PHYSICO-CHEMICAL CHARACTERIZATION AND EVALUATION OF TWO LOCAL CACTUS MUCILAGES (*OPUNTIA* SPP.) AS SUSPENDING AGENTS

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics in the Department of Pharmaceutics, School of Pharmacy

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DEDICATED TO MY BROTHER,
GIRMAY G/SAMUEL (PhD)

DEAR "BIG" BROTHER:

ALBEIT IT IS DIFFICULT TO EXPRESS WITH WORDS, this is a little i can say: YOU SACRIFICED A LOT FOR THE SAKE OF ME AND OUR FAMILY. YOUR ADVICE is CONSTRUCTIVE AND FULL OF STAMINA. YOUR HELP is TREMENDOUS. IN SHORT, YOU ARE REALLY AS YOUR NAME, THE '*GRACE*' OF OUR FAMILY!!!

GOD BLESS YOU!!!

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Acronyms

ASL	Above Sea Level
Conc	Concentration
CFU	Colony Forming Unit
DACA	Drug Administration and Control Authority
EHNRI	Ethiopia Health and Nutrition Research Institute
EPHARM	Ethiopian Pharmaceutical Manufacturing Factory
HCl	Hydrochloric Acid
mPas	milli Pascal Second
mS/cm	milli Siemens per Centimeter
NaCl	Sodium Chloride
NaCMC	Sodium Carboxymethylcellulose
NaOH	Sodium Hydroxide
OFI	*Opuntia ficus-indica*
OS	*Opuntia stricta*
RH	Relative Humidity
SD	Standard Deviation
SP	Swelling Power
TVAC	Total Viable Aerobic Count
TCMYC	Total Combined Molds and Yeasts Count
USP	United States Pharmacopoeia
UV	Ultra Violet
WHC	Water Holding Capacity

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Abstract

Ethiopia is one of the countries where cactus grows naturally. Cactus pear (*Opuntia* spp.), *Cactaceae* family, is a plant with an impressive genetic diversity of over 400 species. *Cactaceae* are well adapted to arid and hot drylands, where the plants have a marked capacity to withstand prolonged drought. The peculiar feature of the plant to adapt such areas is because of the presence of mucilage in specialized cells of the cladodes. Mucilage is a complex carbohydrate 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. It swells in water and form viscous mass. Hence, recently an attention is being paid for its potential use in industry: food, cosmetic and pharmaceutical sectors.

The objective of this study is to determine the physico-chemical properties of mucilages of *Opuntia ficus-indica* and *Opuntia stricta*, the two most abundant cactus species in Ethiopia, and evaluate their suspending ability in pharmaceutical suspensions.

The physico-chemical properties of the mucilages were compared between themselves. The suspending abilities of the mucilages were compared with NaCMC. The dissolutions of the suspensions were performed using the USP paddle method at 25 rpm.

The yield of mucilage from both *Opuntia* spp. was found to be comparable. The solubility and SP of the mucilages were also studied. With increase in temperature the solubility increased and was comparable in both mucilages. However, at all the treatment levels the SP were higher in mucilage of OS than that of OFI the differences of which were statistically significant. At 100% RH the moisture sorption property of OFI and OS were 95.4% and 76.9%, respectively. The pH values of both mucilages were found to decrease upon increasing mucilage concentration which at 12% (w/v) dispersions were 5.57 and 5.87 for OFI and OS, respectively. The results showed that these pH values are to be classified within low acid group. The conductivity, at 12% (w/v), of OFI was found to be 13.12 mS/cm while that of OS was 9.31 mS/cm. The apparent viscosities at 12% (w/v) were 9,017 mPas and 10,060 mPas for OFI and OS, respectively. The apparent

viscosities of the dispersions decreased with increase in shear rates which rendered the dispersions a pseudoplastic flow.

These physico-chemical properties of the mucilages can signal on their potential use in pharmaceutical formulations especially suspensions. Hence, Paracetamol suspensions using the mucilages and NaCMC as suspending agents were formulated. 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. Moreover, the effect of pH on sedimentation volume (%) was addressed. Hence, the suspensions from NaCMC were stable in acidic media while suspensions from OFI and OS were stable in basic media. 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.

Key words: Cactus, Mucilage, *Opuntia* spp., Sedimentation volume, Suspension, Suspending agent

1. Introduction

1.1 Cactus

Cactus (*Opuntia* spp.), *Cactaceae* family, is a plant with an impressive genetic diversity of over 400 species (Stintzing, 2001). Probably no other plant family exceeds *Cactaceae* in diversity of structure: its members include trees, vines, dwarfs, giants, epiphytes and geophytes (Mauseth, 2006). Cactus is treelike, shrubby or caespitose plant; stem-segments cylindrical, club-shaped, subspherical, or more or less flattened, sometimes tuberculate, very rarely ribbed. Leaves are rounded or subulete, usually small, falling quickly; spines are usually many in one, sometimes sheathed and barbed, rarely lacking. Flowers are lateral or subterminal, rarely terminal with yellow, pink, red or whitish color (Hunt, 2000).

1.2 Geographical Distribution

Cactaceae are well adapted to arid and hot drylands, where the plants have a marked capacity to withstand prolonged drought (Cárdenas *et al*., 1997). The main countries where this plant (crop) naturally grows are Argentina, Bolivia, Brazil, Eritrea, Ethiopia, Israel, Italy, Mexico, Morocco, Peru, South Africa, Spain, Tunisia and the United States (Texas) (Sáenz *et al*., 2004).

1.3 Cactus Distribution in Ethiopia

Cactus was introduced to Northern Ethiopia by missionaries (Haile *et al*., 2002) in the mid-19th century through the then part of Ethiopia, Red sea (Mondragon Jacobo *et al*., 2006). Since then cactus has become one of the most common plants in Northern Ethiopia particularly in Tigray Region and its fruits are immensely popular.

Even though there are little efforts made so far to estimate the distribution of cactus in Ethiopia, according to Hunt (2000) the plant is available in the Eastern part of the country stretching from North east to South east with some in the highlands of Shewa. More than 60 cultivars of cactus have been identified so far in Tigray Region (Haile *et al*., 2002). The two most popular cactus plants available in the Region are those that are locally called *"Ashak Beles"* and *"Lommo Beles"* (Fig. 1.1). A survey in the region indicated that about 30,520 hectares (1.88% of the total area of the Region) are covered with *Opuntia ficus-indica* (OFI) (Tegegne, 2001).

(a) (b)

Figure 1.1 The two most common types of *Opuntia* species in Tigray Region, Northern Ethiopia (a) "*Ashak Beles*" (b) "*Lommo Beles*"

1.4 Uses of Cactus

Opuntia spp. and their products serve as food, forage, energy, agronomic, cosmetics, etc (Barber *et al*., 1995). There are reports also on the use of this plant, either the cladode or its fruit, as medicine. The consumption of cactus fruits is traditionally associated with diuretic activity (Bisson *et al*., 2010). In a report by Frati *et al.* (1990) the oral intake of nopal (*Opuntia* spp.) induces an hypoglycemic effect. *Opuntia* spp. cladodes are employed for their anti-inflammatory, cicatrizant and antiulcer activity. In addition, the fibers contained within the cladodes of the plants play an important role in the prevention of many pathological conditions including constipation, haemorrhoids, colon cancer as well as several metabolic diseases as obesity and dyslipidaemias (Galati *et al*., 2007).

It is indeed difficult to find more widespread and better exploited plants, particularly in the subsistence economy of arid and semi-arid zones, where *Opuntia* spp. have become endless source of products and functions, initially as wild plants and, later, as crops for both subsistence and market-oriented agriculture (Barber *et al*., 1995). In addition to its use as vegetable (Nopalitos) and forage, *Opuntia* spp. are being utilized in programs to prevent soil erosion and to combat desertification. They have great capacity for adaptation, growing in severely degraded

soils which are inadequate for other crops and are ideal for responding to global environmental changes such as the increase in atmospheric carbon dioxide levels (Reynolds and Arias, 2001; Habibi *et al*., 2002). *Opuntia* spp. are also important as cover in arid and semi-arid areas because they can survive and spread under conditions of scarce and erratic rainfall and high temperatures and can play an important role in the protection of local fauna (Reynolds and Arias, 2001).

1.5 Cactus Mucilage

The production of mucilages, often referred as pectin polysaccharides, is characteristic of members of the *Cactaceae* family (Matsuhiro *et al*., 2006). The ability of *Cactaceae* to retain water under an unfavorable climatic condition is due in part, at least, to the water-binding capacity of mucilage. The mucilage biosynthesis takes place in specialized cells that excrete it into the apoplast, where it helps regulate the cellular water content during the initial phase of dehydration (Cárdenas *et al*., 1997).

Cactus mucilage is receiving research attention with a focus on yield and some physicochemical characteristics for use in food industry (Goldstein *et al*., 1991; Cárdenas *et al*., 1997; Sepúlveda *et al*., 2007). Unfortunately, the reports do not indicate which types of *Opuntia* spp. have been investigated or it is only that of the OFI species type.

1.5.1 Cactus Mucilage Yield

Different scholars have already determined the mucilage yield from *Opuntia* spp. especially from that of OFI. It was found to be 1.4% from fresh cladode and 17.95% from dried cladode based on a study by Sepúlveda *et al*. (2007). Mucilage content in the stems of four sympatric cactus species varied from none for *Ferocactus acanthodes*, to 19% by dry weight for *Opuntia basilaris*, 26% for *Opuntia acanthocarpa* and 35% for *Echinocereus engelmannii (*Nobel *et al*., 1992*)*. The yield and quality of the mucilage varies and this has been attributed to species types, age of the cladode, season of collection and also topography variation like rain distribution (Sáenz, *et al*., 2004), temperature (Goldstein *et al*., 1991) and soil type (Sepúlveda *et al*., 2007).

1.5.2 Physico-chemical Characteristics of Cactus Mucilages

Assessing the physico-chemical properties of the mucilages is important to know if they are to be qualified for pharmaceutical purposes as the physico-chemical properties of excipients affect the ultimate quality of pharmaceutical formulations.

It was reported that on increasing the pH of aqueous mucilage dispersion of OFI from 2.6 to 6.6, the viscosity increased from 37 to 58 mPas (Sáenz, 2000). In another study, the rheology of the mucilage at different concentration (0.4 to 6% (w/v)) was noticed to have non-Newtonian shearing behavior (Sáenz-Hernander *et al*., 2002).

In a study by Sepúlveda *et al*. (2007), the mucilage extracted from OFI by macerating the cladode for 4 hrs at 40 ^{o}C and using ethanol as precipitating agent, it was revealed the mucilage to be composed of the following: 5.9% moisture; 7.3% protein; and 34.9% ash; 10.53% Ca^{2+}; and 1.81% K^{+}.

1.5.3 Chemical Compositions of Cactus Mucilage

The chemical compositions of OFI mucilage from cladodes (pads), commonly named nopal, have been the subject of various studies (Matsuhiro *et al*., 2006). 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). Further investigations on the mucilage of OFI suggested that the mucilage to have α-arabinofuranosyl, β-xylopyranosyl, β-rhamnopyranosyl, β-galactopyranosyl and α-galactopyranosyluronic acid residues (McGarvie and Parolis, 1979). However, the mucilage composition was found to differ among different cactus species and within species from different areas (Trachtenberg and Mayer, 1981a). For example, the mucilage isolated from *Opuntia aurantiaca* has been shown by methylation and partial hydrolysis studies to possess a highly branched structure. The mucilage is composed of 4-linked galactosyluronic acid, 2-linked rhamnosyl, and 6-linked galactosyl residues. Most of the last-named residues carry branches at C-3 or C-4, or both C-3 and C-4. The branches are composed mainly of (1→3)-linked, (1→5)-linked, end-group arabinofuranosyl

residues and end-group xylopyranosyl residues. The mucilage possesses both acid-stable and acid-labile galactosyluronic acid residues (McGarvie and Parolis, 1981a).

1.5.4 Potential Uses of Cactus Mucilage

Despite lack of rigorous scientific works to corroborate the uses of the cactus mucilage, there are reports that show on the potential uses of the mucilage in pharmaceutical formulations. According to Cárdenas and his co-workers *Opuntia* spp. has been considered as a potential source of industrial hydrocolloid gum which they have indicated the presence of genuine interest among companies to start producing it on a large scale (Cárdenas *et al*., 1997). One of the application areas of these cactus hydrocolloids is in pharmaceutical industry (Del-Valle *et al*., 2005). Stintzing *et al.* (2001) suggested on the possible use of the mucilage in wound creams (cooling cream). This role may be attributed to the humectant nature of the mucilage (Barber *et al*., 1995). Moreover, cactus mucilage swells when dissolved in water to form colloidal and viscous dispersions or jellied masses (Del-Valle *et al*., 2005). Hence, it can be used as a thickening agent in pharmaceutical industries (Sepúlveda *et al*., 2007). This was supported by Young *et al*. (2006). As to their finding *Opuntia* spp. mucilage has shown excellent flocculating ability of water impurities. Hence, proving the flocculating ability of the mucilages to pharmaceutical drugs could be of paramount importance.

1.6 Suspensions

Suspensions constitute an important class of pharmaceutical preparations (Martin, 1961). They are liquid preparations that consist of solid particles dispersed throughout a liquid phase in which the particles are insoluble (Nairn, 2005). Hence, they are included into the group of disperse systems (Dziubinski *et al*., 2004). They often provide a means of supplying insoluble and often distasteful substances in a form that is pleasant to taste. Moreover, the large surface area of the dispersed drug ensures a high availability of drug for dissolution and absorption (Agarwal and Khanna, 2006).

1.6.1 Types of Suspensions

Based on the electrokinetic nature of solid particles, suspensions can be classified as flocculated and deflocculated (Agarwal and Khanna, 2006). In flocculated suspensions there exists

formation of a loose aggregation of discrete particles held together in a network-like structure by physical adsorption of macromolecules with the help of van der Waals force of attraction (Nash, 1996). If the length of polymer extends from the particle surface into the solution is greater than the distance over which the interparticle repulsion acts, the polymer can adsorb onto another particle, thereby bridging the two (Levy *et al*., 1995). This bridging leads to production of loose, voluminous sediments containing large amounts of trapped liquid suspending medium (Schott, 1976). While in deflocculated suspensions, there exists absence of association which occurs when repulsive force between particles predominate (i.e., particles repel each other and remain as discrete, single particles) (WRG, 2009).

1.6.2 Components of Suspensions

During the preparation of physically stable pharmaceutical suspensions, a number of formulation components are used to keep the solid particles in a state of suspension (Nash, 1996). Suspending agents are one component of suspensions commonly used for retarding sedimentation of suspended particles. They can be inorganic materials, synthetic compounds or polysaccharides (Mann *et al*., 2007). Examples are thickeners, polyelectrolytes, flocculating agents (electrolytes) and wetting agents (Nash, 1996).

Thickeners

These are employed to increase the viscosity; even small concentrations have a noticeable effect with the basic disadvantage of solutions of polymeric thickeners being diluted by the application of large shearing forces (Mollet and Grubenmann, 2001). In suspensions, water-soluble hydrophilic colloids work by increasing the viscosity of the aqueous continuous phase, either through simple chain entanglement or formation of network through cross-linking, thereby hindering the sedimentation of the disperse phase. In practice, there is an upper limit for the level of incorporation of any polymer that will allow the formulation of a pourable suspension. This limit will depend on the type of polymer, its mode of action, molecular weight, etc (Moreton, 2009).

Polyelectrolytes

One of the most efficient methods of regulation of properties of suspensions is application of polyelectrolytes (Tkachenko *et al*., 2006), which are known to act as effective flocculant. The formation of bridges between colloid particles and charge neutralization are concepts to explain the mechanism of flocculation of colloids with polyelectrolytes (Adachi and Aoki, 2004).

Electrolytes

The addition of an inorganic electrolyte to an aqueous suspension increases the surface electrostatic repulsion of the suspended particles (Yasueda *et al*., 2004) which alters the zeta potential of the dispersed particles and if this value is lowered sufficiently, flocculation may occur (Billany, 2007). The effectiveness of electrolytes in compression of the electrostatic double layers around the particles and the consequent effect on the stability of colloidal dispersions have been addressed (Wang and Guo, 2007). The presence of electrolytes curtails the range of coulombic interactions and compresses the electrical double layer; this compression by electrolytes eventually results in flocculation of the dispersion. The effect is much more pronounced for multiple charged ions than for those with a single charge (Mollet and Grubenmann, 2001).

The other components of suspensions are grouped under components of the liquid vehicle which include pH controlling agents & buffers and preservatives (Nash, 1996).

pH controlling agents and buffers

pH typically has significant effect on the surface charge of many solids, including drugs and polymers of pharmaceutical interest (Gallardo *et al.,* 2005). According to a study by Arias *et al*. (2009), at lower pH the zeta potential of the particles is low and aggregation of voluminous floccules will be favored by van der Waals attraction. In contrast, at higher pH, the electrokinetic potential of the particles is large, and this will provoke electrostatic double-layer repulsions between them. Hence, maintaining the desired pH through addition of pH controlling agent /buffers is important from suspension stability point of view. Citric acid, Phosphoric acid and their pharmaceutically accepted salts are the most commonly used buffers in suspension formulations (Gohel *et al.*, 2007).

Preservatives

Pharmaceutical suspensions with water as the continuous phase are susceptible to microbial spoilage (Moreton, 2009). They must therefore be protected from large varieties of possible microorganisms (Mollet and Grubenmann, 2001). Examples of preservatives include Benzoic acid, Benzyl acid, Methyl paraben, Propyl paraben, Benzalkonium chloride (Moreton, 2009).

1.6.3 Physical Stability of Suspensions

In this work dispersion and suspension are used interchangeably. A stable dispersion is one in which the total number and size of the particles in the dispersion do not change over time (Mollet and Grubenmann, 2001). Most surfaces acquire a surface electric charge when brought into contact with an aqueous medium (Attwood, 2007). The charge originates by ionization of the surface or by adsorption of ions from the solution (Martin, 1961; Wilson and Ecanow 1963; Attwood, 2007). Moreover, the charge can originate from comminution, as most pharmaceutical suspensions are prepared by it (Mollet and Grubenmann, 2001). Hence, they render greater free energy (owing to their surface area) (Agarwal and Khanna, 2006). This free energy enables the system to have a tendency to revert spontaneously to a state of lower free energy through flocculation of the particles, unless this is prevented by an energy barrier. This energy barrier is the result of the combination of two forces, van der Waals attraction and a repulsive force due to surface charges and adsorption layers (Mollet and Grubenmann, 2001). This was quantitatively expressed by Equation 1.1, considered long ago, which is now known as the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory (Attwood, 2007). This theory applies to colloidal systems, and is not wholly applicable to coarse suspensions. It, however, is applied to them as it does give some insight as to how certain suspension stabilizers function (Moreton, 2009). In this theory, it is assumed that the only interactions involved are electrical repulsion, and van der Waals attraction and these parameters are additive. Therefore, the total potential energy of interaction (V_T) is given by (Matthews and Rhodes, 1970; Ramakrishnan *et al.,* 1998; Amiri *et al.*, 2009):

$$V_T = V_A + V_R \tag{1.1}$$

where V_A is potential energy as a result of van der Waals attraction and V_R is potential energy as a result of electrical repulsion.

The overall charge existing on the suspended particle is the zeta potential and is a measurable indication of the potential existing at the surface of particles. Therefore, flocculation and deflocculation may be considered in terms of zeta potential (Agarwal and Khanna, 2006).

1.6.4 Quality Assessment Parameters of Suspensions

Preparation of pharmaceutical suspension is often associated with problems of physical stability such as the difficulty of redispersibility of sediment and crystal growth (Saeedi *et al*., 2003). Hence, the following parameters are the most commonly employed parameters for the assessment of physical stability of suspensions.

1.6.4.1 Sedimentation Volume and Degree of Flocculation

The extent of flocculation of a system can be defined by the extent of sedimentation volume (Nutan and Reddy, 2009). It is the ratio of ultimate volume of the sediment to the total volume of the suspension which is expressed mathematically by Equation 1.2 (Agarwal and Khanna, 2006):

$$F = \frac{V_U}{V_O} \tag{1.2}$$

where F is the sedimentation volume, V_u is the ultimate volume of the sediment and V_o is the total volume of the suspension.

Hence, sedimentation volume can range from 0 to 1. But some time it may exceed 1 when it forms fluffy floccules (Sinko and Martin, 2006). A more applicable parameter for flocculation is the degree of flocculation which relates the sedimentation volume of the flocculated suspension to the sedimentation volume of the suspension when it is deflocculated which is expressed mathematically by Equation 1.3 (Nutan and Reddy, 2009):

$$\beta = \frac{V_U}{V_\infty} \tag{1.3}$$

where β is degree of flocculation, V_u is total volume of suspension and V_∞ is the ultimate volume of the deflocculated suspension.

1.6.4.2 Redispersibility of Suspensions

Redispersibility is defined as an ability to resuspend settled particles with minimum shaking after a suspension is left standing for a period of time (Manwiwattanagur *et al.,* 2003). It is one of the major considerations in the assessment of a suspension (Saeedi *et al*., 2003). The resuspendability associated with flocculation-deflocculation behavior of drugs has been of great interest since most pharmaceutical suspensions contain hydrophilic polymers as suspending agents (Ntawukulilyayo *et al*., 1996).

1.6.4.3 Rheology of Suspensions

Rheological or the flow behavior of pharmaceutical suspensions is very useful in assessing the state of particle dispersion in powder-liquid suspensions (Wang and Guo, 2007). This is because it affects the settling and redispersion of the dispersed particles (Agarwal and Khanna, 2006) in addition to accurate dosing, ease of flow and application (Nutan and Reddy, 2009). Hence, based on their rheology suspensions can also be classified as those that exhibit plastic or pseudoplastic behavior (for flocculated suspensions). The mucilages of natural and synthetic gums, which include a large majority of suspending agents, are pseudoplastic (Kabre *et al*., 1964). The second type is those that exhibit Newtonian behavior (for deflocculated suspensions) (Billany, 2007).

1.6.4.4 Dissolution of Suspensions

The rate of dissolution of drugs remains one of the most challenging aspects in formulation development of poorly water-soluble drugs (Ambrus *et al.,* 2009). Dissolution may, in fact, be one of the rate-limiting factors for the absorption (Prabhu *et al*., 2001) and bioavailability of suspensions, capsules, and tablets (Howard *et al*., 1979). *In vitro* suspension dissolution has been related to the absorption rate**.** Several investigators have shown that the relationship between dissolution rate and formulation components to be most important. In particular, suspending agents are important in suspension dissolution (Howard *et al*., 1979) as polymers used in suspensions have been found to interfere with the dissolution of drugs from these dosage forms (Azam and Haider, 2008).

1.7 Objectives of the Study

1.7.1 General Objective

To study the physico-chemical properties and evaluate two local cactus mucilages (*Opuntia* spp.) as suspending agents.

1.7.2 Specific Objectives

- To compare mucilage yield of the two cactus species collected from different areas and subareas.
- To study and compare some physicochemical properties of the mucilages
- To evaluate the mucilages as suspending agent in suspension formulations.
- To evaluate the effect of pH and electrolyte concentration on sedimentation volume in the presence of the suspending agents.
- To study *in vitro* drug release profile of suspensions prepared by mucilages of the selected cactus species.

2. Experimental

2.1 Materials

Fresh cactus cladodes were obtained from three different areas of Tigray Region, Northern Ethiopia. Paracetamol powder (China Assoc. Co. Ltd, China) and Trypton Soya Agar Medium (Oxoid Ltd, England) were obtained from EPHARM and Paracetamol reference standard (Batch Nº RM 8051, purity 99.5%) was obtained from DACA. Chloramphenicol Selective Supplement (Oxoid Ltd, England) and Rose Bengal Agar Medium (BDH, England) were obtained from EHNRI. NaCMC–low viscosity grade, Methyl paraben, Propyl paraben (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.

2.2 Methods

2.2.1 Sample Collection, Identification and Mucilage Extraction

Sample Collection

Sample cladodes were randomly collected, in the months of February and March, from three different areas of Tigray Region, Northern Ethiopia where cactus grow naturally: Adigrat (2459 m, ASL), Mekelle (2239 m, ASL) and Maichew (from three different subareas): Wargua (1730 m, ASL), Kankano (2235 m, ASL) and Bokura (2692 m, ASL). The cladodes were kept in refrigerator (LEC pharma, PE202P, United Kingdom) at 4 ^{o}C until extracted.

Sample Identification

Sample cladodes were identified by Mr. Melaku Wondafrash (taxonomist), Department of Biology, AAU, and voucher specimen has been deposited at AAU Herbarium (Nº NGS001 and NGS002 for *Opuntia stricta* and *Opuntia ficus-indica*, respectively). One of the species (Fig. 1.1), locally known as "*Lommo Beles*", was identified to be *Opuntia stricta* (OS) while the second species, locally known as "*Ashak Beles*", was identified to be *Opuntia ficus-indica* (OFI).

Mucilage Extraction

Extraction of the mucilage was done according to the method reported elsewhere (Sepúlveda *et al*., 2007). Briefly, fresh cladodes were washed with distilled water and chopped with knife into pieces. A known amount of cladode was homogenized with distilled water (mixed in a ratio of 1:5) in a blender (Christson, HGP2WPG4, England) for 1 min. The mixture was macerated in an oven at 40 ^{o}C for 4 hrs followed by filtration through muslin. The aqueous extract was centrifuged (Centurion, 6000 series, England) at 3500 rpm for 10 min. The supernatant was decanted and filtered through nylon cloth. The filtered aqueous extract was then mixed with Ethanol 96% in a ratio of 1:3 to precipitate out the mucilage. The aqueous portion was decanted and the precipitate was rinsed with Ethanol 70% and allowed to dry in vacuum oven drier (Genlab, VC 250, England) at 50 ^{o}C for 4 days. Similarly, air dried cladodes were ground into powder with mortar and pestle and extracted following same procedures as in the above.

2.2.2 Yield Determination

From each sample, 100 g of fresh cladode and 20 g of dried cladode (only from Mekelle area) were weighed and mucilages were extracted as described above (Section 2.2.1). Yields recorded are average of three determinations.

2.2.3 Physico-chemical Characterization of the Mucilages

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 and the following properties were determined.

2.2.3.1 Powder Properties

Bulk Density

From each sample, 30 g was carefully poured into a 250 ml graduated glass measuring cylinder. The volumes of the sample powders were then noted. The bulk densities were determined using Equation 2.1. Bulk densities recorded are average of three determinations.

$$\text{Bulk density}\left(\rho_B\right) = \frac{m}{V_B} \tag{2.1}$$

where m is mass of sample (in g) and V_B is volume of sample powder.

Tapped Density

From each sample, 30 g was carefully poured into a 250 ml graduated glass measuring cylinder and tapped 250 times using tapped densitometer (ERWEKA, SVM 20, Germany). The volumes of the sample powders were noted. The densitometer provided a fixed drop of half an inch at a rate of 250 taps/min. The tapped densities were determined using Equation 2.2. Tapped densities recorded are averages of three determinations.

$$\text{Tapped density}\left(\rho_T\right) = \frac{m}{V_T} \tag{2.2}$$

where m is mass of sample and V_T is volume of the sample after tapping.

True Density

True densities of the samples were determined with the principle of liquid displacement using 50 ml Pycnometer. The liquid used for this purpose was Toluene (Density of 0.865 g/ml). Results recorded are averages of three determinations.

Density Related Properties

The Carr's compressibility index and the Hausner ratio were calculated from the bulk and tapped densities of the mucilages using Equations 2.3 and 2.4, respectively.

$$\text{Carr's index (\%)} = \left(\frac{\rho_T - \rho_B}{\rho_T}\right) X 100 \tag{2.3}$$

$$\text{Hausner Ratio} = \left(\frac{\rho_T}{\rho_B}\right) \tag{2.4}$$

where ρ_B is bulk density and ρ_T is tapped density.

Angle of Repose/Flow Rate

The angle of repose/flow rate was determined using a method used elsewhere (Belete *et al.*, 2003). From each sample, 50 g of mucilage powder was allowed to flow from a 10 cm height through a glass funnel with an inner diameter of 15 mm and the flow property was observed.

2.2.3.2 Solubility and Swelling Power

These properties were determined following the method of Takizawa *et al*. (2004), with slight modification. From each sample 0.125 g was dispersed in 10 ml of distilled water in a centrifuge test tube. The dispersion was heated, under mild agitation, in a thermostat (GFL, D 3006, Germany) at 40 ^{o}C, 55 ^{o}C, 65 ^{o}C, 75 ^{o}C and 85 ^{o}C for 10 min. The tube was then removed from the water bath and immediately immersed in cold water for 5 min and centrifuged (K centrifuge, Harmonic series, Taiwan) for 15 min at 3000 rpm. The supernatant was dried to constant weight in oven (KOTTERMANN, D3165, West Germany) at 105 ^{o}C. The precipitated paste and the dried supernatant were then weighed. Similarly, the three properties were also evaluated at room temperature (15±0.5 ^{o}C).

The swelling power (SP) and solubility were calculated using Equations 2.5 (Tattiyakul *et al*., 2006) and 2.6 (Mandala and Bayas, 2004), respectively. Results of these properties recorded are averages of three determinations.

$$\text{Swelling power [g/g mucilage]} = \frac{m_{sw}}{(m_o - m_s)} \tag{2.5}$$

$$\text{Solubility [g/100g mucilage]} = \frac{m_s}{m_o} x100\% \tag{2.6}$$

where m_{sw} is weight of swollen mucilage, m_o is sample weight and m_s is weight of dried supernatant.

2.2.3.3 Moisture Sorption Properties of the Mucilages

The moisture sorption properties of the mucilages were done based on the method described elsewhere (Gebre-Mariam and Schmidt, 1996) with slight modification. Pyrex dessicators containing saturated solutions or appropriate concentrations of NaOH solutions were prepared and stored at room temperature. 2 g of pre-dried mucilage was placed on dried and known weight petri dish and placed in metal frames and transferred to a particular relative humidity chamber. Samples were equilibrated for two weeks at room temperature. The moisture content, expressed as percent of weight of water by weight of solid, of each sample was determined based

on the gravimetric method described in section 2.2.3.4. Moisture sorption, at different RH (%), recorded are average of two determinations.

2.2.3.4 Chemical Compositions

Moisture Content

The moisture contents of the mucilage samples were determined using gravimetric method (Aklilu *et al*, 2002). From each sample, 2 g of mucilage powder was placed in a pre-dried (at 92 ^{o}C for 30 min) and known mass of crucible; and then left to dry in an oven (MEMMERT, Din 40050, West Germany) at 105 ^{o}C for 5 hrs. The crucible was then taken out of the oven and allowed to cool in a desiccator and weighed. The difference in weight is taken as the moisture content. Moisture contents recorded are averages of three determinations.

Ash Value

Ash values of the samples were determined based on the method described elsewhere (Hassan *et al.*, 2005). From each sample, 1 g of powder was weighed in a preweighed ashing dish followed by heating in a furnace (CARBOLITE, OAF 11/1, England) at 550 ^{o}C for 8 hrs. The sample was then removed and kept in a desiccator and weighed. Total ash values in the samples of interest were calculated using Equation 2.7. Ash values recorded are average of three determinations.

$$\% \text{ total ash } = \frac{m_2 - m_1}{m} x100 \qquad (2.7)$$

where m_1 is mass of the ashing dish, m_2 is mass of the crucible plus ash and m is mass of sample.

Crude Fiber Content

Crude fiber contents were determined using the method described elsewhere (Śmiechowska and Dmowski, 2006). From each sample, 2 g of mucilage powder was boiled in 200 ml of 1.25% (v/v) sulfuric acid solution for 30 min and subsequently boiled with 20 ml of 28% (w/v) KOH. Each sample was then filtered through sintered glass crucible and washed with hot water and 1% (v/v) sulfuric acid. Each sample thus prepared was dried in an oven (MEMMERT, Din 40050, West Germany) at 130 °C for 2 hrs; and then removed and allowed to cool in a desiccator for 30 min and weighed. Each sample was then transferred into furnace (CARBOLITE, OFI 11/1, England) at 550 ^{o}C and kept for 30 min and then removed and allowed to cool in a desiccator

and weighed. Finally, crude fiber contents of the mucilage samples were calculated using Equation 2.8. Crude fiber contents recorded are averages of three determinations.

$$\text{Crude fiber content (\%)} = \frac{(m_1 - m_2)(100 - M)}{m_3} \quad (2.8)$$

where m_1 is weight of crucible after drying, m_2 is weight crucible after ashing, m_3 is sample dry weight and M is percent moisture of the sample.

Fat Content

The fat contents of the mucilages were determined by the soxhlet method described elsewhere (Kavishree *et al.,* 2008) with slight modification. From each sample, 2g of mucilage powder was placed in a thimble and plugged into a soxhlet extractor (Soxtec Tecator, HT6, Sweden). 150 ml of diethyl ether was poured into the thimble through an opening on the soxhlet apparatus. A pre-dried and pre-weighed extraction flask was then assembled on the soxhlet as cover to the thimble and collect percolated diethyl ether. The assembled part was allowed to descend to a hot plate and kept for the first 1 hr and then suspended for the next 3 hrs for percolation. The fat contents of the mucilages were determined gravimetrically after oven-drying (at 92 ^{o}C) for 30 min. Fat contents recorded are averages of three determinations.

Protein Content

Protein contents of the mucilages were analyzed using the Kjeldahl method; where the nitrogen content is multiplied by a factor of 6.25 to give protein content (Benhura and Chidewe, 2002). From each sample, 0.5 g mucilage powder was digested at 400 ^{o}C in a digester (TECATOR DIGESTER, I 2020, Sweden) using 6 ml mixture of sulfuric acid and orthophospheric acid (100:5) in the presence of 0.5 ml selenium and 100 ml potassium sulphate. The solution was then distilled with sodium hydroxide (added in small quantity) which converts ammonium salt into ammonia. The ammonia was trapped using 1% (v/v) boric acid and titrated using 0.1 N HCl solution until end point was reached. Both distillation and titration and with subsequent determination of nitrogen and protein content were automatically done by the Microkjeldahl apparatus (Wagtech, 2300 kjeltec analyzer unit, Sweden). Protein contents recorded are averages of three determinations.

2.2.3.5 Aqueous Dispersion Properties

Surface Tension

The surface tension of the mucilage dispersion was determined using Wilhelmy plate method (Muñoz *et al.,* 2007). From each sample, 0.5 g of mucilage powder was first dispersed in distilled water to prepare 1% (w/v) and then placed in a thermostat (Grant, GP200, England) at 80 ^{o}C for 1 hr with continuous stirring. The sample was then removed and cooled to room temperature (20±0.5°C). The bracket (length = 6.5 cm and thickness = 0.3 cm) of the tensiometer was then fully immersed into the dispersion. After equilibrating for 1 hr, the bracket was allowed to ascend with the help of the screw. By the moment the bracket was to emerge out of the dispersion, extent of deflection of the pointer from the reference zero was noted. Hence, equal deflection was produced using diammonium phosphate (DAP) granules whose mass was then determined. Finally, the surface tension was calculated using equation 2.9. Surface tensions recorded are averages of three determinations.

$$\delta = \frac{K}{L} = \frac{m_1 xg}{2x(b+l)} \tag{2.9}$$

Where δ is surface tension (in N/M), m_1 is mass (in kg) of the DAP granule used, g is gravitational acceleration (9.8 m/s^2), b and l are thickness and length of bracket (in meter), respectively.

Conductivity

Different masses of mucilage powder (0.2 g, 0.8 g, 1.6 g and 2.4 g) from each sample were dispersed in distilled water, at room temperature (15±2^{o}C), to prepare 1% (w/v), 4% (w/v), 8% (w/v) and 12% (w/v) dispersions, respectively. The dispersions were stirred using magnetic stirrer for 2 hrs and evaluated for conductivity using conductometer (WTW, LF 300, Germany). Results of the property recorded are averages of three determinations.

pH

Different masses of mucilage powder (0.1 g, 0.4 g, 0.8 g and 1.2 g) from each sample were dispersed in distilled water, at room temperature (15±2^{o}C), to prepare 1% (w/v), 4% (w/v), 8% (w/v) and 12% (w/v) dispersions, respectively. The dispersions were stirred using magnetic

stirrer for 2 hrs. The pH meter (Waterproof, pH Testr 10, USA) was calibrated with a standard solution of known pH. The pH measurement of the mucilage dispersions were read from the instrument. Results of the property recorded are averages of three determinations.

Viscosity

Effect of mucilage concentration on apparent viscosity: Different masses of mucilage powder (2 g, 4 g and 6 g), from each sample, were dispersed in portion of distilled water. The dispersion was adjusted to volume (50 ml) using distilled water with continuous stirring. The preparations were then kept overnight at room temperature. The measurements of the viscosities of the dispersions were made at 20±0.5 °C using spindle number 4 of rotational viscometer (viscostare plus L, KINEMATICA AG, Switzerland) at shear rate of 20 rpm. Results of the property recorded are averages of two determinations.

Effect of shear rate on apparent viscosity: 6 g of mucilage powder, from each sample, was dispersed in portion of distilled water. The dispersion was adjusted to volume using distilled water with continuous stirring. The preparation was then kept overnight at room temperature. The measurements of the viscosities of the dispersion were made at 20±0.5 °C using spindle number 4 of the viscometer (viscostare plus L, KINEMATICA AG, Switzerland). Results of the property recorded are averages of two determinations.

2.2.3.6 Microbial Loads

The microbial loads of the mucilages were done based on the pour plate method described elsewhere (USP, 2009). Total viable aerobic counts (TVAC) of the mucilages were done using 1% Saline-Peptone as diluents and Tryptosan Soya Agar as medium. 1.25 g of each mucilage type was suspended in 12.5 ml of 1% Saline-Peptone solution. Each was serially diluted to get 1:100 and 1:1000. From each of the two dilutions 1 ml was placed in sterile petri dish. Into each of the petri dish 20 ml of Tryptosan Soya Agar Medium, previously sterilized in an autoclave (Raypa® steam sterilizer, CE 1027, Spain) at 121 ^{o}C for 15 min, was added and allowed to conjule. Controls used were Tryptosan Soya Agar Medium and combination of 1% Saline-Peptone and Tryptosan Soya Agar Medium. All were finally incubated (Fisher isotemp® incubator, deluxe model 208, USA*)* for 48 hrs at 37 ^{o}C. Following incubation, the plates

were examined for growth; Total colony forming units (CFU) were counted. TVAC recorded are averages of two determinations. Similarly, the Total Combined Molds and Yeasts Count (TCMYC) was ensued as above, except for using Dichloran Rose Bangal Agar Medium, instead of Tryptosan Soya Agar Medium, and keeping in an incubator (Johanna Otta GmbH, TH 30, Germany) with inverted petri dish for 5 days at 25 ^{o}C.

2.2.3.7 Acute Toxicity

Acute toxicity study was carried out according to the method reported elsewhere (Kumar *et al*., 2009). The animals used in the toxicity studies were female mice, fasted for overnight, which had an average weight of 25 g and age of 6-8 weeks. The mice were divided into three comprising a group of six mice each. The control group received 33.3 ml/kg of distilled water while the second and third groups received 5000 mg/kg of mucilage suspension of OFI and OS, in distilled water, orally. The animals were observed for behavioral changes for the following 4 hrs. The animals were also observed for mortality for the following 48 hrs.

2.2.3.8 Preliminary Phytochemical Screening

The mucilages were screened for secondary metabolites such as alkaloids, flavonoids, saponins, steroidal compounds and tannins using standard procedures.

Test for Steroidal Compounds

Salkowski's test: 0.5 g of the mucilage powder, from each sample, was dispersed in 1 ml chloroform. 1 ml of concentrated sulfuric acid was added down to each of the test tube to form two phases. Formation of a red or yellow coloration was taken as an indication for the presence of sterols (Musa *et al*., 2009).

Test for Alkaloids

The test was done based on the method described by Musa *et al*. (2009). 0.5 g of each mucilage powder was mixed with 20 ml of diluted hydrochloric acid on a water bath. It was continuously stirred and allowed to settle. 12 ml of the filtrate was divided into three. To the first 4 ml, few drops of freshly prepared Dragendorff's reagent were added and observed for formation of orange to brownish precipitate. To the second 4 ml, few drops of Mayer's reagent were added

and observed for formation of white to yellowish or cream color precipitate. To the third 4 ml, few drops of Wagner's reagent were added and observed for a brown or reddish precipitate.

Test for Saponins

Froth test: From each sample, 0.5 g was weighed and dispersed in 10 ml of distilled water in a test tube. The test tubes were tightly closed with stopper and shaken vigorously for about 30 sec; and allowed to stand in a vertical position and observed for 30 min. If a "honey comb" froth above the surface of liquid persists after 30 min the sample is suspected to contain saponins (Muzemil *et al*., 2008).

Test for Tannins

Ferric chloride test: From each sample, 0.5 g of mucilage was dispersed in 30 ml water and boiled. The dispersions were clarified by filtration. Few drops of 10% (w/v) ferric chloride solution were added to each of the clear filtrate. Formation of blue-black or green precipitate was taken as evidence for the presence of tannins (Musa *et al*., 2009).

Test for Flavonoids

Test for free flavonoids: 5 ml of ethyl acetate was added to dispersion of 0.5 g of mucilage powder in water. The mixtures were shaken, allowed to settle and inspected for the production of yellow color in the organic layer which is taken as positive for free flavonoids (Muzemil *et al*., 2008).

Lead acetate test: From each sample, dispersion was prepared using 0.5 g of mucilage powder in water. 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids (Muzemil *et al*., 2008).

Reaction with sodium hydroxide: Diluted sodium hydroxide solution was added to dispersions of 0.5 g of both mucilage powder in water. The mixtures were inspected for the production of yellow color which is considered as positive for flavonoids (Muzemil *et al*., 2008).

2.2.4 Suspensions Preparation

The compositions of the Paracetamol suspensions are given in Table 2.1. The suspensions are grouped into three: A (for NaCMC), B (for OS) and C (for OFI). Each group has five formulations (A2 - A6, B2 - B6 and C2 - C6). The letters A, B and C indicate the type of suspending agent, while the figure 2, 3, 4, 5 and 6 represent the percent concentration 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 till uniform dispersion was obtained.

Table 2.1.Formulation ingredients of the Paracetamol suspensions

Formulation Ingredients	Composition (% (w/v))
Paracetamol	6
Suspending agent*	2, 3, 4, 5 or 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

2.2.5 Physical Stability Assessment of the Suspensions

The suspending ability of the mucilages in comparison with NaCMC was evaluated by assessing the physical stability of the suspension formulations using the following properties.

2.2.5.1 Rheology of the Suspensions

2.2.5.1.1 Effect of Shear Rates on Apparent Viscosity

The effect of shear rates on viscosity was studied using viscometer (viscostare plus L, KINEMATICA AG, Switzerland). The viscosities of the suspension were measured in kPas within 48 hrs of preparation. The measurement was made at 20±0.5 ^{o}C using spindle number 4 at

20, 30, 50, 60, 100 and 200 rpm. Apparent viscosities recorded are average of two determinations.

2.2.5.1.2 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 2.10. Flow rates recorded are average of three determinations.

$$\text{Flow rate} = \frac{V_s}{T} \tag{2.10}$$

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.

2.2.5.2 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. Another batch of suspensions was allowed to settle for four weeks and their sedimentation volumes were recorded every week. The readings of the sedimentation volumes (%) were taken where the clear supernatant start to become cloudy up on descending from the top surface of the suspension. Results recorded are averages of three determinations.

2.2.5.2.1 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.

2.2.5.2.2 Effect of pH on Sedimentation Volume

10 ml suspensions which had pH of 2, 6.5, 8 or 10 were prepared from each of the three suspending agents at concentration of 4% (w/v). The pH of the suspensions was adjusted using

solutions of 0.1 or 1 N HCl and 0.1 or 1 N NaOH. pH meter (SCHOTT, CG 843P, Germany) was used to check the attainment of the desired pH. Each of the preparations was poured to 15 ml of graduated measuring cylinder. The sedimentation volumes (%) of the formulations were noted every day for the following seven days after preparation. Results recorded are averages of three determinations.

2.2.5.3 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. Another batch of suspensions was also kept for four weeks. The measuring cylinders were then manually rotated at 180^{o}. 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.

2.2.5.4 Determination of λ_{max} and Construction of Calibration Curve

Preparation of stock solution

50 mg of pure Paracetamol was weighed and dissolved in 250 ml volumetric flask with about 100 ml of 0.1 N HCl (pH=1.0). Then, the resulting solution was diluted to volume with the solvent and mixed to provide a stock solution with a concentration of $1.32x10^{-3}$M.

Determination of λ_{max}

5 ml sample from the above stock solution was diluted to 100 ml with 0.1 N HCl. The resulting diluted solution was scanned over the wavelength range of 200 nm and 300 nm using UV/Visible spectrophotometer (CECIL, CE 3021, England) to obtain the wavelength of maximum absorption (λ_{max}) of the drug. The solvent was used as blank.

Calibration curve

Different dilutions were made from the already prepared stock solution to provide $1.65x10^{-5}$ M, $3.31x10^{-5}$ M, $4.96x10^{-5}$ M, $6.61x10^{-5}$ M and $8.27x10^{-5}$ M. Then, the UV absorbance of each of

these serial dilutions were measured three times at the λ_{max} (242 nm) and the average absorbance results were used for constructing the Beer-Lambert calibration curve (Fig. 2.1) of the drug in the solvent medium (0.1 N HCl). The Beer-Lambert calibration curve yielded a linear regression equation of A= 9852C+0.13 (where A is the absorbance and C is the concentration in M) with 95% confidence interval and correlation coefficient (R^2) of 0.998.

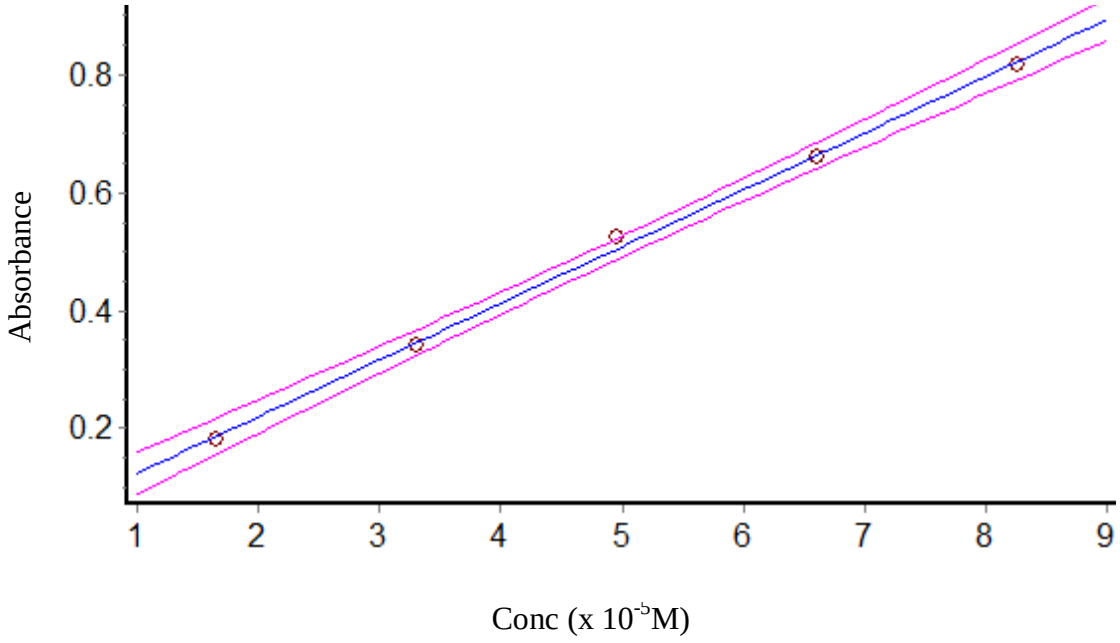

Figure 2.1 UV absorption calibration curve of Paracetamol at 242 nm with 95% confidence interval

2.2.5.5 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 (ERWEKA, type DT 600, Germany). About 495 ml of 0.1 N HCl was placed in the dissolution vessel which was allowed to equilibrate to temperature of 37±0.5 ^{o}C. From each suspension 5 ml was introduced carefully into the bottom of the vessel. The paddle rotation was controlled at 25 rpm. 5 ml of sample was withdrawn using pipette from a fixed position of the vessel at 5, 10, 15, 20, 30 and 45 min (after the start of the dissolution) and 5 ml of fresh 0.1 N HCl solution, equilibrated to 37 ^{o}C, was added immediately as replacement following each withdrawal. The withdrawn sample was filtered through Whatman 5 and appropriately diluted. UV absorbance

reading was read from UV/Visible spectrophotometer (CECIL, CE 3021, England) at the 242 nm. 0.1 N HCl was used as a blank. All the necessary corrections for dilution were made when calculating the drug content from the previously developed calibration equation. Results recorded are averages of three determinations.

2.2.5.6 Statistical Analysis

ANOVA was carried out for the physico-chemical properties of the two mucilages and physical stability test of the suspensions from the three suspending agents using the computer software Sigmastat3.5. Holm-Sidak multiple comparison test was used. At 95% confidence interval, p values less than or equal to 0.05 were considered as significant.

3. Results and Discussion

3.1 Mucilage Yield

The mucilage yields obtained from the two cactus species collected from different areas and subareas are presented in Table 3.1. The reason for collecting the samples from different areas was to look for any significant differences in mucilage yields.

Simple maceration was used for the extraction due to the water soluble nature of the mucilages which are mixture of different polysaccharides (Natale, 2002). Ethanol was preferred to other alcohols to precipitate out the mucilage because it is economical and atoxic (Iturriaga *et al*., 2009). In addition, the use of different types of alcohols was not found to have statistically significant difference in the mucilage yield (Sepúlveda *et al*., 2007).

Table 3.1 Mucilage yield of the two *Opuntia* spp. collected from different areas (Mean±SD, n=3)

Sample collection		Altitude (ASL)	Sample	Yield (%)	
Area	Subarea	(Meter)	type	OFI	OS
Adigrat	-	2459	Fresh	$0.99\pm0.15^{a,b}$	-
Maichew	Wargua	1730	Fresh	0.83 ± 0.35^{b}	-
	Kankano	2235	Fresh	$1.19\pm0.39^{a,b}$	1.47 ± 0.43^{a}
	Bokura	2692	Fresh	1.79 ± 0.26^{a}	2.02 ± 0.49^{a}
Mekelle	-	2239	Fresh	$1.06\pm0.33^{a,b}$	1.62 ± 0.19^{a}
			Dried	17.57 ± 1.52^{a}	19.95 ± 2.11^{a}

N.B. The letters (a, b) indicate significant difference among the different areas (subareas) for a species of same sample type and also between the two species of both types from the same area.

From the above table it can be seen that the yields from fresh cladodes of OS are in the order of Bokura>Mekelle>Kankano. Similarly, the mucilage yields from OFI collected from the different areas and subareas are in the order of Bokura>Kankano>Mekelle>Adigrat>Wargua. Like the yields from OS, OFI did not show statistical significant difference except between Wargua and Bokura. This difference could, among other factors, be due to the effect of altitude. The cladode

mucilage content increases during acclimation to low temperatures; and this could attribute to the adaptation mechanism the species have to a given environment (Sáenz *et al*., 2004).

The yields of the two species from the same area, including mucilage extracted from the dried sample type, were higher in OS than that of OFI but the differences were not statistically significant. Moreover, the yields obtained from fresh and dried cladode of OFI of Mekelle area and that reported by Sepúlveda *et al.* (2007) were found to be comparable.

Samples for further study on physico-chemical properties of the mucilages and their suspending ability were, therefore, collected from Mekelle area.

3.2 Physico-chemical Properties of the Mucilages

3.2.1 Powder Properties

Density and density related properties of the mucilages are presented in Table 3.2. The bulk densities of OS (0.69 g/ml) and OFI (0.68 g/ml) were comparable. The tapped density of OFI (0.85 g/ml) was higher than that of OS (0.81 g/ml), however, the difference was not still statistically significant.

The true density of the mucilage from OFI (1.52 g/ml) was significantly higher than that of OS (1.36 g/ml). This difference might be attributed to the relatively higher fat content in OFI than in OS (Section 3.2.3) and also the high inter-particle space in the powders of OFI than Os.

Table 3.2 Powder properties of the cactus mucilages (Mean±SD, n=3)

Properties		OFI	OS
Density	Bulk density (g/ml)	0.68 ± 0.02^{a}	0.69 ± 0.01^{a}
	Tapped density (g/ml)	0.85 ± 0.02^{a}	0.81 ± 0.03^{a}
	True density (g/ml)	1.52 ± 0.04^{a}	1.36 ± 0.07^{b}
Density related properties	Carr's index (%)	18.82±1.85	15.20±2.52
	Hausner ratio	1.23 ±0.03	1.18±0.04

N.B. The different letters (a, b) indicate the presence of significant difference between the species.

The density related properties are indirect methods to indicate flowability (Aklilu *et al*., 2002). The Carr's index (%) of OFI was 18.82 while that of OS was 15.2. Thus, OFI and OS exhibited fair and good flow properties, respectively, as Carr's values of 5 to 10, 12 to 16, 18 to 21 and 23 to 28 represent excellent, good, fair and poor flow properties in that order (Odeku and Picker-Freyer, 2007). In addition, based on their Hausner ratio values both mucilages of OFI (1.23) and OS (1.18) have good flowability. However, the mucilages generally didn't flow through a funnel (the general method employed for flowability study). This had been expected due to the high moisture content (Table 3.4) and the fineness (< 224 μm) of the mucilage particles.

3.2.2 Solubility and Swelling power

The solubility and swelling power (SP) of the mucilages at different temperatures are presented in Table 3.3. The values of these properties increased with increase in temperature. However, at 75 and 85 ^{o}C SP decreased as compared to the corresponding values at 65 ^{o}C.

The mucilage of OFI was slightly more soluble than that of OS at all the temperatures which may be attributed to the difference in composition. OFI's mucilage is thought to have water soluble sugar molecules (Ginestra *et al*., 2009**)**. However, detailed studies on OS are lacking.

The swelling powers of the mucilage from OS were higher than that of OFI at all temperatures. These might be due to the presence of relatively higher lipid/fats in OFI's mucilage than in OS (Section 3.2.3). Fats are known to inhibit swelling probably by forming insoluble complexes (Tester and Morrison, 1990; Gebre-Mariam and Schmidt, 1996; Odeku and Picker-Freyer, 2007).

The mathematical relationship of swelling power is a unit more than that of Water holding capacity (WHC). Hence, with increase/decrease in WHC, there exists an increase/decrease in SP or the vice versa. Ca^{2+} exists in the cactus mucilage as Ca-oxalate (Garvie, 2006). This biomineral of the mucilage has strong effect in molecular conformation which in turn leads to have positive effect on water holding capacity of the cactus mucilage (Trachtenberg and Mayer, 1981b). Hence, difference in Ca-oxalate content can lead to difference in WHC which in turn brings difference in the SP of the mucilage.

Table 3.3 Solubility and SP of the mucilages at different temperatures (Mean±SD, n=3)

Tem. (^oC)	Solubility (%)		Swelling power (per gram of sample)	
	OFI	OS	OFI	OS
15	54.41 ± 3.22^{c}	49.63 ± 2.41^{c}	3.15 ± 0.17^{b}	4.66 ± 0.38^{b}
40	$57.63\pm1.59^{b,c}$	$54.40\pm1.60^{b,c}$	$3.99\pm0.26^{b,d}$	$5.41\pm0.42^{b,c}$
55	$62.16\pm4.43^{b,c}$	$57.63\pm6.41^{b,c}$	$5.01\pm0.60^{b,c}$	$7.06\pm0.99^{b,c}$
65	$65.42\pm6.40^{a,b}$	$63.24\pm4.82^{a,b}$	$6.78\pm1.59^{a,c}$	$9.01\pm2.96^{a,c}$
75	$68.00\pm3.21^{a,b}$	$64.80\pm3.99^{a,b}$	$6.68\pm0.78^{a,c}$	$8.10\pm1.03^{b,c}$
85	75.19 ± 0.79^{a}	71.22 ± 2.39^{a}	$6.02\pm0.55^{a,c,d}$	$7.86\pm0.63^{b,c}$

N.B. The letters (a, b, c, d) indicate significant difference among the treatments within a property in a species.

3.2.3 Moisture Sorption Properties

Moisture sorption profiles of the two mucilages are depicted in Figure 3.1. Generally, moisture adsorption increased with increase in the % RH. Percent moisture sorbed ranged between 7.7% and 8.55% at low RH (20%) and 95.4% and 76.9% at high RH (100%) for mucilage from OFI and OS, respectively. According to a report by Trachtenberg and Mayer (1981a), at 100% RH, mucilage of OFI sorbed moisture of 75%, a result lower than moisture sorbed by mucilage of OFI used in this study at the same % RH. These differences could be attributed to the mucilage compositional differences the cactus plants from different areas are believed to have. Both species seem to have comparable sensitivity to moisture at lower % RH but at higher % RH OFI mucilage absorbed much moisture than that of OS.

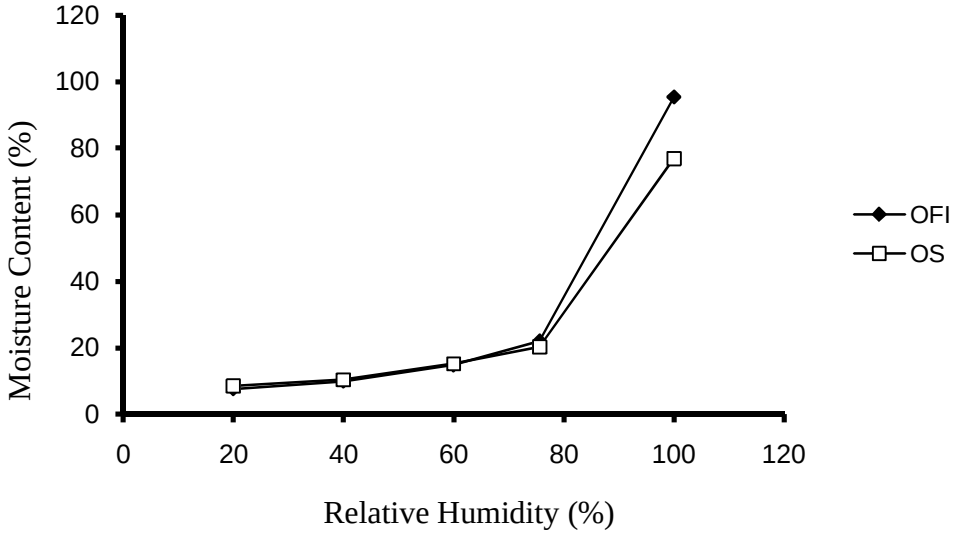

Figure 3.1 Moisture sorption profiles of the mucilages at different % RH.

On comparing the mucilages' moisture sorption properties with that of Godare and Potato starch, reported by Adane *et al.* (2004), both sorbed higher moisture at lower % RH than the two mucilages. Nevertheless, at 100% RH the moisture sorbed by OFI (95.4%) and OS (76.9%) were higher than those of the Godare (45%) and Potato (43%). In general, the hydrophilic nature of the mucilage molecules is probably responsible for the observed higher affinity to moisture. This is in line with the report that the mucilage of OFI has hygroscopic nature (Sepúlveda *et al.*, 2007).

It is important to note that moisture sorption properties of excipients have been correlated with disintegrant effectiveness when used in granule/tablet formulation (Riley *et al.*, 2008). This is because moisture adsorption possibly serves as the primary force for water sorption and tablet permeation. Hence, looking into the possible application of the mucilages in that area is important. Moreover, as mucilages have sticky nature the binder effect shall be also considered.

3.2.4 Chemical Compositions

The functional properties of excipients can be affected by their chemical composition. The compositions of the mucilages from the two *Opuntia* spp. are presented in Table 3.4. As shown in the table below, OFI showed greater ash value, fat and protein contents and lower crude fiber and moisture contents than OS. The differences in the mucilage composition of the two species

could be due to the variation in chemical composition of the soil and the complex phenomenon used by the plants to absorb their nutrients (Sepúlveda *et al*., 2007). The ash values and protein contents of OFI mucilage were comparable to those reported by Sepúlveda *et al*. (2007).

The moisture content of OS (11.72%) was significantly higher than OFI *(*11.57%). This difference could be attributed to the presumed difference in Ca-Oxalate to present in the mucilages of the two *Opuntia* spp. (Section 3.2.2).

Table 3.4 Chemical compositions of the mucilages (Mean±SD, n=3)

Chemical composition	OFI	OS
Ash value (%)	33.96±0.06[a]	29.93±0.05[b]
Crude fiber content (%)	0.06±0.01[a]	0.07±0.03[a]
Fat content (%)	0.42±0.03[a]	0.38±0.06[a]
Moisture content (%)	11.57±0.02[a]	11.72±0.04[b]
Protein content (%)	6.82±0.01[a]	5.18±0.03[b]

N.B. The letters (a,b) indicate significant difference between the species for a given property.

3.2.5 Aqueous Dispersion Properties

Surface Tension

At 20 °C the surface tension of water is 72 mN/M (Fathi-Azarbayjani *et al*., 2009). This surface tension can be decreased by water soluble polymers. Hence, the surface tension of the aqueous mucilage dispersions of OFI (28.71±1.39 mN/M) showed significant decrease compared to that of OS (39.7±2.95 mN/M). This property of the mucilages is very important especially in pharmaceutical suspensions. The decrease in surface tension of dispersions, following addition of polymers, can lead to an increase in the time required for redispersion (Yasueda *et al*., 2004). This is because addition of polymers is followed by lowering of the surface tension which ultimately decreases sedimentation volume (%) (Zatz *et al*., 1979).

Conductivity

The conductivities of mucilage dispersions of OFI were higher than those of OS at all the treatment levels (Table 3.5). These variations may be attributed to the the presence of large amount of the divalent calcium and appreciable amount of the monovalent potassium the OFI's mucilage is known to have; but little is found in the literature about the mucilage from OS. The presence of electrolytes in the mucilages can have an added value in the flocculating ability in suspension formulations.

Table 3.5 Aqueous dispersion properties (Conductivity and pH) of the mucilages at different concentrations (Mean±SD, n=3)

Conc	Conductivity (mS/cm)		pH	
(% w/v)	OFI	OS	OFI	OS
1	2.73 ± 0.11^{a}	2.02 ± 0.01^{a}	6.43 ± 0.06^{a}	6.93 ± 0.06^{a}
4	9.69 ± 0.64^{b}	5.43 ± 0.61^{b}	$6.03\pm0.21^{a,b}$	6.53 ± 0.00^{b}
8	12.04 ± 0.55^{c}	8.09 ± 0.62^{c}	$5.80\pm0.09^{b,c}$	6.37 ± 0.06^{c}
12	13.12 ± 0.05^{d}	9.31 ± 0.08^{d}	5.57 ± 0.06^{c}	5.87 ± 0.12^{d}

N.B. The letters (a, b, c, d) indicate significant difference among the treatments in a given parameter for a given species

pH

On increasing the mucilage concentrations, corresponding decrease in pH values are observed (Table 3.5). The pH values of the dispersions of OS were invariably higher than those of OFI. All the mucilage dispersions had high pH values; and hence, classified within low acid group (pH>4.5) (Sáenz, 2000). This property of the mucilages adds eminence to their quality for their use as pharmaceutical excipient.

Viscosity

Effect of mucilage concentration on apparent viscosity: Viscosity is one of the desired quality parameters of excipients for application in pharmaceutical formulations especially in liquid dosage forms. The viscosities of the dispersions of the two mucilages were found to increase

upon increasing the mucilage concentration (Table 3.6). At all treatment levels, the mucilage of OS had higher viscosities than those of OFI.

Table 3.6 Effect of mucilage concentration on apparent viscosity of their dispersions

Mucilage conc in the dispersions (% w/v)	Apparent viscosity (mPas)	
	OFI	OS
4	1,878	1,728
8	6,119	6,608
12	9,017	10,060

According to Shefter (2002) the viscosity of acacia is about 100 mPas, for 30% (w/v) aqueous solution at 20 ^{o}C, which is a quality used in suspension as thickener and suspending agent. Parsons (2002), who reported NaCMC to have viscosity in the range of 5-13000 mPas at 1% (w/v), ascertained on the possible use of the compound in liquid dosage forms as suspending agent. Hence, despite difference in viscosity between the two mucilage dispersions, the magnitudes are sufficient to impart pharmaceutically desired consistency to liquid formulations especially in suspension preparations. Sáenz (2006) suggested on the potential use of cactus mucilages as natural thickeners.

Effect of shear rate on apparent viscosity: The effect of shear rate on apparent viscosities of the dispersions from the two mucilage samples is depicted in Figure 3.2. From the Figure it can be said that with increase in shear rate the viscosities of the dispersions decreased rendering them pseudoplastic flow.

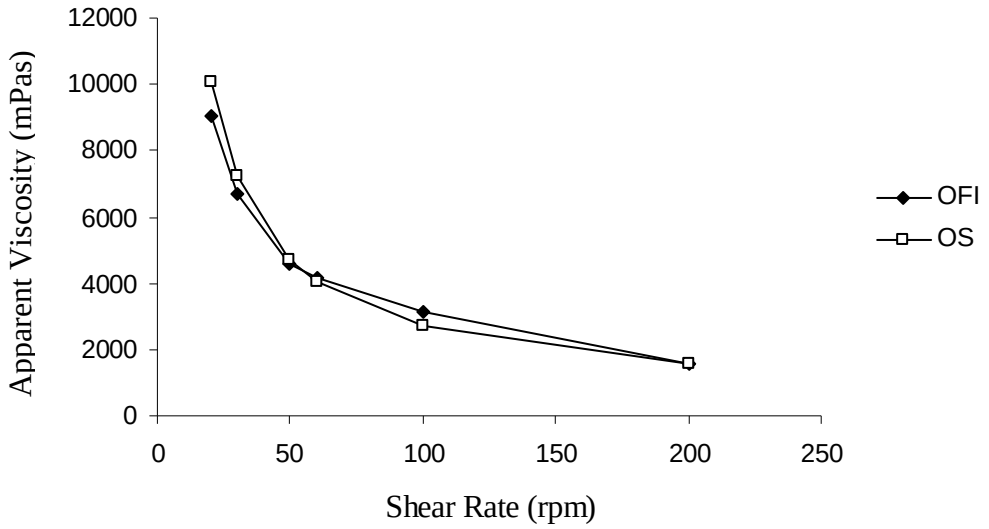

Figure 3.2 Apparent viscosities of mucilage dispersions (12% w/v) at different shear rates.

3.2.6 Microbial Loads

Evaluation of the microbial load (both bacteria and fungi) of pharmaceutical excipients is quite important as the information can signal measures to be taken during storage and formulation preparation especially in liquid dosage forms. Hence, results pertaining to both Total Combined Molds and Yeasts Count (TCMYC) and Total Viable Aerobic Count (TVAC) of the mucilages are presented in Table 3.7. At 100x dilution factor both OFI and OS had too much of TVAC which was difficult to count. However, at 1000x dilution OFI (217 CFU per Petri dish) was found to have higher TVAC than that of OS (4 CFU per Petri dish) which are equivalent to 217000 and 4000 CFU per gram of sample, respectively. However, the controls used, Tryposan Soya Agar and 1% Saline-Peptone, were devoid of CFU. At 100x dilution OFI (16) had higher of TCMYC than that of OS (11), which are equivalent to 1600 and 1100 CFU per gram of sample, respectively. However, both controls (Dichloran Rose Bangal Agar Medium and mixture of medium and 1% Saline-Peptone) were devoid of Molds and Yeasts.

Despite the USP (2009) recommends maximum tolerable limit of 1000 and 100 CFU per gram of sample for TVAC and TCMYC, respectively, for pharmaceutical excipients the extracts showed

much higher values for both TVAC and TCMYC. These might have been so because as reported by Ginestra *et al*. (2009**)** glucose and galacturonic acid are the main sugars of *Opuntia* cladode. Hence, the high moisture content (Section 3.2.4) , high pH value (Section 3.2.5) together with the presumed presence of high content of soluble solids in the mucilages make the mucilage powders very attractive media for micro-organism growth (Sáenz, 2000). Yahia *et al*. (2009) showed that the amount of bacterial colonies presented in media enriched with mucilage of OFI was higher compared with the regular media (without mucilage).

Table 3.7 Microbial load of the mucilages at different dilutions

S/N	Parameter	Dilutions	Average Microbial Load per Petri dish	
			OFI	OS
1	TVAC	1:100	Uncountable	Uncountable
		1:1000	217	4
2	TCMYC	1:100	16	11

3.2.7 Acute Toxicity

It is rare to come across literatures that show toxicity level of the cactus cladode in general and the mucilage in particular. However, there are reports that state on the possible use of *Opuntia* spp. as a source of food for humans, domestic animals and wild life (López-Garcia *et al*., 2001). Hence, the acute toxicity study was addressed to assess the toxicity level and how safe indeed are the mucilages. The findings revealed the mucilages to have very low acute toxicity. This is indeed so as the mice did not show behavioral changes for the following 4 hrs after administration of mucilage dispersions of dose 5 g/kg body weight, equivalent to 300 g for a 60 kg adult human. In addition, no death was recorded in the next two days and even the following two weeks.

3.2.8 Preliminary Phytochemical Screening

These tests are to signify the quality of the cactus mucilages as to whether they were extracted together with other compounds or not. As can be seen in Table 3.8, in both samples, steroidal compounds, saponins and tannins were detected. However, they were devoid of alkaloids and flavonoids. Even though it needs confirmatory tests, the results indicate that the use of ethanol

precipitated other materials too. Mucilage precipitation technique using ethanol tends to provide co-precipitation of other materials such as organic acids, certain salts, proteins, and other similar substances (Natale, 2002).

Table 3.8 Preliminary phytochemical screening of the mucilages

Tested for	Specific test/Reagent used	OFI	OS
Alkaloids	Dragendorff's reagent	(-)	(-)
	Mayer's reagent	(-)	(-)
	Wagner's reagent	(-)	(-)
Flavonoids	Lead acetate	(-)	(-)
	Ethyl acetate	(-)	(-)
	Reaction with NaOH	(-)	(-)
Saponins	Froth test	(+)	(+)
Steroids	Salkowski's test	(+)	(+)
Tannins	Ferric chloride	(+)	(+)

3.3 Physical Stability of the Suspensions

Paracetamol was chosen in this study as it is a typical representative of practically insoluble drug which would require a suspending agent. For such kinds of hydrophobic drugs surfactants and water soluble polymers are usually used to adsorb on the particle surface, form protective colloid, increase the wettability and solubility in water and help the dispersion of the particles in water (Terayama *et al*., 2004; Kuentz *et al*., 2006). Hence, the choice of suspending agent used in this study was based on the good suspension properties it renders in various studies available in the literature (Johnstan *et al*., 1990; Shahjahan and Islam, 1998; Han *et al*., 2006; Azam and Haider, 2008; Kumar *et al*., 2009).

3.3.1 Rheology of the Suspensions

3.3.1.1 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. 3.3). 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. This flow behavior of the suspensions is rendered by the very nature of the suspending agents. Catacalos and Wood have stated that suspending systems tend to be non-Newtonian, specifically pseudoplastic, with increasing shear (Catacalos and Wood, 1964).

As can be seen from the Figure below, 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.

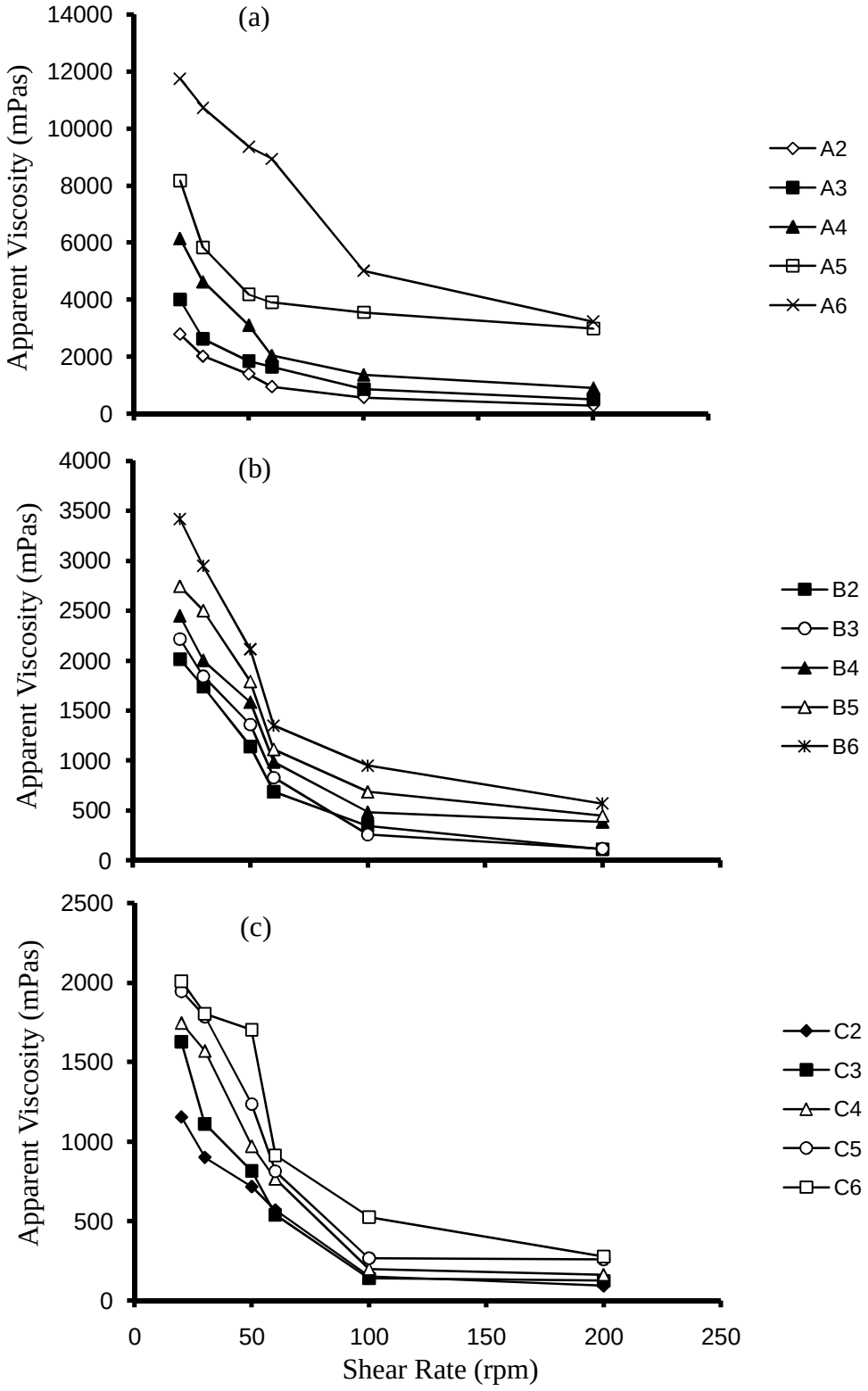

Figure 3.3 Apparent viscosities of suspensions (at different shear rates) having suspending agent of (a) NaCMC, (b) OS and (c) OFI

3.3.1.2 Flowability of the Suspensions

The flow rates of the formulations are presented in Table 3.9. 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 suspensions totally come out of the pipette they are considered as less viscous for which flow rates were calculated. If partly 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. Accordingly, 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 intermediate 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 (see Section 2.2.4 for details of formulation).

Table 3.9 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*

N.B. 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

With increase in the suspending agents concentrations in the formulations, corresponding decrease in flow rates were noticed. The decreases were statistically significant among formulations of the same group except between B4 (0.096 ml/sec) and B5 (0.071 ml/sec). Similarly, 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 in Section 3.3.1.1.

3.3.2 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 Figure 3.4 and 3.5. It can be seen from the profiles of the Figures that the sedimentation volumes (%) of suspensions increased with increase in the concentrations of the suspending agents. In addition to other factors, the results could be due to 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.

From Figure 3.4 (a) quick sedimentation of the drug particles in formulations with low concentrations of suspending agents are observed. Hence, the order of sedimentation rates were A2>A3>A4>A5>A6. From the profiles it can be seen that in formulations A2 and A3 the sedimentation volumes (%) have stabilized starting from day four while in the remaining formulations the decrease has continued until the 7^{th} day. The dispersed particles sediment at faster rate in formulation B2 but the sedimentation volumes (%) are relatively higher for formulations B3 to B6 (Fig. 3.4 (b)). Similarly, formulation C2 had fast sedimentation rate (Fig. 3.4 (c)) probably due to the less viscous nature of the medium while C3 and C4 had intermediate sedimentation rates. Formulations C5 and C6 show slower rate of sedimentation.

The 7^{th} day sedimentation volumes (%) of the formulations, prepared using NaCMC as suspending agent were: A2 (5%), A3 (20%), A4 (66 %), A5 (91%) and A6 (92%). On analyzing all these sedimentation volumes (%) of the formulations, except between A5 and A6, there were statistically significant differences among them. In formulations from the mucilage of OS, B3 (90%), B4 (96%), B5 (97%) and B6 (99%) had significantly higher sedimentation volume (%) than B2 (12%). Similarly, in formulations that had OFI, low sedimentation volumes (%) were recorded in formulation C2 (16%) followed by C3 (72%), C4 (86%), C5 (94%) and then C6 (96%).

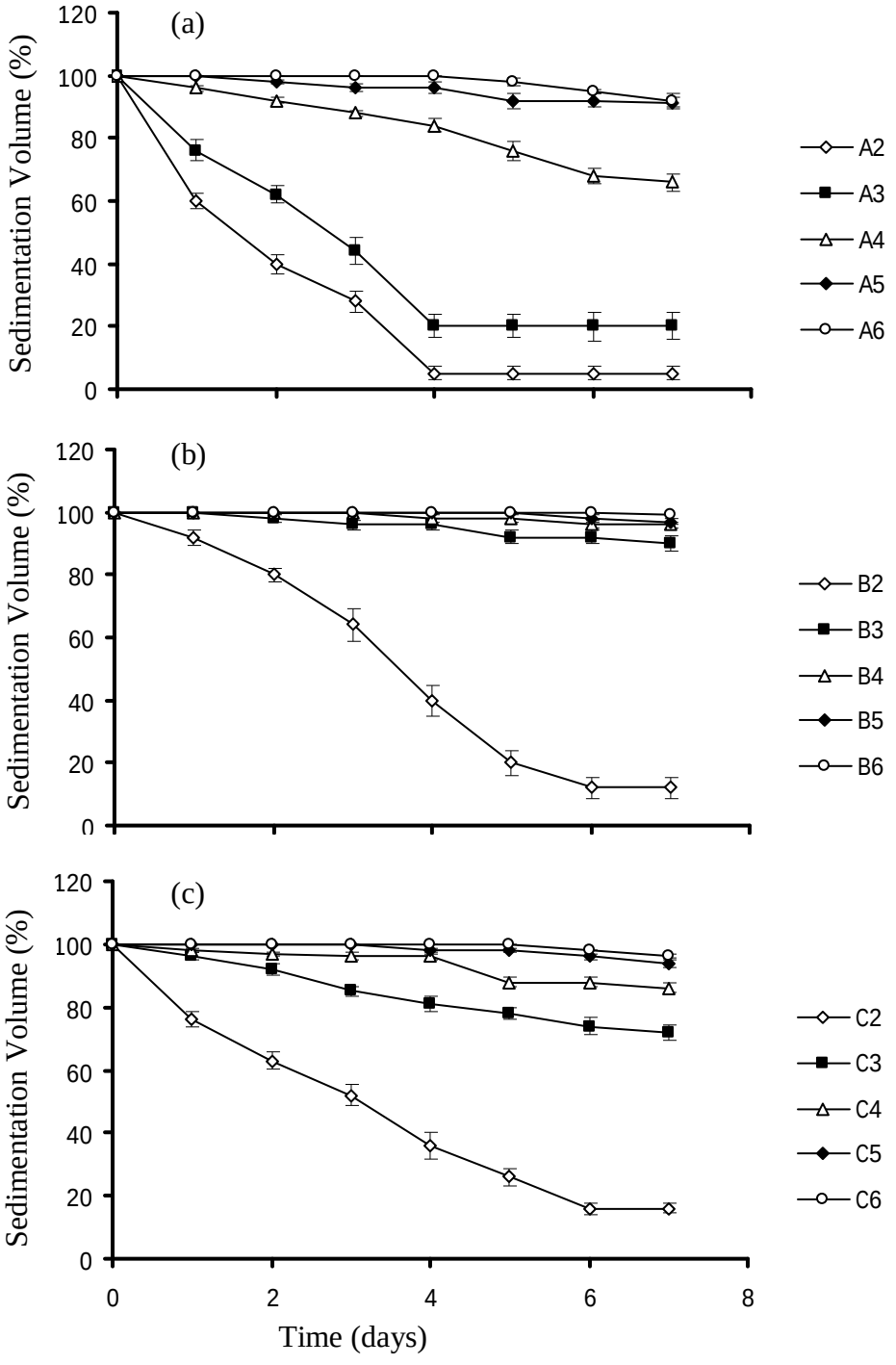

Figure 3.4 One week sedimentation volume (%) profiles of suspensions at different concentrations of the suspending agent (a) NaCMC, (b) OS and (c) OFI

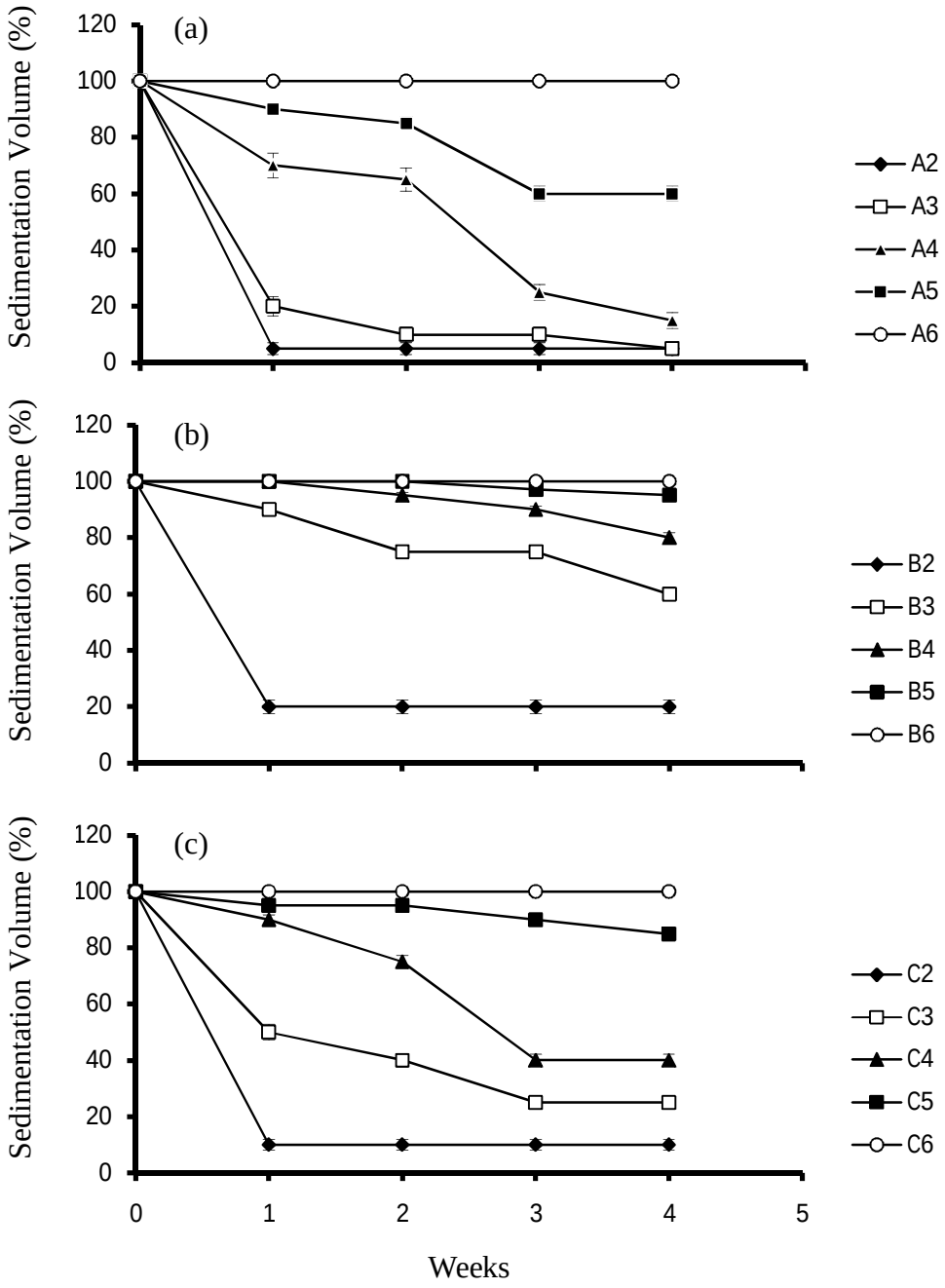

Figure 3.5 Four weeks sedimentation volume (%) profiles of suspensions at different concentrations of the suspending agent (a) NaCMC, (b) OS and (c) OFI

Similarly, from Figure 3.5 the sedimentation volumes (%) of suspensions with low suspending agent concentrations are lower than these with higher suspending agent concentration. A2, B2 and C2 have attained low and stable sedimentation volumes (%) after the first week while A6, B6 and C6 showed no decrease in sedimentation volume throughout the shelf time.

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. In Section 3.3.1.1 it is stated that 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 possible presence of large amount of the divalent calcium and appreciable amount of the monovalent potassium have been stated in Section 3.2.5. Such inorganic minerals (especially the divalents) have the potential in the reduction of zeta potential of dispersed particles. Significant decrease in zeta potential can lead to the flocculation of the suspended particles (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. That was what indeed observed during the experiment.

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.

3.3.2.1 Effect of Electrolyte Concentrations on Sedimentation Volume

The sedimentation volume (%) profiles of suspensions, with different concentrations of NaCl, are presented in Figure 3.6. In all the formulations' profiles stable sediment volumes (%) were not attained until the 7^{th} day after preparation. In Figure 3.6 (a) quick sedimentation of drug particles in the control formulation (devoid of electrolyte) is observed followed by formulation that had low NaCl concentration. However, a different trend of sedimentation volume (%) profiles are noticed in Fig. 3.6 (b) and (C). Hence, for the purpose of simplicity the

sedimentation volumes (%) of the formulations at the 7^{th} day were considered in subsequent paragraphs for discussion.

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%), $1x10^{-3}$ M (91%), $1x10^{-2}$ M (94%) and $5x10^{-2}$ M NaCl (95%). The differences in sedimentation volumes (%) were statistically significant in comparison to the control. Moreover, the sedimentation volume (%) of the suspension at $1x10^{-4}$ M NaCl was significantly higher than the remaining formulations.

Unlike suspensions from NaCMC that have shown continuous sedimentation volume (%) increase, with increase in NaCl concentration, those with OS and OFI have shown decrease in sedimentation volumes (%) at some levels of NaCl concentrations. In suspensions prepared using OS, there were slight increase in sedimentation volumes (%), compared to the control (96%), on addition of electrolyte of $1x10^{-4}$ M (97%) and $1x10^{-3}$ M NaCl (98%). However, suspensions with $1x10^{-2}$ M (95%) and $5x10^{-2}$ M NaCl (90%) showed lower sedimentation volumes (%). But of all the differences in sedimentation volumes (%) produced, only in suspension with NaCl of $5x10^{-2}$ M was statistically significant. Similar trend was observed with suspensions which had mucilage of OFI. This time the increase in sedimentation volume (%) was also noticed in suspensions with NaCl of $1x10^{-2}$ M (91%). However, beyond this NaCl concentration, decrease in sedimentation volume (%) was observed. The differences in sedimentation volumes (%) between the control and the rest of the formulations that had electrolyte were not statistically significant except with the suspension that had NaCl of $1x10^{-2}$ M. Similarly, significant statistical difference was noted only between suspension with NaCl concentrations of $1x10^{-2}$ M and $1x10^{-4}$ 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.

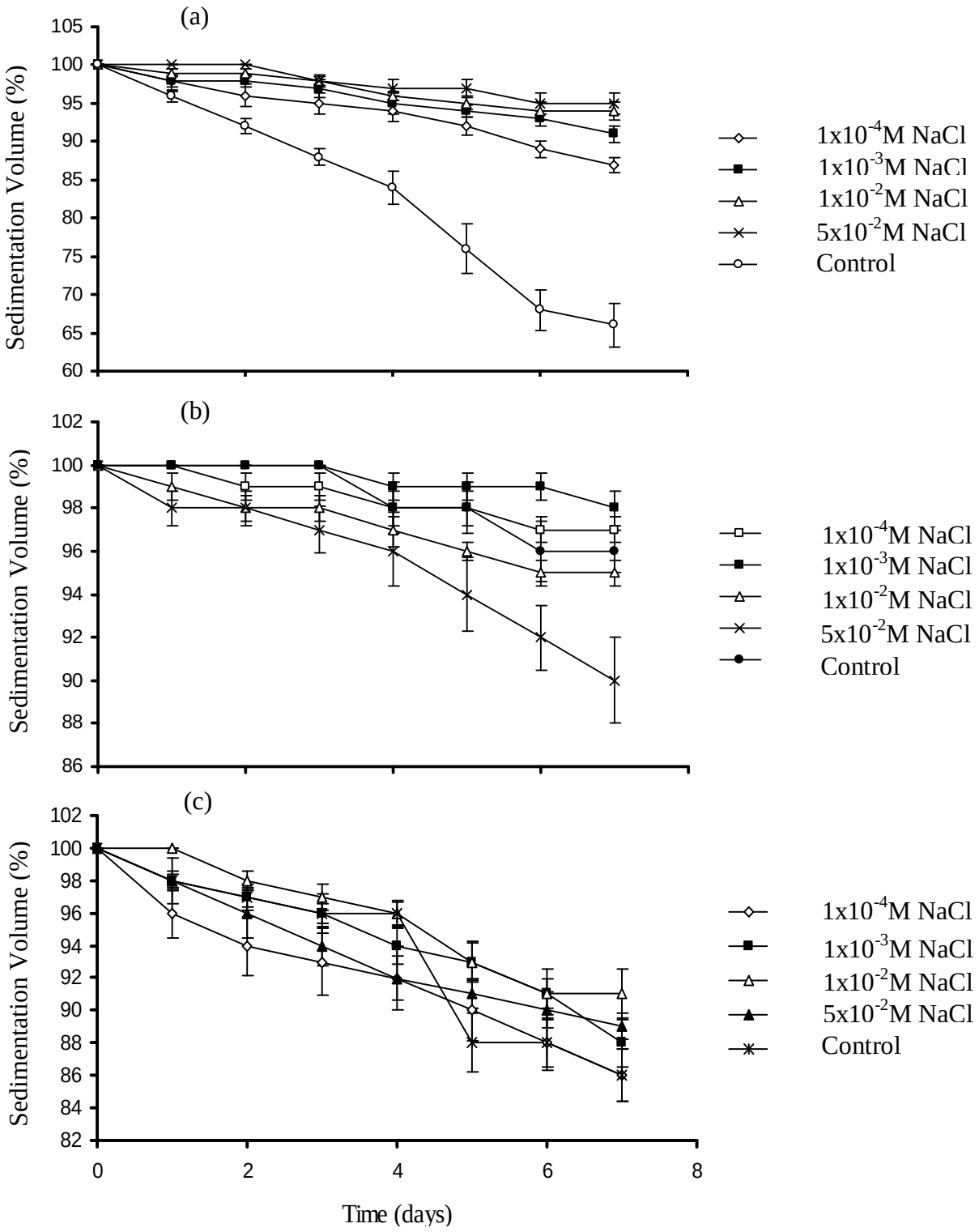

Figure 3.6 Effect of different electrolyte concentrations on sedimentation volumes (%) of suspensions prepared using 4% suspending agent (a) NaCMC, (b) OS and (c) OFI

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.

3.3.2.2 Effect of pH on Sedimentation Volume

The effect of pH on sedimentation volume (%) of the suspensions is depicted in Figure 3.7. From Figure 3.7 (a) it can be seen that formulations with pH of 8 and 10, which have NaCMC as suspending agent, have shown fast sedimentation of the dispersed drug particles. However, in formulation with pH 2, the rate of the sedimentation was low with the sediment volume (%) at the time of preparation persisted for the next seven days without change. In formulations that had mucilage of OS (Fig. 3.7 (b)), the sedimentation rate of the dispersed drug particles were faster in formulations with pH 2 (day 4), pH 6.5 (day 4) and pH 8 (day 5) with the attainment of low and stable sediment at the respective days. In formulation that had OFI (Fig. 3.7 (c)), unlike the control used, they showed quick sedimentation with the attainment of low and stable sedimentation volumes (%) as of day 2.

The suspensions with NaCMC were found stable at lower pH. The sedimentation volumes (%) of the formulations at the 7^{th} day are: 2 (100%), 6.5 (30%), 8 (4%) and 10 (4%). An analysis for variation in sedimentation volumes (%) indicated that there were statistically significant differences among the formulations and also with that of the control (66%) (Fig. 3.7 (a)). The acid stable nature of the formulations from NaCMC might be attributed to the incorporation of the drugs in the hydrophobic aggregates of the polymer. That is the polymer form relatively

hydrophobic aggregates at high hydrogen ion concentrations but at lower hydrogen ion concentration the polymer ionizes and form association with water (Block and Lamy, 1968). In addition, a study by Arias *et al.* (2009) indicates that at low pH, zeta potential of the dispersed particles lowers and formation of voluminous floccules will result. A study made by Nag (2005), on the stability of Barium sulphate suspension using NaCMC as suspending agent, showed similar result to this work's finding. That is, with decrease in pH the sedimentation volume increased with justification that at lower pH dissociation of NaCMC has occurred with subsequent increase in viscosity.

Unlike suspensions of NaCMC, those of OS and OFI were found stable at higher pH. From Figure 3.7 (b) it can be seen that suspensions prepared with OS have higher sedimentation volumes (%), at 7^{th} day after preparation, at alkaline pH than in acidic pH, i.e., 2 (12%), 6.5 (34%), 8 (37%) and 10 (61%). However, the sedimentation volumes (%) of the formulations were significantly lower than the control (96%). Similarly, in suspensions with OFI statistically significant difference in sedimentation volumes (%), at the 7^{th} day, were noticed between the control (86%) and the formulations with adjusted pH (Fig. 3.7 (c)): 2 (5%), 6.5 (6%), 8 (8%) and 10 (21%).

The reasons that can explain the stability of the formulations from OS and OFI at higher pH can be one or more of the following. The viscosity of suspensions of OFI depends on pH, being greatest at alkaline pH (Trachtenberg and Mayer, 1982). That could also hold true for the mucilage of OS so long as the precursor molecular structure is similar. The other important point to be mentioned here is that the mucilage of OFI is found to have acid labile peripheral chains (McGarvie and Parolis, 1981b). Hence, the acid labile branches/chains might have degraded in formulations at pH 2 (and possibly the other formulations). This could have led to lowering of viscosity and also the number of chains that can form bridge between flocs which ultimately lowered the sedimentation volume (%).

When the sedimentation volumes (%) of the preparations with adjusted pH were compared with the controls, NaCMC (66%), OFI (86%) and OS (96%), significant differences existed at all pH levels. This indicates that during the addition of NaOH or HCl, to adjust the pH, degradation of

the polymers might have occurred. Hence, variation in suspension's pH had drastic effect on sedimentation volume (%) of suspensions.

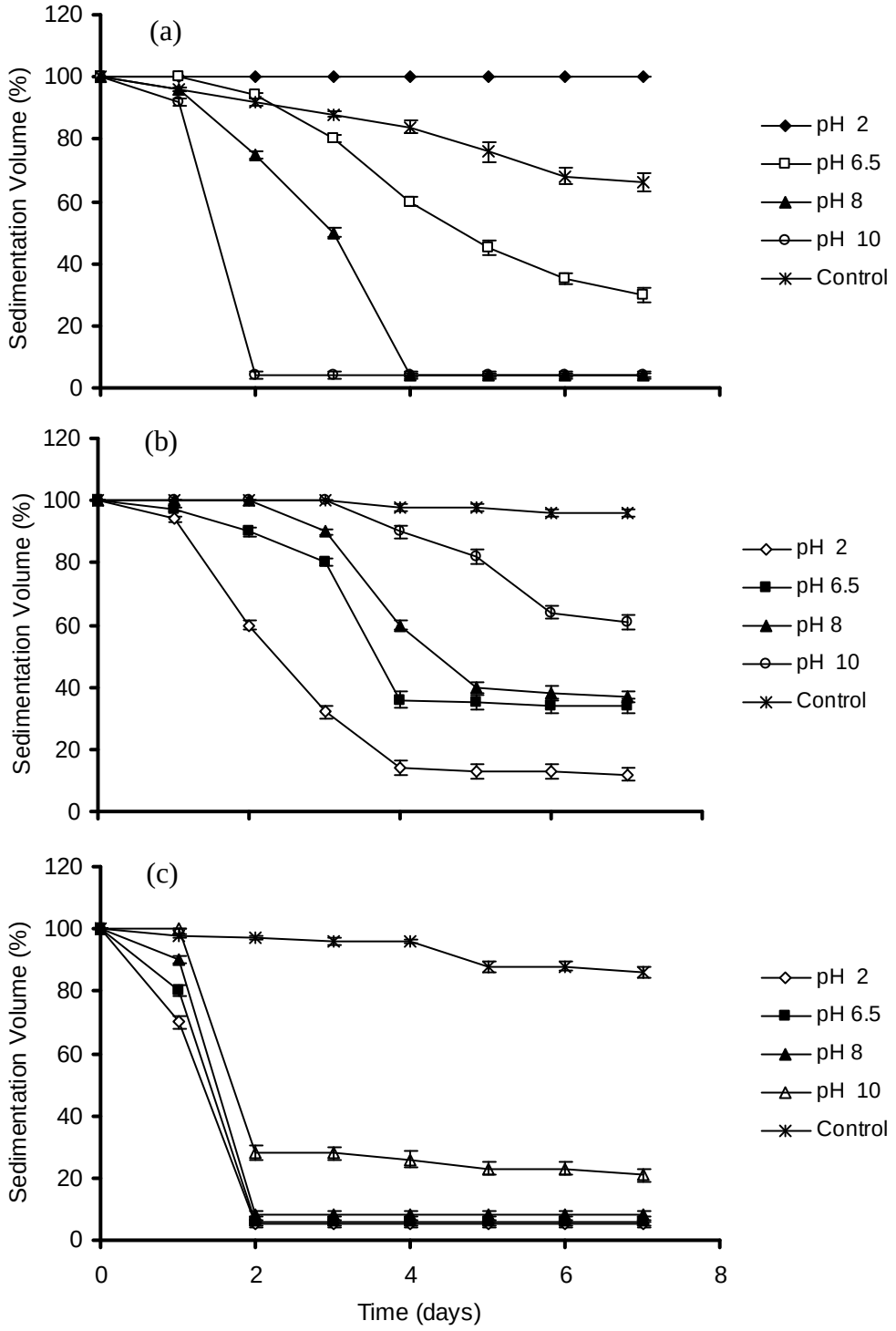

Figure 3.7 Effect of different pH values on sedimentation volumes (%) of suspensions that have 4% suspending agent (a) NaCMC, (b) OS and (c) OFI

3.3.3 Redispersibility of the Suspensions

In Section 3.3.2 it is 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 and OFI while for formulations from NaCMC they were found to be the reverse (Fig. 3.8 and 3.9). From the Figures it can be easily understand that as the level of NaCMC rose, the complete number of turns required to uniformly redisperse (on visual inspection) the suspension has continuously increased. However, for suspensions from OS as the concentration of the suspending agents was increased, i.e., B2 (13), B3 (9), B4 (7) and B5 (6), the complete number of turns required to redisperse the suspensions were decreased. Similar phenomena were observed for suspensions from OFI, i.e., C2 (15), C3 (12), C4 (8), C5 (5). On further increase in suspending agent to 6 % in OFI it was found to level off, i.e., C6 (5) but for OS it increased, i.e., B6 (8).

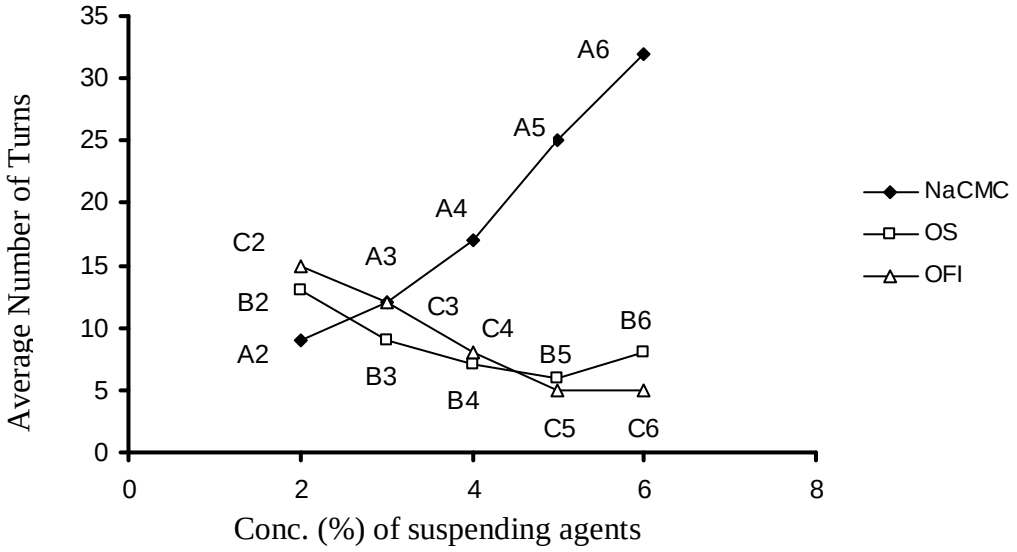

Figure 3.8 Average number of turns required to uniformly redisperse sediment of the suspensions after one week.

As can be seen from Figure 3.9, the average number of turns required to redisperse the sedimented particles, in all the suspensions kept for four weeks, are higher than that of the corresponding formulations kept for a week. That could be due to the decrease in sedimentation volumes that lead to change from region of secondary minimum the region of primery minimum as a result of proximity of particles.

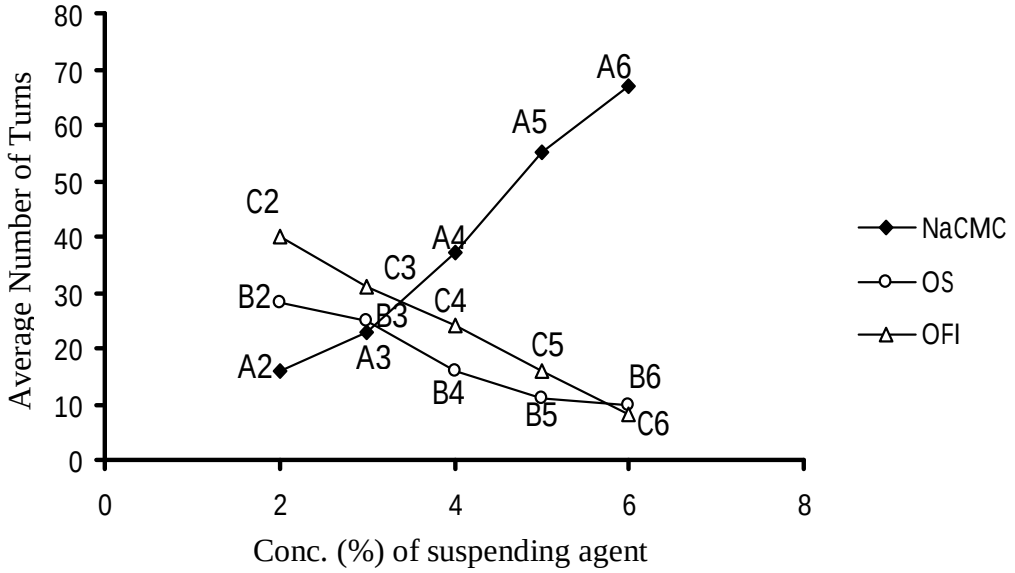

Figure 3.9 Average number of turns required to uniformly redisperse sediment of the suspensions after fours weeks .

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 of the presence of flocculated particles in suspensions 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).

3.3.4 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 Figure 3.10. Suspensions A2 (97%), A3 (92%), A4 (90.3%), A5 (85%), B2 (99.7%), B3 (98.3%), B4 (95.7%), B5 (94.7%), B6 (93%), C2 (98%), C3 (94.1%), C4 (95.7%), C5 (94%) and C6 (91%) 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. The quick release from these formulations could be attributed to the relatively low viscosity of the suspensions as compared to those of NaCMC. Figure 3.10 (a) shows that formulations A2 (98.3%), A3 (97.6%) and A4 (96.7%) have released higher drug content at the 45^{th} min than did formulations A5 (92%) and A6 (87.7%). That is simply because of the viscous nature the latter formulations had. 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.

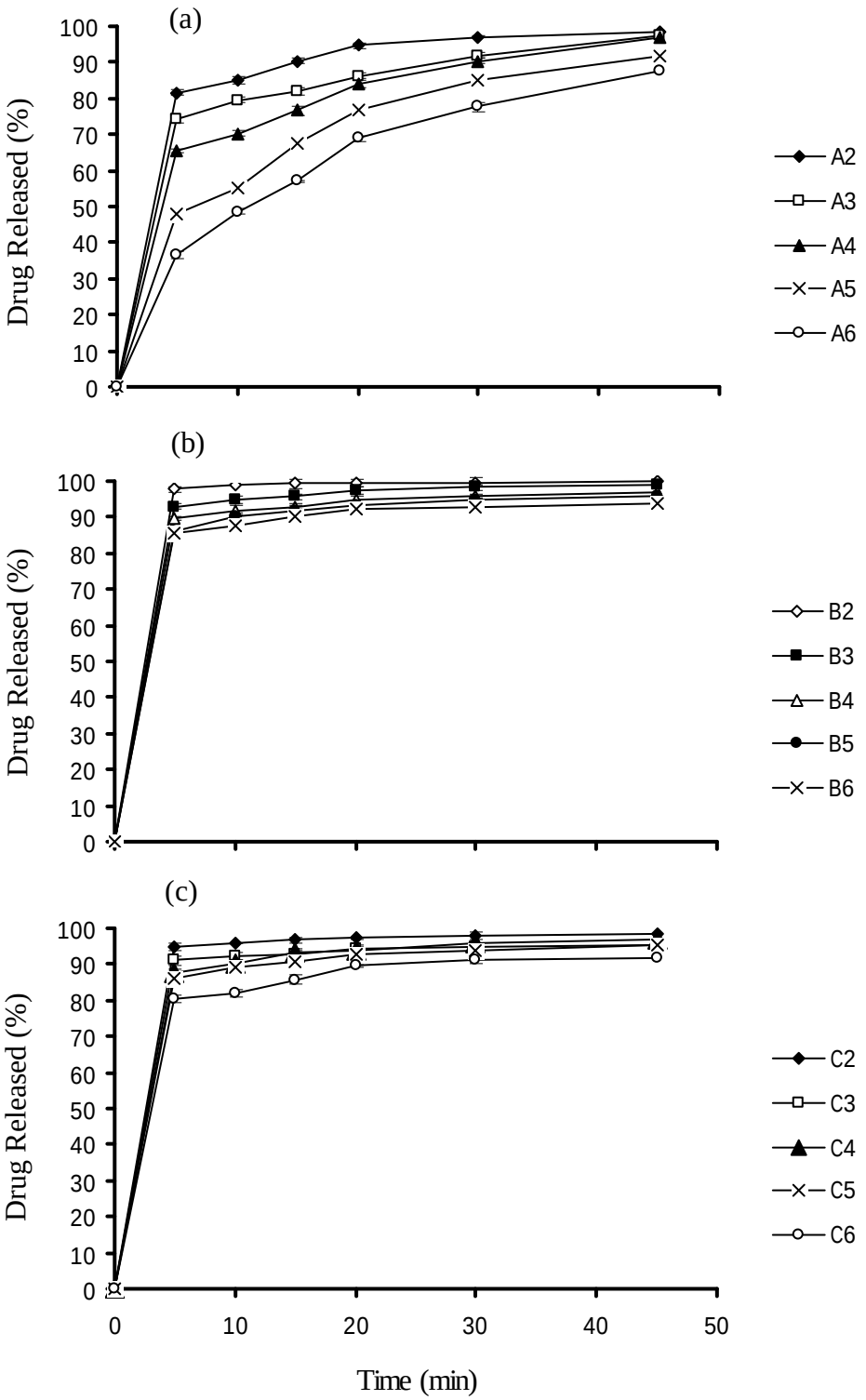

Figure 3.10 Drug release profiles of all the suspension formulations that have suspending agent (a) NaCMC (b) OS and (c) OFI

Despite the quick release of the drug observed in formulations which had mucilage of OS (Fig. 3.10 (b)) and OFI (Fig. 3.10 (c)), most of the formulations did not achieve 100% release of drug content until the 45th min: B2 (100%), B3 (99%), B4 (96.7%), B5 (95.7%), B6 (93.3%), C2 (98.7%),C3 (95.6%), C4 (96.7%), C5 (95.3%) and C6 (92%). 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).

4. Conclusions

Mucilages of the two cactus species, *Opuntia ficus-indica* (OFI) and *Opuntia stricta* (OS), were extracted and characterized. The mucilage yields were found to be comparable between the two species. Characterization of the isolated mucilage revealed comparable results in properties such as bulk density, tapped density, solubility, crude fiber, fat, sorbed moisture content (at lower % RH) and phytochemical screening. Their acute toxicity level is also similar, i.e. both are safe. Mucilage of OFI has shown higher magnitude in properties like true density, ash value, moisture content, conductivity, sorbed moisture content (at higher % RH) and microbial load as compared to OS. However, in properties such as SP, surface tension, pH value and viscosity, the mucilage of OS was found to have higher values than in OFI. Generally, the mucilage from OS had superior quality than mucilage of OFI especially for the application of suspension formulation.

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. With regard to the effect of pH on sedimentation volume (%), the suspensions which had mucilages were found to be stable at higher pH while those with NaCMC the opposite holds true.

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 30^{th} 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.

5. Suggestions for Further Work

The results of the current work necessitate further investigations on the following:

- Different methods of mucilage extraction;
- Detailed study on the chemical compositions;
- Identifying and quantifying the inorganic contents of the mucilages;
- Physico-chemical properties of the mucilages such as particle size distribution, pasting properties, crystallinity, morphology;
- Long-term and accelerated stability studies of suspensions prepared with the mucilages;
- Application of the mucilages as potential tablet disintegrants and binders.

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DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with my approval as a university advisor.

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Place and date of submission: Addis Ababa, Ethiopia, April, 2010.