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RESEARCH ARTICLE

PREPARATION AND EVALUATION OF CARBOXYMETHYL *ENSET* AND CASSAVA STARCHES AS PHARMACEUTICAL GELLING AGENTS

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ABSTRACT

Starch is usually modified either chemically, physically or enzymatically to augment its convenience for industrial use. In the current study, starches from Enset and cassava plants were carboxymethylated, and factors which affect the carboxymethylation process and degree of substitution (DS) were studied. The application of the carboxymethyl starches as alternative pharmaceutical gelling agents for topical delivery of drugs was alsoinvestigated. Accordingly, nine different topical gel formulations of ibuprofen were prepared. All formulations were evaluated with respect to cosmetic qualities, pH, drug content, viscosity, spreadability, extrudability, *in vitro* drug release, anti-inflammatory activity and stability. The results showed that carboxymethylation was significantly affected by the starch source, reaction medium,temperature andtime. All ibuprofen gel formulations showed homogeneous appearance, smooth texture and pleasant odor.The pH values of the formulations ranged from 6.80 to 7.22. Ibuprofen content rangedbetween 98.76 and 100.20% ensuring the uniformity of the drug content. The cumulative percent ibuprofen released over 12 h across cellulose membrane ranged from 43.8% cm^{-2} to 84.5% cm^{-2}. Spreadability, extrudability, the cumulative drug release and diffusion coefficient of ibuprofen were influenced not only by the rheological properties of the formulations but also by the nature of the modified starches. Physicochemically stable ibuprofen gels wereobtained with potent anti-inflammatory activities.

Keywords: *Enset*starch, cassava starch, carboxymethylation, degree of substitution, ibuprofen gel, *in vitro* drug release, anti-inflammatory activity, stability study

INTRODUCTION

Starch is one of the most abundant and renewable biopolymers, which is very promising raw material, available at low cost for preparing various functional polymers. It is usually modified either physically, enzymatically or chemically to augment its convenience for industrial use.Physical modification involves the treatment of starch by physical means such as shear force, blending and thermal treatment[1]. Enzymatic modification usually involves hydrolysis and transglycosylation by carbohydrate enzymes[2] and chemical modification is used to introduce desirable properties to starch for specific applications based on reaction of the free hydroxyl groups of the anhydrous glucose units (AGU) with functional groups of chemicals, resulting in starch derivatives[3].Chemical modification is generally achieved through derivatization such as etherification, esterification, cross-linking and dual modification of starch, oxidation, dextrinization, acid-thinning or hydrolysis[1].

Carboxymethylation is a well-knownderivatization process for polysaccharides, resulting in polyelectrolytes[4]. Carboxymethyl starch (CMS) has unique properties due to the presence of negatively charged functional group (CH_2COO-) and is officially listed in the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP)[5,6].Carboxymethylation involves a two-step reaction: the first step is the alkalization of starch and in the second step, etherification occurs[7,8].

Enset(*Enseteventricosum*(Welw.)Cheeseman) and cassava (*Manihotesculenta*Crantz.) which belong to the family Musaceae and Euphorbiaceae, respectively are starch-rich staple foods widely used in the southern and south-western regions of Ethiopia[9,10]. The application of native starch as pharmaceutical gelling agent has been reported but discouraging mainly because of the need for a high concentration and heating to obtain viscous gel, the opacity of the formed gel, and its poor stability compared to other gelling agents[11]. Several reports indicate that carboxymethylation improves aqueous dispersibility and cold storage stability of starch pastes[12,13].These improved properties suggest the potential application of CMS as a pharmaceutical gelling agent. Thus, the aim of the present work was to prepare and evaluatecarboxymethyl*Enset*and cassava starches as potential pharmaceutical gelling agents for topical applications.

MATERIALS AND METHODS

Materials

Plant materials

*Boulla*was purchased from the local *Enset* cultivating farmers in Durame, KambataTembaro Zone, South Nations Nationalities People Region (SNNPR), Ethiopia and cassava tubers were obtained from local cassava cultivating farmers in Sawula, GamoGofa Zone, SNNPR, Ethiopia.

Chemicals and solvents

Monochloroacetic acid (MCA) (Hopkin& Williams Ltd, England) was obtained from Ethiopian Conformity Assessment Enterprise (ECAE). Triethanolamine (Fischer Chem Alert Guide, USA), sodium metabisulphite and hydrochloric acid (Guangzhou Jinhaunda Chemical Reagent Co. Ltd, China), isopropyl alcohol and glacial acetic acid (Riedel-de Haėn, Germany), methanol (ScharlauChemie SA, European Union), potassium dihydrogen phosphate (Sörensen, Leuren, Denmark), disodium hydrogen phosphate (Fizmerk chemicals, India), sodium hydroxide pellets (UNI-CHEM® chemical reagent, China), ethanol (Joseph Mills (Denaturants) Ltd, Liverpool, England), sodium chloride (Oxford Laboratory, Mumbai, India), and formalin (BDH Chemicals Ltd, England) were all used as received.

Active pharmaceutical ingredients and excipients

Ibuprofen raw material powder and its working standard (Satwik Drugs Limited, India), sodium carboxymethylcellulose(FMC Corporation, USA) and propylene glycol (Research-lab fine Chem. Industries, India) were kindly supplied by Cadila Pharmaceuticals Sh. Co., Ethiopia. Indomethacin and carrageenan (Sigma Chemical Co., USA) were obtained from Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University and Drug Research Directorate, Ethiopian Health and Nutrition Research Institute (EHNRI), respectively. Methyl parabenand propyl paraben (BDH Chemicals Ltd, England) were donated from the Ethiopian Pharmaceuticals Manufacturing Sh. Co. (EPHARM Sh. Co). All the materials were used as received.

Test animals

Mice of either sex (30-45 g) used in anti-inflammatory study were obtained from the animal houses at the School of Pharmacy and Department of Biology, Addis Ababa University, respectively.

Methods

Enset starch isolation

Starch from *boulla* was isolated according to the method described byGebre-Mariam and Schmidt[9].

Cassava starch extraction

Starch from cassava tubers was extracted using the method described elsewhere by Da etal[14].

Preparation of carboxymethyl*Enset* and cassava starches

Carboxymethylenset starch (CMES) and carboxymethyl cassava starch (CMCS) were prepared according to the procedure described by Jieet al[15]with slight modification. Briefly, 764 ml of organic solvent either isopropanol (ISPA) or methanol, was first mixed with water to a total volume of about 955 ml. Subsequently, 250 g *Enset* or cassava starch, which had already been dried in an oven (Kottermann® 2711, Germany) at 50 °C for 24 h, was added to a glass jacketed batch reactor with four necked stoppers fitted with a reflux condenser.The reaction medium was mixed in the glass reactor at room temperature. The reactor content was run at eight different reaction conditions (Table1). Then, 86.4 g NaOH in the form of pellets was added to the reaction mixture. After 15 min, 51 g MCA powder was added to start the etherification reaction. A speed adjustable impeller (Ika-Werke, guni, Hamburg, Germany) was used to stir the reaction medium. The stirrer speed was set at 250 rpm at the beginning of all reaction conditions but was reduced gradually. At the end, the reaction was stopped by neutralization with glacial acetic acid. The liquid supernatant was decanted and the product was washed several times with 80% methanol and finally with analytical grade methanol. The modified starch was finally dried in an oven (Kottermann® 2711, Germany) at 50 °C for 24 h, milled and passed through sieve size of 224 µm.

Table 1: Reaction conditions used for carboxymethylation of *Enset* and cassava starches

Reaction condition	Starch source	Reaction media (80% v/v)	Reaction temp.(°C)	Reaction time (h)
E-M-70-1	Enset	Methanol	70	1
C-M-70-1	Cassava	Methanol	70	1
E-I-70-1	Enset	ISPA	70	1
C-I-70-1	Cassava	ISPA	70	1
E-I-70-0.5	Enset	ISPA	70	0.5
C-I-70-0.5	Cassava	ISPA	70	0.5
E-I-50-1	Enset	ISPA	50	1
C-I-50-1	Cassava	ISPA	50	1

N.B.All reactions were conducted at starch/liquor ratio of 1:3; NaOH/reagent molar ratio of 4.0; and reagent/AGU molar ratio of 0.35.

Determination of degree of substitution (DS)

One gram CMS was converted to the H-form by treating with excess 0.1 N aqueous 80% methanolicHCl in a 100 ml beaker with occasional stirring for 1 h. It was then filtered and washed several times with aqueous 80% methanol solution under suction in a sintered glass funnel (OAKTON®, WP-15-1, Japan),. The resulting sample was dried in an oven (Kottermann®2711, Germany) at 100 ^{o}C for 1 h and cooled in a desiccator. A portion of sample weighing 0.25 g was placed into a 250 ml conical flask and to this 100 ml DW was added, followed by 10 ml of 0.1 N NaOHsolution. The mixture was heated over a boiling water bath (GFL®, D3006, Germany) for 20 min until a clear solution resulted. The hot solution was then titrated with a standard 0.1 N HCl solution to a phenolphthalein end-point. Similarly processed native starch was used as a correction factor for the blank. Each sample analysis was

carried out in triplicate and the mean values were taken. The %Carboxyl and DS were calculated according to the method described byKhalil et al[16] as shown in Eqn. 1and 2, respectively.

$$\%Carboxyl = \left[{}^{(V_b - V)}/_{wt}\right] \times M_{NaOH} \times 0.045 \times 100$$

Eqn. 1

$$DS = {}^{162 \times \%Carboxyl}/_{[4500 - 58 \times \%Carboxyl]}$$

Eqn. 2

where, V_b (ml) is the volume of HCl used for the titration of the blank; V(ml) is volume of HCl used for titration of the sample; wt -is weight in g of sample or native starch; and M_{NaOH}-is molarity of NaOH.

Preparation of polymer gels and drug formulations

Polymer gels were prepared by a method described by Kittipongpatanaet al[11]by mixing the polymer powder at specified concentrations with distilled water (DW), and then allowing it to fully swell overnight before use**.**The modified starches, i.e., CMES and CMCS were prepared at concentrations of 6, 8 and 10% (w/w) and 8, 10 and 12% (w/w), respectively. Na-CMC was used at concentrations of 1, 2 and 3% (w/w) as commercial gelling agent. In addition, nine different medicated formulations (F1-F9) were prepared using the three polymers at the same concentrations as in the blank gels (Table 2). Briefly, the medicated gel formulations were prepared by weighing 2.5 g of ibuprofen on an analytical balance (Mettler Toledo, PR 203, Switzerland) and dissolving it in a co-solvent of ethanol and propylene glycol (PG) (solution A). The preservatives, methyl paraben (MP) and propyl paraben (PP), were also dissolved in solution A. The respective polymers were allowed to swell fully overnight in half portion of the water. Solution A was added into the polymer gel under continuous stirring to yield a homogenous dispersion, which in turn was neutralized with triethanolamine (TEA) to obtain a colorless gel. The weight of the formulation was finally adjusted to final weight of 50 g by adding DW.

Table 2: Percentage composition (w/w) of the different ibuprofen gel formulations

Formula	Ibuprofen	CMES	CMCS	Na- CMC	Ethanol	PG	TEA	MP	PP	DW(qs)
F1	5	6	-	-	10	10	3	0.135	0.027	100
F2	5	8	-	-	10	10	3	0.135	0.027	100
F3	5	10	-	-	10	10	3	0.135	0.027	100
F4	5	-	8	-	10	10	3	0.135	0.027	100
F5	5	-	10	-	10	10	3	0.135	0.027	100
F6	5	-	12	-	10	10	3	0.135	0.027	100
F7	5	-	-	1	10	10	3	0.135	0.027	100
F8	5	-	-	2	10	10	3	0.135	0.027	100
F9	5	-	-	3	10	10	3	0.135	0.027	100

Physicochemical evaluation of the gels

Visual appearance and cosmetic qualities. All prepared gels were physically inspected for clarity/transparency, color, odor, texture, consistency, and homogeneity after they have been filled intoglass jars. The prepared gels were also evaluated for the presence of particles or aggregates.

pH. The pH of each gel formulation was measured using a pH meter (model PH-210, HANNA instruments, Portugal) which has been pre-calibrated with standard buffer solutions of pH 4, 7 and 10. The samples were in contact with the pH electrode until the reading stabilized. The electrode was thoroughly rinsed with DW between each determination to remove all traces of the sample. The averages of three readings wererecorded as pH values.

Drug content determination. Specific quantity (100 mg) from each prepared gel was withdrawn at random from three different sampling points i.e., from the upper, middle and lower portionsof each batch and dissolved in 50 ml of phosphate buffer saline (PBS, pH 7.4) and 2.5 ml aliquot was diluted in 25 ml volumetric flask. The volumetric flask containing the gel solution was vigorously mixed to ensure homogenous dispersion of the formulation ingredients. This solution was filtered and the concentration was estimated spectrophotometrically at 221 nm using pH 7.4 PBS as blank. The drug content was determined from standard calibration curve of ibuprofen in pH 7.4 PBS.

Rheological studies. The viscosities of the gel formulationsat different concentrations were determined at room temperature by a rotational viscometer (Kinematica, AG, Type ViscostarPlus L, Switzerland) using different spindle numbers L1, L2, L3, and L4 at different shear rates: 0.5, 1, 5, 10, 20, 30, 50, 60, 100 and 200 rpm. The gels were placed in the sample holder and the suitable spindle selected was carefully lowered perpendicularly into the sample such that the spindle does not touch the bottom of the container. The spindle was attached to the viscometer and then it was allowed to rotate at a defined speed at room temperature to obtain stable viscosity reading.

Gel clarity. Gel clarity was determined by placing a sample of polymer gel or gel preparation in a disposable cuvette and measuring the absorbance at 700 nm using a

spectrophotometer (CECIL, 1021, 1000 series, England) against water as blank[11].

Spreadabilityof ibuprofen gel formulations. Concentric circles of different radii were drawn on graph paper and a 400 cm^2 glass plate was fixed onto it. Gel (1.0 g) was transferred to the centre of the lower plate and spread over a diameter of 2.4 cm. Another 400 cm^2 glass plate of 185 g was placed gently on the gel and a standardized weight of 170 g was allowed to rest on the upper glass plate for 3 min. The increase in the diameter due to gel spreading was recorded[17].

Extrudability. The method adopted for evaluating gel formulation for extrudability was based on the quantity in percentage of gel extruded from tube on application of certain load. The formulation under study was filled in a clean, lacquered aluminum collapsible tube with a nozzletip of 5 mm opening. It was then placed between two glass slides and was clamped. Extrudability was determined by weighing the amount of gels extruded through the tip when a constant load of 1 Kg was placed on the slides. The percentage of gel extruded was calculated and grades were allotted (++++ excellent, if> 90% of gel extruded; +++ very good, if 80-89.9%; ++ good, if 70-79.9%; + fair, if 50-69.9% and 0 poor, if<50% of gel extruded)[18].

***In vitro*drug release study**

One gram gel sample of each formulation that corresponds to 50 mg of ibuprofen was carefully weighed and placed on cellulose acetate membrane (Sartorius, Goettingen, Germany) with an average pore size of 0.45 µm which was previously soaked in PBS (pH 7.4) for 60 min and fixed to one end and made water-tight with aid of rubber band in an apparatus consisting of cylindrical tube with both ends open, 100 mm in height, 12.9 mm outer diameter and 12.1 mm inner diameter (release area=115 mm^2) as a diffusion cell. The tubes were submerged in a 1000-ml beaker containing 400 ml PBS (pH 7.4) as receptor medium and equipped with stirring and temperature-controlling devices. The whole assembly was fixed insuch a way that the lower end of the cell containing the gel just touched (1-2 mm deep) the diffusion medium.The release test was carried out at a controlled stirring rate of 100 rpm to ensure sink condition and a temperature of 37 ± 1 °C by means of water jacket surrounding each cell. At 5, 15, 30, 60, 120, 180, 240, 300, 360, 480, 600 and 720 min, 5 ml of the buffer containing the released ibuprofen was withdrawn from the beaker and replenished by the same volume of fresh buffer at 37 ± 1 °C to maintain constant volume. The withdrawn sample was diluted to an appropriate volume and the absorbance was measured spectrophotometrically at 221 nm and the amount of ibuprofen released from the gel formulations was calculated based on the established standard calibration curve. Each sample was tested six times and mean values were taken[19].

Kinetics and mechanism of drug release

To analyze the mechanism of drug release from the gel preparations, the release data were fitted to the following equations[20]:

i. Zero – order equation:

$$Q = Q_o - K_o t \qquad \text{Eqn. 3}$$

where, Q_o is the amount of drug present initially, Q is the amount of drug remaining at time t, and K_o is the zero – order release rate.

ii. First – order equation:

$$\ln Q = \ln Q_o - K_1 t \qquad \text{Eqn. 4}$$

where, Q_o is the amount of drug present initially, Q is the amount of drug remaining at time t, and K_1 is the first – order release rate constant.

iii. Higuchi's equation:

$$Q = 2C_o\sqrt{(\mathrm{D}_{app} \times \mathrm{t}/\pi)} \qquad \text{Eqn. 5}$$

where, Q is the amount of drug released per unit area at time t (mg cm^{-2}), t is the time after the application (sec), C_o is the initial drug concentration in the donor chamber (mg cm^{-3}), π is a constant and D_{app} is the apparent diffusion coefficient ($cm^2\,sec^{-1}$); D_{app} is calculated from the slope K (apparent release rate) of the linear plot of Q versus $t^{1/2}$; hence,

$$\mathrm{D}_{app} = {K^2\pi}\Big/{4C_o{}^2} \qquad \text{Eqn. 6}$$

Anti-inflammatory activity study

Anti-inflammatory activity of the medicated gel formulations was performed using carrageenan-induced paw edema method. The mice of either sex weighing 30-45 g were fasted overnight, but water was allowed *ad libitum*. The animals were divided into six groups of five animals each. Group 1-3 (control) received non-medicated polymer gels prepared from 8% (w/w) CMES, 10% (w/w) CMCS, and 2% (w/w) Na-CMC by gently rubbing the gels on the plantar surface of the left hind paw;similarly, groups 4, 5 and 6 (test) received 0.25 g of the medicated ibuprofen gel formulations prepared from the corresponding concentrations of the polymers (F2, F5 and F8, respectively). After 1 h, 0.05 ml of 1% (w/w) suspension of carrageenan was injected into the left plantar surface of the hind paw of all groups using a syringe with needle (Shandong Zibo Shacchuan Medical Instrument Co., Ltd., China). The hind paw volume was measured at different time intervals for 5 h after carrageenan treatment using a plethysmometer(UGO BASILE, 7140, Italy). The percent inhibition in hind paw edema volume was calculated using the formula shown in Eqn. 7 and compared with those recorded for the control group.

$$\%\ Edema\ Inhibition = \left(1 - {V_t}/{V_c}\right) x\ 100 \qquad \text{Eqn. 7}$$

where, V_t-is mean edema volume of test, V_c-is mean edema volume of control[21].

Stability studies

Stability studies on selected formulations were carried out at different conditions, i.e., real time stability conditions (25 °C/60% RH), at accelerated conditions (40 °C/75% RH) in stability chamber (Binder ®, England) and in refrigerator (LG, South Korea) at 4 °Cfor 3 months. The gel formulations were then observed for different evaluation

parameters such as change in the consistency, color, odor, pH, drug contents and phase separation or syneresis and compared with the initial formulations stored at room temperature[22].

Statistical analysis

All data reported in this study are averages of triplicate determinations except for the *in vitro* drug release study (*n*= 6) and anti-inflammatory activity study (*n*= 5). Wherever appropriate, the data were subjected to statistical analysis using Instat +, V. 3.33 (Statistical Services Centre, The University of Reading, UK) and Origin®, version 7.0 SR0 (OriginLab Corporation, MA, USA) assisted by MS Excel 2007. In all cases, individual differences between *in vitro* drug release profile, viscosity of gels, anti-inflammatory study data and all other relevant data were evaluated using a Tukey's test for one-way analysis of variance (ANOVA). *P* value of less than 0.05 was considered to be evidence for a significant difference.

Results and discussion

Factors affecting degree of substitution

The DS is defined as the average number of substituents per AGU, the monomer unit of starch which indicates the amount of carboxymethyl group formed. Since each AGU contains three hydroxyl groups at carbon numbers 2, 3, and 6, the DS lies between zero and three[4,8].Under the same conditions of carboxymethylation, the DS values of the CMS obtained from *Enset* starch were higher than those of the CMS obtained from cassava starch at all reaction conditions investigated indicating that origin influences the DS (Table 3). Highest DS (0.926) was obtained for the *Enset* starch when the reactor content was run at 70 °C for 1 h using ISPA as reaction medium (E-I-70-1); on the other hand, the least DS (0.158) was obtained for cassava starch in methanol as reaction medium at the same reaction condition (C-M-70-1).

Table 3: Effects of starch source, solvent media, reaction temperature and time on DS

Reaction condition	Product behavior 10 min before removal from the reactor	% Carboxyl (mean ± SD)	DS (mean ± SD)	Properties of 6% CMS in water
E-M-70-1	Slightly stirrable	6.51 ± 0.180	0.255 ± 0.008	Slightly turbid and gel particles
C-M-70-1	Easily mixed and stirred	4.14 ± 0.360	0.158 ± 0.015	Paste/dispersion
E-I-70-1	Strong gummy product; not mixed	19.32 ± 0.453	0.926 ± 0.029	Stiff gel
C-I-70-1	Gummy product; not stirrable	16.90 ± 1.803	0.778 ± 0.010	Flowable gel
E-I-70-0.5	Product very firm; slightly mixed	11.03± 0.231	0.463 ± 0.017	Thin flowable gel
C-I-70-0.5	Product moderately firm; slightly mixed	9.74± 0.172	0.401 ± 0.008	Thin flowable gel
E-I-50-1	Product less firm; not easily mixed	6.86± 0.197	0.271 ± 0.001	Weak and easily flowable gel
C-I-50-1	Product less firm; less mixed	5.26± 0.276	0.203 ± 0.021	Weak and easily flowable gel

It is well known that the structural and physicochemical characteristics of starches are related to their botanical sources. Starch characteristics such as amylose content, branch chain length distribution of amylopectin, phosphate monoester, phospholipids and lipid contents affect their functional properties. The granule shape and size also affect the functional properties of starches[23]. The reported amylose content of *Enset* starch is 29%[9]and that of cassava starch is 16.1%[10]. Moreover, *Enset* starch has been shown to have normal granule size distribution with a mean particle size of 46 µm with characteristic morphology of somewhat angular and elliptical[9]while that of cassava starch is 12.71 µm showing spherical morphology[10].The large size differences between *Enset* and cassava starches indicate that the surface area of the latter starch granules is larger rendering more OH groups at the surface which may lead to higher DS. However, on the contrary, *Enset* starch has significantly larger proportion of amylose that contributes to higher rate and extent of reaction and hence DS.

The choice of organic solvent also had a significant influence on DS (Table 3).This could be due to the high ability of ISPA to dissolve the etherifying agent and enhance starch swelling and viscosity when compared to methanol. Moreover, an increase in temperature enhances solubility of the etherifying agent and facilitates both the swelling of the starch molecules and the diffusion of the reactants. The CMS which were prepared using ISPA formed gel and then gummy mass, making stirring difficult; and it was also difficult to remove the products. These observations are consistent with the report of Lawalet al[4]on carboxymethylcocoyam starch at higher temperatures. So, depending upon the DS and rheological properties, modified *Enset* and cassava starches obtained using reaction conditions E-I-70-1 and C-I-70-1were employed as gelling agents in subsequent investigations and designated as CMES and CMCS, respectively.

Physicochemical evaluation of gel formulations

Nine different gel formulations of ibuprofen were prepared using CMES, CMCS and Na-CMC as gelling agents. The physicochemical properties of the gel formulations are shown in Table 4. All the prepared gel formulations shared good and smooth homogeneous appearance, and pleasant smell which was attributed to the presence of alcohol in the formulations. The ibuprofen gel formulations spread smoothly on a clean even glass plate with minimum pressure without any solid or gritty particles. Furthermore, the physical appearance of the gel formulations F7, F8 and F9 were transparent while formulations of the modified starches (F1-F6) were translucent.

The pH values of the gel formulations ranged from 6.80±0.02-7.22±0.06 (*n*=3), within physiologically acceptable pH and, in principle, were devoid of any skin irritation[20,24,25].The uniform distribution of drug was confirmed by content uniformity studies. As depicted in Table 4, the ibuprofencontent in all gel formulations was found to range from 98.76±0.21-100.2±0.42% (*n*=3) of the theoretical value of 5%, w/w which is within the acceptable limits (95.0 to 105.0%)[6]and ensured the uniformity of the drug content in the formulations.

Based on visual observation and absorbance measurementat 700 nm, the clarity of the medicated CMS preparations was found to be much better than those of the corresponding polymer gels as depicted in Table 4. This could be due to the incorporation of TEA in the medicated gel formulations. TEA was incorporated to adjust the pH and to increase the solubility of the drug in the gel formulations[26,27]. However, it appears that TEA also has additional solubilizing effect on the polymer gel matrix. Formulations without TEA produced gels that were whitish cream with simultaneous precipitation of the drug as fine grit dispersions indicating the presence of chemical incompatibility due to pH variation. Further, it was observed that the absorbance readings of the gel formulations increased significantly with increasing concentration of the polymers showing that gel clarity was influenced by concentration and nature of polymer with the rank order of Na-CMC> CMES> CMCS.

The vehicle also plays a key role in the appearance, feel, and successful application of the topical preparations[28].Clear or translucent homogenous gels are often preferred by consumers. Increasing the ethanol content in all formulations precipitated the CMS and Na-CMC that led to the formation of two distinct phases, i.e., a clear solution and semi-solid layer.

Table 4: Physicochemical properties of the different ibuprofen gel formulations

Formula	Physical appearance	Ibuprofen Content (%)	pH	Extrud-ability	Spreading diameter (mm)	Absorbance at 700 nm	
						Polymer gel	Formulation
F1	Translucent	100.2 ± 0.42	7.17 ± 0.09	+++	88.16 ± 1.73	0.323 ± 0.009	0.203 ± 0.016
F2	Translucent	98.96 ± 0.12	7.21 ± 0.01	++	71.43 ± 1.73	0.386 ± 0.004	0.227 ± 0.006
F3	Translucent	99.32 ± 0.64	7.22 ± 0.06	+	53.98 ± 1.16	0.408 ± 0.003	0.301 ± 0.015
F4	Translucent	99.95 ± 0.53	6.86 ± 0.02	++	79.51 ± 0.58	0.456 ± 0.029	0.346 ± 0.009
F5	Translucent	99.81 ± 0.21	6.80 ± 0.02	+	69.32 ± 1.16	0.591 ± 0.019	0.421 ± 0.019
F6	Translucent	98.89 ± 0.49	6.83 ± 0.03	+	56.40 ± 0.58	0.718 ± 0.004	0.568 ± 0.014
F7	Transparent	99.95 ± 0.74	7.01 ± 0.02	+++	87.05 ± 1.16	0.018 ± 0.002	0.017 ± 0.000
F8	Transparent	99.60 ± 0.21	6.98 ± 0.09	++	70.23 ± 1.73	0.022 ± 0.001	0.021 ± 0.000
F9	Transparent	98.76 ± 0.21	7.19 ± 0.09	+	55.74 ± 2.31	0.025 ± 0.001	0.024 ± 0.000

N.B. numerical results are given as mean±SD; +++ very good, ++ good, + fair

The increase in diameter of ibuprofen gel formulations following the spreadability test was found to range from 53.98±1.16 –88.16±1.73 mm (*n*=3). As the concentration of the polymer increased, the spreadability of the formulation decreased. As depicted in Table 4, F1, F4 and F7 showed the highest spreadability among the respective polymers: CMES, CMCS and Na-CMC depending upon the concentration of the polymers. Spreading of topically applied products affect drug delivery as reflected by dermatopharmacokineticstudies[26]. Formulations with higher spreadability values allow ease of application and thereby increased surface area available for drug permeation. A good gel takes less time to spread and will have high spreadability[29,30].

Viscosity is an important physical parameter for characterizing gels as it affects the extrudability, spreadability, release of drug and other physicochemical properties[21].As shown in Fig. 1, the gel formulations showed decreasing viscosity with increased shear rate from 0.5 to 200 rpm exhibiting a shear-thinning behavior. Moreover, ibuprofen gels prepared from CMES had significantly higher viscosity values than those of CMCS, both set of formulations exhibiting increased viscosity with increasing polymer concentration.

The apparent viscosity values of the CMS gels were found to be significantly higher when compared with the corresponding concentrations of the medicated formulations as depicted in Fig. 2.These results might be attributed to the relatively higher concentrations of ethanol and propylene glycol present in the medicated gel formulations that decrease the relative solubility and swellability of the polymers compared to the pure polymer gels[31].

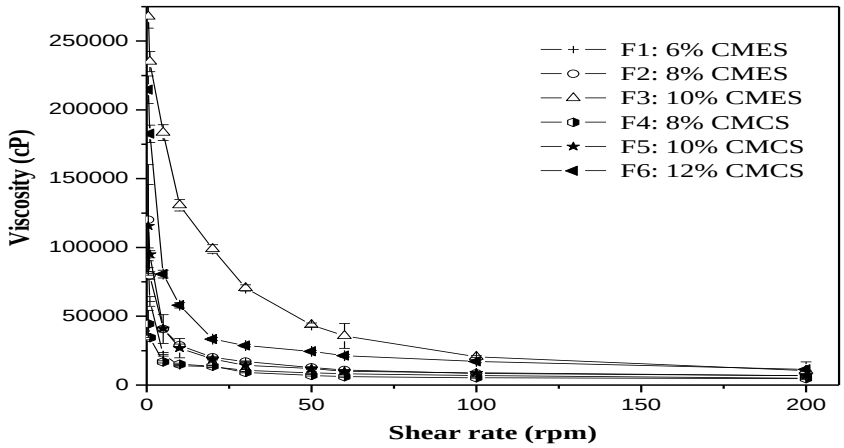

Figure 1: Plot of viscosity vs. shear rate for F1-F6 medicated gel formulations

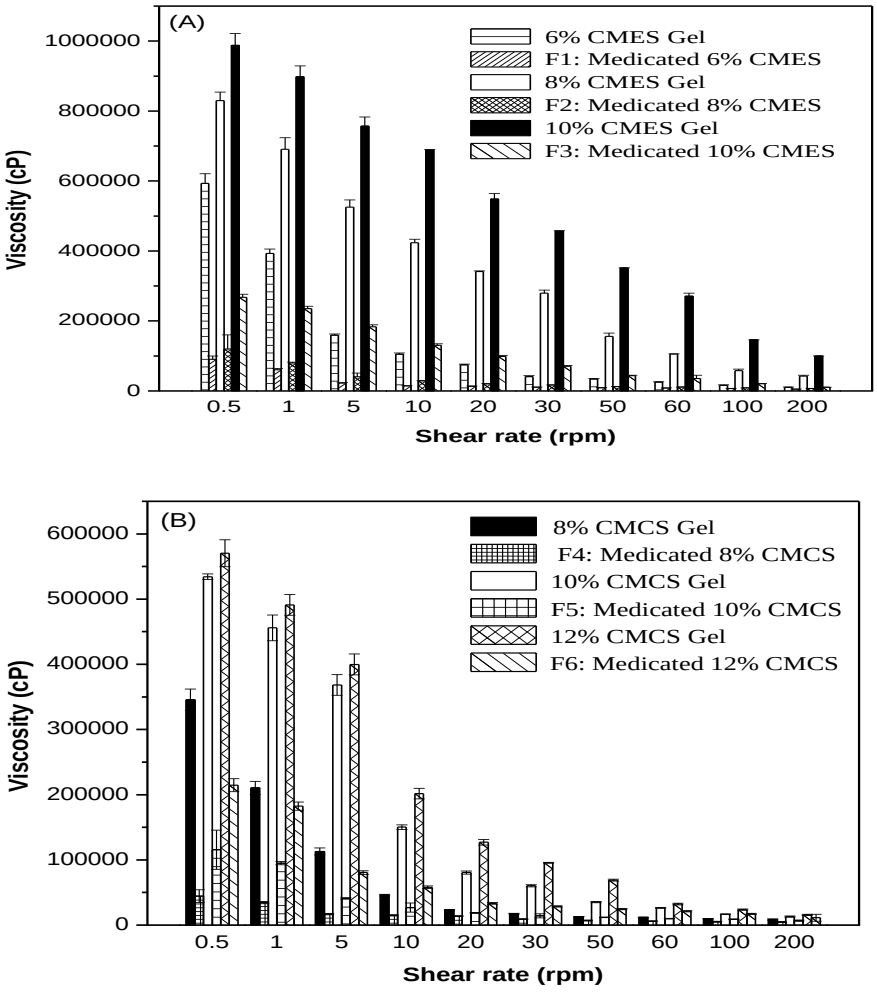

Figure 2: Viscosities of (A) CMES gels and corresponding medicated gel formulations (F1, F2 and F3), and (B) CMCS gels and corresponding medicated gel formulations (F4, F5 and F6) at different shear rates at room temperature.

***In vitro*drug release from gel formulations**

Fig. 3 shows the release profile of ibuprofen from the nine gel formulations using synthetic cellulose acetate as diffusion membrane. The results show that as the concentration of CMES, CMCS and Na-CMC in the gel formulations increased from 6-10% (w/w), 8-12% (w/w) and 1-3% (w/w), the percent of ibuprofen released into the buffer medium gradually decreased from 84.5 to 43.8 %/cm^2, 77.6 to 50.6 %/cm^{2}and 69.1 to 52.4 %/cm^2, respectively. This decrease in the release is likely attributed to increased micro-viscosity, i.e., the environment through which the solute molecules travel as the polymer concentrations increase[32,33].

Ricci et al.(2005) reported that drug release from gels is controlled by gel dissolution and drug diffusion from the gel matrix[34]. In the diffusion process, there are believed be two steps: one is diffusion through the gel, and the other is diffusion through the membrane.The diffusion

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measurements obtained are thus a combination of both these processes[35]. It is possible that at higher polymer concentrations, the active substance will be trapped in smaller polymer cells as it will be structured by its close proximity to the polymer molecules and this increases the diffusional resistance.Rheological properties are known to be related to the gel structure, inter-chain interactions, and polymer chain entanglements, which in turn affect drug release and diffusion processes[36,37]. Further, when the drug diffusion through the vehicle is a rate limiting step, the viscosity of vehicles may play an important role in controlling the drug release[38].

From the release profiles depicted in Fig. 3, it is observed that, initially (~1 h), drug was released rapidly (burst effect) followed by a slow release for the rest of the release study period. The initial burst effect could be due to the release of the drug to the surface of the immediate barrier membrane. Thus, the molecule that is easily accessible to the solvent at the interface immediately diffuses into the receiver compartment establishing a burst effect. As the time advances, there exist greater resistance to the penetration of the solvent to the inside of the gel matrix resulting in a slower release rate thereby exhibiting retarded and steady release profile.

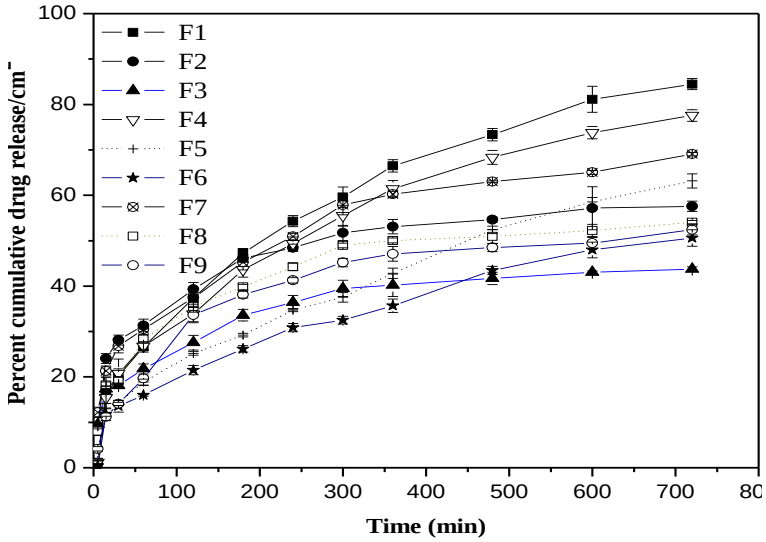

Figure 3: Release profiles of ibuprofen from gel formulations through synthetic cellulose acetate membrane.

Drug release kinetics

The drug release data were fitted to zero order, first order and Higuchi kinetic models and analysed. The results show that the Higuchi kinetic model best fitted the 12 h release profile as confirmed by the highestcorrelation coefficients shown in Table 5. This finding indicates that the rate-controlling mechanism in the drug release process is the diffusion of the dissolved drug through the vehicle network to the external medium, which is in agreement with other studies[20,28,39].

In addition, the drug diffusion coefficient (*D*) was found to be affected by polymer content and nature of the polymer used.The diffusion coefficient values calculated from the Higuchi plots for the different formulations were found to decrease inversely as a function of polymer concentration. The apparent release rate of ibuprofen from F1 (12.7 ± 0.037 mg cm^{-2} $h^{-1/2}$) is the largest of all the formulations and about 2.4 times higher than F3 which has the smallest release rate (5.4 ± 0.018 mg cm^{-2} $h^{-1/2}$) ($p<0.05$). Drug release rate constant of F4 (11.6 ± 0.015 mg cm^{-2} $h^{-1/2}$) is significantly higher when compared to F5 (9.1 ± 0.025 mg cm^{-2} $h^{-1/2}$) and F6 (7.3 ± 0.021 mg cm^{-2} $h^{-1/2}$) ($p<0.05$). These findings support the fact that the release rate is influenced by the rheological properties of the formulations as well as the nature of the polymer composing the vehicle.

Table 5: Kinetic model fitting of the*in vitro* ibuprofen release profiles of the gel formulations.

Formula	R^2			K (mg cm^{-2} $h^{-1/2}$) (mean ± SD)	(D, cm^2 sec^{-1}) x 10^{-5} (mean ± SD)
	Zero-order	First-order	Higuchi		
F1	0.894	0.935	0.986	12.7 ± 0.037	1.48 ± 0.012
F2	0.722	0.753	0.893	6.9 ± 0.047	0.44 ± 0.019
F3	0.772	0.788	0.931	5.4 ± 0.018	0.25 ± 0.003
F4	0.889	0.926	0.984	11.6 ± 0.015	1.23 ± 0.002
F5	0.945	0.966	0.985	9.1 ± 0.025	0.75 ± 0.005
F6	0.919	0.940	0.982	7.3 ± 0.021	0.46 ± 0.004
F7	0.844	0.872	0.967	8.8 ± 0.031	0.72 ± 0.009
F8	0.747	0.771	0.916	7.7 ±0.014	0.54 ± 0.002
F9	0.758	0.782	0.921	7.1 ± 0.017	0.43 ± 0.003

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Formulations F2, F5 and F8 were selected from the ninemedicated gel formulations for furtheranti-inflammatory activity as well as stability studies on the basis of physicochemical parameters evaluation and their representativeness.

Anti-inflammatory activity:

In control groups which received carrageenan alone, rapid andcontinuous increase in paw volume (i.e., inflammation) was observed andthe inflammation was sustained during the entire period of study.The test products (F2, F5 and F8) exhibited anti-inflammatory activity up to 5 h (Fig. 4). The highest anti-inflammatory activity was obtained in F5 (78 ± 0.392%), followed by F8 (68 ± 0.459%), and then F2 (59 ± 0.415%) after the 5thh study. The variation of activities could partly be attributed to the variation in the rate and extent of drug release as discussed in the *in vitro* release study.As shown in Table 5, F5 had the highest drug release rate and diffusion coefficient which contributed to itshighest anti-inflammatory effect.

Stability studies

None of the selected ibuprofen gel formulations (F2, F5 and F8) showed any appreciable change in gel clarity, physical appearance and homogeneity indicating physical stability when inspected at intervals of 1, 2, and 3 months as depicted in Table 6. Furthermore, no obnoxious odor was perceptible from any of the gel formulations. Even after exposure to heat and humidity, no significant change was observed in pH and drug content of the gel formulations. Besides, there was no appearance of air bubbles and crystals or precipitates during the stability study. Thus, all gel formulations were found to be stable under all storage conditions.

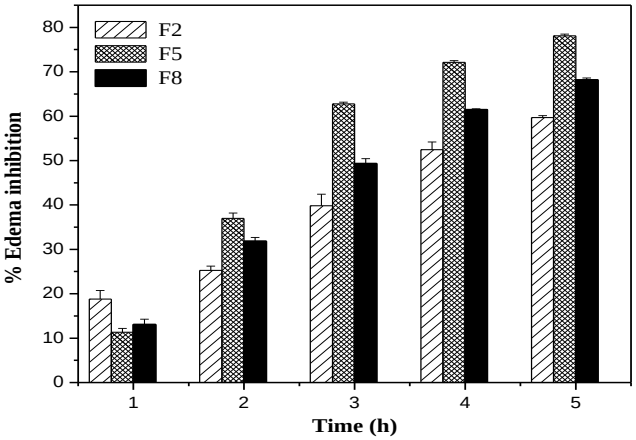

Figure4. Percent inhibitions of hind paw edemafollowing the application of ibuprofen gel formulations.

Table 6: Physicochemicalproperties of ibuprofen gel formulations after 3 months storage under different conditions

Formula	Storage conditions	Color change, precipitate formation	Ibuprofen content (%)	Physical appearance	Homog-eneity	pH
	25 ºC /60 % RH	Nil	99.62 ± 0.53	Translucent	Good	7.17 ± 0.02
F2	40 ºC /75 % RH	Nil	98.72 ± 0.34	Translucent	Good	7.28 ± 0.01
	4 ºC	Nil	98.53 ± 0.67	Translucent	Good	7.24 ± 0.06
	25 ºC /60 % RH	Nil	99.85 ± 0.37	Translucent	Good	6.96 ± 0.04
F5	40 ºC /75 % RH	Nil	99.31 ± 0.51	Translucent	Good	6.88 ± 0.03
	4 ºC	Nil	99.11 ± 0.47	Translucent	Good	6.86 ± 0.02
	25 ºC /60 % RH	Nil	99.71 ± 0.52	Transparent	Good	7.07 ± 0.05
F8	40 ºC /75 % RH	Nil	99.46 ± 0.37	Transparent	Good	7.15 ± 0.09
	4ºC	Nil	98.58 ± 0.31	Transparent	Good	7.11 ± 0.08

CONCLUSIONS

Carboxymethyl starches of *Enset* and cassava were prepared as a product of the reaction of starch and monochloroacetic acid in the presence of sodium hydroxide at different reaction conditions. Subsequently, nine topical gel formulations of ibuprofen were prepared based on the concentration and type of polymers as gelling agents using carboxymethyl*Enset* starch (CMES), carboxymethyl cassava starch (CMCS) and sodium carboxymethylcellulose (Na-CMC).The results showed that carboxymethylation was significantly affected by the source of starch, reaction medium, reaction temperature as well as reaction time. Isopropyl alcohol offered a better medium for carboxymethylation compared to methanol. The drug release data of all gel formulations best fitted to Higuchi model when compared to zero order and first order kinetics following *in vitro* drug release studies over 12 h. The *in vitro* drug release results indicated that the cumulative drug release, the apparent release rate, and the diffusion coefficient of ibuprofen were influenced not only by the rheological properties of the formulations but also by the nature of the polymers. The anti-inflammatory activities of the medicated gel formulations in mouse hind paw edema model revealed that ibuprofen was delivered to the inflammation site at adequate level to significantly lower inflammation induced by carrageenan injection. The medicated gels also showedexcellent stability profile for at least 3 months under different storage conditions.

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