Factors affecting the diffusion of meloxicam from different bases

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الخلاصة

إن أهمية الميلوكسيكام معروفة كمضاد للالتهابات غير الخمجية.ان هذه الدراسة تهدف إلى إيجاد أفضل صيغة تركيبية للميلوكسيكام كمستحضر موضعي . وقد أوضح هذا البحث دراسة تحرر الميلوكسيكام خارج الجسم من عدة قواعد وهي :مستحلب زيت/ماء، مستحلب ماء/زيت، هلام الصوديوم كاربوكسي مثيل سيليلوز و هلام الكاربومير. وهذه الدراسة تضمنت نفاذ الميلوكسيكام خلال قطعة مستأصلة من جلد الفارة بتراكيز مختلفة باستخدام قاعدة هلام الكاربومير. بالإضافة إلى دراسة نفاذ الميلوكسيكام مع عدة عوامل مسرعة للنفاذ مثل البروبلين كلايكول و مثيل سالسيليت واليوريا وحامض الاوليك والدايمثيل سلفوكساید وصمغ الزانثان والبولي اثيلين كلايكول واتضح ان إضافة البولي اثيلين الكلايكول أعطت أعلى نفاذية , وأظهرت النتائج ان التركيبة المختارة غير مهيجة وذلك باستخدام اختبارالتهيج وقد تم دعمه بالاختبار النسيجي . وأثبتت النتائج ان استخدام الداي صوديوم اي دي تي اي كمادة مثبتة اعطت ثبوتية للصيغة التركيبية للميلوكسيكام1% في القاعدة الهلامية حيث ان تاريخ النفاذ سنتان مقارنة 0.8 سنة عند استخدام د-ل-الفا توكوفيرول وقاعدة الهلام للميلوكسيكام لوحده. وكذلك تمت دراسة تاثيرمدة ودرجة حرارة خزن الصيغة التركيبية على سرعة نفاذ الدواء وبعض الخصائص الفيزياوية وقد لوحظ ان سرعة نفاذ الدواء تقل كلما زادت مدة و درجة حرارة الخزن إضافة إلى قلة درجة اللزوجة وتغير طفيف في الاس الهيدروجيني وبدون اي تغير في لون و رائحة الهلام ومن ناحية اخرى تم اجراء دراسة سريرية تمهيدية ل 26 مريض واظهرت النتائج ان 84.6 % من المرضى اعطوا استجابة ايجابية.

ABSTRACT

The value of meloxicam as a non-steroidal anti-inflammatory drug is well established. This study was carried out to formulate a stable and effective meloxicam topical preparation. The in vitro release of meloxicam from different semisolid bases, which are: o/w, w/o emulsions, sodium carboxy methylcellulose gel and carbomer gel base was established. The study also involved the diffusion of meloxicam through excised mouse skin with different concentrations using carbomer gel in addition to the effect of different enhancing agents such as PG (Propylene Glycol), methyl salicylate, urea, oleic acid, DMSO (Dimethyl Sulfoxide), xanthan gum and PEG1000 (Poly Ethylene Glycol) and the last one gave the highest diffusion. The results showed that the selected formula of meloxicam was not irritant and this result was supported by histological examination. In addition, the results showed that the use of disodium EDTA (stabilizer) gave more stable formula where the expiration date was 2 years compared to 0.8 year when using both dl- α tocopherol and blank formula. The results showed that the temperature and the storage period led to decrease both the diffusion rate and viscosity of meloxicam 1% gel but with little change in pH. On the other hand preliminary clinical study was carried out for 26 patients and the results indicated that 84.6% of patients got positive responses.

INTRODUCTION:

Meloxicam is a non-steroidal anti–inflammatory drug (NSAID) of the acidic enol carboxamide class. It is a preferential inhibitor of cyclooxygenase-2(cox-2) which is induced by inflammatory stimuli in pathophysiological conditions.

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It was reported that meloxicam causes fewer gastrointestinal adverse effects compared with standard non-selective NSAIDs, therefore, the topical application is the appropriate mode of achieving therapeutic benefit with less risk of gastrointestinal or other side effects resulting from oral route[1]. However the transdermal drug transport is greatly limited by the relative impermeability of the stratum corneum which provides the principle resistance to percutaneouse permeation of co-administered moieties[2].

This study included the preparation of different meloxicam ointments using many bases and investigated the factors that might affect the release and diffusion of the drug such as the type of bases, concentration of the drug and penetration enhancers in order to get the most suitable topical formula for meloxicam. Histological study, irritancy test and preliminary clinical study were carried out to test the safety and effectiveness of the formula. The stability of the drug was followed at different storage time and temperatures and the expiration date of the drug in the selected formula was determined.

MATERIALS AND METHODS:

Preparation of Bases:

Fusion method was the general method employed for the preparation of the bases. Four types of bases(3,4) were selected to study their effects on the meloxicam 0.5% they were:

1- Water in oil emulsion base (wool fat and water) .

2- Oil in water emulsion base (white bees wax, cetyl alcohol, propylene glycol, sodium lauryl sulphate, water).

3- Sodium carboxy methylcellulose gel (NaCMC, Glycerol, M.H.B, water) .

4- Carbomer gel (Carbopol 941, triethanolamine, M.H.B, P.H.B, water) .

Preparation of the Diffusion Membrane:

The mouse (4-6 week old male) was sacrificed by ether inhalation, and then the skin was shaved lightly with an electrical clipper taking care to prevent any damage to the skin. A rectangular section of abdominal skin was excised from the animal using a sharp blade and then applied the defating procedure[5].

The in Vitro Release and Diffusion of Meloxicam:

A small funnel with 3cm in diameter was modified in order to be filled with 2gm of each base which was containing 0.5% w/w of meloxicam. The mouth of the funnel was covered with filter paper which was secured in place with a rubber bad while in diffusion test a 3gm of base containing 1% w/w meloxicam was introduced in a test tube with a diameter of 1.4cm and the epidermal surface of mouse skin was stretched over the mouth of the test tube and legated with a cotton thread[6,7]. In both testes the release cell and diffusion cell were then inverted and immersed in phosphate buffer (pH 7.4) placed in a beaker of the dissolution apparatus. The system maintained at 37°c and the buffer solution was stirred at 100 r.p.m during the 6 hours of the study. Samples of 5ml were pipetted from the collecting medium after 1, 2, 3, 4, 5, and 6 hours replaced with an equal volume of freshly prepared phosphate buffer (pH 7.4) at 37°c. The samples were then analyzed spectrophotometrically for their drug content at 350nm.

Effect of Meloxicam Concentration:

Different concentrations of meloxicam (0.5%, 1% and 1.5% w/w) were used with carbomer gel base to study the effect of concentration on the diffusion process.

Effect of Penetration Enhancers on Permeation of Meloxicam from the Selected Bases:

The effect of addition of propylene glycol (10% and 40%), Dimethyl sulfoxide(DMSO) (10% and 30%), oleic acid 5%, urea 5%, methyl salicylate10%, PEG1000(1% and 5%) and xanthan gum 0.5% and 1% on the diffusion of meloxicam 1%w/w was investigated.

Irritancy Test:

A group of three males albino rabbits weighing 1Kg were used. The ventral side of animal was carefully shaved, and four circular areas of 2.5cm in diameter were painted with 20% of aqueous solution of formaldehyde. Meloxicam 1% gel, meloxicam powder was impregnated on 2.5cm circular cotton pads and gel base without drug were applied on three of circular area and substance of known irritancy (histamine) was injected intradermally into the fourth of the inscribed circular areas for the purpose of calibration. The back of the animals ear was shaved carefully, xylene was used to dilate the superficial ear viens, 1ml of (0.5%) trypan blue solution was slowly injected through the selected vien of the animal. The degree of irritancy of the substance is estimated by the accumulation of trypan blue at the treated site. The site was observed for 24 hours[8].

Histology:

The selected formula was applied to the abdominal region after hair clipping of abdominal region of 3 male albino mice (4-6 weeks old) and was fixed by special tape. The mouse was then sacrificed and the skin of abdomen was removed after zero time (blank), 6 hours and 24 hours[9]. The skin was fixed in 10% formalin then embedded in paraffin, sectioned and stained with heomatoxilin-eosin and examined by light microscope for histological changes[10,11].

Stability Study:

1- Determination of expiration date of meloxicam in the selected formula:

The study was carried out on the selected formula 1%w/w meloxicam and it was divided into three portions, to the 1st portion added dl-α tocopherol as an antioxidant[12] and EDTA disodium as stabilizer was added to the 2nd portion while the 3rd portion was considered as a blank. All of them were kept in a collapsible tubes in ovens at 40°c, 50°c, and 60°c[13]. Meloxicam concentration in the stored gel was checked every 15 days for 120 days. This was done by HPLC method using the following conditions:
Column: C 18-25cm length.
Wave length: 260 nm, flow rate 1ml/min.
Attenuation: 4
Mobile phase: (50:50) of 0.1%w/v solution of KH_2PO_4, adjust to pH 6 with dilute NaOH: methanol.
Retention time: 2.7 min.
A standard with each reading was used. The height and the area under the curve were measured to calculate the percent remaining of the drug.

2- Effect of Storage Time and Temperature on the Diffusion of Meloxicam from the Selected Formula:

Samples of gel from the selected formula at 40°c and 50°c were subjected to in vitro diffusion study after 15, 30, 45 and 60 days.

3- Effect of Temperature on Viscosity:

Viscosities of the selected formula and blank formula (free from meloxicam) were determined at 6 rpm at room temperature, 40°c, 50°c for 15, 30, 45, and 60 days using rotational viscometer by spindle no. L4.

4- Effect of Storage Time on the pH, color and odor of Meloxicam 1% w/w gel:

The pH was measured at room temperature using pH meter at time intervals of 1st day, 15, 30, 45 and 60 days by taking 2gm of the gel and shaking up with 10ml of water. The color and odor were observed over the storage period..

Preservative Efficacy Test:

Challenge test was used to determine the preservative efficacy[14].

Preliminary Clinical Study:

Twenty–six patients of different cases, age and sex were selected. The gel was applied on the pain area once daily for one week with the observation for pain relief.

Statistical Analysis:

Student t-test was used to determine the relation between 2 parameters.

RASULTS AND DISSCUSION:

Figure (1) showed that the release rate decreased in the following order: carbomer gel >NaCMC gel > o/w emulsion > w/o emulsion.
This could be explained as meloxicam is practically insoluble in water so partitioning of drug is decreased according to the nature of the base and since the gel gave highest release and diffusion therefore it was selected for next studies.

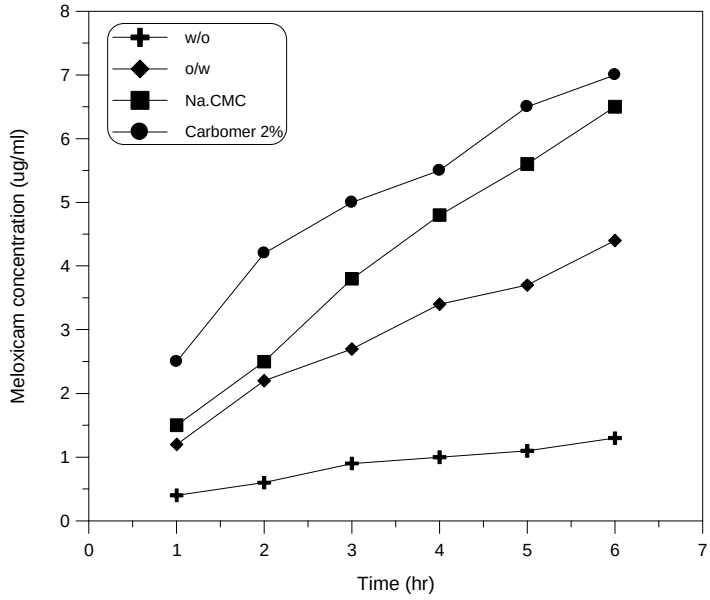

FIG 1 . EFFECT OF DIFFERENT BASES ON THE RELEASE OF MELOXICAM 0.5% W/W.

Figure (2) shows that the amount of meloxicam diffused during 6 hours increased with increasing concentration of the drug.

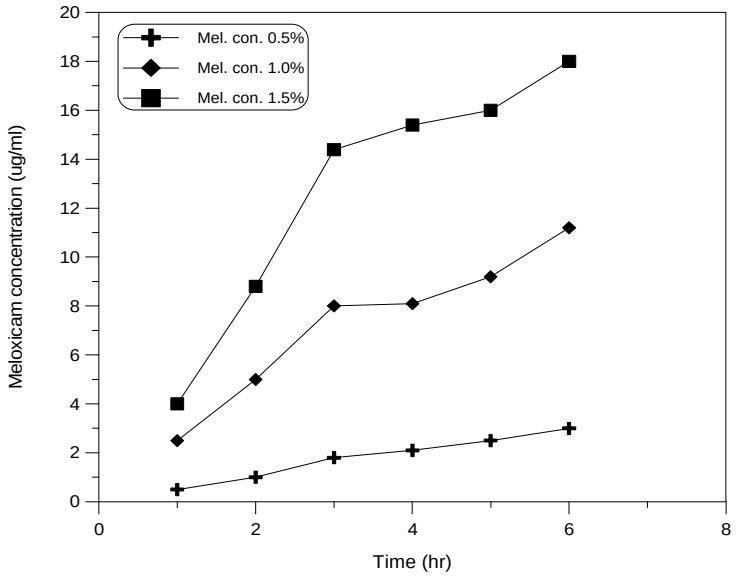

FIG 2 . EFFECT OF MELOXICAM CONCENTRATION ON THE DIFFUSION PROCESS THROUGH MOUSE SKIN FROM CARBOMER GEL.

Figure (3) showed the diffusion order 10% PG> zero % PG > 40%PG. A non significant effect of PG as a co solvent for both concentrations. The decrease in diffusion rate of meloxicam with 40%PG may be due to the decrease in thermodynamic activity of meloxicam in the gel base[(2)].

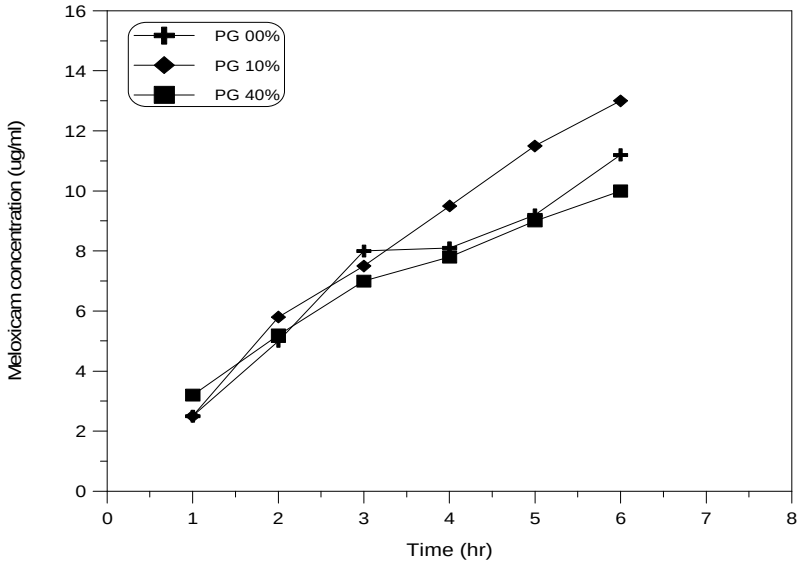

FIG 3 . EFFECT OF PROPYLENE GLYCOL CONCENTRATION ON THE DIFFUSION PROCESS OF MELOXICAM 1% W/W THROUGH MOUSE SKIN.

The effect of addition of 5% oleic acid,10% methyl salicylate and 5% urea on meloxicam diffusion were studied and it was found that addition of 5% oleic acid led to significant ($p<0.05$) decrease in diffusion of meloxicam as shown in figure (4) and this due to the change in whole structure of the gel and form a kind of emulgel with white color instead of transperancy of gel, and addition of 10% methyl salicylate and 5% urea led to a non significant decrease in diffusion and this in agreement with a previous reported date for piroxicam[15].

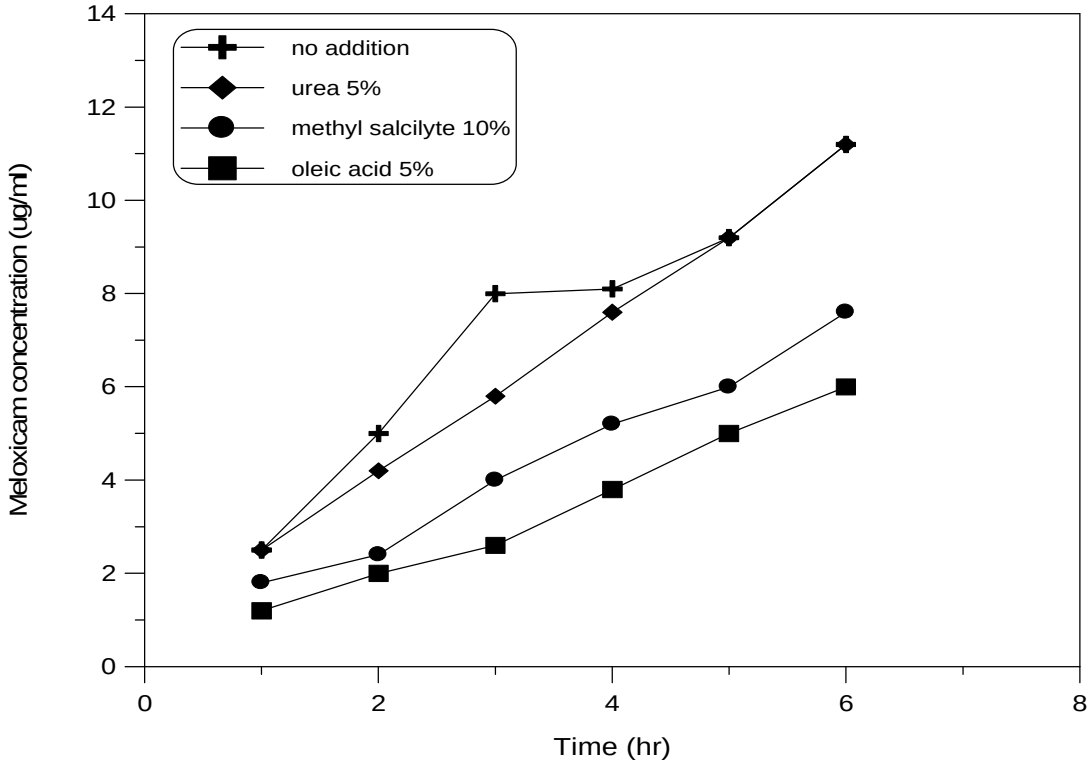

FIG 4 . EFFECT OF OLEIC ACID, METHYL SALICYLATE AND UREA ON DIFFUSION PROCESS OF MELOXICAM 1% W/W THROUGH MOUSE SKIN.

Figure (5) shows a non-significant increase in diffusion of meloxicam from the gel base containing 10% of DMSO, while 30% of DMSO gave a significant increase in diffusion and this due to the property of DMSO as a solubilizer of many lipophilic compound in water[16].
Figure (6) shows a non significant effect of 0.5% of xanthan gum while 1% of xanthan gum led to a significant increase in the diffusion of meloxicam and this may be due to the presence of the cellulose enzyme in the xanthane gum which led to break down in carbomer structure and causes a decrease in the apparent viscosity[17].
Figure (7) shows that the addition of 1% of PEG 1000 led to a significant effect while 5% of PEG1000 gave a very highly significant effect on diffusion of meloxicam.

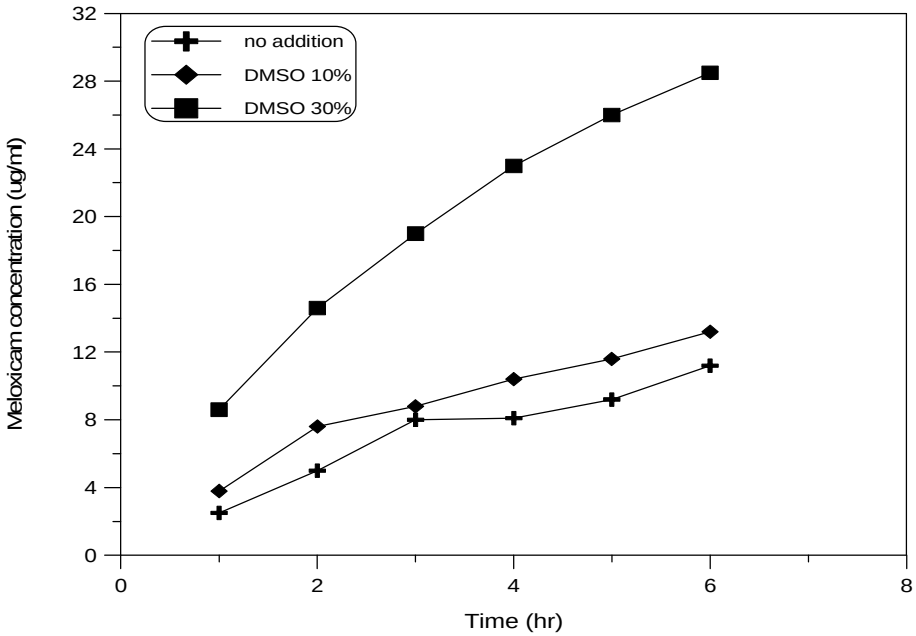

FIG 5 . EFFECT OF DMSO CONCENTRATION ON THE DIFFUSION PROCESS OF MELOXICAM 1% W/W THROUGH MOUSE SKIN.

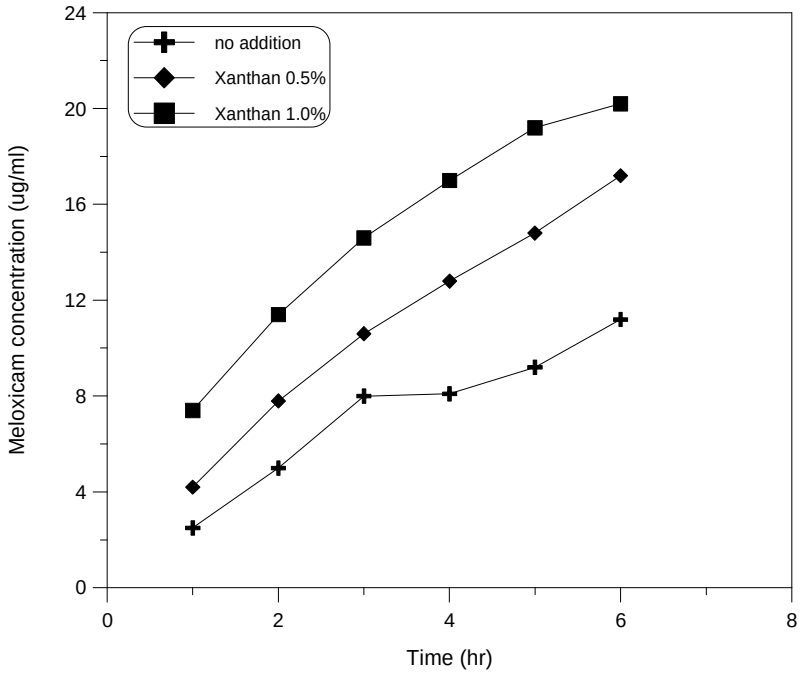

FIG 6 . EFFECT OF XANTHAN GUM CONCENTRATION ON THE DIFFUSION PROCESS OF MELOXICAM 1% W/W THROUGH MOUSE SKIN.

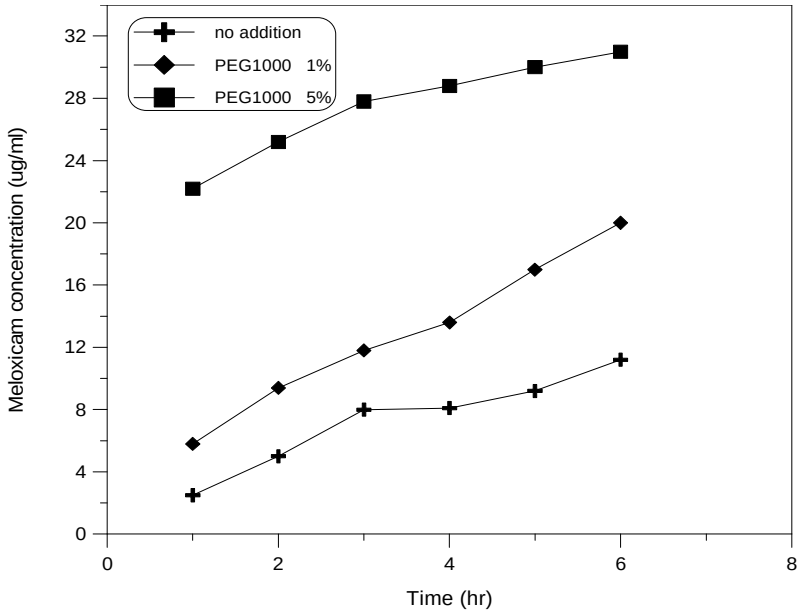

FIG 7 . EFFECT OF PEG1000 CONCENTRATION ON THE DIFFUSION PROCESS OF MELOXICAM 1% W/W THROUGH MOUSE SKIN.

From the overall results, 5% w/w PEG1000 was chosen to be added to the carbomer gel base containing 1%w/w of meloxicam in addition to the presence of 0.15% methyl hydroxyl benzoate and 0.05% w/w propyl hydroxyl benzoate as preservatives. This selected formula was subject for further investigation.

From the irritancy test and histological study, it was found that the selected formula was not irritant and there was no histological changes in the skin occurred.

The degradation of meloxicam in all formulas that contain antioxidant, stabilizer agent and the blank one follows 1st order kinetics, since straight lines were obtained when the logarithm of % remaining of meloxicam was plotted versus time as shown in fig.(8) for formula with EDTA and fig.(9) for formula with dl-α tocopherol and the blank one since the same results were obtained for both formulas.

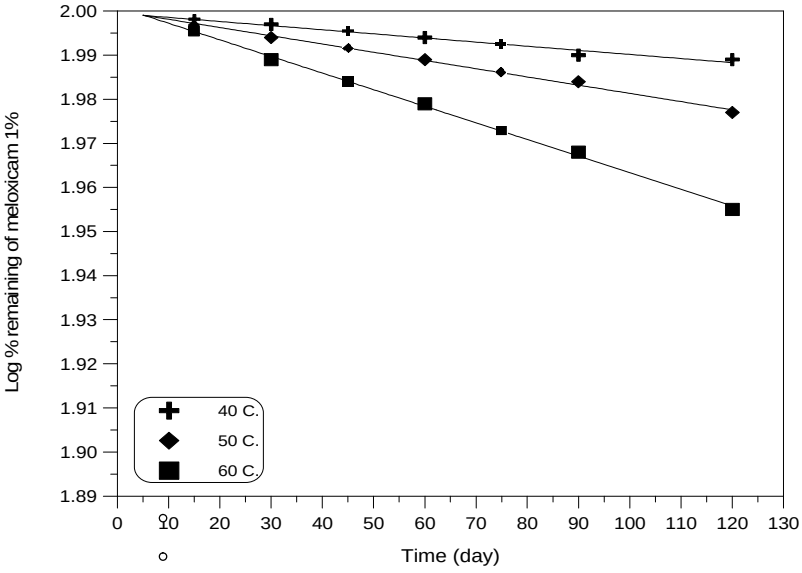

FIG 8 . DEGRADATION CURVE OF MELOXICAM AT 40°C, 50°C AND 60°C FOR FORMULA WITH EDTA.

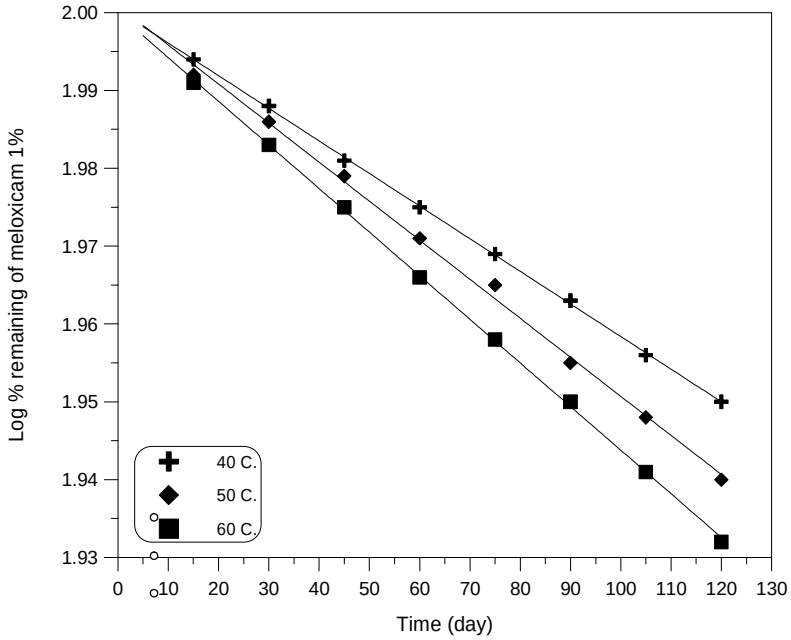

FIG 9 . DEGRADATION CURVE OF MELOXICAM AT 40ºC, 50ºC AND 60ºC FOR FORMULA WITH DL- A OCOPHEROLE AND THE BLANK FORMULA.

The degradation rate constants (k) at 400C, 500C and 600C were calculated from the slopes of the lines as shown in table (1).

Table 1 . Degradation Rate Constants (k) of Meloxicam 1% w/w Gel at 40ºC, 50ºC, 60ºC

Meloxicam 1% w/w Gel with Different Additives	$K_{40} \times 10^{-3}$ (day $^{-1}$)	$K_{50} \times 10^{-3}$ (day $^{-1}$)	$K_{60} \times 10^{-3}$ (day $^{-1}$)
0.1 % EDTA	0.240	0.400	0.806
0.1 % dl-α tocopherole	0.960	1.059	1.266
Blank formula	0.960	1.059	1.266

To determine the expiration date, Arrhenius plot was utilized to predict the degradation rate constant at 25°C (K25) for all formulas and the values are tabulated in table (2).

Table 2 . Degradation Rate Constants (k) of Meloxicam 1% w/w Gel at 25ºC K_{25}

Meloxicam 1% w/w Gel with Different Additives	$K_{40} \times 10^{-3}$ (day $^{-1}$)
0.1 % EDTA	1.40
0.1 % dl- α tocopherole	3.50
Blank formula	3.50

The expiration date was calculated using the following equation:

$$t\ 10\% = 0.104/K_{25}$$

Results showed that the expiration date of meloxicam with EDTA (2 years) is higher than that of formula with dl- α tocopherol and the blank formula (0.8 years). This indicates that utilization of disodium EDTA increase the stability of meloxicam gel and its expiration date. This is in agreement with its use in the formulation of gel like cefazolin to increase the stability of drug 18 folds. In addition, it appears that dl-α tocopherol had no effect on the stability of meloxicam since both formulas with dl-α tocopherol and the blank one had the same expiration date.
The study showed a gradual decrease in the amount of meloxicam with increase in the storage time and temperature (fig.10 and 11), due to the degradation during the storage period.

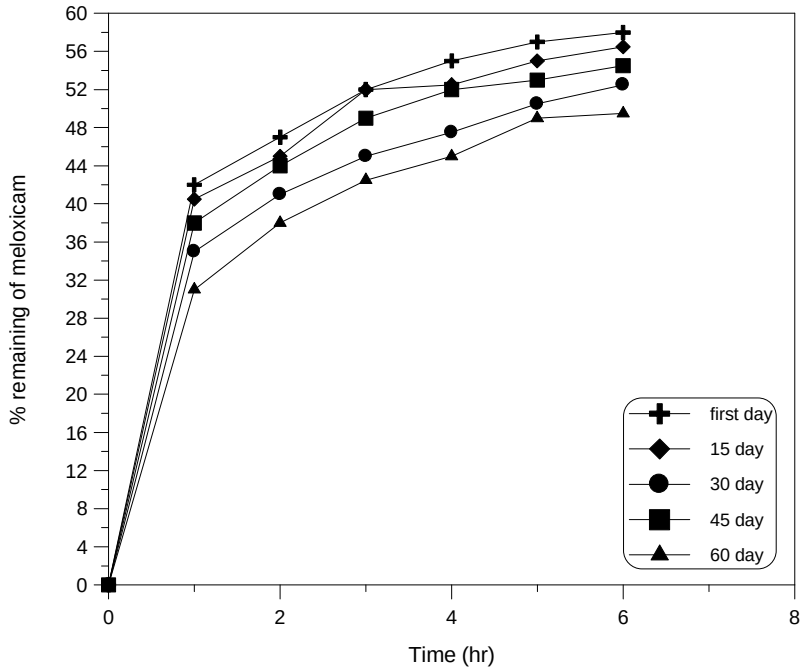

FIG 10 . EFFECT OF STORAGE TIME OF MELOXICAM 1% W/W ON THE DIFFUSION PROCESS THROUGH MOUSE SKIN AT 40°C.

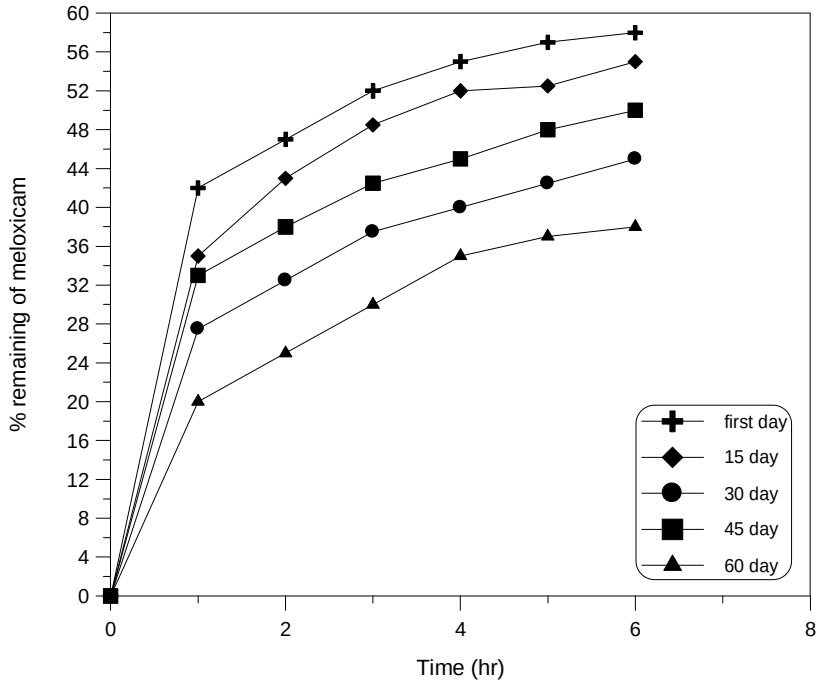

FIG 11 . EFFECT OF STORAGE TIME OF MELOXICAM 1% W/W ON THE DIFFUSION PROCESS THROUGH MOUSE SKIN AT 50ºC.

The results showed that as the temperature increased with storage time the viscosity decreased as shown in table (3). This agreed with results obtained with petrolatum and plastibase[18].

Table 3 . Effect of Storage Time and Temperature on Viscosity of 1% w/w Meloxicam Gel.

Storage Time (days)	Viscosity (centipoise)					
	Room temperature		40ºC		50ºC	
	Test	Blank	Test	Blank	Test	Blank
15	64096	83086	56530	65500	39700	52470
30	62030	74270	42546	57450	25010	44610
45	35210	72210	41630	56190	24446	43746
60	33650	70800	31850	54500	23670	41470

There were no changes in color and odor and undetectable changes in pH within storage period. The preservatives added to the selected formula were effective and met the requirements of the U.S.P.XXIII.

The clinical effects of meloxicam 1% w/w gel have been tested in a group of 26 patients with multiple joint and Rheumatic compliant. Cases of arthritis and inflammatory goint diseases were excluded from the study. Table (4) revealed that 84.6% of the patients showed a positive response (relief of pain and better range of movement). This indicates that meloxicam has good release from the selected topical formula and good diffusion through the skin.

Table 4 . The Preliminary Clinical Study of Meloxicam 1% w/w Gel.

No. of cases	The Case	+ve response	-ve response
4	Disc prolaps sciatica	4	
4	Chondromalacia patalae (CMP)	4	
2	Post traumatic are syndrome	2	
1	Fubromyalgia	1	
10	Osteoarithritise	8	2
1	Tennis elbow		1
1	Frozen shoulder	1	
1	Stiffness knee joint post traumatic	1	
1	Fracture multiple joint	1	
1	Lumbago		1
26	Total patients		

The overall results suggest that stable, non-irritant and effective formula of meloxicam can be prepared to be applied topically.

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