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Lysozyme immobilization via adsorption process using sulphonic acid functionalized silane grafted copolymer

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ABSTRACT

A unique silane based adsorbent material, [stearyl alcohol (SA)-grafted-epichlorohydrin (E)]-grafted-aminoproypyl silanetriol (APST) was synthesized and functionalized with sulphonyl groups via sulphonation process [(SA-g-E)-g-APST/SO_3H]. The adsorbent material characterization was done by FTIR, XRD, and TGA analysis. Immobilization of protein Lysozyme (LYZ) using batch adsorption process was carried out for studying the protein–particle interaction. The most suitable pH for maximum adsorption was found to be 7.0. Pseudo-second-order kinetic model was found to be the best fit and the adsorption equilibrium was attained within 3 h. Studies on diffusion parameters explained that the adsorption mechanism was controlled by film diffusion mode. The adsorption process was then evaluated using the various isotherm models and the Sips isotherm model proved to be the best fit with a maximum adsorption capacity of 37.68 mg/g. The isotherm favorability of the adsorption process was calculated by calculating the separation factor (R_L) and the values confirmed the favorability of the adsorption process. Studies on adsorption percentage with respect to temperature and thermodynamic studies revealed that adsorption process is exothermic, spontaneous with maximum entropy. Batch adsorption/desorption studies in acidic medium, for over six cycles showed the repeatability and regeneration capability of the adsorbent material (SA-g-E)-g-APST/SO_3H.

1. Introduction

Exposure of colloidal particles onto the biological fluids results in the adsorption of proteins on to the particle surface; which may be due to the formation of protein–particle conjugates [1]. It has already been reported that, the proteins that are components of food/drug materials, sometimes adsorbs strongly on the surface of packages and thus becoming ‘organic soilings’ in the food and drug manufacturing industries [2]. And this property of proteins – getting adsorbed on to the surface of the particles, can be utilized for various applications such as drug carriers, signaling (growth factors), targeting (antibodies, opsonins) [3,4], etc. But the protein adsorption onto the adsorbent material is depending on many factors such as protein selection, experimental conditions and adsorbent material itself.

The properties of protein involve its charge, size, amino acid composition and conformation, and the protein stability [5,6]. Proteins with high internal stability can get adsorbed onto hydrophilic surfaces only with the aid of electrostatic interactions and those proteins with less internal stability tends to adsorb onto any surfaces irrespective of any electrostatic interactions [7,8]. Another phenomenon explained was that the major driving force for protein adsorption is the entropy gain which is forming due to the release of surface adsorbed water and salt ions together with structural rearrangements inside the protein [9,10]. Irrespective of these two phenomenons, it can be stated that simple adsorption process cannot be employed for the entrapment of protein molecules, since the protein may get easily desorbed during washing and operation [11–13]. Hence for the present study reversible immobilization technique was employed for conducting the adsorption studies. But for enhancing the reversible immobilization technique the adsorbent material should posses a strong hydrophobic or electrostatic force inducing moieties.

The adsorbent material is to be selected based on its hydrophobicity, structural morphology and the functionality. Variations with respect to physico-chemical surface properties manipulate the electrostatic as well as the hydrophilic/hydrophobic protein–particle interactions, irrespective to the structural morphology. Previous researches have utilized the use of modified cellulose as adsorbents for protein adsorption. But they are highly sensitive to abrasive forces when used in batch or column applications and also leaching of certain water extractives were occurring during the adsorption process [14]. On the contrary, studies on the soil environment by earlier researchers revealed that the activities of biomolecules present in the soil are protected by clay minerals [15,16]. Accordingly clay has been widely used as a drug carrier

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by many pharmaceutical industries. The present study deals with the development of a novel adsorbent material as a drug carrier, an alternative to clay. Many recent research studies exposed that silanol groups could be a better alternative to clay, since it is biocompatible and biologically inert material [17]. Keeping these facts into consideration, 3-aminopropyl silanetriol (APST) was selected as the main component in the synthesis of the novel adsorbent material. The three OH groups and the NH_2 groups present in the main group promote the hydrophilic nature. But for the effective protein adsorption mechanism introduction of hydrophobic moieties are also needed. Hydrophobic nature in the interior part of the adsorbent material and hydrophilic property in the outer surface can enhance the protein adsorption very effectively. Hence an alky glycidyl ether compound was selected since it is being used as an important intermediate in pharmaceutical applications [18]. Stearyl alcohol which is alkyl glycidyl ether, was selected as the compound for providing hydrophobic nature to the adsorbent material. Since the direct linkage of stearyl alcohol (SA) to APST is not possible, double grafting process has been explored. SA was first grafted to epichlorohydrin (E) and this grafted compound was again grafted to APST to obtain stearyl alcohol(SA) grafted epichlorohydrin(E) grafted APST ((SA-g-E)-g-APST). Since the silanol groups get hindered by the long alkyl chain present in SA, a functional group has been introduced to enhance the protein adsorption capacity. Sulphonyl group has been used for functionalizing the (SA-g-E)-g-APST since sulphonyl group will remain unaltered in polar and non-polar media. Hence chloro sulphonic acid was selected and used for functionalizing (SA-g-E)-g-APST resulting in the formation of the adsorbent material, (SA-g-E)-g-APST/SO_3H.

2. Experimental

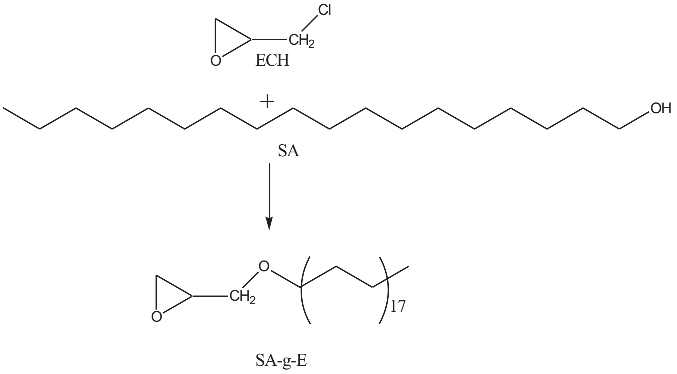
2.1. Materials

The whole chemicals and reagents used for experimental process and analysis were of analytical grade and were used as such. All stock solutions were prepared using double distilled water. Stearyl alcohol (SA) and CTAB were supplied by S. d-fine Chemical Ltd., Mumbai, India. Amino propyl silane-triol (APST) was obtained from ABCR GmbH and Co. KG, Germany. Epichlorohydrin and antimony potassium tartrate were purchased from SISCO Research Laboratories (P) Ltd., Mumbai. Amberlite® SO_3H–polystyrene matrix, sodium hydroxide, toluene, isopropanol, sodium bicarbonate, lyszozyme (LYZ), Follin's reagent, sodium potassium tartarate, and sodium carbonate were afforded by Sigma–Aldrich (Germany). A stock solution of 500 mg/L LYZ solution was prepared and further solutions having different concentrations were freshly prepared from stock solutions for all experimental purpose.

2.2. Synthesis of (stearyl alcohol-grafted epichlorohydrin)-grafted aminopropyl silanetriol/sulphonic acid copolymer ((SA-g-E)-g-APST/SO_3H)

2.2.1. Preparation of SA-g-E

About 0.1 M SA in sodium hydroxide (6.0 g) was taken in a two necked round bottomed flask (100 mL). CTAB of 0.2 g was added as a catalyst to the mixture. About 20 mL toluene was added as a solvent to the mixture. The mixture was then allowed to melt for ten minutes. After the melting process, epichlorohydrin (E) was added to the molten mixture under controlled magnetic stirring. The reaction temperature was positioned at 60 °C for 2 h. On completion of the reaction, the reaction mixture was kept at room temperature for cooling. The cooled product was initially washed with toluene, and then with distilled water; to remove the unreacted reagents. A yellowish precipitate of SA-g-E was obtained and it was then left to cool at room temperature for an overnight. The mechanism is expressed as follows (Schemes 1–3).



Scheme 1. General mechanism for SA grafting onto epichlorohydrin.

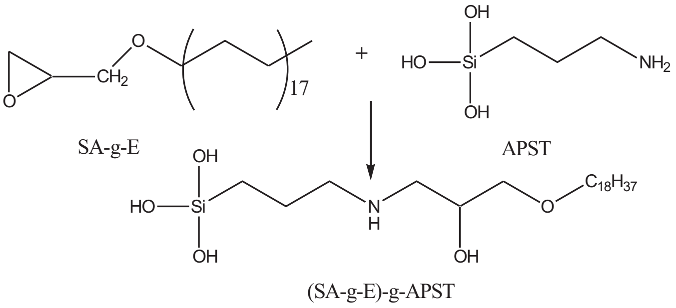
2.2.2. Grafting of (SA-g-E) on APST

The dried SA-g-E was weighed to 12.0 g and it was added to a weighed amount of 8.0 g APST. The whole mixture was taken in a three necked flask containing isopropanol. The whole reaction procedure was done under inert atmosphere and the reaction process continued for 3 h under reflux conditions. The temperature was set at 65 °C. On completion of the reaction a precipitate was formed and it was then filtered off and washed with toluene until the whole unreacted reagents were removed. The pure (SA-g-E)-g-APST thus obtained was then dried in a vacuum oven at 60 °C for 7 h. The mechanism is shown in Scheme 2.

2.2.3. Synthesis of (SA-g-E)-g-APST/SO_3H

An amount of 8.0 mL of chlorosulphonic acid was poured into a solution of formamide under constant stirring at ice cold condition. The chlorosulphonic acid mixture was taken in a three necked flask and to this 2.0 g of (SA-g-E)-g-APST was added under stirring. The whole reaction process was done in an inert atmosphere, under reflux conditions and the reaction process was continued for 6 h at 70 °C. On completion of the reaction a precipitate was formed and it was filtered off and kept at room temperature for cooling. After cooling, 20% NaOH was added to the mixture for neutralization. The precipitate formed was then washed with acetone for 2–3 times. Then it was filtered off and washed with anhydrous ethanol for 4–5 times. A white powder was obtained and this powder was then dried in a vacuum oven for 6 h at 60 °C. The mechanism behind the synthetic process is shown in Scheme 3.

The dried sample was then ground and sieved to obtain −80 + 230 mesh size particles (average diameter of 0.096 mm).



Scheme 2. Ring opening mechanism involved in the grafting of SA-g-E to APST.