*Research article***Extemporaneous formulation and physical stability assessment of piroxicam loaded virgin coconut oil based creamy emulsions**

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Abstract

The aim of this study was to formulate, optimize and evaluate stability of piroxicam loaded virgin coconut oil based nano emulsion with Tween 80® as the surfactant. Piroxicam is a non steroidal anti inflammatory agent with analgesics and antipyretic effect, used for the treatment of rheumatoid arthritis and osteoarthritis. However, it has been associated with gastrointestinal side effects. It is possible to minimize these problems by developing drug carriers to prevent direct contact of drug with gastric mucosa or by topical administration of drug. Ternary phase diagrams for mixtures of Virgin Coconut Oil (VCO), Tween 80® and distilled water were constructed using water titration method previously. Based on those results, appropriate ratios of oil and surfactant which resemble creamy emulsion (O/W) were selected. Nanoemulsions were formulated using both spontaneous and homogenization methods. Stability studies were conducted using visual observation and centrifugation method. Piroxicam was incorporated with most stable sample and studied for characterization of drug loaded formulations. All emulsions were oil in water emulsions. Best formulae was piroxicam 2% (w/w), oil 25.72% (w/w), surfactant 33.24 % (w/w) and water 39.04 % (w/w) .

Key words: Piroxicam, Cream; Virgin coconut oil.

Introduction

Emulsion is a thermodynamically unstable biphasic system consisting of two immiscible

liquids; one phase of which (the dispersed phase) is finely and uniformly dispersed as globules throughout the second phase (the continuous phase), stabilized by presence of emulsifying agent.¹ Emulsions are widely used in food, pharmaceutical, agrochemical, paint and oil industries.² Emulsions are used to make medicines more palatable, to improve effectiveness by controlling dosage of active ingredients, and to provide improved aesthetics for topical drugs such as ointments. Nanoemulsions are type of emulsions with very small and uniform droplets size, in the range of 20-500 nm.³ Nanoemulsions appear transparent or translucent to the naked eye and have stability against sedimentation and creaming due to their small droplet size.⁴ Nanoemulsions have become increasingly important as potential vehicles for controlled delivery of cosmetics and for optimized dispersion of active ingredients in particular skin layers.

The popularity of nanoemulsions for application in personal care, cosmetics as well as in healthcare is due to the following advantages; the very small droplet size causes a large reduction in the gravity force and the brownian motion maybe sufficient for overcoming gravity, therefore no creaming or sedimentation occurs on storage. The small droplet size also prevents any flocculation of droplets and their coalescence, since these droplets are non- deformable surface fluctuations are prevented. In addition, relative to droplet radius, the significant surfactant film thickness prevents any thinning or disruption of the liquid film between the droplets. The small size of the droplets allows them to deposit uniformly on skin. Nanoemulsions can penetrate through the 'rough' skin surface and

this enhances penetration of activities due to their small size.^{5,6} Nanoemulsions can be prepared using lower surfactant concentration. Nanoemulsions are easily used in skin care due to their good sensorial properties and biophysical properties.^{5,6,7}

Piroxicam is a potent non-steroid anti-inflammatory compound with analgesic and antipyretic effects, used for the treatment of rheumatoid arthritis, osteoarthritis, and traumatic contusions.^{8,9} However, it has been associated with gastrointestinal side effects such as upper abdominal pain and ulceration of the gastro-intestinal mucosa and side effects of other systems such as headache, dizziness.^{8,9,10} It is possible to minimize these problems by developing drug carriers that prevent direct contact of drug with gastric mucosa or that allow topical administration of drug.^{8,9} Also piroxicam has a number of undesirable physicochemical properties including its poor solubility in water. Various strategies have been used to overcome the problems arising from its poor aqueous solubility and to improve bioavailability. Piroxicam loaded emulsion is an alternative method to overcome this poor solubility.¹⁰ Some of the many advantages of using piroxicam as an emulsion includes, avoidance of stomach upset, ulcers and bleeding.

Virgin Coconut Oil (VCO) is used as continues phase to prepare the nanoemulsion in this study due to various health benefits and availability of virgin coconut oil in abundance. Coconut oil is produced by either dry or wet processes.^{11,12,13} In the dry process, coconut oil is produced by expelling dry copra and in this process; oil is exposed to high temperatures during refining. Virgin coconut Oil (VCO) is obtained from fresh and mature coconut without thermal treatment and any refining. Traditionally VCO is obtained by a fermentation method, where the white endosperm of freshly harvested coconuts is disintegrated, followed by for 24–36 h at ambient temperature. The disadvantages of this method of extraction are low oil recovery and fermented odor in the oil, which masks

the characteristic coconut flavor.¹⁴

VCO is more beneficial than usually obtained copra oil since the mode of extraction retains more biologically active components in the oil such as vitamin E and polyphenols.^{11,12,14} VCO is rich in medium chain fatty acids and contains nearly 48–53% lauric acid, having antibacterial, antifungal and antiviral properties.^{15,16} It also contains a considerable amount of short chain fatty acids. It is used in skin care products to prevent wrinkles and sagging. VCO is also recognized for its quality of strengthening the structure of damaged, devitalized hair. It lubricates and softens the hair root and conditions the scalp.¹⁶

Emulsions are stabilized by adding an emulsifier or emulsifying agents. These agents have both a hydrophilic and a lipophilic part in their chemical structure. All emulsifying agents concentrate at and are adsorbed onto the oil: water interface to provide a protective barrier around the dispersed droplets. In addition to this protective barrier, emulsifiers stabilize the emulsion by reducing the interfacial tension of the system. Some agents enhance stability by imparting a charge on the droplet surface thus reducing the physical contact between the droplets and decreasing the potential for coalescence.

In pre formulation, studies of “water titration method” was adopted from a previous research.¹⁷ In this study, initially five appropriate formulas which resemble O/W creamy emulsion (Table 1) were selected from the pre-formulation studies by overlapping constructed ternary phase diagrams.

Emulsion formulation methods were adopted by studying previously performed research; study of “Formulation and in vitro Evaluation of Ketoprofen in Palm Oil Esters Nano-emulsion for Topical Delivery”³ and emulsion stability evaluation and characterization methods were adopted from a research study performed on cosmetic multiple emulsion system with macadamia nut oil.¹⁸

The study aimed to formulate, and optimize a VCO based piroxicam loaded creamy emulsion containing Tween 80® as the surfactant and to analyse the stability of optimized formulae with and without incorporation of piroxicam (with and without high sheer homogenization).

Methods

Chemicals and reagents

Tween 80® was obtained from Analytical Instruments (Pvt) Ltd. and Virgin Coconut Oil (VCO) was obtained from Serendipol (Pvt) Ltd. Piroxicam was purchased from Astron Pharmaceutical Pvt. Ltd. Distilled water was used as the external phase. Preparation of emulsion

Method for preparing emulsions were adopted from a published report of Sakeena *et al.*, 2010. Five appropriate formulas which resemble Oil in Water (O/W) creamy emulsions were selected from the pre- formulation studies and 10g of emulsions were formulated using selected ratios of VCO: water: Tween 80® using 2 methods.

Method 1- Magnetic stirrer (Spontaneous emulsification) VCO and Tween 80® were left for 15 min under magnetic stirring 600 revolutions per minute (rpm) (1MLH magnetic stirrer, Rajendra Electrical Industries Limited, Mumbai, India) at 25°C to mix thoroughly. To the resulting mixture, water was added drop by drop while mixing with the aid of a magnetic stirrer at 600 rpm for 5 minutes at 25°C.

Method 2- High sheer homogenization Another set of samples were prepared for each ratio first performing magnetic stirring of VCO and Tween 80® at 600 rpm and 25 °C for 15 minutes and distilled water was added drop by drop with the constant stirring at 600 rpm and 25 °C. The resulting mixture was mixed further under high sheer homogenizer (homogenizer OV5, VELP Scientifica, Italy) at 1200 rpm and 25 °C for 5 minutes.

Stability evaluation

After the optimization of appropriate formulae based on proper oil, water and surfactant ratio, stability was evaluated by visual observation and centrifugation. Stability evaluation and centrifugation tests were done in the laboratory of Pharmaceuticals, Faculty of Allied Health Science (FAHS), University of Peradeniya.

Visual observation

Visual observation was conducted on 1st, 3rd, 5th, 10th, 15th, 30th, 45th, 60th days for phase separation, creaming, sedimentation, flocculation, and coalescence.

Centrifugation

Ten (10) grams of selected stable formulations from phase diagram were placed in the tubes and applied the 1200 'g' centrifugal force by using the centrifuge (C- 854/6, Medico/ Doctor Centrifuge /MiniCentrifuge, Rajendra Electrical Industries Limited, Mumbai, India) for 3 minutes. Stability of the centrifuged formulations was evaluated in 1st, 3rd, 5th, 10th, 15th days.

Drug incorporation and stability evaluation

Piroxicam was incorporated into stable samples obtained via Method 1 and Method 2.

Method 1- Magnetic stirrer (Spontaneous emulsification) VCO and Tween 80® were left for 15 min under magnetic stirring 600 revolutions per minute (rpm) (1MLH magnetic stirrer, Rajendra Electrical Industries Limited, Mumbai, India) at 25°C to mix thoroughly. To the resulting mixture, piroxicam was added. Then distilled water was added drop by drop while mixing with the aid of magnetic stirrer at 600 rpm for 5 minutes at 25°C to the 10 g of 2 (w/w)% piroxicam emulsion.

Method 2- High sheer homogenizer

Another set of samples were prepared for each ratio first performing magnetic stirring of VCO and Tween 80® at 600 rpm and 25 °C for 15 minutes. Piroxicam was added into resulted mixture. Then distilled water was added drop by drop with the constant stirring at 600 rpm and 25 °C to the 10 g of 2 (w/w)%

piroxicam emulsion. The resulting mixture was mixed further under high shear homogenizer (homogenizer OV5, VELP scientifica, Italy) at 1200 rpm and 25 °C for 5 minutes.

Stability was studied through visual observation and centrifugation as described in previously. Visual observation was done for one month 1st, 2nd, 3rd, 5th, 8th, 11th, 14th, 20th, 30th days for phase separation, coalescence and creaming. Centrifugation test was done for 15 days on 1st, 3rd, 5th, 10th, 15th day. Then the most stable drug loaded emulsion was selected and re-formulated via Method 2 (high shear homogenization) for a range of drug concentrations as 1%, 2%, 3% and 4%. Finally, their stabilities were evaluated during 2 weeks on 1st, 2nd, 3rd, 5th, 8th, 11th, 14th days

through visual observation and centrifugation as explained previously.

Results

Evaluation of stability of emulsions without piroxicam

Optimized compositions of the different samples of emulsions tested are given in Table 1. Stability data of visual observation of emulsions formulated via Method 1 and Method 2 are given in Tables 2 and 3.

According to the results given in Table 2, A1 and E1 were unstable at room temperature. However, sample E1 was more stable than A1. Both phase separation and flocculation occurred as instabilities samples A1. Only phase separation occurred in sample E1. B1, C1 and D1 were stable for even 60 days after

Table 1: Emulsions of different compositions after optimization (samples of 10 g)

Sample	VCO (g)	Tween 80®(g)	Water(g)
1	4.860	2.430	2.710
2	2.624	3.392	3.984
3	2.360	3.053	4.587
4	1.771	3.543	4.686
5	4.116	2.232	3.652

VCO, Virgin coconut oil.

Table 2: Stability data of “visual observation” of emulsions formulated via Method 1

Sample	Fresh	3 rd day	5 th day	10 th day	15 th day	30 th day	45 th day	60 th day
A1	P	P	P	P	P,F	P,F	P,F	P,F
B1	S	S	S	S	S	S	S	S
C1	S	S	S	S	S	S	S	S
D1	S	S	S	S	S	S	S	S
E1	S	S	p	P	P	P	P	P

S, Stable; F, Flocculation; P, Phase separation

Table 3: Stability data of “visual observation” of emulsions formulated via Method 2

Sample	Fresh	3 rd day	5 th day	10 th day	15 th day	30 th day	45 th day	60 th day
A2	S	S	S	P	P	P,F	P,F	P,F
B2	S	S	S	S	S	S	S	S
C2	S	S	S	S	S	S	S	S
D2	S	S	S	S	S	S	S	S
E2	S	S	S	S	P	P	P	P

S, Stable; F, Flocculation; P, Phase separation

preparation. In Method 2, samples A2 and E2 were unstable at room temperature but, sample E2 was more stable than A2. Both phase separation and flocculation occurred as instabilities in samples A2. Only phase separation occurred in sample E2. Samples B2, B3 (or C2) and B4 (or D2) were stable for even 60 days after preparation. E2 and A2 samples were more stable than E1 and A1 samples. Stability data of centrifugation of emulsions formulated via Method 1 and Method 2 is given in Tables 4 and 5.

Table 4: Stability data of “centrifugation” of emulsions formulated via Method 1

Sample	1 st day	3 rd day	5 th day	10 th day	15 th day
A1	P	P	P	P	P
B1	S	S	S	S	S
C1	S	S	S	S	S
D1	S	S	S	S	S
E1	S	P	P	P	P

S, Stable; P, Phase separation

Table 5: Stability data of “centrifugation” of emulsions formulated via Method 2

Sample	1 st day	3 rd day	5 th day	10 th day	15 th day
A2	P	P	P	P	P
B2	S	S	S	S	S
C2	S	S	S	S	S
D2	S	S	S	S	S
E2	S	S	P	P	P

S, Stable; P, Phase separation

According to centrifugation data, samples A1 and A2 were unstable even on the date of formulation. E1 and E2 were unstable on the 3rd day and 5th day respectively. Other six samples were stable even after 15 days. Both unstable samples obtained from Method 2 (A2 and E2) were stable for a longer period than unstable samples obtained in Method 1 (A1 and E1) even based on results of centrifugation.

Evaluation of stability of 2 % piroxicam loaded emulsion

Visual observation

Stability data based on visual observation of piroxicam loaded emulsions formulated via Method 1 and Method 2 are given in Tables 6 and 7. All the piroxicam loaded emulsion samples prepared via Method 1 and Method 2 were stable for 14 days upon visual observation.

Table 6: “Visual observation” stability data of piroxicam loaded emulsions formulated via Method 1

Sample name	Fresh	3 rd day	5 th day	10 th day	15 th day	30 th day
B1	S	S	S	S	S	S
C1	S	S	S	P	P	P
D1	S	S	P	P	P	P

S: Stable P: Phase separation

Table 7: “Visual observation” stability data of piroxicam loaded emulsions formulated via Method 2

Sample name	Fresh	3 rd day	5 th day	10 th day	15 th day	30 th day
B2	S	S	S	S	S	S
C2	S	S	S	S	P	P
D2	S	S	S	P	P	P

S, Stable; P, Phase separation

Centrifugation

Centrifugation stability data of 2 % piroxicam loaded emulsions formulated via Method 1 and Method 2 is given in Tables 8 and 9. C1, C2, D1 and D2 centrifuged samples were stable until 15 days, then they become unstable since 15th day. Therefore these samples were removed as unstable samples and B1 and B2 samples were selected as most stable samples.

In this study, centrifugation studies are conducted as accelerated stability studies. Results revealed that piroxicam loaded samples are more stable in Method 2 than in Method 1.

Study on the effect of drug concentration
Stability data of visual observation and centrifugation of piroxicam emulsions in different concentrations formulated via Method

2 is given in Table 10.

Table 8:“Centrifugation” stability data of 2% piroxicam emulsions formulated via Method 1

Sample name	Fresh	3 rd day	5 th day	10 th day	15 th day	30 th day
B1	S	S	S	S	S	S
C1	S	S	S	S	S	P
D1	S	S	S	S	P	P

S, Stable; P, Phase separation

In both visual observation studies and centrifugation stability studies; comparative

stability data on samples with 1%, 2 %, 3% and 4 % of piroxicam were stable for 14 days

Table 9:“Centrifugation” stability data of 2% piroxicam emulsions formulated via Method 2

Sample name	Fresh	3 rd day	5 th day	10 th day	15 th day	30 th day
B2	S	S	S	S	S	S
C2	S	S	S	S	S	P
D2	S	S	S	S	S	P

S, Stable; P, Phase separation

Table 10: Stability data of visual observation and centrifugation of piroxicam emulsions

Table 10: Stability data of visual observation and centrifugation of piroxicam emulsions																
Piroxicam concentration	Number of days															
	1		2		3		4		5		8		11		14	
	V	C	V	C	V	C	V	C	V	C	V	C	V	C	V	C
1 %	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2 %	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3 %	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4 %	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

V, Visual; C, Centrifugation

Discussion

The popularity of nanoemulsions for application in personal care and cosmetics as well as in healthcare is due to the following advantages; the very small droplet size causes a large reduction in the gravity force and the brownian motion maybe sufficient for overcoming gravity, therefore no creaming or sedimentation occurs on storage. The small droplet size also prevents any flocculation of the droplets and their coalescence, since these droplets are non-deformable and hence surface fluctuations are prevented.^{5,6}

The important principle for selection of components is their pharmaceutical acceptability. The solubility of the piroxicam in oils is most important, as the ability of the nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase.¹⁹

Delivering piroxicam through skin is an effective strategy for evading piroxicam related adverse effects in the gastro-intestinal tract as well as improving patient compliance. Virgin Coconut Oil is used as continues phase to prepare the nanoemulsion in this study due to various health benefits and availability of virgin coconut oil.^{11,13} It also contains a considerable amount of short chain fatty acids. It is used in skin care products to prevent wrinkles and sagging.¹⁶ VCO shows anti-inflammatory, analgesic, and antipyretic properties,²⁰ acceleration of wound healing, collagen cross-linking, increase in fibroblast proliferation, neovascularization¹⁴ strengthen skin tissues and repair skin damages,²¹ moisturizing properties,²² anti-wrinkle and anti-sagging properties.¹⁶

Tween 80[®] is used as the surfactant due to its availability and Hydrophile-Lypophile Balance (HLB) value. Tween 80[®] has a HLB value of 15.0. Surfactants exhibiting an HLB between 9 and 16 are most preferable to

produce O/W emulsions (termed O/W emulsifying agents).²³

Stability data of creamy emulsion without drug were shown in the results. Stability of the system is a great concern for the emulsions. Stabilities of the emulsion depend on the preparation method, apparatus used, oils and surfactant selected and their concentration, the sizes of globules obtained and the temperature use, besides other condition.²⁴

Stability data of A1, A2, E1 and E2 centrifuged samples were unstable within 15 days of data collected period. It was due to high percentage of virgin coconut oil and low percentage of Tween 80®. After the stability assessment period, samples B, C and D were stable formulations. No complete phase separation was seen in the centrifuged B, C and D formulations.

There were no any physical changes observed in B, C and D formulations up to two months. It was due to low concentration of virgin coconut oil.

The most common method to increase emulsion destabilization kinetics is by utilizing a centrifuge to accelerate the gravitational separation of dispersed phase particles. By increasing or decreasing the number of rpm and thus the strength of the centrifugal field the kinetics of the emulsion breakdown can be changed.

After the stability evaluation period of drug loaded samples, B is the best formulae from method 1 and method 2 which is consisting with piroxicam 2% (w/w), oil 25.72 % (w/w), surfactant 33.24 % (w/w) and water 39.04 % (w/w). It was due to low percentage of water relative to other two samples. C and D centrifuged samples from method 2 remained as stable up to 15 days from the preparation date. Those samples become unstable due to phase separation thereafter. It was due to high percentage of water content in C and D samples compared to sample B.

Conclusion

Piroxicam loaded nanoemulsion formulations were successfully prepared by the spontaneous emulsification method (titration method) and high shear homogenization method. High shear homogenization leads to substantial reduction of the particle size and increase the stability of the formulation comparing to the conventional emulsion preparation method. The best formulae consisted of piroxicam 2% (w/w), oil 25.72 % (w/w), surfactant 33.24 % (w/w) and water 39.04 % (w/w). Piroxicam loaded, virgin coconut oil based nanoemulsion was physically stable for one month.

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