



STABILITY ENHANCEMENT OF ALOE-GEL BY FORMULATING POLYELECTROLYTE COMPLEX BEADS

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ABSTRACT

The aim of present work was to overcome the stability problem of aloe gel by formulating polyelectrolyte-complex (PEC) beads using chitosan-sodium alginate and suspending them into calamine lotion. Chitosan was used for its sunscreen, moisturizing, antimicrobial and biocompatible properties. For comparison, lotion containing plain aloe gel beads (plain beads) formulated as that of PEC beads except use of chitosan, and conventional lotion containing directly incorporated only aloe gel were prepared. Beads were evaluated for particle size, shape, surface morphology and crushing strength. Lotions were evaluated for pH, color, viscosity, sedimentation volume and stability. Spherical PEC beads and ununiform shaped plain beads were observed in SEM study. Sedimentation ratio was found in order of PEC beads > plain beads > aloe gel, indicating higher suspendibility and stability for PEC beads. pH of all formulations complied with skin pH. Viscosity study of lotions indicated better rheology of PEC bead-lotion. Higher viscosity of PEC bead-lotion and presence of chitosan synergize protection from microbial growth than plain bead-lotion and aloe gel-lotion which was confirmed from stability studies. Aloe gel-lotion and plain bead-lotion is unstable at room temperature showing change in color, pH, viscosity, sedimentation and lumpiness after one and three months respectively, but no change was observed in PEC bead-lotion even after six months. Overall study revealed that the formulation of aloe gel into beads have significantly improved compatibility and stability; even higher for PEC bead-lotion as compared to plain bead-lotion.

Keywords: Polyelectrolyte-complex, Aloe gel, Stability, Chitosan, Sodium alginate, Rheology

INTRODUCTION

Aloe vera (Linn.), also known as *Aloe barbadensis* belonging to family Liliaceae is widely distributed in Asia, Africa and other tropical regions. Aloe contains anthraquinone glycosides mainly barbaloin (C₁₀ glucoside). The fresh mucilaginous juice of the *Aloe vera* has been used for centuries in the treatment of sun burns, deep thermal burns and radiation burns; abrasions and other skin irritations [1]. The proficient properties of the aloe vera plant have created a prerequisite for *Aloe vera* gel and *Aloe vera* products. A foremost apprehension for instability of aloe gel is entrapped oxygen leading to growth of aerobic bacteria [2]. The variable nature of this mucilaginous juice also made it extremely difficult to incorporate in stabilized type of preparation. The mucilaginous gel of aloe vera is incorporated in ointments, creams, lotions and other preparations for topical use. Though there are conflicting reports concerning the efficacy of such preparations [3,4].

Chitosan a natural polysaccharide, having substantiated oxygen scavenging effect [5, 6] will benefit to entrap free oxygen in aloe. Moreover, it has antibacterial [7, 8] antifungal [9] and anthelmintic activities which will avert the attack of several microbes. Chitosan also shows antioxidant activity [10] by blocking free radicals. In addition it has selective permeation for oxygen and carbon dioxide and shown ultra-violet radiation screening behavior, [5] which will in turn help to maintain freshness of aloe gel. Thus by several mechanisms chitosan will help to block the pathways leading to instability of aloe gel.

In addition, chitosan possess wound healing, [11] anti-inflammatory, [12] suncreening, [13] moisturizing [14] and immunomodulatory [15] properties which at least hypothetically will synergize the use of aloe as medicinal and beauty aid.

The conflicting reports concerning the efficacy of aloe gel preparations [3,4] moved our intention to prepare a lotion which contains PEC beads of aloe gel with enhanced stability and efficacy. Keeping this aim we prepared novel lotions containing PEC aloe gel beads (prepared using chitosan and sodium alginate) and plain aloe gel beads (prepared using sodium alginate without chitosan) and conventional lotion containing directly incorporated aloe gel.

MATERIALS AND METHODS

Aloe vera leaves were collected from botanical garden of Govt. College of Pharmacy, Karad, India. Sodium alginate and calamine were

purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Chitosan was a kind gift from Mahatni Chitosan Pvt. Ltd., Ahmedabad, India. Double distilled water was used throughout the study.

Collection of aloe gel

Aloe gel was extracted from leaves by Filet-Cutting Method (removal from the carcass by a cut made parallel to the backbone, usually 2 to 12 oz) and fresh gel was separated.

Preparation of chitosan-alginate aloe gel beads (PEC beads)

Weighed quantity of aloe gel was added to 3% w/v aqueous sodium alginate solution with stirring at high speed. The above solution was mixed homogenously at 1200 rpm using magnetic stirrer (Remi, India) for 20 min. This was then extruded via syringe no. 18 into 5% w/v calcium chloride solution containing 1.5% w/v aqueous chitosan solution with gentle agitation. PEC beads formed by ionic gelation were allowed to stand for 30 min in the solution, filtered through whatman filter paper (size, 0.45 mm), washed with distilled water, and dried at room temperature.

Preparation of plain aloe gel beads (plain beads)

For comparison, aloe gel beads using only sodium alginate in absence of chitosan (plain beads) were prepared by emulsion gelation method. Weighed quantity of aloe gel was added to the 3% w/v aqueous sodium alginate solution with stirring at high speed. The above solution was mixed homogenously at 1200 rpm using magnetic stirrer (Remi, India) for 20 min. This was then extruded via syringe no. 18 into 5% w/v calcium chloride solution with gentle agitation. Formed plain beads were allowed to stand for 30 min in the solution, filtered through whatman filter paper (size, 0.45 mm), washed with distilled water, and dried at room temperature.

Preparation of novel lotions containing beads and conventional lotion

Both types of beads i.e. PEC beads and plain beads were suspended into calamine lotion separately to prepare novel lotions, PEC bead-lotion and plain bead-lotion respectively. Also the conventional lotion containing directly incorporated aloe gel was prepared and compared with the previous ones.

Crushing property

50 gm of PEC beads and plain beads were pressed over butter paper separately, to observe crushing of beads to assess their handling during application of lotion. Beads were observed for extrusion of aloe gel.

Scanning electron microscopic (SEM) study

Surface morphology of the prepared beads was examined using scanning electron microscopy (JSM-6400; Jeol Ltd. Tokyo, Japan). The samples were mounted directly onto the SEM sample holder using double-sided sticking tape and were gold spray-coated.

Particle size analysis

Particle size of beads was determined by using optical microscopy method. The arithmetic mean diameter of total 100 beads for each formulation was calculated by using Edmundson's general equation^[16].

$$d_{\text{mean}} = \left[\frac{\sum nd^{p+f}}{\sum nd^f} \right]^{1/p} \dots\dots\dots (\text{eq. I})$$

Where, 'n' is no. of particles in size range whose mid point is 'd'; 'p' is an index related to the size of an individual particle and 'f' is frequency index. Results of particle size analysis are mean of 3 readings.

EVALUATION OF LOTIONS

pH

pH of all lotions containing beads and aloe gel was determined by using pH meter (Eutech Instruments, India).

Rheological studies

All three lotions were allowed to stand for 24 hr and sedimentation volume was calculated by using standard formula as below^[16]

$$\text{Sedimentation Volume (F)} = \frac{\text{Final volume of sediment (V}_u\text{)}}{\text{Original volume of suspension (V}_o\text{)}} \dots (\text{eq. II})$$

Redispersibility of lotions (15 ml) was observed after 24 hr by its flocculation. Viscosity of all lotions was measured by Brookfield viscometer (Brookfield viscometers Pvt. Ltd. England). Cracking property for 5 ml of each lotion was determined by keeping them at refrigerated temperature (-4°C) for 24 hr and separation of solid and liquid phase was observed.

Degree of flocculation (β) was also determined to relate the volume of flocculated sediment to that in deflocculated system by using following formula^[16]

$$\text{Degree of Flocculation } (\beta) = \frac{\text{Ultimate sediment volume of flocculated suspension}}{\text{Ultimate sediment volume of deflocculated suspension}} \dots (\text{eq. III})$$

Flocculated sediment is the sediment when beads are settled in lotion, while deflocculated sediment is the sediment when beads are dispersed in lotion.

STABILITY STUDY

All the three formulations were kept at different temperatures (0 – 8°C and at room temperature) for four weeks. Formulations were monitored for pH, viscosity, appearance at the interval of one week. All the measurements were performed after allowing the formulations to be equilibrated at 25°C.

STATISTICAL ANALYSIS

Results were analyzed statistically by One Way Analysis of Variance (ANOVA), using software INSTAT. $P < 0.05$ implied significant correlation.

RESULTS AND DISCUSSION

Stability is the major problem of herbal products and encapsulating them in antimicrobial-antioxidant chitosan is the best way to protect them from environmental oxidizing factors. Polyelectrolyte complexation with anionic polymer best describes encapsulation method for cationic chitosan. For comparison, plain beads without chitosan were also prepared by emulsion gelation technique.

Uniform and micronized particle size is an important formulation characteristic for beads. Hence formulation variables were optimized and particle size of optimized batches was studied using optical microscope. Spherical and uniform shaped PEC beads of average diameter $813.30 \pm 52.32 \mu\text{m}$ was observed in microscopic study (Fig. 1). In SEM study, ununiform shaped beads with cracked surface were found in case of plain beads (Fig. 2a), while PEC beads were found to bear uniform and crack free surface with sufficient pores so as to exude aloe gel with ease (Fig. 2b)

Absence of chitosan as a cationic polymer have impacted complexation of plain beads and hence their morphology. While PEC beads showed more uniform and spherical morphology leading to ease in suspendibility which was confirmed from rheological studies. Sedimentation ratio was found in order of PEC beads > plain beads > aloe gel, indicating higher suspendibility and hence higher stability for PEC beads.

Since redispersibility is one of the major considerations in assessing the acceptability of semisolid dosage forms and since the sediment formed should be easily redispersed by moderate shaking to yield a homogeneous system; measurement of sedimentation ratio becomes basic evaluative procedure. Aloe gel lotion was excluded from this study as it is heterogeneous-heterodispersed system^[17]. Sedimentation ratio was determined for fresh lotions as well as after one month and plotted against time (Fig. 3). PEC bead-lotion showed less steeper behavior compared to plain bead-lotion, indicating better redispersibility. Similar results obtained for lotions kept for one month. But PEC bead-lotion showed decrease in sedimentation ratios while plain bead-lotion showed increased sedimentation ratios over time.

Table 1: Morphology of PEC beads and plain beads

Sr. no.	Evaluation parameters	PEC beads	Plain beads
1	shape	spherical	roughly spherical
2	diameter	0.813 ± 0.34	0.985 ± 0.48
3	crushing strength	exudes out	exudes out

Values are in millimeter Each value represents mean \pm SD of experiments (n = 3)

Table 2: Evaluation parameters for lotions

Sr. no.	Evaluation parameters	PEC bead lotion	Plain bead lotion	Conventional lotion
1	pH	6.5 ± 0.02	5.7 ± 0.03	5.8 ± 0.02
2	colour	whitish pink	greenish pink	blackish green
3	redispersibility	easily dispersible	dispersible	not uniform
4	viscosity	$1835 \text{ cp} \pm 8$	$1550 \text{ cp} \pm 12$	$1230 \text{ cp} \pm 6$
5	cracking property	flocculated	flocculated	deflocculated
6	stable for	even after six months	three months	one month

Cp: centipoises. Each value represents mean \pm SD of experiments (n = 3)

Table 3: Short term stability study after 4 weeks

Parameters	PEC bead lotion	Plain bead lotion	Conventional lotion
Temperature (0 - 8°C)			
viscosity	1842 cp ± 5	1578 cp ± 3	1266 cp ± 4
pH	6.48 ± 0.03	5.65 ± 0.05	5.71 ± 0.04
Temperature (room temperature)			
viscosity	1862 cp ± 6	1606 cp ± 4	1315 cp ± 7
pH	6.41 ± 0.02	5.42 ± 0.04	5.32 ± 0.03

Cp: centipoises. Each value represents mean ± SD of experiments (n = 3)

Further degree of flocculation was observed in reverse order to that of sedimentation ratio, assuring higher stability of PEC bead-lotion. Degree of flocculation is dependent on particle size, shape and surface tension of particles. Due to smaller particle size, spherical shape and higher surface tension owing to complexed surface, PEC bead-lotion had shown lesser degree of flocculation.

pH of aloe gel-lotion was found to be 4.1±0.02 while that of plain bead-lotion and PEC bead-lotion was found to be 4.7±0.03 and 5.5±0.02 respectively which complies with skin pH.

Viscosities of PEC bead-lotion, plain bead-lotion and aloe gel-lotion were found to be 1835cp±8, 1550cp±12 and 1230cp±6 respectively, indicating better rheology of PEC bead-lotion during handling. Viscosity results are in accordance with the sedimentation ratio study. Higher viscosity of PEC bead-lotion and presence of chitosan may synergize protection from microbial growth than plain bead-lotion and aloe gel-lotion which was confirmed from stability studies. Results of

short term stability studies as shown in table 3 indicated no significant changes in pH, viscosity and appearance in the optimized formulations of PEC bead-lotion and in plain bead-lotion. However conventional lotion had shown significant change in pH, viscosity, color and appearance with the time.

In ointments, creams, lotions and other preparations for topical use; microencapsulation has been always proved as best method to improve stability of API^[18]. Here plain bead-lotion has significantly improved the stability than aloe gel-lotion. Further, chitosan being antimicrobial, antioxidant, free radical scavenger and complexes with alginate improving strength of beads leading to further significant increase in stability of aloe gel.

Also in concept, chitosan bearing most of the therapeutic properties like aloe gel can synergistically combine to improve its therapeutic properties along with stability and needed to be evaluated.



Fig. 1: Photograph of wet PEC beads

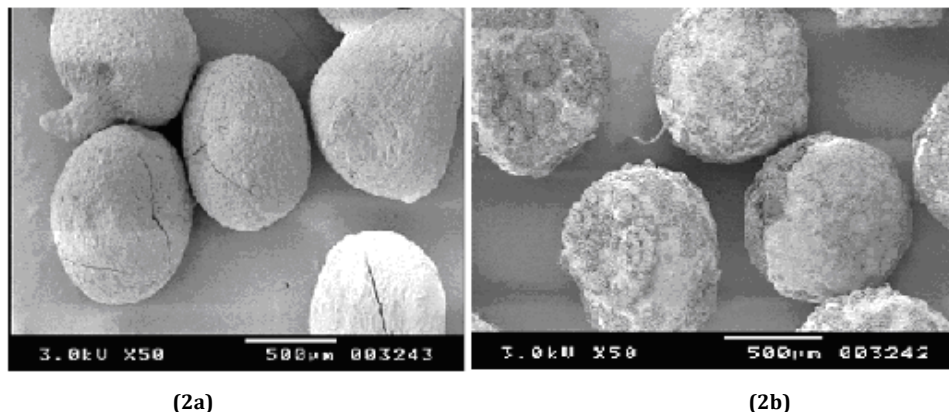


Fig. 2: 2a: Scanning electron microphotograph (SEM) of plain beads; 2b: Scanning electron microphotograph (SEM) of PEC beads

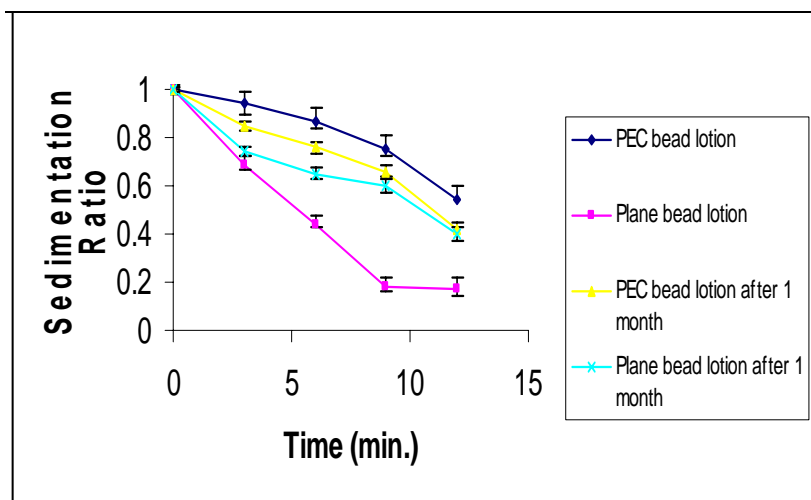


Fig. 3: Sedimentation ratio study

CONCLUSION

The present work concluded that, with the help of chitosan-alginate PEC beads, herbal extract can be incorporated into lotion with greater compatibility. Overall study revealed that, the formulation of aloe gel into beads have significantly improved compatibility and stability in comparison with conventional lotion; even higher for PEC bead-lotion as compared to plain bead-lotion. Further investigations are needed to study the influence of other variables such as molecular weight of chitosan, chitosan: alginate ratio and gelation technique on aloe gel stability.

REFERENCES

- Samuelsson G. Drugs of natural origin: A textbook of Pharmacognosy. 3rd Ed. Sweden: Pharmaceutical Press; 1992. p. 118-20.
- Coats BC. Method of processing stabilized Aloe Vera Gel obtained from the whole Aloe Vera leaf. US Patent 5356811, 18 Oct. 1994.
- Kaufman T, Kalderon N, Ullmann Y, Berge J. Aloe vera gel hindered wound healing of experimental second-degree burns: a quantitative controlled study. J Burn Care Rehabil 1998; 9:156-59.
- Vogler BK, Ernst E. Aloe vera: a systemic review of its clinical effectiveness. Br J Gen Pract 1999; 49:823-28.
- Je JY, Kim SK. Reactive oxygen species scavenging activity of aminoderivatized chitosan with different degree of deacetylation. Bioorg Med Chem 2006; 14(17):5989-94.
- Li Z, Zhong H, Peng X, Li J, Zheng J. Effect of chitosan and CaCl₂ on senescence and membrane lipid peroxidation of postharvest kumquat fruits. Acta Hort (ISHS) 2008; 769:259-64.
- Campos M, Cordi L, Durán N, Mei L. Antibacterial activity of chitosan solutions for wound dressing. Macromol Symposia 2006; 1:515-18.
- Yang TC, Chou CC, Li CF. Antibacterial activity of N-alkylated disaccharide chitosan derivatives. Int J Food Microbiol 2005; 97(1):237-45.
- Hernández-Lauzardo AN, Bautista-Baños S, Velázquez-del Valle MG, Méndez-Montealvo MG, Sánchez-Rivera MM, Bello-Pérez LA, et al. Antifungal effects of chitosan with different molecular weights on *in vitro* development of *Rhizopus stolonifer* Vuill. Carbohydr Polymer 2008; 73(4):541-47.
- Yena MT, Tsengb YH, Lia RC, Mau JL. Antioxidant properties of fungal chitosan from shiitake stipes. Food Sci Tech 2007; 40(2):255-61.
- Ueno H, Mori T, Fujinaga T. Topical formulations and wound healing applications of chitosan. Adv Drug Del Rev 2001; 52(2):105-15.
- Porporatto C, Bianco ID, Correa SG. Local and systemic activity of the polysaccharide chitosan at lymphoid tissues after oral administration. J Leukoc Biol 2005; 78:62-69.
- Chaudhuri RK. Photo stable organic sunscreen compounds with antioxidant properties and compositions obtained therefrom. US Patent 6831191, 14 Dec. 2004.
- Qin C, Du Y, Xiao L, Liu Y, Yu H. TI: Moisture retention and antibacterial activity of modified chitosan by hydrogen peroxide. J Appl Polym Sci 2002; 86(7):1724-30.
- Ayyaru G, Arul V. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquaculture 2006; 255(1-4):179-187.
- Alfred M, Bustamante P, Chun AHC. Physical Pharmacy. 4th Ed. New Delhi: B.I. Waverly, Pvt. Ltd.; 1995.
- Lachman L, Liberman H, Kanig J, The Theory and Practice of Industrial Pharmacy. 3rd Ed. Mumbai, Varghese Publishing House; 1987: 492-494.
- Dinarvand R, Rahmani E, Farbod E. Gelatin Microspheres for the Controlled Release of All-trans-Retinoic Acid Topical Formulation and Drug Delivery Evaluation. Iranian J Pharm Res 2003; 1(2):47-50.