Formulation and characterization of an analgesic ointment indicated for pediatric use

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Abstract

This research work deals with formulation development and characterization of an analgesic pediatric ointment. The ointment was developed with menthol and camphor as active ingredients at lower concentrations than used for an adult formulation. The formulation was novel as it replaced the oleaginous hydrocarbon petroleum jelly base of conventional ointment by olive oil, a more acceptable excipient to the pediatric patients. The developed formulation was compared against iKOOL, a commercial analgesic ointment with similar composition produced by IKOP Sdn. Bhd., IIUM, Malaysia with respect to various quality control tests. The developed formulation showed encouraging results,but needs further investigation to convert it into a commercial product.

Keywords: *Analgesic ointment, Menthol, Camphor, Formulation development.*

Running title: *Analgesic ointment formulation.*

INTRODUCTION

As compared to oral administration, topical administration of analgesic medicine provides some distinct and important advantages. It overcomes gastric irritation and undesirable effects on cognition and level of consciousness associated with its oral administration [1]. Topical administration ensures a more even and continuous application of the dosage for a long period directly to the site of the pain. Topical ointment containing menthol and camphor has been used safely for many decades for analgesic relief of minor aches and pains of muscles and joints [2-4]. Additionally, they are indicated for general relief and suppression of common cold symptoms as cough, sore throat, nasal congestion together with Their natural origin has helped them to be used commercially as safe and efficacious as compared to their synthetic counterparts. Menthol ($C_{10}H_{20}O$; molecular weight, 156; a terpene alcohol) is obtained either from naturally occurring peppermint oil of synthesized by hydrogenation of thymol. In lower concentrations (1% or less), menthol depresses cutaneous sensory receptors, while at higher concentrations (1.25% to 16%), it stimulates sensory receptors and thus it gives its counter-irritant action [5]. Camphor (a terpenoid class of compound) is obtained from the wood of *Cinnamomum Camphora* L. [2]. The analgesic and counter irritant effect of camphor is quite established; however, its pharmacological mechanism is not yet fully understood [6]. In one study, Moqrich et al. [7] found that camphor activates TRPV3, a member of the transient receptor channel that causes excitation and desensitization of sensory nerves. When menthol and camphor are used together as analgesic, they provide synergistic action [8]. In few reports, menthol was found to cause allergic contact dermatitis [9,10] and systemic allergic reactions

[11]. Camphor occurs in in two enantiomer form; natural one is dextrorotatory (D-camphor), while the synthetic one form laevorotatory (L-camphor). As compared to the natural one, synthetic camphor is more toxic orally as claimed by toxicity study in mice [12].

There are many branded products, both as over-the-counter (OTC) and prescription, in the form of ointment, liniment, and cream available in the markets containing the active ingredients like menthol and camphor. However, these commercial products contain high amount of active drugs (5 to 10% menthol and 5% camphor) as well as excipients. As a result, these products are not safe when applied to the pediatric population. Topical therapies, especially, analgesic and counter irritant medications that are widely used for pediatric patients are often misused. Their unique type of skin which is quite immature with respect to barrier property is more susceptible to permeation of drugs as well as excipients as compared to the adult skin; this may often lead to undesirable toxicity [13]. Skin surface area to body weight ratio in infants and children is significantly higher than the adults, which pose a great risk of accumulation of drugs in their body. US Food and Drug Administration (FDA) does not entertain the use of commonly prescribed topical medicines to pediatric patients as around 70% of them are devoid of any pediatric labelling [13].

At this juncture, the objective of the present work was to explore the feasibility of formulation and characterization of an ointment containing a reduced amount of menthol (2% w/w) and camphor (2% w/w) for pediatric patients. An attempt was made to reduce the amount mineral oil as ointment base and replaced it with live oil. This was fundamentally proposed for safety and patient compliance reasons, as olive oil is being historically used to soothe baby skin rashes in addition to its well-known skin moisturizer effect. A analgesic ointment formulation, iKOOL produced by IKOP Sdn Bhd., IIUM, Malaysia was used as reference formulation to compare with the developed formulation.

MATERIALS & METHODS

Materials

Olive oil, menthol crystal, camphor, eucalyptus oil, and peppermint oil were obtained as generous gift from IKOP Sdn., IIUM, Malayisa. For GC analysis, camphor (≥95.50 %), and menthol (≥98.20 %) (Sigma-Aldrich, USA), and petroleum benzine (boiling point 80 - 100 ºC) (Fischer scientific Co., UK) were generously donated by IKOP Sdn Bhd., Malaysia. Analgesic ointment iKOOL used as reference sample was also obtained from IKOP Sdn. Bhd., IIUM, Malaysia. Instrumental facility to prepare the ointment and characterize like water bath, rotational viscometer, centrifuge, stability chamber, gas chromatography etc were also provided by IKOP Sdn. Bhd.

Manufacturing Method

Composition of the ointment formulation is shown in Table 1. Water bath (WNB 8 Memmert GmbH +Co. KG) was adjusted to 75^0 C. Beeswax and olive oil were transferred in a glass beaker placed on that water bath. Beeswax was allowed to melt and mixed with olive oil completely. Then camphor was added to the mixture with consistent stirring. The mixture was cooled down by lowering the bath temperature until 60^0C. Immediately, menthol, eucalyptus oil, and peppermint oil were added to the mixture and allowed to cool down to 28^0C until it forms an ointment with the required consistency.

Table 1: Composition of the analgesic baby ointment.

Material name	Quantity (g/100g)
Olive oil	60 to 73%
Bees wax	17 to 30%
Menthol crystal	2
Camphor	2
Eucalyptus oil	3
Peppermint oil	3
Total	100gm

Optimization of the Ointment Base

In order to optimize the viscosity of the formulation, few experimental trials were carried out with different ratios of beeswax and olive oil as shown in Table 2. iKOOL ointment formulation prepared by IKOP Sdn. Bhd was taken as the reference product to optimize the developed formulation.

Table 2: Various experimental ratios of beeswax and olive oil for analgesic baby ointment formulation.

Number of trials	Ointment base	
	Beeswax % (w/w)	Olive oil % (w/w)
1	30	60
2	25	65
3	20	70
4	15	75
5	17	73

Characterization of the Developed Ointment

Viscosity Measurement

The viscosity of the developed formulation was determined by Brookfield Rotational Viscometer (DV-II+PRO Digital viscometer). A 200 ml test sample was taken in a clean and dry 500 ml beaker and the viscosity of the test sample was determined by using spindle no 6 at speeds of 20,50,100 and 150 r.p.m. During the measurement, the spindle was lowered up to a point where the spindle does not hit the bottom of the beaker. The temperature was kept uniform for all the test samples.

Centrifugation Stressed Test

The stability of the optimized formulated and the reference ointment formulation was evaluated using the forced centrifugation test. This test was carried out as per the method described by Baie & Sheikh [14]. Both the formulations were subjected to speed ranged from (2000 to 14000) rpm for 15 minutes in a centrifuge apparatus (Heraeus Megafuge 8 Centrifuge, Fisher Thermo Scientific, Model No. FB15067) and phase separation of the formulation, if any, was evaluated by visual observation.

Microbial Contamination Test

Developed ointment formulation was tested for both TAMC (total aerobic microbial count) and TYMC (total combined yeast/mould count). An amount of 10 g of the sample was suspended in sodium chloride peptone solution (PH 7). One (1) ml of the prepared previous solution was added to a sterile petri dish. Casein Soya bean digestive agar medium and sabouraud dextrose agar were prepared for cultivation of bacteria and fungi respectively, by pouring them into the prepared petri dishes. Then the petri dishes were incubated at 30^0-35^0 for bacteria and 20^0-25^0 for fungi for five days. One (1) ml of the buffered sodium chloride was used as a negative control.

After the incubation period, colonies were counted using the manual colony counter. Not more than 10^4 for bacteria and not more than 10^2 for fungi per gm /ml was considered as the limit of the microbial contamination test.

Assay for the Developed Formulation for Menthol and Mamphor by Gas Chromatography Both the test and reference formulations were tested for the content of menthol and camphor by gas

chromatography method. According to USP pharmacopeia, the formulation should contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts. A Gas Chromatography (GC) system (Agilent Technologies, 7890B GC System) The test was performed according to the following procedure:

Preparation of Solutions:
Diluent: Petroleum benzene
Blank: 1ml of the diluent was transferred into GC vial after filtration through 0.45 µm filter paper.
Standard stock solution of camphor and menthol: 1 g of camphor was weighted and transferred into a 100ml volumetric flask, containing 60ml of diluent, and after sonication takes place, the volume topped up with the diluent. The same method was adopted for menthol stock preparation using 1g of the menthol material.
Working Standard solution: 1 ml of both camphor and menthol standard stock solution was transferred into 10ml volumetric flask containing 5 ml diluent, mixed well and the volume was topped up, and finally transferred into the GC vials after passing them through a 0.45 µm membrane filter paper
Sample stock solution: 1 g of the ointment sample was transferred into 100ml volumetric flask containing 60ml diluent, then sonicated to ensure sample dissolution, and make up the volume with diluent.
Working sample solution: 1 ml of the sample stock solution is transferred into 10ml volumetric flask containing 5 ml diluent, mixed well and the volume was topped up. Finally, they were transferred into GC vial bypassing a µm 45.0 filter paper.

Chromatographic parameters:
GC detector: Flame ionization detector (FID)
Gas carrier: Helium
Column: VF-WAXms
Length: 30.0 m
Internal diameter: 250.00 µm
Film thickness: 0.25 µm
Mode: Split (Ratio 20:1)
Injection volume: 1µL
Run time: 14 Minute

The content of active ingredient was calculated based on the following equation:

$$\%\ content = \frac{AT}{AS}\ X\ \frac{\text{Standard dilution}}{\text{Test dilution}}\ X\ 100\ X\ P$$

….equation 1

Where, AT: Area of peak response due to analyte content in sample solution

AS: Area of peak response due to analyte content in standard solution

WT: Weight of sample taken

P: % of purity of analyte standard

Stability Study

An accelerated stability study was carried out according to the ICH guidelines at 40^0C/75% RH. Due to time constraint, only one sample of three months was analysed. The formulation was filled in the aluminum collapsible tubes; physical appearance, viscosity, and drug assay were analysed as evaluation parameters.

RESULTS AND DISCUSSION

Several experimental trials were carried out to investigate the best ratio of olive oil and beeswax for the required viscosity of the formulation. The results are shown in Table 3 and Fig. 1 to Fig. 4 based on the highest obtained torque values. It was found that the ratio of 17:73 w/w for beeswax and olive oil was just enough to provide the required viscosity like the reference product. The formulation with this composition of beeswax and olive oil (17:73 w/w) was selected as the optimized

formulation. This selection was based on in its consistency and viscosity resemblance to the commercial iKOOL formulation prepared by IKOP Sdn. Bhd., IIUM, Malaysia. The higher the concentration of beeswax, the higher was the viscosity. The optimized formulation resulted comparable characterization parameters in comparison with the commercial product. Table 4 shows the results of centrifugation stressed test subjected to the optimized test and reference formulation. As such, both the formulations resulted similar type of physical stability profile; they withstand physical separation by centrifugation force exerted at 6000 RPM for 15 minutes, initiate separation at 8000 RPM, but completely separate at 10000 RPM. No bacteria or fungi growth was revealed after microbial contamination test performed on the optimized formulation. 3 months of accelerated stability study at 400°C and 75% RH for three months showed good stability profile for the optimized formulation. The formulation was physical stable without any kind of phase separation. As evident from the GC chromatograms (Fig. 5 and Fig. 6), menthol and camphor were well separated without any kind of interference. Assay results for both active pharmaceutical ingredients after 3 months of accelerated stability study is shown in Table 5. It indicates that both camphor (2.12 %) and menthol (1.96 %) were within the accepted range (±10% of the label claim) of their content percentage (2% w/w).

Table 3: Viscosity measurement results based on the highest obtained torque value.

Number	Ratio (bees wax /Olive oil)	Torque value/ Speed	Viscosity value (cP)
1	(25:65)	93.6/20 r.p.m	46800.00
2	(20:70)	92.3/150 r.p.m	6153.33
3	(17:73)	53.9/150 r.p.m	3593.33
4	(15:75)	61.7/200 r.p.m	3085.00
5	iKOOL	65.6/50 r.p.m	13120.00

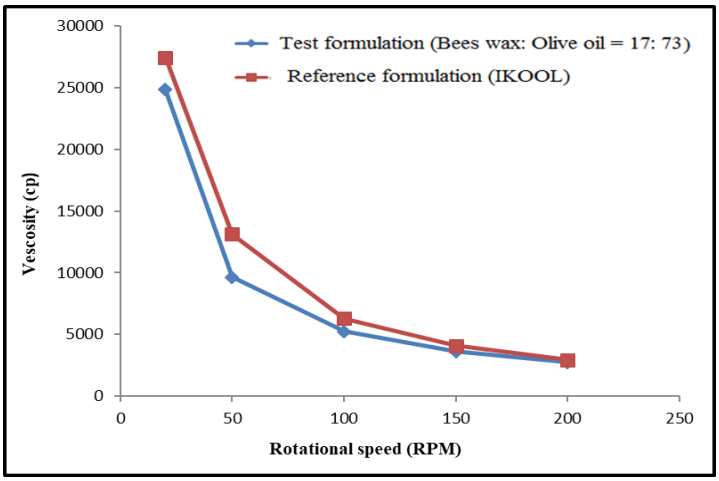

Fig. 1: Viscosity of test formulation (beeswax and olive oil ratio-17:73) and reference formulation (iKOOL).

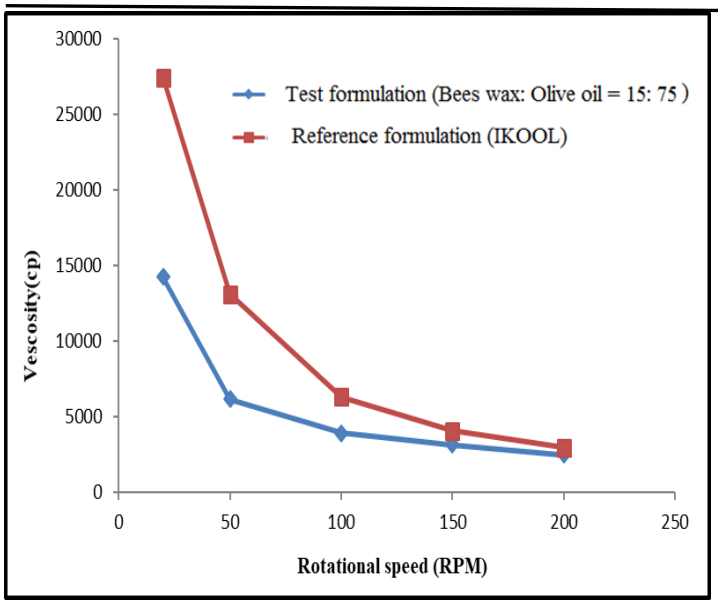

Fig. 2: Viscosity of test formulation (beeswax and olive oil ratio-15:75) and reference formulation (iKOOL).

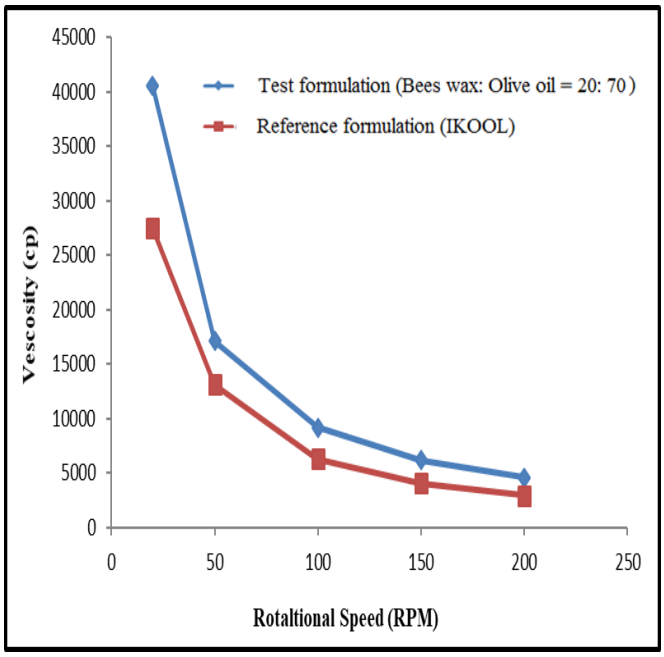

Fig. 3: Viscosity of test formulation (beeswax and olive oil ratio-20:70) and reference formulation (iKOOL).

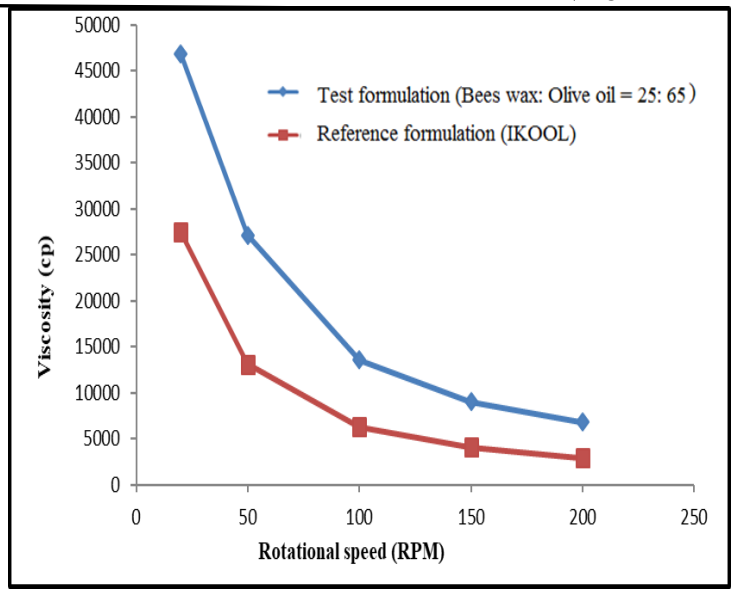

Fig. 4: Viscosity of test ointment (beeswax and olive oil ratio-25:65) and reference product (Original iKOOL)

Table 4: Results of centrifugation stressed test subjected to optimized test and reference formulation

Centrifugation speed	**Observation**	
	Optimized test formulation	**Reference formulation**
2000	No separation	No separation
4000	No separation	No separation
6000	No separation	No separation
8000	Slight separation	Slight separation
10000	separation	Separation
12000	separation	Separation
14000	separation	Separation

Table 5: Assay results for menthol and camphor analytical results in mix standard and optimized test formulation after 3 months of accelerated stability study.

Sample identity	Retention Time (minutes)	Area	Height	Width	Area	Symmetry factor
Menthol in mixed standard	2.756	620.5	773.2	0.0124	47.821	1.132
Menthol in test sample	2.757	642.9	791.1	0.0135	46.748	1.112
Camphor in mixed standard	3.213	677.1	788.7	0.013	52.179	1.192
Camphor in test sample	3.214	732.3	835.2	0.0142	53.252	1.193

subjected to 3 months accelerated stability study.

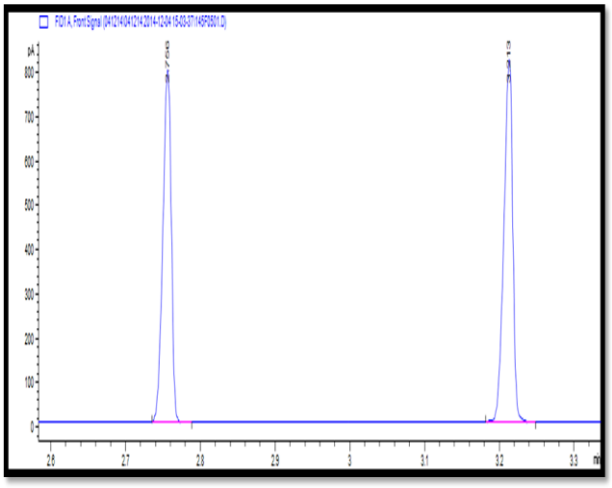

Fig. 5: Representative GC chromatograms showing the separation of menthol (2.758 minutes) and camphor (3.213 minutes) in mix standard.

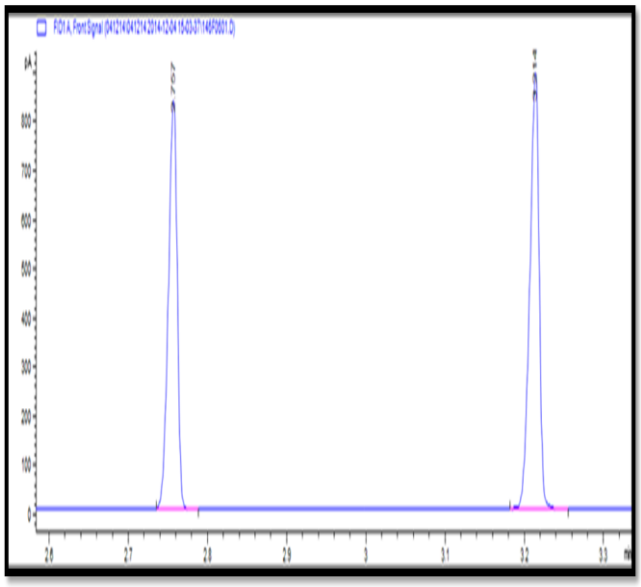

Fig. 6: Representative GC chromatograms showing the separation of menthol (2.757 minutes) and camphor (3.214 minutes) in test sample

CONCLUSION

An optimized analgesic ointment formulation with a composition of 2% menthol and 2% camphor together with beeswax and olive oil mixture (17: 73 w/w) as a base was developed for pediatric patients. The various evaluation parameters carried out to assess the quality of the developed pediatric ointment formulation and they showed satisfactory results when compared with the reference iKOOL ointment prepared by IKOP Sdn. Bhd, IIUM, Malaysia. The viscosity of the optimized formulation was also satisfactory when compared with the reference product. Moreover, the assay results for both the active ingredients after a period of 3 months accelerated stability study was within the accepted range for the active pharmaceutical ingredients. The developed analgesic pediatric ointment containing menthol and camphor may be further investigated to commercialize in future as a

replacement of the conventional analgesic ointments available in the market.

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