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Short communication

Chromatographic resolution of racemic α-amino acids: Chiral stationary phase derived from modified xanthan gum

Sadanand Pandey*, Shivani B. Mishra

Department of Chemical Technology, University of Johannesburg, P.O. Box 17011, Doornfontien 2028, Johannesburg, South Africa

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ABSTRACT

Enantiomeric resolution of α-amino acids into L-amino acid and D-amino acid via column chromatography using chiral stationary phase was performed. For this purpose, a dynamic chiral stationary phase prepared by grafting of methylmethacrylate onto xanthan gum (XG) was successfully employed in resolving various α-amino acids racemates. The peculiarities of the chromatographic behaviour of xanthan gum–graft-poly(methylmethacrylate)–amino acid interaction and the mechanism of their retention in column are discussed. The enantioselective properties of the xanthan gum–graft-poly(methylmethacrylate) in the separation of enantiomers of α-amino acids were studied using acidic solution of alanine, leucine, valine and tryptophan. The procedure is characterized by simplicity, efficiency and relatively low cost to analyze enantiomers of some amino acids.

1. Introduction

Effective methods for the separation and recovery of the particular enantiomers of biochemicals such as amines, amino acids as well as other types of biochemicals is of great importance in modern technology. The importance is exemplified by the growing need and desire to produce and use optically pure pharmaceuticals and other biochemical for human and other use. In most of the cases, only one of the enantiomer of a chemical compound is biologically active or produces a desired result. Thus, in order for a recipient of a pharmaceutical to receive enough of the biologically active enantiomer, twice the amount of pharmaceutical is generally given. Whereas the other form is either without effect or may also show side effects (Ahuja, 2000; Ariens, 1987; Beesleey & Scott, 1998, chap. 1). For example, the undesired enantiomer of thalidomide has been known to cause severe malformation in children born to pregnant women who took the drug by prescription for the benefits of the desired enantiomer (Pirkle, 1987). Therefore, much research has been conducted to produce enantiometrically pure pharmaceuticals such that the biologically active or desired enantiomer may be used in pure form to eliminate the drawbacks due to undesired enantiomer.

Generally two mirror images of a chiral molecule are called enantiomers or optical isomers. The term *optical activity* is derived from the interaction of chiral materials with polarized light. There are two forms of enantiomer (D)/(+) and (L)/(−) form. These two forms of enantiomer do not differ in their physical properties, but they rotate the plane of linearly polarized light.

There are essentially three theoretical methods that may be used to obtain optically pure compounds. First, the desired enantiomer may be synthesized in the desired enantiomeric form. Unfortunately, this method is often impractical because, in many cases, these types of synthesis methods have not been discovered, the production cost of making the pure enantiomer has been prohibitive. The second method involves crystallization. For example, tartaric acid as a crystallization platform has been used for such a separation. Though this is a cost effective method, it is useful in only a minority of cases. Chromatographic separation of enantiomers using chiral stationary phases (CSPs) (Danankov, Kurganov, & Bochkov, 1983; Shinbo, Yamaguchi, Nishimura, & Sugiura, 1987; Shinbo et al., 1992; Sogah & Cram, 1979; Sousa, Sogah, Hoffman, & Cram, 1978; Subramanian, 1994) has been widely used as a powerful means of separation.

Naturally occurring polysaccharides and their derivatives provide useful chiral stationary phase (CSP) materials. Microcrystalline triacetyl cellulose forms versatile column on which a number of compounds have been resolved but the method could not result in to total resolution due to salvation substrate (Wolf, Köunig, & Roussel, 1995). Resolution of racemic mixtures of amino acids (AAs) into their enantiomeric forms is a topic of active research, where practicable uses range from the control of peptide synthesis to geological dating (Bada, 1985; Hare, Hoering, & King, 1980). Chiral separation of amino acids by chiral octamide derivatives of calixarenes derived from resorcinol by

* Corresponding author. Tel.: +27 11 559 6163; fax: +27 11 559 6425.
E-mail address: spandey.uj@gmail.com (S. Pandey).

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impregnation on a polymeric support has been reported, where phenylalanine–tryptophan and phenylglycine–trytophan mixtures were separated by column chromatography (Seyhan, Özbayrak, Demirel, Merdivan, & Pirinççioğlu, 2005). Capillary electrophoresis with 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate as chiral selector has also been used in chiral separation of amino acids (Tran & Kang, 2003). Optical resolution of α-amino acid by Chiral poly(crown ether)s 2 and 5; and Chiral crown ether-coated reversed-phase packings have been reported by Kakuchi, Takauka, and Yokota (1990) and Shinbo et al. (1987) respectively. Hirose et al. (2005) reported a CSP by immobilizing a chiral pseudo-18-crown-6-type host on 3-aminopropyl silica gel for enantiomer separation of amino compounds using a normal mobile phase.

But in present work, an attempt has been made to synthesis and to exploit poly(methylmethacrylate) functionalized xanthan gum (XG–g-PMMA) for the separation of α-amino acids. Since potentially such materials are highly stereoselective due to the presence of the chiral centres in the polysaccharide as well as the methylmethacrylate grafts. It was first time any natural renewable polysaccharide (xanthan gum) is exploited for the separation of aminoacid. For the first time the enantioselective properties of the XG–g-PMMA in the separation of enantiomers of α-amino acids was studied using acidic solution of alanine, leucine, valine and tryptophan. The procedure is characterized by simplicity, efficiency, nontoxic, eco-friendly and relatively low cost to analyze enantiomers of some amino acids.

2. Methods and materials

2.1. Column chromatography

Column chromatography was performed by using column of length 20 cm and diameter 20 mm (made: Borosil) using XG–g-PMMA as CSP. To recognize the pure optical isomer polarimeter was used. Optical rotations were measured on a Rudolph instrument Sirius 251 visual polarimeter, USA. Enantiomeric and racemic pure amino acid, DL-alanine, DL-leucine, DL-valine and DL-phenylalanine (from Sigma) with 99.9% purity were used. All the chemicals used in this study were of analytical reagent grade and was used as received without further purification. DI water was used throughout the study. XG and MMA were purchased from (Merck, South Africa). MMA was washed with 5% aqueous alkali to remove phenolic inhibitor and then distilled before use. Infrared (IR) spectra were recorded on a Nicolet 5700 of FTIR spectrophotometer using KBr pellet The XRD measurements were carried out using X' Pert Pro MPD PANalytical powder diffractometer operating in the reflection mode with Cu Kα radiation. SEM analyses of the modify XG were performed using a JEOL JSM-5600 instrument (JEOL, Pleasanton, CA) operated at 20 mA with an accelerating voltage of 20 kV equipped with a EDS detector, for low resolution images. In order to avoid charging, XG–g-PMMA was samples with coated carbon. Samples with different grafting ratio were also analyzed as CSP.

The percentage (%G) and efficiency (%E) of grafting were calculated according to Eqs. (1) and (2) as given below (Kojima, Iwabuchi, Kojima, & Tarumi, 1971).

$$\text{Percent grafting}\,(\%G) = \frac{W_1 - W_0}{W_0} \times 100 \quad (1)$$

$$\text{Percent efficiency}\,(\%E) = \frac{W_1 - W_0}{W_1} \times 100 \quad (2)$$

where W_1, W_0, and W_2 denote respectively, the weight of the grafted XG, the weight of original XG and weight of the monomer used.

2.2. Chiral stationary phase preparation

2.2.1. Synthesis of XG–g-PMMA

To a solution of XG (0.1 g in 25 mL water), calculated amount of MMA and ascorbic acid (AA) were added and the reaction mixture was thermostated on thermostatic water bath at 35 ± 0.2 °C. After 30 min calculated amount of $K_2S_2O_8$ (KPS) was added and this time of addition of KPS was taken as zero time. Graft copolymerization was allowed for 1 h. similar method as reported earlier (Pandey & Mishra, 2011, 2012). Grafted XG samples (having different % grafting) were separated from the respective reaction mixtures by pouring them into excess of acetone. The copolymer samples thus obtained were finally extracted with acetone in a soxhlet apparatus for 4 h to dissolve all the homopolymer and the copolymer samples thus obtained were finally dried under vacuum at 50 °C for >24 h to a constant weight and results were shown in (Fig. 3A–D).

2.3. Optical resolution of amino acids

Optical resolution of DL-alanine, DL-valine, DL-leucine and DL-phenylalanine (α-amino acids) was done through XG–g-PMMA column (Fig. 1). Eluent used was 0.001 N HCl and elute collected at the rate of 0.5 mL/min. The collected elute is measure by using polarimeter. Polarimeter measures the rotation of polarized light as it passes through an optically active fluid. The measured rotation can be used to calculate the value of solution concentrations; especially substances such as sugars, peptides and volatile oils. It consists of a polarized light source, an analyzer, a graduated circle to measure the rotation angle, and sample tubes. The polarized light passes through the sample tube and exhibits angular rotation to the left L (−) or right D (+). On the side opposite the polarizer is the analyzer. Using optics, visual fields are manually adjusted by the user to measure the optical rotation angle. The schematic diagram of chirality separation of amino acid and detection through polarimeter is provided in Fig. 1.

3. Result and discussion

Varying various reaction parameters for grafting such as MMA, KPS, AA, and XG, chiral stationary phase (XG–g-PMMA) samples with different %grafting (%G) were synthesized using KPS/AA as a redox initiator at 35 ± 0.5 °C in presence of atmospheric O_2 (Fig. 2A–D). Apart from above, some other parameter such as Reaction time and temperature was also varied (figure provided in supporting file S1 and S2). The maximum %G that could be reached was 300% using [MMA] = 17×10^{-2} M, [KPS] = 40×10^{-3}, [AA] = 2.3×10^{-2} M, [XG] = 4 g/L and total reaction volume 25 mL and reaction time 1 h. The sample synthesized at optimum conditions was fully water insoluble and was used for optical resolution of α-amino acids. Under same condition, samples with different grafting ratio were also analyzed as CSP.

3.1. Characterization of the stationary phase

The used chiral stationary phase was characterized with FTIR, XRD and SEM to see their chemical structures.

3.1.1. FTIR

XG showed characteristic polysaccharides absorptions including O—H stretching (at 3427 cm^{-1}) and C—H stretching (2923 cm^{-1}). In XG–g-PMMA, O—H and C—H stretching are seen at 3442 cm^{-1} and 2930 cm^{-1} respectively.

In the copolymer, additional sharp peaks due to —C=O, C—O stretching are seen at 1730 cm^{-1} and 1120 cm^{-1} respectively while O—H bending peak is 1023 cm^{-1} respectively. The presence of ester C=O and C—O stretching in the graft copolymer provided

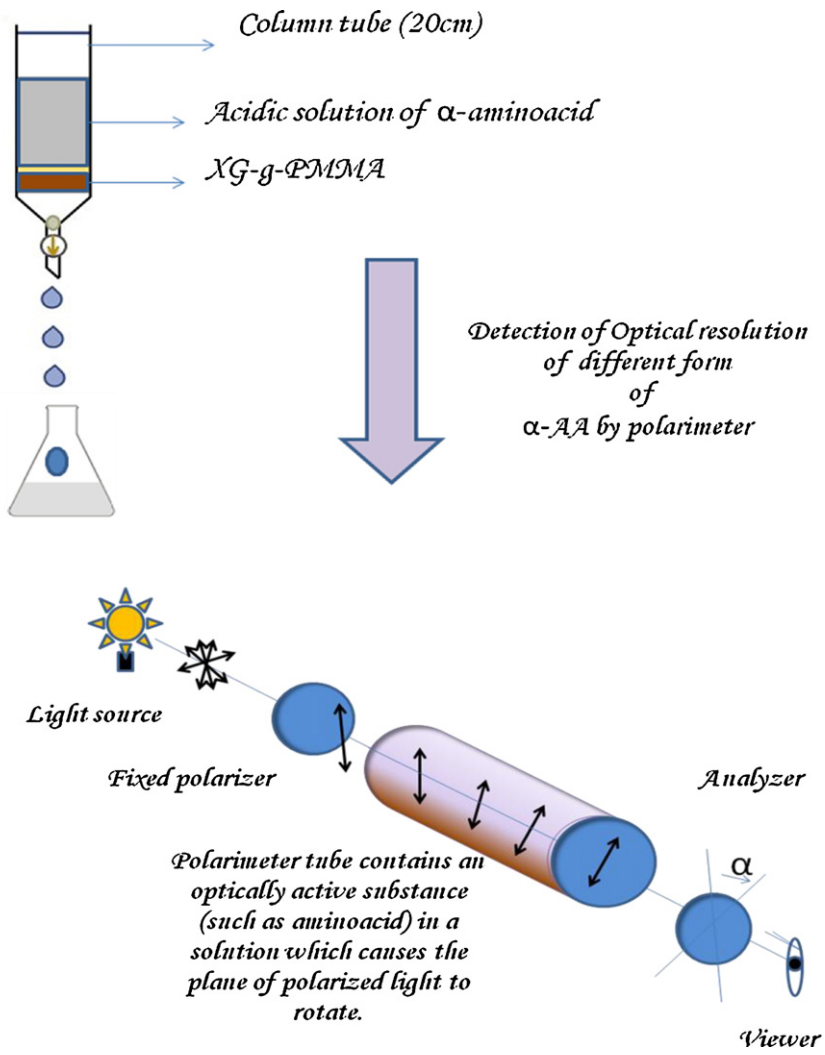

Fig. 1. Schematic diagram of the chirality separation of amino acid by loading in coloumn containg XG–g-PMMA (CSP) and detection by polarimeter.

substantial evidence of PMMA grafting onto XG gum (Fig. provided in supporting file S-3.).

3.1.2. XRD

X-ray diffraction (XRD) of the XG showed a broad hallow with a broad hump at 2θ 17° like amorphous materials while XG–g-PMMA showed sharp crystalline peaks in the region of 2θ 29–32° which indicates increase in the crystallinity on PMMA grafting on XG backbone. Change in the XRD pattern upon grafting evidenced the incorporation of PMMA grafts in the copolymer (figure not provided).

3.1.3. SEM

The scanning electron microscope (SEM) picture of XG displayed a homogeneous, microporous and network structure, whereas XG–g-PMMA exhibited a dense and nonporous flaky structure. A change in the SEM picture confirms grafting (figure not provided).

3.2. Optical resolution of amino acids

With the exception of glycine, all α-amino acids are asymmetric molecules. They can be obtained in two forms called enantiomers. Enantiomers differ considerably in their biological activity. Efficient optical resolution of (±) DL-alanine, (±) DL-valine, (±) DL-leucine and (±) DL-phenylalanine was done by employing a process of chromatography using chiral column of XG–g-PMMA. The difference between the accumulated amounts of D- and L-isomers penetrated was large indicating that the diffusion rate of L-isomer was much faster than that of D-isomer.

Separation of the optical isomers consisting of exactly same chemical structure except its stereochemical structure through the enantioselective stationary phase can be explained by the interaction among the chiral environment and isomers being separated. The interaction among the isomers and the chiral environment of stationary phase will be different depending on the characteristics of the isomers and chiral environment of stationary phase that cause differences in the diffusion rate of isomers. The different diffusion rates of isomers will be the key to the optical resolution.

XG itself contain a large amount of chiral active carbons on the ring backbone structure but its solubility & biodegradability limits its application as CSP. The numbers of chiral active centres increase on grafting of PMMA chain onto XG. The interaction between the optical isomers and chiral active centres in XG–g-PMMA are responsible for optical resolution.

3.3. Effect of %G on resolution

The percentage of resolution was found to be increase with increase in %G from 70 to 300 and maximum % resolution was observed at %G 300 (Fig. 3A), indicating that the %G onto XG is

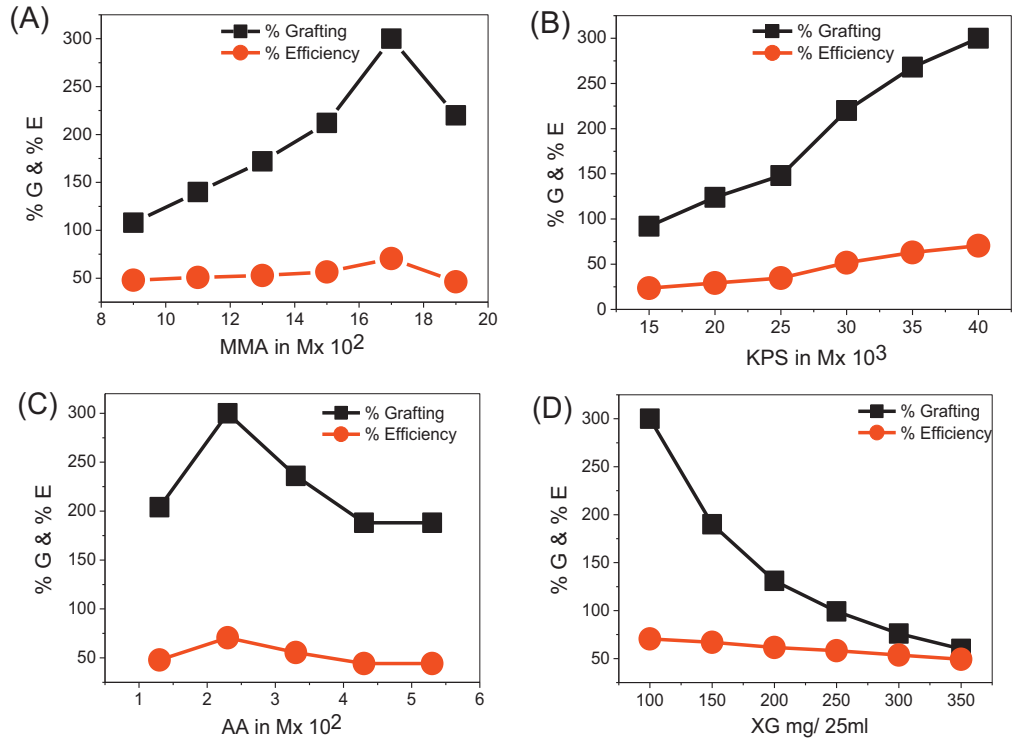

Fig. 2. (A) %*G* and %*E* at different MMA concentrations at [XG] = 4 g/L; [KPS] = 40×10^{-3} M; [AA] = 2.3×10^{-2} M, keeping the total reaction volume and grafting time fixed at 25 mL and 1 h respectively. (B) %*G* and %*E* at different KPS concentrations at [XG] = 4 g/L; [MMA] = 17×10^{-2} M; [AA] = 2.3×10^{-2} M, keeping the total reaction volume and grafting time fixed at 25 mL and 1 h respectively. (C) %*G* and %*E* at different AA concentrations at, [MMA] = 17×10^{-2} M; [XG] = 4 g/L; [KPS] = 40×10^{-3} M; [XG] = 4 g/L keeping the total reaction volume and grafting time fixed at 25 mL and 1 h respectively. (D) %*G* and %*E* at different gum concentration (mg/25 mL) at fixed [MMA] = 17×10^{-2} M; [KPS] = 40×10^{-3}; [AA] = 2.3×10^{-2} M, in a total reaction volume of 25 mL on thermostatic water bath at 35 °C in 1 h (MMA, methylmethacrylate; KPS, potassium persulfate; AA, ascorbic acid; XG, xanthan gum).

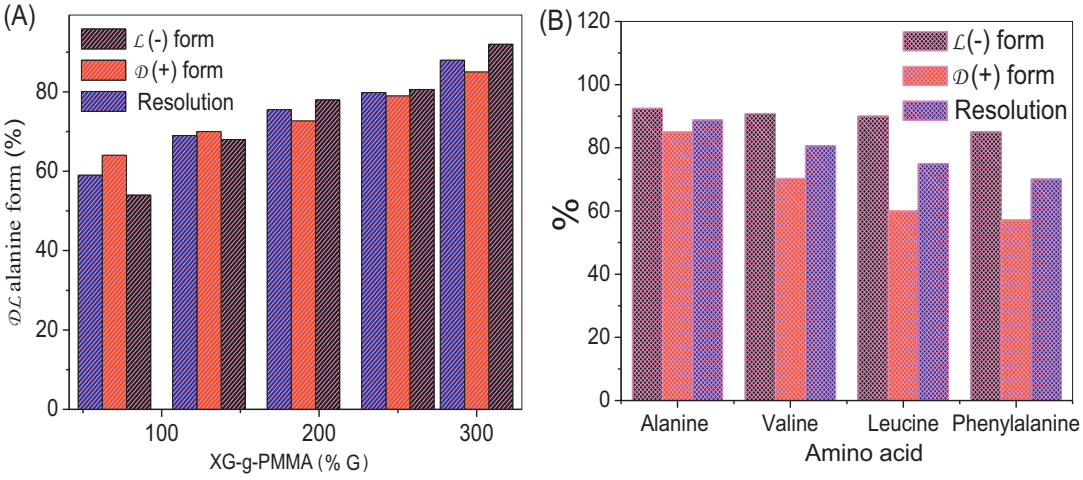

Fig. 3. (A) Graph showing percentage of L-form, D-form and percentage of resolution DL-alanine using CSP, XG–g-PMMA with different %*G*. (B) Graph showing percentage of L-form, D-form and percentage of resolution different amino acids using CSP, XG–g-PMMA with highest %*G* i.e. 300.

an important factor in the resolution of α-amino acids. As with increase in %*G*, number of chiral active centres increases which are responsible for resolution of optical isomers.

3.4. *Effect of size of side chain in amino acids on resolution*

XG–g-PMMA used as CSP contains large numbers of active centres on the backbone structure possible to form helical structures as generally the polysaccharides have the helical structures. The helical structure has the small chiral active spaces responsible for resolution of optical isomers. So, percentages of resolution of optical isomers decrease with increase in size of side chain in α-amino acids and it follows the following order: Alanine > Valine > Leucine > phenylalanine (Fig. 3B).

4. Conclusion

XG–g-PMMA can be synthesized by using redox initiator by thermostatic conventional method. These synthesized XG–g-PMMA can efficiently used for the optical resolution of α-amino acids especially DL-alanine, DL-valine, DL-leucine and DL-phenylalanine. The presence of chiral centerson ring structure of

XG and additional chiral centres added through grafting making XG–g-PMMA enantioselective stationary phase. Enantioselectivity increases with increase in %*G* i.e. chiral active centres.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.11.102.

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