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Biodegradable polymer based encapsulation of neem oil nanoemulsion for controlled release of Aza-A

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ABSTRACT

Azadirachtin a biological compound found in neem have medicinal and pesticidal properties. The present work reports on the encapsulation of neem oil nanoemulsion using sodium alginate (Na-Alg) by cross linking with glutaraldehyde. Starch and polyethylene glycol (PEG) were used as coating agents for smooth surface of beads. The SEM images showed beads exhibited nearly spherical shape. Swelling of the polymeric beads reduced with coating which in turn decreased the rate of release of Aza-A. Starch coated encapsulation of neem oil nanoemulsion was found to be effective when compared to PEG coated encapsulation of neem oil nanoemulsion. The release rate of neem Aza-A from the beads into an aqueous environment was analyzed by UV–visible spectrophotometer (214 nm). The encapsulated neem oil nanoemulsion have the potential for controlled release of Aza-A. Neem oil nanoemulsion encapsulated beads coated with PEG was found to be toxic in lymphocyte cells.

1. Introduction

Neem (*Azadirachta indica*) a tropical plant was used for the development of medicine and industrial products (Biswas, Chattopadhyay, Banerjee, & Bandyopadhyay, 2002). Neem contains medicinal applications in cancer (Mahapatra et al., 2011), skin disorders (Abdel-Ghaffar & Semmler, 2007), diabetes (Dixit, Sinha, & Tank, 1986), ulcers (Maity, Biswas, Chattopadhyay, Banerjee, & Bandyopadhyay, 2009), antibacterial (Dhayanithi, Kumar, & Kathiresan, 2010; Upadhyay, Dwivedi, & Ahmad, 2010), antifungal (Wang, Li, Cao, & Jiang, 2010) and antiviral (Tiwari, Darmani, Yue, & Shukla, 2010). Neem oil can act as an effective, natural pesticide which has strong insect antifeedant and growth regulating substance (Judge, Neal, & Derr, 2005; Wu, Zhao, Taylor, & Shelton, 2006). Azadirachtin a tetranortriterpenoid limonoid found in neem. Azadirachtin a biologically active compound has applications in herbal medicine, healthcare products and biopesticides (Liu, Chen, Chena, & Linb, 2005). Aza-A contributes about 80% of the azadirachtin (Devakumar & Kumar, 2008).

Nanoemulsions are thermodynamically stable liquid dispersion of oil, water and surfactant with a droplet size of 20–200 nm (Sadurní, Solans, Azemar, & García-Celma, 2005). Phase inversion temperature method, high-shear stirring, high-pressure homogenizers and ultrasonic generators are used for the preparation of emulsion (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). Nanoemulsions are prepared using pharmaceutically ingredients since it has medicinal applications (Shakeel et al., 2008). Neem oil nanoemulsion has larvicidal property against *Culex quinquefasciatus*. For controlling vector borne diseases neem oil nanoemulsion will be a good alternative to other pesticides (Anjali, Sharma, Mukherjee, & Chandrasekaran, 2012).

Microencapsulation and controlled delivery technology seems to be the most convenient method to reduce toxicity to non target organisms in case of pesticides. This technique makes a convenient as well as efficient for targeted delivery. There are various reports on encapsulation of neem oil Kulkarni, Soppimath, Aminabhavi, Mehta, and Dave (1999) studied the preparation of polymeric granules containing natural liquid pesticide viz., *A. indica* A. Juss. (Neem) seed oil. A patented invention has been developed which relates to an improved granular formulation of neem seed extract containing neem Aza-A having enhanced storage stability (Sreenivasa, Davendra, Liza, & Chandrasekaran, 2006), and the potential for gradual release of neem Aza-A for application to the plant rhizosphere. Encapsulation of a natural liquid pesticide using sodium alginate (Na-Alg) as a controlled release polymer after cross linking with glutaraldehyde (GA) has been reported (Kulkarni, Soppimath, Aminabhavi, Dave, & Mehta, 2000). Sodium alginate (Na-Alg) has been used as a controlled release matrix material in medicine (Nochos, Douroumis, & Bouropoulos, 2008; Mehyar, Liu, & Han, 2008) and agriculture (Fernandez-Perez, Gonzalez-Pradas, Villafranca-Sanchez, & Flores-Ceíspedes, 2000; Guan, Chi, Yu, & Li, 2008) after cross linking it with calcium chloride and glutaraldehyde. Alginate polysaccharides are identified to be

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hemocompatible and do not build up in any organs of the human body. Encapsulating nanoparticle layers at the emulsion droplet interface may be engineered to increase droplet stability and control of release kinetics. But there are hardly any reports on encapsulation of nanoemulsion.

In this study, Na-Alg beads have been prepared to use glutaraldehyde as cross linking agent. To the best of our knowledge, this is the first study to report on encapsulation of varying amounts of neem oil nanoemulsion in Na-Alg beads using glutaraldehyde as cross linking agent. The effect of coating agents (starch and PEG) on beads was compared. Release kinetics of Aza-A from beads were reported. The optimum conditions for release of neem Aza-A from such beads were investigated.

2. Experimental

2.1. Materials

Neem oil was procured from Vellore, Tamilnadu and stored at room temperature. Tween 20 (polyoxyethylene (20) sorbitan monolaurate) was purchased from Sigma–Aldrich, USA. Starch and AR grade methanol samples were purchased from S.D.-Fine Chem. Limited, Mumbai. Sodium alginate, glutaraldehyde (25%, w/v) solution and polyethylene glycol M.W. 400 was supplied from HiMedia, Mumbai. Deionized water (Milli-Q water, Millipore Corporation) was used for all experiments. All chemicals were of analytical grade.

2.2. Methods

2.2.1. Neem oil nanoemulsion formulation

Neem oil, Tween 20 and Milli-Q water were used in the preparation of emulsion. The emulsions thus formed were sonicated for 1–3 h using high energy sonication in a sonicator (Sonics Vibracell Ultrasonicator, 130 W, and 20 kHz). To delineate the phase boundaries precisely formed in Phase diagram, maximum ratios were covered for study at temperature 25 °C. The physical condition of nanoemulsion was marked on a Ternary Phase diagram with one axis representing oil, the second one representing surfactant and the third one representing aqueous phase. The 1:3 ratio of neem oil nanoemulsion was stable with a size of 31.03 nm (Anjali et al., 2012).

2.2.2. Preparation of nanoemulsion encapsulated beads

Sodium alginate solution (4%, w/v) in distilled water was prepared in a heating mantle. After complete cooling, neem oil nanoemulsion containing neem Aza-A (2000 ppm) was added at different concentrations and mixed thoroughly using a magnetic stirrer. The polymer solution containing nanoemulsion was added dropwise into methanol containing 2% (w/v) glutaraldehyde as a cross linking agent and 0.1% (w/v) of 1 N HCl as a catalyst, using a 25 ml hypodermic syringe (1.0 mm diameter) with constant stirring. The beads formed in methanol were kept for overnight. The beads were washed with water and then dried. The entrapment efficiency was calculated as the ratio between the initial mass of Aza-A to be encapsulated and its mass in the final product.

2.2.3. Coating of capsules with starch and PEG

Five grams of starch and PEG were dissolved in distilled water (50 ml) in a beaker (250 ml). Briefly, the dried beads of cross linked sodium alginate mixed with nanoemulsion were dipped for 5 min in starch and PEG solution (5%, w/w). Then, the coated beads were dried at 30 °C for 24 h.

2.2.4. Bead size range

Completely dried beads from the different formulations were selected and their sizes were measured using a micrometer screw gauge with an accuracy of ±0.01 mm.

2.2.5. Fourier transforms infrared (FTIR) measurements

FTIR measurements were done to detect any chemical interactions between neem oil nanoemulsion and Na-Alg or the cross linking agent i.e., GA. This was done by taking following samples: (a) pure neem oil, (b) neem oil nanoemulsion, (c) Nanoemulsion encapsulated bead, (d) Nanoemulsion encapsulated bead coated with starch and (e) Nanoemulsion encapsulated bead coated with PEG. The beads were crushed with potassium bromide and pellets were formed under a hydraulic pressure of 600 kg/cm. The FTIR measurements were done using Perkin Elmer spectrum-1 FTIR in the range of 4000–450 cm^{-1}.

2.2.6. Phase contrast microscopy

Phase contrast microscopic examination was done to achieve morphology of the beads. Sample was placed on a slide and observed under magnification (50×). Phase contrast microscopic images were taken in Leica DM 2500 microscope.

2.2.7. Scanning electron microscope (SEM)

Topographical characterization of beads was obtained by SEM study. The sample was deposited on brass hold and sputtered with gold. SEM photographs were taken with FEI Quanta 200F Scanning Microscope at the required magnification at room temperature.

2.2.8. Drying rate study of the beads

Drying rate study was carried out by selecting few samples of the beads formed after cross linking with GA. The beads were allowed to dry in an oven maintained at 30 °C (the initial mass of the beads should be nearly equal). The masses of the beads were taken at fixed intervals of time until the constant mass was achieved. All the mass measurements were done on a Mettler single pan balance. In order to obtain reproducible results, experiments were performed in triplicate, and the average values were used for the calculation and plotting of the data vs. time.

2.2.9. Swelling study of the individual beads

Swelling of the beads was carried out by the percentage of water uptake by the beads at a definite time interval. Three different beads exposed to GA were selected and incubated with distilled water in a watch glass. The mass of three different beads were taken at different time intervals, and the average value was calculated. During this method, handling of the swollen beads should be smooth so as to avoid mass loss by breaking or erosion of the beads. All mass measurements of the swollen beads were done on a Mettler single pan balance. Experiment was carried out in triplicate.

$$\%\ \text{swelling ratio} = \frac{(\text{wet weight} - \text{dry weight}) \times 100}{\text{Dry weight}}$$

2.2.10. Content uniformity

Beads were evaluated for the azadirachtin content and this was done by refluxing a known mass of the beads with 100 ml of methanol at 65 °C. Refluxing was continued for 1 h, to ensure complete extraction of azadirachtin from beads. The absorbance of methanol containing the extracted amount of azadirachtin was taken at a wavelength of 214 nm in a UV spectrophotometer (Systronics, India) using absolute methanol as a blank.

2.2.11. Dissolution studies

The dissolution study was carried out in 250 ml conical flasks containing the dissolution media (0.1% Tween 20 solution in

distilled water) with the closure caps which were kept at 35 °C in an incubator. Beads weighing about 10 mg of different formulation and coated ones were taken in the dissolution media. At definite intervals of time, the conical flasks were shaken well, and a 10 ml aliquot was taken for the analysis of azadirachtin content using UV spectrophotometer at 214 nm. Experiments were performed in triplicate in order to minimize the experimental error. The average values were used for further data analysis and plotting.

2.2.12. Cytotoxicity testing of the nanoemulsion encapsulated beads

The cytotoxicity of neem oil nanoemulsion was estimated by the international standard for biological evaluation of medical devices (ISO 10993-5) (Seo et al., 2012). Nanoemulsion encapsulated alginate beads and nanoemulsion encapsulated beads coated with starch and PEG (1%, 4% and 8%) was incubated in RPMI 1640 medium (HiMedia, Mumbai) at 37 °C for 24 h under shaking. The beads were filtered to remove insoluble material residues by 0.2 μm membrane filter (Millipore™ MF). Human lymphocytes were isolated using lymphocyte separation media (HiSep™, HiMedia, Mumbai) and cultured in RPMI 1640 supplemented with 1% PHA, 1% penicillin, 1% streptomycin and 10% FBS at 37 °C in 5% CO_2. In each well of the 96-well culture plates, 1.0×10^6 cells in RPMI 1640 medium was seeded and incubated for 24 h. Nanoemulsion encapsulated beads with RPMI 1640 medium was added to the culture medium and the cells were further incubated for 24 h. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich, USA) solution (5.0 mg/ml) was added to each well and incubated for 4 h. The absorbance was measured at 570 nm using a microplate reader (Biotek devices, USA).

2.2.13. Statistical analysis

Data was subjected to statistical analysis by one way analysis of variance (ANOVA) using Graphpad Prism 5 software. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Preparation of neem oil nanoemulsion capsules and cross linking

Neem oil nanoemulsion encapsulation was prepared using sodium alginate as a controlled release polymer after cross linking with glutaraldehyde, and then the capsule was coated with starch and PEG. It was observed that coating with starch was found to be more effective compared to PEG. The optimum conditions for encapsulation of neem oil nanoemulsion such as the time left in the glutaraldehyde solution were investigated. Overnight incubation resulted in significant cross linking. In order to optimize the drying conditions, some samples of the beads of equal weights but with different extents of cross linking were selected for their testing rate of drying.

The crossing linking mechanism of glutaraldehyde and alginate could be through coacervation reaction between the hydroxyl groups of glutaraldehyde and sodium alginate. Aza-A with glutaraldehyde would increases the entrapment efficiency in sodium alginate beads and the cross linking reactions might be stable due to covalent bonds. Glutaraldehyde is a good crosslinking agent for the preparation of neem encapsulated beads for the controlled release of neem Aza-A. This parallels the observation of Riyajan and Sakdapipanich (2009).

The chemical interactions amongst the ingredients i.e., GA, Na-Alg and neem oil nanoemulsion are assessed in order to understand the stability of nanoemulsion in the matrix system. This was confirmed by FTIR data compiled in Fig. 1 for neem oil alone (curve a), neem oil nanoemulsion (curve b), beads containing neem oil nanoemulsion (curve c), starch coated beads (curve d) as well as PEG coated beads (curve e). The characteristic sharp peaks of neem oil are at 3465 (O—H), 2925 (aromatic and/ or vinylic C—H), 2854 (aliphatic C—H) and 1037 (various C—O). These observations support the findings reported by Siddiqui et al. (2004). These peaks were found with slight variation even after the formation of the beads, thereby indicating the absence of chemical interactions between nanoemulsion and the Na-Alg polymer. Kulkarni et al. (2000) reported the absence of chemical interaction between neem oil and Na-Alg after bead formation. This confirms that nanoemulsion was successfully encapsulated in Na-Alg beads.

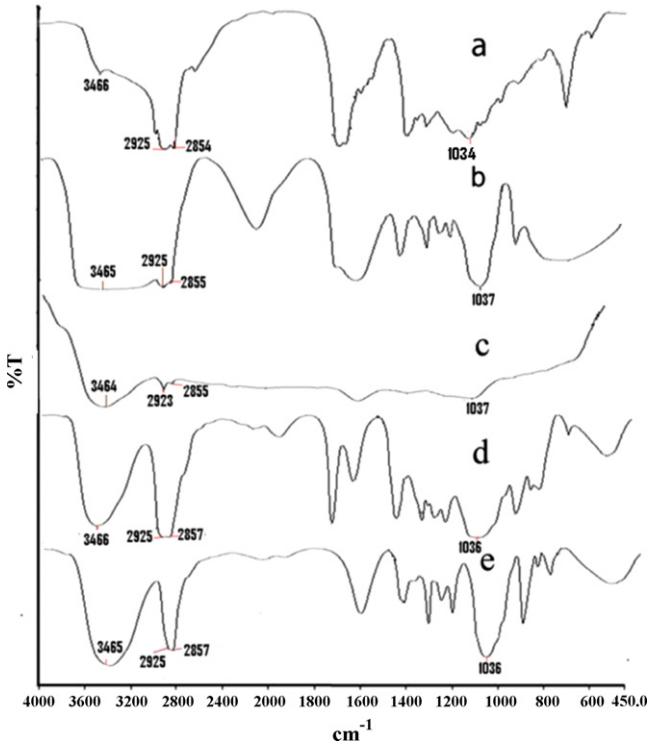

Fig. 1. FTIR spectra of (a) pure neem oil, (b) neem oil nanoemulsion, (c) nanoemulsion encapsulated bead, (d) nanoemulsion encapsulated bead coated with starch and (e) nanoemulsion encapsulated bead coated with PEG.

The results of percentage entrapment efficiency and bead diameter are presented in Table 1. The beads formed are nearly spherical in shape with the particle size ranging from 1.28 to 1.49 mm in diameter. The particle size did not differ significantly with increasing percentage loading of neem oil nanoemulsion. But, the percentage entrapment efficiency varied considerably with the percentage loading. The entrapment efficiency decreased with an increase in neem oil nanoemulsion loading. This might be attributed to the release of nanoemulsion to the external methanol.

Table 1
Effect of the neem oil nanoemulsion Aza-A content on bead diameter and entrapment efficiency of beads.

Neem oil nanoemulsion loading (%)	Bead diameter (mm)	Entrapment efficiency (%)
1 ml (without coating)	1.28 ± 0.006	81.8 ± 0.06
1 ml (starch coated)	1.32 ± 0.004	72.4 ± 0.12
1 ml (PEG coated)	1.36 ± 0.006	71.7 ± 0.06
4 ml (without coating)	1.36 ± 0.004	79.9 ± 0.06
4 ml (starch coated)	1.41 ± 0.004	70 ± 0.12
4 ml (PEG coated)	1.44 ± 0.006	69.7 ± 0.06
8 ml (without coating)	1.42 ± 0.006	76.2 ± 0.06
8 ml (starch coated)	1.46 ± 0.004	67.7 ± 0.12
8 ml (PEG coated)	1.49 ± 0.004	66.5 ± 0.15

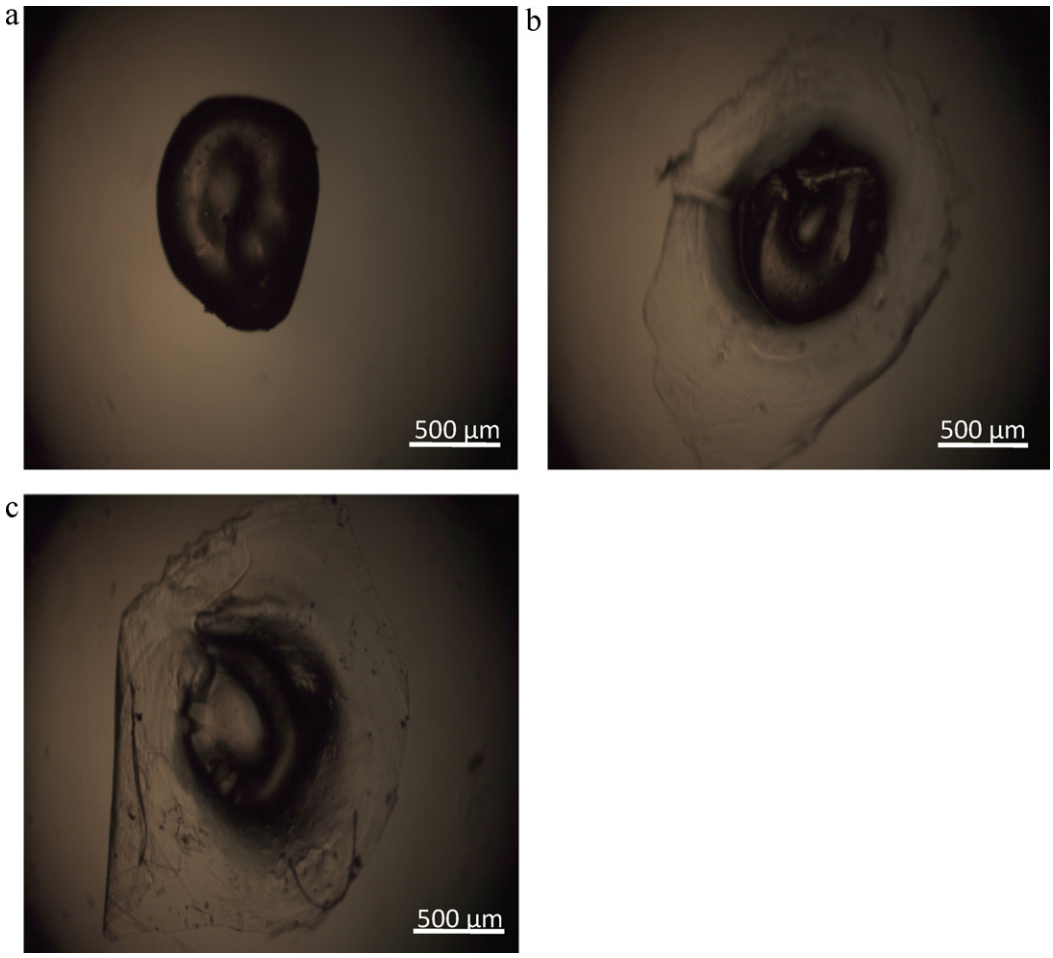

Fig. 2. Phase contrast microscopic image (a) control, (b) starch and (c) PEG.

3.2. Phase contrast microscopy

The phase contrast microscopic images of the encapsulated nanoemulsion beads are shown in Fig. 2. Fig. 2a represents control one, (b) represents starch coated bead and (c) shows PEG coated bead. A protective coating can be seen around bead.

3.3. SEM

The beads formed were almost spherical in shape with smooth surface as revealed by the SEM image shown in Fig. 3. The mean particle size was 1.21 mm. After the coating of beads with starch and PEG, their diameter drastically increased to 1.32 mm and 1.34 mm and had a smoother bead surface.

3.4. Swelling study

The effect of cross linking on the percentage of swelling ratio by beads exposed to glutaraldehyde in case of individual formulation is shown in Fig. 4a–c. Maximum uptake of water was shown by the beads during the first hour. Aza-A release from the beads was measured after subjecting them to a set of parameters (both physical and chemical) including those related directly to the release medium and those resulting from changes to the characteristics of the structures controlling release (beads). The bead in glutaraldehyde solution kept at overnight was coated with starch and PEG. It was observed that the percentage of swelling ratio decreased in case of starch coated ones compared to PEG.

It is observed that the release rate of neem Aza-A from the beads can be improved by modifying the bead structure by coating them with starch and PEG. Vanderaer (1974) reported that diffusion in polymers is a powerful mechanism in pharmacy for controlling the release of drugs. They found that diffusion in polymeric systems is passive, if the driving force is merely a Brownian, molecular motion. However, diffusion can also be modified by various external factors, either by the influence of the release medium through swelling or biodegradation, or by the effects of physical forces such as electrical, osmotic or convective forces. A suitable coating material must be nonreactive, and capable of being rapidly used to produce a film. It should be essentially immiscible with the material being encapsulated. Starch and PEG were selected as the coating agent of neem Aza-A-sodium alginate beads to provide an adequate barrier wall. The dry beads of cross linked sodium alginate mixed with neem Aza-A (2000 ppm) were dipped into starch and PEG solution (5%, w/w) for 5 min. Then, the coated beads were dried at 30 °C for 24 h. In order to achieve the effect of cross linking on the release rates of neem oil nanoemulsion from the matrix, swelling was studied in terms of percentage of water uptake by the beads. However, the swelling property of the beads was measured in terms of percentage of water uptake by the beads at a specified time interval.

It is clear that the rate of swelling decreased dramatically after coating neem oil nanoemulsion encapsulated beads with starch and PEG compared with that of the beads without coating. Swelling ratio of the beads dramatically decreased in case of starch coated beads compared to PEG. The swelling ratio of neem oil nanoemulsion encapsulated beads in aqueous medium for 1 ml formulation

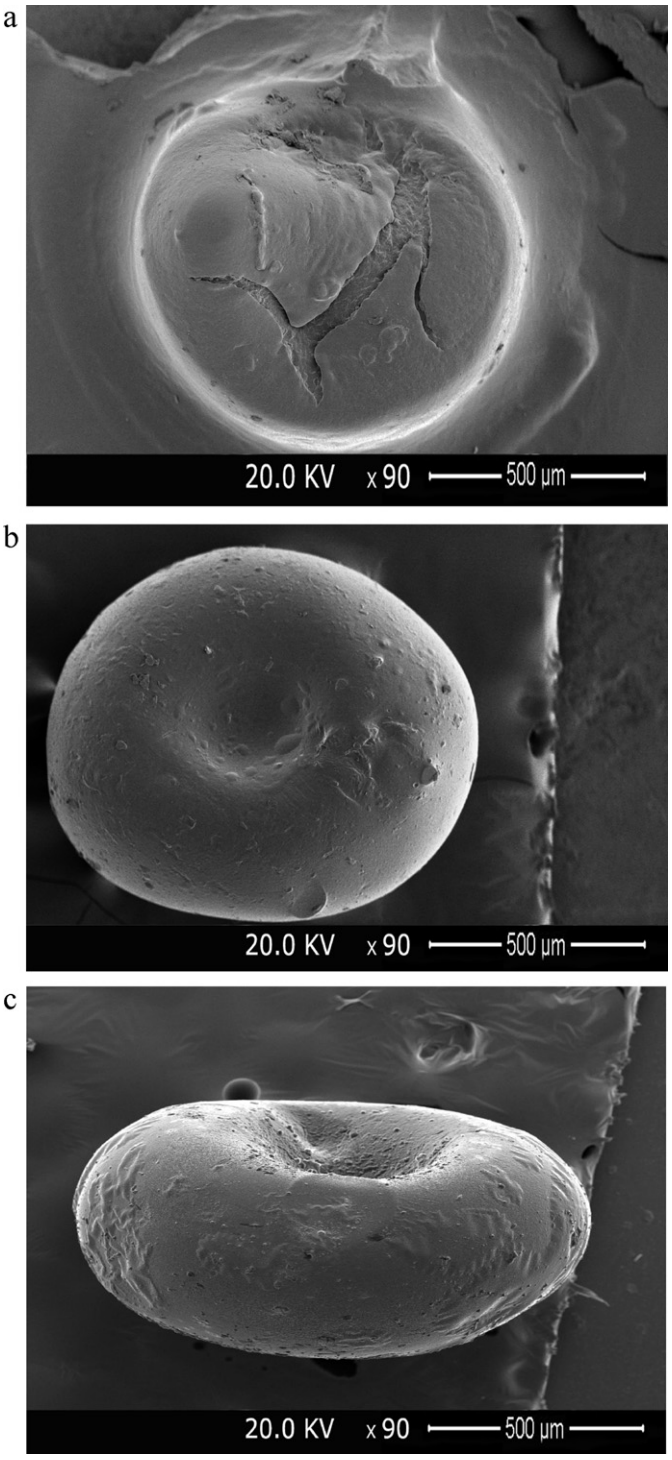

Fig. 3. Scanning electron microscopic image (a) control, (b) starch and (c) PEG.

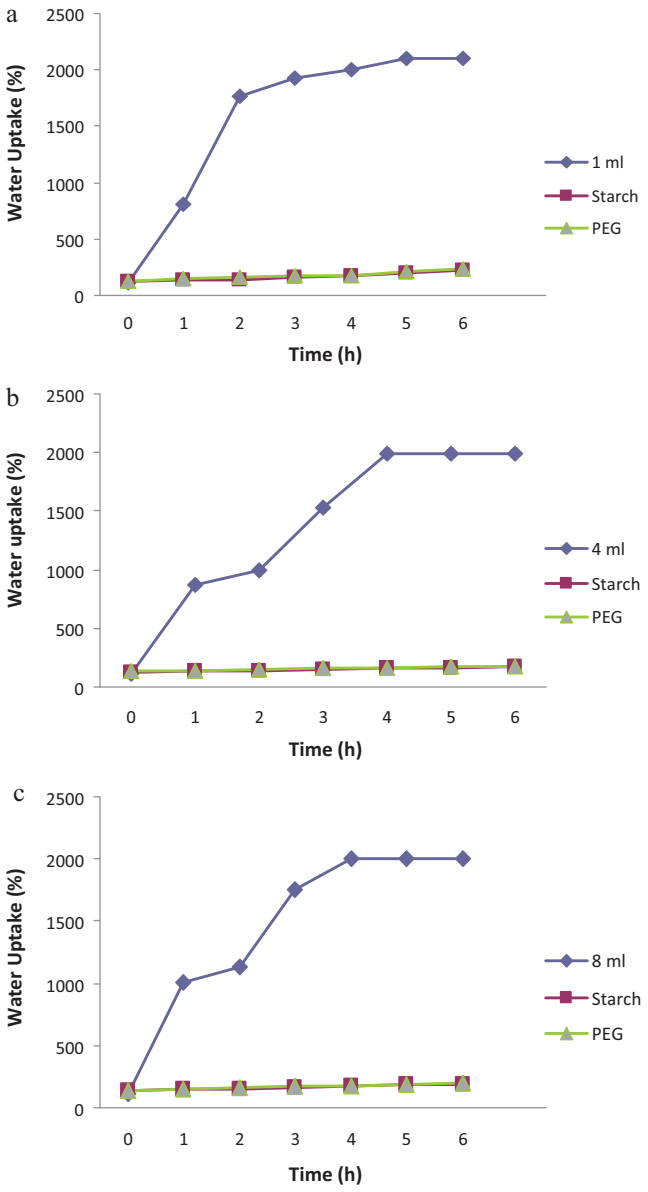

Fig. 4. Effect of varying loading on percentage of water uptake (a) 1 ml nanoemulsion along with starch and PEG coated, (b) 4 ml nanoemulsion along with starch and PEG coated and (c) 8 ml nanoemulsion along with starch and PEG coated.

at 2, 4, 6 and 24 h of storage neem Aza-A was 1129.51%, 2000.41%, 2000.54% and 2000.54%, respectively. The swelling ratio of the nanoemulsion encapsulated beads decreased in case of starch and PEG coated ones. Swelling ratio of starch coated ones at 2, 4, 6 and 24 h of storage was 153.87%, 172.32%, 190.2% and 191.45% respectively. In case of PEG coated ones for 2, 4, 6 and 24 h; it was 160.43%, 174.52%, 197.56% and 200.31% respectively. Similar results were observed in case of 4 ml and 8 ml nanoemulsion formulation encapsulated beads. The transport of water through Na-Alg polymer is dependent upon the rigidity of the polymer as well as the extent of cross linking, since it is a hydrophilic one (Kulkarni et al., 1999).

3.5. Dry weight

In order to optimize the drying conditions, beads with different proportions of cross linking were selected such that the initial weight is almost equal. Results of drying are shown in Fig. 5 which indicate that the beads encapsulated with 1 ml neem oil nanoemulsion formulation dried within 4 h compared to one with 4 ml nanoemulsion and 8 ml nanoemulsion (5 and 6 h respectively). The drying data can be further treated to calculate the diffusion coefficients for desorption of the liquid from the beads.

3.6. Static dissolution study

Azadirachtin release from the beads were subjected to a number of physical and chemical parameters including those related

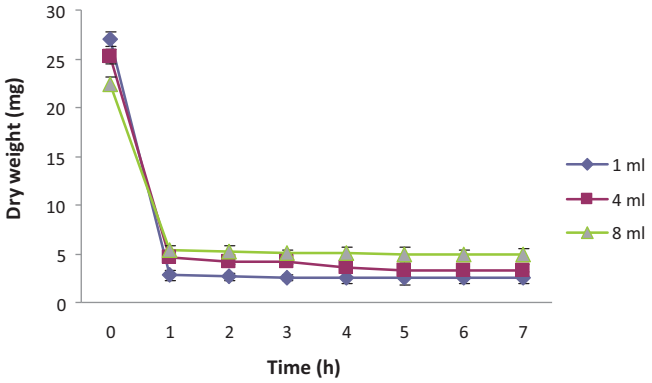

Fig. 5. Effect of varying loading on drying of beads.

directly to the release medium (Tween-20 solution), the release conditions such as temperature and those resulting from change in the characteristics of the release controlling device (beads). The effect of degree of cross linking of Na-Alg beads on the kinetics of Azadirachtin A release is depicted in Fig. 6a–c. The effect of

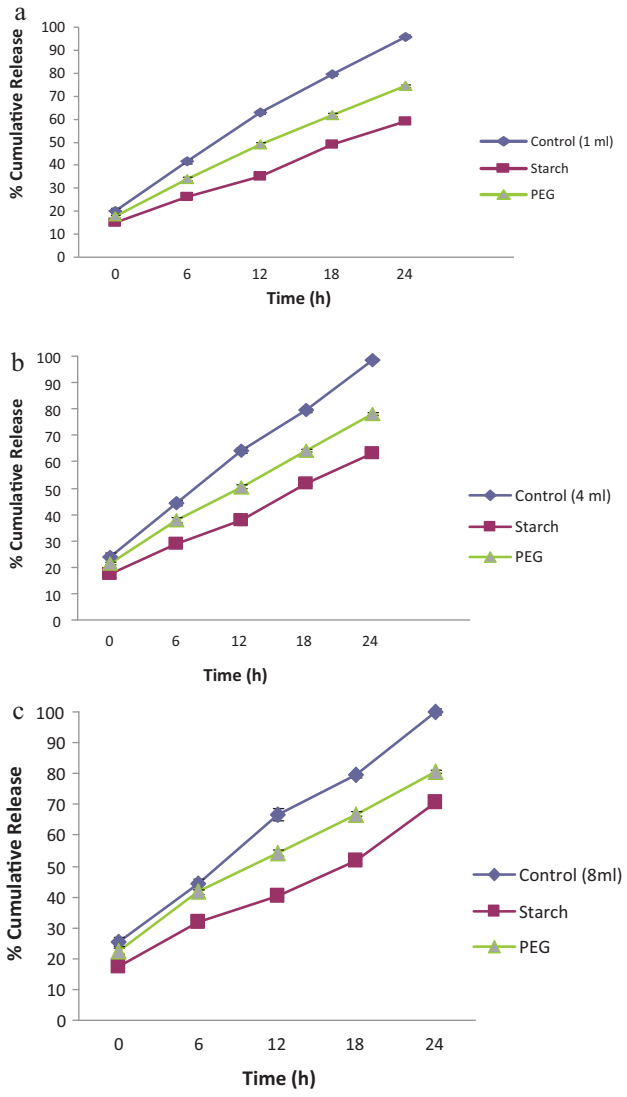

Fig. 6. Effect of varying loading on release of neem Aza-A (a) 1 ml nanoemulsion along with starch and PEG coated, (b) 4 ml nanoemulsion along with starch and PEG coated and (c) 8 ml nanoemulsion along with starch and PEG coated.

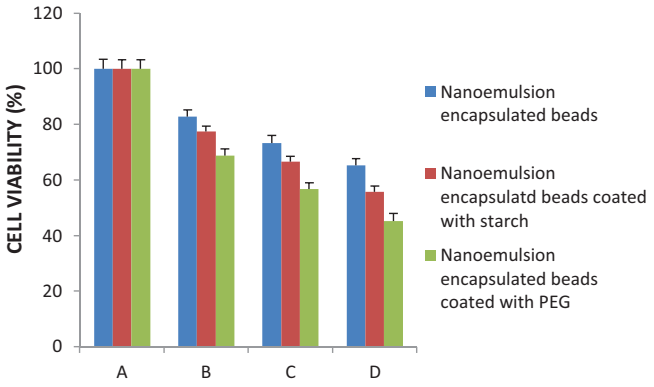

Fig. 7. Cytotoxicity of the nanoemulsion encapsulated beads. Cell viability of the nanoemulsion encapsulated beads was assessed in human lymphocyte cell ($n = 3$, $p < 0.05$). (A) Control and (B) 1% of the nanoemulsion encapsulated beads coated with starch and PEG C) 4% of the nanoemulsion encapsulated beads coated with starch and PEG D) 8% of the nanoemulsion encapsulated beads coated with starch and PEG.

release rate of azadirachtin from the beads loaded with different amounts of neem oil nanoemulsion and those beads coated with starch and PEG is presented in Fig. 6a–c. It was observed that the higher the amount of nanoemulsion loaded the higher the release rate. The release rate of 8% of neem oil nanoemulsion loaded beads has shown 100% release in the 24th hour. In case of 4% and 1% neem oil nanoemulsion loaded beads, they showed only 98.4% and 95.8% release after 24th hour. 100% release rate was obtained with in small concentration of nanoemulsion itself which makes this process more effective. These findings correlate with the observations reported by Kulkarni et al. (2000).

Beads loaded with different amounts of neem oil nanoemulsion (1%, 4%, and 8%) were selected for starch and PEG coating, sine in all cases they showed effective release. It is clear that the neem Aza-A release rate is reduced significantly by starch and PEG coating, which is consistent with the results of the swelling study. The effect of release rate of Aza-A from the beads coated with starch was found to be more effective compared to PEG. The release of azadirachtin from starch coated beads were particularly less compared to PEG coated ones. The neem Aza-A cumulative release in case of 8% neem oil nanoemulsion loaded beads without coating exposed for 6, 12, 18 and 24 h in aqueous medium was 44.2%, 66.8%, 79.4% and 100% respectively. But when same beads were coated with starch in the same condition, the release was observed to be 31.6, 40.4, 51.6 and 70.6 respectively. In case of PEG coated ones, it was 41.6%, 54.2%, 66.8% and 80.6% respectively. It should be noted that with coating of starch and PEG, the bead matrix becomes more compact and rigid, resulting in reduction in rate of diffusion of neem Aza-A through swollen beads. Starch coated beads showed decreased rate of diffusion compared to PEG coated ones.

3.7. Cytotoxicity testing of the nanoemulsion encapsulated beads

The cytotoxicity of the nanoemulsion encapsulated beads was assessed in lymphocyte cells (Fig. 7). 1% of neem oil nanoemulsion encapsulated beads were comparatively less toxic to the lymphocyte cells than 4% and 8% of neem oil encapsulated beads. The viability of the treated cells was found to be 83% in 1% nanoemulsion encapsulated beads. PEG coated beads were toxic to the lymphocyte cells than the starch coated beads and nanoemulsion encapsulated beads were comparatively less toxic than the starch and PEG coated beads.

Sodium alginate is a biodegradable polymer and generally regarded as safe substance because of its non toxic properties and biomedical applications (Shalumon et al., 2011).

Glutaraldehyde has applications in many fields such as histochemistry, microscopy, biomedical and pharmaceutical applications (Migneault, Dartiguenave, Bertrand, & Waldron, 2004). 2.5% of glutaraldehyde was less toxic to human fibroblast cell line (WI-38) (Sun, Feigal, & Messer, 1990). Azadirachtin at 10^{-4} M concentration did not affect the L929 murine fibroblast cells over 72 h but was toxic to the Sf9 insect cell lines (Salehzadeh et al., 2003). Starch based polymers are drug delivery carriers and is less toxic to the L929 murine fibroblast cells (Marques, Reis, & Hunt, 2002). PEG is commonly used in cosmetics and pharmaceutical preparations. Low molecular weight PEG 200 was toxic to Chinese hamster epithelial livercell line when compared to PEG 400 (Biondi, Motta, & Mosesso, 2002).

This is the first work to be reported on neem oil nanoemulsion encapsulation. Controlled and optimized release of neem oil nanoemulsion from beads plays a crucial role in agriculture as pesticide and in various other fields. Therefore, further work is going on in our laboratory to evaluate the effect of encapsulated neem oil nanoemulsion beads in medicine, aqua culture, mosquito larvae, etc. and to optimize the release kinetics in these systems.

4. Conclusions

These preliminary results indicate that neem oil nanoemulsion in varying amounts can be successfully encapsulated into the sodium-alginate capsules cross linked with glutaraldehyde. SEM images showed encapsulated beads were nearly spherical in nature. Coating of neem oil nanoemulsion encapsulated sodium alginate capsules with starch and PEG had a marked effect on slowing the release of neem Aza-A. Coated beads exhibited more efficient release of neem Aza-A as compared to the uncoated beads. Higher release rate of Aza-A was observed in beads with higher amount of neem oil nanoemulsion. The FTIR results indicated that there are no chemical interactions among the ingredients in formulated matrices. Controlled release of the formulated beads can be of more effective use in medicine, soil agriculture field, aquaculture and various other fields.

Conflict of interest

There is no conflict of interest.

Acknowledgment

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