Research Article

Encapsulation of Omega-3-rich Oil and Anti-anxiolytic Properties of Flax Seed Oil

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ABSTRACT

The objective of the present study was to encapsulate Linum usitatissimum (Flax seed) oil and to evaluate the anxiolytic property of the oil. The mean diameter of the nano encapsulated flax seed oil by Alginate method was 173 ± 35nm; by lecithin method was 1947 ± 198nm and by chitosan method was 308 ± 16nm. In addition, we have also seen the effect of omega-3-rich oil on anxiety assessed using two behavioral paradigms employed to specifically assess anxiety, the Open field test and Elevated plus maze test. Experiments were carried out on white inbred female mice (30-35 g). Flax seed oil also increased anti-conflict effect. Thus, the seed oil possesses a marked anti-anxiolytic profile. GABA system is probably involved in realization of anti-anxiolytic action of flax seed oil.

Keywords: Flax seed, omega-3-fatty acid, the Open field test, Elevated plus maze test.

INTRODUCTION

Flaxseed has recently gained attention primarily because it is the richest known source of □□linolenic acid (ALA) and the phytoestrogen, lignans, as well as being a good source of soluble fiber. Flaxseed can modestly reduce serum total and low-density lipoprotein cholesterol concentrations, reduce postprandial glucose absorption, decrease some markers of inflammation, and raise serum levels of the omega-3 fatty acids, ALA and eicosapentaenoic acid. Oils rich in ω-3 have a positive effect on human health, acting in the prevention of cardiovascular diseases (Hasler, 1998 and Zhao et al., 2004). However, during processing, distribution and handling, oils can easily oxidize, due to their high in saturation degree. Oxidation leads to the formation of unpleasant tastes and odors. Consequently the products shelf life is decreased and free radicals are formed which affects negatively to the organism (Ahn et al., 2008). Microencapsulation of oils is an alternative that has been used in order to protect unsaturated fatty acids against lipid oxidation, thus increasing their shelf life. Besides protecting oils against oxidative damage, it also offers the possibility of controlled release of lipophilic functional food ingredients and can be useful for supplementation of foods with polyunsaturated fatty acids (Drusch et al., 2007).

In the present study we have encapsulated omega-3-rich flax seed oil by different methods to prevent oxidation. In addition, we have also seen the anti-anxiolytic effect of omega-3-rich oil in mice using standard methods such as open field and plus maze tests.

METHOD

Preparation of flaxseed oil encapsulated particle

One by **Alginate method**; 1.5grams of alginic acid sodium salt from brown algae (SIGMA ALDRICH) ,WHICH IS KNOWN AS ALGINATE, was dissolved in 100ml of distilled water in a 200ml beaker. 1ml of 1.5% alginate was taken in a 50ml beaker and 10ml distilled water was added.1ml of extracted flaxseed oil bought from the local market was added in 5 ml of distilled water with 1ml of polyethylene glycol sorbiton monooleate(tween80,Sigma Aldrich) to make oil and water miscible.In 10 ml of the dilute alginate solution, 1ml of oil emulsion was added in a 100ml conical flask and mixed properly.This solution was slowly dropwise added in a 100 ml of 3% calcium chloride (MERCK INDIA) solution.

Two by **lecithin method;** 5ml of extracted flaxseed oil was added in 2.5 grams of soya lecithin (HIMEDIA), chloroform was added to dissolve .mix properly. 2.5gm Chitosan(SIGMA

ALDRICH) was dissolved in 100ml of water and filtered through whatmann paper in a conical flask. Both solutions are mixed and evaporated using FLASH EVAPORATOR till butter like consistency reached. Three by **chitosan method;** 1g of oil was added in 5 ml of water, the mixture was made sparingly soluble with the help of tween80 (SIGMA ALDRICH).1% chitosan solution was made in 1% acetic acid solution.In 50ml of 5% TPP solution the above mixture was added dropwise.

Characterization of encapsulated flaxseed oil

One by using **dynamic light scattering (particle size analysis);** the dispersions were characterized in terms of particle size and size distribution. Particle size analysis measurements were performed using ZetaSizer Nano ZS (Malvern Instrument Ltd, Malvern, UK.) Storage stability was assessed by monitoring the size as a function of time (up to 1 month). The mean diameter measuring the fluctuation of the intensity of the scattered light which is caused by the particle movement was measured. The particle size of the various nanoparticles were prepared encapsulating flax seed oil in chitosan and lactate products purchased from M/s Everest Biotech, Bangalore was described by the volume weighted mean diameter . By definition, the volume weighted mean islet volume is the mean islet volume if particles are weighted proportional to their volume. This parameter can be estimated without assumptions regarding the shape of the particles and provides unbiased information of three-dimensional size, in contrast to the commonly used two-dimensional estimates of mean droplet profile area. The polydispersity index (PI) is a measure for the width of the distribution. It is a measure for the width of the distribution ranging from 0 (monodispersed) to 0.500 (relatively broad distribution). Two by using **uv-visible spectroscopy;** UV-visible spectra of nanocapsules were recorded on nanodrop spectrophotometer at room temperature. The region from 200-800 nm was employed for scanning about 2mg sample dissolved in 20 ml methanol was used for recording the spectrum.

Animals

Laboratory-bred, eight- to ten-week-old balb C mice (Defence Food Research Laboratory, Mysore) of both sexes were used in the toxicity study and only males were used for ant-anxiety study. The animals were housed at 21–25 ◦C, a relative humidity of 30–70% and a 12 h/12 h day/night cycle. Pelleted mouse feed (M/s Tetragon Pvt Ind ltd, Bangalore) and reverse-osmosis water was provided *ad libitum*. All procedures used in the study were approved by the Institutional Animal Ethics Committee.

The seven-day acute oral toxicity test

Standard acute toxicological evaluations of the mice were performed in the initial assessment of the effects of nanoencapsulated flax seed oil using different methods. Following overnight fasting, mice were weighed and randomly divided into four groups. Placebo (deionised water), encapsulated oil using three different methods (one by Alginate; two by lecithin and three by chitosan); all at a concentration of 10ml/kg body weight were administered in a single dose by gavage using a gastric intubation tube. On day seven, all the animals were weighed and any signs of toxicity were noted.

ANTIANZIETY PROPERTYOF THE ENCAPSULATED FLAXSEED OIL

One by using **open field test**; A whole board with a white painted wooden board (40 cm x 40 cm) with four equidistant holes (1 cm diameter x 2 cm depth). Using two thick coloured lines which intersect at the centre, the board was divided into 4 equal sectional squares of 20 cm x 20 cm (our modification to facilitate the determination of effect on locomotion, along with exploration). One hour after oral treatment (10 ml/kg) with distilled water, flax seed oil and diazepam (1 mg/kg), each mouse was placed in turn at one corner of the board with the animal subsequently moving about and dipping its head into the holes. The number of sectional crossings in 5 min was recorded for individual mouse (File and Wardill, 1975; Yemitan et al., 2001; Dhara et al., 2002). Two by using **elevated plus-maze;** Plus-maze test was performed according to the method described by Pellow et al. Briefly, the apparatus was made of wood and consisted of two open arms (50 × 10 cm) painted in black, and two closed arms (50 × 10 × 40 cm) painted in white but wooden walls had their natural color, with an open roof. The apparatus was elevated 50 cm above the floor. Two open arms were opposite to each other and were illuminated by a 40 W bulb positioned Plus-maze test was performed according to the method described by Pellow et al. Briefly, the apparatus was made of wood and consisted of two open arms (50 × 10 cm) painted in black, and two closed arms (50 × 10 × 40 cm) painted in white but wooden walls had their natural color,

with an open roof. The apparatus was elevated 50 cm above the floor. Two open arms were opposite to each other and were illuminated by a 40 W bulb positioned 20 cm below each open arm. Each rat was placed individually in a new cage which was similar to home cage for 5 min immediately before the test (this procedure results in an increase in the total number of arm entries during the test). Each rat was then placed in the centre of plus-maze facing one of the open arms. Time spent in open arms was measured during the five-minute test. The maze was cleaned with a paper towel after each trial. Experiments were performed between 9.00 and 17.00. Three by using **antianxiety instrument;** the test was performed by the method described earlier by Vogel et al. with small modifications. The test box (50 × 12 × 40 cm) was made of plexiglass,had grid floor of stainless steel and contained drinking bottle with water (Anxio-Meter, Model102; Columbus, USA). Electric shock (0.4 mA, 1 s duration) was applied between the grid floor and the drinking spout. The animals received the first shock after 30 s of drinking. During the subsequent 3 min test period shocks were delivered every 20th lick. The number of shocks accepted was recorded and the procedure was controlled by a microcomputer. Rats were deprived of water for 2 days before the test. After the first 22 h of water deprivation each rat was placed individually in the test box and was allowed to drink water for 10 min without an electric shock. Then rats were given water *ad libitum* in their home cage for 2 h. After another 20 h of water deprivation each rat was placed once again into the test cage and animals which did not start to drink during the first 2 min or which did not drink continuously for 2 min were excluded from further experiment. Again animals had free access to water for 2 h in their home cage. On the third day, after another 20 h of water deprivation the test was performed. The effects of each drug in all experiments were measured in groups of 8–10 animals. Experiments were performed between 9.00 and 17.00.

RESULTS AND DISCUSSION

Characterization of the particles

DLS was used to quantitatively measure the size and size distribution of the particles. The mean diameter of the nano encapsulated flax seed oil by Alginate method was 173 ± 35nm; by lecithin method was 1947 ± 198nm and by chitosan method was 308 ± 16nm. The findings are seen in figure 1-3. These findings provide a direct measure of particle size and size distribution in solution. According to the National Nanotechnology Initiative (2006), 'Nanotechnology is the understanding and control of matter at dimensions of roughly 1100 nm'. However, this definition of nanoparticle size was established for chemistry, physics and electronics; in the fields of nanofood and nano herb research, the particle size of interest is 1–1000 nm. Preetz *et al* developed stable polyelectrolyte nanocapsules with an average size of 130 nm. These nanocapsules could be extremely useful to the food.

UV-VIS CHARACTERIZATION

Figure 4 shows the UV-visible spectrum of the nanocapsules. According to the UV/Vis spectra of controls of all the methods were having no or less absorption at 220nm was observed, but in test with the oil encapsulated the absorbance increased at 220nm, which is evident in figure 4. The phenomenon resulted in the conclusion that the encapsulation is successful.

The seven-day acute oral toxicity test

Table 2 indicates that the no-observed-adverse-effect level (NOAEL) of chitosan encapsulated flax seed oil. But there was a sever mortality (100%) in case of alginate and few in lecithin encapsulated capsules at 10ml / kg body weight. Throughout the study, no unusual behaviour were observed, including labored breathing, difficulties moving, hunching or unusual interactions with cage mates in case of chitosan encapsulated capsule treatment.

Open Field Test

The oil also significantly ($p<0.05$) increased the locomotor activity/the total ambulatory distance thereby suggesting that flax seed oil is working effectively as an anxiolyte and ruling out the fact that the mouse due to anxiety crouched and stayed in one corner of the apparatus. The oil also showed effective results comparable with diazepam (Table 3).

Elevated plus maze test

The standard drug Diazepam showed significant anxiolytic behavior by increasing the percentage of time spent as well as the number of entries into the open arm. Pretreatment of mice with flax seed oil alleviated anxiety levels by significantly ($p<0.05$) increasing the number of entries on the open arm, time spent on open arms, percent time spent on open arms and decreasing the

percent time spent on closed arms and the number of entries into the closed arms (Table 4).

Vogel conflict test

All groups caused a significant increase in punished drinking; the number of shocks was increased in Group C (Control with picrotoxin). The elevated values for the test group (Flax seed oil) also did not differ significantly from control animals (Table 5).

In the present study we have encapsulated omega-3-rich flax seed oil by different methods to prevent oxidation. In addition, we have also seen the effect of omega-3-rich oil on anxiety assessed using two behavioral paradigms employed to specifically assess anxiety, the Open field test and Elevated plus maze test. And reveal the possible involvement of GABA-ergic system in this effect. Experiments were carried out on white inbred female mice (30-35 g). Diazepam as reported increased the number of entries into the open arms as well as the time spent on the open arms and also caused a significant increase in the total ambulatory activity as observed by the open field test. Oil also showed a significant increase in the number of entries on the open arm, time spent on open arms, percent time spent on open arms and decreasing the percent time spent on closed arms and the number of entries into the closed arms in the elevated plus maze test. An increase in the time spent in the open arms suggests the anxiolytic effects of the oil at that concentration. This also suggests that the oil is effective in decreasing the sensitivity of mice to aversive stimuli such as an open arm. Diazepam increased the number of punished responses in Vogel's conflict test. Flax seed oil also increased anti-conflict effect (Table 5). Thus, the seed oil possesses a marked anti-anxiolytic profile. GABA system is probably involved in realization of anti-anxiolytic action of flax seed oil.

These behavioral changes produced by flax seed in the behavioral tests elucidate the anxiolytic effects of the oil similar to the positive control i.e diazepam. The major thrusts of current work dealing with anxiety disorders have centered around the gamma amino butyric acid mechanisms, the serotonergic system, noradrenergic mechanisms and neuropeptides (Barchas & Altemus, 1999). GABA works to regulate the neuronal excitability and thereby serves as a 'brake' on the neuronal circuitry during stress and is the brain's natural stress reliever (Weeks, 2009). However, Hovatta *et al* took a deviation and linked oxidative stress with anxiety by correlating the expression levels of two genes – glyoxalase 1 and glutathione reductase 1 both of which take part in the oxidative stress pathway. Several other reports have also suggested for the role of oxidative stress in anxiety [Masood *et al*. (2008), Bouayed *et al*. (2007), Oliveira *et al.* (2007), (Atmaca *et al.*, 2004; 2008). All these reports provide new insights into current work focusing on anxiety disorders and stress on the beneficial role the antioxidants have in anxiety disorders.

CONCLUSION

The preparation formulation (nanocapsules) can be used to fortify omeg 3 oil rich food products. These nanocapsules can be fortified with any food products because the capsules are tast less. These food products provide additional nutrition supplementation with high stability with respect to temperature , p^H and digestive juices. Bioavalibility of the product prepared based on this formulation will help in fighiting with degenerative diseases with slow and effective release of omega 3 rich oil in the intestine. The results from the present study enunciate the anxiolytic properties of the flax seed oil. The oil can be used to incorporate into nutraceuticals promises to provide solace to the anxious.

Table 1: DLS results of the nanocapsules prepared

Method of preparing nanocapsules	Poly disperse indexPDI	z-Avg (nm)
By chitosan	0.41 ± 0.01	308 ± 16
By lecithin	0.59 ± 0.05	1947 ± 198
By Alginate	0.30 ± 0.05	173 ± 35

Table 2: Body weight and mortality during the seven-day acute toxicity test that assessed the acute toxicity of the nonocapsules prepared, which was administered orally to the mice. Data are mean ± SD values. Dose Sex (n)

	Initial body weight (g)	Final body weight (g)	Mortality dead/treated
Control (10ml water kg−1 body weight) Female (5)	32.5 ± 3.7	34.2 ± 3.8	0/5
By chitosan(10ml emulsion kg−1 body weight) Female (5)	32.7 ± 3.4	34.3 ± 3.6	0/5
By lecithin (10ml emulsion kg−1 body weight) Female (5)	33.1 ± 2.9	34.9 ± 3.8	3/5
By Alginate (10ml emulsion kg−1 body weight) Female (5)	32.4 ± 3.6	NA	5/5

Table 3: Effects of flax seed oil on locomotion in the open field test

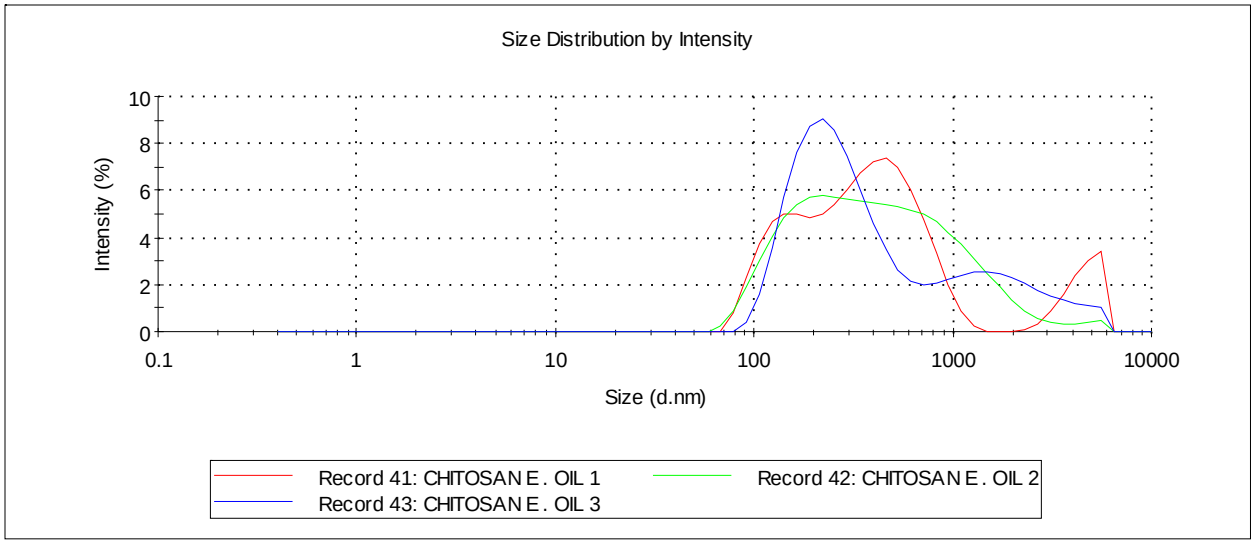
Treatment	No of sectional crossings
Group a (oil with picrotoxin)	50.4
Group b (control without picrotoxin)	27.4
Group c (control with picrotoxin)	32.6
Group d (diajapam with picrotoxin)	59.2

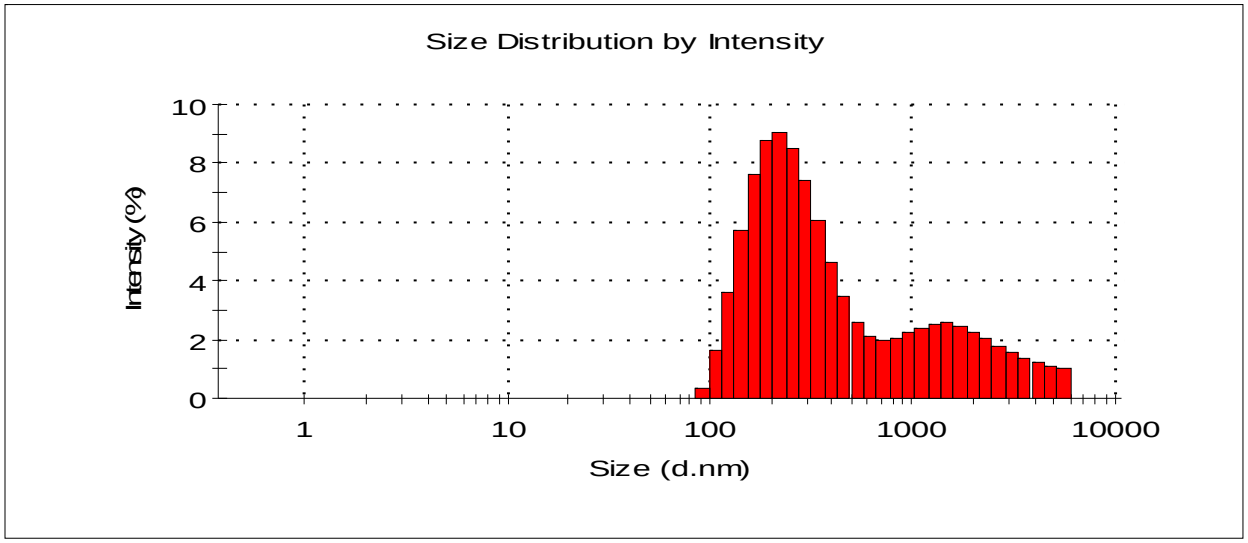
Table 4: Effects of flax seed oil on the elevated plus-maze test in mice

Treatment	Time spent in (sec)	
	Closed arm	open arm
Group a (oil with picrotoxin)	2.4	2.2
Group b (control without picrotoxin)	3.0	1.6
Group c (control with picrotoxin)	3.8	1.1
Group d (diajapam with picrotoxin)	2.0	2.8

Table 5: Anxiolytic effects of gold preparations using Vogel's conflict test in rats

Treatment	Number	
	Licks	Shocks
Group A (Flaxseed oil with picrotoxin)	43	1.2
Group B(Control without picrotoxin)	30	1.2
Group C (Control with picrotoxin)	52	3.4
Group D (Diazepam with picrotoxin)	56	1.8





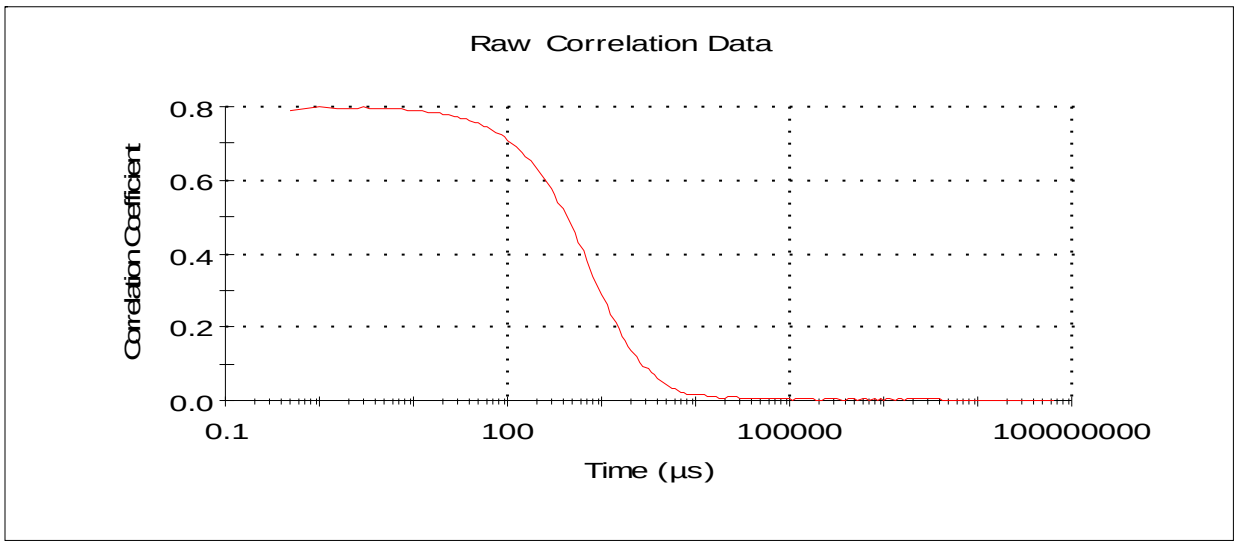
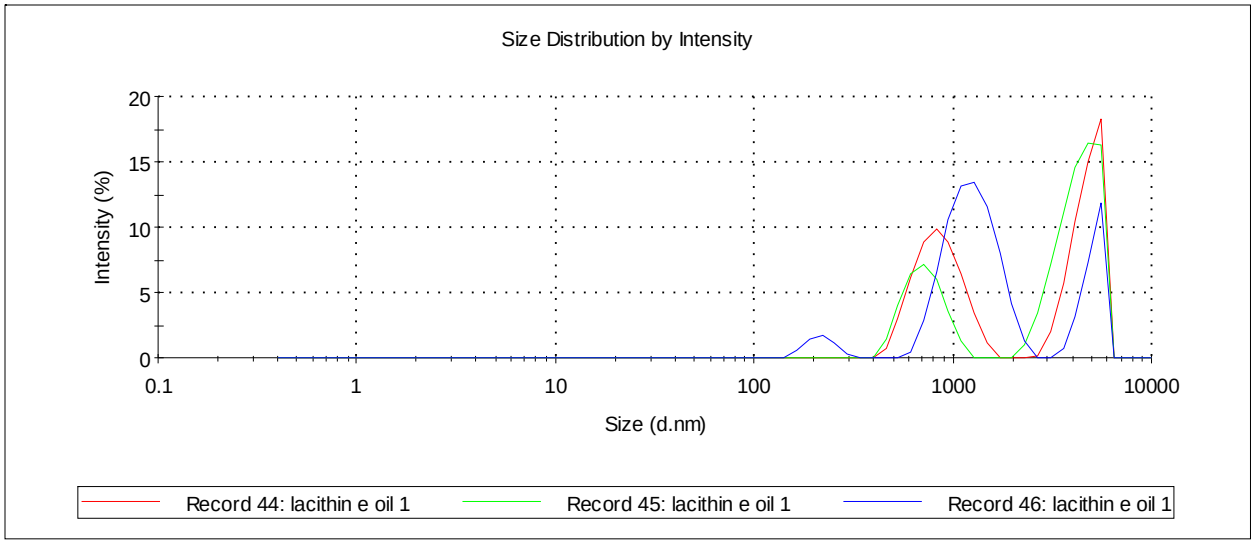
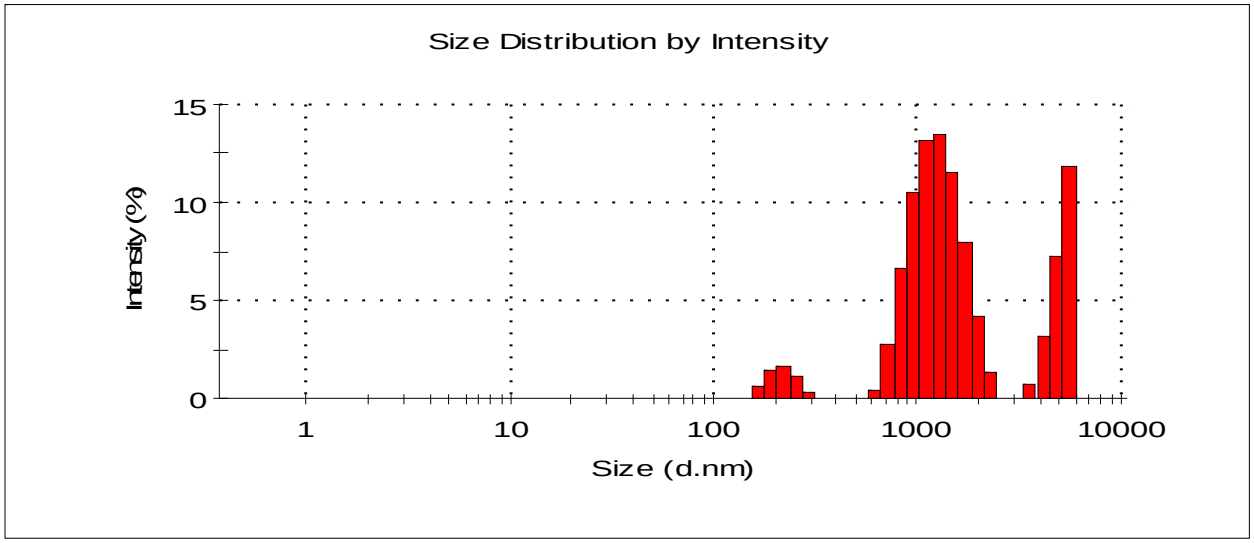


Fig. 1: Data of flax seed oil nanocapsules prepared by chitosan method





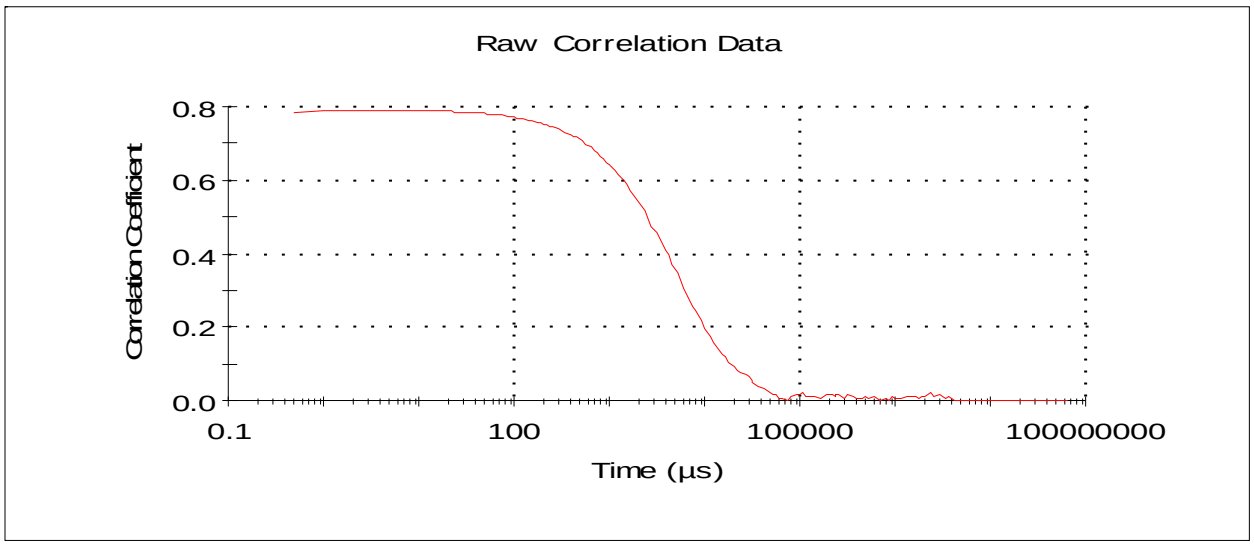
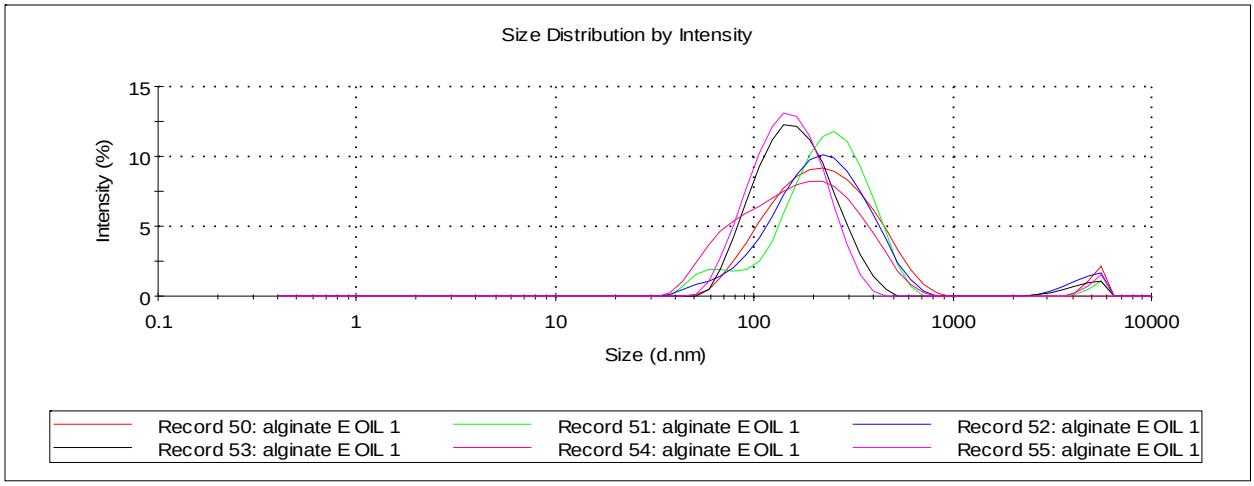
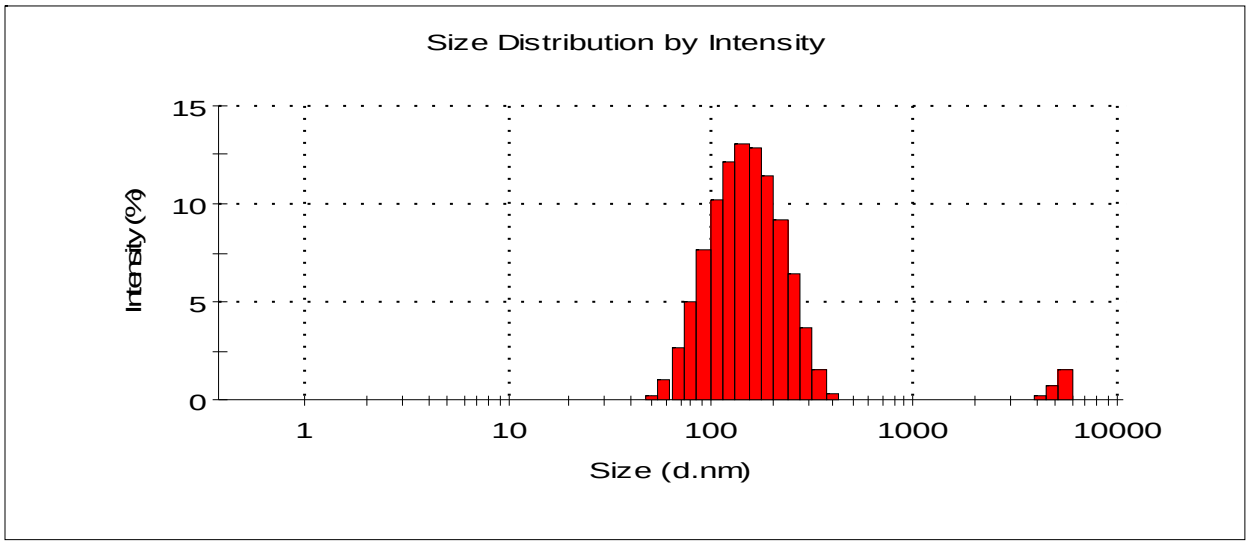


Fig. 2: Data of flax seed oil nanocapsules prepared by lecithin method





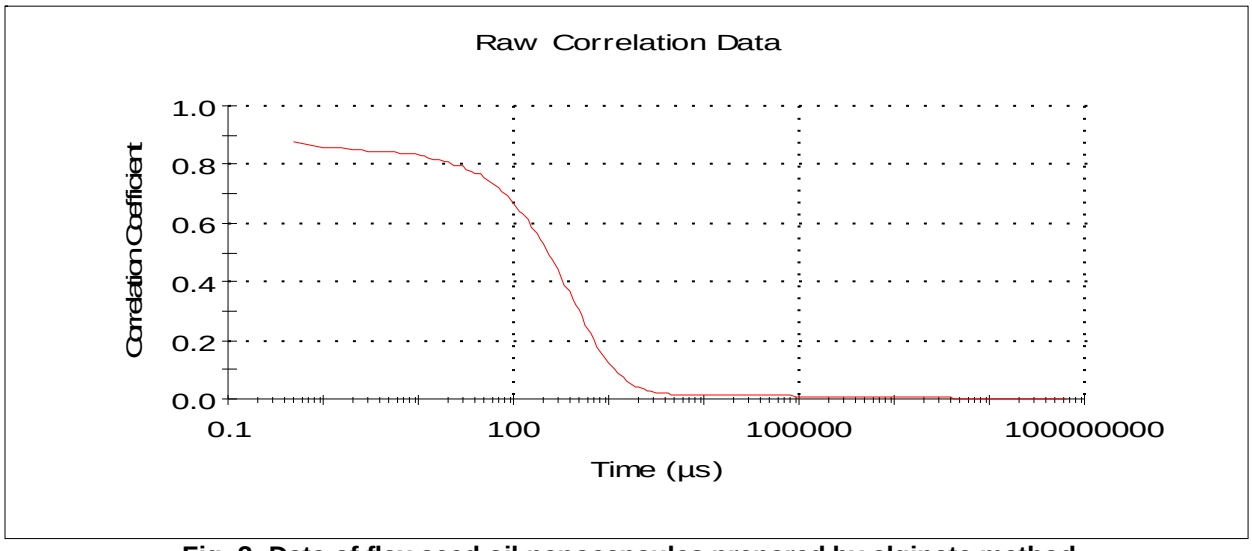
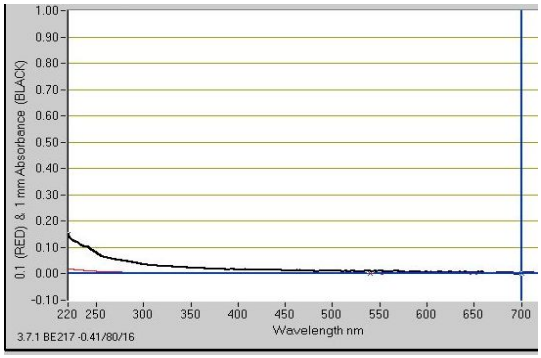


Fig. 3: Data of flax seed oil nanocapsules prepared by alginate method



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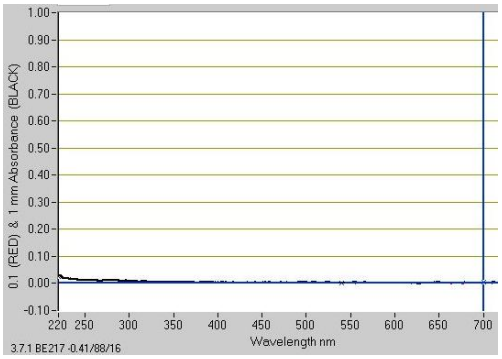
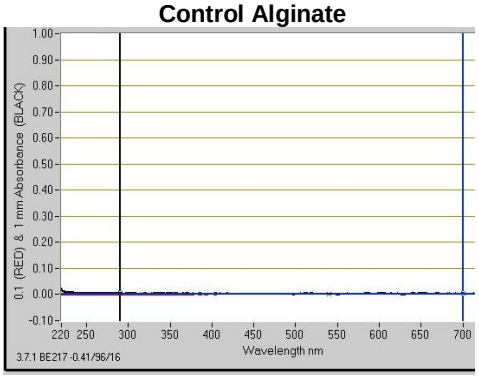
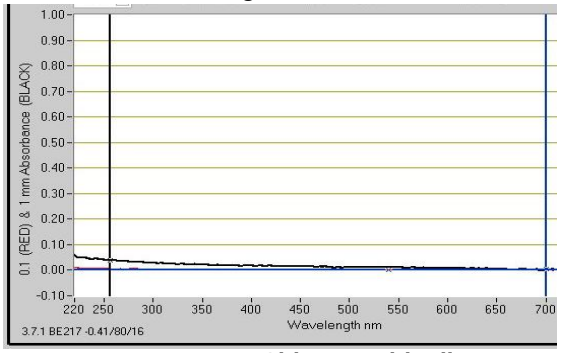


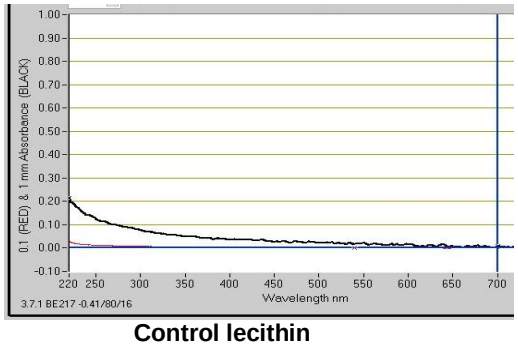
Alginate with oil



Control Citosan



Chitosan with oil



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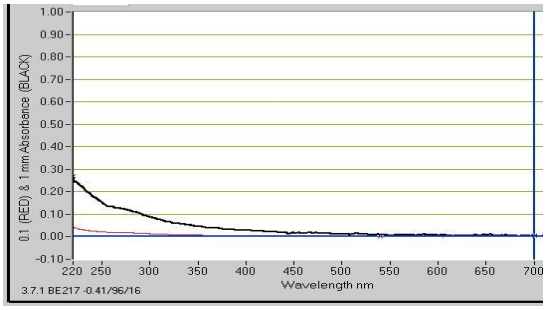

lecithin with oil

Fig. 4: UV-VIS spectra of the encapsulated nanocapsule

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