COMPARATIVE STUDY OF CHARACTERISTICS OF EMULSIONS

LIVER OIL PREɾARED WITH VARIOUS EMULSIFYING AGENTS

Submitted By:

jjxIKORE, SOLOMON KARANJA

UNDER THE SUPERVISION OF

MR. D. S. KARANJA

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF THE BACHELOR OF PHARMACY DEGREE

DEPARTMENT OF PHARMACY

FACULTY OF MEDICINE

UNIVERSITY OF NAIROBI

NAIROBI KENYA.

D £ D I C A T I O N

Mom and Dad and their family to which I belong.

A C K N O W L E D G E M E N T S

TO MR. D. S. KARaNJA

Head of pharmaceutics Division,

Department of pharmacy, University of Nairobi.

His consistent evaluation of this project and dedicated scrutiny of the work after completion was a source of encouragement and hope. His contribution to the whole project is invaluable as a project supervisor.

To the Technical staff of pharmaceutics, Mr. Ben Kinai especially, his readiness to cooperate in times of need was very helpful. Not to mention the willingness of Mr. Njoroge, the chief technician to assist with the reagents and materials.

To Janerose Njoki for her dedicated secretarial work of typing this manuscript. To you I say thank you very much.

CONTENTS Page

A B S T R A C T

The characteristics of emulsions were studied on a comparative basis , Acacia, tragacanth, sodium Carboxymethyl cellulose, Polyoxyethylene Sorbitan monooleate and sorbitan monolaurate were used as emulgents to prepare the emulsions of Cod liver oil.

Phase separation, colour changes, behaviour of emulsion at elevated temperature, the content of vitamin **A** and microscopic observations were recorded.

Observations revealed unstable emulsions with Sodium Carboxymethyl cellulose, and relatively stable emulsions with £r*gacanthJ and acacia and sorbitan monolaurate with polyoxyethylene sorbitan monolaurate.

INTRODUCTION AND LITERATURE SURVEY

An emulsion is a comparatively unstable preparation consisting of two immiscible liquids one of which is dispersed as small globules in the other. Emulsifying agents are added to stabilise the globules and prevent their coalescene. An emulsion in which the oil is dispersed as globules in the water phase is an oil - in - water emulsion. One where water is dispersed in oil a water-in-oil emulsion.

Emulsions are used pharmaceutically for external and internal preparations. Externally they are applied topically on the skin and mucous membranes while internally administered as oral or parenteral preparations. Orally oil-in-water emulsions are pref erred. The medicinal oil is enveloped in a film of emulsifying agent which helps in masking the disagreeable taste and oily sensation which often accompany oil administration -

Emulsified oils are rapidly assimilated when the particles are sufficiently small (1, 2, 3). This may explain why oils are administered as emulsions.

The stability of emulsions depends on the method of preparation and the emulsifying agent. Creaming and breaking of emulsions occurs in unstable emulsions, occurs in unstable emulsions. This tends to reduce the elegance of the emulsion. In absence of a yield stress, creaming can be controlled according to strokes equation.

$$V = d_{st} X (P - p\hat{}) \times g$$

$_{st}$ = stokes diameter of globule

Velocity of globule

= viscocity

g = acceleration due to gravity

Pi $-P_2$ = density difference

Increasing the viscosity of an emulsion reduces the velocity of separation. This is achieved by use of thicke^ing agents. Reduction in globule diameter helps in emulsions stabilisation and it depends on the method of preparation.

Choice of emulsifiers is geared towards those which produce an emulsion with the desired stability and possess the appropriate flow characteristics. Thermodynamic unsta^iiity differs from consumers definition of stability on the basi$ of- entirely subjective judgement. Acceptable pharmaceutical stability does not require thermodynamic stability. If an emulsion creams or sediments, it may be pharmaceutically acceptable as long as it can be reconstituted by a modest ^mount of shaking to enable dosage measurement. This may be unacceptable to the consumer due to unsightly separation which makes the preparation cosmetically inelegant.

A stable emulsion is one that maintains the same size& of globules of the dispersed phase per unit volume of the continuous phase (4). A list of in vitro requirements for liquid **Preparation** in quality control includes assay of the active ingredient, visual appearance, colour, odour, taste, light stability, contain^ closure compatibility, redispersibility, suspendibility, pourability, viscosity, isotonicity, particle size agglomeration and distribution, clarity, crystallisation and precipitation &as evolution, microbial stability, specific gravity, PH, surface tension, sterility testing and storage conditions (5)* To screen emulsions, simple tests have been suggested (6). These i"ciuides. heating to 50 - 70 degrees celsius and observing gross physical stability of the emulsion visually and turbidometrically• -j^e most stable emulsion to heat being one stable at room temperature> Coalescence test to detect gross difference in emulsion stability

room temperatureo

COD LIVER OIL

This is the destearinated oil obtained from fresh livers of Gadus morrhua and other species of the family Qadidae. It contains in each gram not less than 255 micrograms of vitamin A and 2.125 micrograms of vitamin D. (u.s.p.)

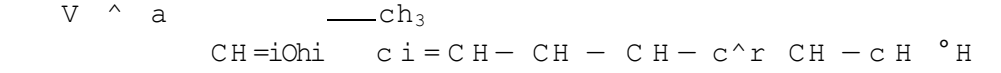
The oil is a thin liquid having a characteristic slightly fishy but not rancid odour, and fishy taste. This necessitates the formulation of Cod liver oil as an oil-in-water emulsion to mask its disagreable taste to infants to whom the oil is usually administered as a source of vitamins.

The oil consists of glycerides of:

- Saturated fatty acids mainly palmitic and myristic acid
- Unsaturated fatty acids most abundant jecclic and therapic acids.
- Lower fatty acids - acetic, butyric and capric acids

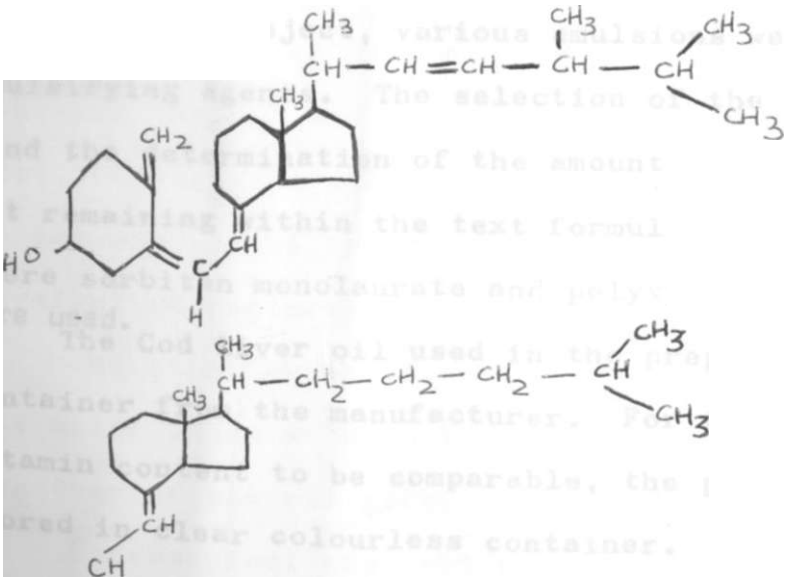
The presence of a wide range of fatty acids makes the preparation when formulated as an emulsion susceptible to oxidation Cn exposure to U.V. light it decomposes by free radical mechanism and saturation of unsaturated fatty acids occurs.

It is a good source of vitamin A, empirical formula of the vitamin 0 and the structural formula of the alcohol is as shown below.



The two stereoisomers A, (retinol) and A^ (3, ^ - dehydroretinol) are present in the oil and are active. Deficiency of the vitamin causes poor dark adaptation due to poor visual purple regeneration. The vitamin possess various unsaturated double bonds. These are susceptible to free radical oxidation. The vitamin is affected by heat light and air. Oxidation is catalysed by traces of metal ions and is the most important degradation process. (7)* Antioxidants are therefore necessary and those which have been added includes alphatocopherol, hydro^uinone, propylgallate. (8). Esters of vitamin A are more stable under unfavourable conditions such as elevated temperatures, exposure to U. V. light and air.

Vitamin D is an ingredient of Cod liver oil. It consists of D,, - (ergosterol) or D^ (7 - dehydrocholesterol). Both are structurally close and are produced from provitamins by energy application in form of Ultra violet light. The structural formula of D2 and D^ are as shown and their molecular formulae are C^g H^ 0 and C^ H^ 0 respectively.



Gnu

7 A so ck blester I

H o

Vitamin D deteriorates due to increased exposure to air **(9).** Stabilisers have been used in preparations of the vitamin eg ethylgallate, butylhydroxytoluene, citraconic acid, citric acid and ascorbic acid, Ethylgallate inhibits the oxidation of the vitamin while citric acid, ascorbic acid and citroconic acid accelerates the inversion of sugars in vitamin syrups.

Cod liver oil therefore contains ingredients susceptible to degradation. The fatty acids and vitamin constituents are degraded by free radical oxidation catalysed by exposure to light and air. Formulation of the oil as an emulsion greatly enhances the process due to increased decomposition by the suspending agents such as acacia which has been shown to contain oxidase (10)•

Cod liver oil emulsion is not cited as a formulation in any recent official compendia. The National Formularly has an emulsion which contains acacia as the suspending agent. Apothecary (1913) page *2k* has an emulsion of Cod liver oil similarly suspended in acacia. b.P.(1953) has the emulsion containing tragacanth and lime. An emulsion containing acacia, tragacanth, Irish moss, glycerrin has been suggested (11).

In this project, various emulsions were prepared using varying emulsifying agents. The selection of the emulgents was random a nd the determination of the amounts to be added was also random but remaining within the text formulations where possible except where sorbitan monolaurate and polyxyethylene sorbitan monooleate were used.

The Cod liver oil used in the preparation was in a colourless container from the manufacturer. For the studies carried out on vitamin content to be comparable, the prepared emulsions were also stored i_n clear colourless container.

There were studies carried out on the emulsions to compare their physical stabilities. Very little literature of basic techniques of comparing emulsions physical stability which could be suitably carried out in the laboratory exists; The procedures adapted for this project were original and some adaptations of other procedures previously carried out elsewhere. Adaptation was necessary to suit the apparatus available in the laboratories of the department.

The various emulsifying agents used to prepare emulsions were acacia, tragacanth, polyoxyethylene sorbitan monooleate, sorbitan monolaurate, sodium carboxymethylcellulose. Polyoxyethylene sorbitan monooleate and sorbitan monolaurate although considered in the experiment have not been shown to be safe enough for wide use internally.

When gum acacia and tragacanth are used together, the acacia acts as the primary emulgent and tragacanth as auxiliary einulgent assisting in raising the viscosity of the emulsion thus acting as a thickening agent.

Emulsions are usually white due to the different refractive indices of the two phases. The emulsions of Cod liver oil contains the oil and water which have differing refractive indices (12). Changes in colour may be used to evaluate the stability of an emulsion,,fast colour changes signifying faster changes in physical characteristics.

The other observation was the separation of the oil phase and the water phase at room temperature. This promotes the coming together of the oil globules hence facilitates further coalescence.

Microsopical observation of the oil globules of the emulsions were recorded. The globule size and characteristics were compared With the °ther physical stability characteristics shown by the emulsion$_0$

The emulsions were centrifuged at room temperature and the results of any separation occuring were recorded.

Any changes occuring to the emulsions on warming at 50 - 52 degrees celcius were also recorded.

An adapted method of analysis of vitamin A content of the emulsion was used (13)« Various methods are available for the determination of vitamin A in pharmaceutical preparations. These methods includes Ultra Violet, chromatographic, coiourimetric, and polarographic methods (1^). The spectrophotometric method of assaying vitamin A has been appraised due to its precision but has the drawback of lack of specificity. The main difficulty being interference by ingredients in the formulation and isomers of vitamin A such as 2 -cis, 2, 6 - dicis and 6 cis which are inactive while only the trans isomer is active (15). This method was adopted in the experiment as the y^S'P also adopts a spectrophotometric method.

MATER1ALS AND REAGENTS

Reagents

Acacia

Tragacanth

Glycerrin

4-methyl hydroxybenzoate

Sodium carboxymethylcelldlose

Polyoxyethylene sorbitan Monoleate. (Tween 80)

Sorbitan monolaurate (span 20)

Cyclohexane

Cod liver oil

Materials and apparatus

Emulsions 1 - V11

Spectronic - 21

Centrifuge

Testtubes

Graduated centrifuge tubes

Clear colourless glass bottles with screw caps

Pipettes

Measuring cylinder

Homogeniser

Testtube shaker

Lsion (D - (7)

COMPOSITION

TA3LS A

INGREDIENTS		QUANTITIES						
!od Liver Oil	(In mis)	50	50	50	50	ko	50	50
ragacanth	(g)				1.5	o		1.5
cacia	(g)		0		12.5	0	12.5	12.5
.ycemm	(ml)	15		0				
ween 80	(g)	0			0	o . • •	0	0
•an 20	(g)							
me	(g)				0	0		
jdium Carboxy methyl cellulose	(g)					2.0	0	
•methyl hydroxy benzoate	(g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
.stilled Water (mis) to		100	100	100	100	100	100	100

PREPARATION OF EMULSIONS

a) EMULSION 1

gms of finely powdered acacia were weighed on a balance and mixed with similarly weighed 1 gm of tragacanth and lime in a dry mortar. The powders were triturated until fine with a dry pestle.

50 mis of cod liver oil was measured using a dry measuring cylinder and poured into the mortar and triturated using a dry pestle. When homogeneous **25** mis of distilled water measured with a measuring cylinder was added all at once and emulsified with trituration. When a thick homogenous emulsion was obtained 15 mis of glycerrin were added and further triturated to disperse thoroughly.

0.1 gms of 4—methyl hydroxyben^ate was weighed accurately and dissolved in 10 mis of water and this was added to the emulsion and stirred. The emulsion was transferred to a final container, and made to the 100 mis mark with distilled water and shaken.

b) EhULSION, 2

6 gms of Tween 80 was weighed and transferred to a dry mortar with 50 mis of cod liver oil.

7 gms of span 20 was weighed and mixed with the Tween 80 in the mortar.

25 mis of distilled water was added and triturated. The emulsion **was transferred to the final container and made to 100 mis** mark with **distilled water some of which had the preservative** dissolved.

EMULSION 3

2.0gms of sodium carboxy methylcellulose was weighed and transferred to a dry mortar, 30mls of hot water was added to produce a mucilage and triturated.

0.1gms of 4-methyldroxy benz.oate was added to the mucilage.

40mls of cod liver oil were added in aliquots of 5mls with trituration to emulsify.

When all the oil was trasfered, the emulsion was made to 100mls by adding purified water.

The emulsion was transferred to a final container and stored at room temperature.

The other emulsions 3* 6 and 7 were prepared as in emulsion 1

Emulsion 4 was passed through a homogeniser after preparation.

INVESTIGATIONS

The emulsions were allowed to stand overnight for 2k hours before any observations or studies were made. This allowed the emulsion components to reach a pseudo equilibrium state (16). After the 2.k hour period studies were done on coalescence, centrifugation, microscopy changes on warming and absorbance at 326 nm«

EXPERIMENTAL:

P H Y S I C A L C H A R A C T E R I S T I C S

(i) COALESCENCE

An emulsion either sediments or creams when oil globules dispersed in water come together to form larger globules depending on the/density of the oil. This means the emulsion is no longer homogenous and this brings the particles closer togetner hence facilitating further coalescence (1?)

The extent to which separation of the two phases occured was recorded at room temperature.

PROCEDURE

5 ml of each of the emulsion was withdrawn using a clean and dry pipette.

i

Each of the emulsion was transferred to a clear and clean dry testtube.

The testtubes were labelled for identification 1 - 7 and kept closed in a locker under laboratory conditions.

The observations made on the emulsions were as recorded in table C.

(ii) CENTRIFUGATION

Despite little evidence to suggest that instability of an emulsion under stress can be related to normal sfielf, life comparative shelf stability can be assessed by such studies. Recording the amount of separation of an emulsion after centrigu^ation was done. (18)

PROCEDURE:

3mls of each of the emulsion was pipetted with a dry pipette into a dry clear graduated tube. The tubes were labelled 1-7.

The tubes were centrifuged using a Labofuge I type of centrifuged using aonstant speed for 5 minutes.

The tubes were removed from the centrifuge and Volume of separation if any was noted by recording off from the grad ation marks.

(iii) MICROSCOPICAL OBSERVATION:

Studies have shown that, changes do occur in emulsion globule size on storage (19). The change is initially rapid but s.'.ows down even in emulsions showing appreciable Coalescence (6)

PROCEDURE:

2mls of each of the emulsion was pipetted from the bottle where stored and transferred onto a petridish. A few grains of Sudan III dye were added and using a glass rod was slowly mixed to disperse the dye. A drop of the coloured emulsion was transferred using the glass rod onto a slide and covered with a coverslip.

Observations were made under the microscope of the characteristics of the globules.

The results were as shown on diagram I

(if) OBSERVATIONS ON WARMING TO 50-52 DEGREES CELSIUS

In an attempt to set up a realistic stability programme to assess the shelf life of an emulsion, it has been suggested (l8) the emulsion should survive heating at a temperature of ^{J}+5-50°C degrees Celsius,

PROCEDURE

The water bath was adjusted with the thermostat to 50°C«

5ml6 of each of the emulsion was pippetted and transferred into a test tube. The test tubes were marked 1-7 and placed in a beaker kept filled with warm water at 50°C in the water bath ahd- supported by clamps.

The times taken for the emulsions to show signs of separation were recorded. The results are as shown in table F.

(v) COLOUR CHANGES ON STORAGE:

To the Consumer a stable emulsion is one that appears "stable" on subjective basis of colour, separation and Viscosity. It was therefore necessary to record Some of the characteristics of the emulsion such as colour- Changes at various times intervals.

The results are as shown in table D.

CHEMICAL ANALYSIS

SPECTRQNIC ANALYSIS OF EMULSION BY USE OF VITAMIN A ABSORPTION PEAK OF 326nm

The fact that vitamin A absorbs in the U.Y range at 326nm and the peak disappears on degradation is a useful fact which can be used to quantitate or compare the amount of vitamin in preparations (13)

In cold liver oil, this was used to compare the absorbances of freshly prepared emulsions and that which had been prepared earlier and stored. Three of the emulsions have subjected to this study. The other four emulsions were omitted after observations previously recorded showed that they had deteriorated in colour and separated. They were therefore phased out from the study.

Wert

PROCEDURE

0.25ml of each of the emulsion was pipetted and transfered to a separate clean dry testtube.

10ml of cyclohexane was added and the mixture was shaken using a testtube shaker at a constant speed for 5 minutes.

The supernatant was allowed to stand for 5 minutes and slowly decanted into a clean spectronic cell and the absorbance was observed at 326nm. Using spectronic -21.

Fresh emulsions of emulsions 2, 6 and 7 were prepared and allowed to stand overnight for 24 hrs after which the same Procedure was followed and absorbance read off at 326nm.

The results were as recorded on table G.

RESULTS

(a) OBSERVATIONS AFTER PRu PARAT I DM

After preparation, all the emulsions were white in colour uith no signs of separation.

On allowing them to stand overnight they uere observed to be similarly white with no signs of separation except preparation 5 which was completely separated,.

Preparation 1 and 3 were viscous and not easily pourable.
Preparation 2, ***k,*** 6 and 7 were easy to pour as seen by tilting the glass container.

(b) TABLE 3

CENTRIFUGE RESULTS

PREPARATION	FRACTION OF DHL SEPARATED		
	2 days	ID days	^•days
1	• .36	***0.1*3***	***Q.U5***
2	•	0.2	• .32
3			D. ^3
5	Separated	Separated	Separated
6	•	0.12	• .2
7	• .1	0.2*4-	D.3

(c)

TABLE C

COALESCENCE AT ROOM TEMPERATURE

	PROPORTION OF OIL SEPARATED		
PREPARATION	2 days	10 days	60 days
1	No separation	0.2	0.3
2	No separation	0.04	0.1
3	No separation	0.06	0.3
k	No separation	0.1	0.2
5	Separated	Separated	Separated
6	No separation	No separation	0.09
7	No separation	0.1	0.15

The values were the ration of thickness of layer of oil and thickness of the emulsions.

TABLE D

OBSERVATIONS ON COLOUR CHANGES ON THE EMULSIONS

	COLOUR OF EMULSION		
PREPARATION	2 days	14 days	60 days
1	White	Cream	Yellow
2	White	White	White
3	White	Cream	Yellow
	White	White	Cream
5	-	-	-
6	White	White	White
7	White	White	White

(e) **TABLE E**

RESULTS OF MICROSCOPICAL OBSERVATION

	GLOBULE SIZES		
PREPARATION	2 days	14 days	60 days
1	Heterodisperse	Heterodisperse	Heterodisperse
2	Fine	Fine	Fine
3	Large	Large	Large
4	Fine	Fine	Fine
5	Separated	Separated	Separated
6	Fine	Fine	Fine
7	Monodisperse Medium	Monodisperse Medium	Monodisperse Medium

Schematic diagram of Globules as seen after 14 days.

DIAGRAM 1

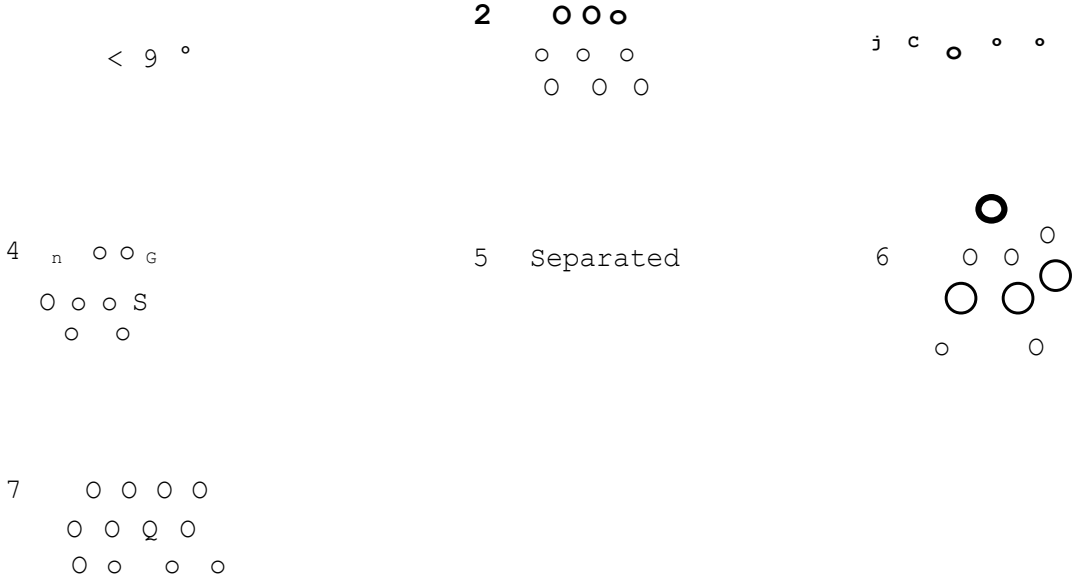


TABLE F

RESULTS OF TINE TAKEN TO SHOW SIGNS

Off SEPARATION AT 5Q-52°C

PREPARATION	TIME TAKEN FOR SIGNS TO BE SEEN		
	2 days	24 days	50 days
1	2 hrs	1.5 hrs	0.67 hrs
2	1.5 hrs	1 hr	0.08 hrs
3	1.67 hrs	0.67 hrs	0o5 hrs
4	1.5 hrs	0.83 hrs	o.25 hrs
	Not Studied	Not Studied	Not Studied
6	0.67 hrs	2 hrs	Not separated
7	1 hr	1 hr	1.67 hrs

(g) TABLE G

THE SPECTRONIC READINGS OF THE EMULSIONS

ABSORBANCE TAKEN AT 526 nm USING SPECTRONIC-21

FRESH EMULSIONS	READINGS			
	1	2	3	Average
2	0.21	0.22	0.23	0.22
6	0.15	0.155	0.15	-.0.16
7	0.15	0.15	0.14	0.15
EMULSION STORED AT ROOM TEMPERATURE FOR 95 DAYS				
2	0.2	0.21	0.21	0.21
6	0.12	0.13	0.13	0.13
7	0.12	0.12	0.11	0.12

DISCUSSION

The separation of preparation 5 on storage at room temperature for two days suggests that Cod liver oil cannot be formulated using sodium carboxy methylcellulose. This emulsion showed such a rapid rate of separation that no other test was performed to assess its physical stability.

Emulsion 1 and 3 were observed to be viscous and not easily poured. This increase in viscosity could have been due to the presence of lime in the emulsion which was absent in the other emulsions. Therefore lime increases the viscosity.

On centrifugation, it was observed that there was an increase in the proprotion of oil separated with storage. The volume of oil separated when an emulsion was centrifuged after two days storage was less than that observed after a kO days storage time. Preparation 1 and 3 showed the greatest fractional separation of the oil phase despite their observed high viscosity. On this basis, the emulsions are less stable than the others on centrifugation.

Except emulsion 5, no separation of emulsion was observed on storage at room temperature for upto 10 days. This suggests that the formulations can be freshly prepared during dispensing and used without showing any signs of separation. On storage for 60 days emulsion 3, 1 and k showed the greatest amount of eparation and therefore can be considered as being the more stable emulsions.

All the emulsions were white on preparation. However emulsion 1 and 3 changed colour to cream in 1k days and in 60 days were yellow together with observed precipitate sediment in preparation k. This showed that this emulsions were forming large globules whose colour was observed since cod liver oil is yellowish in colour.

Microscopically, emulsions 2, k and 6 showed monodisperse globules while 1 and 3 showed polydisperse globules. The emulsion k had been homogenised, its globules were fine and therefore a yield stress existed in the emulsion caused by the shearing action of the hand homogeniser. This may explain why the globules coalesced faster causing colour change to yellow to be faster in this emulsion.

At elev«tecl temperature, emulsions 1, is anu 3 ana 1 showed faster separation while 6 and 7 showed a slower rate of separation after storage. This is as shown in emulsion 6 which showed a separation time of 0.67 hrs after two days storage and did not separate at all after fifty days storage. This result was difficult to explain but it showed a stabilisation of emulsion 6 [a]nd 7 on storage. This suggests a form of equilibriation of this emulsions which is not adversely affected by heating whereby the [r]ate of coalescence of the oil globules was same as the rate of breaking of the large oil droplets due to stress in the globules.

Spectronic observations showed there was a difference in the absorbances of the fresh emulsions and those which had been stored, at 326 nm. There was a decrease in the absorbance on storage. This is consistent with the observation that, on oxidation, vitamin A loses its absorbance at this wavelength. Emulsions 6 and 7 showed a greater difference in tne reading as compared to the difference in reading observed in emulsion 2. This suggests that vitamin A is less rapidly degraded in emulsion stabilised by Tween 80 and span 20 as compared to one of acacia and tragacanth combined. This is due to the presence of oxidase enzyme present in acacia which enhances degradation of the vitamin (20).

The procedures used to assess the properties of the emulsions are not standard procedures. They are experiments based on the principles of the behaviour of emulsions. In 1976, Akers J.M. and Lach J (16) investigated emulsion stability by considering coalescence measurements and globule size analysis. There procedure was adapted in the present study of emulsions in this project. Lachman L suggested that an emulsion should be stable with no signs of separation at 50° Celsius. This suggestion was adopted for experimental procedure in the project.

Since the results obtained were to assist in comparing the various emulsions, they were considered sufficient as it was not Necessary to obtain absolute values.

C O N C L U S I O N

It can be concluded that, sodium carboxy methyl cellulose produced an unstable emulsion as shown by its separation within 24 hours.

Lime increases the viscosity of emulsions 1 and 3*

The rate of degradation of vitamin A was higher in emulsions which contained acacia than that which contained 'Tween 80• and 'span 20•.

After screaming the emulsions, emulsion 2, 6 and 7 were observed to possess a higher stability than the others. However further investigations of the emulsions is necessary before they can be considered to be released as market products.

Reference S

(1) Jones C. M., Culver P. J. Drummey <*• D- and Ryan A- E- Ann. Int. Med. 29: 1 19^8

(2) Shoskes M. et al Proc. Soc.. SxP- 3iol« Med 75i 680, 1950

(3) Shoskes M. et al Ame. Dietet. A 27, 191 1951

(if) Garette E. R. J. pharm. Science 1557 1965

(5) Pernarowski M. 1970 Biopharmaceutics and relevant pharmacokinetics p. 170

(6) Bernstein D. F. et al 1971 J- Phar- Science 60 690

(7) Debodard M et al J. pharm. pharmacol. 3, » 19S1

(8) Macet J. J. Ame. J. pharm 132 ^33 19^0

(9) Nelson E. M. J. Ameri. Med. Ass. 171 1°3 1959

(10) Griffiths et al; Analyst 1933

(11) Spatton L. M. "Pharmaceutical emulsions and emulsifying agents" 2nd Edition Pg 82

(12) Shotton S. "Physical pharmaceutics" Oxford Clarendon press.

(13) Specifications for quality control of pharmaceutical preparations page 5^8.

(l*i) Macet J. T. Ame. J. pharin. 132, ^33 (I960)

(15) Methods of vitamin assay 3*"d edition Freed rayer 1966 page 90

(16) Michael J. K. ; Lach J. J. pharin. Sci• 65 (2) 216 - 22 1976

(17) Remmington Pharmaceutical science pg. 3^7 l^th Edition

(18) Laciiraan L. "Theory and practise of Industrial Pharmacy" page 18^.

(19) Garett E. R. J. pharm. Sci. 5^ 1557 1965