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Original Article

CROSS-LINKED CARBOXYMETHYL MUNG BEAN STARCH AS PHARMACEUTICAL GELLING AGENT AND EMULSION STABILIZER

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ABSTRACT

Objectives: This study aims to improve and expand the performance of carboxy methyl mung bean starch (CMMS) as a gelling agent and an oil-in-water (o/w) emulsion stabilizer *via* cross-linking reaction with dichloroacetic acid (DCA).

Methods: Mung bean starch was carboxymethylated with chloroacetic acid and subsequently cross-linked with 0.1-10% DCA. Fifteen cross-linked carboxy methyl mung bean starches (CL-MBs) were obtained and the viscosity, clarity and pH of freshly-prepared gels, gels subjected to freeze-thaw cycles, and gels stored for 3 months at 8ºC and 45ºC were evaluated. The best CL-MB was selected and employed as gelling agent and as emulsion stabilizer in the pharmaceutical formulations of *Capsicum* gel and emulsion gel.

Results: The gel formulation containing 5%w/w CMMS cross-linked with 8% DCA (CL-MB-8) as gelling agent was found to be comparable to the formulations using commercial gelling agents. CL-MB-8 was shown to tolerate up to 30%w/w alcohol with no significant effect to the gel characteristics. In the emulsion gel formulation, the use of 3%w/w CL-MB-8 helped stabilizing the o/w emulsion prepared from a mixture of *Capsicum* oil extract and water (1:3), with 2%w/w Tween®80 as emulsifying agent. The formulation using wet-gum method yielded smooth, creamy gel with consistent color and good viscosity. Microscopic evaluation of the formulation revealed mostly small-sized o/w droplets evenly dispersed in the texture.

Conclusion: Cross-linkage with DCA enhanced the viscosity of CMMS gel network and broadened the application of CMMS as gelling agent for herbal extract gel formulation with high alcohol content and also as a stabilizer for o/w emulsion.

Keywords: Carboxymethyl starch, Cross-link, Mung bean, Gelling agent, Emulsion stabilizer.

INTRODUCTION

Carboxymethyl mung bean starch (CMMS) is an etherified starch prepared by a reaction between native mung bean starch and chloroacetic acid. An aqueous solution of 3-5%w/w concentration has been shown to produce good viscosity and rheological properties suitable for use as a gelling agent in topical pharmaceutical products [1]. However, a number of topical gel formulations contained high amounts (30-50%) of alcohol, e. g., gel of water-insoluble drugs and gel prepared from plant extracts, which could decrease the gelling ability of CMMS. Reactions with cross-linking agents have been reported to increase the viscosity of several carboxymethyl starches [2-5] and, therefore, could be used to improve alcohol tolerance of CMMS gel and broaden the application of CMMS in the medium- to high- alcohol-containing gel formulation.

Capsicum extracts (CE) is one of the most commonly formulated topical preparations and is currently available commercially in forms of gel, emulgel, cream, ointment, and patch for relief of pains and aches [6,7]. CE can be prepared in the forms of both alcoholic extract (tincture) and oil extract of powered fruits of *Capsicum annuum, C. frutescens* and other related species. Unlike the preparation in the laboratory or research scales, however, commercial-scale production of CE and many other plant extracts usually do not involve solvent evaporation. Thus the extracts carry a large amount of alcoholic solvents to the formulations. In case of the gel dosage form, this high alcohol content causes problems in the performance of most gelling agents, resulting in the decrease in the viscosity and the instability of the products. The preparation of high-viscosity gelling agent is a rationale way to solve this problem. Among commonly used cross-linking agents, dichloroacetic acid (DCA) was reported to yield modified starches with good hydrogel-forming property [3,5], while other cross-linking agents such as sodium trimetaphosphate (STMP), epichlorohydrin (ECH), phosphorous oxychloride, and citric acid showed a combination of decreased solubility and increased swelling, which resulted in the products being more suitable as a superdisintegrant [8-11].

In addition to the use as gelling agent, high-viscosity modified starches have been reported to possess emulsion stabilizing property. A non-ionic surfactant, 2-hydroxyalkyl carboxy methyl starch, has been reported as a thickening agent and polymeric oil-in-water (o/w) emulsifier [12]. In the continuing development of starch-based excipients for pharmaceutical and food products, this study investigates the viscosity, clarity and pH of aqueous and alcoholic aqueous gels prepared from CMMS cross-linked with varied concentrations of DCA. The best DCA-crosslinked CMMS was then tested as a gelling agent in the formulation of alcoholic *Capsicum* extract gel, and also investigated as an emulsion stabilizer in an o/w emulsion containing oil extract of *Capsicum*.

MATERIALS AND METHODS

Chemicals

Mung bean starch was from Sitthinan Company Ltd. (Bangkok, Thailand). Chloroacetic acid (MCA) and dichloroacetic acid (DCA) were products of Merck (Darmstadt, Germany). All other chemicals and solvents used in the preparation and analysis of modified starches were AR grade or equivalent. Double-distilled commercial-grade methanol was used in the washing of the final products, except for of the final wash in which the AR grade methanol was used.

Starch modification

Cross-linked carboxymethyl mung bean starches (CL-MB) were prepared using method and conditions modified from those described by Kittipongpatana et al. [13] And Heß et. al. [3]. MCA (40 g) was dissolved in 254 g MeOH. Then, while stirring, 138 g of mung bean starch powder was added into the solution followed by 78.5 g of 50% w/w NaOH solution. The mixture was maintained at 70ºC with continuous stirring. After 60 min, varied amounts of DCA (0, 0.1, 0.3, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10%w/w of starch) were then added into the mixture, and the reaction was allowed to continue for 45 min. At the end, the reaction was terminated by neutralization

with glacial acetic acid. The liquid supernatant was decanted, and the powder product was washed several times with 80% methanol until the filtrate gave no precipitate when tested with silver nitrate solution and a final wash with 100% methanol. The products, designated as CL-MB-0 (CMMS), and CL-MB-0.1 to CL-MB-10, were oven-dried at 50°C for 24 hours before passing through sieve no.60 (60 mesh, 0.250 mm).

SEM Analysis

Scanning Electron Microscopy (SEM) experiments to analyze the granule surface, shape and size were conducted using a Phillip XL 30 ESEM (FEI company, Hillsboro, Oregon, USA) equipped with a large field detector. The acceleration voltage was 15 kV under low vacuum mode (0.7 torr).

Free-Swelling Capacity

Free swelling capacity (FSC) was determined using described by Heβ et. al. [3]. Sample (1.0 g) was accurately weighed into a pre-weighed, dry tea bag. The bag was tied and submerged in a beaker containing an excess amount of water at room temperature. After 1, 5, 15, 30 and 60 min, the bag was taken out, the excess water was wiped out until no visible droplets were observed. The weight of the tea bag and the content was determined. FSC value was calculated as;

$$FSC = \frac{m_t - m_{tb} - m_w}{m_s}$$

when m_t is the weight of the tea bag with water-absorbing gel, m_{tb} is the weight of the empty, dry tea bag, m_w is the weight of the water, absorbed by the empty, wet tea bag., and m_s is the weight of the dry sample.

Preparation of Gel samples

Modified starch powders were dissolved in 3 types of solvents, water (0% alcohol), 7:3 water-ethanol mixture (30% alcohol), and 5:5 water-ethanol mixture (50% alcohol), at concentrations of 1, 3 and 5 %w/w to form a gel. Concentrated parabens solution was used as preservative. Each gel sample was divided into four even portions and stored in four different conditions – (1) freshly prepared (no storage), (2) eight (8) cycles of heating-cooling cycles (48 h each at 8°C and 45°C), (3) 3 months at 8°C, and (4) 3 months of 45°C. The viscosity, clarity, and pH of each sample from each storage condition were tested.

Viscosity, Clarity and pH evaluations

Viscosity, clarity and pH evaluations were conducted on the 5%w/w gel samples. The viscosity test was conducted using a Brookfield R/S-CPS rheometer, with a plate-to-plate geometry. The measuring system was SP25 with the controlled shear rate mode (CSR) at a controlled temperature of 25±1°C. Brookfield Rheo 2000 software was used to analyze triplicated data, and the viscosity was expressed in Pa. s. For clarity test, a sample (2.5 ml) was placed in a disposable cuvette, and the absorption was measured at 700 nm on a Jasco™ V-530 spectrophotometer against a water blank. The pH of the gel samples was determined using a pH Scan™ WP2.

Preparation and evaluation of *Capsicum* Gel Formulation

Capsicum gel formulation (100 g) contained 45.5 g *Capsicum* tincture (ethanolic extract of 1 g Capsicum powder/1 mL EtOH), 5 g of a selected CL-MB as gelling agent, with or without addition of silicone oil. The gel product was subjected to the viscosity test.

Oil Absorption Capacity (OAC)

OAC was determined using a method modified from that described by Bhosale & Singhal [14]. In brief, sample (0.5 g) was placed in a pre-weighed centrifuge tube. Mineral oil (7.5 g) was added and mixed vigorously with the vortex mixer for 1 min, then allowed to settle at room temperature for 30 min. The tube was centrifuged at 4000 rpm for 20 min. The oil was decanted, and the final weight of the oil-absorbing sample was determined. Oil absorption capacity was calculated as gram of oil absorbed per gram of sample.

Preparation and evaluation of *Capsicum* emulsion gel formulation

Capsicum emulsion gel formulation (80 g) contained 15 g of *Capsicum* extract in mineral oil (1 g *Capsicum* powder/1 mL mineral oil), 1.6 g Tween®80 (HLB 15.0), 2.4 g of a selected CL-MB, and 1 mL of concentrated parabens as preservative. Using the wet-gum method, the extract was levigated with Tween®80 and modified starch. Water was then added and levigation was continued until a homogenous, creamy emulsion was obtained. The emulsion product was subjected to microscopic evaluation to determine the stability.

Statistical Analysis

All tests were performed at least in triplicate. The statistical significant tests were performed using analysis of variance (ANOVA) at 95% confidence level (p<0.05). Significant differences among mean values were determined by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Fig.1 showed that while all CL-MBs retained their granular shape, with the size and shape of granules remained unchanged from those of non-cross linked CMMS, there were noticeably small cracks and fissures on the granule surface of CL-MBs. These fissures, possibly a result of cross linking, became more prominent in samples treated with high DCA concentrations. Cross-linking reaction was known to affect the structure and surface of starch granules, causing physical changes such as the rough surface, black zone, fragmentation and the formation of a deep grove [15].

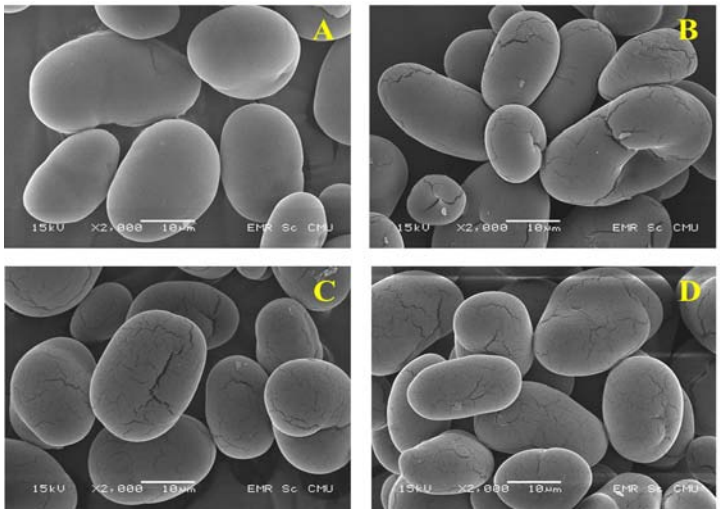

Fig. 1: SEM images of (A) CMMS; (B) CL-MB-2; (C) CL-MB-8; (D) CL-MB-10

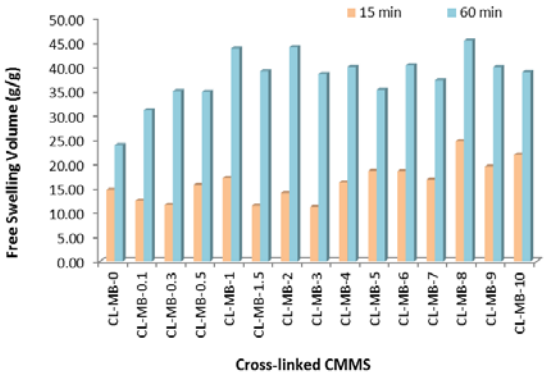

Fig. 2: Free Swelling Capacity (FSC) of CMMS and CL-MB at 15 and 60 min

FSC values of non-crosslinked CMMB were 15.0 and 24.8 g/g after 15 and 60 min, respectively, of submersion in excess water at RT. Cross-linked samples exhibited values in the range of 0.8-1.4 times higher after 15 min, and 1.2-1.6 times higher after 60 min (Fig.2). In all samples, the gel texture remained intact and no phase separation was observed. This was different from previously reported CMMS cross-linked with sodium trimetaphosphate (STMP) and epichlorohydrin (ECH) in which certain levels of crosslinking caused a separation of water from the gel network [1,8].

The viscosity at shear rate 100 s^{-1}, clarity and pH of 5% aqueous were determined, and the data are compiled in table 1. The viscosity of non-crosslinked CMMS (CL-MB-0) was not significantly different from that of low (0-0.5%) CL-MB samples. As the DCA concentration was raised above 1%, the viscosity increased steadily and significantly. The maximum viscosity for a 5% gel was achieved in the CL-MB-8 sample (10.25±0.64 Pa. s). This value was statistically comparable to that of commercial gelling agents, CP940 (1%), MC (3%), HPMC (4%) and SCMC (5%). Increases of DCA concentration to 9 and 10% resulted in samples with slight but significant drops in viscosity, indicating that 8% DCA was an optimum concentration for achieving the highest viscosity. All gels exhibited good clarity, which was a superior characteristic of mung bean starch gel over other starches [1]. The pHs of 5% w/w CL-MB gels ranged narrowly between 6.8 and 7.1.

Table 1: Viscosity, clarity and pH of 5% w/w gels of carboxymethyl mung bean starches cross-linked with varied amounts (0-10%) of dichloroacetic acid (DCA), compared to commercial gelling agents, determined freshly after preparation

Samples	Viscosity (Pa. s, ±SD)	Clarity (Abs@700 nm)	pH
CL-MB-0	3.73±0.05^{a}	0.31±0.03	6.8
CL-MB-0.1	3.85±0.07^{ab}	0.30±0.04	6.9
CL-MB-0.3	3.92±0.10^{ab}	0.35±0.03	6.8
CL-MB-0.5	3.84±0.11^{ab}	0.33±0.07	7.0
CL-MB-1	4.52±0.15^{c}	0.37±0.05	7.0
CL-MB-1.5	4.05±0.18^{b}	0.34±0.02	7.1
CL-MB-2	4.65±0.12^{c}	0.38±0.08	7.1
CL-MB-3	4.44±0.20^{c}	0.42±0.07	7.0
CL-MB-4	6.29±0.37^{d}	0.45±0.11	6.9
CL-MB-5	7.41±0.40^{e}	0.37±0.12	6.8
CL-MB-6	9.27±0.55^{g}	0.47±0.09	7.0
CL-MB-7	6.81±0.38^{de}	0.43±0.11	7.1
CL-MB-8	10.25±0.64^{h}	0.41±0.05	7.0
CL-MB-9	8.25±0.47^{f}	0.45±0.12	6.9
CL-MB-10	8.56±0.40^{f}	0.56±0.07	7.0
CP 940 (1%)	11.07±0.87^{h}	0.12±0.02	7.4
MC (3%)	9.88±0.76^{gh}	0.26±0.04	7.2
HPMC (4%)	10.42±0.55^{h}	0.41±0.06	6.9
SCMC (5%)	11.23±0.97^{h}	0.38±0.09	6.9

Values in the same column with the same superscript are not significantly different ($P < 0.05$), CP 940 – Carbopol 940; MC – methylcellulose; HPMC – hydroxypropylmethylcellulose; SCMC – sodium carboxymethylcellulose

Table 2: Viscosity, clarity and pH of CL-MB-8 gels (5%w/w) in water, 7:3 water-alcohol, and 1:1 water-alcohol, determined freshly after preparation, after 8 freeze-thaw cycles, 3 months at 8°C and 3 months at 45ºC

Alcohol	Conditions	Viscosity (Pa. s, ±SD)	Clarity (Abs@700 nm)	pH
0	Freshly prepared	10.25±0.64^{a}	0.41±0.05	7.0
	8 Freeze-thaw cycles	9.77±0.87^{ab}	0.39±0.06	7.0
	8ºC, 3 months	8.52±0.65^{c}	0.56±0.06	6.9
	45ºC, 3 months	8.44±0.72^{c}	0.40±0.07	7.0
30	Freshly prepared	10.07±0.81^{ab}	0.50±0.06	6.9
	8 Freeze-thaw cycles	9.50±0.54^{b}	0.57±0.05	7.1
	8ºC, 3 months	9.32±0.76^{b}	0.61±0.04	7.0
	45ºC, 3 months	9.17±0.49^{b}	0.47±0.08	7.0
50	Freshly prepared	8.59±0.43^{bc}	0.76±0.06	6.9
	8 Freeze-thaw cycles	8.33±0.50^{c}	0.88±0.05	7.0
	8ºC, 3 months	8.72±0.61^{bc}	0.81±0.05	7.0
	45ºC, 3 months	8.85±0.57^{bc}	0.67±0.06	7.0

Values in the same column with the same superscript are not significantly different ($P < 0.05$).

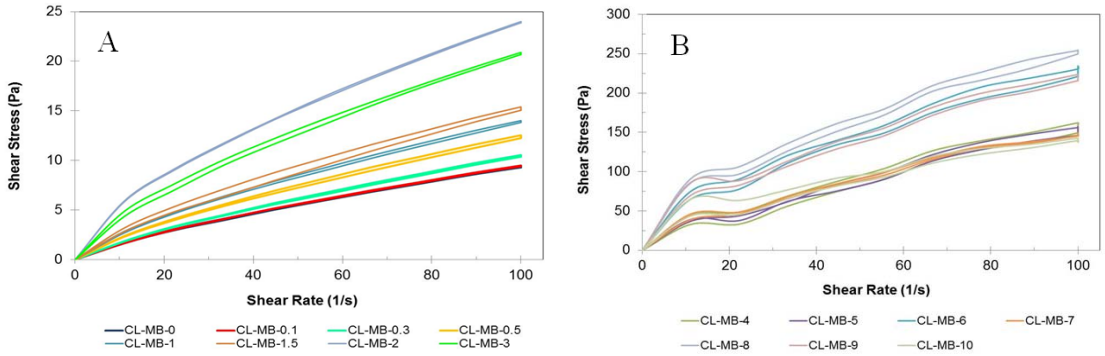

Fig. 3: Rheological profiles of carboxy methyl mung bean starch cross-linked with varied concentrations of dichloroacetic acid (A) 0-3%, (B) 4-10%

Rheological profiles of CL-MB-0 and low DCA cross-linked gel samples (CL-MB-0.1 to CL-MB-3) were essentially no different, exhibiting pseudoplastic flow patterns with a small degree of thixotropy (Fig.3A). These are consistent with previous reports on CMS [13,16]. At DCA concentrations 4% and above, gels showed high stress at low shear (up to 10 s^{-1}) before assuming a typical shear-thinning flow. Larger values of thixotropy were observed, indicating that DCA crosslinking not only altered the viscosity but also affected the flow properties of the cross linked starch gel (Fig.3B).

Based on the FSC and viscosity results, CL-MB-8 was selected for the subsequent studies. The viscosity, clarity and pH of CL-MB-8 gel prepared in 30% alcohol were not significantly different from those of freshly prepared gel, when compared under the same storage conditions. In 50% alcohol, the gel showed only approximately 15% loss in viscosity, despite a significant decrease in clarity (Table 2) compared to the non-alcohol gel. Still, the values remained significantly higher compared to that of the non-crosslinked gel, of which the viscosity and clarity decreased from 3.73±0.05 Pa. s and 0.31±0.03 in water, to 3.21±0.04 Pa. s and 0.61±0.04 in 30% alcohol, and to 3.02±0.08 Pa. s and 0.83±0.05 in 50% alcohol, respectively. Gels prepared in all three water-alcohol ratio exhibited good stability against freeze-thaw cycles, and 8°C and 45°C for 3 months. *Capsicum* gel formulation prepared using 5% CL-MB-8 as a gelling agent (Fig.4B) showed comparable appearance and viscosity to that of commercial CP940-based product (Fig.4C) and were evidently superior to the formulation using non-crosslinked CMMS as gelling agent (Fig.4A).

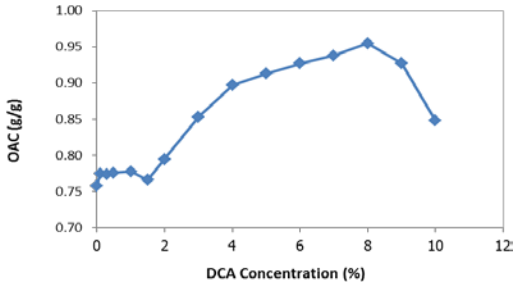

Fig. 5: Oil absorption capacity (OAC, g/g) of CL-MBs prepared using varied DCA concentrations

Oil-absorption capacity (OAC) is one of the preferred properties of modified starches used in the food industry. OAC, usually improved upon chemical modification [17], plays a crucial role in many food processings, including flavor entrapment and shelf life extension [18]. Low DCA-crosslinked samples showed OAC values (0.76-0.79 g/g sample) that were not significantly different from that of the non-crosslinked sample (0.76 g/g). DCA concentrations at above 2%, however, yielded CL-MB samples with increased OAC. A maximum OAC value of 0.95 g/g, a 26% increase, was obtained from CL-MB-8

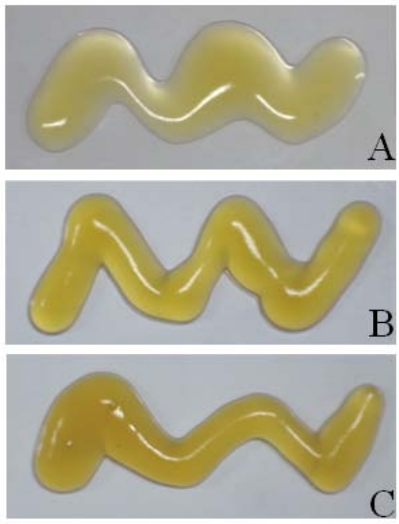

Fig. 4: Appearances of *Capsicum* gel prepared using (A) 5%CMMS (CL-MB-0), (B) 5% CL-MB-8, compared to (C) commercial *Capsicum* gel using 1% CP940 as gelling agent

(Fig. 5). The higher OAC value of cross-linked samples was likely a result of an increase in the absorption surface area of granules due to small fissures, as observed under SEM. The oil could be physically entrapped in these fissures by capillary attraction [16]. This property, together with the increase of solution viscosity, suggested potential application of CL-MB as an emulsion stabilizer.

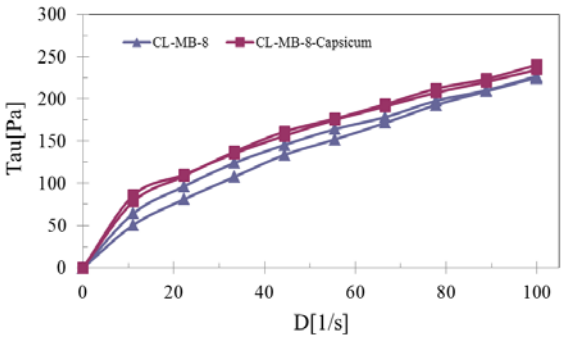

Fig. 6: Rheological profiles of emulsion formulations using CL-MB-8 as stabilizer, with (CL-MB-8-*Capsicum*) and without (CL-MB-8) *Capsicum* extract

In emulsion formulation, the use of CL-MB-8 at 3% helped stabilizing the emulsion between mineral oil and water to yield a smooth, creamy emulsion with good viscosity, without addition of Tween 80. The size of o/w droplets, however, appeared to be large and varied. Using *Capsicum* oil extracts in the formulation, the color of the emulsion became orange, while the viscosity was not affected. The viscosities of the emulsion base and *Capsicum* emulsion formulation were not significantly different. At 2.17±0.84 and 2.72±0.87 Pa. s, respectively. The rheological profiles were almost identical (Fig. 6).

Microscopic evaluation of the formulation (Fig. 7) revealed that the emulsion based on CL-MB-8 was oil-in-water (o/w) type. The droplet size was rather big with broad size variation. The addition of Tween® 80 into the formulation of *Capsicum* emulsion resulted in a more evenly size distribution.

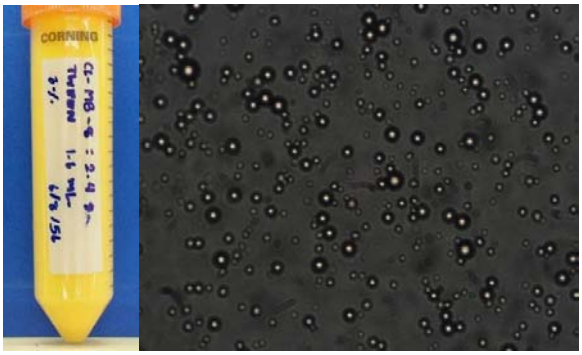

Fig. 7: Microscopic images of *Capsicum* o/w emulsion at 40X

CONCLUSION

Carboxymethyl mung bean starch cross-linked with 8%w/w dichloroacetic acid (CL-MB-8) was, at concentration 5%w/w, effective as a gelling agent in *Capsicum* gel formulation having an alcohol content as high as 30%. It can also be used, at a lower (3%w/w) concentration, as a stabilizer in the *Capsicum* oil-in-water emulsion formulation containing 2% Tween® 80 as an emulsifier.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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