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Multiple spectroscopic studies of the structural conformational changes of human serum albumin—Essential oil based nanoemulsions conjugates

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ABSTRACT

Nanoemulsions have numerous biomedical applications. For the first time, we have investigated the effects of orange and eucalyptus essential oil based nanoemulsions towards the structural aspect of human serum albumin (HSA). Quenching effect of nanoemulsion against the intrinsic fluorescence potential of tryptophan and tyrosine residues were evidenced from the fluorescence spectroscopic analysis. Static quenching mechanism was found to lead the binding of HSA–nanoemulsion systems. Synchronous and three dimensional spectroscopic studies have revealed the possible changes to the aromatic environment of HSA by the nanoemulsion. UV–Visible spectroscopic studies have confirmed the existence of the ground state complex formation between HSA and the surface of nanoemulsions by exhibiting the hyper-chromic effect in a concentration dependant manner. FTIR spectroscopy revealed the slight alteration in the Amide I, II and III bands of HSA after interaction. FT-Raman spectroscopy showed the decrease in the Raman intensity of the aromatic amino acid residues and shift in the amide bands of HSA upon binding with the nanoemulsion. Dichoric band obtained from the far UV-CD spectra at 208 and 222 nm of HSA showed the corresponding decrease in the alpha-helical contents upon interaction with nanoemulsions. Near UV-CD spectra also showed the prominent changes in the aromatic positions of the amino acid residues of HSA on binding with nanoemulsions. The above study has extrapolated the side effect analysis of the nanoemulsions in pharmaceutical applications in vitro in reference to their interaction with serum proteins.

1. Introduction

Drug delivery defines the process flow, in which the proper distribution of the administered active compound in the aqueous environment and their availability onto the site of action through the whole body is carried out by the drug-delivery vehicles. Numerous colloidal systems have been developed as the drug delivery vehicles such as the oil-in-water emulsions, nanoemulsions and micro emulsions to nanoparticles systems which include liposomes, solid lipid nanoparticles, etc. [1]. Among them, Oil/water nanoemulsions have great potential in terms of the delivery of poorly water-soluble drugs [2,3] due to the major advantages such as the ease of fabrication, increased drug loading, enhanced drug solubility and bioavailability, reduced patient variability, controlled drug release, and protection from enzymatic degradation [4,5]. In general, nanoemulsions are defined as the heterogeneous systems comprising of two immiscible liquids in which one liquid is dispersed over another in the form of droplets whose diameters ranges from ten to a few hundred nanometers [4]. The major advantages of nanoemulsion over conventional emulsion are the possibility to dilute them with water without changing the droplet size distribution and utilization of reduced amount of surfactant [6].

Emulsion-based delivery systems involve the encapsulation, protection and delivery of the poorly water soluble nutraceuticals and drugs through the oral route for both functional food and pharmaceutical applications [7]. Emulsions of varying compositions, structures, and functional performances were formulated from the commercially available ingredients (such as lipids, emulsifiers, and water). Simple unit operations such as mixing and homogenization are the basic working phenomena behind it [8]. However, there is a lack of utilization of the plant based oils in the nanoemulsion formulation and exploration of their potential biomedical application.

Essential oils derived from plants are considered to be safer compared to synthetic products for application in the food and pharmaceutical industries, because of the bioactive components present in them. Essential oils have shown to possess insecticidal, antifungal, and antibacterial properties [9]. For example, *Eucalyptus*

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globulus essential oil contains 45.4% 1,8-cineole (eucalyptol) with strong antimicrobial activity against human and food borne pathogens [10]. Their intra-dermal administration was reported to increase the capillary permeability and favours wound healing [11]. Similarly, Orange peel essential oil is also significant due to their application in food, cosmetics, and pharmaceutical industries. They contain limonene (94%), myrcene (2%), linalool (0.5%), octanal (0.4%), decanal (0.4%), neral (0.1%), geraniol (0.1%) and several other compounds. Their constituents such as the aldehydes (octanal, decanal), alcohols (linalool), esters and terpenoids (limonene) are low molecular weight compounds [12], making the essential oil more volatile. Hence, in our study, the nanoemulsions were formulated with above mentioned oils containing significant bioactive compounds and investigated for their interaction with biomolecules. This would reveal information on the side effects of utilizing the above mentioned nanoemulsion systems as the drug delivery vehicles inside the body.

Because, the best transmission pathway for a drug or the drug delivery vehicles to its target tissue is through the blood-circulatory system that infiltrates most body tissues. It is in this medium that biomolecules such as the proteins would be exposed to exogenous compounds such as drugs, and also to the nanoemulsions that are being used as drug carriers [13]. Polypeptides of a protein deserve distinct structural conformation. Hence, its adsorption at the nano-bio interface depends not only on the characteristics of the nanoemulsion, but also on the interacting proteins and the surrounding medium [14–18]. The affinity of the protein towards the nanoemulsion surface favors the complete capture of their binding sites. However, the pattern at which the protein molecules adsorb over the larger surface of the nanoemulsion decides the effect on the structure and biological reactivity at the cellular level [19].

Serum albumin such as the human serum albumin (HSA) is the most abundant extracellular protein predominant in the blood plasma. It contains about 585 amino acids [20,21] and remains to be the most important blood protein. They perform several biological functions such as the regulation of pH, osmotic pressure and in the transportation of metabolic and exogenous compounds [22]. They possess a half-life period of 19 days [23]. They show higher affinity to various compounds such as fatty acid, cations and drugs [24] through two main binding sites [25]. They serve as a model protein for studies involving protein folding and ligand-binding approaches [26]. Hence, the presence of changes in the secondary and tertiary structure of biomolecules, due to their binding with nanoemulsion, would answer the questions raised on the biosafety issues of nanoemulsion based systems.

To our knowledge, there are no literature reports available on the interaction of plant based essential oil nanoemulsions with human serum albumin. Hence, multiple spectroscopic techniques such as the UV–Visible and fluorescence spectrophotometry that evaluates the nanoemulsion protein corona in coupling with possibilities of disturbances in the aromatic residues environment, FTIR and FT-Raman spectroscopy for evaluating the changes in the secondary structural conformations along with the amino acid residues shift was done. Circular Dichorism studies were also taken in account of the alteration in the alpha-helical contents and aromatic amino acid residues position with respect to the nanoemulsion binding.

2. Materials and methods

2.1. Materials

Human serum albumin, Tween 80 and Bioxtra were purchased from Sigma-Aldrich, USA. Eucalyptus Oil (*E. globulus*) and Orange Oil (*Citrus sinensis*) is obtained from Hi media, India. All other chemicals were of analytical grade.

2.2. Nanoemulsion formulation

Oil in water nanoemulsion (1:1%v/v) was formulated using essential oil (6% v/v) and non ionic surfactant, Tween 80 as dispersed phase and water as the continuous phase. Dispersed phase was added drop wise to the continuous phase at room temperature with the use of magnetic stirrer at 250 revolutions per minute for 30 min. Further coarse emulsion was subjected to high energy emulsification using 20 kHz ultrasonic processors with maximum power output of 750 W for 10 min (Sonics, USA). Sonication process was carried out in a beaker containing ice cubes to minimize the heat effect, in which each cycle consisted of 30 s pulses off and 30 s pulses on with fixed amplitude of 40%.

2.3. Characterization of formulated nanoemulsion

2.3.1. Thermodynamic stability study

Formulated nanoemulsion was analyzed for the thermodynamic stability study to ensure physical stability of the emulsion system. Different stress tests include 1. Centrifugation: Formulated emulsion was subjected to centrifuge at 3000 rpm for 30 min and observed for phase separation if any. Stable samples were further analyzed to heating–cooling and freeze-thaw cycle. 2. Heating–Cooling cycle: This was carried out at 45 and 4 °C for 48 h and the cycle repeated for thrice.3. Freeze-Thaw cycle was at −20 and 25 °C for 48 h and repeated thrice.

2.3.2. Measurement of droplet size, morphology and polydispersity index

Dynamic Light Scattering analysis (Brookhaven Instruments Corporation, USA) measures the droplet size distribution and polydispersity index (PI) of formulated essential oil nanoemulsion. The samples were diluted in the ratio of 1:30 with double distilled water to reduce the multiple scattering effects prior to analysis. Transmission Electron Microscopy of both the nanoemulsions was taken to identify the morphology of emulsion droplets.

2.3.3. Turbidity measurement

The turbidity of essential oil nanoemulsion was assessed using UV–Visible spectrophotometer (Systronics, India) at 600 nm. The samples were diluted prior to experiment in the ratio of 1:10 with double distilled water to reduce the turbidity of the samples.

2.4. Interaction studies with human serum albumin

0.01% of HSA in phosphate buffer of pH 7.2 of 0.1 M was allowed to react with different volume of the diluted nanoemulsions (1:200 ratios) for 10 min at 25 ± 3 °C in the orbital shaker working at 120 rpm. The interacted samples were subjected to UV–Visible and fluorescence spectral measurements. For FTIR and FT-Raman measurements, the interacted samples were allowed to lyophilize to obtain a fine powder and kept in a dessicator until the spectrum is carried out. For CD spectra measurements, the interacted samples were analyzed using buffer as the blank and the percentage contents were evaluated.

2.4.1. UV–Visible spectroscopy

The absorbance of HSA was measured by using Double Beam spectrometer at a resolution of 0.1 nm (Systronics, India) in the spectral range from 250 to 350 nm. Samples were allowed to interact with diluted nanoemulsion concentrations such as 2–10 μl in the orbital shaker at 120 rpm at 25 ± 3 °C for 10 min.