A multilayered supramolecular self-assembled structure from soybean oil by
in situ polymerization and its applications

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The present study emphasizes in situ transformation of soybean oil to self-assembled supramolecular multilayered biopolymer material. The said polymer material was characterized and the entrapment efficacy of both hydrophilic and hydrophobic moieties was studied. In brief, soybean oil at varying concentration was mixed with mineral medium and incubated under agitation (200 rpm) at 37 °C for 240 h. Physical observations were made till 240 h and the transformed biopolymer was separated and subjected to physical, chemical and functional characterization. The maximum size of the polymer material was measured as 2 cm in diameter and the cross sectional view displayed the multilayered onion rings like structures. SEM analysis illustrated the presence of multilayered honeycomb channeled structures. Thermal analysis demonstrated the thermal stability (200°C) and high heat enthalpy (1999 J/g). Further, this multilayered assembly was able to entrap both hydrophilic and hydrophobic components simultaneously, suggesting the potential industrial application of this material.

Keywords: Biopolymer, Multilayered structures, Polymerization, Self-assembly, Soybean oil, Supramolecular structures

Polymers and polymer products find extensive applications in various fields. Compared to synthetic sources, renewable resources are highly preferred for polymer synthesis for environmental sustainability. In recent years, there has been a substantial growth of biopolymers especially from plant oils. Soybean oil, a triglyceride made up of a complex multi-component mixture of functionalized oleic, linoleic and linolenic acid methyl esters as well as saturated fatty acids is widely studied for varied applications. Presence of enough unsaturation in soybean oil makes it highly compatible for engineering and other related applications.

Recently, studies on self-organization of molecules or utilization of self-assembled molecules are in lime light, since, these molecules are able to encapsulate the compounds and release them through their layer opening system. For example in the field of drug delivery, identification of suitable matrix is highly challenging. However, exploitation of self-assembled molecules having supramolecular structures of bio-macromolecules (carbohydrates, proteins and lipids) found suitable, because of high biocompatibility. Self-assembly of molecules display varied structures and shapes depending on the interacting groups and their specificity. According to Shanks most of the organic molecules exhibit shapes with low inter-conversion energy, called conformations and the control on these conformations enables the self-assembly structures to supra-molecular structures. When the components of polymer compositions contain harmonizing chemical and symmetrical form they can self-assemble into supramolecular structures with specific properties. Studies on construction of self-assembled supramolecular structures demand intelligent materials, which are responsive to the external stimuli. Macromolecules are well preferred for the construction compared to molecules of synthetic origin.

The present study exemplify in situ transformation of fatty acids of soybean oil to biopolymer and in detail emphasizes the physical and chemical characteristics of the polymerized product, the possible hypotheses involved during the transformation of biopolymer and finally detailed the simultaneous entrapment of hydrophilic and hydrophobic moieties.

Media composition and experimental setup—The medium used in the present study contains (g/L distilled water) soybean oil (at different

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percentages (w/v); 1.0 NH$_4$NO$_3$; 2.55 NaH$_2$PO$_4$; 0.5 MgSO$_4$ \(7\)H$_2$O; 0.1 CaCl$_2$ H$_2$O; 0.02 MnSO$_4$ H$_2$O; 1.0 Peptone and 0.5 Glucose; pH 7.4 ± 0.2. All the ingredients were mixed except soybean oil and autoclaved at 121 °C for 15 min. Soybean oil at various concentrations added to the sterile medium individually and incubated at 37 °C at 200 rpm for 168–240 h. Physical observations on transformation of soybean oil to self-assembled multilayered supramolecular biopolymer material were observed during the experimental period. On the day of completion, biopolymer was separated and subjected to characterization.

Characterization of biopolymer—Nature, colour, size, shape and solubility, were the parameters analyzed for the self-assembled biopolymer material. Size of the biopolymer was measured using metric scale; solubility was assessed using both polar and non-polar solvents. FT-IR analysis was made by Spectrum one Perkin-Elmer Co., USA model. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis made using TGA Q50 V20.6 Build 31 (USA) and DSC Q200 V23.10 Build 79 (USA) respectively. Structural morphology of self-assembled biopolymer was examined using scanning electron microscope (Hitachi S 4000, Japan at 10 keV voltage). In order to assess the reproducibility of formation of macro-sized self-assembled multilayered biopolymer material by the said method, the experiments were replicated at 30 days interval in the similar manner.

Entrapment studies—Water and oil entrapment by the self assembled multilayered material was assessed by exposing biopolymer to the mixture containing equal volume of water and oil under agitation (200 rpm) at 37 °C for 24 h. After scheduled time, biopolymer was removed and squeezed to remove the trapped molecules and the released molecules were kept without any disturbances for separation of oil and water phase. The volume of each phase was measured.

Results and Discussion
Results revealed, transformation of soybean oil to macro-sized self-assembled multilayered biopolymer material in aqueous medium containing mineral salts, glucose and peptone. Experiments were carried out at 37 °C under agitation (200 rpm) for 240 h. Though different concentrations [(0, 2.5, 5.0 and 10% ; w/v)] of soybean oil was examined, the formation of ball like self-assembled multilayered biopolymer material was observed only with 10% concentration and the flasks with lower concentrations of oil displayed only the micellar structures (Fig.1 a-d). In the first stage, transformation of soybean oil to micelle (Fig. 1b), and the initial vesicle formation could be due to agitation and the mineral salts present in the medium.\(^5\,^6\). Gelation was observed after 6 days of incubation (Fig. 1c). When the incubation period increased beyond 9 days, a single ball like self-assembled multilayered biopolymer material was observed (Fig. 1d). Ladet \textit{et al}.\(^\text{4}\) reported onion ring like multi-membrane structured biopolymer material using chitosan and sodium alginate. Similarly existence of onion like structures in block copolymer nanoparticles was observed by Higuchi \textit{et al}.\(^\text{7}\).
Microscopical examinations (Fig. 2a-d) of samples obtained on different days (day 2 and 4) of incubation demonstrate, micelle formation, aggregation of micelles followed by stabilization, thickening of boundary wall with structured network. Despite the availability of instrumental procedures for the determination of micelle formation and aggregations, in the present study, only the microscopical observations were displayed because of the interference due to the oil present in the medium during spectrophotometric determinations.

The size of the biopolymer was measured as 2±0.2 cm and Fig. 3 gives the cross sectional view of the biopolymer. The inner structure of the cross-sections displayed a multiple layered onion ring like with no hallow space and with tissue like appearance and feel in the centre core. The thickness of the outer layer of the biopolymer was measured as 1 mm.

The formation of vesicle and thickening of oil (gellation) could be due to the aggregation of micelles. According to Vauthey et al., presence of mineral salts, glucose, peptone, shaking conditions and the prevailing temperature (37 °C) may initiate the primary aggregations. In addition, presence of higher percentage of unsaturated fatty acids, lecithin and phosphate moiety in soybean oil induces gellation and stabilization of micelles. Further, according to Lee, micelle formation proceeds whenever there was a balance between the attractive and repulsive forces in addition to the molecular dynamic property and an increase in hydrophobicity (because of higher concentration of soybean oil) intensify the primary aggregations.

Followed by primary aggregations, sequential secondary aggregations proceeds and these secondary aggregations may be initiated by the hydrophobic interactions, which result with the layering and continuation of self-assembly process. According to Ai et al. and Liu et al., the layered assembly has molecular scale thickness and a few tens of layers can be easily achieved and the total layered area can be extended above the micrometer scale. Similarly, in the present study it was observed that continuous layering results with a self-assembled biopolymer of 2 cm in diameter. Since, the directional and differential orientation of self-assembly decides the final size and structure of the secondary aggregates, layering continues with directional orientation resulting with spherical size. Additional hydrogen bonding and the external agitation force may be responsible for the directionality, and the weak intermolecular forces (Van der Waals attractions) play the major role in the force balance, which results with multiple layering of bilayers in the secondary aggregations. Other than the above said driving forces, pH of the medium (6.0 ± 0.5) and presence of divalent cations and temperature may also instigate the secondary aggregations and the co-operation between the primary and the secondary aggregations.

Fig. 2—Microscopical observations on micellar aggregations and layering of micelle, gellation and network structure. [a: formation of bilayer micelles, b: fusion of multilayer vesicle through self assembly, c: aggregation of multilayered structured structure, d: structured honeycomb network structure]

Fig. 3—Cross sectional view of biopolymer exhibit onion like multilayered network structure.
According to Israelachvili\textsuperscript{19}, higher valence cations act as an effective promoter for self-assembly. The prevailing environmental conditions (mineral salts, glucose, peptone, temperature, agitation) further take the process to the next level (tertiary aggregations). These tertiary structures finally transform to supramolecular structures with the available weak intermolecular forces. When the directional bonding between the molecular buildings units were perfectly coordinated with the geometrical matching, a self-assembled aggregate with precise defined structures can be formed\textsuperscript{16}. According to Lee\textsuperscript{16} an additional weak intermolecular force induces an even larger size of self-assembled aggregates and this kind of observations matches with the self-assembly of collagen in the tissues. In addition, the layered structures observed in the cross-sectional view of multilayered biopolymer formed suggest the occurrence of single plane symmetry packing.

Scheme 1 gives the hypothesis behind the formation of self-assembled biopolymer and suggests that the initial micelle formation acts as a precursor for the primary aggregations (bilayer formation), followed by secondary aggregations which results with the formation of a multilayered structure with the space in between them able to entrap either hydrophilic or lipophilic compound depending on the nature of the layer. Finally, a spherical macroscopic multilayered biopolymer observed after tertiary aggregations. Further, the co-ordination between the molecular building units and the geometrical matching decides the shape of the self-assembly structures.

With reference to the physical characteristics feature of the biopolymer, the colour was pale creamy-brown with thick outer layer and a soft tissue like multilayered network inside and sparingly soluble to both polar and non-polar solvents. FT-IR spectral analysis of the biopolymer showed (Fig. 4a) peaks at 3411, 2924, 2855, 1739, 1709, 1637, 1546, 1458, 1075, 719 cm\textsuperscript{-1} corresponding to -NH stretching, antisymmetric, symmetric stretching of -CH\textsubscript{3}, C=O stretching, C=O stretching, primary amide, secondary amide, -CH\textsubscript{2} bending, -CO-O-C symmetric stretching, alkane -CH rocking respectively.

Compared to the substrate (soybean oil) biopolymer was up to 200 °C and decomposed when the temperature increased further (Fig. 4b). With respect to differential scanning calorimetry (DSC), sharp melting for multilayered biopolymer was observed at 117.8 °C, whereas no phase transition was observed for the substrate (insert figure). The heat of enthalpy for the biopolymer was calculated as 1999 J/g (Fig. 4c) and this could be due to the multilayered structures.

Scanning Electron Microscopy analysis of the portion of the multilayered biopolymer demonstrates a typical honeycomb structure with mesopores and micropores (Fig. 5 a-c). At higher magnification, a clear interconnected channel structures were observed.

![Scheme 1](image_url)—Schematic representation on transformation of soybean oil to self-assembled multilayered biopolymer.
The honeycomb network provided an additional exploration on the existence of aggregations.

**Application studies**

Separate experiments on assessment on entrapment of both oil and water at different time intervals demonstrated, irrespective of the oils (soybean, sunflower, peanut) taken for the study and the time of exposure, the material can entrap $2.7 \pm 0.3$ mL from a mixture containing equal amount of oil and water. The release of both oil and water during cross sectioning of the multilayered biopolymer is shown in Fig. 6. When calculating the volume of oil and water entrapped, it was observed that the entrapment of water volume was higher (2:1; water: oil) than the oil. In addition, the biopolymer was able to entrap wastewater containing toxic pollutants (recalcitrant, xenobiotic) and heavy metals.
Conclusion
In the present study, a self-assembled multilayered biopolymer material was prepared using soybean oil as substrate. Biopolymer formation starts with primary aggregations of micelles and ends with tertiary aggregations. Shaking, mineral salts and other components present in the medium mediates the transformation of soybean oil to biopolymer with multilayered honeycomb structures which can trap both oil and water simultaneously. Biomedical application of this biopolymer is suggested.

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