Covenant Journal of Physical & Life Sciences (CJPL) Vol. 5 No. 2, Dec 2017

An Open Access Journal available online

Development of oil-in-water (o/w) nanoemulsion formulations for spontaneous transdermal delivery of ciprofloxacin

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***Abstract*:** Nanoemulsions have attracted attention in delivery of therapeutically active agents since most of the new chemical entities are hydrophobic in nature and the delivery of poor water soluble drugs is a challenge. This study was carried out to adopt nanoemulsion as a means of entrapping ciprofloxacin in the oil phase of the emulsion for transdermal drug delivery. Nanoemulsions were formulated as oil in water (O/W) type and prepared by self-mild mechanical nanoemulsification method. The formulation consisted of Sandbox (*Huracrepitan)* and Sesame seed (*Sesamumindicum*) as the organic phase of the emulsion, Polyethylene (20) sorbitanmonooleate (Tween 80) and Polyethylene (20) sorbitanmonolaurate (Tween 20) as the surfactants and Polyehtylene glycol (PEG 400) as co-surfactant. The formulations were tested and characterized. Ciprofloxacin (0.075 g) was incorporated into the oil phase of the most stable nanoemulsion formulation prior emulsification and tested on *Escherichia coli*. Transdermal application was done on male Wister rats (R) followed by biopsification. The result showed the zones of inhibition of HCa3+Ciprofloxacin (Ciprofloxacin-loaded, *Huracrpitan* oil based nanoemulsion) and SSA3+Ciprofloxacin (Ciprofloxacin-loaded, Sesame oil based nanoemulsion) to be 26.00 and 25.00 mm respectively. The HPLC results showed that, out of 75000 µg of ciprofloxacin loaded in the oil phases of HCa3 and SSA3 formulations, 6.0076 (R2), 0.4112 (R3) and 6.7241 µg (R6) were absorbed in HCa3 while 1.9519 (R1), 1.2631 (R4) and 2.1801 µg (R5) were absorbed in SSA3. The SEM images revealed an encapsulation with globule size diameter of 94 and 63 nm respectively. The findings of this work showed that sandbox and Sesame seedoil based nanoemulsions are effective for transdermal drug delivery.

Keywords: Nanoemulsion, Transdermal, Hydrophobic, Emulsification, Biopsification.

Introduction

The use of drugs or vaccines to fight disease-causing microorganisms can be dated back to the existence of man on planet earth. However, the mode and efficiency of drug delivery has undergone series of modifications over the years in order to surmount the barrier of poor bioavailability owning to poor solubility of drugs. Thereby increasing and improving the therapeutic abilities of drugs by providing alternative routes such as the nose, skin, eyes, and ears, through which the drugs can be delivered more effectively and efficiently into the body of living organism. This research work is centered on the use of colloids (oil-in-water nanoemulsion) as a means through which drugs can be delivered transdermally into the body system and as well retain its potency even in the oil phase of the colloid. As such, nanoemulsions; a thermodynamically metastable (long lived) non-homogenous (comprising of O/W) liquid phase [1] have been proposed as a delivery system in nanomedicine which is the application of technologies on a scale of 1 to 600 nm to diagnose and treat diseases [2,3] capable of controlling the release of functional ingredients [4] like tamoxifen citerate [5] and glycyrrhizin [6] through skin [7] owing to their large surface area to volume ratio. Transdermal drug delivery may offer an alternative for efficient delivery of drugs [8, 9] because it avoids the problem of gastrointestinal intolerance, avoid first pass liver metabolism and eliminate the need for intravenious access [10].

Materials and methods

Analytical grade Ciprofloxacin powder was obtained from FIDSON Pharmerceuticals, Polyethylene (20) sorbitanmonooleate (Tween 80), Polyethylene (20) sorbitanmonolaurate (Tween 20) and Polyehtylene glycol (PEG 400) were purchased from Evergreen Chemical industry, Idumota Lagos. Petroleum Ether and Chloroform were purchased from Leo Chemicals, Abeokuta, Ogun State, Strain of *Escherichia coli* was also purchased. Distilled deionized water was prepared at Department of Chemistry, Federal University of Agriculture Abeokuta, Ogun State.

Extraction of oil: A 100 g dry milled *Huracrepitan* seeds was weighed on an analytical balance (KERN ABS 220-NM) and the seeds were poured into the column of a Soxhlet extractor; petroleum ether (40 - 60ºC) was poured into the seed from the top of the column. The apparatus was set up by connecting the condenser to the top of the extractor while distillation flask was connected to the lower base of the set-up. The set-up was then placed on a heating mantle and heated. The vapour moved to the top of the extractor where it percolated into the sample and extracted the oil by simple solubility process. This process was allowed to continue for 4 hours for complete extraction of oil from the seeds and the extracted and processed Sesame seed oil was provided by research team using Piteba Extractor (Mechanical device)

Preparation of Nanoemulsion Formulation: The preparation was carried out based on the modification of the work done by Tri Suciati and co-workers [3].

The formulation was optimized in two (2) steps.

1 Optimization of total amount of surfactant and co-surfactant at a fixed ratio (1:1).
2 Optimization of total amount of surfactant and co-surfactant at varying ratio (1:2, 1:3, 3:1 and 2:1).

Subsequently, the emulsification was carried out with distilled de-ionised water at a constant value of the oils, 3 % wt/wt. All measurements were carried out in %wt/wt (grams) as shown in Tables 1, 2, 3 and 4.

Table 1: Formulation of surfactant and co-surfactant (T80) at constant ratio

Materials	Amount of material (%wt/wt)			
Oil	3	3	3	3
Tween 80	10	12	14	15
PEG 400	10	12	14	15
Water	77	73	69	67

Table 2: Formulation of surfactant and co-surfactant (T80) at varying ratio

Materials	Amount of material (%wt/wt)			
Oil	3	3	3	3
Tween 80	8	6	18	16
PEG 400	16	18	6	8
Water	73	73	73	73

Table 3: Formulation of surfactant and co-surfactant (T20) at constant ratio

Materials	Amount of material (%wt/wt)			
Oil	3	3	3	3
Tween 20	10	12	14	15
PEG 400	10	12	14	15
Water	77	73	69	67

Table 4: Formulation of surfactant and co-surfactant (T20) at varying ratio

Materials	Amount of material (%wt/wt)			
Oil	3	3	3	3
Tween 20	8	6	18	16
PEG 400	16	18	6	8
Water	73	73	73	73

Emulsification: The emulsification processes were carried out according the ratios given in Tables 1 – 4. (*Huracrepitan* and Sesame seed oils (3 g, since the value of the oil is constant) was weighed into a beaker, surfactant and co-surfactant were also added, the mixture was stirred gently using a magnetic stirrer (Faithful Huanghua SH-4C) at 400 rpm before 73 g weight of water was added gently, and the mixture was stirred continuously at about 800 rpm for 50 minutes.

Physical appearance test: The test was carried out to determine the physical properties of the emulsion such as colour changes, clarity and cloudiness.

pH test: The pH of the emulsion after emulsification was determined to know the level of acidity or basicity using a digital pH meter, the values obtained was recorded.

Stability test: This test was carried out immediately after emulsification to observe the separate stability of the emulsion at room temperature over time and note if some will be thermodynamically unstable.

Viscosity index measurement: Viscosities of the stable and unstable emulsion were measured (Brookfield DV-E viscometer, spindle size 06, 500 rpm) at room temperature. The viscosity indexes ($Cpx10^3$) and Torque value (%) were recorded.

Freeze-Thaw test: This test was carried out on some selected formulation with Torque value between 1 and 20%, moderate viscosity index value with a good visual appearance that falls on the category of stable emulsion by observing the stability of the emulsions after each circle for 4 cycles. To complete a circle, the emulsion was kept in a freezer for 24 hours and subsequently placed on the shelf at room temperature for 24 hours. It takes 2 days to complete a circle.

Drug incorporation: In brief, the drug was dissolved in a mixture of surfactant and co-surfactant in the oil phase by magnetic stirrer for 5 minutes. The spontaneous nanoemulsion was prepared as mentioned previously by adding deionized water with gentle stirring.

Drug potency test: This test was done in order to determine the potency of the antibiotics (ciprofloxacin) in the oil phase of the emulsion on *Escherichia coli* using agar well diffusion method [10].Microbial growth was determined by measuring the diameter of zone of inhibition. Control plate was set up in similar manner without the test isolate.

Morphology characterization: The emulsion morphology (size, shape etc.) was determined using a scanning electron microscope (SEM) using a cryofixation method. This was done on *Huracrepitan* and Sesame oil based formulation before incorporating the drug and after the drug has been incorporated into the emulsion to determine the globule diameter of the dispersed phase of the nanoemulsion and also to verify the effects of the drug on the morphology of the emulsion.

Transdermal application: This is the application of the 2 mL drug-loaded O/W nanoemulsion formulations on the scraped portion of skin of a male Wister albino rats for 3 days followed by biopsification.

High Performance Liquid Chromatography (HPLC) analysis: High performance liquid chromatographic analysis was done witAgilent 1260 infinity liquid chromatographic system (Agilent Technologies, Santa Clara, USA) fitted with variable (200-800 nm) Ultraviolet-visible detector and a quartinery pump. The column was Hypersil ODS (C_{18}) 3.5 µm, 4.6 x 100 mm reversed phase stainless steel type (Agilent Technologies, Santa Clara, USA) in order to determine majorly the presence and the concentration of the drug absorbed into the rat skin.

Results and Discussion

Huracrepitan oil, a brown-coloured oil, was extracted from its seed using Sohxlet apparatus, the oil was suitable for this work because it contains short chain polyunsaturtaed fatty acid (Carbon content between C12 and C18, which includes linoleic acid, stearic acid, palmitic acid and lauric acid among others [11] thus providing point of anchorage for the surfactant molecule. The oil from the seed was also cyanide free [12] while the yellow-coloured, sweet-smelling Sesame seed oil extract showed a high fatty acid of low molecular short-chain proportion

containing 41 % linoleic acid, 39 % palmitic acid, 5 % stearic acid and 8 % oleic acid and of good medicinal values [13]. However, physical appearance and stability test was carried out to determine the physical properties in relation to the stability of the emulsion such as colour changes, clarity and cloudiness which is shown in Figures 1. The formulation shows a characteristic colour, translucent, slightly creamy to yellowish based on the surfactant used. The physical appearance of the nanoemulsion produced is determined by the colour and the concentration of the surfactant. Tween 20 is slightly yellow while Tween 80 is colourless. It was observed that the formulation produced from Tween 20 was slightly yellowish in colour while the formulation produced from Tween 80 was creamy. The shades of the colour vary with the concentrations of the surfactant [14]. In terms of stability test, the best formulations of the nanomulsions were selected after a month based on their stability on shelf by considering some of the physical properties such as appearance, colour changes and clarity. The formulations that passed the stability test showed it has potentials to serve as a means for spontaneous transdermal drug delivery due to their level of stability [1] and the concentration of the surfactant used [8]. Table 5 showed the formulations and their respective stability test while Figures 1a and b showed stable and unstable formulation respectively showed a stable formulation while Figure 5 showed an unstable formulation.

Table 5: Observed stability of nanoemulsions

S/N	Stable	Mildly Stable	Unstable	Very Unstable
1	SSB3	HCB3	SSB1	HCa1
2	HCB1	SSB4	SSb2	SSa1
3	SSA2	SSb4	HCb1	HCa4
4	HCb3	SSa4	SSb1	HCA2
5	HCa3	SSa3	SSA1	
6	HCB4	SSB2	SSA4	
7	HCb4	SSb3	HCA1	
8	HCB2			
9	SSA3			
10	SSa2			
11	HCa2			
12	HCb2			
13	HCA3			
14	HCA4			

SS* Sesame seed oil based nanoemulsion

HC* *Huracrepitan* oil base nanoemulsion

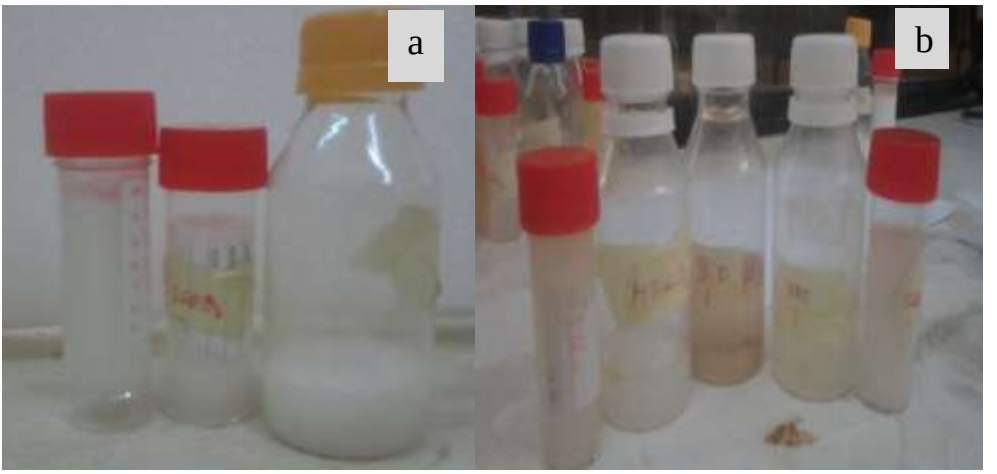


Figure 1: Nanoemulsiom Formulation (a) Stable O/W; (b)Unstable O/W

Furthermore, Viscosity index measurement and pH value are very important parameters while considering the use of oil in water nanoemulsion for transdermal drug delivery. The formulation with low viscosity index value and a pH (Table 6) range between 4.2 and 6.2 is best for this purpose (transdermal delivery), for easy penetration, easy flow and less toxic to the skin [3]. Therefore, formulation with viscosity value between 1.0 and 4.0 Cps x 10^3 and pH value between 4.2 and 6.2 were selected.

Table 6: Viscosity index measurement and pH value

S/N	Formulations	Cps x 10^3	pH
1	HCa2	2.00	4.4
2	HCA3	3.98	4.69
3	HCa3	2.84	4.39
4	SSA2	1.34	4.71
5	HCA4	2.86	3.86
6	SSA3	1.72	4.55

While Freeze-Thaw test result showed that HCa3, SSA3 and HCa2 formulations tends to be stable amongst others. Though minute instability (mild coalescence) was observed but not so obvious compared to others and HCA4 showed a marked level of stability but was not included because of its high level of acidity (3.86) which may cause skin irritation [3, 15] The result shows that the nanoemulsion formulations, retains its stability.

Research has shown that Sesame seed oil has some level of antimicrobial activities. Similarly, *Huracrepitan* oil [12]. To determine if these oils actually retains its antimicrobial activity in the oil phase of the emulsion, drug potency test was carried out in order to determine the antimicrobial activity of the oil in the emulsion (before drug loading) and the potency of the antibiotics (Ciprofloxacin) in the oil phase of the emulsion on *Escherichia coli*.

Appreciable zones of inhibition (ZI)

were observed when the emulsion containing the drug as shown in Figure 6A-E with average zone diameter as follows:

Cp = 27.00 mm
HCa3 + Cp = 26.00 mm
SSA3 + Cp = 25.00 mm.
*Cp: Ciprofloxacin

The result showed that the Sesame seed oil-based (SS) and *Huracrepitan* oil-based (HC) nanoemulsions formulations without the drug does not have any effect on the microorganism (*E. coli)* growth, that is, they do not possess any anti-bacterial activity in the emulsion phase. It can be inferred that the antimicrobial activities of the oils have been masked in the emulsion phase (Figure 2b and d) while formulations with the drug showed a remarkable zone of inhibition (Figure 2c and e) thereby showing that the drug in the oil phase of the nanoemulsions is potent as shown in Figure 2.

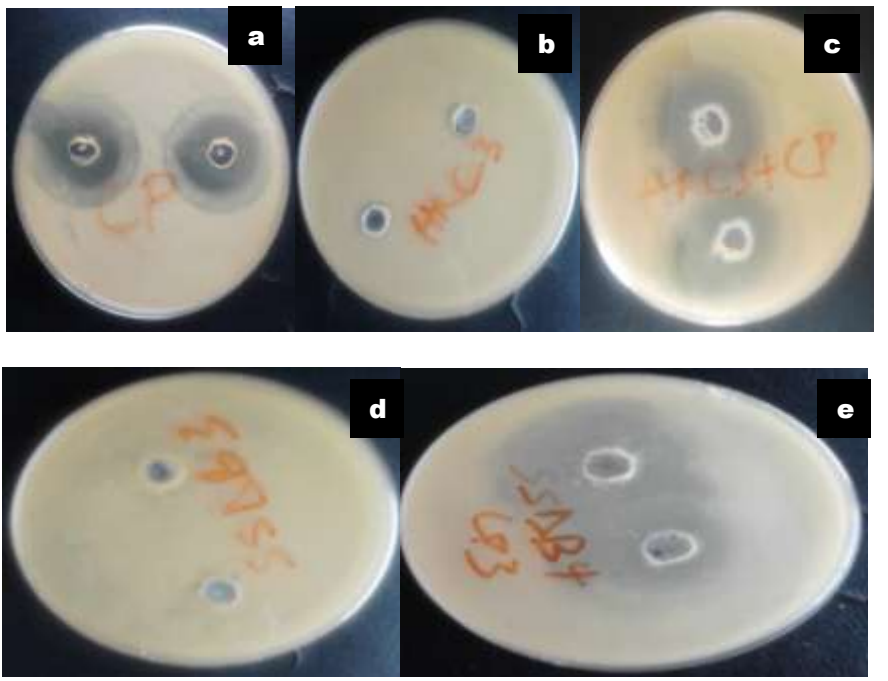


Figure 2: (a) ZI of Ciprofloxacin (Cp); (b) ZI of HCa3; (c) ZI of HCa3+Cp; (d) ZI of SSA3 (e) ZI of SSA3+Cp

Furthermore, the emulsion morphology (size, shape etc.) was determined using a scanning electron microscope (SEM), (TESCAN, USA) on HCa3 and SSA3 emulsion. An image with a good morphological characteristic can be seen in SEM image of samples A and C before and also after drug incorporation, B and D i.e. HCa3 + Cp and SA3 + Cp. This was done to determine the globule characteristic of the nanoemulsion thus verifying the effects of the drug on its morphology and also to know the possibility of entrapment of the antibiotics in the oil phase of the nanoemulsion. The results obtained gave clear information of the globule characteristic of the emulsion formed.

[16] corroborated by [17] reported that nanocapsules suspensions are a colloidal system in their metastable state which helps to confirm that the SEM image of the sample C is actually a colloid. However, there is a clear difference in the morphology of the drug-loaded and the non-drug loaded formulation suggesting that the presence of drug in the oil phase of the drug-loaded nanoemulsion actually has an effect in restructuring the nanoemulsion morphology in which an encapsulation can be seen in the image formed in sample B and D (red-marked region) unlike sample A and C thus showing encapsulation had occurred [18]. The average globule diameter/size of drug loaded nanoemulsion formulation of HCa3 and SSA3 was 94 nm and 63 nm respectively thus confirming the formation of nanoemulsion and thus can be adopted for effective transdermal drug delivery as reported earlier [19, 3].

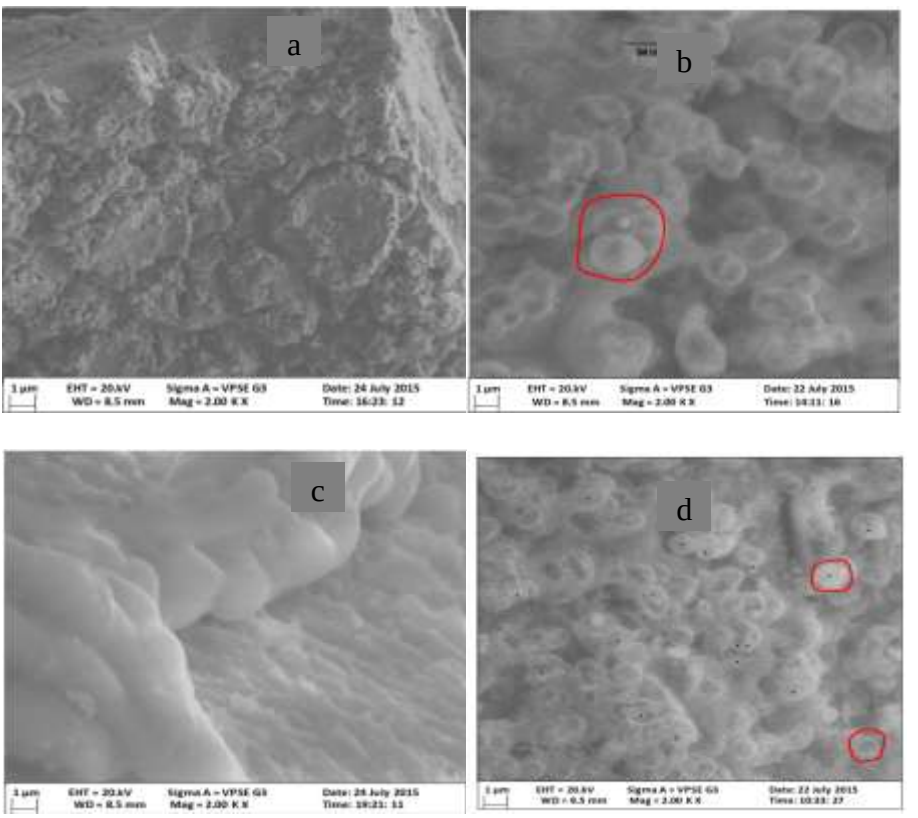
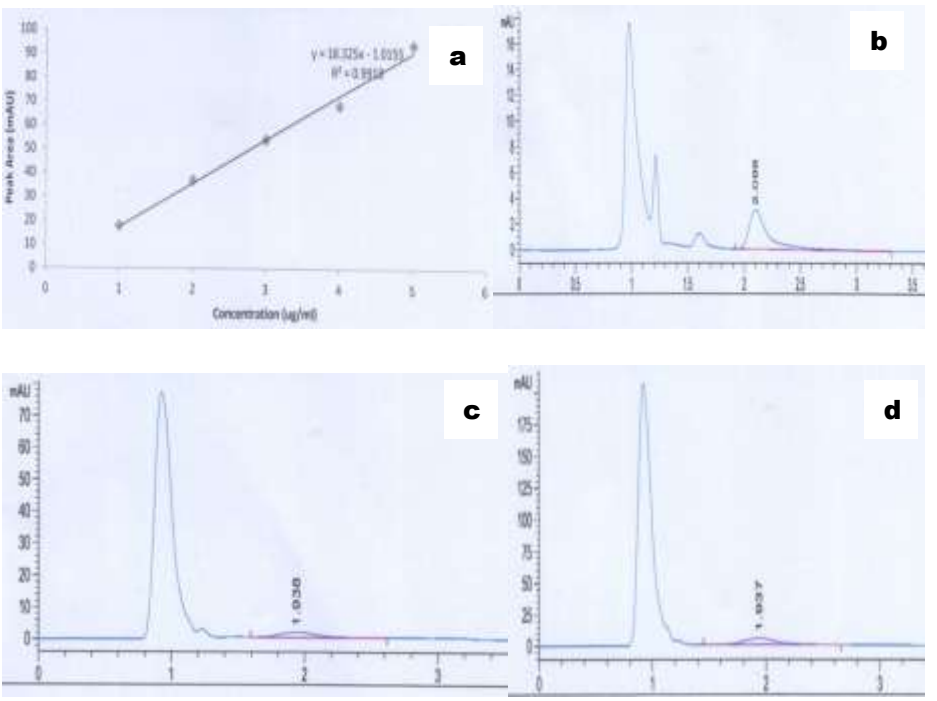


Figure 3: Scanning Electron Microscope image of (a) HCa3; (b) HCa3+Cp; (c) SSA3; (d) SSA3+Cp

The Transdermal application result carried out on rats (R) in which 2 mL/day of the drug loaded nanoemulsion formulations were administered transdermally daily for three (3) days prior sacrificing in which the degree of penetration was determined using High performance liquid chromatography (HPLC) in order to ascertain if actually the drug loaded-in O/W nanoemulsion has actually been absorbed into the rat skin. The Chromatogram in Figure 4a showed the calibration curve of the working

standards of ciprofloxacin and the equation of the curve while Figure 4b showed the chromatogram of ciprofloxacin standard with a prominent peak around 1.5 and 2.5 min (retention time) and as such, the peaks of the samples (Figures 4c - h) analyzed is expected to appear in that range of time which was definitely so. The values of Y_1 and Y_2 are the peak areas of each of the chromatogram which were used to calculate the average peak area for each of the samples. Thus,Mean peak area (Y) = Y_1+ Y_2 / 2**.** The values obtained were used to calculate the value of X which is the concentration (µg/mL) of the absorbed Ciprofloxacin in the sample by using the equation of the linear graph calculated from the plot of the working standard against the peak area of Ciprofloxacin (Figure 4a) viz; Y = 18.325X – 1.015**.**

Thereby substituting the values of the mean peak area (Y) into the equation to give the values of X**.**



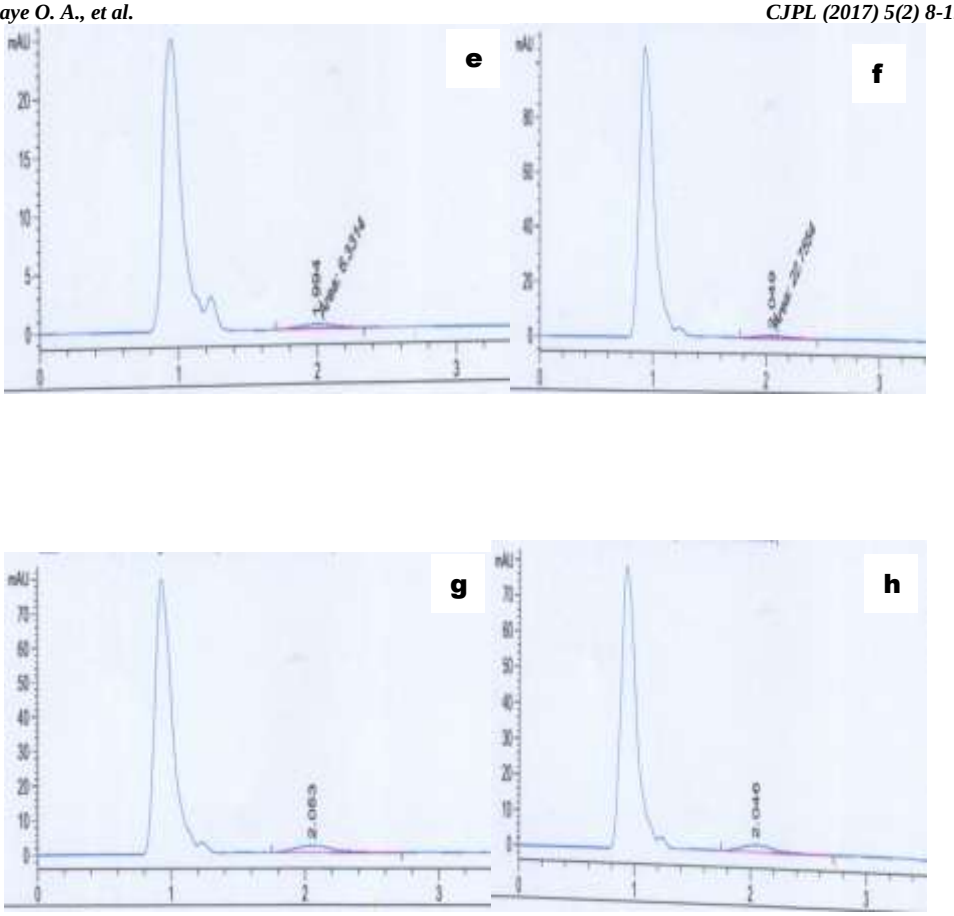


Figure 7: (a) Calibration curve of Ciprofloxacin; (b) Chromatogram of Ciprofloxacin; (c) Chromatogram of Sample 1; (d) Chromatogram of Sample 2; (e) Chromatogram of Sample 3; (f) Chromatogram of Sample 4; (g) Chromatogram of Sample 5; (h) Chromatogram of Sample 6

However, the absorption determination was done by considering the amount (grams) of the drug (Ciprofloxacin) introduced into the oil phase of the nanoemulsion prior emulsification and the results obtained from the HPLC analysis of the rat skin is analyzed as follows; 0.5 g of Ciprofloxacin = 5 g of oil, X g of Ciprofloxacin = 1 g of oil

X g = 0.5 g/5 g = 0.1 g/g.

This means that, 0.1 g of Ciprofloxacin was dissolved in 1 g of oil i.e. 0.1 g/g. Therefore, the values obtained in Table 7 (column X (ug/mL) shows the amount of the drug absorbed into the rat skin out of 0.075 g/g (750000 µg) per gram of *Huracrepitan* oil.

Table 7: The peak area, peak height, average peak area, absorbed concentration and the oils

Samples	Y_1(mAU*s)	Height(mAU)	Y_2(mAU*s)	Y_1+Y_2	$Y_1+Y_2/2$	X(µg/mL)	Oils
R1	35.2453	1.7273	34.2397	69.4850	34.7425	1.9519	SS
R2	109.7163	5.2711	108.4304	218.1468	109.0734	6.0076	HC
R3	6.7072	3.6867	6.3314	13.0386	6.5193	0.4112	HC
R4	21.5044	1.1060	22.7554	44.2598	22.1299	1.2631	SS
R5	39.1694	2.0072	38.7000	77.8694	38.9347	2.1801	SS
R6	122.0358	5.9207	122.3701	244.4060	122.2030	6.7241	HC

Conclusion

The findings showed that the oils especially *Huracrepitan* oil is most suitable for the production of oil-in-water nanoemulsions with Tween 80 and PEG 400 at 1:1 ratio for effective transdermal drug delivery in terms of stability. The Sesame seed oil formulation at ratio 3:1 shows a better degree of penetration which can be accrued to the smaller globule size of the dispersed phase [3, 15].

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