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Adsorption property and affinity chromatography of polystyrene derivative sorbent towards cow's milk xanthine oxidase

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ABSTRACT

On the basis of the biospecific molecular recognition between complementary chemical groups of xanthine oxidase (XO) and their ligands particularly sulphated glycoaminoglycans and heparin. Poly (styrene chlorosulfonyl) particles modified by sulfonate sodium groups was synthesized and its adsorption property towards cow's milk XO was established. The adsorption of XO onto this functional polymer was performed in batch at 4 °C and at pH 6.0 during 30 min. of incubation. The adsorbed XO content at the interface allows establishing the chemisorption isotherm curve. The affinity association estimated from this adsorption isotherm according to the Langmuir equation was found to be significantly high in the magnitude of 1.25×10^{6} M^{-1}. Affinity chromatography on column using this functional polymer as a stationary phase confirms its high ability to adsorb XO at low ionic strength. In fact, the xanthine oxidase of the crude extract is strongly adsorbed onto the sorbent and is eluted at high ionic strength with out any significant loss of its biological activity. The purified enzyme possesses a protein flavin ratio (PFR) of 6.05 with a specific activity of 1.78 UI/mg. On the other hand, the electrophoresis of XO fraction showed a single band with a molecular weight of about 150 kDa. Thus, the synthesized beads functionalized by sulfonate group could be used efficiently and advantageously in the purification of XO instead of other conventional chromatographic methods which need several steps.

1. Introduction

In bio-separation sciences the development of a new selective support packing for protein purification constitutes an important field of research in order to improve the efficiency of the methods and the purity of protein products. On the other hand, the choice of a suitable corresponding ligand is very important for obtaining a highly effective adsorbent. Styrene-divinylbenzene copolymer, one of the many synthetic polymers, has long been used in liquid chromatography as adsorbent. This support received an increasing interest as a result of the modification of their hydrophobic property in protein purification in aqueous media. Cross-linked polystyrene substituted by L-tyrosine and its derivatives have been successfully employed in removing, *in vitro*, antibodies anti-FVIII from hemophiliac A patients on which they are highly adsorbed [1,2]. In the same order, polystyrene modified by serine used as a stationary phase in high performance affinity chromatography has been shown to develop a specific and strong affinity towards basic fibroblast growth factor in solution. The solute has been adsorbed on the resin in low ionic strength and eluted by raising the salt concentration [3]. Furthermore, phosphorylated polystyrene was found to be an interesting chromatographic support in the fractionating of RNA polymerase II transcription factor [4,5]. On the same functional polymer, Migonney et al. [6,7] have demonstrated that this support exhibits phospholipid-like surface which are able to interact with factors II and IX in presence of calcium with a high affinity and can be used as a stationary phase of both factors by highly specific liquid chromatography. On the other hand, we have demonstrated that the poly (styrene sodium sulfonate) and the poly(styrene cysteine sulfamide) gel beads possess a high affinity toward human serum albumin and the association constants estimated from the obtained isotherm curves were in the order of 7.5×10^{5} and 1.45×10^{6} M^{-1} respectively [8]. Finally, in recent study, using polystyrene matrix functionalized by sulfonate group constitute an effective heparin-like adsorbent in the purificatin process of human vitamin K-dependent coagulation factor IX [9].

Mammalian xanthine oxidoreductase (XOR) is a complex molybdo-flavoenzyme composed of two identical subunits of approximately 150 kDa; each monomer contains one molybdenum center, one FAD center and two Fe2/S2 sites. The enzyme is readily available from cow's milk and represents a major component of the fat globule membrane [10,11]. XOR catalyses the hydroxylation of hypoxanthine to xanthine and xanthine to uric acid, excreted in urine, in man's purine catabolism [12,13]. Furthermore, it has been reported that the human xanthine oxidase bind to sulphated glycoaminoglycans on the endothelial cell plasma membrane [14]. In purification procedure, it has been demonstrated also that xanthine oxidase from different species interact tightly with heparin coupled to cross-linked Sepharose [15].

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On the basis of the biospecific molecular recognition between complementary chemical groups of xanthine oxidase on one hand and their ligands on the other one, the present study reports the synthesis of cross-linked polystyrene adsorbent beads functionalised by suitable chemical groups in particularly sulfonate groups, which confers this polymer an adsorbing property similar to heparin. The adsorption ability of this functional polymer towards xanthine oxidase from cow's milk was also investigated. Furthermore, this functional polymer particle was used as a stationary phase in affinity column chromatography for purification of xanthine oxidase from the crude extract.

2. Material and methods

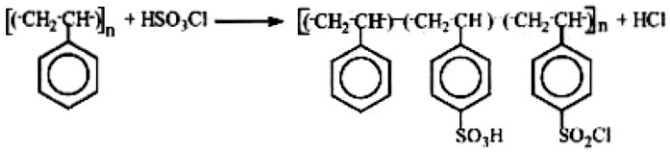
2.1. Material

Fresh raw milk was obtained from farmer of Sétif region (East part of Algeria). Reagents for synthesis of poly (styrene sodium sulfonate) adsorbent, extraction and assay of xanthine oxidase and electrophoresis are analytical grade and were purchased from Sigma-Aldrich Chemie GmbH (Germany) and Fluka Riedel-de Haën (Germany).

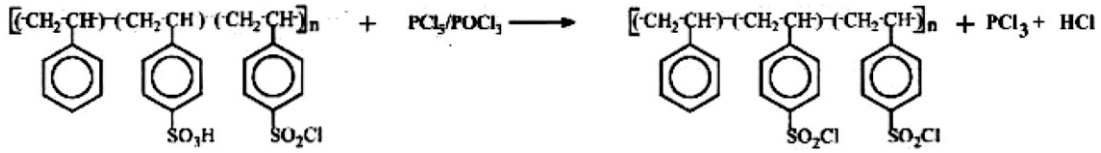
2.2. Preparation of the polystyrene derivative adsorbent

The poly (chlorosulfonyl styrene) resin adsorbent in the form of spherical beads was synthesized by free radical suspension polymerisation in three steps using chlorosulfonic acid as sulfonating agent and PCl_5 as chlorinating agent according the reported procedure [16–22] as described briefly below.

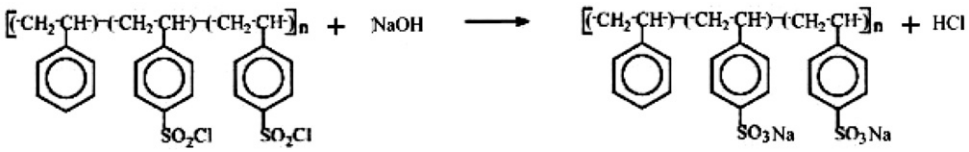
First step: the chlorosulfonation of the copolymer gel of styrene 98% and 2% divinylbenzene, porous spherical beads, in the form of 200–400 mesh (33–75 μm) was done in the excess of chlorosulfonic acid in 1,2 dichloroethane. The reaction was achieved during 4 hours at 40 °C under stirring and the filtered, washed and dried particles were finally obtained.



Second step: in order to obtain an uniform chlorosulfonyl ($-SO_2Cl$) groups substitution on the polystyrene chain a chlorination was processed at 105 °C during 2 hours using a mixture of chlorinating agent $PCl_5/POCl_3$ (7,5 g of PCl_5 dissolved in 20 ml of $POCl_3$). The chlorinated particles were filtered, washed and dried in the same way.



Third step: the homogeneous chlorosulfonyl co-polymer styrene-divinyl benzene resin was hydrolysed with 1 M NaOH, and then equilibrated with 10^{-2} M NaOH until sodium ion exchange equilibrium was reached. Finally, the resin particles were washed and dried under vacuum at 50 °C and stored until its use in the different studies.



2.3. Chemical and physical characterization

The content of chlorosulfonyl groups ($-SO_2Cl$) was estimated by argentimetric method. Firstly, 0.3 g of the chlorosulfonated copolymer adsorbent was treated with an excess of 2 M NaOH aqueous solution, then the free chlorine in the supernatant was titrated with a standard 5 mM silver nitrate as titrant using chromium potassium as indicator. On the other hand, the exchange capacity of the obtained product was determined by flame photometry by displacing ion sodium by $CuCl_2$ solution (10^{-3} M). Finally, qualitative identification of the main groups of the poly (styrene sodium sulfonate) particles was assessed by infrared spectroscopy. The infrared spectrum of the sample (few mg) was recorded on a Perkin Elmer spectrophotometer using anhydrous KBr (2%) pellet.