Formulation Characterization and Evaluation of Meloxicam Nanoemulgel to be Used Topically

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Abstract

Meloxicam is a non-steroidal anti-inflammatory drug. It is practically insoluble in water. It is associated with gastrointestinal side effects at high doses on long term treatment. The aim of this investigation to formulate and evaluate gellified nanoemulsion of meloxicam as a topical dosage form to enhance meloxicam therapeutic activity and reduce systemic side effect.

The pseudo ternary phase diagrams were made, including the oil mixture which is composed of almond oil and peppermint oil at a ratio (1:2), variable surfactant mixture (S mix) which are tween 80 and ethanol at ratios of (1:1, 2:1, 3:1, and 4:1) and double distilled water. Slow dripping of double distilled water to the combination of the oil mixture and Smix was performed to get nanoemulsion. The pseudo ternary phase diagram that has a greater nanoemulsifying area was contained Smix ratio (3:1) from which seven formulas of nanoemulsion (NE1-NE7) were taken for characterization of prepared nanoemulsions and to prepare nanoemulgel formulas (NF1-NF7). The seven nanoemulgel formulas were subjected to various evaluations. NF1 was the selected formula and investigated for its stability and morphology.

Atomic force microscopy (AFM) indicated that the optimized formula (NF1) was nanosized particle nearly spherical in shape and smooth surface globules, this indicates stability of optimized nanoemulgel (NF1). It can be concluded that the selected formula (NF1) is an effective alternative for the topical delivery of meloxicam.

Keywords: Meloxicam, Nanoemulgel, Arthritis.

تحضير وصياغة وتقييم هلام المستحلب النانوي للميلوكسيكام للأستخدام الموضعي

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الخلاصة

الميلوكسيكام هو من الأدوية غير الستيرويدية المضادة للالتهابات. أنه غير قابل للذوبان عمليا في الماء. ويرتبط مع الآثار الجانبية المعدية المعوية في الجرعات العالية للعلاج لفترة طويلة.الهدف من هذا البحث هو تحضير وصياغة وتقييم هلام المستحلب الدقيق للميلوكسيكام كشكل دوائي موضعي لتحسين النشاط العلاجي وتقليل الآثار الجانبية للميلوكسيكام.

وقد تم رسم المخطط الثلاثي الأطوار والمكون من مزيج الزيت المكون من زيت اللوز وزيت النعناع بنسبة (١: ٢)، وخليط من مواد مقلل الشد السطحي وهو توين ٨٠ و الإيثانول في نسب (١: ١، ٢ :٣، ١،و ٤: ١)، والماء المضاعف التقطير.بالتقطير البطيء للماء المضاعف التقطير الى خليط من مزيج الزيت وخليط مقلل الشد السطحي تم الحصول على المستحلب الدقيق. تم اختيار المخطط ثلاثي الأبعاد الذي يحتوي على نسبة (١:٣) من خليط مقلل الشد السطحي والذي لديه مساحة اكبر لتكوين المستحلب الدقيق وتم أختيارمنه سبع صيغ لتوصيف المستحلب الدقيق وإعداد سبعة من صيغ هلام المستحلب الدقيق(NF1-NF7) والتي خضعت لمختلف التقييمات. كانت الصيغة (NF1) هي الصيغة المختارة وتم اجراء أختبار الأستقرار والشكل السطحي.

مجهر القوة الذرية يشير إلى أن الصيغة الأمثل ذات جزيئات نانوية الحجم وتقريبا كروية الشكل وسطح ناعم وهذا يؤشر انها مستقرة إلى حد كبير. نستنتج أن الصيغة المختارة (NF1) هي بديل فعال للتسليم الموضعي للميلوكسيكام.

الكلمات المفتاحية : الميلوكسيكام ، هلام المستحلب النانوي، التهاب المفاصل.

Introduction

The nanotechnology implementation in the domain of health sciences has great interest at the present time. The a noscale drug delivery system that has nano size particles or molecules can improve drug solubility and permeability due to nano oily droplets provide greater surface area for drug libration and faster absorption from biological barrier and this will lead to increase bioavailability of both hydrophilic and hydrophobic drugs that enhance the therapeutic efficacy of the drug [1].Meloxicam is a non-steroidal anti-inflammatory drug utilized to relieve the symptoms of inflammatory arthritis such as (rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, systemic lupus erythematosus) and degenerative arthritis such as osteoarthritis. It is a pale yellow powder, practically insoluble in water; very slightly soluble in alcohol [2].

In spite of meloxicam preferentially activity that inhibits COX-2 (cyclooxgenease-2) over COX-1 (cyclooxgenease-1), in practice it still has higly disturbe gastrointestinal side effects at high doses on long term treatment [3]. Therefore, there is a high requisiteness of to deliver meloxicam by another route to increase its solubility and modifying gastric adverse effect and delivers the drug to the inflammatory site.

Nanoemulsion (NE) which is nanocarrier delivery systems in transdermal drug delivery has great interest as a part of nanotechnology. They are optically transparent with the range of particle sizes between 100 to 500 nm. It is compose of the oil, surface active agent, co-surfactant and aqueous phase [4]. Anyway, the low viscosity of nanoemulsion make it unsuitable for application to the human skin [5]. By use gelling agent with nanoemulsion, this make more convenient for transdermal application [6]. When gelling agent and nanoemulsion are utilized in combination form the dosage form is called nanoemulgel. Most of the hydrophobic drugs cannot be incorporated directly into the gel base because the solubility problem that emerge during the drug release. Nanoemulgel assist in the hydrophobic drugs merger into the oil phase and then oil droplets are dispersed in aqueous phase leading to oil in water (o/w) nanoemulsion then this nanoemulsion can be combined into gelling agent to get attractive delivery system which is nanoemulgel [7].

Thus, the aim of this investigation is to formulate and evaluate gellified nanoemulsion of meloxicam used topically to increase solubility of meloxicam and decreasing the oral side effect of meloxicam that lead to enhance meloxicam local bioavailability , therapeutic activity and patient compliance.

Materials and Methods
Materials
Meloxicam supplied by Wadi Al-Rafidian factory for pharmaceutical products(Baghdad, Iraq). Almond oil, peppermint oil were purchased (BAR-SUR-loop Grasse A. M Franc), Carbopol 940 was purchased (Hengshui Taocheng Chemicals Auxiliary Co., Ltd. China). Tween 80 (SD fine Chemlimited (SDFCL) Mumbai, India). Methanol and ethanol (grin land chemical comp, United). KH_2Po_4 and Na_2HPo_4 (Merck & Co., Inc. Germany).

Methods
Preparation of meloxicam nanoemulsion and construction of pseudo ternary phase diagrams
The oil mixture was made by mixing two oils which are almond oil and peppermint oil in a ratio (1:2) to get oil mixture. The oil mixture, surfactant mixture (Smix) and aqueous phase are components of the pseudo ternary phase plot that were selected by employing the aqueous phase titration technique. The surface active agent and co-surfactant was mixed in different weight ratios (1:1, 2:1, 3:1, 4:1) to get Smix. The oil mixture that was loaded by meloxicam blend with Smix in different weight ratios for each phase diagram plot, so that all areas of the pseudo ternary phase plot were covered. Slow dripping of double distilled water to the combination of the oil mixture and Smix was performed to know the borderline of phases and visual searches were made for transparency when the double distilled water was added drop by drop untill the nanoscale blend was a clear liquid to the eyes, then stop further adding double distilled water and different o/w nanoemulsions were formed. The pseudo ternary phase diagram was constructed. The drawn area of nanoemulsion represented by the shaded area and the greater area refer to better nano emulsifying activity. From pseudo ternary phase plot that has a wider area of emulsifying activity we took seven formulas to formulate nanoemulgel of meloxicam [8].

Preparation of meloxicam nanoemulgel and gel
The aqueous phase and oil phase were prepared. The aqueous phase was made by combining double distilled water and tween80. The oil mixture that was previously prepared represented the oil phase. The oil mixture was sonicated for 15 minutes. The oily phase was heated to 70°C while the aqueous phase to 80°C on a hot plate. The oil phase gradually was added to the aqueous phase with continuous stirring until cool . Stirring continuously with magnetic stirrer at 2000 rpm and stirring was continued for 5-10 min until becoming unwarm at room temperature [9]. The gel bases were prepared by dispersing carbopol 940 with specified concentration in double distilled water with constant stirring at a moderate speed using mechanical stirrer. Few drops of triethanolamine were added in preparation until get a pH of about 6.5. The nanoemulsion obtained was then mixed with the gel in (1:1) ratio to get homogenous nanoemulgel [10]. On the other hand, meloxicam gel 1 % (w/w) was prepared by dissolving meloxicam in a previously

prepared mixture of propylene glycol and ethanol (1:1). Carbopol 940 was dispersed in distilled water under continuous shaking using a stirrer at 1000 rpm until it produce a homogenous dispersion. The carbopol 940 added to meloxicam solution 1% (w/w) with continuous stirring to get a homogenous dispersion of meloxicam gel. Methylpapaben and propylparaben were used as preservatives. Sodium sulphite function as antioxidant that prevent or slow the deterioration of formulas caused by chemical reactions with oxygen. The formulas of 100 g of meloxicam nanoemulgel and meloxicam gel were prepared for evaluation [9,10].

Table(1): The Meloxicam Nanoemulgel Formulations.

Formulation components (%w/w)	Formulation code							
	NF1	NF2	NF3	NF4	NF5	NF6	NF7	Meloxicam gel
Meloxicam	1	1	1	1	1	1	1	1
Carbopol 940	1	1	1	1	1	1	1	1
Oil mix(1:2)	2.5	5	7.5	10	12.5	15	17.5	0
Smix(3:1)	22.5	22.5	22.5	22.5	22.5	22.5	22.5	0
Propylene glycol	0	0	0	0	0	0	0	5
Ethanol	0	0	0	0	0	0	0	5
Methylparaben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Propylparaben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sodium sulphite	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Double distilled water up to	100	100	100	100	100	100	100	100

Characterization of the prepared meloxicam nanoemulsion
Droplet size measurement

The samples of 5 ml nanoemulsion sonicated at the 37 ^{o}C for 30 min and measured using ABT-9000 nanolaser particle size analyzer. The average of droplets size and droplets size distribution diagrams were obtained. All measurements were taken in triplicate [8].

Poly dispersity index (PDI) assay

It estimates the homogenicity of droplets size in nanoemulsion formulation. It can get by ABT-9000 nanolaser particle size tester. The measurements were occuring in triplicate. PDI range from 0.0 to 1.0. As the polydispersity index value is closer to zero, the droplets are higher homogenous. The higher value of the poly dispersity value indicates the lowest homogenicity of droplets of nanoemulsion formulations [11].

Evaluation meloxicam nanoemulgel
Physical analysis

The formulated nanoemulgel formulations were visually tested for color, appearance and uniformity. The tested samples were occurred in triplicate [12].

pH estimation

One of the high consequential parameters in the evaluation of nanoemulgels is the pH. The pH values affect on ionization of the drug i.e. ionized and unionized forms of the drug. The human skin is naturally acidic with a normal pH of 4-6. The women have been attested to have more acidic skin than men. The pH of all the nanoemulgel formulations was tested by dissolving 1g of nanoemulgel in 100 ml of distilled water and stored for 2h then use a digital pH meter at room temperature [13,14].

Swelling index measurement

The separation of formulation molecules of water molecules causes an expansion in volume of the mass called swelling index. It related to the area of application of nanoemulgel. One gram of meloxicam nanoemulgel formulations was wrapped with aluminium foil that is punched to produce holes and put in phosphate buffer pH 6.8 for 6 hours, then the swollen samples wiped with the filter paper to eliminate absorbed distilled water on the surface and then it was rapidly weighed on an electronic balance. The estimations were performed in triplicate.

The swelling index was measured by utilizing the following equation (Eq):

$$Sw = [(Wt - Wo) / Wo] \times 100 \dots (Eq1)$$

Where, SW = percentage of swelling index of meloxicam nanoemulgel

Wt = the weight(g)of the nanoemulgel at time t

Wo = initial weight (g) of the meloxicam nanoemulgel [15].

Viscosity measurement

The viscosity and rheology behavior of meloxicam nanoemulgel formulations were measured using NDJ-55 digital viscometer by utilization a spindle no. 3 at room temperature. The measurements were taken in triplicate [8].

Measurement of the drug content

By UV-visible spectrophotometer utilization, the drug content was estimated [16]. One gram of the samples was mixed with methanol up to 100mL then take 1mL and read in the UV spectrophotometer after second dilution with methanol. The samples were made in three trails. The absorbance was observed at 263 nm and the percent of drug content was obtained by the following equation:

% Drug content = (Analyzed content/Theoretical content) x 100 ….. (Eq2)

In vitro release study

A United States Pharmacopeia (USP) dissolution tester apparatus II is the device that was used to measure the in vitro release of meloxicam from formulations. One gram of meloxicam nanoemulgel formulations (NF1-NF7) or meloxicam gel was put in a glass tube with 1.5cm diameter, covered with a cellulose acetate membrane which was formerly steeped in phosphate buffer of pH (6.8) for about 24 hours. The membrane adequately sealed and inverted under the surface of 900 mL of phosphate buffer of pH 6.8, containing 1% polysorbate 80, at 37 ± 0.5 °C with stirring speed of 100 rpm. The samples of 5 mL were withdrawn at 0,15,30, 60, 90,120, 150,180,210, 240 min and replaced with an equivalent quantity of dissolution medium. The drug content of the samples was estimated by a UV spectrophotometer at 263 nm. All samples were taken in three trails [17,18].

Atomic force microscopy (AFM) analysis

The morphology of optimized meloxicam nanoemulgel formulation was obtained by AFM angstrom advanced inc. AA3000 USA. Atomic force microscopy analysis was performed by placing drops of the selected sample onto a glass slide and then estimated [19].

Statistical analysis

The results of the investigation were given as an average of three trail estimation of samples and use analysis of variance (ANOVA) at level (P<0.05) for interpretation of the results [20].

Results and Discussion

Preparation of meloxicam nanoemulsion and Construction of pseudo ternary phase

The pseudo ternary phase plots were made by plotting oil mixture (1:2) with variable Smix ratios as 1:1, 2:1, 3:1, and 4:1 and double distilled water as shown in figures (1-4). The shady part of pseudo ternary phase digram offer the region of nanoemulsion, whereas the nonshady part offer the region of emulsion. The pseudo ternary phase diagram that has a greater nanoemulsifying area was contained Smix ratio (3:1) from which seven formulas of nanoemulsion (NE1-NE7) were prepared for characterization in order to prepare nanoemulgel formulas (NF1-NF7).
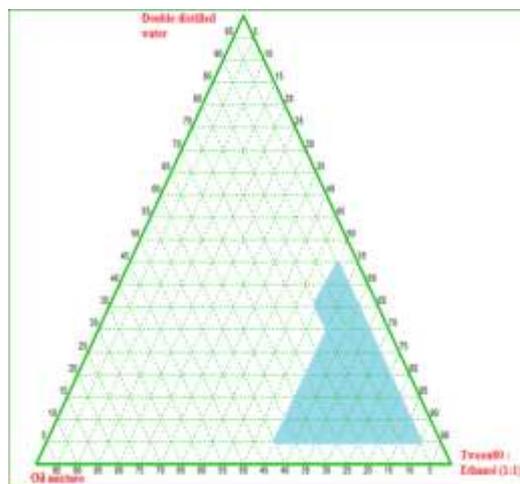

Figure (1): The pseudo ternary phase diagram of oil mixture of almond oil and peppermint oil in a ratio (1:2), Smix (1:1) and double distilled water.
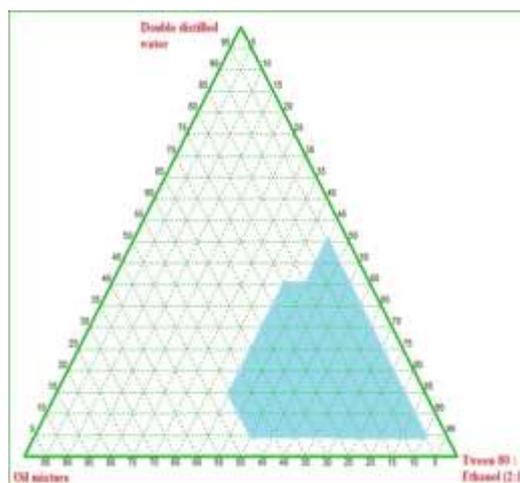

Figure (2): The pseudo ternary phase diagram of oil mixture of almond oil and peppermint oil in a ratio (1:2), Smix (2:1) and double distilled water.
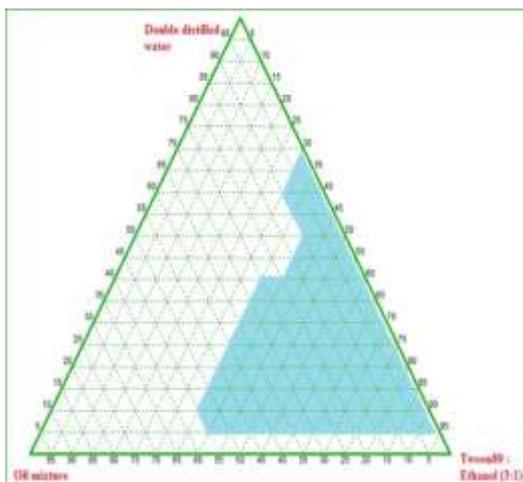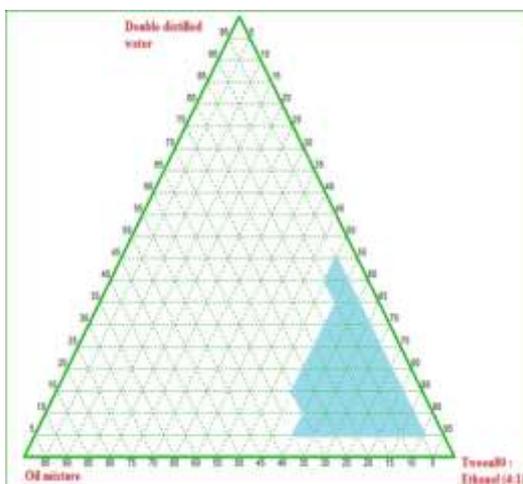
Figure (3): The pseudo ternary phase diagram of oil mixture of almond oil and peppermint oil in a ratio (1:2), Smix (3:1) and double distilled water.

Figure (4): The pseudo ternary phase diagram of oil mixture of almond oil and peppermint oil in a ratio (1:2), Smix (4:1) and double distilled water.

Characterization of the prepared meloxicam nanoemulsion
Droplet size measurement
The results of globule size range were NE1 (5-5.1nm), NE2 (5-6.29 nm), NE3 (6.29-7.92 nm), NE4 (11.1-15.8 nm), NE5 (15.8-22.3 nm), NE6 (25-28.1 nm) and NE7 (5.1-39.7 nm) that contained an oil mixture (%w/w) concentrations of 5, 10, 15, 20, 25, 30, 35 respectively. The outcomes refer to all the preparations had droplets in the nanometer size. Analysis of variance indicated significant differences between droplet size values and the concentration (%w/w) of oil mixture where ($p < 0.05$).

Poly dispersity index (PDI) assay
PDI was from (0.011 to 0.188). The results of PDI referred to that nanoemulsion formulations had a high alliterative and constrict size distribution as shown in figure (5).
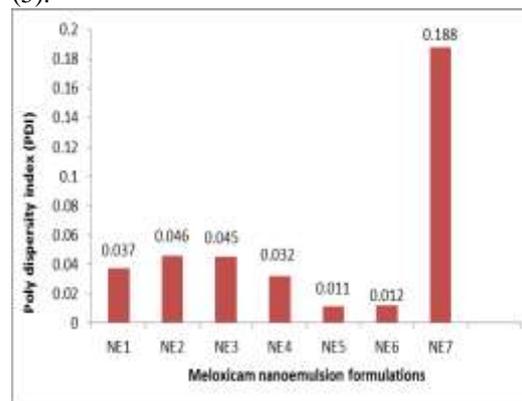

Figure (5): The poly dispersity index (PDI) of meloxicam nanoemulsion formulations (NE1-NE7).

Evaluation meloxicam nanoemulgel formulations
Physical analysis
The physical appearance of the prepared nanomulgels formulas was yellow in color, transparent with excellent homogenicity as shown in table (2).

pH estimation
The pH of all the nanomulgels formulas was found to have a range between 5.45 to 5.88 as shown in table (2) which are within the normal pH value of human skin.

Table (2): The Physical Appearance, pH, Percent of Swelling Index and Percent of Drug Content in Meloxicam Nanoemulgel Formulations.

| Formulation code	Color	Clarity	Uniformity	Phase separation	pH	% Swelling index	% Drug content
NF1	Yellow	Transparent	Excellent	None	5.88	40	96.921
NF2	Yellow	Transparent	Excellent	None	5.73	38	97.044
NF3	Yellow	Transparent	Excellent	None	5.76	37	97.66
NF4	Yellow	Transparent	Excellent	None	5.81	35	98.522
NF5	Yellow	Transparent	Excellent	None	5.52	33	98.891
NF6	Yellow	Transparent	Excellent	None	5.79	30	99.384
NF7	Yellow	Transparent	Excellent	None	5.45	25	99.5
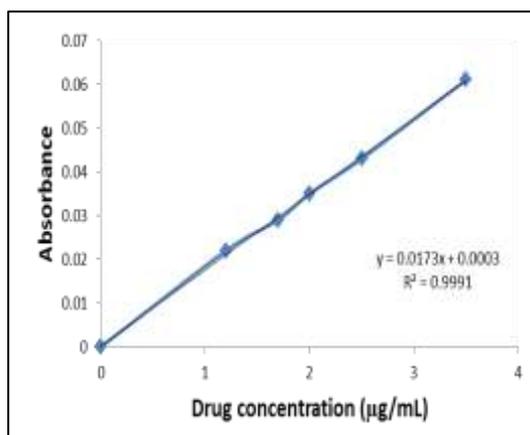
Figure (6): Calibration curve of meloxicam in methanol.

Swelling index measurement

    The outcome described in the table (2). The results showed the effect of oil mixture concentration on swelling index at constant gelling agent concentration. As the oil mixture concentration increase that lead to create more hydrophobic media that retard water molecule from enterance to formula therefore it was found NF1 exhibited significantly higher swelling index that it contained the least oil mixture quantity while NF7 with higher oil mixture concentration had significantly lower swelling index ($p < 0.05$).

Measurement of the drug content

    The drug content was in a range of (99.5 – 96.921%) as shown in table (2). These results were found within normal outline range.

Viscosity measurement

    The outcome of viscosity for meloxicam nanoemulgel (NF1-NF7) and meloxicam gel was found between (248 mPa-293 mPa). There is a significant difference ($P < 0.05$) between formulations. With these results the formulas can removed rapidly from the site of application and provide good spreadability and application of formulations on the skin surface. When a viscosity plot against rpm figure (7) a pseudoplastic diagram will be obtained. It was indicated that increase oil mixture concentration at a constant Smix concentration contributes to produce pseudoplastic system.
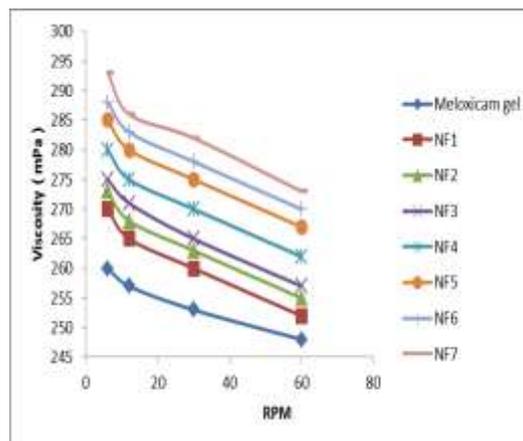

Figure (7): Viscosity analysis of meloxicam nanoemulgel and meloxicam gel formulations

In vitro release study

    By utilizing the united states pharmacopeia (USP) dissolution apparatus type II, the analysis of drug release was done for the meloxicam nanoemulgel (NF1-NF7) and meloxicam gel formulations in phosphate buffer pH (6.8).

    The drug release diagram in figure (8) indicates that the NF1 was significantly highest in release rate while meloxicam gel formula has lower in dissolusion rate which was significantly lowest ($P < 0.05$) .

    The comparability drug release profile of meloxicam nanoemulgel (NF1-NF7) and meloxicam gel show that the drug release was followed the descending order: NF1>NF2 >NF3> NF4>NF5>NF6>NF7> meloxicam gel.

    The drug release analysis from meloxicam nanoemulgel formulations (NF1-NF7) showed the effect of oil mixture concentration at constant concentration of Smix and gelling agent,as the concentration oil mixture increase this lead decrease drug release this may be due to the high concentration of oil mixture increase hydrophobicity of formulations and this will provide retarding forces for dissolution media to pass through hydrophobic matrix and liberate the drug also the meloxicam molecules will have greater diffusional pathway to reach the dissolution media therefore it was found NF1 has greater release profile compare to other of meloxicam nanoemulgel formulations due to it had the least concentration of oil mixture that make less retarding effect for dissolution molecules and lower diffusional pathway to drug molecules to reach the dissolution media.

    On the other hand, the meloxicam gel dissolution profile has lowest dissolution rate in comparison to other meloxicam nanoemulgels this due to that nanoemulsion

when present in nanoemulgels provide many nanosize droplets and enhance solubility of meloxicam hydrophobic drug this will enhance enterance of dissolution media to hydrophobic matrix and lower diffusional pathway for meloxicam molecules to reach the dissolution media that make meloxicam nanoemulgels superior and highly prefer in comparison to meloxicam gels [8, 21].
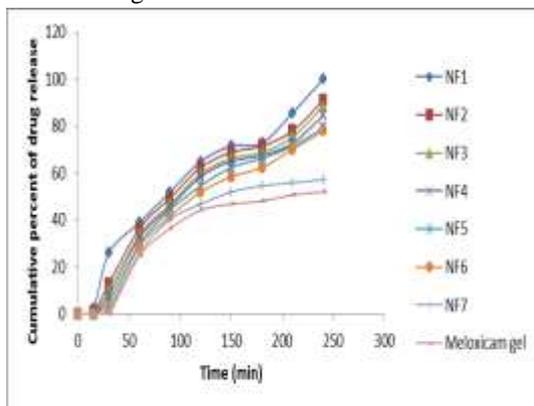

Figure (8): Drug release profile for meloxicam nanoemulgel and meloxicam gel formulations.

advantages in comparison to gel such as

Selection of the optimized formula

From a study of the characteristics of nanoemulsion which are globule size analysis and PDI also from evaluation of nanoemulgel formulations through study physical appearance, pH, swelling index measurement, viscosity measurement, measurement of the drug content and in vitro release study, it was found that (NF1) is an optimized formula where it had lower globule size (5-5.1nm) as shown in figure (9) , low PDI (0.037), excellent physical appearance, the normal value of pH (5.88), a higher percent of swelling index (40), encourage viscosity, accepted percent of drug content (96.921%) and higher release rate makes it faster in relieving inflammatory disorder that improve therapeutic efficacy. The selected formula (NF1) subject for further analysis of atomic force microscopy (AFM) study.
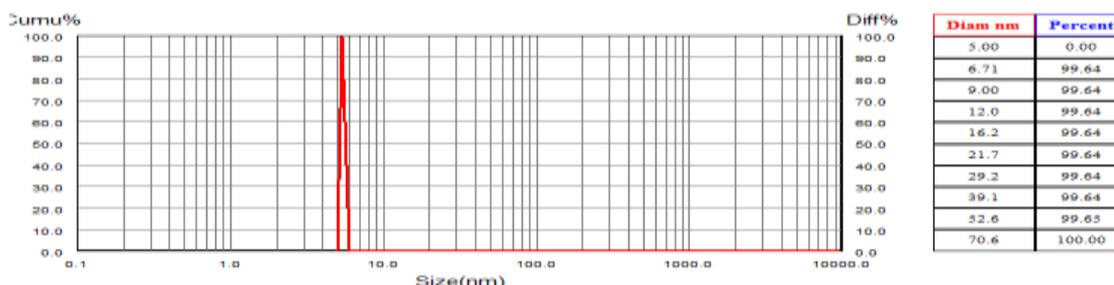

Figure (9) The particle size distribution for NF1.

Atomic force microscopy (AFM) analysis

The outcome of AFM indicate that the optimized formula (NF1) had nanosized particle nearly spherical in shape and smooth surface globules, this indicates stability of optimized nanoemulgel (NF1) as shown in figure (10).
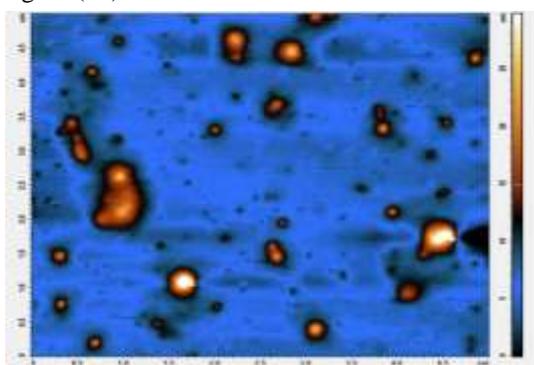

Figure (10) The AFM image of NF1 where scanning area is 5 μm * 5 μm.

Conclusion

The aqueous phase titration method is a low energy emulsification method, easy and cheap that used in the preparation of nanoemulsion that made a nanoemulgel.

The prepared meloxicam nanoemulgel formulations have a greater release rate when compared to meloxicam gel that indicate it is better than meloxicam gel.

From the study of AFM, the selected formula (NF1) with its nanoscale droplet has excellent stability that makes it promising formula to local and systemic delivery of meloxicam that lead to avoid side effect that associated with oral administration.

The meloxicam nanoemulgel (NF1) with its attractive physical appearance and higher in vitro release rate enhance bioavailability that lead to increase therapeutic activity of meloxicam and improve patient compliance.

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