565	9.553175	8	5.96E-06	21.8
F12	10	0.165	13.23448	8	5.07E-07	24.6
F13	10	0.1585	15.0123	8	1.91E-07	28.8

Significant:($P<0.05$; $T_{stat.}>2.23$) ; Not Significant:($P>0.05$; $T_{stat.}<2.23$) D.F:degree of freedom ; P: Probability ; $T_{stat.}$: T statistics, COV:Coefficient of variation

Solubility Studies

MX depicted poor aqueous solubility due to its inherited property. Its PMs with different solubilizers enhanced the solubility of the drug. The enhanced solubility was due to incubation, mixed micelles and wetting effects.[14,15] The solubility of MX in PM prepared with LM showed higher solubility compared to PM prepared with other solubilizers. This is due to incubation, wetting of drug particles and mixed micelles effects. Whereas β-CD induced its effect through incubation only. The solubility of drug in its PM (F2) prepared with LM (1:3) was about 32 times as greater as the solubility of the drug. The solubilizing power of LM in ratio 1:3 was about 20 times as greater as that of β-CD(F5) in the same ratio. The solubility of MX in its PMs with SBS were also increased to an extent less than PMs prepared with both LM and β-CD. In PM of MX with LM and SBS (F10) (1:1:1) MX showed lowest solubility when compared with F2 and F5. This may be due to incompatibilities between micelles in LM & SBS. The solubility behavior of all formulae is shown in figure 4.

Dissolution Studies

Figure 4, revealed the slow dissolution rate of MX, due to its poor inherent solubility in aqueous medium. By reviewing the results of the solubility studies it is clear that, the dissolution of MX in its different formulations is a function of the solubility.

Increased LM concentrations improved the dissolution rate of MX especially in ratio (1:3) where the drug is completely dissolved after 30 minutes, the PM of drug with LM and SBS gave the same results. This may be due to formation of inclusion complex between amino acids molecules which are the host and the guest molecules of the drug beside the wetting properties of surfactants present in both LM and SBS more over the solubilizing effect of their mixed micelles.[10,11] MX depicted a high dissolution rate in all formulations. This is due to solid-solid transition of MX from crystalline to amorphous state when formulated with LM and SBS. The results of dissolution were in accordance with DSC, XRPD, IR and SEM.

Kinetic analysis

Table 4 illustrates the kinetics of the drug release. The drug dissolved in all formulae, followed first order kinetics and diffusion model. $T_{50\%}$ clarifies the variations present between the different formulations of drug, its PMs in the solubility and dissolution rate, F_2 depicted the shortest T_{50}.

Statistical Analysis

The statistical results showed the significance difference between F_2 and other formulae, COV showed the vast variation in dissolution of drug in F_2 and other formulae especially F_{MX}.

CONCLUSION

The results of this work revealed that the used LM and SBS enhanced the solubility by dual effects, mixed micelle and incubation of drug. This finding would rationalize the use of such natural substance as a promising additive for the development of optimal formulation conditions of poorly water soluble drugs and avoidance of side effects which may result from synthetic solubilizers.

REFERENCES

1. Neil. M.J.O, The Merck Index. 13th edn. Merck Research Laboratories Station, NJ.2006 P.1040.
2. Farzana, S., Bandarkar., Pradeep, R and Vavia.; A Stability Indicating HPLC Method for the Determination of Meloxicam in Bulkand Commercial Formulations, Tropical Journal of Pharmaceutical Research, 8(3), 2009, 257-264.
3. Syed M. Muhammad H., Shoaib F., and Inam U.;Bioequivalence studies of two brands of Meloxicam tablets in healthy Pakistani volunteers by RP – HPLC, Pak. J. Pharm. Sci, 22(2), 2009, 199-204.
4. Namita Desai., Purnima Amin; Stability indicating HPTLC determination of meloxicam, Indian Journal of Pharmaceutical Sciences, 63(3), 2001, 245-247.
5. Reddy, M.N., Murthy, T.K., Rajita, K and Shankar, D.G.;New spectrophotometric methods for the determination of meloxicam, Indian Journal of Pharmaceutical Sciences, 65(6), 2003, 655-658.
6. Seedher Neelam, Garg, A and Bhatia Sonu; Spectrophotometric method for estimation of some COX-2 inhibitors in pure form and in pharmaceutical formulations, Indian Journal of Pharmaceutical Sciences, 65(7), 2003, 685-688.
7. Mahmoud M Omar, Omiya A Hasan. Improving dissolution and oral bioavailability of fenofibrate by microwave induced fusion technique using HPMC E5 LV., 39(3), 2019, 587-598.
8. Mahmoud M Omar, Omiya A Hasan, Amani M El Sisi. Preparation and optimization of lidocaine transferosomal gel containing permeation enhancers: a promising approach for enhancement of skin permeation, 14, 2019, 1551-1562.
9. Mura,P., Characterization and Dissolution Properties of Ketoprofen in Binary and Ternary Solid Dispersions with Polyethylene Glycol and Surfactant**.** Drug Dev. and Ind. Pharm., 30, 2005, 425-434.
10. Shahira F. El-Menshawe, Rasha M. Kharshoum and Amani M. El Sisi; J Nanomed Nanotechnol, 2017, 8-2.
11. Amani M. El Sisi, Rasha M. Kharshoum, Adel A. Ali, Khaled M. Hosny, Ahmed Abd-Elbary; Int. J. Pharm. Sci. Rev. Res., 30(2), Jan.-Feb. 2015, Article No. 01, 1-6.
12. Hala M. Alkhalidi, Ghada H. Naguib, Mallesh Kurakula, Mohamed T. Hamed, Moaz H. Attar, Ziyad H. Almatrook, Ahmed Y. Aldryhim, Rahaf H. Bahmdang, Rasha A. Khallaf, Amani M. El Sisi and Khaled M. Hosny; Journal of Drug Delivery Science and Technology, 48, 2018, 450-456.
13. "Milk analysis". North Wales Buffalo. Archived from the original on September 29, 2007. Retrieved August 3, 2009. (Citing McCane, Widdowson, Scherz, Kloos, International Laboratory Services.)
14. USDA National Nutrient Database for Standard Reference. Ars.usda.gov. Retrieved November 24, 2011. Archived August 21, 2008, at the Wayback Machine.
15. Higuchi T, Connors KA. Phase-solubility techniques. In: Rilly CN, ed. Advances in Analytical Chemistry and Instrumentation. New York, NY: Wiley Interscience; 1965, 117-212.
16. Majumdar, S, Rao, M E B. Reparation of Solid Dispersions of NSAIDS and Its Mechanism of Drug Polymers Interaction, Indian J. Pharm. Sci., 1, 2008, 167-174.
17. Nafady M.; Pharmaceutical study on extended release lyophilized milk treated ibuprofen beads; WJPR, 4(1), 2014, 658-671.

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