WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 15, 1534-1561. Research Article ISSN 2277– 7105

# DEVELOPMENT AND EVALUATION OF INJECTABLE IN SITU IMPLANT FOR SUSTAINED RELEASE OF MELOXICAM BASED ON BIODEGRADABLE POLYMERS (MUSK AND MASTIC)

**Mahfood Omer Otaq*[1,2], Gamil Qasem Othman[1] and Khaled Abdullah Al-Tahami[3]**

[1]Department of Pharmaceutics, Faculty of Pharmacy, University of Science and Technology, Sana'a, Yemen.

[2]Shphaco Pharmaceutical Industry, Sana'a, Yemen.

[3]AL Khaled Pharma and Medical Appliances, Sana'a, Yemen.

Article Received on
20 July 2020,

Revised on 10 August 2020,
Accepted on 06 Sept. 2020,

DOI: 10.20959/wjpr202015-18501

***Corresponding Author**
**Dr. Mahfood Omer Otaq**
Department of Pharmaceutics, Faculty of Pharmacy, University of Science and Technology, Sanaa, Yemen.

## ABSTRACT

The main objective of this study was to develop and to evaluate meloxicam in situ implant from biodegradable polymeric substances to attain sustained release of meloxicam, which help to manage pain for long time and reduce side effect. **Methods:** meloxicam in situ implant or precipitate was prepared by phase separation method employing musk and mastic as biocompatible and biodegradable polymers in several concentrations of polymers and solvent. The formulated meloxicam implant / precipitate were evaluated for implant / precipitate morphology, viscosity of solution or suspension and syringability, drug entrapment efficiency, loading of drug, drug-polymer combination compatibility, the drug release in- vitro study and accelerated stability study. **Results:** The proportion entrapment efficiency of drug was existing within the acceptable limit and found to be higher in case of mixed biodegradable polymer (Musk, Mastic) with xanthan gum as compared to low concentration of musk alone. From Fourier transform infra-red spectrums of the implant or precipitate observed no interference between meloxicam with the polymers applied. Scanning electron microscope examined of implant observed small coarse spherical or gel precipitate. The release in vitro study of meloxicam from the all formulations was different polymers with different concentration. Long meloxicam release was showed in formulation has musk concentration or mixed two polymers with medium viscosity after injected in phosphate buffer media at PH 7.4 throughout eight days. The proportion of entrapment efficiency and loading of drug

elevated whereas meloxicam sustained release from polymeric implant or precipitate, when the drug used with different types and concentration of polymer ratio increased. The initial burst release was acceptable in most formulation and showed acceptable stability of best formula exactly in F12 with good correlation coefficients $R^2$. **Conclusion:** The results which it obtained from this study the Meloxicam, implant / precipitate from biodegradable polymers were performed to be appropriated delivery of meloxicam or any active drugs as safe and efficient sustained delivery system.

**KEYWORDS:** Parenteral Sustained release, Meloxicam, Musk, Mastic, biodegradable polymers, Phase Separation Method.

## INTRODUCTION

Sustained release drug delivery systems provide several advantages over the regular formulations such as better patient comfort and compliance, prolonged drug delivery, decreased dosing frequency, and minimum side effects. In situ implant system is a special class of polymeric sustained release systems that is manufactured as a gelling and solidifies after administration.[1]

One of the major disadvantages associated with the phase separation method is the initial drug burst.[2] It is characterized by an initial high drug release rate at the beginning of the administration process, an effect that results in a plasma elevated drug concentration that may exceed the maximum effective concentration and may cause tissue irritation. The main cause for this phenomenon is the fast distribution of the administered drug during the sol/gel (solidification) or precipitate transformation process.[3,4] Other factors that could contribute to this behavior are uneven distribution of the drug inside the polymeric matrix[5] and the rapid diffusion of the drug adsorbed on the surface of the polymer.[6] Some efforts have been made to overcome this problem. Among these is the use of organic solvents miscible with water.[7,8] Choices of musk and mastic polymers, and I use of different polymer quantities and solvent concentration, and incorporation of other excipient.[9,10]

Implant delivery systems has an advantage over some other drug delivery systems as release of drug is sustained for a longer period of time (weeks to months), which may increase patient compliance and improve therapeutic index as constant blood drug level is maintained. It also prevents body exposure to unnecessary high drug concentration, which is observed in conventional dosage forms.[11,12]

In situ implant systems are based on a drug-containing biodegradable polymer solution in a biocompatible organic solvent. After injection in the body, the solvent diffuses into the aqueous environment leading to polymer precipitation and the formation of an in situ implant or precipitate inside the injection site. However, in situ implant systems suffer from many disadvantages like the initial rapid release and difficult injectability of highly concentrated polymer solution due to high viscosity.[13]

As an alternative to other in situ implant systems, in situ forming implant system has been developed. These systems are consisting of an internal polymer phase of the drug and the biodegradable polymer (Musk, Mastic) dissolved in a biocompatible solvent such as Dimethyl Sulfoxide (DMSO). Once injected, DMSO diffuses into the surrounding tissue fluid leaving the drug loaded polymer precipitated in the form of implant formed in situ.[14]

It consists of a biocompatible biodegradable hydrophobic polymer that is dissolved in a water miscible/partially miscible, biocompatible solvent.[15,16] The drug is dissolved or suspended in the polymeric matrix. The musk and mastic is used as polymers utilized in this system. It possesses many useful properties among which are the biocompatibility, biodegradability, and the availability of musk and mastic, drug loaded with biodegradable polymeric substances such as musk and mastic have been widely used in development of drug delivery systems due to their biodegradation properties, ability the drug to formed implant or precipitate and biocompatibility are well understood and a number of delivery systems based on these polymeric materials have been FDA approved.[16,45]

Meloxicam is a potent non-steroidal anti-inflammatory drug used for the treatment of rheumatoid arthritis, osteoarthritis or other musculoskeletal disorders.[1] Due to ability to cause severe gastric damages like irritation or bleeding upon exposure to stomach at high concentration, Meloxicam is regarded as good candidate for the formulation of sustained release dosage regimens.[17]

Therefore, the aim of this study was to develop and evaluate an optimized in situ forming implant system with sustained release properties therapy of meloxicam formulation with a low initial drug burst using a biodegradable polymers musk and mastic with different types of solvent in different concentrations. Subcutaneous administration of this formulation could be considered as an alternative for the commercially available drug oral daily tablets or aqueous injection. For post-operative and rheumatoid arthritis, osteoarthritis or other musculoskeletal

disorders pain management in order to decrease dosing frequency and increase patient compliance, avoid the drug per oral first pass effect and enhance the drug bio-availability. The effect of different formulation parameters on the release of meloxicam from the implant formulae was investigated. A 12 experimental formula were performed to investigate the influence of formulation variables on the release profile of the drug.

## MATERIALS

Meloxicam was providing from Globela pharma PVT. LTD- India, Natural polymer (Musk, Mastic) were obtained from Market (Bin Yasin), dimethyl sulfoxide (DMSO) HPLC grade by Scharlau (Spain), polyethylene glycol 400 (PEG 400) Analytic grade by (JPM), Disodium hydrogen orthophosphate by Kimia international Ltd- UK( Hayman), Potassium dihydrogen orthophosphate by Dr chemicals Co., LTD, methanol by Fisher (UK), acetonitrile by Scharlau (Spain), Sodium Lauryl Sluphate by Globela pharma PVT. LTD- India, Methyl paraben by Globela pharma PVT. LTD- India and Propyl paraben by Globela pharma PVT. LTD- India. All other reagents were obtained analytical grade from UST research center, Yemen.

## METHODS

### Preparation of the in situ implant drug delivery systems:

**Preparation of injectable polymer solution:** 500 grams for each polymers were immersed in pure water for 24 hours, then filtered and leave drying for many days. Different concentrations of each non water soluble biodegradable polymer (musk, mastic) were dissolved into different solvent mixtures [dimethylsulfoxid (DMSO), polyethylene glycol (PEG)] in a glass vial and stirring was kept in water bath (37°C, 35 rpm) over one day. The polymers solubility was checked by visual inspection. The polymer solution injectability was tested by passing through a 21-gauge needle. The mixed solvent was formulated by two integrated solvents; dimethylsulfoxid (DMSO) and polyethylene glycol (PEG)] in different ratios.[27,41]

### Preparation of polymer–drug solution:

The therapeutic agent (Meloxicam) was integrated into an in situ implant polymers' liquid solution. This polymer's solution was formulated by integrating biodegradable polymers in different concentrations and adding them into solvents in different concentrations (DMSO, PEG) as shown in Table 1, in glass vials until a clear solution or suspenision is formed by homogenizing at 2200 rpm for 2 min at 30° C. The formulations with injectable properties were passed through a 21-gauge needle. This study showed different concentations of

polymers in twelve formulations, and used an organic solvent composition which is formulated as elaborated in the steps.[18]

**Table 1: Formulation of meloxicam in different ratios of biodegredable polymer:**

| Formulation code | API (mg) | Musk (mg) | Mastic (mg) | Xanthan gum (mg) | Carbomer 497 (mg) | DMSO (ml) | PEG (ml) | Drug/ polymer ratio | Injection volume (ml) |
|---|---|---|---|---|---|---|---|---|---|
| F1 | 50 | - | 250 | 25 | - | 1 | 0.5 | 1 : - : 5 | 1.5 |
| F2 | 50 | - | 250 | - | - | 1 | 0.5 | 1 : - : 5 | 1.5 |
| F3 | 50 | - | 300 | - | - | 1.5 | - | 1 : - : 6 | 1.5 |
| F4 | 50 | 125 | 125 | - | - | 1.5 | - | 1 : 2.5 :2.5 | 1.5 |
| F5 | 50 | 125 | 125 | - | - | 1 | 0.5 | 1 : 2.5 :2.5 | 1.5 |
| F6 | 50 | - | 250 | - | 0.015 | 1 | 0.5 | 1 : - : 5 | 1.5 |
| F7 | 50 | 250 | - | - | 0.015 | 1 | 0.5 | 1 : 2.5 : - | 1.5 |
| F8 | 50 | 400 | - | - | - | 1.5 | - | 1 : 8 : - | 1.5 |
| F10 | 50 | 125 | 125 | 25 | - | 1 | 0.5 | 1 : 2.5 : 2.5 | 1.5 |
| F11 | 50 | 250 | - | 25 | - | 1 | 0.5 | 1 : 5 : - | 1.5 |
| F12 | 50 | 300 | - | - | - | 1.5 | - | 1 : 6 : - | 1.5 |

F: Formulation, API: Active Pharmaceutical Ingredient, mg: milligram, ml: milliliter, DMSO: Dimethyl sulfoxide, PEG: Polyethylene glycol.

**Chemical compatibility study**

Studying the compatibility of drug-polymer in the phase sensitive preparation was performed via measuring Fourier Transform Infra-Red (FTIR) spectra (FTIR-8400, Shimadzu, Asia Pacific Pvt. Ltd. Singapore) of pure model drug, natural polymer and model drug loaded polymers. FTIR spectra were recorded by putting a drop of the sample to be examined with potassium bromide (KBr) powder (40mg) at room temperature using FTIR spectrophotometer. Spectra were recorded in the scan range of 400-4000 cm-1.[28] The IR spectrum of drug was compared to that present in literature after that the IR results of mixture (meloxicam and polymers) was compared to that of pure drug with noting any changes and shifting in different peaks.

**Surface morphology**

The morphological characteristics of the implant prepration were examined by scanning electron microscopy (SEM). The particles showed neither circular shape nor smooth regular preciptate surface. For both musk and mastic in different concentrations, I was examined the morphology and pores on the surface of the in situ implant. Then the effect of this characterization on release was studied.[29]

**PH test**

The PH of polymeric solution and suspension was tested using digital PH meter.

***Drug content:***

Five milliliters of the appropriately diluted sample/standard were withdrawn from the prepared formulations and used for measuring the absorbance in uv–vis spectrophotometer at 361 nm (UV 1700, Shimanzu, and Kyoto, Japan). Samples from formulations without drug were used as absorbance blank control samples. To a volume of injection containing 50 mg of meloxicam (1.5 ml), add 5 ml of pure methanol and add 0.7 ml of 0.4M sodium hydroxide and add adequate methanol (40 %) to form 25 ml. Dilute 5 ml of the resulting solution to 100 ml with methanol (40%). Note add pure methanol because the formulation precipitate in pipette when withdrawal. In vitro quantification of meloxicam was done by applied high pressure liquid chromatography (HPLC) instrument which contain ultra violet detector. The column used for separation was C18 reverse-phase column. The UV detection of the samples will be recorded at wavelength (λ) of 361 nm. The column used for separation was ACE C18 reverse-phase column with dimensions of 150×4.6 mm (length × internal diameter) (USP 2013). Chromatographic system which consist of mode: LC, detector: UV 361 nm, column: 4.6-mm × 15-cm (internal diameter * length), column temperature: 45C$^{o}$, flow rate : 1.0 ml/ min, Injection size : 10 uL.

**Drug entrapment efficiency**

Drug entrapment efficiency (DEE) was studied for all the formulations to find the best one. The DEE was obtained as an amount ratio between the actually quantities of therapeutic agent(meloxicam) as well as the integrated polymers to that theoretically quantity used throughout the in situ `implant prepration. This ratio was used in the polymers preparation. The capability of polymer to load the therapeutic agent was exactly determined, and the weighed quantities of polymer in the formulation were dissolved in a solvent (0.1 N NaOH) and then this mixture was diluted with phosphate buffered saline (PBS) (*PH* 7.4).

The produced formulation was shaked over 1 hour until the solvent was completely eliminated. The drug content was calculated by measuring its absorbance at an assigned wavelength at (361nm) using uv-vis Spectrophotometer of the USP (2013). Drug quantities were calculated by changing the measured absorbance using calibration curve. The actual meloxicam of polymeric implant in each preparation was examined at the average peak area that was offered on the ultra-violet. The results were compared with the drug standard.[30,31]

The DEE and drug loading were calculated according the following formulas:

$$\mathbf{DEE\%} = \frac{\text{Actual drug content}}{\text{Theoretical weight of drug and polymer}} \times 100$$

$$\mathbf{Loading\ \%} = \frac{\text{total Actual amount of meloxicam} - \textit{free meloxicam}}{\text{implant drug/polymer weight}} \times 100$$

**Syringability and Viscosity of solution**

The syringabilty of the solution (drug/polymer) was determined using 21-gauge needle. Viscosity is an essintial parameter for the in situ implant, to be estimated the viscosity and rheological charactraization of the polymeric formulations in solution or suspension using a Fall-ball rheometer. The viscosity properties of these developed prepration should be easy to be administered by the patient.[26,32]

**In -vitro drug release study**

In vitro release tests were carried out with Dissolution Tester device by using the USP, Apparatus I. model drug loaded in phase sensitive formulation injected in glass flask containing 25 ml of isotonic phosphate buffered saline (PBS) of pH= 7.4The formulation immediately changed into precipitate or implant. Then, the flask was kept in water bath at 37C$^{o}$ ± 0.5 C and 50 rpm for the entire period of study. Five milliliters of aliquot are withdrawn at specified time points which are used for determining quantity, the drug releasing media is withdrawn at various time interval recent after injected then after every of 30 min, 1, 2, hours and then after 2,3,5,6,7,8 days replaced by an equal volume of freshly prepared release medium. In vitro data analysis of release profiles for two different polymers formulations are compared with each other.[22] Samples were withdrawn and the absorbance determined by UV spectrophotometrically at 361nm.

The quantity of the meloxicam released (mg) at every time as follows: Ar (mg) = Cr µg/ml × 5 ml/100 where 5 was the volume of the drug release vehicle. The percent of meloxicam release (% Ar) at every time was determined from following equation: %Ar =100 × Ar (mg)/50 (mg) where 50 mg was the quantity of meloxicam present in implant was tested. The cumulative meloxicam release% (Σ Ar% t) at every time was determined as follows: (Σ Ar% t=Ar% t + Σ Ar% (0 to (t-192 hr)), where Σ Ar% t, Ar% t, Σ Ar% (t2-t)) were the cumulative drug release at time (t), the percent of meloxicam release at time and the cumulative of meloxicam release at previous times where 0.5 hr. and also day was the time interval, respectively.

To determined meloxicam releases kinetics, the cumulative release data were fitted to orders (mechanism) represent in, zero, first order and Higuchi equations.

**Stability Studies**

The stability study, the shelf life for 3 months was determined, the best formulations was investigated for stability test. The stability tests were done by storing the formulations in a glass container at 40C°/75% RH for 3 months according WHO and ICH guidelines. The samples were taked at different interval period (2 week, one month, two months and three months) and examined of physical test ( PH, Viscosity, Clarity, Apperance, Particles Size). Meloxicam assay was also evaluated and estimated by UV spectrophotometer at 361 nm.

**Kinetics and Statistical analysis**

All calculations were done by Microsoft Excel 2013 program. Whenever necessary descriptive statistical limits including the average, standard deviation (SD), Range, 95% confidence limit The best formulations were done kinetics calculations by Microsoft Excel 2013 program. Whenever necessary descriptive statistical limits including the average, standard deviation (SD), Range and 95% confidence limit were determined. With regard to calibration curve the validation of data depended on the linearity of the curve determined as regressions coefficient ($R^2$).

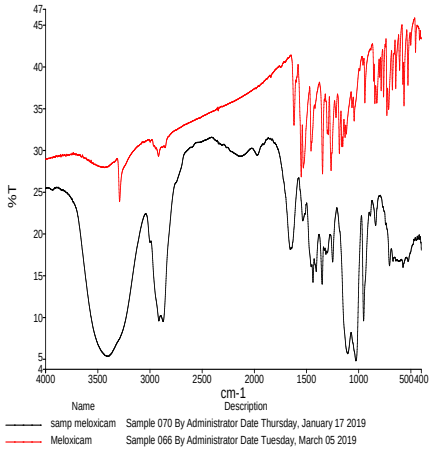
**RESULTS and DISCUSSION**

**Chemical compatibility study by Fourier-transform infrared spectroscopy:**

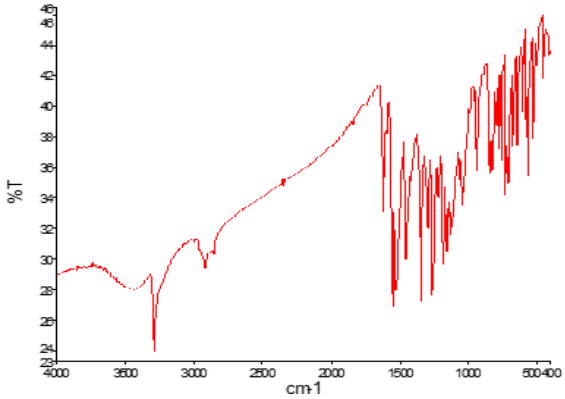
Drug-polymer compatibility investigation was carried out by using Fourier transform infrared spectrometry (FT-IR) spectra of pure components to examine meloxicam-polymer compatibility. Their physical mixture and the preparation are exhibited in Figure 1 where showed the pure meloxicam (B), meloxicam with mastic and musk as (C, D) and sample of formulation component F12 (A). The absorption bands of meloxicam were shown at 3294, 2919, 1622 and 1525cm for NH, CONH, $SO_2$ and C=C, respectively, as shown in Figure1. The aromatic ring made an absorption peak between 527 and 1652cm.

In the in situ implant of mixed polymers (Musk, Mastic) with the meloxicam formulation, additional bands were observed combining to the peaks of the individual meloxicam component. A very broad band was also visible at 3286 $cm^{-1}$ in all formulations, which can be attributed to the presence of hydroxyl group (COOH). Moreover, a long peak was observed at 2911 $cm^{-1}$ due to the presence of aliphatic ($CH_4$), which were found in many

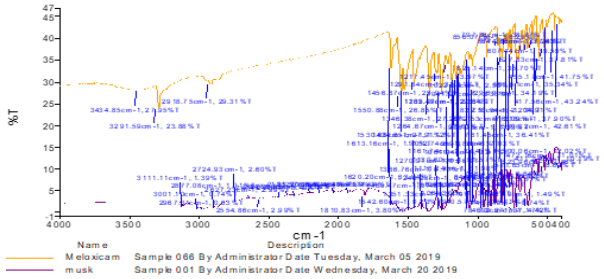
polymers' components especially in the mastic formulation, as shown in Table (2). This indicates that there is no chemical interaction between the components of the formulations they have different polymers.[46]



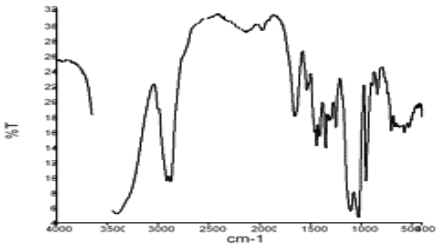

**Meloxicam, Sample of Formulation F12 (A)**




**Meloxicam (B)**




**Meloxicam, Musk (C)**




**Meloxicam with Mastic (D)**

**Figure 1: Drug Polymer Compatibility**

**Table 2: Chemical compatibility by using FT-IR**

| Functional group of absorption peaks | Secondary amine stretch | C-H stretch aromatic | Aliphatic $CH_3$ | $NH_2$ scissoring vibrations | C=N stretch | S=O stretch |
|---|---|---|---|---|---|---|
| FTIR Spectra of meloxicam STD ($cm^{-1}$) | 3286 | 3091 | 2911 | 1620 | 1536 | 1150 |
| FTIR Spectra of meloxicam in (F) with mastic ($cm^{-1}$) | Broad 3284 | 3092 | Long 2911 | 1622 | 1538 | 1151 |
| FTIR Spectra of meloxicam in (F) with musk ($cm^{-1}$) | Broad 3285 | 3092 | Long 2911 | 1620 | 1536 | 1150 |

**Surface Morphology**

The surface morphology of the meloxicam implant or the precipitation was examined by scanning the electron microscope (SEM). Figure 2 shows the microphotographs of formulated implant or precipitate. The photographs of SEM demonstrated that the implant is

approximately irregular shaped with gel in the outer layer. Additionally, the photographs can exhibit the implant and precipitates formulated by the solvent phase separation method. Some implant and precipitates are irregular shaped in some formulations and some precipitates are coarse in other formulations. This result agrees with those obtained by other studies.[14] SEM photographs indicate that in case the polymer amount is elevated, two polymers are mixed or there is a high viscosity of formulation, products are of clear implant or precipitate with gel coarse surface[43], as shown in Figures (2- 3- 4).

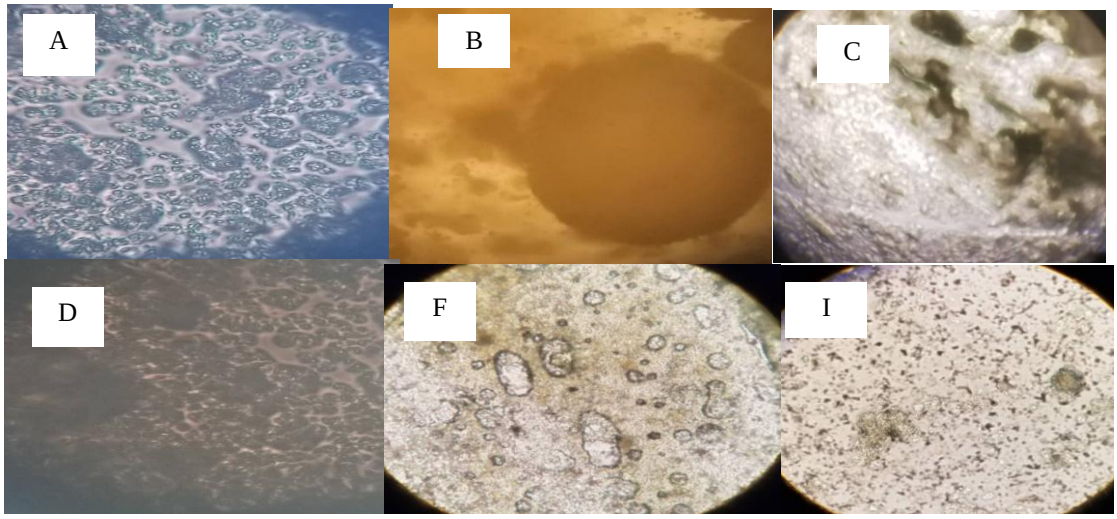



**Figure 2: Scanning the electron microscopy of formulations' erosion of all Musk, Mastic, Mixed polymers after 30 minutes; (A) F2, (B) F5 (C) F7, (D) 10, (E) 11, (F)**

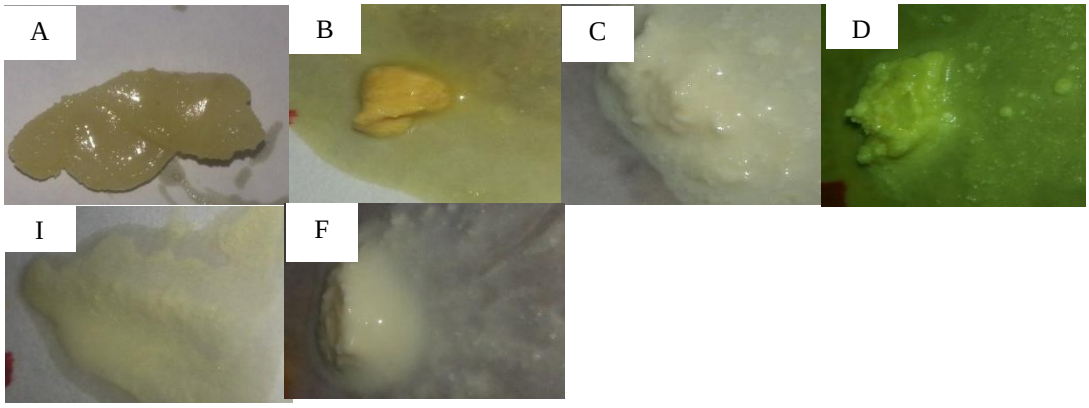



**Figure 3: In-vitro in situ implant of formulations of all Musk, Mastic, Mixed polymers after injecton; (A) F2, (B) F5, (C) F7, (D) F10, (E) F11, (F) F12**

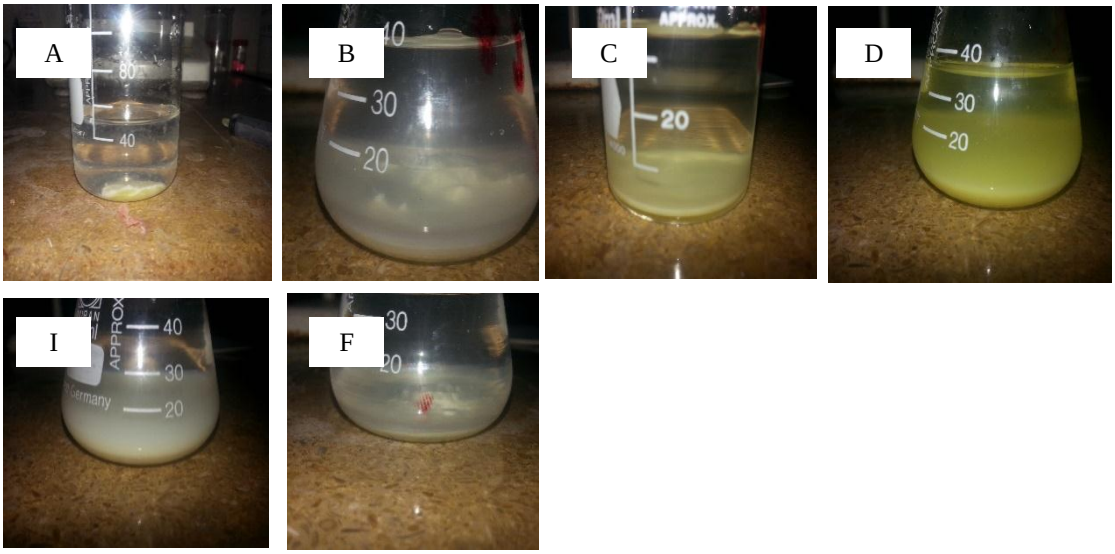



**Figure 4: In-vitro in situ impant of formulations of all Musk, Mastic, Mixed polymers after 24 hrs in PH of 7.4; (A) F2, (B) F5, (C) F7, (D) F10, (E) F11, (F) F12**

**Drug Content**

The results of meloxicam drug content of all formulations observed different drug contents that may be because some formulations have high viscosity and reqire long time to be mixed prefectly. However, all the meloxicam formulations' retention time was recorded as 7.5 min. In order to shorten this time, some modifications were made in the mobile phase ratio. By keeping the components constant, the ratio was shifted to 50:25:25 (v/v/v) (Table 4.2). The good drug content is in formulation (F4, F5, F6, F7, F10, F12) were shown in Table 4.2, (89.7%, 87.5%, 104%, 90%, 91.8%, 96.5%) respectively. That is mean the meloxicam distributed prefect in last formulation, the F4, F5 & F10 is have two mixed polymers (mastic, musk) and F12 is have musk polymer alone, and F6 is have mastic alone. The F4 & F5 is formed by two mixed polymers (mastic, musk) without any other polymers, low viscosity and good drug release. But F5 with PEG and slightly high viscosity compered to F4 so the drug content reduces from 89.7% in F4 to 87.5% in F5. The results also became good when vigorous mixed the component, despite of different main polymers types and high viscosity as we show in F10, F7& F6 (91.8%, 90%&, 104%). In F12 which have moderate concentration of musk (300 mg) without any other additional polymers and solvent and it has low viscosity the drug content is good (96.5%). The F4, F7, F10, F12 are the best formulation in this study when examined other tests. The implant of meloxicam when we mixed with biodegradable polymers (mastic & musk) can be get good drug content at different type and concentration of polymers, with addition of other polymers and solvent (e.g. PEG). The viscosity plays main roll in results drug content because reduce the drug distribution,

however an overcome this challenge by vigorous mixed the all component as we found when examined in F10.

**Table 3: Drug content.**

| Formulation | Drug content % ± RSD |
|---|---|
| F1 | 69.5% ± 0.62 |
| F2 | 62.6 % ± 0.99 |
| F3 | 77.7 % ± 0.42 |
| F4 | 89.7% ± 0.31 |
| F5 | 87.5% ± 0.39 |
| F6 | 104% ± 0.08 |
| F7 | 90% ± 0.25 |
| F8 | 81.4% ± 0.54 |
| F10 | 91.8% ± 0.36 |
| F11 | 80% ± 0.66 |
| F12 | 96.5% ± 0.07 |

**Entrapment efficiency**

From various preparations of meloxicam in situ implant, the drug entrapment efficiency (EE) percentages were found to be varied from 64% - 86% and loading drug capacity increased from 30.5 % - 42.7 % as shown in Table 4. Which effected by many factors.

Generally, all the formulated preparations showed good percentages of entrapment efficiency that may be due to poor solubility of meloxicam in water phase, and the insoluble polymers which enhance making implant and precipitate in the aqueous phase and accelerate the solid gel sedimentation when injection with DMSO & PEG in PH buffer at 7.4.[21,22]

Another factor which employed a significant effect on EE% is related to the properties of the selected solvent for meloxicam and polymers (Musk, Mastic) during the preparation of in situ implant by organic solvent phase separation techniques. DMSO in all formulations or PEG in some others are used as organic solvents miscible with water to increase the solubility of polymers and their viscosity through the preparation of meloxicam loaded implant. Therefore, in situ implant was found to have high water miscibility and little high viscosity thus improving the entrapment efficiency of F1, F6, and F10 as shown in table 4.[19]

From the results, it was observed that the entrapment efficiency was increased in F4, F5 and F10 with EE% of 78, 81, and 82, respectively because the polymer concentration was increased and two polymers were mixed. These results comply with those of Kumar *et al.* (2013). This indicates that the polymer concentration, high viscosity and two mixed polymers play a vital important role in therapeutic agent entrapment efficiency and sustained release.

The entrapment efficiency was decreased in F3 to F2 with EE% of 68 and 72 respectively because they had lower ratios of Mastic polymer to drug content (250 mg and 300 mg, respectively). Moreover, the entrapment efficiency was decreased in F7 to F12 with EE% of 64 and 76 respectively because they had lower ratios of musk polymer to drug content (250 mg and 300 mg, respectively) when compared to other formulations with higher ratios of musk polymer (e.g. F8 with 400 mg and EE% of 85).[20]

The high entrapment efficiency is related to the increase in the polymer ratio which may be due to some reasons. The first reason is because of the increase in the polymer concentration which increases in the speed of forming coarse precipitate or gel in situ implant which may reduce meloxicam loss from the coarse precipitate or gel implant surface after the injection step in PH 7.4 buffer. This indicates that the rapid precipitation of the polymer due to its high concentration prevents diffusion of meloxicam across the phase border. The second reason is because of the increase in the viscosity of the suspension or solution which reduces the diffusion within the polymer in situ implant gel or precipitate.[23,24]

The results exhibited that the therapeutic agent entrapment efficiency was found to be higher in case of two mixed polymers with gelling agent carbomer in situ implant formulations (e.g. F6) when compared to those of an individual polymer (e.g. F2 and F3) because the solubility of polymers in buffer solution at PH 7.4 was decreased and the precipitation rate during the phase separation process was enhanced. Musk and mastic showed a high solubility in DMSO, therefore they had an affinity to precipitate in the buffer solution at PH 7.4. Their solidification rates were decreased, leading to low drug entrapment efficiency values.[25]

**Table 4: Formulation design, drug entrapment efficiency and size distribution of different sustained release injection formulations.**

| Formulation code | Meloxicam:Mastic:Musk | Morphology | Entrapment Efficiency % ± RSD | Drug loading % ± RSD |
|---|---|---|---|---|
| F1 | 1 : 5 : - | implant | 86 % ± 0.6 | 42.7% ± 0.21 |
| F2 | 1 : 5 : - | precipitate | 68 % ± 0.59 | 32.7 % ± 0.36 |
| F3 | 1 : 6 : - | precipitate | 72 % ± 0.33 | 34.9 % ± 0.32 |
| F4 | 1 : 2.5 : 2.5 | precipitate | 78 % ± 0.91 | 38.26 % ± 0.08 |
| F5 | 1 : 2.5 : 2.5 | implant | 81 % ± 0.64 | 39.9 % ± 0.90 |
| F6 | 1 : 5 : - | precipitate | 86 % ± 0.82 | 42.4 % ± 0.66 |
| F7 | 1 : - : 2.5 | precipitate | 64 % ± 1.2 | 30.5 % ± 0.85 |
| F8 | 1 : - : 8 | implant | 85 % ± 0.29 | 42.2 % ± 0.75 |
| F10 | 1 : 2.5 : 2.5 | implant | 82 % ± 0.54 | 40.4 % ± 0.24 |
| F11 | 1 : - : 5 | implant | 73.6 % ± 0.64 | 35.8 % ± 0.38 |
| F12 | 1 : - : 6 | implant | 76 % ± 0.88 | 37.15 % ± 1.3 |

Furthermore, the results showed that the mastic polymer had a higher viscosity rate in the majority of formulations compared to that of the musk polymer. Therefore, the entrapment efficiency in F1 is higher (86%) than in F7 (64%) because F1 has a mastic polymer with PEG as co-solvent but F7 has a low concentration of musk polymer. Besides, the entrapment efficiency in F10 was better (82 %) than that in F11 (73.6%) because the former has two mixed polymers with xanthan gum in DMSO with PEG which help to form solidification and reduce the initial burst release and prolong release, but the latter only contains a musk polymer with xanthan gum. However, the entrapment efficiency in F12 was lower (76%) because it only contains a musk polymer without xanthan gum.

**Syringeability and viscosity of solution**

This examined was conducted for all formulations and the results were showed in Table 5 that the formualtions with mastic polymer or mastic polymer, viscosity agent (xanthan gum and carbomer 497), and PEG solvent have high viscosity as shown in F1, F6, F7, F10, and F11 which examined by fallball viscometer. All formulations was examined relative density and viscosity at 25 $C^o$ & 37 $C^o$ .

We obsorved reduce the viscosity when increase the temperture in all formulation. Additionally, viscosity plays an important role in the meloxicam entapment efficacy,drug content and intial drug release as we obsorved in F1, F6, and F10. All formulation was syringeable and injectable under the skin through needle 21 gauge syring.

**Table 5: Syringeability and viscosity at 37 & 25 $C^o$ of different implant Meloxicam formulations.**

| Formulation code | Syringeability | Viscosity (pa-s) | | Relative Density |
|---|---|---|---|---|
| | | At 25 $^O$ C ± RSD | At 37 $^O$ C ± RSD | |
| F1 | Pass | 91 ± 0.36 | 90.80 ± 0.70 | 1.108 % |
| F2 | Pass | 89.61 ± 0.09 | 89.41 ± 0.39 | 1.083 % |
| F3 | Pass | 89.87 ± 0.04 | 89.67 ± 0.08 | 1.084 % |
| F4 | Pass | 89.56 ± 0.31 | 89.36 ± 0.45 | 1.078 % |
| F5 | Pass | 89.75 ± 0.07 | 89.31 ± 0.35 | 1.090 % |
| F6 | Pass | 91.38 ± 0.29 | 91.08 ± 1.1 | 1.109 % |
| F7 | Pass | 91.15 ± 0.51 | 90.95 ± 0.27 | 1.097 % |
| F8 | Pass | 89.86 ± 0.60 | 89.66 ± 0.43 | 1.105 % |
| F10 | Pass | 91.24 ± 0.36 | 91 ± 0.9 | 1.103 % |
| F11 | Pass | 91.2 ± 0.72 | 91 ± 0.78 | 1.101 % |
| F12 | Pass | 89.23 ± 0.08 | 89 ± 0.12 | 1.075 % |

**The in vitro-release**

The result of examineted in vitro-release of meloxicam injection which performed by measuring the percentage of the cumulative release of different formulations in PBS of pH =7.4 is obsorved sustined drug release for eight days as shown in Figures 5,6,7.

Table 6 shows that the cumulative release rates of meloxicam from the various formulations were found to be dependent on different types of polymers and their concentrations. In F8 and F12, lower release rates were observed to be 8.2% and 9.62%, respectively. However, the sustained drug release was observed in all formulations. This result agrees with those of Bindu *et al.* (2018) and Geng *et al.* (2016).

The study showed that the meloxicam implant released the drug very low in F8 over the 8 days because F8 has a high concentration of musk (400 mg) with a high entrapment efficiency rate (85%). However, the drug release was sustained through a period of five days in F12 then totally decreased because of the low viscosity medium concentration of musk polymer (300 mg) leading to completely destroying the drug delivery system.

**Table 6: In vitro drug release of different formulations.**

| Time (T) in hrs. | F1 % ±RSD | F2% ± RSD | F3% ± RSD | F4% ± RSD | F5% ± RSD | F6% ± RSD | F7% ± RSD | F8% ± RSD | F10% ± RSD | F11% ± RSD | F12% ± RSD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **0.50** | 12.2 ± 1.6 | 17.9 ± 1.8 | 19.2 ± 1.3 | 13.3 ± 1.3 | 20 ± 0.91 | 12.5 ± 1.4 | 15.8 ± 0.9 | 8.2 ± 1.7 | 12.6 ± 0.9 | 12.7 ± 1.1 | 9.62 ± 0.8 |
| **2** | 29.84 ± 0.98 | 38.1 ± 0.8 | 43.4 ±1.1 | 32.2 ± 0.6 | 42.4 ± 1.1 | 22.2 ± 0.7 | 41.6 ± 1.1 | 0.44 ± 1.2 | 22.75 ± 0.07 | 27.92 ± 0.4 | 17.68 ± 0.31 |
| **3** | 46.2 ± 0.58 | 44 ± 0.2 | 46.12 ± 0.6 | 41.6 ± 0.7 | 55.2 ± 0.8 | 40.7 ± 0.05 | 44.2 ± 0.05 | 1.68 ± 0.9 | 30.63 ± 0.03 | 31.5 ± 0.56 | 28.8 ± 0.03 |
| **48 hrs (2 days)** | 51.5 ± 0.35 | 59.3 ± 0.4 | 42.72 ± 0.09 | 64.6 ± 0.02 | 60.8 ± 0.05 | 42.7 ± 0.7 | 47.2 ± 0.08 | 4.6 ± 02 | 33.35 ± 0.06 | 46.2 ± 0.06 | 67 ± 0.06 |
| **72 hrs (3 days)** | 61.68 ± 0.66 | 61.6 ± 0.08 | 47.72 ± 0.85 | 70.7 ± 0.05 | 65.44 ± 0.2 | 44.7 ± 0.6 | 49.28 ± 0.1 | 10.4 ± 0.07 | 44.35 ± 0.1 | 51.86 ± 0.04 | 67 ± 0.03 |
| **120 hrs (5 days)** | 68.6 ± 1.3 | 69.1 ± 0.54 | 52.92 ± 0.9 | 72.6 ± 1.7 | 68.88 ± 0.5 | 48.54 ± 1.1 | 50.4 ± 0.9 | 15.64 ± 1 | 58.63 ± 0.3 | 61.2 ± 0.09 | 67.2 ± 0.4 |
| **144 hrs (6 days)** | 75.72 ± 0.43 | 71.2 ± 0.3 | 56.52 ± 0.05 | 79.2 ± 0.71 | 81 ± 0.8 | 53.5 ± 0.5 | 76.2 ± 0.2 | 23.6 ± 0.8 | 75.11 ± 0.07 | 77.5 ± 0.07 | 86.6 ± 0.08 |
| **168 hrs (7 days)** | 86.2 ± 0.55 | 73 ± 0.28 | 60.92 ± 0.04 | 84.5 ± 0.04 | 84.5 ± 0.6 | 61.4 ± 0.06 | 81.7 ± 0.3 | 25.5 ± 0.9 | 80.15 ± 0.03 | 82.44 ± 0.2 | 95.9 ± 0.1 |
| **192 hrs (8 days)** | 95.4 ± 0.78 | 75.4 ± 0.08 | 68.8 ±0.8 | 92.3 ± 0.05 | 90.91 ± 0.07 | 66.1 ± 0.05 | 92.4 ± 0.8 | 40.9 ± 0.7 | 92 ± 0.08 | 91.2 ± 0.13 | 97.1 ± 0.14 |

The results also revealed that the release profile of all formulations showed that the initial burst release rates of meloxicam ranged from 8.2% to 20% followed by a moderate to slow release related to the concentration of polymers or mixed polymers obtainable followed by the burst effect and resulted from the rate of drug release and diffusion before meloxicam in situ implant gel depot was formed.

Table 6 also shows that all prepared implant/precipitate formulations exhibit good burst release with a maximum initial burst release of 20% in F5 which continues release after the 8 days because it has mixed polymers (musk, mastic) with similar concentration of 125 mg with medium viscosity, entrapment efficiency and good drug content (87.5%).

This release is decreased by the increase in the biodegradable polymer amount which complies with the result found by Guo *et al.* (2018). However, the lowest burst release was observed in F8 (40.9%) after 8 days because it has a high viscosity rate and carbomer 497as showed in previous published study.[33,34]

Moreover, the cumulative release of meloxicam in F8 is higher than that in F12 because F8 has a higher musk polymer concentration (400 mg) with a lower burst release rate (8.2%), whereas F12 has a lower musk polymer concentration (300 mg) with a slightly higher burst release rate (9.62%) (Figure 7).

These results indicate that the meloxicam sustained release from polymeric in situ implant or precipitate depends on the polymer type and its concentration ratio which may be attributed to the increase in the wall thickness of the polymeric in situ implant or coarse precipitate elevated, therefore, leading to an extension in the length of drug diffusion through the layer of polymer these results are in line with those of some previous studies.[35,36,37]

Meloxicam release observed in the state of musk in situ implant depot formulations was slower (F8, F12) than that of mastic in situ implant depot formulations. This may be attributed to the higher hydrophobicity of musk polymer as a result of a lot of methyl groups on its chemical structures which decrease the penetration through the aqueous media and reduce the degradation of the musk polymer and finally result in slower degradation time as compared to that of more hydrophilic mastic polymers (Figures 5,6,7).

The burst release was reported to be controlled by the rate of precipitation or gelation. The higher the rate of gelation, greater was the burst release and more hydrophobic (or less hydrophilic) a formulation is, the sooner it was form the gel depot in situ. After this initial burst release, the rest of the release is predominantly controlled by rate of degradation of polymer by hydrolysis. The more hydrophobic a formulation is, the greater it would resist the entry of water and therefore, the slower rate of degradation as found in previous study.

To increase the viscosity and help to form implant, the release was sustained in F1 and F11 because the two polymers (Musk, Mastic) were mixed severally with the xanthan gum as well as in F4 and F10 because the two polymers (Musk, Mastic) were mixed together (Figure 6 ) as found in published studies.[38,39]

The release profiles of different concentrations of mastic polymer from meloxicam in situ implant depot are shown in Figure (5), those of musk polymer are shown in Figure (7), while those of mixed polymers are shown in Figure (6). The results of this released in first time high amount then decrease released gradually with time and carried out summarized all cumulative results.

In general, all the prepared meloxicam formulations made of meloxicam implant or precipitate with different types and concentrations of both polymers (musk, mastic) exhibited sustained drug release in phosphate buffer saline (pH 7.4) for eight days. The best formula was F12 with $R^2$ 0.98 with long and constant release.

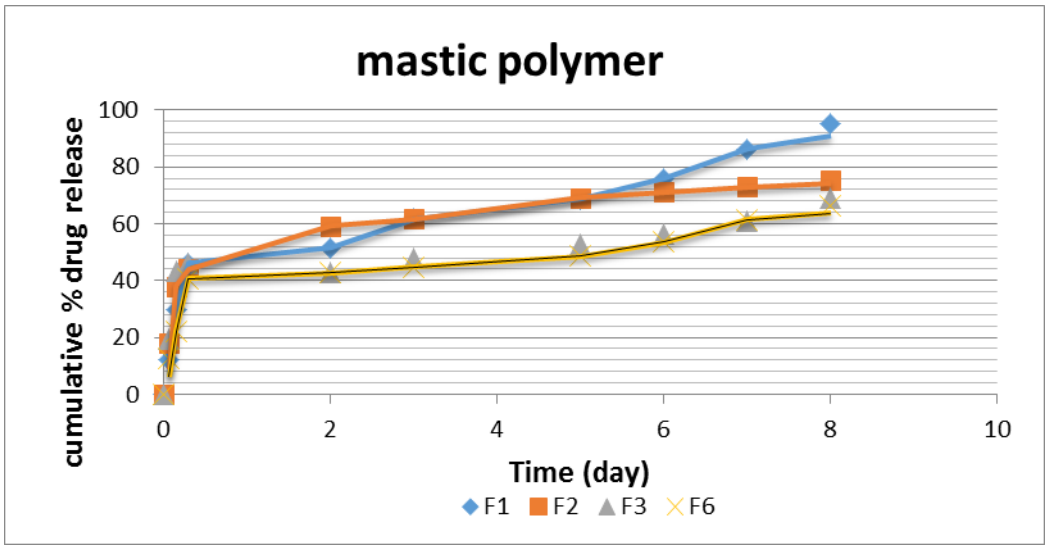



**Figure 7: In-vitro release profiles of different concentration mastic polymer from Meloxicam in situ implant depot.**

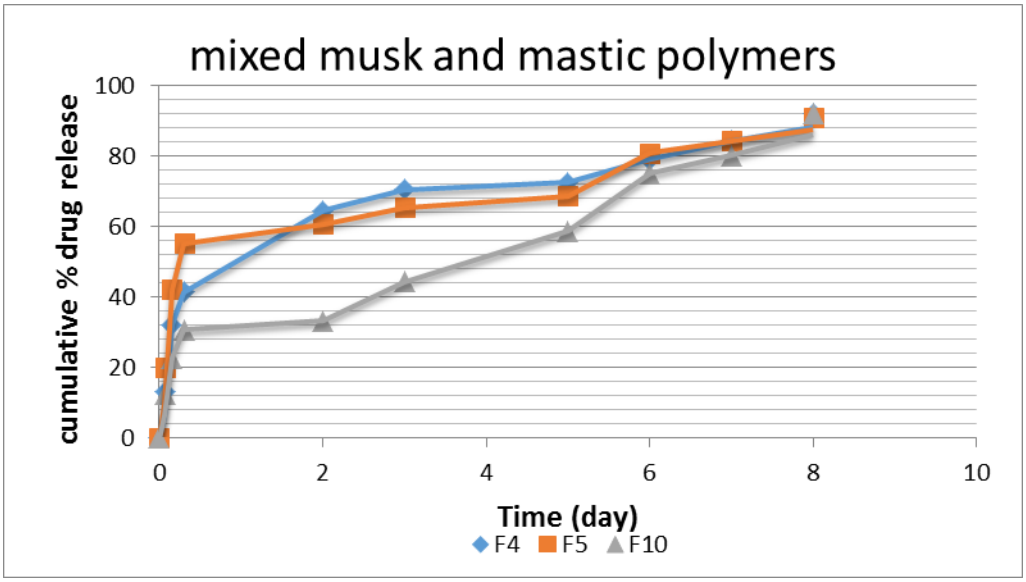



**Figure 6: In-vitro release profiles of different concentration of mastic and musk mixing polymer from Meloxicam in situ implant depot.**

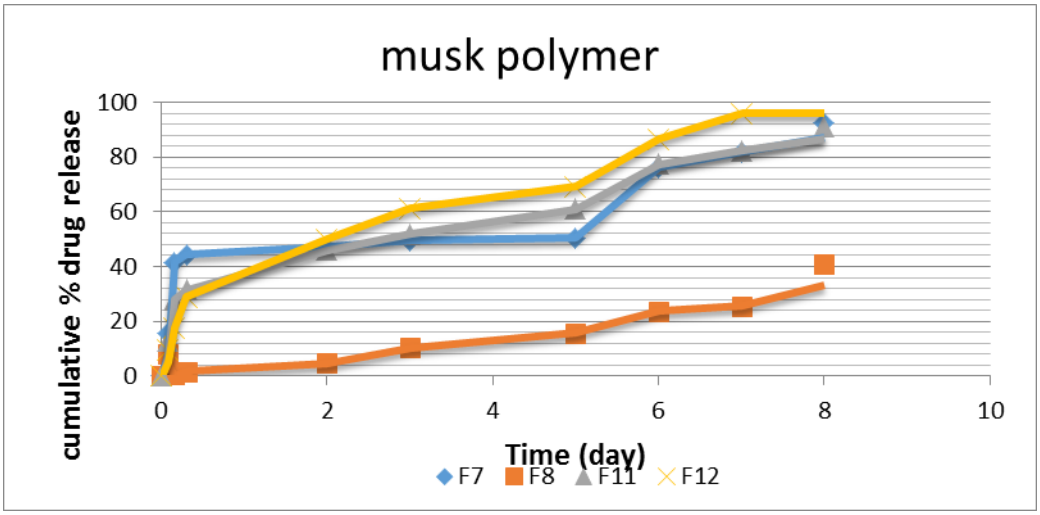



**Figure 7: In-vitro release profiles of different concentration musk polymer from meloxicam in situ implant depot.**

**Kinetic analysis of the release data**

The in vitro release profile was used by several kinetic models (zero order, first order and higuchi). It was showed that the release in some different formulations which followed diffusion controlled mechanism as shown in Table (7). Generally, diffusion, erosion and degradation are the most essential mechanisms for solute transmit from polymeric matrices. The $t_{50}$% of the formulations is extended from 24 h to 192 h referring the effect of the different formulation variables in sustaining the release of the drug this result comply with published study.[40] The F10, F11, F12 is show is controlled diffusion in several kinetic

model, and the best one was F12 especially in Higuchi kinetics and zero order ($R^2$=0.98) as showed in in Figure 8, 9, 10 respectively. Results showed that formulations drug had correlation coefficients for higuchi kinetics rather than zero and first order as shown in table 7. These finding results imply that the drug release by higuchi kinetics for most formulations with different $R^2$ which mean many factor can effect on drug release. This is essential significant in vitro release test between most implants formulations. In general, most formulation of meloxicam implants made of mastic or musk polymer with different ratios of polymers in formulation showed sustained meloxicam liberation in PBS at pH equal 7.4 for eight days and this result consider preferred of research published.[11,12] The best formulation is F12 with $R^2$ 981.

**Table 7: Kinetic parameters of the release of Meloxicam from implant formulations.**

| Formulation code | Zero order | | First order | | Higuchi kinetics | |
|---|---|---|---|---|---|---|
| | $K_0$ ($hr^{-1}$) | $R^2$ ± RSD | $K_1$($hr^{-1}$) | $R^2$ ± RSD | $K_H$ ($hr^{-1}$) | $R^2$ ± RSD |
| F1 | 0.2 | 0.70 ± 0.08 | 1.2648 | 0.24 ± 0.51 | 0.004 | 0.71 ± 0.21 |
| F2 | 0.2 | 0.80 ± 0.35 | 1.2095 | 0.81 ± 0.25 | 0.002 | 0.82 ± 0.23 |
| F3 | 0.16 | 0.84 ± 0.52 | 1.1682 | 0.38 ± 0.13 | 0.002 | 0.82 ± 0.36 |
| F4 | 0.185 | 0.13 ± 0.07 | 1.0625 | 0.48 ± 0.62 | 0.007 | 0.42 ± 0.55 |
| F5 | 0.2 | 0.27 ± 0.91 | 0.9822 | 0.29 ± 0.22 | 0.008 | 0.31 ± 0.08 |
| F6 | 0.19 | 0.69 ± 0.63 | 0.9684 | 0.21 ± 0.48 | 0.004 | 0.70 ± 0.72 |
| F7 | 0.23 | 0.73 ±.12 | 0.7558 | 0.72 ± 0.85 | 0.005 | 0.75 ± 0.28 |
| F8 | 0.26 | 0.78 ± 0.53 | 0.5314 | 0.72 ± 1.3 | 0.44 | 0.73 ± 0.92 |
| F9 | 0.256 | 0.27 ± 0.08 | 0.4313 | 0.12 ± 1.1 | 0.028 | 0.26 ± 0.88 |
| F10 | 0.21 | 0.90 ± 0.43 | 1.2648 | 0.83 ± 0.07 | 0.004 | 0.98 ± 0.14 |
| F11 | 0.21 | 0.97 ± 0.05 | 1.2095 | 0.81 ± 0.24 | 0.009 | 0.98 ± 0.27 |
| F12 | 0.25 | 0.98 ± 0.03 | 1.1682 | 0.83 ± 0.23 | 0.007 | 0.981 ± 0.2 |

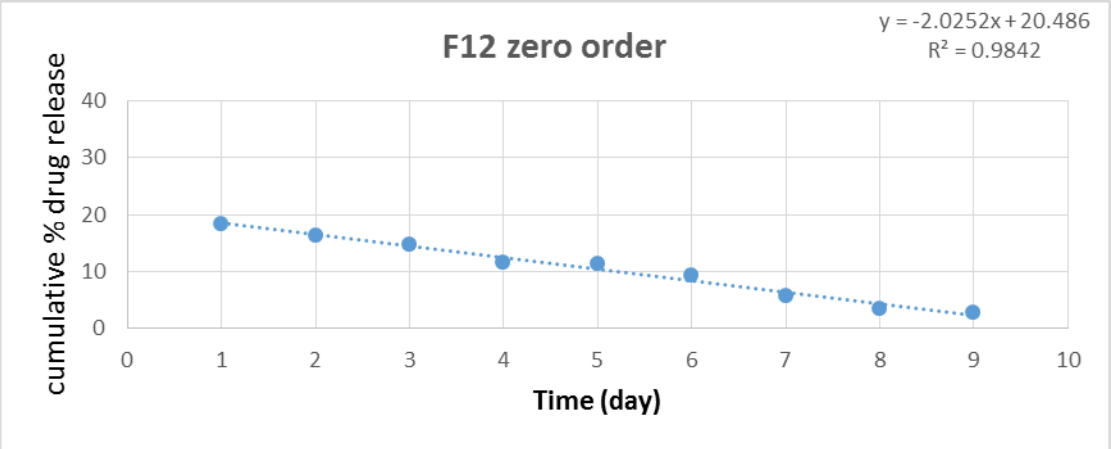



**Figure 8: In vitro drug release kinetics of meloxicam implant (zero order F12).**

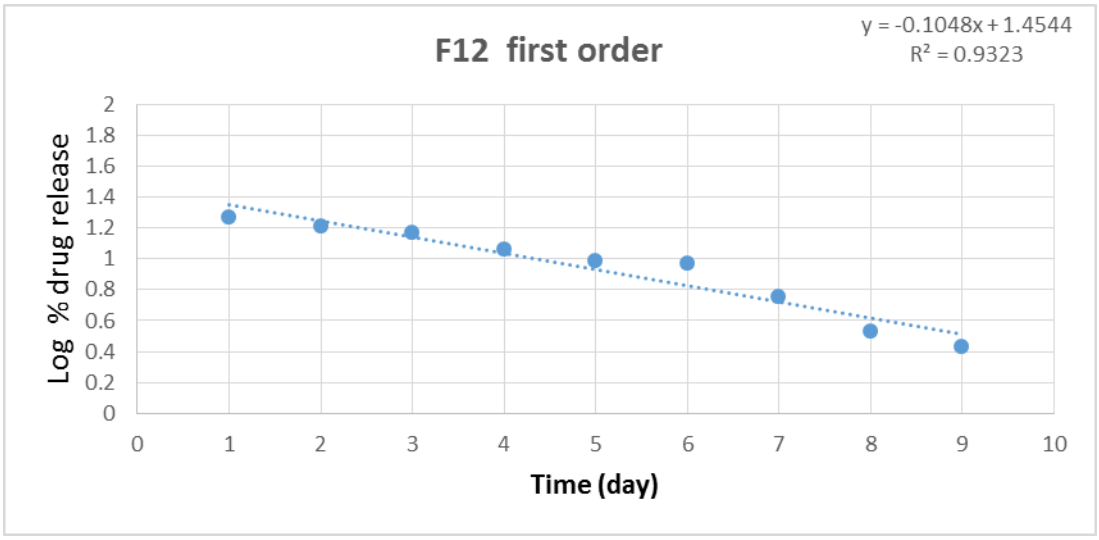



**Figure 9: In vitro drug release kinetics of meloxicam implant (first order F12).**

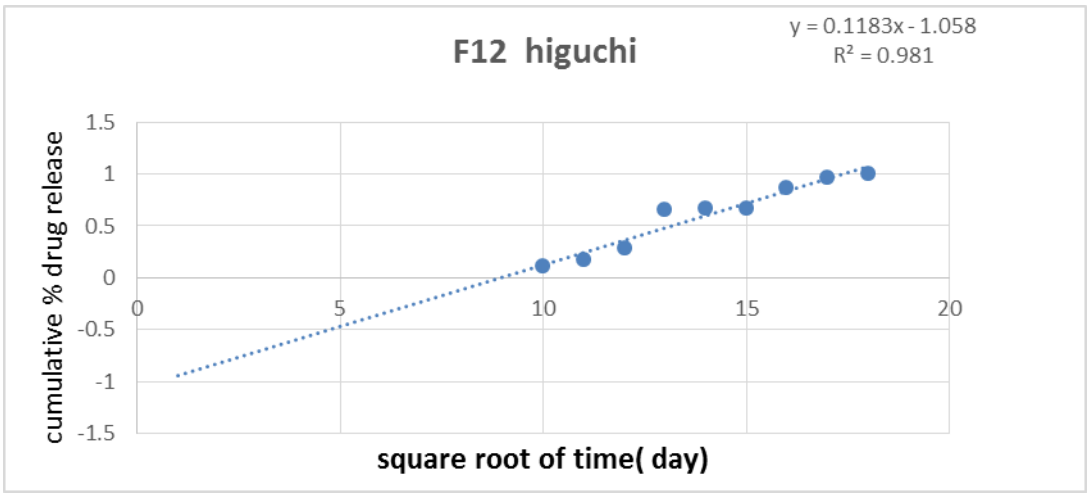



**Figure 10: the in vitro drug release kinetics of meloxicam implant (higuchi order F12)**

**Stability Studies.**

The stability study was achieved for best formulations at 40°C ± 5°C temperature & 75% humidity according WHO & ICH. In the Table (8) showed different parameters were analyzed at different time over three months. There was no difference showed in appearance of polymeric solution or suspension was able to pass through 21-gauge needle so there was no difference found with syringeability. Drug content was found to stable during 3 month of stability study. It can be said that there was no significant alteration in drug (meloxicam) content or other physical properties during 3 month of stability study according the results. Here by it can be said that the formulation was found to be stable at 40°C ± 5°C. The best samples will be determined by the method discussed previously as same as this study.[43,44]

As shown in the stability study results which tested as Table (8) no significant different between results at begging of study and after three months.

**Table 8: Stability study data over 3 month of optimized formulation.**

| Formulation | 2W | | | | Drug content % | | | | 3M | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Appearance | Particle in sol. | PH | Clarity | 2W | 1M | 2M | 3M | Appearance | Particle in sol. | PH | Clarity | discoloration |
| F1 | Yellow suspension | Re-dispersion particle | 7.23 | Clear | 69.5 | 69.2 | 69 | 68.5 | Yellow suspension | Re-dispersion particle | 6.82 | - | No change |
| F4 | Yellow suspension | Re-dispersion particle | 6.88 | Clear | 89.7 | 89.4 | 89.1 | 88.7 | Yellow suspension | Re-dispersion particle | 6.65 | - | No change |
| F7 | Yellow solution | No particle | 6.8 | Clear | 90 | 89.8 | 89.7 | 89.5 | Yellow solution | No particle | 6.51 | Clear | No change |
| F10 | Yellow suspension | Re-dispersion particle | 7.12 | - | 91.8 | 91.5 | 91.1 | 90.8 | Yellow suspension | Re-dispersion particle | 6.69 | - | No change |
| F11 | Yellow solution | No particle | 6.78 | - | 80 | 80 | 79.6 | 79.4 | Yellow solution | No particle | 6.58 | Clear | No change |
| F12 | Yellow solution | No particle | 6.75 | - | 96.5 | 96.1 | 96 | 95.8 | Yellow solution | No particle | 6.55 | Clear | No change |

W= Week, M= Month, T1/2 = Half-life

After the stability study was accomplished for three months at 45°C ± 5°C& humidity 75 % as shown Table 8 showed several parameters were analyzed at different time interval from two weeks during three months. There was no difference showed in appearance or discoloration of formulations (solution or suspension), it was able to pass through 21-gauge needle so there was no difference found with syringeability, the PH no significant different in initial study in both formulation solution or suspension and drug content was found to stable through three month of stability study. The solution was also estimate for transparency yellow color, clarity without particle and for any physical preparation and suspension still easy re-dispersion particle. The release studies of best formulation after stability study three months as shown in Table (9) good release of drug over eight days. As the results it has been showed that the preparation observed no alteration in PH, clarity, appearance, and meloxicam release over eight days and in drug content. The F12 was the best formulation as showed in Figure (11).

**Table 9: In vitro release studies of best formulation after stability study (3 months).**

| Time (T) in hrs. | F1% ± RSD | F4% ± RSD | F7% ± RSD | F10% ± RSD | F11% ±RSD | F12% ±RSD |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.50 | 17 ± 0.59 | 19 ± 0.82 | 20.1 ± 0.65 | 16.9 ± 1.2 | 18.7 ± 0.87 | 13.1 ± 0.68 |
| 2 | 34 ± 0.33 | 39.1 ± 0.78 | 46.3 ± 0.75 | 22.4 ± 0.59 | 39.8 ± 0.65 | 18.1 ± 1.3 |
| 3 | 50 ± 0.06 | 42.3 ± 0.09 | 50.9 ± 0.13 | 25.7 ± 0.22 | 46.7 ± 0.09 | 28.1 ± 0.06 |
| 48 hrs (2 days) | 55 ± 0.08 | 52.9 ± 0.22 | 55.7 ± 0.32 | 45.7 ± 0.36 | 55.8 ± 0.37 | 51 ± 0.52 |
| 72 hrs (3 days) | 65.2 ± 0.12 | 56.2 ± 0.13 | 60 ± 0.35 | 54.4 ± 0.05 | 60 ± 0.49 | 59.3 ± 0.23 |
| 120 hrs (5 days) | 68.8 ± 0.08 | 58.1 ± 0.41 | 62.9 ± 0.08 | 76.1 ± 0.07 | 64.5 ± 0.06 | 71.6 ± 0.08 |
| 144 hrs (6 days) | 74.7 ± 0.05 | 69.5 ± 0.24 | 75.6 ± 0.16 | 81 ± 0.24 | 75.9 ± 0.11 | 86 ± 0.28 |
| 168 hrs (7 days) | 81.2 ± 0.09 | 75.2 ± 0.12 | 82.9 ± 0.08 | 86 ± 0.38 | 81.5 ± 0.09 | 91.4 ± 0.18 |
| 192 hrs (8 days) | 88.3 ± 0.03 | 86.1 ± 0.18 | 87.3 ± 0.14 | 90.6 ± 0.27 | 89.1 ± 0.06 | 95.1 ± 0.07 |

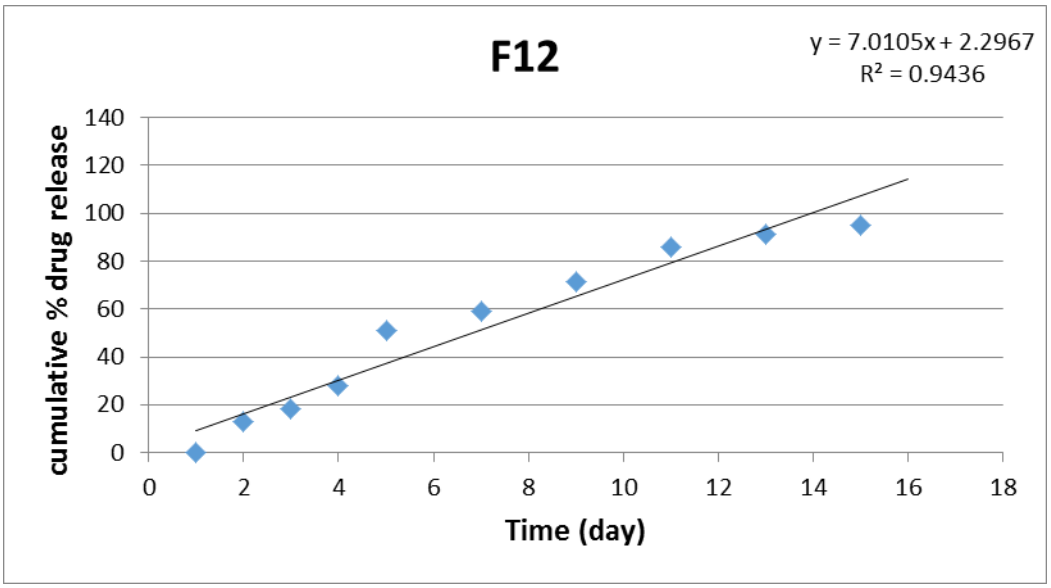



**Figure 11: In-vitro release profiles of different concentration polymer from best Meloxicam implant formulation (F12).**

## CONCLUSION

This study was perform to develop and evaluate sustained release in situ implant injection using meloxicam as a model drug, also in this research explained that solvent phase separation technique has been successfully employed to formulate meloxicam implant with two polymers musk and mastic or mixed both with desirable drug entrapment efficiency, drug loading, viscosity, drug polymer compatibility and extended release profile. The formulation

variables as drug-polymer ratio and the type of polymer used exerted a significant influence on the drug entrapment efficiency as well as drug release pattern exactly in F12 with good correlation coefficients $R^2$.

The percentage of entrapment efficiency (EE) of the implant or precipitate was good. It was observed that as the biodegradable polymer concentration increased, the EE as well as the drug release was sustained over a period of eight days in a phosphate buffer saline (pH 7.4).

The study of drug polymer compatibility by FTIR showed that there are no significant changes in the position of the characteristic peaks of meloxicam when mixed with musk and mastic polymers with different concentration. The viscosity paly main roll in reduce burst release and sustained release.

It can be concluded from the obtained data that the prepared polymeric in situ implant of meloxicam may represent a promising approach for safe and effective sustained drug delivery which can be used to overcome the adverse effects. In addition, it was concluded that suggested formulation could be applied musk and mastic to formulate the sustained release drug delivery system.

**ACKNOWLEDGEMENT**

The authors are thankful to Research center in Sciences and Technology University for providing all the facilities to carry out the work. The authors are highly thankful to Shaphaco Pharmaceutical industries, Yemen for providing gift sample of meloxicam, other excipient and all the facilities to carry out the work.

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