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Novel material; non catalytic functioning of linear polystyrene with diaminopropane-β cylodextrin that will be used in α-amylase separation and purification

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ABSTRACT

The functionalizing of linear polystyrene with diaminopropane-β-cyclodextrin that will be used as stationary phase in affinity chromatography on α-amylase purification is described. Polystyrene is altered to (chloromethyl)polystyrene by substituting one hydrogen of aromatic ring with methylene chloride. Substitution reaction between chloride and amine functional groups in diaminopropane was carried out to bind the diaminopropane to linear polystyrene. β- cyclodextrin was reacted with tosylchloride to produce 6-O-(paratosyl)-β-cyclodextrin before incorporating to diaminopropane-polystyrene. It was grafted without catalyst using DMF as solvent to the polystyrene-diaminopropane and novel material polystyrene-diaminopropane-β-cyclodextrin was produced. The characterization of all materials involved was carried out using FTIR, $_{13}C$ and $_{1}H$ NMR, elemental analysis, TEM, and SEM.

Keywords β-cyclodextrin, diaminopropane, linear polystyrene, α-amylase

I. INTRODUCTION

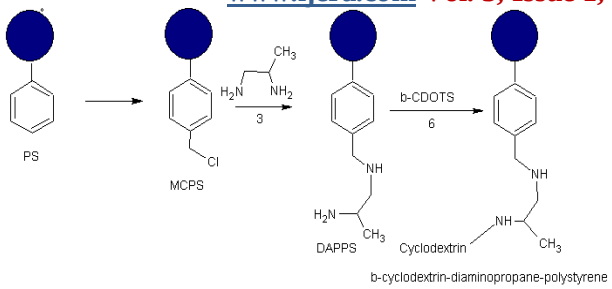
Downstream processing can amount to 80-90% of the total manufacturing cost of biotechnology production. Shorten the process can reduce the expense. Affinity chromatography is believed could shorten the downstream processing route. This method based on specific and reversible interaction between stationary phase and product target in column. α-Amylase is an enzyme that used in food and beverages, textile, bio-ethanol and pulp industry[1]. It has specific and reversible interaction with its inhibitor. One of α-amylase inhibitor is β-cyclodextrin. The use of β-cyclodextrin as a ligan in affinity chromatography of amylase purification is interested to explore. Silica gel is one of commercial solid support widely used for β-cyclodextrin. Unfortunately silica gel is not stable at pH more than seven [2]. Other solid support for β-cyclodextrin as stationary phase is sepharose. This stationary phase exhibited high selectivity in α-amylase purification but it is limited due to its low mechanical rigidity even though in heavy cross-linked [3]. These drawbacks head off its application in preparative scale operation.

Polymeric resin as a solid support has advantages such as convenience, chemical stability, easy regeneration and better selectivity by functional modification and controlling pore structure. Some polymeric that functionalized by cyclodextrin was reported such as poly(methylmethacryate) [4], polyurhetane [5], polyamidoamino [6], and polyethylene oxide [7].

Polystyrene is one of polymers that has been available widely, and not expensive. It possesses mechanical rigidity and covers wide range of pH value. Polystyrene was used as solid support in protein [8] and antibody [9] purification using affinity chromatography methods. Due to its hydrophobic property, polystyrene gets interest as a solid support in protein purification in aqueous media.

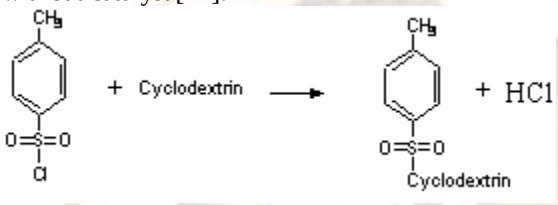
Recent research has shown that technique to incorporate β-cyclodextrin to polystyrene is being developed. Inclusion of β-cyclodextrin to fiber linear polystyrene by electro spinning technique was reported [10]. The advantage of this method is the process can be completed in short time, but unfortunately the process and equipment are more complicated. Other simple technique to graft β-cyclodextrin into polystyrene is reaction in homogenous system. Reaction in homogenous system might run without catalyst [11].

The present paper describes catalyst-free functionalizing of linear polystyrene with diaminopropane-cyclodextrin in homogenous system. Some precursor compounds have been prepared before covalently binding. To attach the diaminopropane that acts as nucleophilic function group, the linear polystyrene (PS) has been prepared to have an electrophilic function group. The aromatic hydrogen was substituted by methylenechloride. Chloride atom in chloromethylated polystyrene (CMPS) can be substituted by amine group of diaminopropane to produce diaminopropane functionalized polystyrene (DAPPS), Scheme 1.



Scheme 1. Route of PS to functioning linear polystyrene with diaminopropane-β- cyclodextrin

Before incorporating β-cyclodextrin to DAPPS, it is altered to 6-O-(paratosyl)-β-cyclodextrin (β-CDOTS) by tosyl chloride, Scheme 2. Carbon atom in β-CDOTS can act as electrophilic function group. DAPPS can be functioned by β-CDOTS by means of substitution reaction in base condition. This reaction can be done also in homogenous system without catalyst [11].



Scheme 2. Reaction between β-cyclodextrin and tosylchloride to produce β-CDOTS)

II. EXPERIMENTAL

2.1, Instrumentation and reagents

The intermediate and end material were characterized using spectroscopy method such as FTIR (Shimadzu, Japan), $_{13}C$ and $_{1}H$ NMR ECS 400 (JEOL,Japan), microscopy method like TEM (JEOL, JEM-1400, Japan), and SEM S-4800 (Hitachi, Japan). After drying carefully, the sample composition (C, N and H) was measured by elemental analysis equipment, MT-6 CHN Corder (Yanaco, Kyoto, Japan).

Experiments were performed with commercially available linear polystyrene kindly supplied by Abestyrindo, Co. (Indonesia). β-cyclodextrin, p-toluenesulfonyl chloride or tosylchloride and 1,2-diaminopropane and cetyltrimethylammonium bromide (CTAB) were obtained from sigma. Pyridine, dimethyl formamide (DMF), were obtained from Wako Pure Chemical Industries (Osaka, Japan). All reagents was used without further purification.

2.2, Functionalizing of linear polystyrene

Linear polystyrene containing β-cyclodextrin was synthesized according to reaction shown in scheme 1 and 2. The reaction in Scheme 1 consists of three steps. First step was chloromethylation of linear polystyrene. The reaction was carried out according to the procedure described by Gao and the typical process is as follows [12]. 10 g PS was washed by 1 M NaOH, 1 M HCl, distilled water and alcohol, respectively. Dried polystyrene was solved in carbon tetrachloride in three neck flask following by addition of 0.1 M aqueous CTAB. After mixture was agitated for 5 hours it was added by formaldehyde and concentrated HCl. Temperature of reaction mixture was risen to 65^{o}C. Phosphorous chloride was added drop-wise carefully under stirring condition. Temperature was kept constantly at 65^{o}C during 8 hours. CMPS was precipitated by using ethanol. It was washed with distilled water until there were no chloride ions in the filtrate (tested using $AgNO_3$ solution). After drying at 50^{o}C, the chloride contained of CMPS was measured by Volhard methods and CMPS was identified by FTIR and elemental analysis methods.

The second step was functioning of CMPS by 1,2-diaminopropane. 1 g of CMPS was poured to three neck flask that contained 80 ml 1,2-diaminopropane. The mixture was refluxed at 80^{o}C for 12 hours. The yellow solid was cut to small pieces and washed by distilled water until all un-reacted 1,2-diaminopropan removed (the pH of the water was 7). The DAHPS was diluted in DMF, added drop wise by sulphate acid 0.9% to remove trapped diaminopropane. White precipitated was filtered and washed by NaOH solution till pH of solution was 7, distilled water, and alcohol. The amine contained in DAPPS was determined by FTIR and elemental analysis methods after drying at 50^{o}C.

The monotosyl derivative of β-cyclodextrin, β-CDOTS, was prepared according to the procedures described by Crinni [11]. 0.4378 g of tosylchloride was added to solution of 5.1832 g β-cyclodextrin in 40 ml dehydrated pyridine in erlenmeyer flask. The mixture was stirred and kept at 0^{o}C for 48 h. Distilled water was added to the mixture and followed by evaporation of the solvent. Oil obtained was poured into cold water. The white precipitate that obtained was filtered and washed with water and acetone and dried in vacuum at room temperature. The precipitate was dissolved in DMF and purified using column chromatography, using silica gel 60 N as stationary phase. 13 fractions were checked by TLC and the solvent was evaporated. The structure of white powder was checked by NMR.

The last step was functioning of DAPPS by β-CDOTS. The β-CDOTS was bound to DAPPS resin by following reaction. 1.0031 g of DAPPS resin was dissolved in 35 ml DMF in three necked flask. 0.2834 g β-CDOTS was added to the solution. The mixture was stirred and refluxed at 80^{o}C. The reaction mixture was taken along 5 days every 24 hours. 1 ml of reaction mixture was poured into 20 ml distilled water while stirred. The white precipitate was filtered, washed with distilled water and dried at 50^{o}C for 7 hours. The resin obtained and alcohol was identified by elemental analysis,

$_{13}$C solid NMR and the morphology was seen by TEM and SEM.

III. RESULT AND DISCUSSION

The reaction between MCPS and diaminopropane was run in homogenous systems using diaminopropane as media and as reactant. The product structure was checked by FTIR. The PS, CMPS, and DAPPS FTIR spectra can be shown in Figure 1.

It shows that both spectra for CMPS and DAPPS show the same peaks at 3024, 1492, 756 and 700 cm^{-1}. These peaks are found usually in PS spectra. Two peaks at 698 and 756 cm^{-1} in PS spectra become three peaks in MCPS. New peak at 787 cm^{-1} relates to stretching vibration of C-Cl, and peak at 702 and 759 cm^{-1} relates to stretching vibration C-H in para-substituted of aromatic. At DAPPS spectra, three peaks at CMPS become to two peaks at the same (wavelength)$^{-1}$ as PS. The new broad peak appears at 3500 cm^{-1} that related to NH bond.

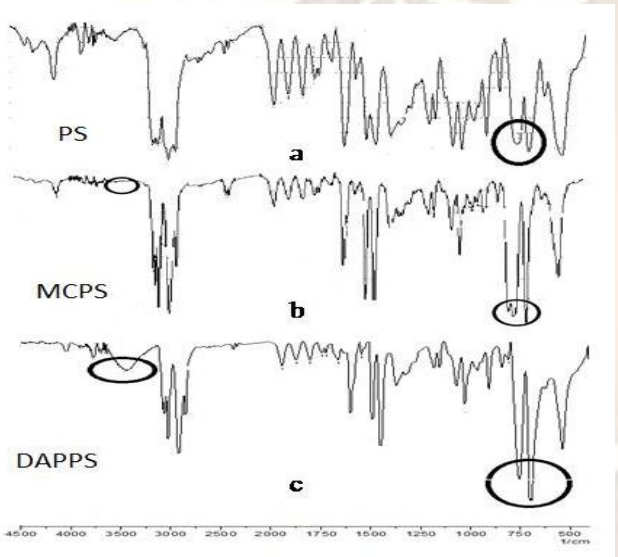


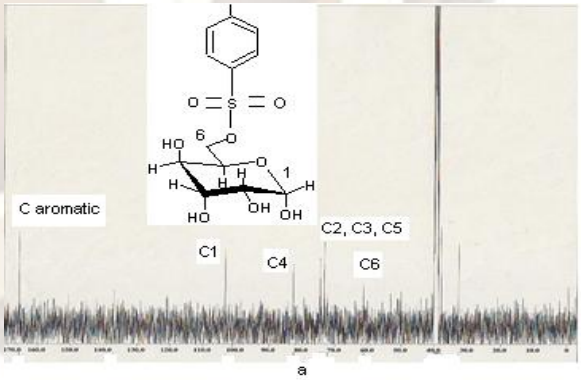
Figure 1. FTIR spectra of PS (a), CMPS (b), and DAPPS (c)

The elemental analysis of PS, CMPS and DAPPS can be used to support the structures beside FTIR spectra, Table 1. In CMPS, the weight percentage of rest (except C, H, N), 6.14%, increases compare to PS (2.58%). It indicates some chlorides have attached to PS (3.56% or 1 mmol/gr resin). Volhards method that digested chloride from MCPS also can be used qualitatively as supporting data to IR spectra. The nitrogen percentage increase from 0.05 in MCPS to 0.35% in DAPPS, relates to the attachment diaminopropane to resin MCPS. It supported the appearance peak at 3500 cm^{-1} in DAPPS FTIR spectra. The substitution degree of diaminopropane is 25% or 25 mmol/gr resin.

Table 1. Elemental analysis of PS

	% Weight			mmol/gr				
	H	C	N	Rest mean	Cl	Cl	N	Cyclo dextrin
PS1	7.78	90.01	0.1	2.58				
PS2	7.73	89.13	0.09					
MCPS1	7.28	87.2	0	6.14	3.56	1		
MCPS2	7.16	85.98	0.1					
DAPPS1	7.7	91.16	0.37	0.785			0.25 (25%)	
DAPPS2	7.69	91.18	0.33					
DAPPS -Cyclo1	7.75	91.8	0.13	1.61				0.205 (82%)
DAPPS -Cyclo2	7.37	89.73	0					

The NMR ($_{13}$C and $_{1}$H) spectra of β-CDOTS is in agree with NMR spectra of 6-O-(paratosyl)-β-cyclodextrin that has been reported [11]. Shifts of $_{1}$H NMR spectra are at 7.6 (d), 7.3 ppm (d). These shifts relate to two aromatics hydrogen from tosyl. Two shifts at 3.5 ppm (m) and 3.7 ppm (m) relate to methylene hydrogen in cyclodextrin. Shift at 4.9 ppm (d) relates to hydrogen in methyl at cyclodextrine (C6) that is bound to tosyl, and at 3 ppm (s), H without neighbor, relates to methyl aromatic from tosyl. The $_{13}$C NMR spectra of β-CDOTS (Figure 2) shows shifts at 165.6 ppm, (C aromatic), 6 shifts at 103.3, 82.6, 74.4, 73.3, 73.2, 61.4 ppm (6 C from cyclodextrin), and at 30 ppm (C methyl from tosyl).



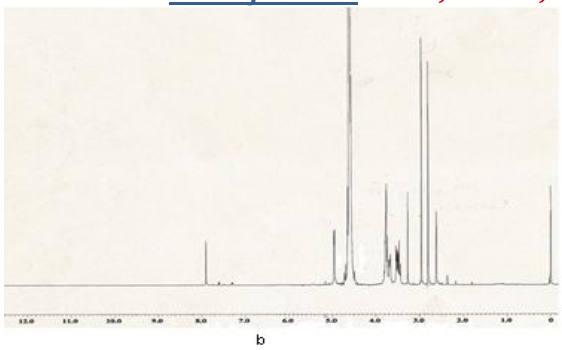


Figure 2. β-CDOTS $_{13}C$ (a) and $_{1}H$ (b) NMR spectra in DMSO

To react β-CDOTS with DAPPS, the solvent that can dissolves both is found out. β-CDOTS dissolves in DMSO and DMF, whereas DAPPS dissolves only in DMF. The reaction between β-CDOTS and DAPPS was carried out without catalyst using DMF as a solvent. The reaction was estimated by elemental analysis. After 24 hours reaction, the percentage of nitrogen decreases in the resin resulted. The decreasing of nitrogen percentage, from 0.35% to 0.065%, and increasing carbon percentage relates to increasing of carbon due to carbon in cyclodextrin (Table 1). The increasing of rest, 0.785 to 1.61%, relates to oxygen in β-cyclodextrin. The nitrogen per carbon percentage ratio was calculated every 24 hour from elemental analysis data. The conversion was calculated by equation below.

$$\text{Conversion} = \frac{(\text{N/C DAPPS} - \text{N/C(time)})}{\text{N/C DAPPS}} \times 100\%$$

The relation between time consumption and conversion can be shown on Figure 3. The highest conversion (82%) was achieved in 24 hours. After that conversion decreases and increases again after 96 hours. The conversion states the degree of substitution. Eighty two percentage of conversion is as same as 0.205 mmol β-cyclodextrin attached to 1 gr of DAPPS. Substitution degree of β-cyclodextrin to DAPPS is 82%

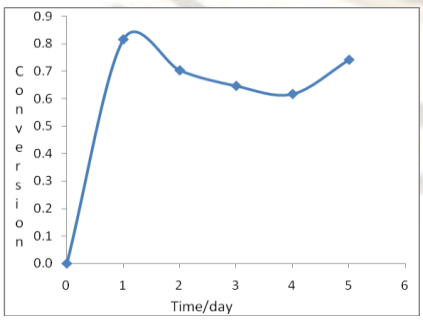


Figure 3. Conversion reaction between PSDAP and β-CDOTS in time

The DAPPS-cyclodextrin solid $_{13}C$ NMR CP MAS spin 5 kHz spectra shows (Figure 4) board shifts at 182-210 ppm (spinning side band, SSB), 165-175 ppm (C=O, polymethyl methacrylate in rotor cap), 145-150 ppm (C aromatic quaternary in polystyrene), 125-135 ppm (C-H aromatic or C aromatic tertiary) and 38-45 ppm (CH_2CH-Ar) and 10 ppm (CH_3-CH_2-Ar). These shifts agree with polystyrene $_{13}C$ solid NMR that has been reported [13]. Board shifts at 104-110 ppm (C1), 85-95 ppm (C2, C3, C5), 65-70 ppm (C6) come from cyclodextrin [14] and shift at 45-55 ppm comes from aliphatic carbon in diaminohexane that overlap with alkane group in polystyrene backbone. Shift that related to C4 in cyclodextrin appear at 82 ppm when spin was increased to 7 KHz.

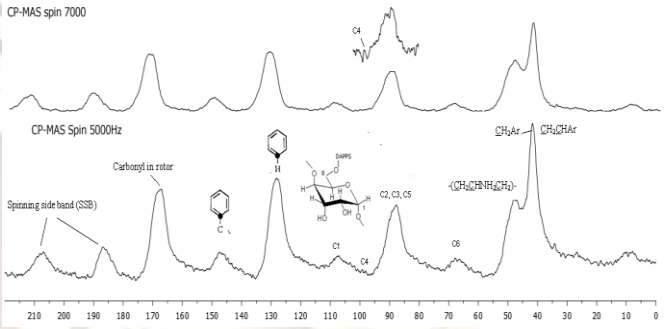


Figure 4. solid $_{13}C$ NMR CP MAS spin 5 kHz spectra of DAPPS-cyclodextrin

The morphology of resin can be shown in Figure 5 and Figure 6. TEM photograph showed that bead of polystyrene was non-porous [9] but TEM photograph of functioned polystyrene by diaminopropane successive β-cyclodextrin shows some pores. Significant deformation of polystyrene resin occurred after it was functioned. SEM Photograph shows that the particle is spherical shape and agglomeration has non-homogenous size. The particle size is in between 5-20 µm. In chromatography, particle size influences efficiency of separation and pressure drop. Practically particle size also influences the inner diameter of the column to be used.

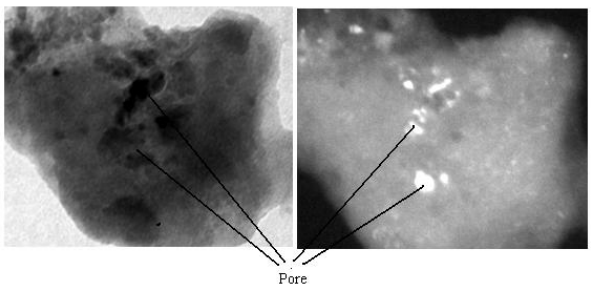
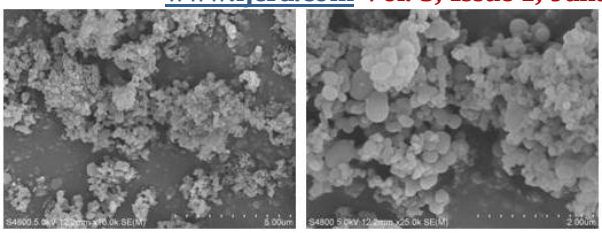


Figure 5. TEM photograph of functionalized linear polystyrene with diaminopropane and β-cyclodextrin



a b

Figure 6. SEM photograph of functionalized linear polystyrene with diaminopropane and β-cyclodextrin (a) 10 x (b) 25 x

IV. CONCLUSION

This paper described the functioning of polystyrene with diaminopropane successive β-cyclodextrin. Some stages were done without catalyst through choosing adequately condition of reaction. The functioned polystyrene was characterized by elemental analysis, spectroscopy such as FTIR, $_{13}$C solid NMR and microscopic methods. Chromatographic application of functioned polystyrene will be described elsewhere.

ACKNOWLEDGEMENTS

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