Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **7(12),** 1-10, December **(2017)** *Res. J. Chem. Sci.*

Microencapsulation and characterization of astaxanthin prepared using different agents

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Available online at: www.isca.in, www.isca.me
Received 20th October 2017, revised 16th December 2017, accepted 18th December 2017

Abstract

The present study was implemented to check the best methods for encapsulation of astaxanthin and characterized. Astaxanthin is present in microalgae, yeast, salmon, crayfish, shrimp, crustaceans etc. It has wide application as an animal feed, food colorant and dietary supplement for human to cure many diseases. Encapsulated astaxanthin was prepared using sodium alginate, chitosan, TPP and liposomes as various encapsulating agents. The encapsulated Astaxanthin prepared using different methods were analysed for Morphological changes, percentage yield, drug content, entrapment efficiency and in vitro drug release. SEM view of encapsulated astaxanthin was predicted in which all the encapsulated beads formed showed minimum size of 1.522 to 15.21 µm. FT-IR analysis showed the presence of aldehyde, ketone, amines related to both chemicals used and astaxanthin. The percentage yield of ME 1, ME 2, ME 3 and ME 4 was founded to be 87.67±0.577%, 87.00±1.000%, 90.67±0.577% and 93.67±2.08% respectively. From drug content estimation it is established that maximum drug content was achieved by ME 4 (91.33 ± 1.528%) and minimum by ME 2(74.00±1.000%). In vitro drug release was performed in both Stimulated gastric fluid and Stimulated intestinal fluid. Both showed better results. Astaxanthin encapsulated microspheres were prepared by different methods. The encapsulated astaxanthin was analyzed for size, morphology and drug release behavior was studied. Thus astaxanthin loaded microspheres can be used in novel drug delivery systems.

Keywords: Sodium alginate, Chitosan, TPP, Liposomes, SEM analysis, FT-IR, *In vitro* drug release.

Introduction

Microencapsulation is a quickly extending innovation in which extremely tiny droplets or particles of fluid or solid material are encompassed or covered with a consistent film of polymeric material[1]. The microencapsulation technique was introduced by Bungen burg de Jon and Kan[2]. Microencapsulation are associated with changing fluids to solids, which modify colloidal and surface properties, offer ecological protection and control the discharge attributes of various coated materials[3-5]. The vast majority of microencapsulated items have diameters between 1 to 1000 µm[6]. An extensive number of core materials like live cells, adhesives, flavors, agrochemicals, enzymes, pharmaceuticals etc., can be epitomized. The scanning electron microscopy is utilized to reveal the structural types of microencapsulated compound[7].

Different methods are accessible for microencapsulation of carotenoid pigments. Truly outstanding and simple techniques are ionotropic gelation technique. Ionotropic gelation relies upon the capacity of polyelectrolytes to cross connection within the sight of counter ions to frame hydrogel beads which is called as gelispheres. Gelispheres are round crosslinked hydrophilic polymeric component accomplished of expansive gelation and swelling in virtual biological liquids and the arrival of medication through it controlled by polymer unwinding. The hydrogel beads are transported by dropping a drug stacked polymeric arrangement into the fluid arrangement of polyvalent cations. The cations diffuses into the drug stacked polymeric drops, molding a three dimensional network of ionically crosslinked moiety. Biomolecules can similarly be stacked into these gelispheres under mild conditions to hold their three dimensional structure[8,9]. In Ionotropic gelation system, there has been a creating excitement for the usage of characteristic polymers as drug bearers in light of their biocompatibility and biodegradability. The characteristic or semisynthetic polymers such as Alginates, Gellan gum, Chitosan, Pectin and Carboxymethyl cellulose are usually used for the epitome of medication by this technique[10]. These systematic polyelectrolytes contain certain anions/cations on their substance structure, these anions/cations shapes meshwork structure by joining with the counter particles and incite gelation by cross connecting. Notwithstanding having a property of covering on the drug center these usual polymers like manner goes about as release rate retardant[11].

Astaxanthin is universal in nature, particularly in the marine environment[12] and is a red pigment basic to numerous marine animals, for example, salmonids, shrimp, lobsters, and crayfish, donating to the pinkish-red color of their flesh[13]. Its fundamental role is to afford the desirable reddish-orange color in these organisms since they do not have access to natural sources of

carotenoids[12]. The ketocarotenoid astaxanthin, 3, 3′-dihydroxy-β,β-carotene-4, 4′-dione, belongs to the family of xanthophyll's, the oxygenated derivatives of carotenoid. The production of xanthophyll's in plants is derived from lycopene. Similarly, like other carotenoids, astaxanthin is water insoluble, which brings about low absorption and bioavailability[14]. To conquest such disadvantages, exemplification advances that capture the dynamic specialist inside a biopolymer vehicle for oral conveyance have been explored. Joining of astaxanthin into a polymer system may shield astaxanthin from light, high temperatures, and oxygen, notwithstanding enhancing its dissolvability and bioavailability[15,16].

Calcium alginate gel is usually consumed in light of the fact that it is harmless, extremely biocompatible, mechanically strong, and acid stable[17-20]. Sodium alginate is a sodium salt of alginic destructive a characteristic polysaccharide and a direct polymer made out of 1,4-connected β-D-Mannuronic corrosive (M) and α-D-gluronic corrosive (G) developments in fluctuating degrees and courses of action. Sodium alginate is dissolvable in water and shape a reticulated structure which can be cross-connected with divalent or polyvalent cations to mount insoluble meshwork. Calcium and zinc cations have been represented for cross-connecting of corrosive gatherings of alginate. In addition, the Calcium alginate gel of the supposed egg-box structure can be easily made by ionotropic gelation within the sight of calcium particles[21]. To decrease the porosity and increase stability, alginate microparticles have been covered with chitosan by electrostatic interaction[22]. The negatively charged carboxylic acid groups of alginate bind ionically with positively charged amino groups of chitosan, a cationic polymer, to procedure a polyelectrolyte complex on the basis of their opposite charges[23].

Chitosan and its byproducts are the natural poly cationic polysaccharides containing glucosamine and N-acetyl-glucosamine. They have been broadly studied for applications in food, agriculture, biochemistry, cosmetic, pharmaceutical industries and waste water treatment since they are quickly available by means of cationic polyelectrolyte in acid solution. Moreover, they have multipurpose properties and ecologically friendly crude material, non-toxic, biocompatible and biodegradable. The types of the chitosan biopolymer could be fluid crystals, membranes and microcapsules[24,25]. In spite of several advantages, chitosan has some essential drawbacks including poor releasing capability and poor solubility in water. These disadvantages could be enhanced by reducing the particle size. The positively charged groups in chitosan can be chemically cross-linked with TPP.

TPP is a non-toxic composite that has been renowned as an acceptable food additive by the US Food and Drug Administration[26]. The speedy gelling capacity of TPP is the important property that make it a potential cross-linker for an ionic gelation of chitosan[27,28]. The chitosan–TPP matrix has been already examined as the chelating resin of copper (II) ion[29] and the carrier of tea catechins[30]. Based on the polar properties of the chitosan–TPP matrixes, carotenoids encapsulated in the chitosan–TPP nanoparticles have aggregate water solubility. These altered properties could profit numerous food industrial applications. Essentially, the procedure of ionic gelation of chitosan with TPP is possible for scaling up in a particle processing operation[31].

Liposome encapsulated astaxanthin was produced by using L-phosphatidylcholine. Liposomes are synthetic vesicles comprising of an aqueous core enclosed in one or more phospholipid layers; water soluble compounds can be encapsulated in the aqueous core, while lipid-soluble compounds can be integrated into the lipid bilayer of the liposome. Encapsulation of astaxanthin in liposomes enhances their therapeutic potential against oxidant-induced tissue injuries, since liposomes obviously encourage intracellular delivery and prolong the retention time of entrapped agents inside the cell[32].

The main objectives of the present study is to prepare microencapsulated astaxanthin using different agents and to compare the evaluation parameters such as morphological studies, percentage yield, drug content, entrapment efficiency and *in vitro* drug release behavior in stimulated gastric fluid and intestinal fluids.

Materials and methods

Preparation of encapsulated astaxanthin using different agents: Preparation of sodium alginate encapsulated astaxanthin (ME 1): Astaxanthin was purchased from Rudra Bio ventures Pvt Ltd, Bangalore. Encapsulated astaxanthin was prepared by extrusion method using sodium alginate beads. 1% of astaxanthin was dispersed in 2% of sodium alginate solution containing 50 ml of 1.5% Tween 20 and mixed for 45 minutes in magnetic stirrer. The polymer solution was then added dropwise into 10 mL of 3% calcium chloride solution using syringe of 22G needle under constant stirring at 150 rpm for 15 min at room temperature. The beads were hardened for 30 mins and the resulting beads were centrifuged to remove the liquid part and then allowed to dry at room temperature in a dust-free chamber[33,34,57].

Preparation of alginate-chitosan encapsulated astaxanthin (ME 2): To form alginate-chitosan encapsulated astaxanthin beads, 1% of astaxanthin suspension was mixed with 2% of sodium alginate solution, 1 ml of vegetable oil (Canola or olive oil) and 0.5 % of Tween 80 solution[35] with modification. Then the mixture was dropped through a syringe into 60 ml of calcium chloride solution (3%). The beads were left to harden at room temperature for 15 minutes. According to Krasaekoopt *et al.*[36], Chitosan (Low molecular weight) was dissolved in 90 mL distilled water and acidified with 0.8 ml glacial acetic acid to achieve a final chitosan concentration of 0.4% (w/v). The pH was then adjusted to between 5.7 and 6 by adding 1 m NaOH

and total volume was adjusted to 100 ml. The mixture was filtered through filter paper (Whatman No. 41) and autoclaving at 121°C for 15 min. Alginate beads prepared were transferred to 100 ml of Chitosan solution and mixed gently using magnetic stirrer at a speed of 100 rounds for 50 min. The resulting alginate coated-chitosan beads were washed twice with peptone water solution (1.0%) and stored at 4°C until further use[37].

Preparation of chitosan-TPP encapsulated astaxanthin (ME 3): Encapsulated astaxanthin was prepared by combining 2 % (w/v) of astaxanthin within the chitosan-tripolyphosphate (TPP) matrix. Initially, 40 ml of astaxanthin solution was dropped by syringe in the beaker containing 200 ml of 1.5% (w/v) chitosan solution while stirring at 750 rpm for 2 hours. At that time, 1 ml of Tween 80 was added slowly and the mixture was stirred constantly for 2 hours. Afterward, 20 ml of 2% (w/v) TPP solution was dropped into the mixture and constantly stirred for another 2 hours. At last, the mixture was homogenized for 30 minutes at 5000 rpm. The mixture gained was freeze dried at -40°C.The dried out encapsulated astaxanthin were powder with a blender at 60 Hz, 37°C for 3 minutes and stored at -20°C for further analysis[38,39].

Preparation of liposome encapsulated astaxanthin (ME 4): The method for production of liposome-encapsulated astaxanthin was done as follows: L- phosphatidylcholine (0.04 g/ml of DMSO) and cholesterol (0.01 g/ml of DMSO) were liquefied in 5 mL of mixed solvent of chloroform/methanol (2:1 v/v). To the above mixture, an appropriate amount of astaxanthin was added. Lastly, concentration of astaxanthin was made to 1.0 mM. This solution was ultrasonicated and concentrated under decreased pressure to totally drive off the organic solvents until a membranous product formed on the inner wall of the concentrator. The desiccation process was done for another 2 hours in vacuum drier. The product membrane was dissolved in 15% of ethanol/double distilled water. The solution was sonicated for 30 min with a probe Sonicator. The dispersal was filtered through a 0.2-µm membrane. The filtrate was lyophilized to obtain Liposome- encapsulated Astaxanthin[40].

Morphological studies of encapsulated astaxanthin: The size circulation of the encapsulated astaxanthin was done by a particle size analyzer. Filed Emission- Scanning Electron Microscope (FE-SEM) was used to study the morphological properties of dried encapsulated astaxanthin. Fourier transform infrared (FT-IR) was carried out to determine the functional groups that mediate binding between astaxanthin and coated micro particles[40,41,34].

Percentage Yield: The yield of microspheres was determined by comparing the whole weight of microspheres formed against the combined weight of the copolymer and drug[42].

Percentage yield = Mass of microspheres obtained / total weight of drug and polymer used × 100

Determination of drug content: 50 mg of Encapsulated astaxanthin were suspended in 15 mL 0.05 mol/L phosphate buffer solution (pH 7.4) having 0.75 g/100 mL Tween 80 with magnetic stirring for 12 hours. Then, the suspension solution was mixed with chloroform. Astaxanthin was removed by ultrasonication for 1 hour and isolated by centrifugation at 6372×g for 15 min. At that point, the lower chloroform phase having Astaxanthin was measured at 478 nm by a spectrophotometer[34]. Free astaxanthin is used as standard.

Astaxanthin content = sample absorbance/ standard absorbance × 100

Determination of entrapment efficiency: The entrapment efficiency of the encapsulated astaxanthin was calculated as follows:

% of Entrapment Efficiency=[Estimated drug content/ Theoretical drug content] ×100 [43,44].

Theoretical drug content(%) =Initial drug weight/ Microspheres weight × 100

Estimated drug content(%)=Entrapped drug weight/ Microspheres weight × 100

Amount of Entrapped Drug=Initial Drug Weight in Microspheres Preparation - Drug Weight Escaped in Medium.

***In vitro* release behavior:** The quantity of encapsulated astaxanthin released was tested in Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF); 0.05 mol/L sodium chloride solution adjusted to pH 1.5 with 1 mol/L HCl as an SGF and 0.05 mol/L sodium dihydrogen phosphate buffer solution adjusted to pH 6.8 with 1 mol/L NaOH as an SIF[45]. 20 mg of encapsulated astaxanthin were mixed to 5 mL media and incubated at 37°C for a preset time in a horizontal rotator. At prearranged time intervals, samples were centrifuged at 200×g for 10 min. The fractional release of the entrapped astaxanthin was calculated as follows:

Fractional release (g/100g) = M_t/ M_0

Where: M_0 is the amount of astaxanthin initially entrapped, and Mt is the amount of astaxanthin released from encapsulated astaxanthin into the simulated fluid[34,57].

Results and discussion

Scanning Electron Micrograph (SEM) Analysis: SEM images of encapsulated astaxanthin were showed in Figure-1 to Figure-4. Scanning Electron Microscope of all the samples showed that the morphology of all microsphere was generally spherical and uniform with dents surface which is due to dehydration followed by shrinkage of microspheres during air drying and lyophilization (freeze drying) over the period of storage.

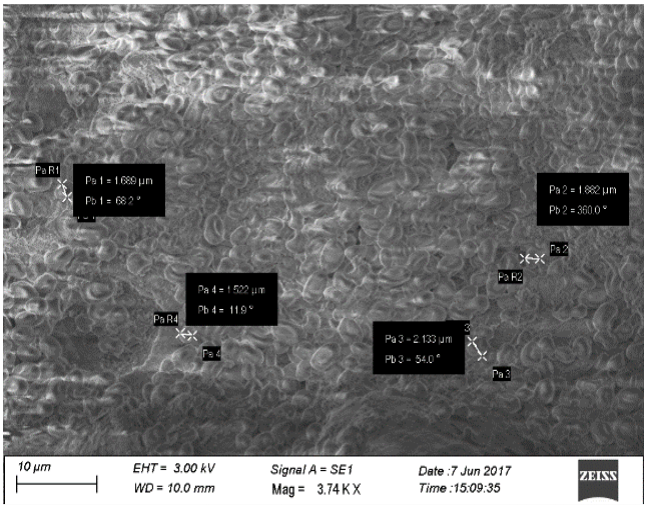

Figure-1: SEM image of ME 1.

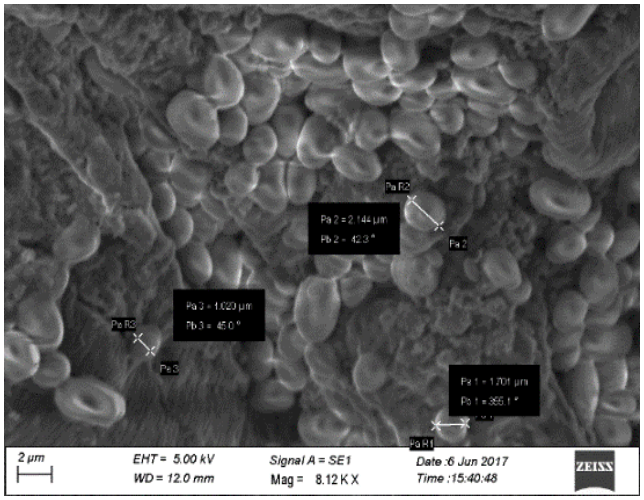

Figure-2: SEM image of ME 2.

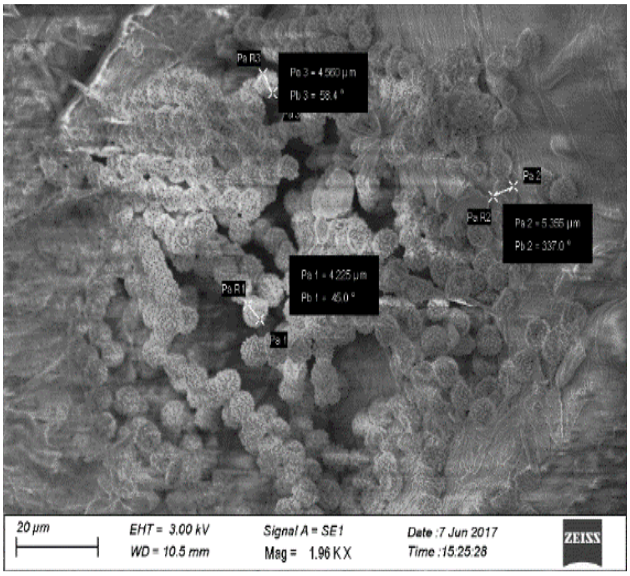

Figure-3: SEM image of ME 3.

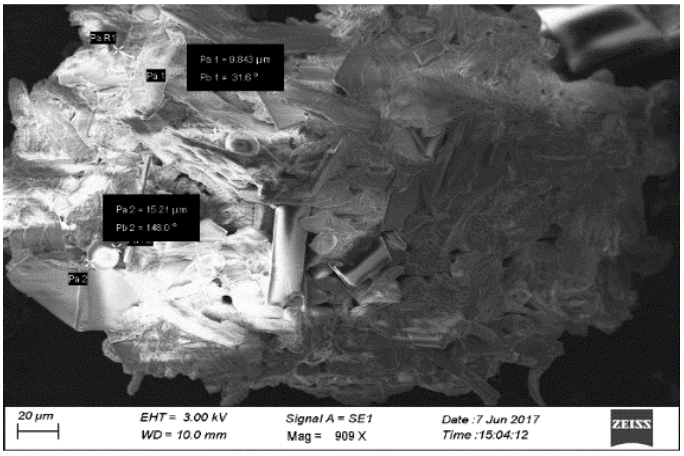

Figure-4: SEM image of ME 4.

The size of the astaxanthin – sodium alginate microspheres (ME 1) ranged from 1.522 to 2.133µm and the mean particle size was about 1.806 µm were seen which is represented in Figure-1. The microparticles in the range of 1.020µm to 2.144µm were seen in encapsulated astaxanthin using alginate and chitosan (ME 2) which is shown in the Figure-2 with the mean size of 1.701 µm. The SEM analysis of encapsulated astaxanthin using TPP-chitosan (ME 3) were given in the Figure-3 in which the size of microspheres produced higher particle size than astaxanthin encapsulated using sodium alginate and alginate-chitosan. The size in the range of 4.225 µm to 5.355 µm with the mean size of 4.560µm was observed. Chitosan coating changed morphology and shape of microspheres and modified the surface of alginate beads. The size of the microsphere increased with the addition of chitosan-TPP coating. This result suggests that the chitosan layer has been successfully deposited onto the external surfaces of the microspheres.

The liposomal structure of microencapsulated astaxanthin using L- phosphatidylcholine and cholesterol was predicted in Figure-4. From the SEM view of ME 4 the size of the microencapsulated astaxanthin was found to be 9.843 µm and 15.21 µm which is very higher than that of ME 1, ME 2 and ME 3. It shows that when compared to other agents (sodium alginate, alginate-chitosan, TPP-chitosan) the microencapsulated astaxanthin size increases when liposomal agents (L-phosphatidylcholine and cholesterol) were used.

The SEM view of present study was compared with other research papers. The study of Lee *et al.*[46] generated ovoid shape and smooth surface for astaxanthin- calcium alginate bead. The size of the astaxanthin-calcium alginate microsphere extended from 10 to 800 mm, and the average particle size of about 210.26 µm was reported[34]. Choi *et al.*[54] identified the dents surface of microparticles due to water loss during shrinkage which is correlated with our present study. In our previous study, the mean size of microencapsulated astaxanthin produced 5.355 µm and microencapsulated astaxanthin isolated from *Portunus sanguinolentus* (Three Spotted Crab), *Callinectes sapidus* (Blue Crab) and *Paralithodes brevipes* (Spiny King

Crab) produced 7.315 µm, 6.03 µm and 12.89 µm when astaxanthin is entrapped with sodium alginate[57].

Fourier Transform –Infrared Spectra (FT-IR) Analysis: The variation of the shape, shift, and intensity of the IR absorption peaks of the guest or host can provide enough information for the occurrence of the inclusion. The characteristic functional groups associated with astaxanthin are with carbonyl and hydroxyl groups located on the hexatomic rings at both ends of the molecule.

Characteristic frequencies of non-encapsulated astaxanthin are around 1000 cm-1, 1600 cm-1 and 3400 cm-1 , these correspond to aromatic C-H bending, C=O stretching and OH stretching, respectively[58,59]. These groups allow astaxanthin to perform its functional abilities, e.g. antioxidant activity and solubility in both aqueous and non-aqueous solvents.

The infra-red vibrational spectra of the encapsulated samples ME 1 to ME 3 reveals the presence of both functional groups with a sharp increase in vibrational mode at 1600 cm-1 which is a result of the C=O stretching and the mode at 3400 cm-1 is the result of the OH stretching. However, the absorption bands at all the frequencies of the sample ME 4 was greatly weakened, indicating that a majority of hexatomic ring of astaxanthin was included by liposomes.

The changes during the synthesis of encapsulated complexes can be an indication of the inclusion of the hexatomic ring of Astaxanthin in the complex formation[59]. The IR spectrum of astaxanthin showed its characteristic bands in agreement with the previous report[60].

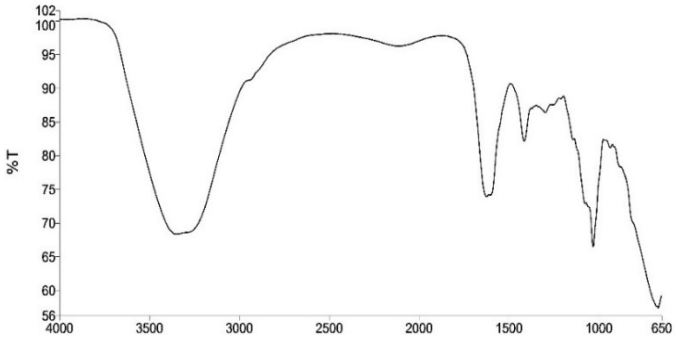

Figure-5: FT-IR spectra of ME 1.

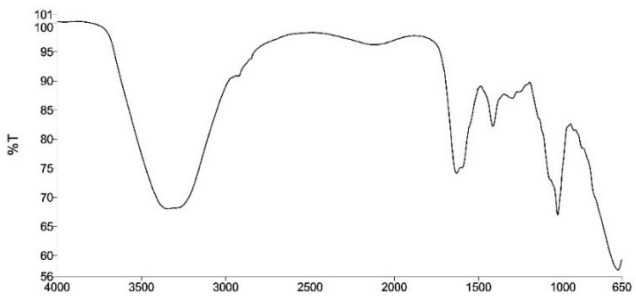

Figure-6: FT-IR spectra of ME 2.

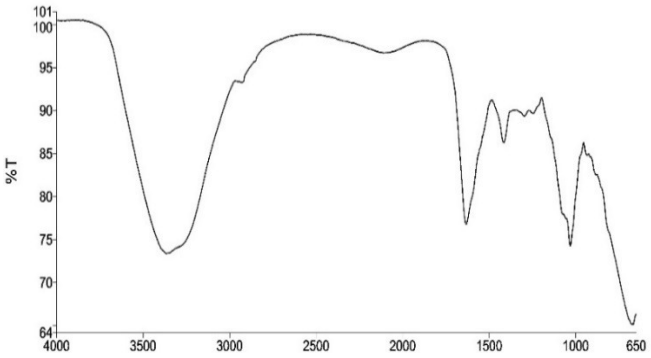

Figure-7: FT-IR spectra of ME 3.

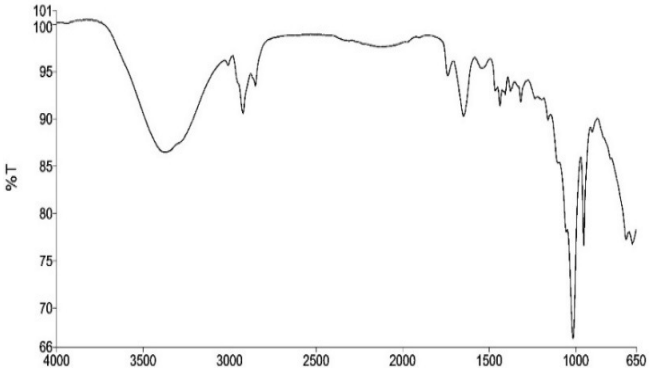

Figure-8: FT-IR spectra of ME 4.

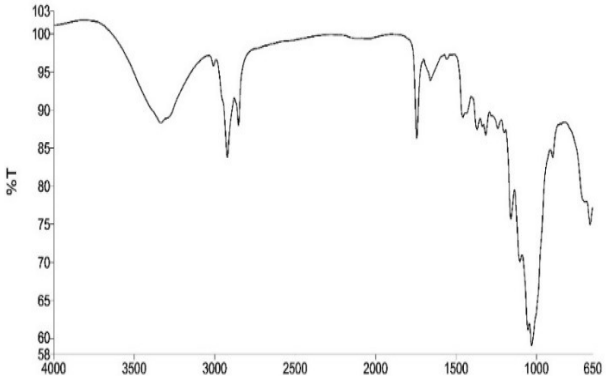

Figure-9: FT-IR spectra of Non-encapsulated astaxanthin.

Percentage of yield: In present study, Figure-10 represents the percentage of yield was calculated for four different types of microencapsulated astaxanthin samples. The percentage of yield ranges from minimum of 87% to maximum of 93.67% (Table-1). It was found that higher percentage yield was produced by the ME 4 sample (93.67%) followed by ME 3 sample (90.67 %). Similar yield was gained by ME 1 (87.67%) and ME 2 (87 %).

The present study was compared with the study of Chintagunta Pavanveena *et al.*[48] which shows three unique details of medication: polymer proportions (1:1, 1:2, 1:3) and marked as

F1, F2 and F3. The percentage yield of F1, F2 and F3 was 50%, 67 % and 76%. It was detected that as the polymer ratio in the formulation increases, the product yield also increases. The little percentage yield in some formulation may be because of microspheres loss in the washing process. Another studies showed that the item yield of the astaxanthin oleoresin tests ran from 33.40% to 70.1% which was gained[47]. They have used those biopolymers as divider material to make microcapsules of oleoresin carotenoids with high return efficiencies. When compared with similar research works, microencapsulation of astaxanthin using sodium alginate, alginate-chitosan, chitosan-TPP and liposomes showed higher percentage of yield.

Table-1: Percentage Yield of Microencapsulated Astaxanthin using different agents.

Content	Mass of Microsphere Obtained (g)	Total Weight of Drug And Polymer (g)	% Yield Mean ± SD
ME 1	2.63	3	87.67 ± 0.577
ME 2	4.39	5	87.00 ± 1.000
ME 3	4.51	5	90.67 ± 0.577
ME 4	1.84	2	93.67 ± 2.08

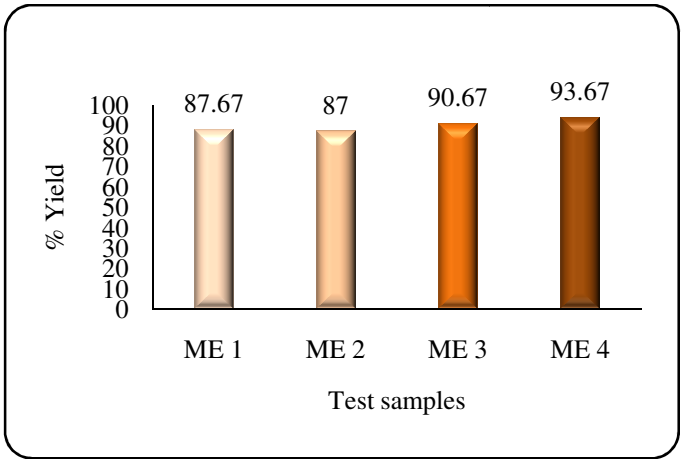

Figure-10: Percentage yield of Astaxanthin encapsulated by different methods.

Drug content: The microencapsulated beads prepared from various methods were analyzed for drug content. The maximum percentage of drug content was obtained by ME 4 (91.33%). Other types of microspheres showed maximum percentage of 77.33% (ME 1), 74% (ME 2) and 75.67% (ME 3) respectively, which was represented graphically in Figure-11. Therefore, it is found that in encapsulated astaxanthin ME 4 using liposomes, astaxanthin content was encapsulated in higher concentration than other three types of methods.

Table-2: Estimation of drug content of microencapsulated astaxanthin using different agents.

Content	OD at 478 nm	% of Drug content Mean ± SD
Ast free (Std)	1.210	-
ME 1	0.898	74.33 ± 1.155
ME 2	0.901	74.00 ± 1.000
ME 3	0.937	75.67 ± 1.154
ME 4	1.103	91.33 ± 1.528

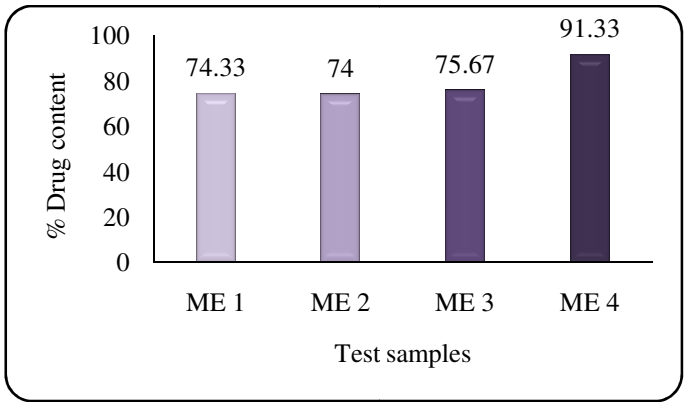

Figure-11: Percentage of Drug Content of Different Microencapsulated Astaxanthin.

Entrapment efficiency: The entrapment efficiency of encapsulated astaxanthin using different methods were determined (Table-3). The highest entrapment efficiency of 94.67% was gained by astaxanthin encapsulated using liposomal method (ME 4) followed by ME 3 (91.67%) in which astaxanthin was encapsulated using TPP- chitosan. Similarly, astaxanthin encapsulated via sodium alginate (ME 1) and alginate- chitosan (ME 2) showed 88.67% and 89.67% respectively which means that the entrapment efficiency is mainly based on the physical factors such as the properties of core and wall materials, emulsion viscosity, solid content and atomization type. The graphical representation of each samples were given in Figure-12.

The present investigation was related with other findings. The higher entrapment efficiency of 89.09% was obtained by chitosan – TPP encapsulated carotenoids was reported[39]. Higher entrapment efficiency was not provided by higher concentration of carotenoids[49,50]. 99% entrapment efficiency was gained when astaxanthin was encapsulated using chitosan matrix which was reported[51]. The maximum initial Entrapment Efficiency (91.34%) was observed with alginate and chitosan[53]. Similar results were reported in the present study. The results were also similar to those of Chan[52], who reported the Entrapment Efficiency of palm oil in calcium-alginate beads up to 90%.

Table-3: Entrapment efficiency of different microencapsulated astaxanthin by different agents.

Content	Estimated drug content (%)	Theoretical drug content (%)	% EE Mean ± SD
ME 1	34	38	88.67 ± 2.309
ME 2	36	40	89. 67 ± 0.577
ME 3	42	45	91.67 ± 1.528
ME 4	48	50	94.67 ± 1.155

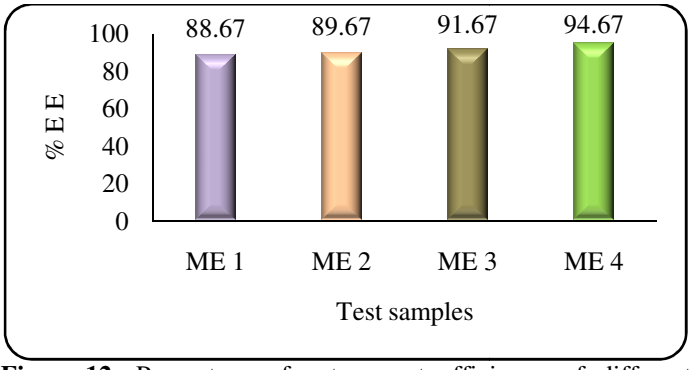

Figure-12: Percentage of entrapment efficiency of different microencapsulated astaxanthin.

In vitro drug release: Stimulated Intestinal Fluid (SIF): *In vitro* drug release was tested for all types of microencapsulated astaxanthin over 3 hours incubation period in SGF and SIF. For all types of microspheres, the release profiles of the entrapped astaxanthin were much higher in SIF than in SGF (Table-4 and Figure-13). This might be because of the distinction in dissolvability of particles under soluble and acidic arrangement. At the point when alginate break down in basic arrangement SIF, the microparticles swell, and the ensnared materials are discharged quickly[55]. Normally, alginate is changed over into insoluble alginic corrosive in SGF, averting swelling of the microparticles, and along these lines restrain the dispersion of the stacked Astaxanthin into the media[34].

Table-4: Estimation of Stimulated Intestinal Fluid by different microencapsulated astaxanthin samples.

Time (mins)	Fractional release g/100 g			
	ME 1	ME 2	ME 3	ME 4
30	15	12	14	18
60	25	20	24	26
90	38	31	37	32
120	44	41	43	46
150	60	55	58	62
180	74	71	73	79

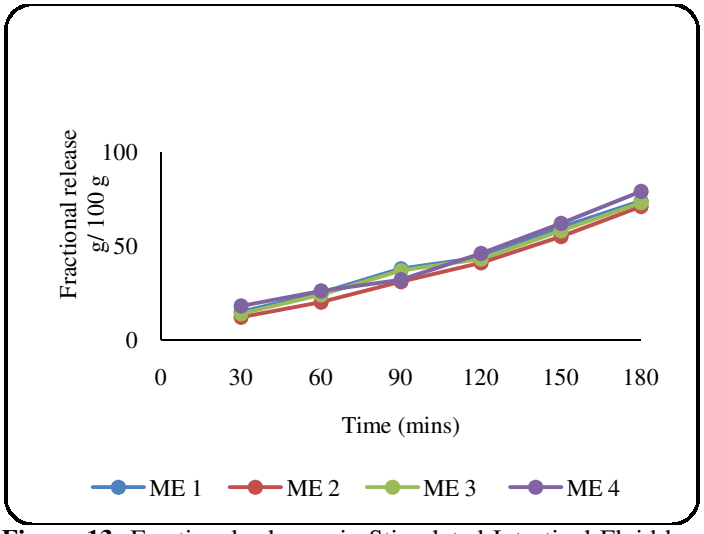

Figure-13: Fractional releases in Stimulated Intestinal Fluid by different microencapsulated astaxanthin.

Stimulated Gastric Fluid (SGF): The release content of entrapped astaxanthin in SIF for 3 hours significantly increases from 15 to 74g/100g for astaxanthin-sodium alginate encapsulated beads (ME 1). Whereas, other types of encapsulated astaxanthin such as ME 2, ME 3 and ME 4 ranges from 12 to 71g/100g, 14 to 73g/100g and 18 to 79g/100g respectively. The drug release in Stimulated Gastric Fluid (SGF) was performed for all types of microencapsulated astaxanthin which is given in Table-5 and Figure-14. The highest release content was founded in ME 1 and ME2 which ranges from 14 to 64g/100g and 12 to 61g/100g. The minimum release content was obtained for ME 3 (10 to 60g/100g) and ME 4 (8 to 59 g/100g). When the particle size increases, the surface area will also increase. Thus, when there are increases in interaction between microparticles and media, the content of drug release will also increases56.

Table-5: Estimation of Stimulated Gastric Fluid by different microencapsulated astaxanthin samples.

Time (mins)	Fractional release g/100g			
	ME 1	ME 2	ME 3	ME 4
30	14	12	10	8
60	24	22	18	16
90	35	34	29	24
120	41	40	38	33
150	58	53	49	48
180	64	61	60	59

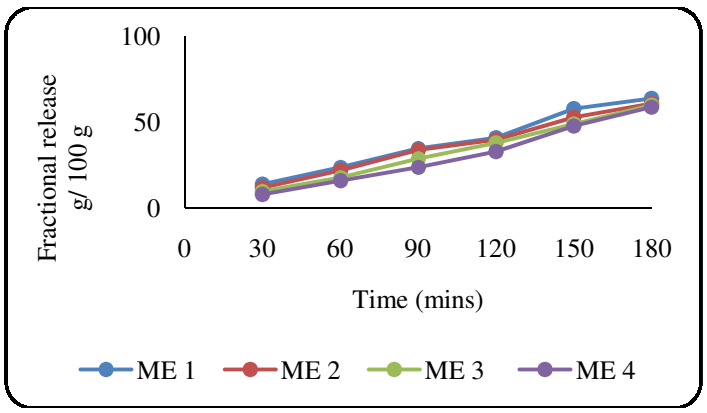

Figure-14: Fractional releases in Stimulated Gastric Fluid by different microencapsulated astaxanthin.

Conclusion

Astaxanthin encapsulated beads were successfully synthesized and characterized using different methods. The yield of encapsulated microspheres was also abundant. Encapsulation of drug into the microspheres and entrapment efficiency is appreciable in case of encapsulation as liposomes. There is sustained drug release which increases exponentially with time and particle size. Encapsulated astaxanthin could be promoted as novel drug for anticancer therapy which requires sustained drug release with better efficiency. Thus, Astaxanthin loaded microspheres can be explored for various biopharmaceutical purposes.

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