Evaluation of the suspending properties of two local *Opuntia* spp. mucilages on Paracetamol suspension

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Abstract: Some excipients are currently available for the formulation of pharmaceutical suspensions. The purpose of this study is to develop cheap and effective natural excipient that can be used as an effective alternative for the formulation of pharmaceutical suspensions. The suspending properties of *Opuntia ficus-indica* and *Opuntia stricta* mucilages (family Cactaceae) were evaluated comparatively with that of NaCMC at concentration range of 2-6% (w/v) in Paracetamol suspension. Sedimentation volume (%) (with and without electrolyte), rheology, redispersibility, and dissolution rate of the suspensions were employed as evaluation parameters. The values obtained were used as basis for comparison of the suspending agents. The apparent viscosities of the suspensions in all the suspending agents concentration levels and applied shear rates were in the order of NaCMC>OS>OFI with non-Newtonian flow and accordingly the flow rates of the suspensions were in the order of OFI>OS>NaCMC. The sedimentation volumes (%) of the suspensions in all the suspending agent concentration levels were higher for OS followed by OFI and then NaCMC. The high sedimentation volumes (%) of suspensions, in turn, were accompanied by ease of redispersibility of that order. The effect of electrolyte on sedimentation volume (%) had dual effect. It was only the suspensions that had NaCMC that showed increase in sedimentation volume (%) in all molar NaCl concentration. However, in suspensions that had mucilages of OS and OFI, an initial increase in sediment volumes (%) were accompanied by decrease after $1x10^{-3}$M and $1x10^{-2}$M of NaCl, respectively. Dissolution of the suspensions which had mucilages attained the acceptable ranges (≥ 80% drug release in 30 min) in 5 min. Similarly, except A6 formulations A2, A3, A4 and A5 have attained the limit but the release was not as quick as the previous formulations. Hence, it can be concluded that mucilages of *Opuntia* spp. (*Opuntia ficus-indica* and *Opuntia stricta*) can be used as alternatives to NaCMC as suspending agent in suspension formulations.

Keywords: Mucilage, *Opuntia ficus-indica, Opuntia Stricta*, sedimentation volume, suspension, suspending agent, viscosity, dissolution.

INTRODUCTION

Excipients are additives, used to convert active pharmaceutical ingredients into pharmaceutical dosage form suitable for administration to patients (Patel *et al.,* 2007). New and improved excipients continue to be developed for conventional drug delivery systems and also to meet the needs of modern and better formulations. Mucilages are most commonly used as adjuvants in the manufacturing of different pharmaceutical dosage forms. They possess variety of pharmaceutical properties, which include binding (Kulkarni *et al.,* 2002), disintegrating (Patel *et al.,* 2007), suspending, emulsifying (Kumar *et al.,* 2009), and altering release properties (Kulkarni *et al*, 1997) at different proportion in different pharmaceutical dosage forms. Natural mucilages are preferred to semi-synthetic and synthetic materials because of their low cost, free availability, emolliency, non-irritating nature and non toxicity (Jani *et al*., 2009; Patel *et al*., 2011).

Among the naturally available materials that have potential use as suspending agents is the mucilage of cactus, a plant that belongs to cactaceae family, which grow in arid and semi arid regions of the globe. Mucilage is a complex carbohydrate (Sáenz *et al*., 2004) with a highly branched structure, which contains varying proportions of L-arabinose, D-galactose, L-rhamnose and D-xylose, as well as galacturonic acid in different proportions (Paulsen and Lund, 1979; Sepúlveda *et al*., 2007). The present work aims to compare the suspending ability of mucilages of two *Opuntia* spp. (*Opuntia ficus indica* and *Opuntia stricta*) with sodium carbo-xylmethylcellulose.

MATERIALS AND METHODS

Materials

Paracetamol powder and Paracetamol reference standard were kindly gifted from Ethiopian pharmaceutical Manufacturing Factory and Drug administration and control authority (DACA), Ethiopia, respectively. NaCMC–low viscosity grade, Methyl paraben, Propyl paraben were purchased from BDH, England. Glycerin purified (Research-lab fine Chem. Industries, India), Tween® 80 (Atlas Chem. Industrial INC, USA), Sodium Chloride and Sodium Hydroxide (LABMERK CHEMICALS, India) and Ethanol 96% (DELF, Ethiopia) were used as received. Cactus mucilage was extracted

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from fresh cladode of both *Opuntia ficus-indica* (OFI) and *Opuntia stricta* (OS) from Mekelle area, Tigray Region, Ethiopia.

METHODS

Mucilage Extraction

Extraction of the mucilage was done according to the method by Sepúlveda *et al*. (2007). The dried mucilages were finely comminuted in mortar and pestle. The particles were then allowed to pass through a sieve of mesh size 224 μm.

Preparation of paracetamol tablets using isolated mucilage

The compositions of the Paracetamol suspensions are given in Table 1. The suspensions are grouped into three: A, B and C. Each group has five formulations (A2-A6, B2-B6 and C2-C6). The letters A, B and C indicate the type of suspending agent which corresponds to NaCMC, OS and OFI, respectively, while the figs. 2-6 represent the percent concentration (w/v) of the suspending agents. All the suspensions were prepared based on the method described elsewhere (Saeedi, *et al*., 2003). The suspending agent and Tween 80 were initially dispersed in distilled water containing the preservatives. Then, the Paracetamol which was wetted with glycerin was added to the vehicle and stirred continuously until uniform dispersion was obtained.

Table 1: Formulation ingredients of the Paracetamol suspensions

Formulation Ingredients	Composition (% (w/v))
Paracetamol	6
Suspending agent*	2, 3, 4, 5, 6
Methyl paraben	0.04
Propyl paraben	0.02
Tween 80	0.4
Glycerin purified	5
Distilled water, qs**,	100 ml

*The suspending agents used are mucilage of OFI, OS and NaCMC each at the specified conc.

**Quantity sufficient

Effect of shear rates on apparent viscosity of the suspensions

The effect of shear rates on viscosity was studied using rotational viscometer. The viscosities of the suspension were measured in kPas within 48 hrs after preparation. The measurement was made at room temperature using spindle number 4 at 20, 30, 50, 60, 100 and 200 rpm. Apparent viscosities recorded are average of two determinations.

Flowability of the suspensions

The flow rates of the suspensions were measured based on the method described elsewhere (Femi-Oyewo *et al*., 2004). The time required for each 10 ml suspension sample to flow through a 10 ml pipette was used to calculate the flow rate using Equation 2.1. Flow rates recorded are average of three determinations.

$$\text{Flow rate} = \frac{V_s}{T} \qquad (1)$$

where V_s volume of sample in the pipette (in ml) and T is time (in sec) required for the 10 ml suspension to totally elute out of the pipette.

The suspensions are classified as less viscous, intermediate viscosity and very viscous based on their rate and extent of flow out of the pipette. If the suspension totally comes out of the pipette, with the aid of only gravitational force of attraction, they are considered as less viscous for which flow rates are to be calculated. If partly to come out but not totally, they are considered as having intermediate viscosity. If, however, the suspensions do not totally come out of the pipette, they are considered as very viscous.

Sedimentation volume

20 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 where the clear supernatant start to become cloudy up on descending from

Table 2: Flow rate of the suspension formulations (Mean±SD, n=3)

Suspending agent conc. in suspensions	Flow rate (ml/sec)		
	NaCMC	OFI	OS
2% (w/v)	0.09±0.02[a]	0.48±0.02[a]	0.36±0.02[a]
3% (w/v)	0.06±0.01[b]	0.37±0.01[b]	0.17±0.04[b]
4% (w/v)	Interm. Viscosity*	0.24±0.05[c]	0.10±0.01[c]
5% (w/v)	Very viscous	0.15±0.01[d]	0.07±0.00[c]
6% (w/v)	Very viscous	0.07±0.03[e]	Interm. Viscosity*

The letters (a, b, c, d, e) indicate the presence of significant difference in flow rate among suspensions that had same suspending agent but different concentration level. - * intermediate viscosity

the top surface of the suspension. Results recorded are averages of three determinations.

Effect of electrolyte concentrations on sedimentation volume

10 ml suspensions which had NaCl concentration of $1x10^{-4}$ M, $1x10^{-3}$ M, $1x10^{-2}$ M or $5x10^{-2}$ M were prepared from each of the three suspending agents at concentration of 4% (w/v). The sedimentation volumes (%) of the formulations were noted every day for the following seven days after preparation. Results recorded are averages of three determinations.

Redispersibility of suspensions sediment

The redispersibility of suspensions was evaluated according to a method described elsewhere (Saeedi *et al*., 2003). 20 ml of each suspension formulation was poured into 25 ml measuring cylinder and allowed to settle for a week. The measuring cylinders were then manually and genteelly rotated at 180°. The formulations were evaluated based on the number of turns (one complete cycle) required to uniformly redisperse the sedimented Paracetamol particles throughout the suspension. Results recorded are averages of two determinations.

Dissolution of the suspensions

Dissolution studies of the Paracetamol suspensions were performed based on the method described elsewhere (Azam and Haider, 2008) using the USP paddle method in dissolution tester.

STATISTICAL ANALYSIS

ANOVA was carried out for physical stability test of the suspensions from the three suspending agents using the computer software Sigmastat 3.5. Holm-Sidak multiple comparison test was used. At 95% confidence interval, p values less than or equal to 0.05 were considered as significant.

RESULT

Rheology of the suspensions

Effect of shear rates on apparent viscosity of the suspensions

The rheological behavior of the suspensions prepared with *Opuntia* spp. mucilages and NaCMC were pseudoplastic and their viscosities decreased with increase in shear rates (fig. 1). As can be seen from the figure, the viscosity increased with increase in the concentrations of suspending agents in all the formulations. The viscosities of the formulations were in the order of NaCMC>OS>OFI. These differences in viscosities had implications on many parameters of the suspensions with flowability and sedimentation rate in the forefront.

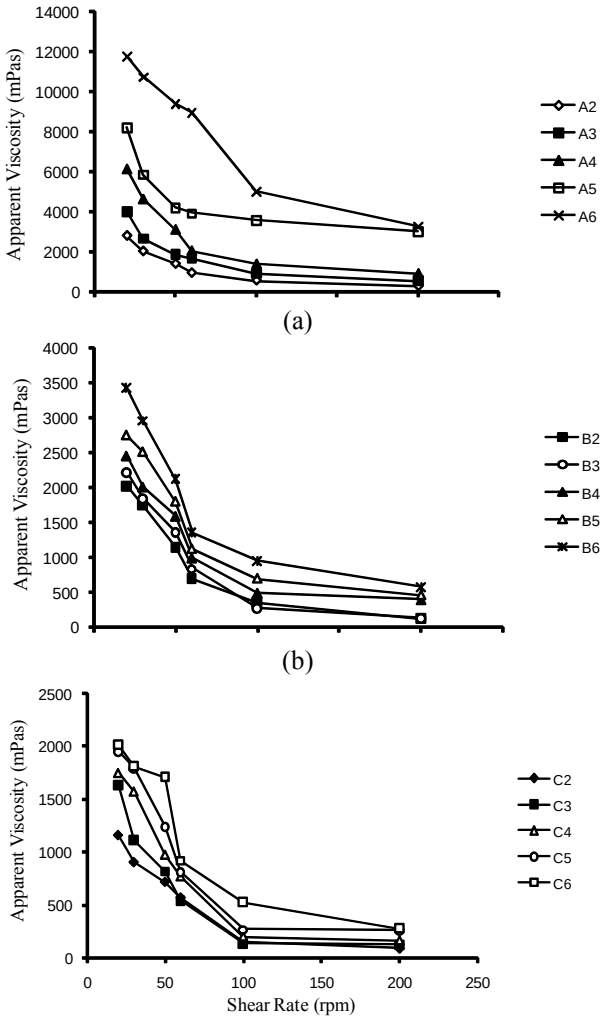


Fig. 1: Apparent viscosities of suspensions (at different shear rates) having suspending agent of (a) NaCMC, (b) OS and (c) OFI

Flowability of the suspensions

The flow rates of the formulations are presented in table 2. The flowability of the suspensions, at all concentration levels of the suspending agents, were in the order of OFI>OS>NaCMC with suspension A4 having inter-mediate viscosity. A5 and A6 were too viscous to flow through the 10 ml pipette with the help of gravitational force. Like A4 formulation B6 was found to be with intermediate viscosity.

Sedimentation volumes of the suspensions

It is quite understood that the better is the suspending medium, the higher the sedimentation volume (%) which is an indication of slower rate of sedimentation. The sedimentation volumes (%) of the suspensions are presented in fig. 2. It can be seen from the profiles of the figs. that the sedimentation volumes (%) of suspensions increased with increase in the concentrations of the suspending agents.

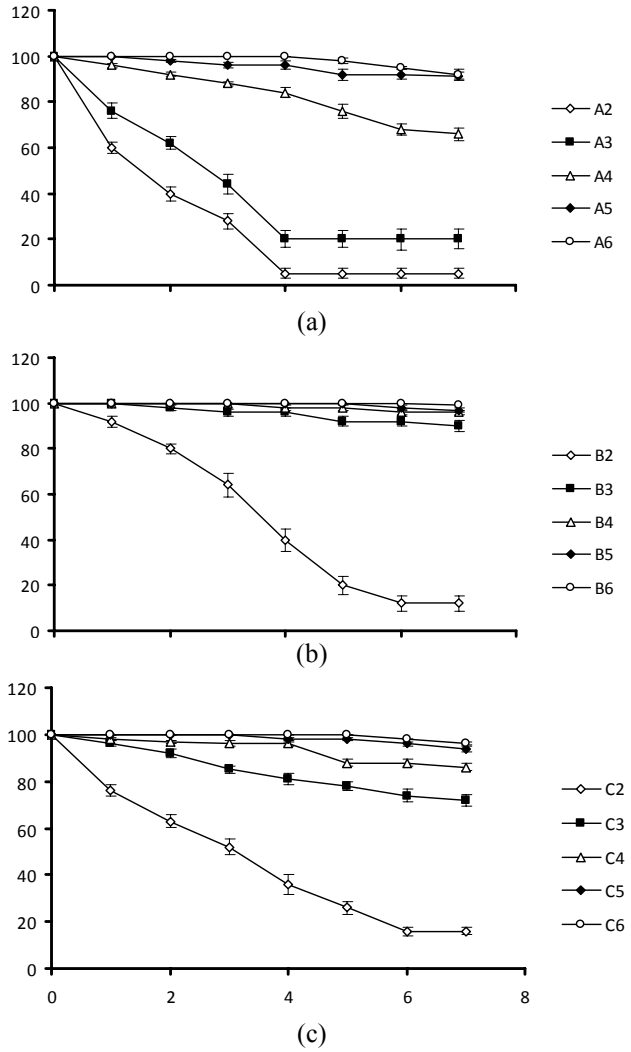
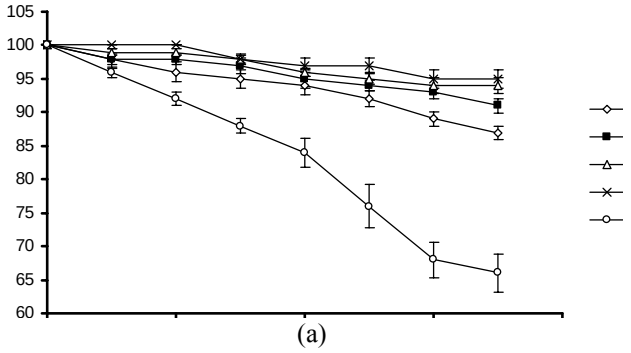


Fig. 2: One week sedimentation volume (%) profiles of suspensions at different concentrations of the suspending agent (a) NaCMC, (b) OS and (c) OFI.

Effect of electrolyte concentrations on sedimentation volume

The sedimentation volume (%) profiles of suspensions, with different concentrations of NaCl, are presented in figs. 3 (a, b and c) and 4. In all the formulations' profiles sedimentation volume get decreased till the seventh day.



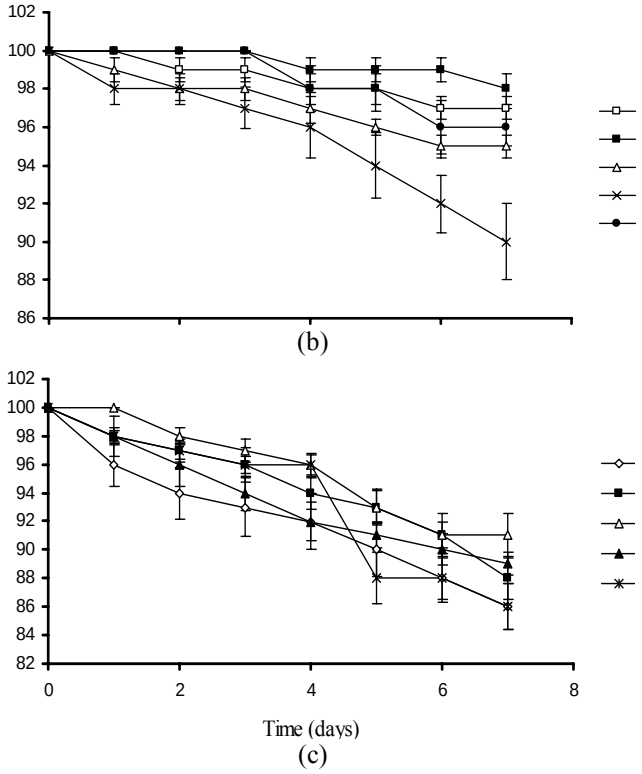


Fig. 3: Effect of different electrolyte concentrations on sedimentation volumes (%) of suspensions prepared using 4% suspending agent (a) NaCMC, (b) OS and (c) OF.

Fig. 4 shows the seventh day sedimentation volume of all the formulations. Suspensions that had NaCMC as suspending agent have shown continuous increment in sedimentation volumes (%) up on increasing NaCl concentrations in the formulations: Control (66%), $1x10^{-4}$ M (87%), 1×10^{-3} M (91%), 1×10^{-2} M (94%) and 5×10^{-2} M NaCl (95%). The differences in sedimentation volumes (%) were statistically significant in comparison to the control.

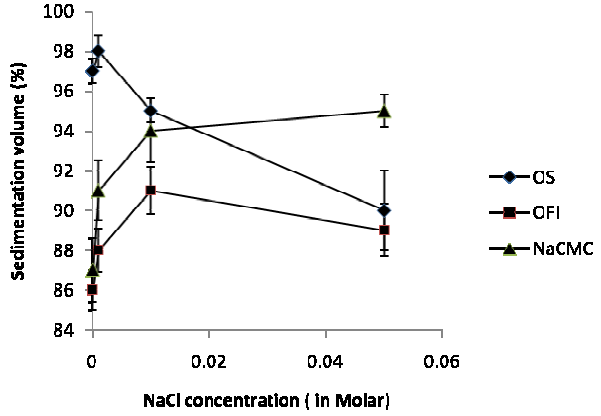


Fig. 4: seventh day sedimentation volume (%) of suspensions containing different molar concentrations of NaCl.

However, those with OS and OFI have shown increase in sedimentation volume (%) at lower NaCl concentration and then decrease at higher electrolyte concentration (fig. 4). In suspensions prepared using OS, significant sedimentation volume (%) difference was noted in suspension with NaCl of 5×10^{-2} M. Similar trend was observed with suspensions which had mucilage of OFI. This time the suspension with significant sedimentation volume (%) that had NaCl of 1×10^{-2} M.

From these results, it can be said that the concomitant use of electrolytes as flocculating agent in suspension formulations together with NaCMC is recommendable but not with mucilages of OS and OFI.

Redispersibility of the suspensions

It was stated that with increase in concentrations of suspending agents, progressive rise in sedimentation volumes (%) resulted. These increases were associated with ease of redispersibility in all formulations from OS (except B6) and OFI while for formulations from NaCMC they were found to be the reverse (fig. 5).

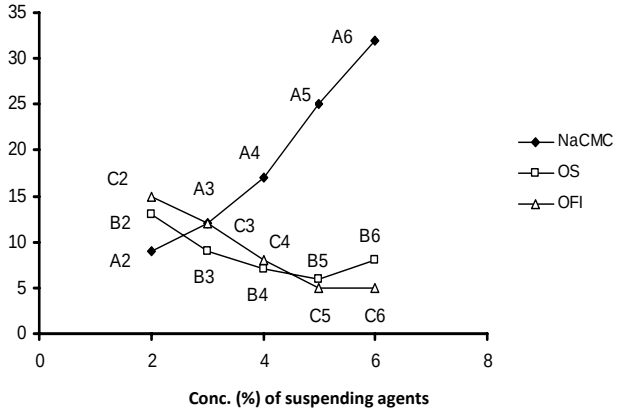


Fig. 5: Average number of turns required to uniformly redisperse sediment of the suspensions after one week.

Dissolution profiles of the suspensions

There is no official specification for the acceptable range of dissolution of Paracetamol suspensions within a specified period of time. However, the USP (2009) specifies drug release acceptable range of not less than 80% within 30 min for Paracetamol tablets.

The dissolution profiles of the suspensions are shown in fig. 6. All formulations from the three suspending agents, except A6 (87.7%) have achieved the acceptable range within the specified time limit. However, from suspensions that had mucilage suspending agents the acceptable limit was attained much earlier than expected.

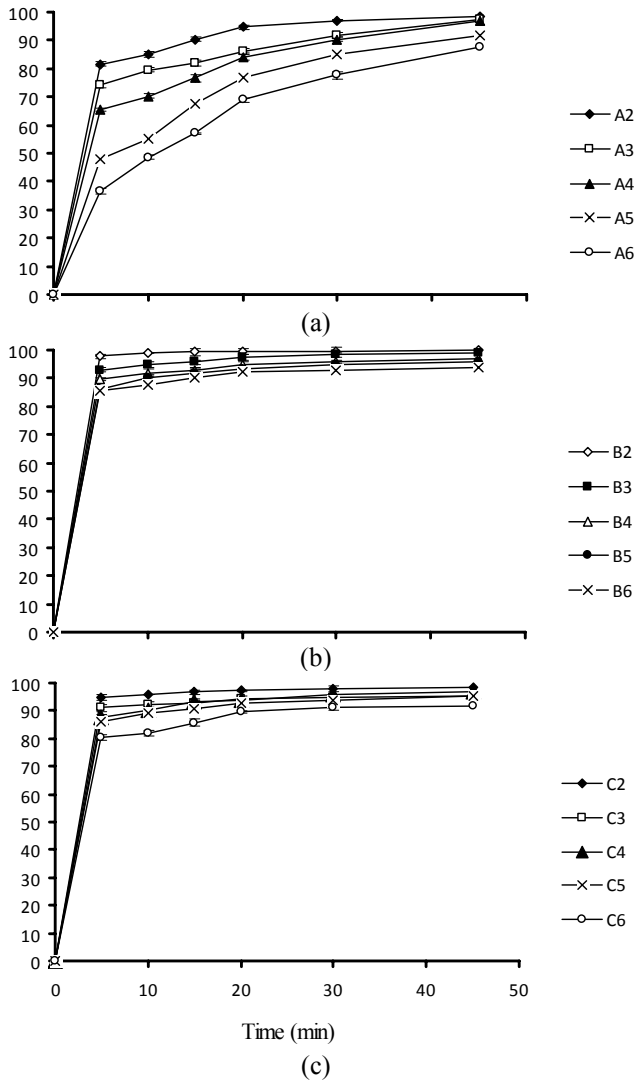


Fig. 6: Drug release profiles of all the suspension formulations that have suspending agent (a) NaCMC (b) OS and (c) OFI.

DISCUSSION

It was stated that suspending systems tend to be non-Newtonian, specifically pseudoplastic, with increasing shear (Catacalos and Wood, 1964). The pseudoplastic nature of the suspensions is an essential property in the formulation of suspensions which during shaking can form uniform dispersion and allow ease of pourability.

Statistically significant differences existed in flow rates among formulations that had different types but same concentration of suspending agents. The very reason for these differences was due to the differences in viscosities of the formulations observed.

The increase in sedimentation volume with increase in sedimentation volume could be due to, in addition to other

factors, the increment in viscosities with increase in suspending agents' concentrations. These increased consistency of the media lead to the retardation of sedimentation of suspended particles.

On comparing among the sedimentation volumes (%) of formulations that had same suspending agent concentration but different types, they were in the order of OS>OFI>NaCMC. As stated earlier the suspensions have viscosities of the order of NaCMC>OS>OFI. But the sedimentation volumes (%) are in the order of OS>OFI>NaCMC. This signifies the presence of additional factor, other than viscosity, to produce higher sedimentation volume (%). To uphold this, the presence of large amount of the divalent calcium which exist as Ca-oxalate (Garvie, 2006) and appreciable amount of the monovalent potassium are the contributing factors for high sedimentation volum as they have something to do with reduction of zeta potential (Billey, 2007). In addition, unlike NaCMC which is a linear polymer, the presence of many branched chains in OFI and OS having different functional groups can form bridge among the different flocs to form voluminous suspension.

Hence, the mucilages seem to have a dual nature to render the purpose of both polymers (as a thickeners) and electrolytes (flocculants); as reported elsewhere (Trachtenberg and Mayer, 1982). According to them the purified mucilage from OFI is a high molecular weight polysaccharide which behaves as a polyelectrolyte.

In suspensions that have mucilages as suspending agent, the reduction in sedimentation volumes (%) at higher NaCl concentration might have occurred due to reduction in the number of bridges between floccules. This could be due to the higher affinity electrolyte might have towards the drug particles. Hence, little space was left for the mucilage to get adsorbed and form bridge among floccules. This is because polymer adsorption is a key requirement for bridging (Zatz *et al*., 1979). Indeed, Miller *et al.* (2008) have corroborated the flocculating/coagulation mechanism of mucilages of the *Opuntia* spp. to be adsorption of dispersed particles and forming bridge among them. With regard to suspensions from NaCMC that showed an increase in sedimentation volume (%) with increase in molar NaCl concentration may, however, be due to the low natural electrolyte concentrations the polymer has. So the added electrolyte might have been adsorbed on the surface of the drug particles to induce flocculation. Hence, together with the viscosity imparting effect of the polymer synergistic effect is noticed.

The governing factors for redispersibility of suspensions are nature of dispersed particles and viscosity. However, in this particular redispersibility study the effect of nature of dispersed particles outweighed that of viscosity in formulations with the two mucilages. This is so because with mucilage suspending agents while in suspensions from NaCMC the effect of viscosity outweighed.

It was indicated earlier that sedimentation volumes (%) of the suspensions from NaCMC were basically attributed to the retardation of sedimentation rate by thickening the medium. While for OS and OFI, in addition to the viscosity imparting ability of the mucilage, the high sedimentation volumes (%) were attributed to the flocculating ability of the suspending agents. Thus, fluffy nature of the suspensions promoted the ease of redispersibility since relatively small forces can accomplish destruction of the loosely bound floccules. The sedimentation volumes (%) of flocculated systems are high which is accompanied by ease of redispersibility as relatively small forces can accomplish destruction of the non impacted flocs (Jones *et al*., 1970; Zatz *et al*., 1979).

The quick release from these formulations could be attributed to the relatively low viscosity of the suspensions as compared to those of NaCMC. This is in agreement with reports by Azam and Haider (2008) whereby viscosity increased with increase in concentration of suspending agent, which in turn, decreases the dissolution rate.

Despite the quick release of the drug observed in formulations which had mucilage of OS (fig. 6b) and OFI (fig. 6c), all the formulations, except B2, did not achieve 100% release of drug content until the 45^{th} min. This may be due to, in addition to the viscosity of the formulations with high suspending agents concentration levels, the floccules formed that hinder the release of the Paracetamol particles. The percentage of drug dissolved from suspensions is slow as drug particles in the suspension can form floccules (Boonme *et al*., 2002).

CONCLUSION

The formulations of Paracetamol suspensions containing mucilages of the two *Opuntia* spp. and NaCMC as suspending agents showed pseudoplastic flow and the viscosity were in the order of NaCMC>OS>OFI and accordingly the flow rates of the suspensions were in the order of OFI>OS>NaCMC. At the same suspending agent concentration, the sedimentation volumes (%) and ease of redispersibility of the suspensions were in the order of OS>OFI>NaCMC.

The concomitant use of electrolytes, as flocculant, in formulations with mucilages of OS and OFI was found to be worthless, but with NaCMC it is recommendable as the increment in sedimentation volumes (%) is significant compared to the control used.

Dissolution studies of suspensions with the mucilages of *Opuntia* spp. as suspending agent released more than 80 % of the Paracetamol within 5 min after the start of the dissolution tests while formulations with NaCMC at concentration of 3% (A3), 4% (A4) and 5% (A5) have attained the limit within 30 min. However, the formulation with 6% NaCMC (A6) did not attain the acceptable limit even at the 30th min.

Hence, it can be concluded that mucilages of *Opuntia* spp. (*Opuntia ficus-indica* and *Opuntia stricta*) can be used as alternatives to NaCMC as suspending agents in suspension formulations.

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