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Research article

Preparation and evaluation of soft gelatin capsules loaded with astaxanthin, niacin and garlic oil for removal of plague in atherosclerosis

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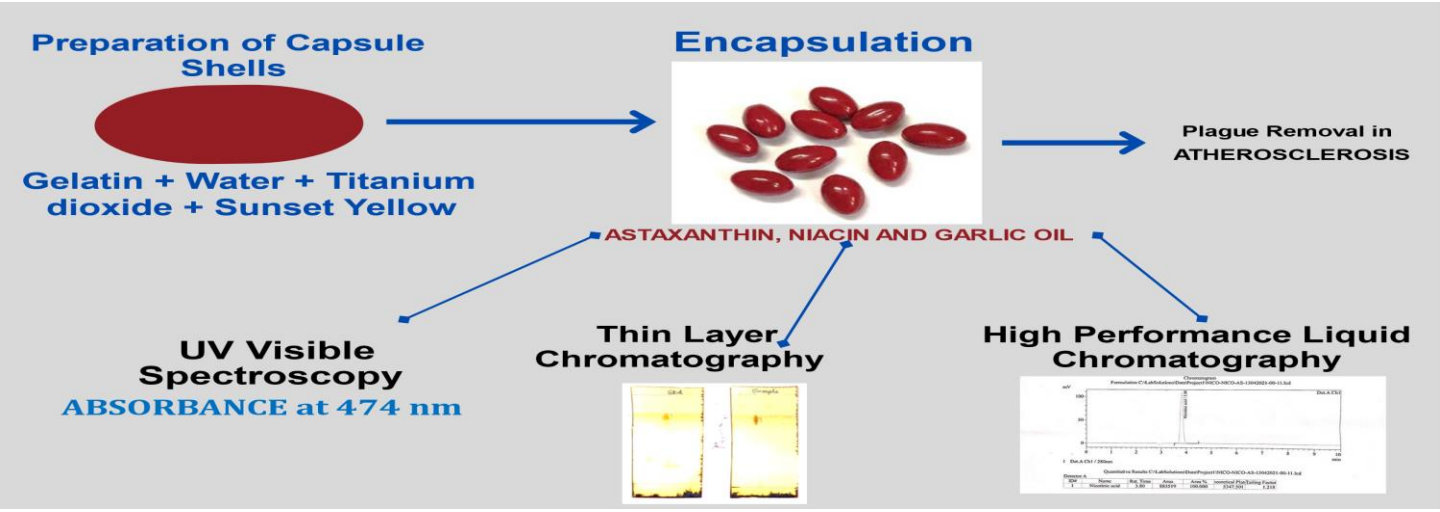
ABSTRACT

Atherosclerosis, a chronic inflammatory disease, is the leading cause of cardiovascular diseases. In atherosclerosis, the arterial walls are characterized by plaque deposits on the inside of arteries. This study focuses on preparing a fill formulation to remove plaque in atherosclerosis, then analyse the pharmaceutical ingredients used and later produce soft gelatin capsules. Soft gelatin capsules are hermetically sealed, one-piece capsule which has a suspension or semi-solid as fill. Oxidative stress and inflammation are factors responsible for the development of atherosclerosis. Hence, the active pharmaceutical ingredients (APIs) chosen for fill formulation need to possess effective anti-oxidant and anti-inflammatory properties. A novel combination of APIs: Astaxanthin, Niacin and Garlic oil were chosen for the removal of plaque in atherosclerosis. Each of these APIs is known to possess a specific function in the control of plaque development. The fill formulation prepared was analysed for its recovery using various analytical techniques like UV spectrophotometry, high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). This fill formulation was encapsulated within the gelatin shell prepared to produce soft gelatin capsules. The encapsulated soft gelatin capsules were evaluated based on various parameters which include average net weight, disintegration test, loss on drying and dissolution.



Keywords: Soft gelatin capsules, Plaque removal, Oxidative Stress, Cardiovascular Diseases, Encapsulation.

INTRODUCTION

Atherosclerosis is a chronic cardiovascular condition characterized by the deposition of plaques within the arterial walls. These plagues are also known as atheromas and are composed of fats, cholesterol, calcium and other substances that are present in the blood. Plagues can harden over the time and narrow the arteries, limiting the flow of oxygen-rich blood to various organs and tissues. The process of plague formation is known as atherogenesis, which involves the following possibilites like endothelial injury, lipid accumulation, oxidation of low density lipoproteins (LDL), inflammatory response and fibrous cap formation. Plagues may restrict the blood flow leading to the condition called ischemia, which can be recognised by chest pain and shortness of breath. Other life threatening complications include plague rupture and blockage in blood flow to other organs, the stroke as they obstruct blood flow to the brain. The reduced blood flow to the limbs can also cause pain and mobility issues and may result in gangrene in severe cases. The condition of atherosclerosis can weaken the walls of arteries, leading to the formation of aneurysms, which can rupture and cause life-threatening internal bleeding. In order to prevent severe complications such as heart attacks, strokes and peripheral artery diseases, surgical interventions can be done. Effective management through life style changes and medications can also reduce the significant risks to cardiovascular health. This research work aims to prepare a soft gel capsule containing Astaxanthin, niacin and garlic oil for the removal of plague.

Role and Potential Benefits of Chosen Ingredients

Astaxanthin

This is a potent antioxidant found in microalgae, yeast, salmon, trout, krill, shrimp and crayfish. It reduces oxidtive stress by scavenging free radicals, which can prevent the oxidation of low density lipoproteins, which is a key factor in the development of atherosclerosis [1-3]. Astaxanthin also inhibits the production of pro-inflammatory cytokines, which can help in reduction of inflammation in arterial walls. The clinical and preclinical studies have shown that Astaxanthin supplementation can reduce markers of oxidative stress and inflammation [4].

Niacin (Vitamin B3)

Niacin is a water soluble vitamin involved in metabolic processes and also helps in managing the lipid levels. Niacin effectively raises HDL cholesterol levels and lowers triglycerides. It reduces the inflammation by reducing the production of inflammatory cytokines. A study has demonstrated the niacin's effectiveness in improving lipid profiles [5, 6].

Garlic Oil

Garlic oil contains sulfur compounds like allicin and is derived from the garlic cloves. It has antioxidant properties and also reduces inflammation by inhibiting the synthesis of pro-inflammatory cytokines. It is also beneficial in preventing the blockage of arteries by reducing the blood clot formation. Studies have been reported that garlic supplementation can improve the lipid profiles and hence beneficial for atherosclerosis prevention and management [7, 8].

Other Excipients chosen for Fill Formulation

Yellow Bees wax – It acts as a stabilizer to maintain the consistency of the softgel, viscosity modifier ensuring the equal distribution within the softgel. Beeswax provides a natural moisture barrier, protecting the active ingredients from humidity and prolonging the shelf life of the sofgels [9].

Soya Lecithin Liquid – Lecithin is composed of glycolipids, triglycerides and phospholipids being obtained from egg yolk, soy or rice beans. This is used as an emulsifying agent in the preparation of soft gelatine capsules to stabilize the semulsion. It also enhances the flow of the material during the encapsulation process.

Sesame Oil – Sesame oil was chosen as a base or diluent for the soft gelatine capsule formation. Being a rich source of poly unsaturated fatty acids (PUFA), it possesses both anti-inflammatory and anti-oxidant properties, which in turn helps in reducing atherosclerosis

MATERIALS AND METHODS

Astaxanthin – 5% oil was procured from India Glycols, Odourless Garlic oil from Xiamen Boten Biological Technology Ltd, Nicotinic acid from Veer – chemie & aromatics pvt ltd. Other ingredients such as Lecithin, yellow bees wax, and sesame oil were procured from Arjun wax refinery.

The amount of active pharmaceutical ingredients namely Astaxanthin, Niacin and Garlic oil to be added in the fill formulation was determined based on the Recommended Dietary Allowance (RDA) chart and also based on the label claim of existing products in the market. The recommended amount of Niacin is 18mg per day for men and 14mg per day for women. The amount of other excipients to be added is determined by trial and error method to get a perfect oily mass with the adequate flow property for encapsulation [10, 11].

Preparation of fill formulation

Table 1: Composition of Fill Formulation

Components	RDA	Label Claim	Potency	mg/capsule*
Astaxanthin (5%)	4 mg	4 mg	5.3%	75.47 mg
Vit B3/Niacin	18/14 mg	14 mg	99.5%	14.07 mg
Garlic oil	2-5 mg	5 mg	100%	5.00 mg
Yellow Beeswax	q.s.**	Trial-and-Error Method		10.00 mg
Soya lecithin				20.00 mg
Sesame oil				50.46 mg
	TOTAL FILL WEIGHT			**175 mg**

mg per capsule is calculated by Label claim*100/Potency

** q.s. is Quantum satis which means the substances are added until the necessary purpose or the desired result is achieved.

Appropriate fill formulation was prepared by mixing the fixed quantity of API and other ingredients as mentioned in the Table

1. The suitability of the formulation was decided on the basis of flow, pH and sedimentation properties. Yellow beeswax was weighed and and the thoroughly mixed with sesame oil. Required quantity of soya lecithin was weighed and mixed with the oil mixture until it dissolved completely. Astaxanthin, Niacin and garlic oil were then weighed and mixed with the oil mixture to get a homogenous mixture. The entire mixture was transferred to a large container and mixed thoroughly with the help of a homogenizer.

Preparation of Capsule Shells

For preparing 50 soft gelatin shells, 8 Gms of glycerine and 32 Gms of water were transferred in to a gelatin melting tank, whose temperature was maintained at $70^0C \pm 5^0C$. About 20 Gms of gelatin was added slowly with a continuous stirring. The whole mixture was stirred for 45 mins at 34 RPM at $70^0C \pm 5^0C$ till the gelatin flakes melted completely. The suitable quantity of Ponceau 4R, Titanium dioxide and Sunset Yellow FCF were dissolved in purified water, filtered through the nylon cloth and added into the gelatin melting tank. Entire mixture was then mixed for 30 minutes to ensure uniform mixing of color. Vaccum was applied at a pressure of 600 to 700 mm Hg at $70^0C \pm 5^0C$ for 20 to 30 min to remove any bubbles formed. The samples were then tested for its homogeneity of gelatin mass and uniformity of color mixing. The mixture was filtered and the gelatin mass was unloaded into the preheated gelatin service tank. It was then maintained at the temperature of 55±5°C and allowed to maturate it for NLT 8.0 hrs.

Encapsulation

Encapsulation refers to the technique which is used to enclose the fill formulation in the capsule shell to produce soft gelatin capsules. In this process, a pump is used to deliver warm gelatin, which was delivered over two chilled drums that are present at both sides of the encapsulation machine. The drums were made up of stainless steel and are maintained at a temperature of 57-59°F. Once the warm gelatin flows over the hot drums, the liquid gelatin gets converted into solid gel ribbons. These ribbons later pass over small rollers which are known to feed them to the die rolls. The die rolls are used to adjust the size and shape of soft gelatin capsules to be made. A wedge delivers the fill material into the space formed between gelatin ribbons and die rolls cut it right to seal the two halves together forming the capsules.

Drying and Polishing

Soft gelatin capsules were initially dried in a hollow drum which had perforated walls known as the tumble drier and later transferred on to trays kept in a drying tunnel. The first stage of drying, known as the rotary drying process was carried out by pumping the dry air at a temperature less than 35°C. The second stage of drying was done by the tray drying process in which the capsules are spread on trays and many trays together were stacked in a drying tunnel. In which the temperature was maintained at 21-24°C with low humidity. After the drying process, the soft gelatin capsules were sorted based on their size and then polished to remove glycerin from the exterior surface of the capsule by washing with solvent. Figure 1 shows the prepared soft gelatin dark red colored capsules with 3 minim size.

Figure 1: Encapsulated Soft gelatin capsule

Evaluation of Encapsulated Soft Gelatin Capsule

Physical Characteristics

Appearance

Capsules were screened for uniformity in color and shape, with no visible defects such as cracks, bubbles or deformations.

Odor and Taste

Capsules were checked for the consistency with product specifications and free from any off-putting smells or tastes.

Weight Variation and Content uniformity

20 capsules were chosen randomly and weighed. The contents of the capsules were emptied and the empty shells were weighed. The difference in weight for all was calculated and the average weight was determined. The content uniformity was determined by comparing the individual weight of each capsule with that of average weight.

Disintegration Test

Disintegration test was carried out by placing 6 capsules in the basket rack assembly of the disintegration apparatus. Discs were placed over the capsules and suspended the assembly in the beaker consisting of water as the disintegration medium. Time taken by the capsules to disintegrate completely in the medium was noted.

Dissolution Test

Dissolution test was conducted using a simulated gastric fluid as a dissolution medium. The samples were withdrawn at different time intervals and analysed for the amount of niacin released by HPLC method.

Loss on Drying

Loss on drying was done to measure the moisture content of the shell. In this process, the capsules were cut open and the contents were removed. The shells were then washed with acetone and 1g of the shell was weighed and kept in hot air oven for 2 hrs. Later, it was kept in the desiccator for 20 – 30 mins. The weight of the shell was measured and the loss of drying was calculated.

Assay of Astaxanthin using UV Spectrophotometer

A standard solution of Astaxanthin was prepared by dissolving 25 mg of pure Astaxanthin (5%) in 5 ml DMSO. The solution was kept in water bath at 45 – 50^0C for 30 mins with intermittent shaking. After 30 mins, the solution was brought to room temperature and the volume was made up to 100 ml with acetone. About 5 ml of the resulting solution was diluted to 25 ml with acetone

to get a solution of concentration of 2.5 µg/ml. The absorbance of the resulting solution was measured at 474nm using acetone as blank.

The sample solution was prepared by 1093.75 mg (Equivalent to 25 mg of Astaxanthin 100%) in a 200 ml volumetric flask. 5 ml of DMSO was added and the solution was kept for shaking in a water bath for 30 mins at 45 – 50^{0}C. The solution was then cooled and diluted to volume with acetone. The resulting solution was then filtered through Whatman filter paper and 2ml of the filtrate was diluted to 100 ml with acetone. Absorbance was measured at 474 nm and compared with standard absorbance to determine the amount of Astaxanthin present in the capsules.

Identification of Garlic Oil using Thin Layer Chromatography

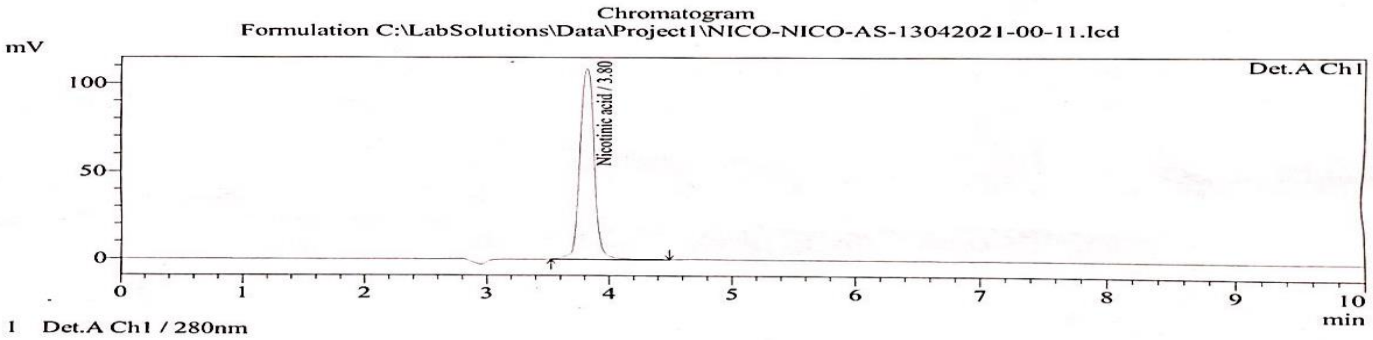
The standard solution of garlic oil was prepared by dissolving 50 mg of garlic oil in 50 ml of ethanol. For sample, 175 mg of capsule content was transferred in to a 10 ml volumetric flask and the volume was made up to the mark with ethanol. The solution was filtered through 0.45 µm nylon filter and marked as sample. The adsorbent used was 0.25mm layer of chromatographic silica gel plates. About 10 µl of standard and sample solutions were spotted on the glass plates. Development was made with the mobile phase solvent system consisting of ethyl acetate and methanol in the ratio of 7:3 v/v. After development, the detection of yellow color spots was carried out using the iodine chamber and the Rf values were calculated and compared.

Estimation of Niacin / Nicotinic acid using HPLC

About 150 mg of nicotinic acid reference standard was weighed and transferred in to 100 ml volumetric flask. The sample was dissolved in 50 ml water and the volume was made up to the mark. The 10 ml of the standard solution was then diluted to 100 ml with water to get 150 µg/ml. The mobile phase used was methanol and acetate buffer pH 3.0 in the ratio 80:20 v/v. Buffer was prepared by dissolving 1.4 g of Hexane sulphonic acid in 20 ml of glacial acetic acid, the pH was adjusted to 3.0 using glacial acetic acid and the volume was made up to 2000 ml with water.

For sample preparation, the contents of 5 samples were transferred in to a 500 ml volumetric flask and sonicated for 20 mins with 200ml of water. The solution was finally made up to 500 ml with water and filtered through 0.45 µ nylon filter. About 20 µl of each sample was injected in a C18 HPLC column (4.6 x 250 mm x 5 µm) with a flow rate of 1ml/min. The column temperature was maintained at 30^{0}C and the chromatogram was recorded using the PDA detector. The run time was 30 minutes for each sample and the drug was found to get eluted at 3.81 mins (Figure 2). The peak area of standard and sample were compared to determine the content of niacin present.

Figure 2: Chromatogram of Niacin showing Retention time at 3.81 mins



Quantitative Results C:\LabSolutions\Data\Project1\NICO-NICO-AS-13042021-00-11.lcd

Detector A

ID#	Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
1	Nicotinic acid	3.80	883519	100.000	5347.501	1.218

RESULTS AND DISCUSSION

The fill formulation prepared had desirable basic properties when tested. The flow of the fill prepared was a perfect oily mass with desired flow consistency for encapsulation. The pH was observed to be 5.93. The fill formulation once prepared must not sediment, i.e., the sesame oil and contents added must not separate into two different layers. The formulation had no sedimentation when observed after about 6 hours.

Once the basic properties are tested, the fill formulation was analysed for each active pharmaceutical ingredient. Astaxanthin assay by UV spectrophotometer yielded 96.8% release which is well within the expected release range obtained by the standard procedure, i.e., 94.5 – 115.5%. The necessary system suitability values also gave desired results. The percentage relative standard deviation values obtained from the six estimations was 1.99% (not more than 2%). The percentage recovery was between (97.7% to 100.1 %.) Garlic oil was analysed by TLC method and the spots of standard and test were found to be identical.

Nicotinic acid or Niacin was analysed by the HPLC method. The assay values were calculated to be 106.46% for five different samples. These release percentage values are within the desired range obtained by the standard procedure, i.e., 99.0 – 121.0%. The system suitability calculations yielded expected results. The %RSD of areas obtained from the five standard preparations of nicotinic acid was not more than 2.0, i.e., 0.205%. The percentage system drift between

standard and bracketing standard was calculated to be 100.2% and the percentage recovery between standard and check standard preparations was 99.4%. The tailing factor value of nicotinic acid was 1.41 (not more than 2.0). The theoretical plate values of nicotinic acid were observed to be more than 4000 (Table 2).

The capsules obtained after encapsulation, drying and polishing can be described as red colored, oval-shaped capsule with 3 minim size containing dark red colored oily liquid. Encapsulated capsules were evaluated for various parameters which include average net weight, dissolution test, disintegration test and loss on drying. The average net weight of the soft gelatin capsule was 173.7 mg. The dissolution test of niacin yielded a release percentage of 106.13%. The time required for disintegration was 4 minutes 12 seconds. The percentage loss on drying was 10.4%. Assay of Astaxanthin, Garlic oil and Niacin were also carried out by UV, TLC and HPLC methods respectively.

Table 2: Summary of Results

Tests	**Specification**		**Observation**	
Description	Red colored oval shaped opaque, soft gelatin capsules containing dark red colored oily liquid.		Red colored oval shaped opaque, soft gelatin capsules containing dark red colored oily liquid.	
a) Identification of Astaxanthin by UV spectrophotometer b) Identification of Garlic oil by TLC c) Identification of Niacin by HPLC	The absorbance of sample should correspond to the absorbance of standard measured at the specific wavelength of 474 nm. The principal spot obtained in the standard solution should match with the sample solution. The Rt of sample should match the standard with optimized HPLC conditions such as C18 column Column – C18, Mobile Phase – Methanol and Buffer (80:20 v/v) and detection wavelength at 280 nm.		The absorbance of sample was found to match with the absorbance of standard measured at 474 nm. The principal spot obtained by the standard solution was found to match with that of sample solution The retention time was found to be 3.81 mins with the tailing factor of 1.41 and the theoretical plates were 5870.	
Average Net Weight (in mg)	175 ± 7.5% (161.9 mg – 188.1 mg)		173.7 mg	
Disintegration time	NMT 20 minutes		04 minutes 12 seconds	
Loss on drying of capsule shells	8.0% to 12.0% w/w (at 105ºC for 2 hours)		10.4% w/w (at 105ºC for 2 hours)	
Assay of fill formulation	**Label Claim (mg)**	**Release (%)**	**Observation**	
Astaxanthin	4	94.5 – 115.5	3.8715 mg (96.8%)	
Niacin	14	99.0 – 121.0	14.9046 mg	106.46%
Dissolution for Niacin	NLT 70%		106.13%	

CONCLUSION

Soft gelatin capsules are a unique dosage form that is known to have notable advantages over the existing dosage forms such as tablets and hard gelatin capsules. The developed formulation strongly promises to improve the bioavailability of poorly soluble drugs. The fill formulation prepared and soft gelatin capsules produced in this study showed desirable results when evaluated. This work requires further research on the stability and in vivo parameters of the produced soft gelatin capsules.

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