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Formulation and Characterization of Metformin Emulsions Using Locally Sourced Materials

Okoye Godwin Ejike[1], Ngwuluka Ndidi Chinyelu[1], Okafor Ugochukwu Chukwuma[2, *], Ben-Umeh Kenechukwu Chijioke[3]

[1]Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria
[2]Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
[3]Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

Abstract

Over the years, diabetes mellitus has been a disease plaguing the developed nations largely because of their lifestyle and their diet. In recent times, in Nigeria, the incidence and prevalence of diabetes has become of profound concern with about 90% of diabetic patients having type 2 diabetes mellitus. The first-line drug used for the management of this kind of diabetes is metformin. However, the conventional dosage forms are fraught with challenges such as after taste of the metformin, and bioavailability of 40% due to its low permeability. This research work focused on developing metformin into an emulsion so as to improve bioavailability of the drug, encourage adherence and reduce difficulty in ingestion as well as after taste of the drug. Using locally sourced materials would serve as a means of saving cost for both the manufacturers and consumers of this drug. As a result, emulsions were formulated using unmodified dika gum and a microwaved assisted modification of the dika gum as emulsifying agents (with or without preservatives) and melon oil as the oil phase. The formulated emulsions were characterized for 28 days in 2 batches employing such tests as pH, acid value, saponification value, globule size and viscosity. Emulsions with unmodified dika gum as emulsifying agent (without preservative) flocculated, creamed and had an offensive odour after 14 days. Emulsions with modified dika gum (without preservative) were stable as long as 21 days but deteriorated before the 28th day. Emulsions with unmodified dika gum with preservative were stable until the 21st day while emulsions with modified dika gum were stable after the study period of 28 days. However, all emulsions were slimy as the days progressed; a characteristic nature of dika gum. Dika gum and melon oil hold promise as excipients for the pharmaceutical industry. However, the studies should be undertaken to limit the sliminess of dika gum.

Keywords

Diabetes Mellitus, Emulsions, Dika Gum, Metformin, Melon Oil

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1. Introduction

According to World Health Organization (WHO), Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is described as a group of metabolic disorders characterized and identified by the presence of hyperglycaemia in the absence of treatment. [1] Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.

There are two principle forms of diabetes: Type 1 diabetes (formerly known as insulin-dependent) in which the pancreas fails to produce the insulin which is essential for survival. This form develops most frequently in children

* Corresponding author
E-mail address: uc.okafor@unizik.edu.ng (O. U. Chukwuma)

and adolescents, but is being increasingly noted later in life and Type 2 diabetes (formerly named non-insulin-dependent) which results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well. Certain genetic markers as well as environmental factors have been found to be major causative agents. [1].

Diabetes in pregnancy may give rise to several adverse outcomes, including congenital malformations, increased birth weight and an elevated risk of perinatal mortality. Strict metabolic control may reduce these risks to the level of those of non-diabetic expectant mothers.

Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) refer to levels of blood glucose concentration above the normal range, but below those which are diagnostic for diabetes. Subjects with IGT and/or IFG are at substantially higher risk of developing diabetes and cardiovascular disease than those with normal glucose tolerance. The benefit of clinical intervention in subjects with moderate glucose intolerance is a topic of much current interest.

The symptoms of diabetes may be pronounced, subdued, or even absent in Type 1 diabetes, the classic symptoms are excessive secretion of urine (polyuria), thirst (polydipsia), weight loss and tiredness. These symptoms may be less marked in Type 2 diabetes. In this form, it can also happen that no early symptoms appear and the disease is only diagnosed several years after its onset, when complications are already present.

A recently compiled data show that approximately 150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles. By 2025, while most people with diabetes in developed countries will be aged 65 years or more, in developing countries most will be in the 45-64 year age bracket and affected in their most productive years [1].

The mainstay of non-pharmacological diabetes treatment is diet and physical activity. However, several classes of drugs have been discovered and formulated in mostly oral and parenteral dosage forms. The classes of drugs used include insulin (which is the mainstay in type 1 diabetes), sulfonylureas (e.g. tolbutamide, glipizide, glibenclamide); biguinides (e.g. metformin, phenformin); meglitinides (e.g. nateglinide repaglinide); thiazolidenediones (e.g. pioglitazone, rosiglitazone) [2].

2. Materials and Methods

2.1. Materials/Solvents

Beakers (250 mL, 500 mL and 1000 mL), Measuring cylinder (100 mL), Conical flask (250 mL), Porcelain mortar and pestle, Burettes, Pipettes, Soxhlet apparatus, rotary evaporator, Funnel, Water bath, Whatman Filter paper, Glass stirring rod. Melon oil (obtained from a local extractor in Jos North, Plateau state), dried dika nuts (obtained from the local market in Ogbadibo, Benue state), pure metformin hydrochloride USP (Medisbca Inc. Plattsburgh, New York, USA), Tween-80 (Sigma Aldrich Laborchemikalein, Seelze, Germany), phenolphthalein (C.O.O. Germany), benzoic acid, sodium metabisulphite, sodium hydroxide and alcobholic potassium hydroxide (British Drug House, poole, England), hydrochloric acid, 96% alcohol, diethyl-ether (Hopkin and Williams Ltd, Chadwell heath. Essex, England), Amaranth solution, arachis oil and acacia gum (locally sourced).

2.2. Methods

2.2.1. Cold Extraction of Melon Oil

A 500 g mass of melon seed was weighed and pulverized. This was transferred to 1 L of n-Hexane and left for 48 hours stirring intermittently. The mixture was sieved using a cloth sieve to obtain the filtrate which was passed through a whatman filter paper (Xinxing qualitative filter paper 103 slow and 240 mm). The oil was retrieved from the n-Hexane by using a rotary evaporator. [3]

The oil yield was then calculated using Equation 1.

$$\text{Percentage Yield of Oil} = \frac{\text{Weight of Oil Extracted}}{\text{Weight of Melon Seed Used}} \times 100 \quad (1)$$

2.2.2. Extraction of Dika Fat and Polysaccharide (*I. gabonensis*)

I. gabonensis kernels were pulverized to powder and screened using a mesh in order to remove impurities. Then, 1.5 kg of the pulverized and screened powder was weighed into a glass bowl. Four (4) liters of n-hexane was poured into it and stirred periodically for 48 hours using a glass rod to de-fat the milled kernels. Thereafter, the mixture was filtered using a muslin cloth. The n-hexane filtrate was cooled periodically for 15 minutes in a refrigerator to precipitate the fat. The precipitated fat was scooped out of the n-hexane, air dried for 2 weeks, weighed and packed into a plastic bag. [4]

I. gabonensis kernels were pulverized to powder and screened using a mesh in order to remove impurities. Then, 1.5kg of the pulverized and screened powder was weighed into a glass bowl. Four (4) litres of n-hexane was poured into it and stirred periodically for 48 hours using a glass rod to de-fat the milled seed. Thereafter, the mixture was filtered using a muslin cloth. The n-hexane filtrate was cooled periodically

for 15 minutes in a refrigerator to precipitate the fat. The residue was collected and dried, the dried powder was macerated in 8 litres of distilled water for 2 hours, the menstrum was filtered using a muslin cloth. The gum was precipitated using 12 litres of ethanol. [4] The precipitate was collected, dried, weighed and stored in a plastic bag. The gum yield was determined using the equation 2:

$$\text{Percentage Yield of Polysaccharide} = \frac{\text{Weight of Polysaccharide Extracted}}{\text{Weight of Crude Dika Powder Used}} \times 100 \quad (2)$$

2.2.3. Preparation of Modified Dika Gum by Microwaving

An amount (3g) of dika gum was weighed and transferred into a dry porcelain mortar and blended to get fine particles of the dika gum and then transferred into a 1L beaker. Then, 500 ml of water was measured and added to the dika gum. This was stirred using a glass rod until the gum was evenly dispersed and then microwaved at Power 100 for 15 minutes. After using the microwave oven, the beaker was brought out and 1 L of ethanol was used to precipitate the gum. The precipitate was filtered and washed several times with ethanol. The modified dika gum was then air dried and weighed to determine percentage yield employing equation 3. [5]

$$\text{Percentage Yield of Modified Gum} = \frac{\text{Weight of Microwaved Dika Gum}}{\text{Weight of Dika Gum Used}} \times 100 \quad (3)$$

2.2.4. Preparation of Metformin Emulsion (Oil in Water Emulsion)

Wet Gum Method

The quantities of the materials required to prepare the primary emulsion were calculated using a suitable ratio of oil-water-gum. Distilled water was added to the gum and mixed to form a mucilage in a mortar. Then the oil was added little by little while triturating the mucilage with a pestle in a uniform direction until the primary emulsion was formed (clicking sound was heard). Distilled water was added little by little while still triturating in a uniform direction until the primary emulsion was diluted to the required volume. [6]

Dry Gum Method

The quantities of the materials required to prepare the primary emulsion were calculated using a suitable ratio of oil-water-gum. The oil was mixed with the gum in a mortar. The distilled water required to formulate the primary emulsion was poured into the mortar directly at the middle. Then the pestle was placed at the middle of the mortar and the mixture was triturated in uniform direction until the primary emulsion was formed (clicking sound was heard). Distilled water was added little by little while still triturating in a uniform manner until the primary emulsion was diluted to the required volume. [7]

Several excipients/materials were combined and their quantities varied in order to formulate a desired emulsion. Formulations with promising potentials were selected, preservatives and metformin were added in some emulsions, and the emulsions were characterized for stability using 2 batches over a period of 28 days each to allow for testing the reproducibility of this research work.

2.2.5. Characterization of Emulsions

The following methods as employed by [8] were used to characterize the oil in water emulsions:

Determination of pH: The pH meter was calibrated using standard buffer solution (pH of 4 and 7). About 0.5 g of the cream was weighed and dissolved in 50.0 mL of distilled water and its pH measured.

Determination of Viscosity: The viscosity of the cream was determined using an Ostwald Viscometer, (Model: BDV-1S by Biobase biodustry, Shandong co. Ltd. and Biobase biotech, Jinan Co. Ltd.) at room of temperature of 25-27°C. Water was poured into the open end of the wide arm of the viscometer till water rose via capillary action to the lower mark. Time was recorded for when water moved from the lower mark to the upper mark. This was repeated to get the mean time. This method was used for the emulsion whose viscosity was to be determined. [9] Viscosity of the emulsion was then determined using Equation 4:

$$\eta e = \eta w\ (de.te/dw.tw) \quad (4)$$

Where, ηe = viscosity of emulsion; ηw = viscosity of water

de = Density of the emulsion te = Time taken for emulsion to move to upper mark of viscometer

dw = Density of water; tw = Time taken for water to move to upper mark of the viscometer.

Determination of Emulsion Type Using Dye Test: A drop of the emulsion was placed on a microscope slide. A drop of amaranth solution (scarlet red) was mixed with it, covered with a cover slip and examined under a microscope. If the disperse globules appear red and the dispersion medium remains colorless, the cream is o/w type. The reverse condition occurs in w/o type emulsion i.e. the disperse

globules appear colorless in the red dispersion medium.

Determination of Globule size: This is done by switching on the microscope and setting the objective lens to ×4. The slide rule is then positioned so that the outer edge of one of the black hash marks is in line with the widest edge of the field of view. The diameter of the field of view was then determined. This was done for ×10, ×40 and ×100 objective lenses. 3 drops of the emulsion was then placed on the stage with the appropriate objective lens that allows for the most field of view. The sizes of 10 globules were measured and the average taken.

Appearance of Emulsion: The appearance of the emulsion was assessed by its color and texture

Type of Smear: After application of emulsion, the type of film or smear formed on the skin were checked. This was checked for greasiness or non-greasiness on the skin.

Acid Value of Emulsion: Acid value is defined as the number of sodium/potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are usually present during the decomposition of oil glycerides.

A 10 g of the emulsion was dissolved in a 50 mL mixture of equal volume of alcohol and solvent ether (diethyl ether) in a 250ml beaker. This mixture was heated for 30 minutes to dissolve the emulsion. After this, 2 drops of phenolphthalein was added and the solution titrated with 0.1 N NaOH until faintly pink color appeared after shaking for 30 seconds. [10] [11] Acid value was calculated using Equation 5:

$$Acid\ value = \frac{n\ x\ 5.6}{W} \quad (5)$$

Where;

n = the volume of NaOH in mL required.

w = the weight of cream in g used.

Saponification Value of Emulsion: It is defined as the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from complete hydrolysis of 1g of the sample.

A 2 g sample of the emulsion was heated with 25 mL of 0.5 N alcoholic KOH in a 250ml beaker for 30 minutes. After this, 2 drops of phenolphthalein was added and titrated immediately with 0.5 N HCL. [10][11] The saponification value was calculated using Equation 6:

$$Saponificaton\ value = \frac{(b-a)\times 28.05}{W} \quad (6)$$

Where;

a = the volume of titrant (0.5 N HCL) in mL,

b = the volume of titrate (volume of titrant consumed in blank titration) in mL,

w = Weight of cream used in g.

Irritancy Test:

An area (1 sq.cm) was marked on the dorsal surface of the left hand. The cream was applied to the specified area and the time was noted. Irritancy, erythema, edema, was checked at regular intervals up to 24 hours and reported [12]

2.2.6. Preparation of Reagents

Sodium Hydroxide Solution

To prepare 500 mL of 0.1N NaOH solution, 2 g of pure NaOH crystals was weighed and dissolved in about 10 mL of distilled water. This was poured into a 500 mL standard volumetric flask and made up to volume with distilled water.

Phenolphthalein Solution

To prepare 100 mL of 0.5% phenolphthalein solution, 0.5 g of pure phenolphthalein was weighed and dissolved in 50 mL of 95% alcohol. This was poured into a 100 mL standard volumetric flask and made up to volume with distilled water.

Alcoholic potassium hydroxide

To prepare 500 mL of 0.5 N alcoholic KOH, 14.25 g of pure KOH powder was dissolved in about 10 mL of distilled water. This was then poured into a 500 mL standard volumetric flask and made up to volume with 95% alcohol. The flask was stoppered and allowed to stand for 24 hours. After this, the clear supernatant liquid was decanted into another tightly stoppered container for use.

Hydrochloric acid

To prepare 1000 mL of 0.5 M HCL from a stock solution of 36%, 42.925 mL of the stock was measured and poured into a 1000 mL standard volumetric and then made up to volume with distilled water. [13]

3. Results

3.1. Percentage Yield of Melon Oil

The yield of melon oil extracted from melon seeds stored for one year was 72%

$$\text{Percentage Yield of Oil} = \frac{\text{Weight Of Oil Extracted}}{\text{Weight Of Melon Seed Used}} \times 100 \quad (7)$$

Cold Extraction = 360/500 x 100 = 72%

The average percentage recovery of modified dika gum obtained from microwaving of the defatted polysaccharide and recovering the precipitate with alcohol was found to be 38.06%.

3.2. Percentage Yield of Microwaved Dika Gum

$$\text{Percentage Yield of Modified Gum} = \frac{\text{Average Weight of Microwaved Dika Gum}}{\text{Average Weight of Dika Gum Used}} \times 100 \quad (8)$$

Average Weight of Dika Gum Used

$$\frac{152.23}{400}\ x\ 100 = 38.06\%$$

3.3. Tables Showing Results

As shown in Table 1, Arachis oil was initially used to obtain a workable ratio so as not to waste the melon oil that was extracted. However, emulsions using Arachis oil and dika gum as emulsifying agent in the ratios as shown did not form. At such, the use of melon oil was employed. Based on the results obtained during the formulation of the emulsion, it can be seen that the conventional 4:2:1 ratio for fixed oils failed for both wet gum method and dry gum method. Other ratios that failed include 2:4:1, 2:2:1, 40:20:1, 4:6:1 4:4:1, 4:10:1, 4:1:1, 5:3:1, 6:3:1, 6:4:1 and 6:8:1 (O:W:G). Then, the ratios 2:4:1 and 2:2:1 formed, before creaming and thereafter cracking were observed. The ratio 6:8:1 had visible floccules seen from the dispensing bottle. The rest deteriorated by creaming and with emission of an offensive smell. Following subsequent trials, 8:8:1 formed but failed almost immediately for both the unmodified and modified dika gum. A ratio of 4:8:4 formed an emulsion that solidified after 1 hour for the unmodified dika gum and 3 hours for the modified dika gum. Ratios of 5:5:1 and 5:6:1 produced stable emulsions for both wet and dry gum method but this stability only lasted for hours. Ratios for 6:5:1 lasted for 1 day. On addition of 1 mL of tween 80, the resultant assumed a more whitish appearance and lasted for 5 days. Addition of 2 mL of tween 80 improved appearance and stability for up to 7 days. A ratio of 6:6:1 derived produced an emulsion that lasted for 9 days. On addition of 1 and 2 mL respectively of tween 80, stability was seen for 12 and 15 days respectively. The major mode of deterioration was seen to be emission of an offensive smell. This led to the need for the addition of antioxidants and antimicrobial agents. This caused an extended stability of up to 34 days. Addition of up to 3 mL of tween 80 caused the emulsion to thicken after 21 days.

Mode of deterioration ranged from upward creaming (this was corrected with the addition of tween 80), Cracking (was corrected by looking for much more stable ratios to form primary emulsion), malodourous smell - this was corrected by addition of an antioxidant (0.05% sodium metabisulphite) and a preservative (0.5% Benzoic acid). When preservatives were added to the 6:6:1 ratio, the emulsion lasted for more than 28 days but less than 40 days.

Table 1. Compositions of Emulsions Formed and Outcomes.

S/N	Arachis Oil (m)	Melon Oil (ml)	Water (ml)	Dika Gum (g)	Microwaved Dika Gum (g)	Ratio (O:W:G)	Tween 80 (ml)	Method	Outcome
1.	2	-	1	0.5	-	4:2:1	-	Dry	Failed
2.	2	-	1	0.5	-	4:2:1	-	Wet	Failed
3.	2	-	1	-	0.5	4:2:1	-	Dry	Failed
4.	2	-	1	-	0.5	4:2:1	-	Wet	Failed
5.	2	-	4	-	0.5	2:4:1	-	Dry	Failed
6.	2	-	4	-	0.5	2:4:1	-	Wet	Failed
7.	4	-	4	0.5	-	8:8:1	-	Dry	Formed, unstable
8.	4	-	4	-	0.5	8:8:1	-	Dry	Formed, unstable
9.	6	-	8	1	-	6:8:1	-	Dry	Failed
10.	4	-	4	2	-	2:2:1	-	Dry	Failed
11.	12	-	12	1.5	-	8:8:1	-	Dry	Failed
12	12	-	12	1.5	-	8:8:1	-	Wet	Failed
13	4	-	2	0.1	-	40:20:1	-	Dry	Failed
14	2	-	2	0.5	-	4:4:1	-	Dry	Failed
15	2	-	3	0.5	-	4:6:1	-	Dry	Failed
16	2	-	3	0.5	-	4:6:1	-	Wet	Failed
17	2	-	4	2	-	4:8:4	-	Dry	Formed, solidified after 1 hour
18	2	-	4	-	2	4:8:4	-	Dry	Formed, solidified after 3 hours
19	2	-	5	0.5	-	4:10:1	-	Dry	Failed
20	2	-	5	-	0.5	4:10:1	-	Dry	Formed, unstable
21	-	4	2	-	1	4:2:1	-	Dry	Failed
22	-	4	2	-	1	4:2:1	-	Wet	Failed
23	-	4	1	-	1	4:1:1	-	Dry	Failed
24	-	5	3	-	1	5:3:1	-	Dry	Failed
25	-	6	3	-	1	6:3:1	-	Dry	Failed

S/N	Arachis Oil (m)	Melon Oil (ml)	Water (ml)	Dika Gum (g)	Microwaved Dika Gum (g)	Ratio (O:W:G)	Tween 80 (ml)	Method	Outcome
26	-	12	8	-	2	6:4:1	-	Dry	Failed
27	6	6	8	2	-	6:4:1	-	Dry	Failed
28	6	6	8	1	1	6:4:1	-	Dry	Formed, stable for 1 hour
29	6	6	8	1	1	6:4:1	-	Wet	Failed
30	-	10	12	-	2	5:6:1	-	Dry	Formed, stable for 1 hour
31	-	10	10	-	2	5:5:1	-	Dry	Formed, stable for 1 day
32	-	10	10	-	2	5:5:1	-	Wet	Failed
33	-	6	5	-	1	6:5:1	-	Dry	Formed, stable for 1 day
34	-	6	5	-	1	6:5:1	1	Dry	Formed, creamy, stable for 5 days
35	-	6	5	-	1	6:5:1	2	Dry	Formed, creamy, stable for 7 days
36	-	6	6	-	1	6:6:1	-	Dry	Formed, stable for 9 days
37	-	6	6	1	-	6:6:1	1	Dry	Formed, stable for 12 days
38	-	6	6	1	-	6:6:1	2	Dry	Formed, stable for 15 days
39	-	6	6	-	1	6:6:1	1	Dry	Formed, creamy, stable for 30 days
40	-	6	6	-	1	6:6:1	2	Dry	Formed, creamy, stable for 34 days.
41	-	6	6	-	1	6:6:1	3	Dry	Formed, thickens after 21 days
42	-	6	7	-	1	6:7:1	2	Dry	Formed, stable for 7 days
43	-	7	6	-	1	7:6:1	2	Dry	Formed, stable for 4 days
44	-	5	12	-	1	5:12:1	2	Dry	Formed, stable for 5 days
45	-	4	10	-	1	4:10:1	2	Dry	Formed, stable for 5 days

The ratio that provided the most stable emulsion was 6:6:1 (O:W:G) in addition to the addition of tween 80 because it helped with stability as well as enhancing appearance of the emulsion. Characterization was done for 4 different formulations (Table 2) based on this ratio for both the unmodified and modified sample. Two of the formulations contained preservatives (An antioxidant - sodium metabisulphite and an antimicrobial agent - Benzoic acid).

Table 2. Compositions of Selected Metformin Emulsion.

Code	Melon Oil (ml)	Water (ml)	Dika Gum (g)	Microwaved Dika Gum (g)	Tween 80 (g)	Ratio	Benzoic Acid (g)	Sodium metabisulphite (g)	Metformin Hydrochloride (g)
U-	6	6	1	-	2	6:6:1	0.5	0.05	5
U+	6	6	1	-	2	6:6:1	0.5	0.05	5
M-	6	6	-	1	2	6:6:1	0.5	0.05	5
M+	6	6	-	1	2	6:6:1	0.5	0.05	5

Key: Emulsions formulated based on:
U- = Unmodified dika gum without preservatives
U+= Unmodified dika gum with preservatives
M- = Modified dika gum without preservatives
M+ = Modified dika gum with preservatives

The unmodified dika gum preparation without preservatives started showing signs of deterioration from day 14 with relatively high acid values and saponification values, low pH, a progressive increase in globule size as compared with the start of characterization, malodourous smell, creaming, coalescence and a rough texture of the emulsion with presence of visible particulate matters (Tables 3 and 4). Addition of preservatives to the unmodified dika gum preparation further enhanced stability. However, signs of deterioration were seen from Day 28.

Table 3. Characterization of Emulsion U - 1st batch for 28 days.

Day	pH	Acid Value (ml/g)	Saponification Value (mL/g)	Globule Size (µm)	Globule Shape	Type	Irritancy	Smear	Odour	EOR	Compact-ness	Appearance
1	5.60	5.02	177	47	Spherical	O/W	-	-	-	+	Dispersed	Milky coloured, smooth.
7	5.49	5.47	182	48	Spherical	O/W	-	-	-	+	Dispersed	Milky coloured, smooth.
14	5.35	5.94	189	53	Spherical	O/W	-	-	+	+	Compacted	Milky coloured, rough
21	5.21	6.24	191	55	Spherical	O/W	-	-	+	+	Compacted	Milky coloured, slimy.
28	4.81	6.78	195	56	Spherical	O/W	-	-	+	+	Compacted	Brown coloured, slimy.

Key:
EOR: Ease of removal (+ indicates ease of removal, - indicates non-ease of removal)
Odour: (- indicates absence of odour, + indicates presence of odour)
O/W: Oil in Water type of emulsion.

Table 4. Characterization of Emulsion U+ 1st batch for 28 days.

Day	pH	Acid Value (ml/g)	Saponification Value (ml/g)	Globule Size (µm)	Globule Shape	Type	Irritancy	Smear	Odour	EOR	Compact-ness	Appearance
1	5.71	5.01	173	45	Spherical	O/W	-	-	-	+	Dispersed	Milky coloured, smooth.
7	5.68	5.08	174	45	Spherical	O/W	-	-	-	+	Dispersed	Milky coloured, smooth.
14	5.62	5.14	177	46	Spherical	O/W	-	-	-	+	Dispersed	Milky coloured, smooth
21	5.58	5.27	179	47	Spherical	O/W	-	-	-	+	Dispersed	Milky coloured, smooth
28	5.21	5.90	186	49	Spherical	O/W	-	-	+	+	Compacted	Milky coloured, slimy.

Characteristics of emulsion with modified gum are shown in Tables 5 and 6 for batch 1. Modified dika gum preparation without preservatives started showing signs of deterioration from Day 21 with an eventual sliminess of the emulsion, odour and creaming. The preparation with modified dika gum and preservatives was relatively stable with lesser acid values and saponification value and a more stable globule size. However, it became slimy on Day 28.

Table 5. Characterization of Emulsion M- 1st batch for 28 days.

Day	pH	Acid Value (mL/g)	Saponification Value (mL/g)	Globule Size (µm)	Globule Shape	Type	Irritancy	Smear	Odour	EOR	Compact-ness	Appearance
1	5.63	4.92	171	46	Spherical	O/W	-	-	-	+	Dispersed	White coloured, smooth.
7	5.57	5.02	173	47	Spherical	O/W	-	-	-	+	Dispersed	White coloured, smooth.
14	5.51	5.11	182	50	Spherical	O/W	-	-	-	+	Dispersed	White coloured, smooth
21	5.44	5.29	188	52	Spherical	O/W	-	-	-	+	Compacted	Milky coloured, rough
28	5.20	5.73	190	52	Spherical	O/W	-	-	+	+	Compacted	Milky coloured, slimy.

Table 6. Characterization of Emulsion M+ 1st batch for 28 days.

Day	pH	Acid Value (ml/g)	Saponification Value (ml/g)	Globule Size (µm)	Globule Shape	Type	Irritancy	Smear	Odour	EOR	Compact-ness	Appearance
1	5.75	4.89	169	45	Spherical	O/W	-	-	-	+	Dispersed	White coloured, smooth.
7	5.75	4.90	169	45	Spherical	O/W	-	-	-	+	Dispersed	White coloured, smooth.
14	5.71	4.96	171	46	Spherical	O/W	-	-	-	+	Dispersed	White coloured, smooth
21	5.70	4.99	173	46	Spherical	O/W	-	-	-	+	Dispersed	White coloured, smooth
28	5.66	5.05	177	47	Spherical	O/W	-	-	-	+	Dispersed	White coloured, slimy.

The second batch of emulsions was prepared and the characteristics are shown in Tables 7 – 10. This was reproduced like the 1st batch. However, viscosity was carried out for this batch and there was a drastic decrease in the viscosity observed for emulsion prepared with unmodified dika gum. By Day 28, the emulsion became brown in colour. Addition of preservative stabilized the viscosity to a relative extent. The results were duly reproduced however; the values obtained were different from the 1st batch characterized. The results obtained for the preparation with modified dika gum and preservatives was reproduced from the first batch. However, the values obtained were different. Viscosity was relatively stable over a period of 28 days.

Table 7. Characterization of Emulsion U- 2nd batch for 28 days.

Day	pH	Acid Value (ml/g)	Saponification Value (ml/g)	Globule Size (µm)	Globule Shape	Type	Viscosity	Smear	Odour	EOR	Compact-ness	Appearance
1	5.59	5.02	180	47	Spherical	O/W	923	-	-	+	Dispersed	Milky coloured, smooth.
7	5.50	5.47	184	49	Spherical	O/W	615	-	-	+	Dispersed	Milky coloured, smooth.
14	5.39	5.84	189	53	Spherical	O/W	379	-	+	+	Compacted	Milky coloured, rough
21	5.10	6.27	193	56	Spherical	O/W	142	-	+	+	Compacted	Milky coloured, slimy.
28	4.63	6.71	203	59	Spherical	O/W	86	-	+	+	Compacted	Brown coloured, slimy.

Table 8. Characterization of Emulsion U+ 2nd batch for 28 days.

Day	pH	Acid Value (ml/g)	Saponification Value (ml/g)	Globule Size (µm)	Globule Shape	Type	Viscosity	Smear	Odour	EOR	Compact-ness	Appearance
1	5.74	5.00	173	45	Spherical	O/W	991	-	-	+	Dispersed	Milky coloured, smooth.
7	5.69	5.10	175	46	Spherical	O/W	854	-	-	+	Dispersed	Milky coloured, smooth.
14	5.60	5.18	179	48	Spherical	O/W	722	-	-	+	Dispersed	Milky coloured, smooth
21	5.51	5.37	181	48	Spherical	O/W	604	-	-	+	Dispersed	Milky coloured, smooth
28	5.23	5.88	188	50	Spherical	O/W	428	-	+	+	Compacted	Milky coloured, slimy.

Table 9. Characterization of Emulsion M- 2nd batch for 28 days.

Day	pH	Acid Value (ml/g)	Saponification Value (ml/g)	Globule Size (µm)	Globule Shape	Type	Viscosity	Smear	Odour	EOR	Compact-ness	Appearance
1	5.64	4.91	170	45	Spherical	O/W	1150	-	-	+	Dispersed	White coloured, smooth.
7	5.56	5.05	174	46	Spherical	O/W	1012	-	-	+	Dispersed	White coloured, smooth.
14	5.53	5.19	183	48	Spherical	O/W	803	-	-	+	Dispersed	White coloured, smooth
21	5.45	5.33	188	49	Spherical	O/W	547	-	-	+	Compacted	Milky coloured, rough
28	5.20	5.90	190	51	Spherical	O/W	336	-	+	+	Compacted	Milky coloured, slimy.

Table 10. Characterization of Emulsion M+ 2nd batch for 28 days.

Day	pH	Acid Value (ml/g)	Saponification Value (ml/g)	Globule Size (µm)	Globule Shape	Type	Viscosity	Smear	Odour	EOR	Compact-ness	Appearance
1	5.75	4.89	169	45	Spherical	O/W	1229	-	-	+	Dispersed	White coloured, smooth.
7	5.74	4.90	169	45	Spherical	O/W	1170	-	-	+	Dispersed	White coloured, smooth.
14	5.74	4.91	170	46	Spherical	O/W	1092	-	-	+	Dispersed	White coloured, smooth
21	5.70	4.98	172	46	Spherical	O/W	1001	-	-	+	Compacted	White coloured, smooth
28	5.64	5.07	173	47	Spherical	O/W	921	-	-	+	Compacted	white coloured, slimy.

Figures 1 – 9 are charts showing the comparative data at a glance of the characteristics of the emulsions prepared with unmodified dika gum and modified dika gum with or without preservatives.

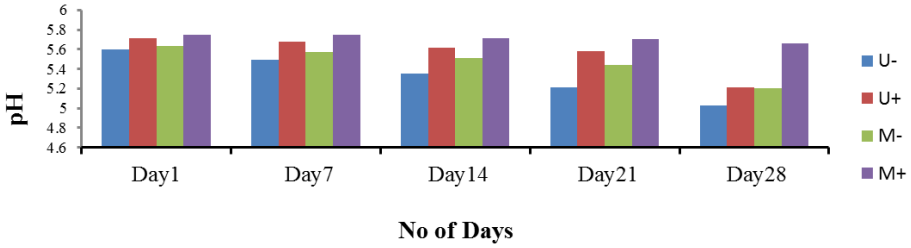

Figure 1. Comparative pH Values of the Emulsions (1st batch).

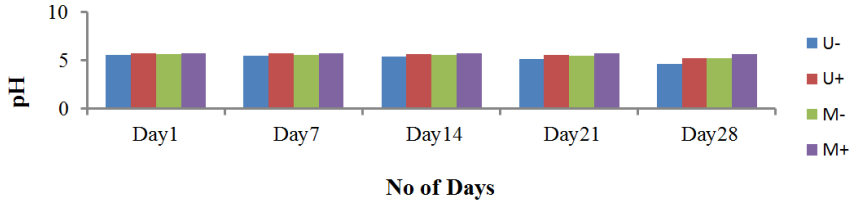

Figure 2. Comparative pH Values of the Emulsions (2nd batch).

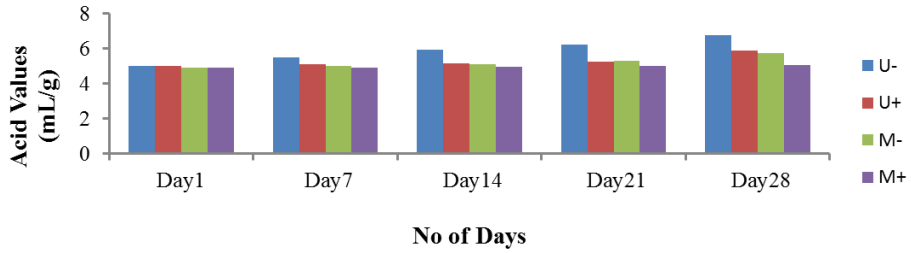

Figure 3. Comparative Acid Values of the Emulsions (1st batch).

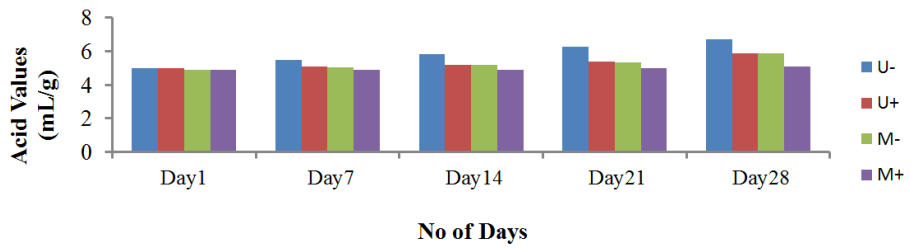

Figure 4. Comparative Acid Values of the Emulsions (2nd batch).

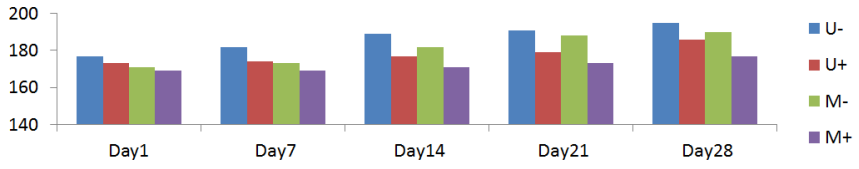

Figure 5. Comparative Saponification Values of the Emulsions (1st batch).

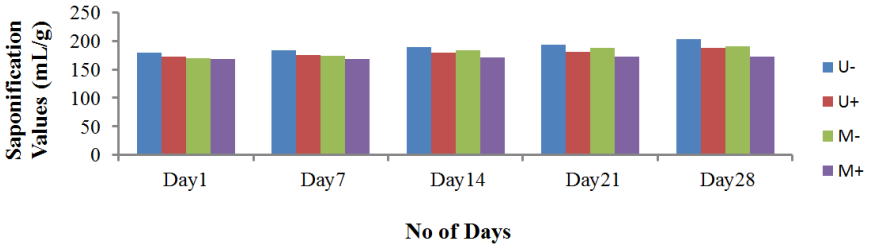

Figure 6. Comparative Saponification Values of the Emulsions (2nd batch).

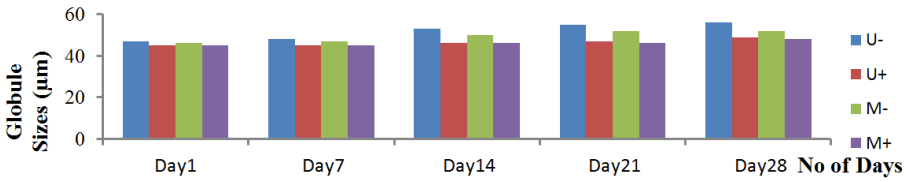

Figure 7. Comparative Globule Sizes of the Emulsions (1st batch).

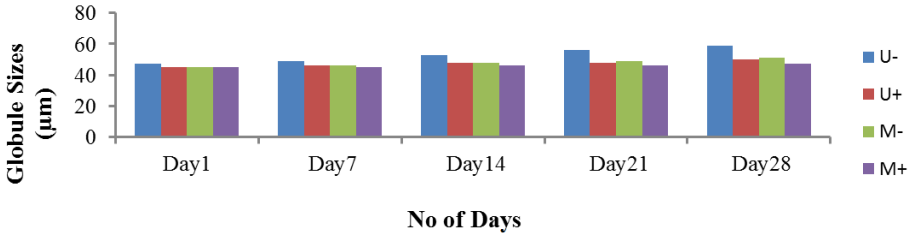

Figure 8. Comparative Globule Sizes of the Emulsions (2nd batch).

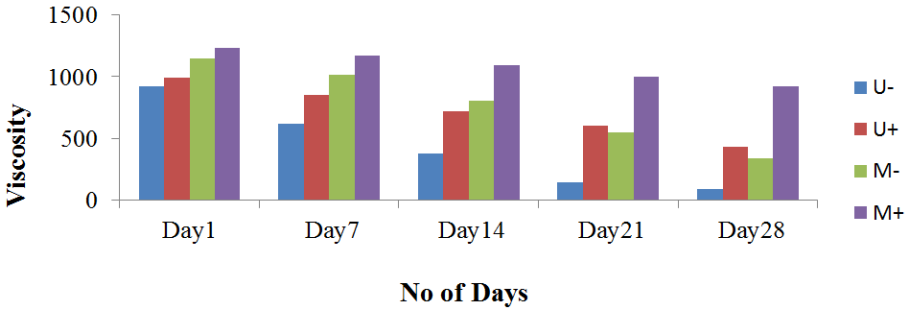

Figure 9. Comparative Viscosity of the Emulsions (2nd batch).

The results obtained from the 1st 28 days of characterization tallied with that from the 2nd 28 days of characterization.

4. Discussion

Various ratios were tested to ensure that the emulsions formed and were stable. Special techniques were also employed to ensure that the formed emulsions were stable over long periods of time. The most stable ratio for primary emulsion (O:W:G) obtained was 6:6:1. With an equal amount of oil and water volumes. However, the emulsions formed were having an unattractive brown colour and were unstable. This necessitated the addition of a surfactant that is compatible with o/w emulsions. Tween 80 (HLB value of 15) was added and a 2mL volume was found to be the most effective for the formulation as it increased stability of emulsions by enhancing the ability of insoluble ingredients to be soluble in a given medium especially in the case of oil and water.

The emulsions deteriorated by various ways such as

creaming, which occurred when the oil phase (the globules) started to rise up to the surface of the emulsion due to difference in density between the oil and water phase but this was easily reversed by shaking it. This was probably due to low viscosity of the emulsion formed, large globule size of droplets, great disparity between globule size and difference in the density between the oil and water phase [14]. Measures were taken to ensure proper globule size distribution and homogenization during formulation and to also ensure solubilization of metformin in both polar and non-polar phases of the emulsion by addition of a non-ionic surfactant like tween 80 that is compatible with o/w emulsions.

Flocculation was seen in some of the creams formulated where globules present in the dispersed phase come closer to each other forming colonies in the continuous phase (water). This usually happened before creaming. It is reversed by shaking the emulsion.

Cracking was seen clearly as a complete separation of oil and the water phase. This was clearly seen and was irreversible. Factors such as addition of an incompatible emulsifiers e.g cationic surfactant was avoided by using non-ionic surfactants like tween 80. Dika gum acts by increasing the viscosity of the continuous phase however, microbial growth occured as a result of use of dika gum (a polysaccharide emulsifier) and could have precipitated cracking. Dika gum would encourage bacterial growth because of the presence of essential nutrients present in the polysaccharide that could enhance growth of microbes.

Offensive Odour emanated after some days of creaming indicating microbial growth. Addition of an antioxidant (sodium metabisulphite) and an antimicrobial agent (Benzoic acid) increased the stability of the emulsions to over 30 days. Offensive odour observed was due to the increased rancidity of the melon oil and bacterial growth as a result of the dika gum both of which caused malodourous smell and other emulsion instability.

Oil in water emulsions generally favours microbial growth relative to Water in oil emulsions.

The pH levels of the emulsions were seen to be almost equal at the start of Day 1 but as the days progressed, that of unmodified without preservative (U-) had a drastic fall in pH from 5.59 to 4.63 indicating weakly acidic emulsion. While that of the modified with preservative (M+) fell from (5.75-5.66) which is a less than 10 unit fall over a 28 day period. The range of acidity for the 4 preparations was seen to be: U->M->U+>M+.

The acid values (indicates presence of free organic fatty acids from oil phase) increased over the 28 day period of characterization. The order of increasing value was seen to be U->M->U+>M+. With M+ being the most stable.

The saponification values (indicates rate of hydrolysis of emulsion to produce fatty acids mainly) at the start of Day 1 was different for all 4 preparations. Preparations with the unmodified gum had higher values. This could be as a result of presence of more water in the polysaccharide which could enhance the process of hydrolysis to yield fatty acids. For U- (unmodified gum without preservatives), it kept increasing significantly and progressively. M+ (Modified gum with preservatives) remained almost constant for the stipulated 28 days of assay. This indicates that the microwaved modified dika gum and preservatives used played a role in maintaining stability. At the start of characterization, the rate of decreasing saponification values was seen to be: U->U+>M->M+ but on Day 28, the sequence changed to U->M->U+>M+ indicating that preservatives helped to stall the process of hydrolysis of the oil phase.

The average globule size of the preparations varied significantly for U- and M- but was seen to be more stable when preservatives were added to U+ and M+. This explains that preservatives prevent an undue increase in globule size to prevent the formation of floccules that could lead to creaming or cracking. At the start, all the globule sizes were seen to be 45μm except for U- which had an average globule size of 47 μm. U- ranged from 47-59 μm while M+ ranged from 45-47 μm with no changes between Day 1 and 7 as well as Day 14 and 21.

The globule shape remained spherical and there was no phase inversion given that the use of amaranth solution yielded a positive o/w kind of emulsions.

The emulsion became malodorous for emulsions without antioxidants and antimicrobial agents. For U-, it started from Day 14 for both batches. But for M-, the smell was detected from Day 28 of assay. This indicated the superiority of microwaved modified dika gum over unmodified dika gum. No odour was perceived at the 28^{th} day indicating the benefit of modification of dika gum as well as the importance of addition of preservatives to emulsions.

There was no sign of smear when applied to the skin as well as it was easy to wash away the emulsion with water for all 4 preparations. This further confirmed the emulsion to be oil-in-water. The globules were properly dispersed in the continuous phase however; compaction was seen as the cream deteriorated except for M+ which was never compacted**.**

The viscosity of the emulsions was seen to reduce drastically over 28 days for the unmodified dika gum formulation. By Day 7, the unmodified gum with preservative (U+) and the modified gum without preservative (M-) preparations had an

almost equal viscosity but reduction in viscosity was seen to be drastic for M- at Day 21 owing to the absence of preservatives which could enhance deterioration of the emulsion leading to decreased viscosity of the preparation. The modified dika gum with preservative preparation was seen to have the most stable viscosity profile. The higher the viscosity, the more stable an emulsion tends to be.

The emulsions exhibited colour change with time. With U- changing from a milky coloured emulsion to brown. M+ maintained a white colour over 28 days. The sliminess is largely due to the dika polysaccharide's nature to draw as well as the effect of time and decreased viscosity of the preparations. [15].

5. Conclusion and Recommendation

Stable emulsions were formulated using microwaved modified dika gum as emulsifying agent and melon oil as the oil phase. This formulation was characterized and ascertained to be stable over a period of 28 days given various parameters used. The characterization parameters were able to be reproduced over an extra period of 28 days further establishing the accuracy and precision of the various methods and materials used. Indeed, the locally sourced materials, melon oil and microwave-assisted modified dika gum have shown promise as pharmaceutical excipients.

The Following Recommendations were made from the Research:

1) The sliminess of the emulsion after a few weeks is due to the polysaccharide used as the emulsifying agent-dika gum. Special techniques such as soaking the gum in a combination of vinegar, tamarind and lemon juice for 30-60 minutes and washing afterwards with ethanol, salt can be added to enhance release of excessive moisture.
2) A combination of more emulsifying agents as well as surfactants for increased stability.
3) In vivo bioavailability studies should be undertaken to assess the plasma concentration after oral administration of this emulsion.
4) Toxicological studies can be carried out to ensure safety levels of the emulsion formulated.
5) This material should be further explored and further characterized in order to use them in the pharmaceutical industry thereby using locally sourced material to reduce cost.

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