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# Improvement of the solubility and dissolution of ketoprofen using natural bile salts

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**ABSTRACT**

*The aim of this work was to improve the solubility and dissolution of the poorly water soluble ketoprofen(KP) using natural solubilizers, The physical mixture(PM) and lyophilized solid dispersion(LSD) of drug were prepared using different carriers namely shark bile salts(S.B) , fresh water fish(Tilabia nilotica) bile salts(F) , polysorbate 80(P.S) and sodium lauryl sulphate(SLS) . The solubility and dissolution of KP in distilled water were investigated as a function of solubilizer concentration(0-3%w/w) in the prepared PMs and LSDs . In a concentration of 2%w/w solubilizer , the drug solubility and dissolution were improved dramatically. In LSDs the solubility increased from 14 mg/ml to 81 mg/ml and 63 mg/ml when using shark bile and polysorbate 80 respectively . Dissolution was found to be a function of solubility especially in case of natural solubilizers and polysorbate 80 . Simple and mixed micellar solubilization was the main mechanism suggested for the increased solubility and dissolution of the drug. Results were confirmed by surface tension measurements of the aqueous solution of the plain drug , its PM and LSD . DSC , IR and XRPD studies suggested possible interactions between KP and some of the used solubilizers. The results suggested that shark bile salts revealed promising technique in enhancing the solubility and dissolution of the poorly water soluble drugs as KP and application in the field of dosage form design in digestive therapy. The kinetic study revealed that , the drug release from all formulations followed zero order kinetics.*

**Keywords:** ketoprofen , solubility , shark bile salt , surface tension , lyophilized solid dispersion

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## INTRODUCTION

KP is a nonsteroidal anti-inflammatory drug which has good analgesic properties, but KP has a low solubility in water so that it can cause problems in formulating and limiting the bioavailability[1]. The improvement of drug solubility thereby its oral bio-availability remains one of the most challenging aspects of drug development process especially for oral drug delivery system. These in vivo and in vitro characteristics and the difficulties in achieving predictable and reproducible in vivo/in vitro correlations are often sufficiently difficult to develop formulation on many newly synthesized compounds due to solubility issues [2,3] . One way to increase the solubility of poorly soluble drugs is through the formation of solid dispersion. Numerous solid dispersion systems have been demonstrated in the pharmaceutical literature to improve the dissolution properties of poorly water-soluble drugs [4] . Other methods, such as salt formation [5], complexation with cyclodextrins [6-10] enhances solubilization of drugs in solvents [11 , 12] and particle size reduction [13] have also been utilized to improve the dissolution properties of poorly water-soluble drugs; however, there are substantial limitations with each of these techniques. On the other hand, formulation of drugs as solid dispersions offers a variety of processing and excipient options that allow for flexibility when formulating oral delivery systems for poorly water soluble drugs .

In this study natural and synthetic solubilizers have been used to enhance the solubility through micellar solubilization . Thus, the study opens the chances of preparing such solid dispersion formulations of poorly water soluble drugs with natural surfactants which adds a promising technique for enhancing solubility and dissolution. If chemical stability of the drug remains unaffected, it opens a new era of more stable economic and safe products in the market. In this work , attempts were made to enhance the solubility of the poorly soluble KP to improve its dissolution and hence its bioavailability

## MATERIALS AND METHODS

### Materials

Freeze-dried gall bladder contents of shark and fish , KP, micronized polysorbate 80 , sodium lauryl sulphate , absolute alcohol were purchased from Sigma Chemical Co., St.Louis, MO. All water used was distilled de-ionized water. All other chemicals were of reagent grade and used as received.

### Preparation of Lyophilized Solid Dispersion

The drug and each of the following solubilizers(0-3%w/w) : shark , fish gall bladder contents(freeze-dried) , polysorbate 80 , SLS were dissolved in absolute alcohol . Alcohol was evaporated using rotary evaporator ( Rotavapor RII , Buchi , Switzerland) . The solid dispersion obtained was dispersed in a suitable amount of water then transferred to a freezer at −22°C and kept in the freezer for 24 h. The frozen SDs were placed in a lyophilizer for 24 h using a Novalyphe-NL 500 Freeze Dryer (Savant Instruments, Holbrook, NY) with a condenser temperature of −45°C and a pressure of 7 × 10−2 mbar. The lyophilized solid dispersion(LSD) were kept in a desiccators over calcium chloride (0% relative humidity) at room temperature until further used.

### Preparation of Physical Mixture

KP was uniformly mixed with different solubilizers in concentrations used in the LSDs using a mortar and pestle. The prepared mixtures were kept in a desiccator until used.

### Drug Content

An amount of LSD and PM equivalent to a theoretical KP content of 25 mg was accurately weighed and allowed to disintegrate completely in 100 ml of absolute alcohol. After filteration , the solution was assayed spectrophotometrically for drug content at 262 nm.

### Surface Tension Studies

Solutions of KP and its LSDs and PMs , equivalent to 25 mg in 100 ml distilled water were subjected to surface tension measurements using ring tensiometer (Kruss K26 , Germany) at ambient temperature . The surface tension was expressed in millimeter Newton/ meter ( mN/m) . All measurements were done in triplicates.

### Solubility Studies

KP (25 mg), its LSDs and PMs equivalent to 25 mg were placed in glass stoppered flasks and 100 mL water was added to each flask. The flasks were shaken in a water bath at 25°C for 15 h (USP XIX). The solutions were filtered through a membrane filter (0.45 μm) and the dissolved drug was measured spectrophotometrically at 262 nm. This experiment was done in triplicate.

### Dissolution Studies

The dissolution profiles of KP LSDs( KP lyophilized with 2%w/w solubilizer) and PMs (KP mixed with 2%w/w solubilizer) and the plain drug, 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 distilled water maintained at 37± 0.5°C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 25 mg. After specified time intervals, samples of dissolution medium were withdrawn, filtered , and assayed for drug content spectrophotometrically at 262 nm after appropriate dilution with water.

### 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 50°C/min in the range of 50–300°C. Thermograms for KP , LSDs, and 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. Diffraction patterns for KP, LSDs, and 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 KP , KP LSDs and KP PMs were prepared with 2%w/w of each of the previously mentioned solubilizers was mixed separately with 400 mg dry potassium bromide powder , compressed into transparent discs and their IR spectra were recorded.

**Kinetic Analysis**

The release data of plain drug , PMs and LSDs were subjected to kinetic analysis according to zero order , first order kinetics and Higuchi diffusion model.

## RESULTS

**Table (1): Surface tension of aqueous solution of ketoprofen in LSDs and ketoprofen in PMs prepared with different solubilizers at ambient temperature.**

| Solubilizer Concentration(%w/w) | Surface Tension(mN/m) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LSD | | | | PM | | | |
| | Sharkbile | Fishbile | Polysorbate80 | SLS | Sharkbile | Fishbile | Polysorbate80 | SLS |
| 0.0 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 |
| 0.5 | 54 | 61 | 58 | 63 | 56 | 62 | 57 | 63 |
| 1.0 | 40 | 59 | 49 | 60 | 41 | 60 | 51 | 62 |
| 1.5 | 33 | 55 | 45 | 55 | 37 | 56 | 47 | 58 |
| 2.0 | 32 | 54 | 43 | 53 | 35 | 55 | 46 | 57 |
| 2.5 | 32 | 53 | 42 | 51 | 34 | 55 | 45 | 58 |
| 3.0 | 31 | 53 | 42 | 50 | 33 | 55 | 45 | 57 |

**Table (2) :Solubility of ketoprofen PMs prepared with different solubilizers in water at 25°C±0.5**

| Solubilizer concentration %w/w | Solubility of Ketoprofen mg/ml | | | |
|---|---|---|---|---|
| | Shark Bile | Fish | Polysorbate 80 | SLS |
| 0 | 14±1.221 | 14±1.221 | 14±1.221 | 14±1.221 |
| 0.5 | 25±1.304 | 14±1.581 | 19±2.167 | 14±1.581 |
| 1.0 | 28±1.581 | 18±3.162 | 24±1.871 | 15±1.483 |
| 1.5 | 37±3.162 | 20±1.580 | 29±1.583 | 16±0.442 |
| 2.0 | 48±1.480 | 21±1.140 | 33±1225 | 16±1.414 |
| 2.5 | 46±1.481 | 21±1.36 | 34±1.280 | 16±2.121 |
| 3.0 | 48±1.421 | 21±1.47 | 34±1.517 | 16±0.707 |

**Table(3) : Solubilityof ketoprofen LSDs prepared with different solubilizers in water at 25°C±0.5**

| Solubilizer concentration %w/w | Solubility of Ketoprofen mg/ml | | | |
|---|---|---|---|---|
| | Shark Bile | Fish | Polysorbate 80 | SLS |
| 0 | 14±1.221 | 14±1.221 | 14±1.352 | 14±0.982 |
| 0.5 | 64±0.871 | 31±1.812 | 36±1.144 | 18±0.865 |
| 1.0 | 71±1.268 | 36±2.123 | 45±0.970 | 22±1.224 |
| 1.5 | 75±.967 | 38±0.580 | 57±1.330 | 26±0.851 |
| 2.0 | 81±0.542 | 41±1.611 | 63±1.186 | 31±1.686 |
| 2.5 | 80±1.35 | 40±0.760 | 62±1.532 | 30±1.323 |
| 3.0 | 80±1.66 | 41±1.145 | 63±1.781 | 30±0.864 |

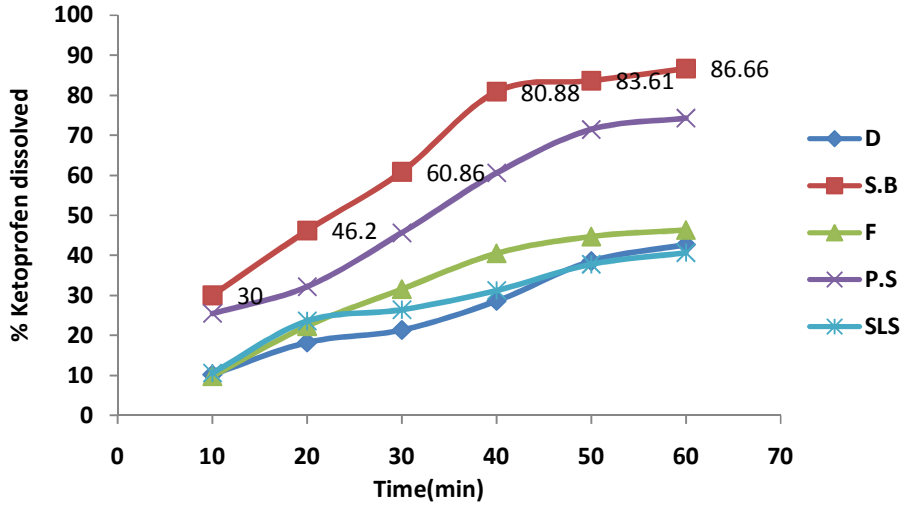


**Fig 1. Dissolution profiles of KP powder and Its PMs containing 2%w/w solubilizers in distilled water at 37ºC . N=3 with SD.**

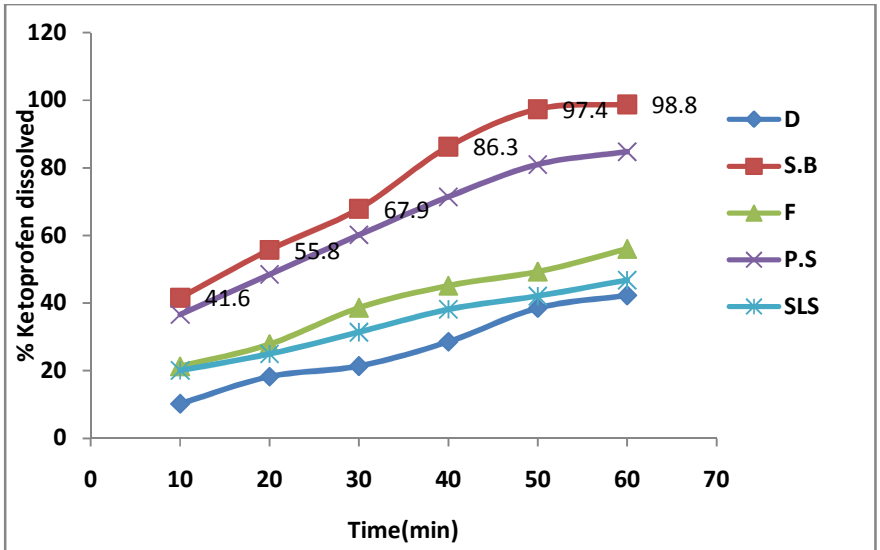


**Fig 2.Dissolution profiles of KP powder and Its LSDs containing 2%w/w solubilizers in distilled water at 37oC . N=3 with SD.**

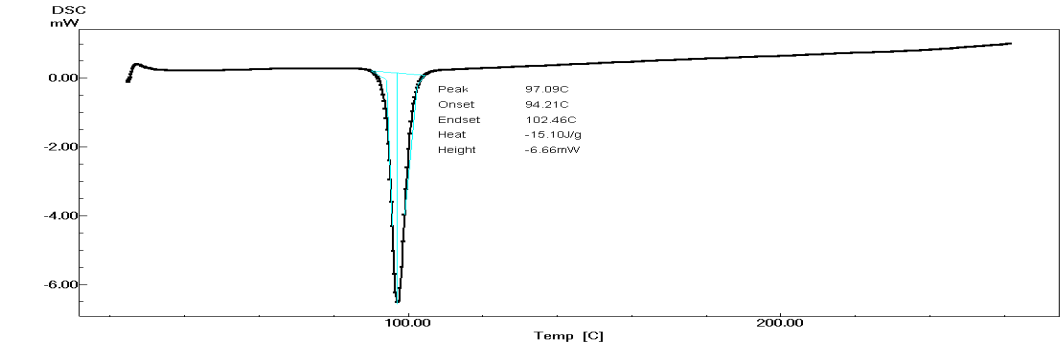


**Fig 3a . DSC of plain Drug**

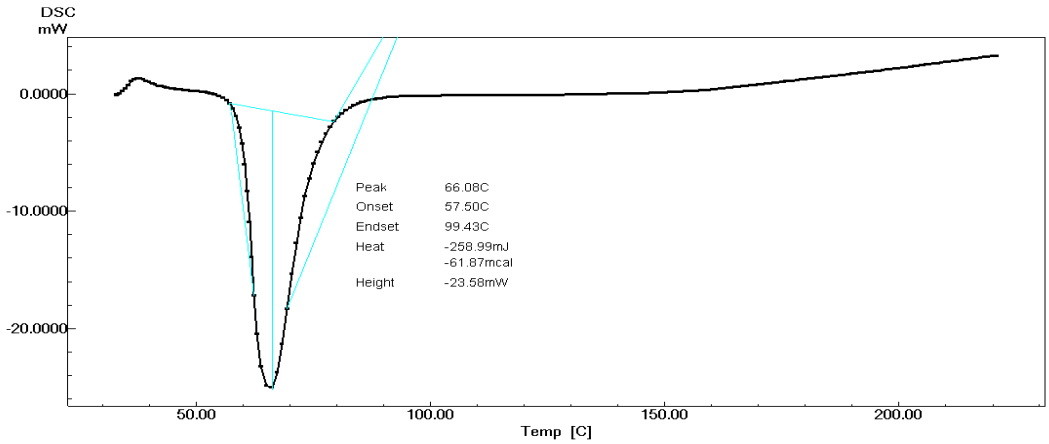


**Fig3b .DSC of KP in PM (2%w/w shark bile)**

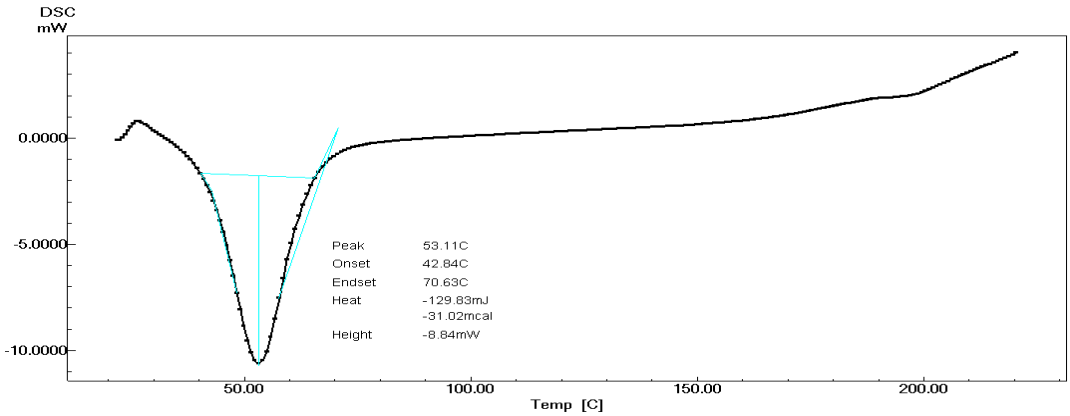


**Fig3c. DSC of KP in LSD (2%w/w shark bile)**

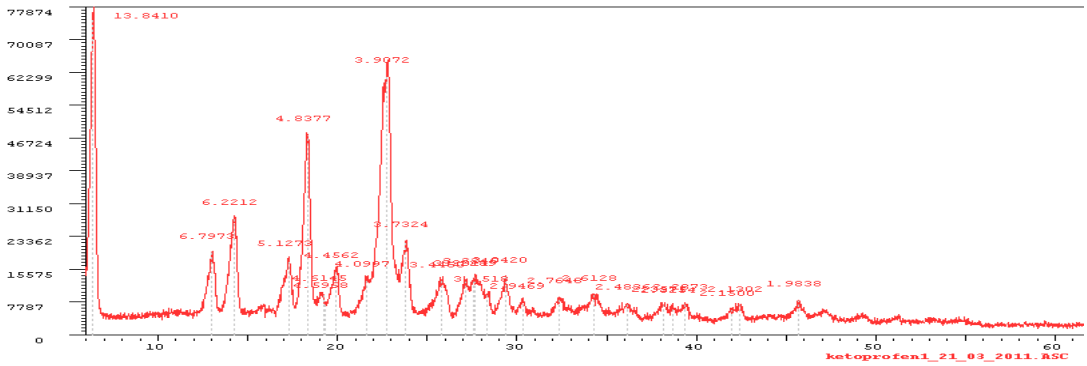


**Fig 4a. XRPD of plain KP**

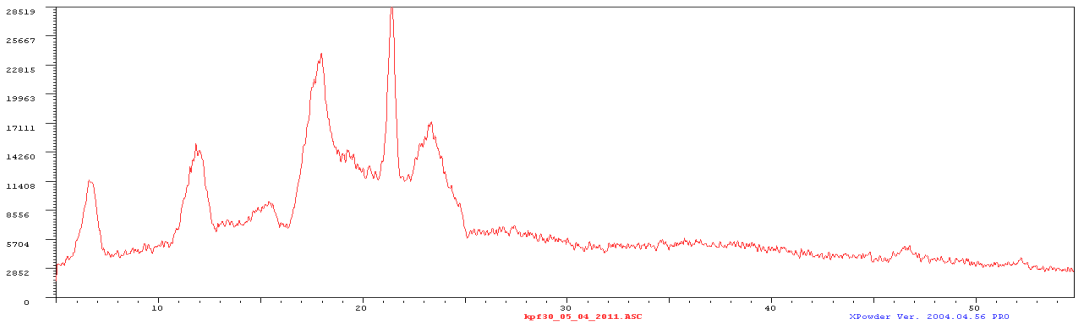


**Fig 4b .XRPD of KP in PM (2%w/w shark bile)**

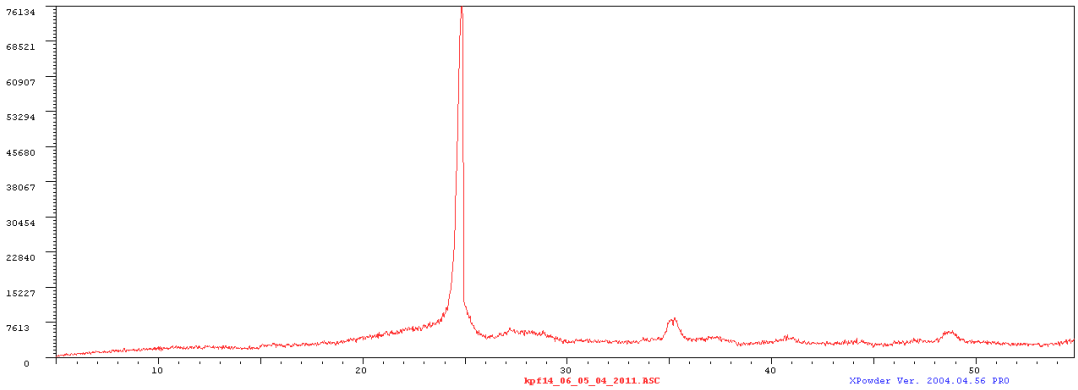


**Fig4c . XRPD of KP in LSD (2%w/w shark bile)**

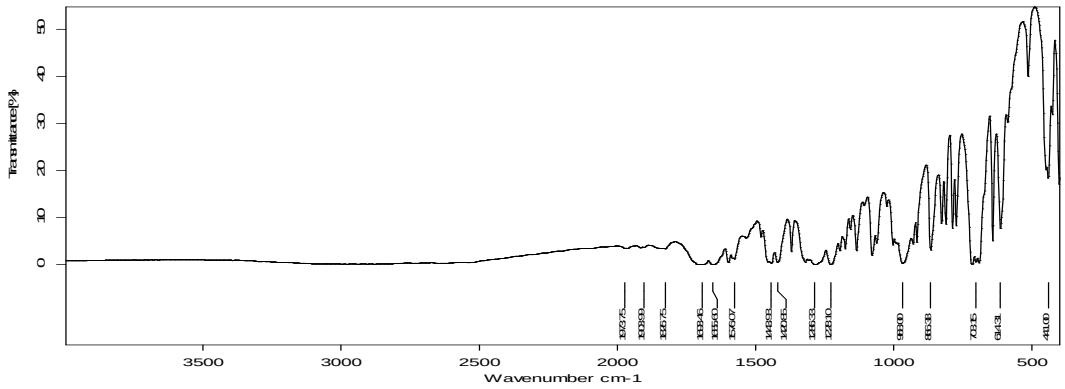


**Fig 5a. XRPD of plain KP**

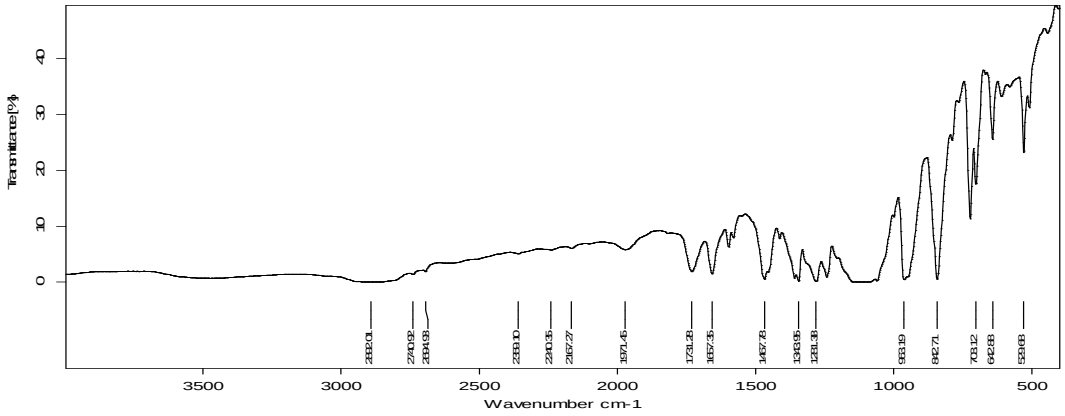


**Fig 5b. FTIR of KP in PM (2%w/w shark bile)**

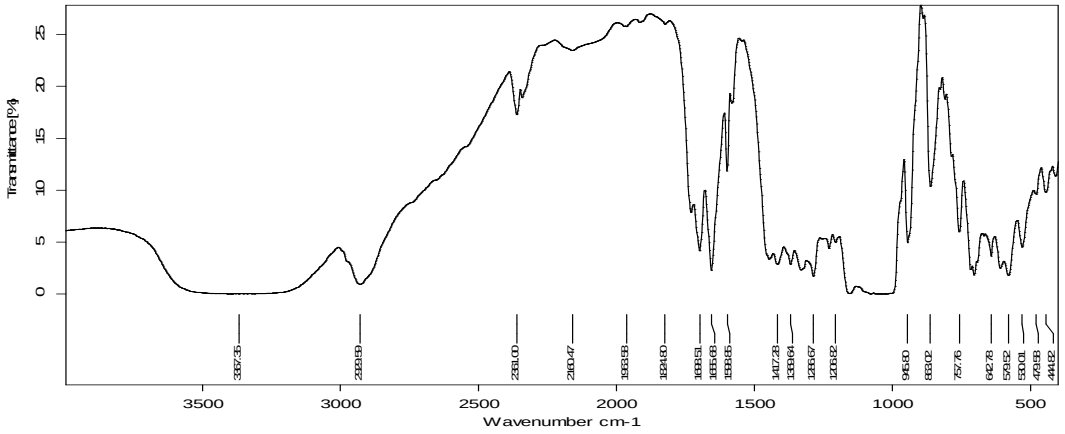


**Fig5c . FTIR of KP in LSD (2%w/w shark bile**

**Table (4): Kinetic analysis of release data of ketoprofen , its PMs and LSDs prepared with different solubilizers**

| Formulation | Kinetic order | $R^2$ | Slope | Y-intercept | $T_{50\%}$ (min) | Order of release |
|---|---|---|---|---|---|---|
| Ketoprofen | Zero | 0.991 | 0.6588 | 3.5527 | 70.50 | Zero |
| | First | 0.975 | | | | |
| | Diffusion | 0.954 | | | | |
| PM(S.B) | Zero | 0.965 | 1.1873 | 23.1470 | 22.62 | Zero |
| | First | 0.964 | | | | |
| | Diffusion | 0.939 | | | | |
| PM(P.S) | Zero | 0.973 | 1.0766 | 13.9310 | 33.50 | Zero |
| | First | 0.970 | | | | |
| | Diffusion | 0.971 | | | | |
| PM(F) | Zero | 0.987 | 0.7391 | 6.6733 | 58.62 | Zero |
| | First | 0.965 | | | | |
| | Diffusion | 0.887 | | | | |
| PM(SLS) | Zero | 0.968 | 0.5651 | 8.6047 | 73.27 | Zero |
| | First | 0.964 | | | | |
| | Diffusion | 0.932 | | | | |
| LSD(S.B) | Zero | 0.9998 | 1.2263 | 31.7130 | 14.91 | Zero |
| | First | 0.997 | | | | |
| | Diffusion | 0.988 | | | | |
| LSD(P.S) | Zero | 0.981 | 0.9987 | 28.7920 | 21.24 | Zero |
| | First | 0.979 | | | | |
| | Diffusion | 0.980 | | | | |
| LSD(F) | Zero | 0.983 | 0.6989 | 15.2390 | 49.74 | Zero |
| | First | 0.889 | | | | |
| | Diffusion | 0.784 | | | | |
| LSD(SLS) | Zero | 0.994 | 0.5470 | 14.7760 | 64.39 | Zero |
| | | | | | | |
| | First | 0.679 | | | | |
| | Diffusion | 0.543 | | | | |

*$R^2$: coefficient of determination , $T_{50\%}$ : time required for dissolution of 50% of drug*

## DISCUSSION

### Drug Content

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

### Surface Tension Study

By reviewing the data of table 1 it was obvious that , the concentrations of the used solubilizers were beyond their critical micelle concentrations(CMC) as indicated by an almost constant value of the surface tension especially at 1.5 and 2%w/w . Solutions of the treated drug showed remarkable decrease in surface tension . The surface tension of solutions of the drug solid dispersions prepared with 2%w/w of any of the used carriers ranged from 32 to 57 mN/m versus 65 mNm for the plain drug.

### Solubility Studies

The solubility of KP using different natural and synthetic solubilizers in water is shown in tables 2 and 3 . The used solubilizers with their surface activity property enhanced the wettability of the hydrophobic drug , thus resulted in improved drug solubility. This increase in drug solubility could be attributed to the micellar solubilization of large amount of the unionized drug in the hydrophobic interior of the micelles of different solubilizers moreover the increased surface area of contact in case of LSDs . The difference in the solubility capacity of the used solubilizers may depend on a specific interaction between KP and different micellar species. Each type of the micellar species has different number of hydroxyl groups which could form hydrogen bonds with the polar part of drug molecule . This trend was pronounced with shark bile and polysorbate 80 . The micelles of these solubilizers may offer a larger number of hydroxyl groups and hence , larger areas for interaction . Moreover , the dramatic increase in solubility of KP associated with the bile of skark is probably due to the presence of some agents other the bile salts as lecithin and cholesterol suggesting formation of mixed micelles which have hydrophobic core in which the poorly soluble KP can dissolve [14] .

**Dissolution Studies**

Figures 1,2 reveal a very slow dissolution rate for the plain drug , due to its low inherent solubility in aqueous medium . By reviewing the results of the solubility studies it is clear that, the dissolution of KP in its LSDs and PMs is a function of the solubility especially in case of natural soubilizers and polysorbate 80 . Increased solubilizer concentration can improve KP dissolution by increasing the saturated solubility of the drug and/or by increasing surface area of the powder via improved wetting [15] . The percent of drug dissolved after 60 min for LSDs prepared with 2%w/w solubilizer increased from 42..27% to 98..8 % , 84.77 % , 56 % and 46 % of the initial drug content in case of shark bile , polysorbate 80 , fish bile and SLS respectively. It is obvious that higher drug dissolution was obtained from LSD and PM prepared with solubilizers especially that of shark bile . This is in agreement with the results of the solubility study. These promising results will potentiate formulations of improved bioavailability . This may be due to reduction in particle size under the effect of mixed micelles [16 , 17] , hence accelerate the anti-inflammatory effect of KP[18]

**Differential Scanning CalorimetryStudies(DSC)** [19]

DSC studies were performed on KP powder, its LSD, and PM (Fig. 3a). The DSC curve of KP showed a sharp endothermic peak at nearly 90°C, corresponding to its melting transition point. The thermogram of the PM showed the endothermic peak of KP shifted to the right at melting transition point at 66$^{o}$C(Fig3b) , indicating that the crystalline state is maintained in the PM. However, the melting endotherm was sharply shifted to the right at 53$^{o}$C on the DSC thermogram of the LSD(Fig 3c), suggesting absence of crystallinity and presence of amorphous state of the drug.

**X-ray Powder Diffraction Analysis(XRPD)**

These results were further confirmed by x-ray diffraction studies (Fig. 4a). The x-ray diffraction pattern of the pure drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The diffraction study of the PM of drug and solubilizer showed the peaks corresponding to the crystalline drug molecules present in the mixture, although their intensity was lower due to the high solubilizer–drug ratio employed(Fig 4b) . The diffraction pattern of the LSD of drug showed absence, broadening, and reduction of major KP diffraction peaks indicating that mostly an amorphous form (disordered state) existed in the LSD(Fig 4c). These results could explain the observed enhancement of solubility and rapid dissolution of KP in LSD.

**Infrared Spectroscopy (FTIR)**

Infrared spectroscopy was used to study the interactions between the drug and the solubilizers. KP has a carboxylic acid group, which can interact with the functional groups of the polymers. The carbonyl peaks in the IR spectra of KP were recorded at 1694 $cm^{-1}$ and 1654 $cm^{-1}$, and have previously been assigned to dimeric carboxylic acid carbonyl group and ketonic carbonyl group stretching vibrations, respectively(Fig 5a) . IR spectra of PM( Fig5b) and LSD( Fig 5c) revealed no prominent change in functional group region whereas, an obvious change in fingerprint region especially with LSD of drug with shark bile . The fingerprint regions of drug and drug in LSD and PM are not superimposed which confirm the change in physical characteristics of KP. These results are in accordance with DCS and XRPD.

**Kinetic Analysis**

Table 4 illustrates the kinetics of drug release . The drug dissolved in all formulations , followed zero order kinetics . $T_{50\%}$ clarifies the variations present between the different formulations of drug , its PMs and LSDs in the solubility and dissolution rate .

## CONCLUSION

The results of this work revealed that the used micellar system enhanced the solubility of the drug as well as its dissolution . The enhancement achieved by shark bile was comparable to that obtained by the synthetic solubilizer polysorbate 80 . In this respect , the enhancement due to the bile of shark was superior to other solubilizers . These findings would rationalize the use of such natural substances as promising additives for the development of optimal formulation conditions of poorly water soluble drugs.

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