

## RESEARCH ARTICLE

# Evaluation of carboxymethylated *Plectranthus edulis* starch as a suspending agent in metronidazole benzoate suspension formulations

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## Abstract

Some excipients are currently available for the formulation of pharmaceutical suspensions. The objective of this study is to develop cheap and effective starch-based excipient that can be used as an effective alternative for the formulation of pharmaceutical suspensions. Carboxymethylated *Plectranthus edulis*, Vatke (*P. edulis*) [fam., Lamiaceae], starch was evaluated as a suspending agent in metronidazole benzoate suspensions in comparison with sodium carboxymethyl cellulose (NaCMC) at a concentration range of 1–4% (w/v). The resulting suspensions were evaluated for their sedimentation volume (%), degree of flocculation, rheology, redispersibility, and dissolution rate. Stability studies were performed for 3 months. The apparent viscosities of the formulations prepared with carboxymethylated *P. edulis* starch at reaction condition E (CMPS-E) was significantly lower than that of NaCMC ( $p < 0.05$ ). The flowability of the suspensions, at all concentration levels of the suspending agents, were in the order of CMPS-E > NaCMC. AT 1% concentrations, carboxymethylated *P. edulis* starch ( $76 \pm 1.5\%$ ) provided significantly higher ( $p < 0.05$ ) sedimentation volume than NaCMC ( $40 \pm 1.5\%$ ). At 3% and 4%, both gave comparable sedimentation volume (100%). Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) employed as a flocculating agent significantly increased ( $p < 0.05$ ) the sedimentation volume of the suspensions prepared with carboxymethylated *P. edulis* starch and NaCMC. The redispersibilities of CMPS-E were better than those of NaCMC. All suspensions showed a release of greater than 85% of drug within 1 h. The results of stability studies showed that all suspension formulations were stable. From the foregoing, it can be concluded that carboxymethylated *P. edulis* starch could be used as an alternative suspending agent.

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## Introduction

Excipients are additives, used to convert active pharmaceutical ingredients into a pharmaceutical dosage form suitable for administration to patients. New and improved excipients continue to be developed for conventional drug delivery systems and also to meet the needs of modern

and better formulations. Starch is the most commonly used excipient in the manufacturing of different pharmaceutical dosage forms.

Some of the Ethiopian plants which have been shown to possess starch of commercial value include Enset [1]; Ethiopian Yam [2]; Godare [3]; Anchote [4]; Cassava [5] and Kottee Harree [6]. *P. edulis* is locally known by various vernacular names such as *Wolaita donuwa*, *Dincha Oromo*, *Gurage* potato, *Agew* potato and generally, as Ethiopian potato [7–8]. The plant was one of the traditional root crops, especially to Wolaita and Hadiya people, before the introduction of other tuberous plants like cassava and sweet potato to the region. It is cultivated in mid and high altitudes (1880 to 2200 meters above sea level) in the north, south and western Ethiopia primarily for its edible tubers [7, 9]. Starches are inherently unsuitable for most applications and, therefore, must be modified to enhance their positive attributes and/or to minimize their defects. The inherent objectionable characteristics of native starches include their poor aqueous dispersion and poor freeze-thaw stability [10]. Modified starches possess some unique properties, not found in natural starches, which are suitable for the development of new products. Examples are solubility in unheated water, specific changes in rheological profiles, lower gelatinization temperature, less retrogradation, and greater pH stability [11–12].

Several modified starches have been also used in the pharmaceutical field. Chemically modified starches have shown promise in the pharmaceutical industry as a disintegrant, thickening agent and sustained-release matrices. For example, starches substituted with groups like carboxymethyl [13–14] or hydroxypropyl [15] or acetate [16], and starches cross-linked by various agents such as epichlorohydrin and sodium trimetaphosphate have all retarded drug release from solid dosage forms at various levels [17]. Also, carboxymethyl starch showed the ideal properties of a gelling agent [18] and tablet disintegrants [12].

A large amount of pharmaceutical suspension marketed and prescribed in Ethiopia each year implies that a large quantity of suspending agent is required. Most of the suspending agents currently used in the preparation of such dosage form, for example, acacia, sodium alginate, sodium carboxymethylcellulose, tragacanth, and xanthan gum, are imported and are generally expensive. The development of new suspending agents based on domestically and abundantly available materials, such as starch, proposes a way to decrease the number of imported materials, and the price of the dosage form. It also benefits the cultivators of starch-containing plants. Furthermore, it may add value to the body of existing evidence in the literature as far as suspension is concerned.

The present work aimed was to modify *Plectranthus edulis* starch with monochloroacetic acid to prepare carboxymethylated starch and evaluate it as a pharmaceutical suspending agent in metronidazole benzoate suspension. NaCMC is the most widely commercially available pharmaceutical suspending agent and was included in the study for comparative purposes. Metronidazole benzoate, practically insoluble in solid, was selected as a model drug for this study.

## Materials and methods

### Materials

Fresh *P. edulis* tubers were purchased from the local tuber cultivating farmers (Shashigalle Kebele, Wolaita Zone, Ethiopia) at its maturity age (7 months after planting) and authenticated by the Department of Biological Sciences, College of Natural Sciences, Addis Ababa University. Metronidazole benzoate, propylene glycol (Research-lab fine Chem. Industries, India), NaCMC powder (Dharyal Polymers Pvt. Ltd, India), saccharin sodium (Tianjin Changjie Chemical Co, LTD, India), Methyl para hydroxybenzoic acid, propyl para hydroxybenzoic acid (BDH Chemicals Ltd, England), Sodium metabisulphite and hydrochloric acid

(Guangzhou Jinhaunda Chemical Reagent Co. Ltd, China), glacial acetic acid, methanol and isopropyl alcohol (Sigma-Aldrich, Germany), monochloroacetic acid (Hopkin & Williams Ltd, England), sodium hydroxide (Sigma-Aldrich, Sweden), potassium dihydrogen phosphate (Sørensen Leuren, Denmark), sodium chloride (Oxford Laboratory, Mumbai, India) silver nitrate (Hemanshu, chemicals, India) sorbitol (BDH Chemical Ltd, England), sucrose (Nolab Life Science Co, India) tween 80 (UNI-CHEM® chemical reagent, China) were used as received.

## Methods

**Starch isolation.** The extraction of starch from dried tubers of *P. edulis* was undertaken for the determination of the percent yield of starch from the tubers. The method employed in the extraction of starch from fresh *P. edulis* tubers was used here with some modifications [19]. The modifications being that after the tubers were reduced to small sizes, they were air-dried before grinding. The ground tuber flour was then weighed accurately and the same procedure was followed for the extraction of starch. The resulting starch was weighed and the percent yield noted.

**Chemical composition.** The following analytical determinations were performed in triplicate: fat by acid hydrolysis; ash by measurement of the residue left after combustion in a furnace at 550°C; and Protein was estimated from the determination of N<sub>2</sub> by elemental analysis using Carlo Erba Elemental Analyser, Mod. 1106. A conversion factor of 6.25 was used for the estimation [20].

**Carboxymethylation of *Plectranthus edulis* starch.** Carboxymethylated *Plectranthus edulis* starch (CMPS) was prepared following the method described by Nattapulwat *et al.*, [12] with slight modification at different reaction conditions as depicted in Table 1. The modified starch was dried in an oven (Kottermann® 2711, Germany) at 50°C for 24 h. The dried modified starch was milled and passed through a sieve size of 224 µm.

**Determination of degree of substitution.** The degree of substitution (DS) was determined according to the method described by Nwokocha and Ogunmola [21]. The dried modified starch (0.25 g) was weighed into a 250 ml conical flask and then 100 ml of distilled water was added, followed by 10 ml of standard NaOH solution. This was heated over a boiling water bath (GFL®, D3006, Germany) for 20 min. When a clear solution resulted, the hot solution was titrated with a standard HCl solution to a phenolphthalein endpoint. Native starch processed similarly as above was used as a correction factor for the blank. Each sample analysis

**Table 1. Reaction conditions used for carboxymethylation of *Plectranthus edulis* starch.**

Reaction condition	Reaction media (80% v/v)	nMCA/nAGU	nNaOH/nAGU	Reaction time (h)	Reaction T° (°C)
A	ISPA	1	3	4	50
B	ISPA	1.5	3	4	50
C	ISPA	2	3	4	50
D	ISPA	1	2	4	50
E	ISPA	1	2.5	4	50
F	ISPA	1	3	2	50
G	ISPA	1	3	3	50
H	ISPA	1	3	4	30
I	ISPA	1	3	4	40

ISPA: isopropyl alcohol, nMCA/nAGU: molar ratio of mono chloroacetic acid to anhydrous glucose unit, nNaOH/nAGU: molar ration of sodium hydroxide to anhydrous glucose unit, T°: temperature.

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was carried out in triplicate and values were averaged. The percentage of carboxyl was calculated as follows:

$$\%Carboxyl = \left[ \frac{(Vb - V)}{Wt} \right] \times M_{NaOH} \times 0.045 \times 100 \quad (1)$$

where, Vb (ml) is the volume of HCl used for the titration of the blank; Wt is weight in a gram of sample or native starch; V (ml) is the volume of HCl used for titration of the sample,  $M_{NaOH}$  is the molarity of NaOH.

The DS was calculated as follows:

$$DS = \frac{162 \times \%Carboxyl}{[4500 - (58 \times \%Carboxyl)]} \quad (2)$$

**Preparation of metronidazole benzoate suspensions.** The composition of the metronidazole benzoate suspensions is given in Table 2. The suspensions are grouped into two: FCMC (for NaCMC), and FCMPSE (for Carboxymethylated *Plectranthus edulis* starch at reaction condition E). Each group has four formulations (FCMC1-FCMC4, and FCMPSE1-FCMPSE4). The letters FCMC, and FCMPSE indicate the type of suspending agent, while the numbers 1, 2, 3, and 4 represent the percent concentration of the suspending agents. For the preparation of metronidazole benzoate suspensions, polymer powder and metronidazole benzoate were triturated together with 10 ml of a solution containing 15 g sucrose, 0.05 g Tween 80, and 0.07 g sodium saccharine to form a smooth paste. 70% sorbitol solution (30 ml) was added gradually with constant trituration followed by preservative solution (methylparaben and propylparaben) in propylene glycol. The mixture was transferred into an amber-colored bottle, made up to volume with distilled water and then shaken vigorously for 5 min [22].

**Rheology of the suspensions.** The effect of suspending agent concentration on viscosity was studied using a vibrational viscometer (VIBRO Viscometer, SV-10, Japan). The viscosities of the suspensions were measured in mPas within 24 hrs after preparation. The measurement was made at a constant shear rate and—room temperature (20 °C). Apparent viscosities recorded are average of three determinations.

**Flowability of the suspensions.** The flow rates of the suspensions were measured based on the method described elsewhere [23]. The time required for each 10 ml suspension sample

**Table 2. Compositions of metronidazole benzoate suspension formulations.**

Formulation ingredients	Composition
Metronidazole benzoate	6.43 (% w/v)
Suspending agents*	1,2,3 and 4% (w/v)
Methyl paraben	0.18 (% w/v)
Propyl paraben	0.02 (% w/v)
Propylene glycol 2	2 (% v/v)
Tween 80	0.05 (% v/v)
Sodium saccharine	0.07 (% w/v)
Sucrose	15 (% w/v)
Sorbitol	(70%, w/v) 30 ml
Distilled water	Qs 100 ml

\*The suspending agents used were sodium carboxyl methyl cellulose, and Carboxymethylated *Plectranthus edulis* starch each at the specified concentrations.

Qs: Quantity sufficient

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to flow through a 10 ml pipette was used to calculate the flow rate using Eq 3. Flow rates recorded are average of three determinations.

$$\text{Flow rate} = \frac{V_s}{T} \quad (3)$$

Where  $V_s$  volume of sample in the pipette (in ml) and  $T$  is time (in a sec) required for the 10 ml suspension to—elute out of the pipette?

The suspensions are classified as low viscosity, intermediate viscosity and high viscosity based on their rate and extent of flow out of the pipette. If the suspension—comes out of the pipette, with the aid of only gravitational force, it is considered as low viscosity for which flow rates are to be calculated. If it partly comes out, it is considered as having intermediate viscosity. If, however, the suspension does not—come out of the pipette, it is considered as high viscosity.

**Sedimentation volume.** Twenty ml of each of the formulations was poured into 25 ml of graduated measuring cylinder and kept at room temperature. The sedimentation volumes (%) of the formulations were noted every day for the following seven days after preparation. The readings of the sedimentation volumes (%) were taken when the particles settle down and the cloudy supernatant started to clear up on descending from the top surface of the suspension. The results recorded are averages of three determinations.

**Degree of flocculation.** The degree of flocculation was evaluated according to the method described by Kumar *et al.*, [24] using the following equation.

$$B = \frac{V_u}{V_\infty} \quad (4)$$

Where  $B$  is the degree of flocculation,  $V_u$  is the total volume of flocculated suspension and  $V_\infty$  is the ultimate volume of the deflocculated suspension. To determine the degree of flocculation, flocculated suspensions were prepared using 0.004 M  $\text{KH}_2\text{PO}_4$  as a flocculating agent.

## Redispersibility of suspensions

The redispersibility of suspensions was evaluated according to a method described elsewhere [25]. A 20 ml of each suspension formulation was poured into a 25 ml measuring cylinder and allowed to settle for a week and up to one month. The measuring cylinders were then gently rotated at  $180^\circ$ . The formulations were evaluated based on the number of turns (complete cycles) required to uniformly redisperse the sedimented metronidazole benzoate particles throughout the suspension. Results recorded are averages of three determinations.

**Dissolution of the suspensions.** The dissolution studies were performed following the method described by Zietsman *et al.*, [26] which is based on the United States Pharmacopeia (USP) monograph for metronidazole tablets, with modification of the method to include apparatus II (ERWEKA, DT600, Germany). The dissolution medium was 0.1N HCl (900 mL), and the paddle rotation speed was 100 rpm. Samples (5 ml) were withdrawn at 10, 20, 30, 45, and 60 min. Dissolution samples were assayed by UV spectrophotometer (CECIL, 1021, 1000 series, England) at 232 nm. Results recorded are averages of six determinations.

**Assay of suspensions.** The suspensions were assayed for total metronidazole benzoate concentration using the High-performance liquid chromatography (HPLC) method described in British Pharmacopeia (BP) [27]. A sample (5 ml) of each metronidazole benzoate suspension was dissolved in 1 ml of dimethylformamide and mixed with 150 ml of methanol; sufficient water was added with mixing to produce 250 ml mixture. The mixture was cooled by running tap water and then centrifuged. One milliliter was diluted to 10 ml with methanol

(60%). These samples were then analyzed in triplicate using HPLC (SHIMADZU, CBM-20A, Japan). The chromatographic procedure was carried out using (a) a stainless-steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 μm), (b) as a mobile phase a mixture of 40 volumes of a 1.25% w/v solution of ammonium acetate, adjusted to pH 7.0 with dilute acetic acid, and 60 volumes of methanol at a flow rate of 1.0 ml per minute and (c) a detection wavelength of 310 nm.

**Stability studies.** Metronidazole benzoate suspensions were placed in the stability chamber and maintained at the real-time condition of 30°C/65% Relative humidity (RH), and accelerated condition of 40°C/75% RH and in a refrigerator at 4°C for 3 months. Samples were collected at 0, 30, 60 and 90 days. The analysis comprised testing of certain parameters, which could change during storage, such as pH, drug content, particle size (optical microscopy), physical appearance and odor. The appearance of each sample was evaluated by visual inspection of the suspension [28–29]. The suspensions were assayed for total metronidazole benzoate concentration using the HPLC method described in BP [27]. Results recorded are averages of triplicate determinations.

### Statistical analysis

ANOVA was carried out for the physical stability test of the suspensions from the three suspending agents using the computer software Origin version 8.5.1 (Origin Lab Corporation). Tukey test at a 95% confidence level ( $p < 0.05$ ) was used. At a 95% confidence interval,  $p$  values less than or equal to 0.05 were considered significant.

## Results

### Starch isolation

The starch yield on a dry weight basis was found to be  $85.74\% \pm 1.45$ . Thus, starch is the major constituent of *P. edulis* tuber.

### Chemical composition

The chemical composition of the *P. edulis* starch on dry weight basis was found to be 0.14% ± 0.01 ash, 0.21% ± 0.03 fat, 0.43% ± 0.03 protein, and 99.2% ± 0.02 starch.

### Carboxymethylation of *Plectranthus edulis* starch and degree of substitution

In this study, the effects of the parameters including the amount of MCA and NaOH, time of reaction, and temperature on DS were investigated. In the reaction, different quantities of MCA (the molar ratios of MCA to AGU ranging from 1 to 2) were used while the other conditions were kept constant. The DS increased significantly ( $p < 0.05$ ) with increasing MCA/AGU ratio from 1 to 1.5. However, at higher MCA/AGU ratio (2), the DS decreased significantly ( $p < 0.05$ ).

Different quantities of NaOH (the molar ratios of NaOH to AGU ranging from 2 to 3) were used, while other reaction conditions were kept constant. The increase in the molar ratio of NaOH to AGU from 2 to 2.5 significantly ( $p < 0.05$ ) increased the DS. But, with the increase in the molar ratio of NaOH/AGU from 2.5 to 3, there was no significant increase in the DS. A significant increase ( $p < 0.05$ ) in DS was observed as the duration of the reaction increased within the time frame studied (2–4 h), while the other conditions were kept constant. The degree of substitution increases significantly ( $p < 0.05$ ) with an increase in temperature from 30 to 50°C.



The DS of the modified starches is presented in Table 3. The DS values obtained for the CMPS ranged between 0.285 and 1.06. The lowest DS value was obtained at reaction conditions C and H and the highest DS value was obtained at reaction conditions B and E.

### Rheology of the suspensions

The viscosity of the suspension is a factor of great importance for the stability and pourability of suspensions. As the viscosity of the suspension increases, the terminal settling velocity decreases. Thus, the dispersed phase settles at a slower rate and remains dispersed for a longer time yielding higher stability to the formulated suspension. The viscosity of the suspension formulations was measured at 20 rpm and the result is presented in (S1 Fig). The viscosity of the suspension formulations prepared with different concentrations of CMPS-E and NaCMC -was significantly ( $p < 0.05$ ) different from each other.—Viscosity was directly proportional to the concentration of the suspending agent in both formulations as indicated in Fig 1. The apparent viscosity of the formulations prepared with CMPS-E was significantly ( $p < 0.05$ ) lower than that of NaCMC.

### Flowability of the suspensions

The flowability of suspension is important for the uniformity of dosing. As the flowability of the suspension is optimum, the dispersed phase remains dispersed for a longer time yielding a better distribution of the drug throughout the formulated suspension. The flow rates of formulations prepared with CMPS-E and NaCMC as suspending agents decreased with increased concentration of the suspending agents. The flow rates of the formulations are presented in Table 4. The flowability of the suspensions, at all concentration levels of the suspending agents, were in the order of CMPS-E > NaCMC.

### Sedimentation volumes of the suspensions

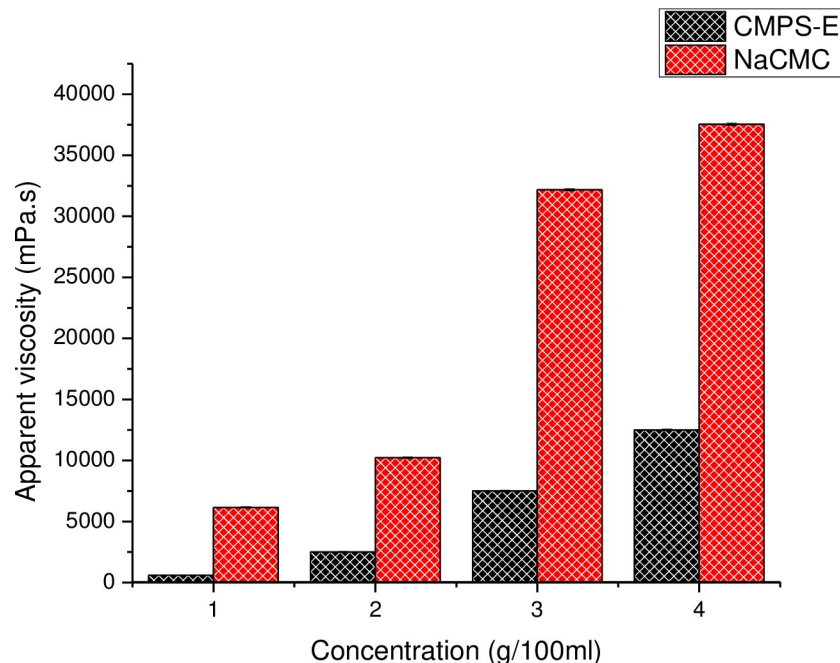
The sedimentation volumes (%) of the suspensions are depicted in (S2 Fig). Analysis of variance among the formulation showed that there was a significant difference ( $p < 0.05$ ) among the formulations. The results also showed that as the concentration of the suspending agents increased the sedimentation volume increased significantly ( $p < 0.05$ ) as depicted in Fig 2.

At lower concentration of the suspending agents (1%), FCMPS-E1 provided higher sedimentation volume than FCMC1 but at 2% concentration FCMPS-E2 had lower ( $p < 0.05$ ) sedimentation volume (94.71%) than FCMC2 (97.84%). At 3% and 4% concentration, both gave

**Table 3. Percent carboxyl, and degree of substitution (DS) of carboxymethylated *Plectranthus edulis* starch prepared under different reaction conditions (n = 3, mean  $\pm$  SD).**

Modified starch	% Carboxyl (mean $\pm$ SD)	DS (mean $\pm$ SD)
CMPS-A	12.600 $\pm$ 0.259	0.542 $\pm$ 0.018
CMPS-B	21.34 $\pm$ 0.315	1.06 $\pm$ 0.021
CMPS-C	7.188 $\pm$ 1.620	0.285 $\pm$ 0.038
CMPS-D	10.740 $\pm$ 0.022	0.437 $\pm$ 0.019
CMPS-E	21.34 $\pm$ 1.013	1.06 $\pm$ 0.038
CMPS-F	13.83 $\pm$ 0.103	0.606 $\pm$ 0.012
CMPS-G	12.600 $\pm$ 0.259	0.542 $\pm$ 0.018
CMPS-H	7.188 $\pm$ 0.089	0.285 $\pm$ 0.009
CMPS-I	13.83 $\pm$ 0.103	0.415 $\pm$ 0.000

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**Fig 1. Apparent viscosities of suspensions at various concentrations of suspending agents: sodium carboxyl methylcellulose (NaCMC), and Carboxymethylated *Plectranthus edulis* starch (CMPS-E) in metronidazole benzoate formulations.**

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comparable sedimentation volume although the suspensions of NaCMC were more viscous than those of CMPS-E.

### Degree of flocculation

The flocculation characteristics of metronidazole benzoate suspension formulations at 1% and 2% w/v of the suspending agents were studied using  $\text{KH}_2\text{PO}_4$  as a flocculating agent for a month and the results are presented in (S3 Fig). At 1% and 2% w/v concentration of FCMPSE and FCMC, there was a significant difference ( $p < 0.05$ ) in the degree of flocculation between the metronidazole benzoate suspension formulations. The results suggest that the sedimentation volume increased significantly ( $p < 0.05$ ) with a flocculating agent in the case of 1% w/v concentrations of FCMPSE and FCMC as shown in Fig 3.

### Redispersibility of suspensions

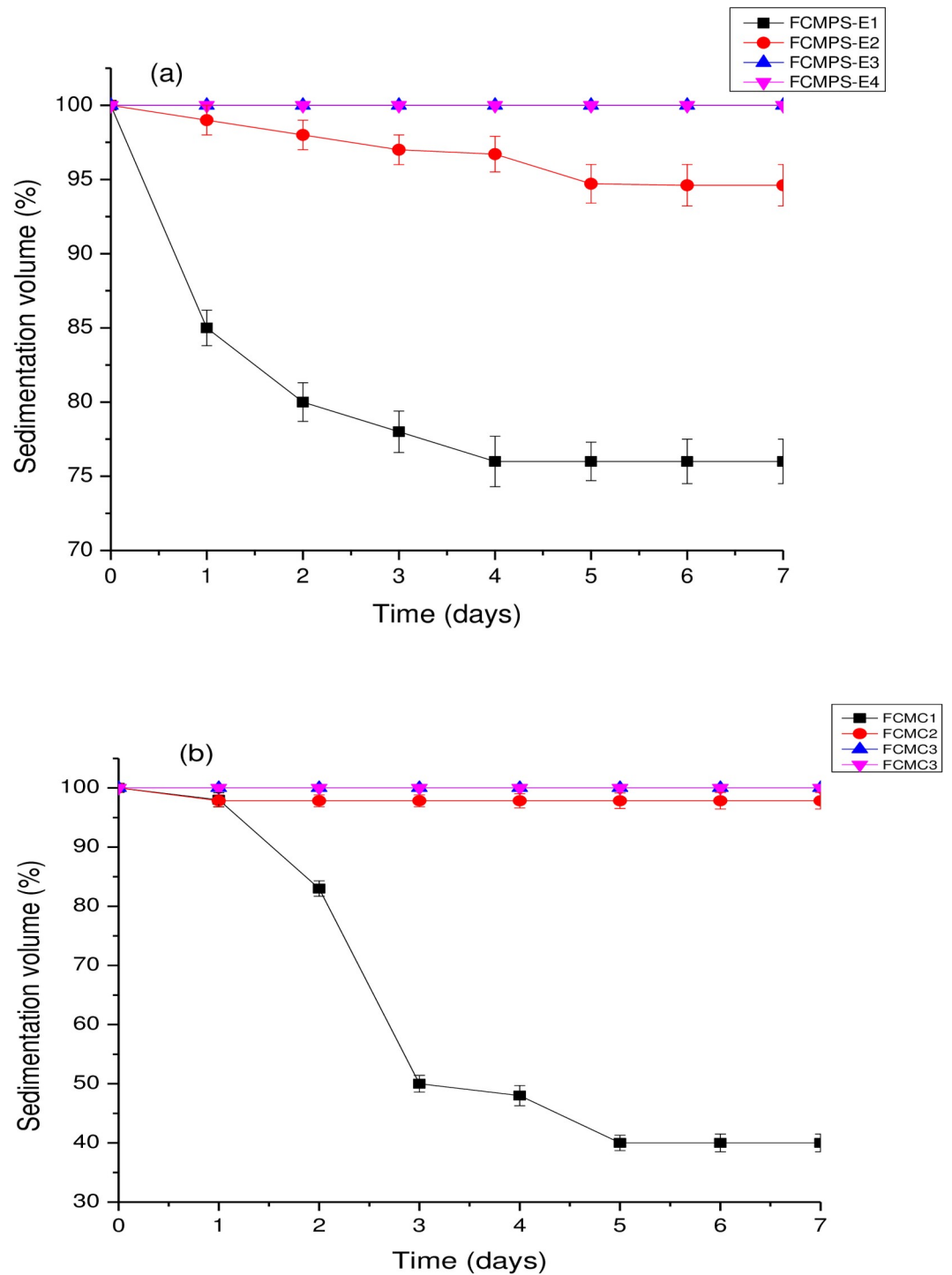
The number of inversions required to redisperse the formulation after a week and a month is given in Table 5. After a week, the number of inversions required to redisperse the suspension

**Table 4. The flow rate of the suspension formulations (n = 3, mean  $\pm$  SD).**

Suspending agent concentration (%)	Flow rate (ml/sec) (mean $\pm$ SD)	
	CMPS-E	NaCMC
1	0.626 $\pm$ 0.038	0.022 $\pm$ 0.014
2	0.379 $\pm$ 0.011	Intermediate viscosity
3	Intermediate viscosity	Very viscous
4	Very viscous	Very viscous

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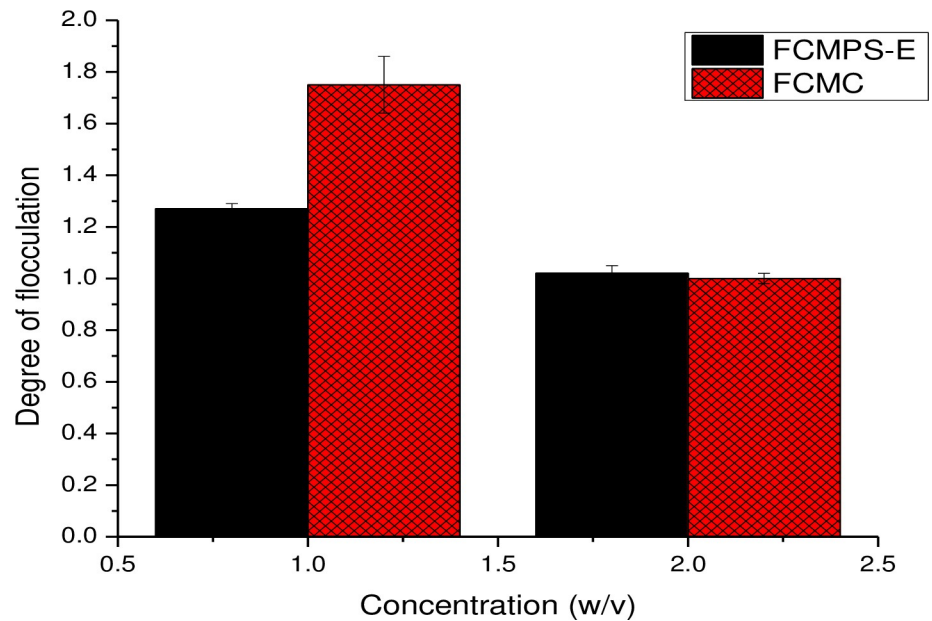




**Fig 2.** One week sedimentation volume (%) profiles of suspensions at different concentrations of the suspending agents, (a) carboxymethylated *P. edulis* starch at reaction condition E (FCMPS-E) and (b) sodium carboxyl methylcellulose (FCMC).

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formulations prepared with 1% (7 cycles) CMPS-E was significantly ( $p < 0.05$ ) lower than those prepared with and 1% (23 cycles) NaCMC. At the concentration of 2%, the number of inversions required for the formulation prepared with CMPS-E after a week (4 cycles) was



**Fig 3.** The degree of flocculation for metronidazole benzoate suspension formulations containing 1% and 2% w/v of suspending agents: carboxymethylated *Plectranthus edulis* starch (CMPS-E) and sodium carboxyl methylcellulose (FCMC).

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significantly ( $p < 0.05$ ) greater than that of NaCMC. At a concentration of 3% and 4%, both required no inversion after a week.

After a month, the number of inversions required to redisperse the suspension formulations prepared with 1% (14 cycles) CMPS-E was significantly ( $p < 0.05$ ) lower than those prepared with 1% (25 cycles) NaCMC. At the concentration of 2%, there was no significant difference ( $p > 0.05$ ) in the number of inversions required for redispersion for CMPS-E (6 cycles) and NaCMC (5.67 cycles). At a concentration of 3% and 4%, both required no inversion after a month as the formulations were completely suspended. The results revealed that there were significant differences among the formulations containing different concentrations of the same suspending agent ( $P < 0.05$ ).

### Dissolution of the suspensions

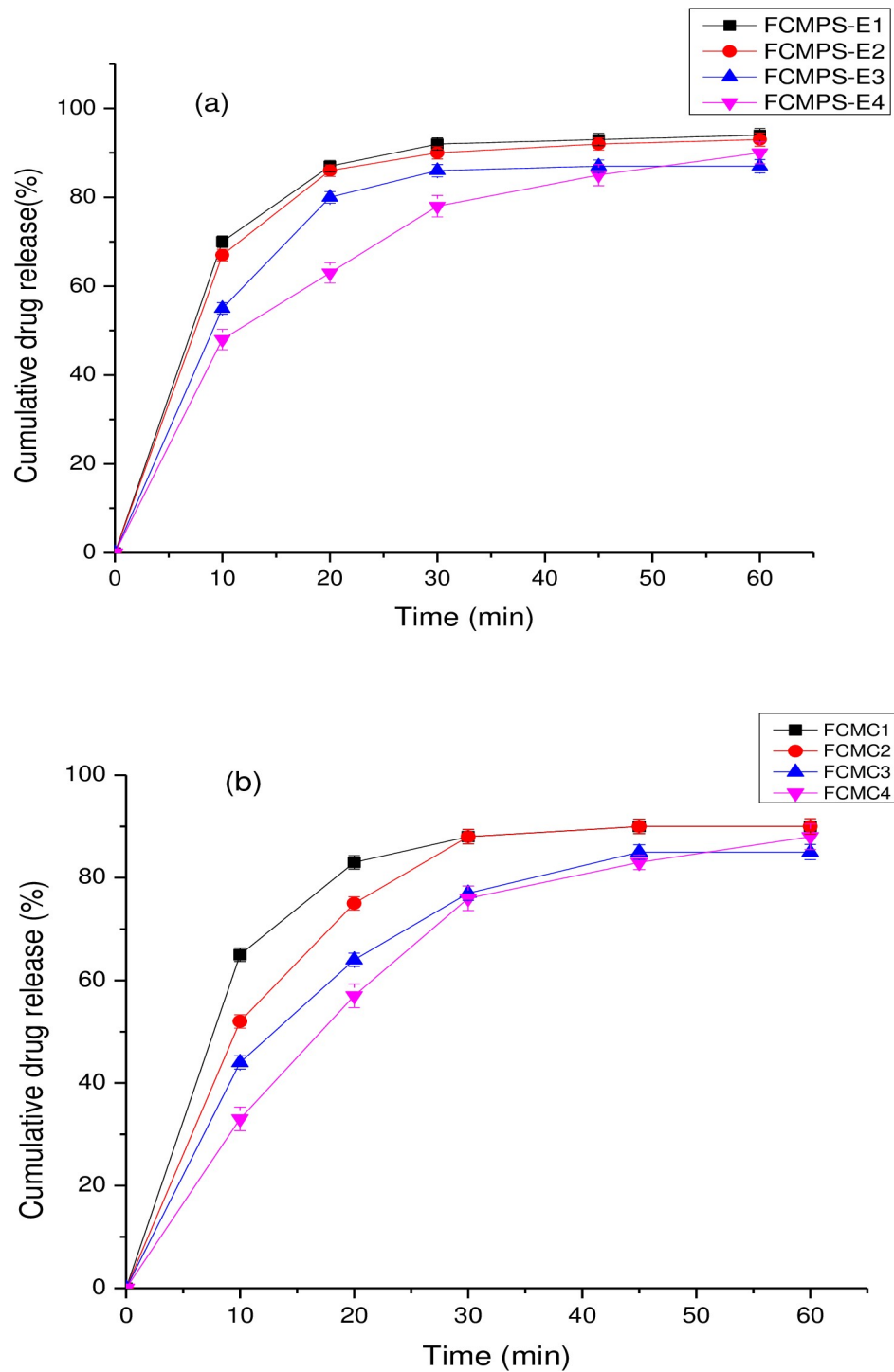
The dissolution profiles of metronidazole benzoate suspensions formulated with different concentrations of suspending agents are presented in [Fig 4A and 4B](#). The result showed that

**Table 5.** Redispersibility of the suspension formulations after a week and a month (mean  $\pm$  SD).

SA concentration (% w/v)	Rate of redispersibility (cycles)			
	After a week		After a month	
	CMPS-E	NaCMC	CMPS-E	NaCMC
1	7 $\pm$ 1.00	23 $\pm$ 2.00	14 $\pm$ 1.6	25 $\pm$ 2.00
2	4 $\pm$ 0.00	INR	6 $\pm$ 0.60	5.67 $\pm$ 1.04
3	INR	INR	INR	INR
4	INR	INR	INR	INR

CMPS-E: carboxymethylated *Plectranthus edulis* starch, NaCMC: sodium carboxyl methylcellulose, SA: Suspending agent, INR: Inversion not required because the suspension was homogenously dispersed.

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**Fig 4.** Dissolution curves ( $n = 6$ ) of metronidazole benzoate suspensions containing (a) carboxymethylated *P. edulis* starch at reaction condition E (FCMPS-E) and (b) sodium carboxyl methylcellulose (FCMC) as the suspending agents in 0.1 N HCl.

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FCMPS-E1 and FCMPS-E2 released more than 85% of the metronidazole benzoate within 20 min whereas FCMPS-E3, FCMC1, and FCMC2 attained the limit within 30 min. FCMC3 and

**Table 6. Assay and pH values of metronidazole benzoate suspensions stored at 4°C, 30°C / 65% relative humidity and 40°C / 75% relative humidity for 3 months.**

Formulations	Storage Conditions	Time 0 month		Time 3 months	
		pH (mean ± SD)	% Recovery (mean ± SD)	pH (mean ± SD)	% Recovery (mean ± SD)
FCMPS-E1	30°C /65% RH	6.03 ± 0.07	100.02 ± 0.26	6.14 ± 0.02	100.17 ± 0.05
	40°C /75% RH	6.03 ± 0.07	100.02 ± 0.26	6.27 ± 0.01	98.28 ± 0.06
	4°C	6.03 ± 0.07	100.02 ± 0.26	6.35 ± 0.03	99.31 ± 0.06
FCMC1	30°C /65% RH	6.19 ± 0.01	98.97 ± 0.49	6.41 ± 0.01	97.95 ± 0.14
	40°C /75% RH	6.19 ± 0.01	98.97 ± 0.49	6.45 ± 0.02	97.65 ± 0.11
	4°C	6.19 ± 0.01	98.97 ± 0.49	6.47 ± 0.01	97.54 ± 0.13

RH: Relative humidity

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FCMPS-E4 attained the limit within 45 min whereas FCMC4 attained the limit within 60 min. Therefore, all the formulations prepared with CMPS-E or NaCMC as a suspending agent released the drug within the USP acceptance range.

### Stability studies

The bulk visual appearance, as well as the odor of all suspension formulations, remained the same (creamy-white and odorless, respectively) throughout the 3 months stability study. There was no color change in all metronidazole benzoate suspension formulations stored at all storage conditions. The changes in pH of all metronidazole benzoate suspension formulations were within the acceptable range as indicated in Table 6.

The suspension formulations stored under the same condition also showed no significant change ( $p > 0.05$ ) in mean particle size throughout the study period as shown in Table 7.

### Discussion

The increase in DS values as the amount of MCA is increased could be reasonably attributed to increasing contact between the starch molecules and the etherifying agent. It is assumed that increasing the nMCA/nAGU ratio, after a certain value, could lead to the usage of NaOH and the formation of sodium glycolate [30–31]. During the carboxymethylation process, the NaOH provides the alkaline environment for the reaction and it also serves as the swelling agent to facilitate diffusion and penetration of the etherifying agent to the starch granular structure [32]. The increase in DS with time could be attributed to the enhanced swelling of

**Table 7. Mean particle size values of metronidazole benzoate suspensions stored at 4°C, 30°C / 65% relative humidity and 40°C / 75% relative humidity for 3 months.**

Formulations	Storage conditions	Time 0 month	Time 3 months
		Particle size (µm) (mean ± SD)	Particle size (µm) (mean ± SD)
FCMPS-E1	30°C /65% RH	53.34 ± 0.49	53.57 ± 2.31
	40°C /75% RH	53.34 ± 0.49	53.67 ± 2.22
	4°C	53.34 ± 0.49	53.88 ± 2.22
FCMC1	30°C /65% RH	63.82 ± 2.75	63.91 ± 1.22
	40°C /75% RH	63.92 ± 2.75	64.11 ± 1.33
	4°C	63.92 ± 2.75	64.21 ± 2.14

FCMPS-E: formulation containing carboxymethylated *P. edulis* starch at reaction condition E, FCMC; formulation containing sodium carboxyl methylcellulose.

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the starch with time which improved the accessibility of the etherifying agent [21, 33–35]. The increase in DS with an increase in temperature could be due to enhanced solubility of the etherifying agents, the swelling of the starch molecules and the diffusion of the reactants as temperature increases and which in turn provide the highest reaction rate, leading to the highest DS values. This finding is consistent with the findings reported elsewhere [11, 30, 34, 36–39].

As the concentration of the suspending agents increased the sedimentation volume increased which is due to the increase in the viscosity of the medium with a concentration that retards the rate of sedimentation. This finding is consistent with the results reported in the literature [22, 40–41]. At the lower concentration of the suspending agents, CMPS-E provided higher sedimentation volume than NaCMC. This signifies the presence of additional factors, other than viscosity, to contribute to higher sedimentation volume (%) of a suspension formulation of CMPS-E. One factor may be the presence of many-branched chains in CMPS-E that can form a bridge among suspended particles to form a flocculated suspension. Unlike CMPS-E, NaCMC is a linear polymer and it has a lower tendency to form flocs. Reports indicated that flocculation by polymer could be attributed to the simultaneous interaction of a polymer molecule with more than one particle [42–43]. The good flocculating properties of CMPS-E are also supported by literature that reported that anionic starch derivative [44] and cationic one [45] have good flocculating properties.

Dose uniformity and precision of pharmaceutical suspensions depend critically upon their homogeneity at the time of administration. Since dilute suspensions tend to settle, redispersibility is an important aspect of their pharmaceutical quality [46–47]. The number of inversions required for redispersion of the suspension formulation decreased as the concentration of the suspending agent increased and increased with the period of storage. The results are in agreement with the findings reported elsewhere [48–49]. Formulations prepared with CMPS-E showed better redispersibility than those of NaCMC. This might be attributed to the lower viscosity of the formulations with CMPS-E.

Dissolution testing is a routine test for quality control of pharmaceutical solid dosage forms. However, it has been noted that heterogeneous systems including suppositories and suspensions may also have problems and the dissolution (the rate-limiting step in the absorption process) of their active substances could be inconsistent and hence their dissolution properties should be determined [50]. There is no official specification for the acceptable range of dissolution of metronidazole benzoate suspensions within a specified period. However, the USP [51] specifies drug release an acceptable range of not less than 85% within 60 min for metronidazole tablets. Fig 4A and 4B depicted that the release rate was faster in formulations containing lower concentrations of suspending agent. This could be attributed to increased viscosity of the formulations with an increase in the concentration of the suspending agent. The more viscous the preparation, the slower the release of the API is likely to be. Similar results have been reported in the literature [52]. Fig 4A and 4B also showed that the drug release profiles from suspension formulations containing NaCMC as a suspending agent were slower than those containing CMPS-E as suspending agents at the same concentrations. This might also be due to the higher viscosity in formulations containing NaCMC compared to those prepared with CMPS-E.

The pH range stated in BP [27] for metronidazole benzoate suspension formulation is in the range of 5.0–6.5. Similarly, at all storage conditions, the changes in average assay recovery of the suspension formulations were within the acceptable range (90.0 to 110.0%) as shown in Table 6 [53–54].

## Conclusion

Metronidazole benzoate suspension containing CMPS-E as a suspending agent was evaluated and compared with that of suspension containing NaCMC as a suspending agent. The flow rates of the suspensions were found to be according to the following sequence CMPS-E > NaCMC. Suspension from CMPS-E showed comparable sedimentation volume with that of NaCMC. On the other hand, suspensions from CMPS-E showed better redispersibility than that of NaCMC at a lower concentration. In the case of 1% w/v concentrations of CMPS-E and CMC the concomitant use of potassium dihydrogen phosphate, as a flocculant, results significant ( $p < 0.05$ ) increment in sedimentation volumes (%) compared to the control.

Dissolution studies of the suspension formulations showed that all suspensions prepared with CMPS-E or NaCMC as suspending agent have achieved  $\geq 85\%$  drug release within a 1 h period. Also, stability studies showed that the suspension formulations were stable at all storage conditions. Hence, it can be concluded that carboxymethylated *P. edulis* starch can be used as an alternative suspending agent to NaCMC in suspension formulations.

## Supporting information

**S1 Fig. Apparent viscosities of suspensions at various concentrations of suspending agents: sodium carboxyl methylcellulose (NaCMC), and Carboxymethylated *Plectranthus edulis* starch (CMPS-E) in metronidazole benzoate formulations.**

(DOCX)

**S2 Fig. One-week sedimentation volume (%) profiles of suspensions at different concentrations of the suspending agents, (a) carboxymethylated *P. edulis* starch at reaction condition E (FCMPS-E) and (b) sodium carboxyl methylcellulose (FCMC).**

(DOCX)

**S3 Fig. The degree of flocculation for metronidazole benzoate suspension formulations containing 1% and 2% w/v of suspending agents: Carboxymethylated *Plectranthus edulis* starch (CMPS-E) and sodium carboxyl methylcellulose (FCMC).**

(DOCX)

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