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A novel β-cyclodextrin polymer/tungsten carbide composite matrix for expanded bed adsorption: Preparation and characterization of physical properties

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ABSTRACT

A series of matrices named CroCD–TuC are prepared by the reversed-phase suspension crosslinking technique for expanded bed adsorption. The matrices have regular spherical shape, and follow logarithmic normal size distribution with the range of 80–320 μm and mean diameter of 143–168 μm. The densifier of tungsten carbide embedded within the β-cyclodextrin polymer skeleton causes a significant increase of particle density. The maximum wet density of matrices is 2.09 g cm^{-3}, which shows a potential for the operation in an expanded bed at high flow rate. The matrices also have suitable water content and pore properties for the chromatographic separation use. Moreover, the addition of tungsten carbide improves the rigidity of the matrices, which can tolerate high pressure at high flow rate and show low compressibility in the column. The new matrices can also be ionized in NaOH solution, which may provide some beneficial information for peculiar usage.

1. Introduction

Expanded bed adsorption (EBA), a promising and practical technique for integrated bioseparation, has been applied to capture bioactive molecules directly from unclarified suspension liquid and purify the bio-molecules in high efficiency (Anspach, Curbelo, Hartmann, Garke, & Deckwer, 1999; Chase, 1994; Hjorth, 1997; Hubbuch, Thömmes, & Kula, 2005). EBA processes should use specially designed adsorbents, which expand in the column with upward fluid and perform as a stably classified fluidized bed (Thömmes, 1997). Therefore, the matrices for expanded bed have some special requirements, such as regular spherical shape, proper size and density, size and/or density distribution, which can ensure the stability of expanded bed and excellent hydrodynamic properties in the fluid (Pai, Gondkar, & Lali, 2000; Pålsson, Gustavsson, & Larsson, 2000). Moreover, the EBA matrices should have good mechanical strength and chemical stability for repeating use, and they should be modified to fabricate functionalized adsorbents. Polysaccharides like agarose and cellulose are most commonly used as the supporting materials for the chromatographic matrices. These hydrophilic materials with large amounts of hydroxyl groups can be easily activated by epoxides, allyl bromide, divinylsulfone (DVS) etc. and coupled with the ligands to produce ion-exchange, hydrophobic interaction, affinity chromatography adsorbents for various applications (Zhao, Yao, & Lin, 2009).

Cyclodextrins (CD) are a series of oligosaccharides containing six, seven, and eight D-glucopyranose units, named as α-, β-, and γ-CD, respectively. A CD molecule has a hydrophobic hollow cavity which can form inclusion complexes with many guest compounds, with a requirement that the guests should fit into the cavity, even if only partially (Harada, 2001; Li & Purdy, 1992). CDs also have plenty of hydroxyl groups on the outer surface of the molecule that can be selectively modified with various reagents to form functionalized derivatives (Khan, Forgo, Stine, & D'Souza, 1998). CD polymer beads are a hydrophilic water-insoluble material having been used as a supporting matrix in the chromatographic separations (Wiedenhof, 1969b; Smolková-Keulemansová, 1982). Solms and Egli (1965) reported the preparation of CD polymers in concentrated sodium hydroxide solution with epichlorohydrin. After drying, comminuting and sieving, the compressible powders in an amorphous form were obtained for the packed bed separation. Wiedenhof, Lammers, and van Eck (1969a) prepared CD polymer microspheres by the reversed-phase suspension crosslinking technique with *o*-xylene or methyl isobutyl ketone as the dispersed phase containing nonionic detergent of Nonidet P-40. The CD polymer beads were packed in the column to separate a mixture of amino acids (Wiedenhof, 1969b). Generally, the microspheres show better hydrodynamic properties and mass transfer than the amorphous granules, and might reduce the operating pressure. However, it is found that the CD polymer beads prepared above cannot be used at high flow rate because of the compression and deformation caused by very high pressure drop (Li & Purdy, 1992; Nussstein, Staudinger, & Kreuzer, 1994). Some efforts have

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Nomenclature

a_{MB}	occupied surface area of one MB molecule (m^2)
C_{eq}	equilibrium concentration of MB (mg ml^{-1})
$d_{particle}$	particle diameter (μm)
d_{pore}	mean pore diameter (nm)
F_{compr}	bed compression factor (dimensionless)
H	bed height (cm)
H_0	sedimented bed height in quiescent fluid (cm)
k	dissociation constant in Langmuir adsorption equation (mg ml^{-1})
M	molecular weight of MB (g mol^{-1})
N_{AV}	Avogadro's number (mol^{-1})
P	porosity (dimensionless)
R^2	decision coefficient (dimensionless)
$R_{\mathrm{TuC/CD}}$	mass ratio of TuC/CD (dimensionless)
S	specific surface area (m^2 g^{-1})
Sr	shrinkage of the beads (dimensionless)
U	superficial flow rate (cm min^{-1})

Greek letters	
ΔP	pressure drop (kPa)
Γ	equilibrium adsorption capacity (mg g^{-1})
Γ_∞	saturated adsorption capacity in Langmuir adsorption (mg g^{-1})
ρ_p	wet density of the particles (g cm^{-3})
ω	water content (dimensionless)

Abbreviations	
EBA	expanded bed adsorption
CD	cyclodextrin
DVS	divinylsulfone
MB	methylene blue
PVA	polyvinyl alcohol
TuC	tungsten carbide

been made to overcome the shortcoming of poor rigidity. The first method is to prepare co-polymers with other polymers, such as poly-CD/polyvinyl alcohol (PVA) co-polymeric matrix reported by Szejtli, Fenyvesi, Zoltan, Zsadon, and Tudos (1980). The second method is to use CD derivatives, like hydroxyalkyl-CD polymer beads according to Nussstein et al. (1994), which show preferable rigidity under the pressure as high as 1 MPa. The third one is to cover CD polymers onto the rigid granules (e.g. silica (Carbonnier, Janus, Lekchiri, & Morcellet, 2004; Thuaud, Sebille, Deratani, & Lelievre, 1991) and zirconia (Kim, Choi, Yoon, & Park, 2006)) to form organic–inorganic composite beads. The last is considered as one of the best methods to prepare the rigid CD polymer beads.

CD polymer beads have been applied in expanded bed for protein refolding by Mannen, Yamaguchi, Honda, Sugimoto, and Nagamune (2001). With CD as an artificial chaperone, the monomeric enzyme α-glucosidase was refolded with a yield of two folds at a protein concentration higher than five folds, which indicated a potential application of CD polymer beads as efficient refolding matrix. However, the operation flow rate was relatively low (*ca.* 200 cm h^{-1}) because of low density of the beads. Till now, the applications of CD polymer beads are scarce in the preparative chromatography due to few CD-based matrices available, specially for EBA.

In this work, a novel β-CD polymer/tungsten carbide composite matrix will be developed for the application in EBA. With β-CD polymer as the skeleton, the tungsten carbide (TuC) powder will be used as the densifier to prepare a series of high-density CD-based matrices. The physical properties and mechanical strength of the matrices prepared will be characterized for the potential use as the chromatographic matrix.

2. Experimental

2.1. Materials

β-Cyclodextrin (β-CD, DRAMAX®W7 Pharma) was provided by Maxdragon BioChem. Ltd. (Guangzhou, China). Tungsten carbide (TuC) with the density of 15.1 g cm^{-3} and mean particle diameter of 2–5 μm was obtained from Ganzhou TEJING Tungsten & Molybdenum Co., Ltd. (Ganzhou, China). High-speed vacuum pump oil GS-1 was provided by Beijing SIFANG Special Oil Factory (Beijing, China). Paraffin liquid was purchased from Hangzhou GAOJING Fine Chemical Industry Co., Ltd. (Hangzhou, China). Epichlorohydrin (≥98.5%) was provided by Jiangsu YONGHUA Fine Chemical Co., Ltd. (Wuxi, China). Tween-80 was obtained from Wenzhou QINGMING Chemical Co., Ltd. (Wenzhou, China). Methylene blue (MB) trihydrate was obtained from Shanghai SSS Reagent Co., Ltd. (Shanghai, China). Deionized water was purchased from Hangzhou WAHAHA Group Co., Ltd. (Hangzhou, China). Other chemicals used are of reagent grade or higher quality.

2.2. Preparation of composite beads

The β-CD polymer/TuC composite beads are prepared by the reversed-phase suspension crosslinking technique similar to Nussstein et al. (1994). Generally, 20 g β-CD are dissolved in 40 g 25% (w/w) NaOH solution at 80 °C, and 10 ml epichlorohydrin is added dropwise within 30 min with stirring. The mixture is kept at 80 °C with stirring for a further 1 h. After that, TuC powder is added, and the mixture is dispersed in the mixed-oil containing 2.9 g tween-80, 120 g paraffin liquid and 170 g pump oil GS-1 in a 500 ml flask under agitation at 350–400 rpm for 15 min at 60 °C. Subsequently, 10 ml epichlorohydrin is added rapidly and the mixture is agitated at the same speed for a further 2.5 h at 60 °C. Having been cooled to room temperature, the beads are filtered off and successively washed with 500 ml petroleum ether (bp 60–90 °C), 300 ml of water, and then neutralized with 1 mol L^{-1} HCl. Finally the beads are washed with water and screened with standard sieves, and the β-CD polymer/TuC composite beads with a particle size range of 80–320 μm are obtained, named CroCD–TuC.

2.3. Measurement of physical properties

The particle size distribution and mean particle diameter (d_m) are determined by laser particle size analyzer MASTERSIZER 2000 (Malvern Instruments, UK). The morphology of beads is observed with the microscope Nikon ECLIPSE E200 (Nikon Instruments, Japan).

The main physical properties of CroCD–TuC matrices are measured as follows. Water content (ω) and Shrinkage (Sr) are obtained by dehydration at 110 °C to a constant mass, which are calculated by the decrease of the mass and volume of the sedimented particles in a 10 ml measuring cylinder, respectively. Wet density (ρ_p) is determined by water replacement in a 10 ml gravity bottle. Other physical properties, including porosity (P) and the mean pore diameter (d_{pore}) of the matrices are measured with the methods as reported previously (Xia, Lin, & Yao, 2007).

2.4. Adsorption of MB and calculation of specific surface area

A series of 100 ml conical flask containing 30 ml MB aqueous solution (0.5–10 mg·ml^{-1}) and 3 g wet matrix are shaken on a thermostat oscillator (Taicang Instruments, China) at 25 °C for 30 h. After centrifuging, the supernatant is diluted and analyzed by a spectrophotometer (Ultrospec 3300 *pro*, Amersham Biosciences, Sweden) at 660 nm. The concentration of MB in the supernatant is calculated from the calibration curve, and the adsorption isotherm is fitted by Langmuir adsorption equation:

$$\Gamma = \Gamma_\infty \frac{C_{eq}}{k + C_{eq}} \tag{1}$$

where Γ and C_{eq} are the equilibrium adsorption capacity and MB concentration in supernatant, respectively. Γ_∞ represents the saturated adsorption capacity and k is the dissociation constant. Γ_∞ can be correlated by the data of adsorption isotherm, and thus the specific surface area (S) can be calculated as

$$S = \frac{a_{MB} N_{AV} \Gamma_\infty}{1000 \times M} \tag{2}$$

where a_{MB} is the occupied surface area of one molecule of MB assuming as flat adsorption (135×10^{-20} m^2 (Kipling & Wilson, 1960)), N_{AV} is Avogadro's number (6.022×10^{23} mol^{-1}), M is the molecular weight of MB (373.9 g mol^{-1}, trihydrate).

2.5. Characterization of mechanical properties

The mechanical strength of the matrices is evaluated by determining the curve of pressure drop *vs.* flow rate in a packed-column (Nussstein et al., 1994). The experiments are conducted with ÄKTA Explorer 100 chromatographic system (Amersham Biosciences, Sweden). A XK16/20 column (16 mm diameter, 20 cm length, from Amersham Biosciences, Sweden) is used in which 20 ml CroCD–TuC beads are packed. The mobile phase is deionized water, and the maximum flow rate and pressure are set at 50 ml min^{-1} and 0.65 MPa, respectively. Proper column vertical alignment is assured in all experiments. The corresponding pressure drop (ΔP) and bed height (H) at the setting superficial flow rate (U) are measured and evaluated.

The bed compression factor (F_{compr}), another parameter to characterize the mechanical strength of matrix, is calculated as,

$$F_{compr} = \frac{H}{H_0} \tag{3}$$

where H_0 is the height of sedimented bed in quiescent fluid, and H is the height of sedimented bed in the mobile phase at different flow rates. The value of F_{compr} will be less than 1.0 if the bed has been compressed.

2.6. Measuring of chemical stability in acid or alkali

3.00 g wet matrix CroCD–TuC 4 is weighed precisely and added into 100 ml conical flasks containing 30 ml HCl or NaOH solution (0.01, 0.1, and 1 mol L^{-1}). The flasks are shaken on a thermostat oscillator (Taicang Instruments, China) at 30 °C for 10 h, 20 h and 30 h, respectively. After washed with 20 ml deionized water twice and filtered with 3$^\#$ sintered-sand filter (pore diameter of 15–40 μm), the matrix is weighed precisely and compared with the initial mass. The relative mass is calculated, and the chemical stability in acid or alkali is evaluated by mass decrease of the matrix.

3. Results and discussion

3.1. Preparation of the composite beads

β-CD monomers can be crosslinked with epichlorohydrin in alkaline solution to form the CD polymer, and the reaction has been described by Fenyvesi, Szilasi, Zsadon, Szejtli, and Tudos (1981). Generally, a suitable ratio of *β*-CD to epichlorohydrin is between 1:1 and 2:1 (w/w). The concentration of NaOH in the reaction mixture is depended on the amount of crosslinking reagent added, and preferably ranged from 20% to 30% (w/w) (Nussstein et al., 1994). The *β*-CD polymer obtained is bright solution of golden color when the mass percentage of epichlorohydrin is not more than 20% in the mixture. Whereas higher concentration of epichlorohydrin and NaOH will make the solution cloudy with lots of micro-solids formed during the reaction process. In our present work, 25% (w/w) NaOH solution is used, and the concentrations of CD and epichlorohydrin are 28% (w/w) and 16% (w/w), respectively.

It is found that the dispersing medium and the preparation conditions show significant influences on the shape and size distribution of composite particles prepared. In the present work, several kinds of oil phases are chosen as dispersing medium, and most are not so viscous that the beads formed are too large. But the pump oil GS-1 has much higher viscosity that the beads size is suitable at about 120 μm, indicating that increasing the viscosity of the dispersing medium results in the increase of droplet stability by decreasing the droplet collision frequency (Dowding & Vincent, 2000). In order to control the viscosity of dispersion medium, the mixture of pump oil GS-1 and paraffin liquid is used, and the results are shown in Fig. 1. It is found that the particle size increases with the increase of paraffin liquid percentage in the mixture. The proper particle size distribution can be obtained at the optimal paraffin liquid percentage of 27.8% with the particle size of about 70–400 μm before sieving. The dispersant tween-80 is added into the dispersing medium to prevent the conglutination of the beads, and its concentration is optimized as 1.0% by weight based on the mixed oil. Agitating speed is another important factor on the shape and size distribution of composite beads and it is found the particle size diminishes as agitation becomes more violent. Moreover, the droplets easily agglomerate at high agitation speed and the shape of particles becomes elliptic, rod-like or other unexpected irregular shapes. Therefore, the proper agitating speed is chosen as 350–400 rpm for the mean diameter of about 150 μm.

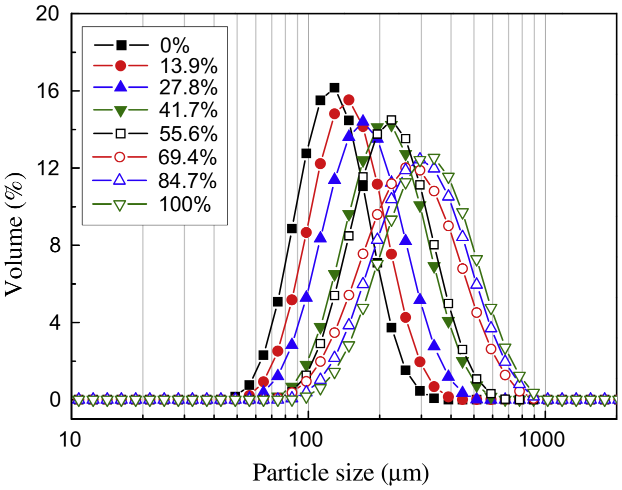

Fig. 1. Particle size distribution of composite beads as the function of the mass percentage of paraffin liquid in the mixture oil.

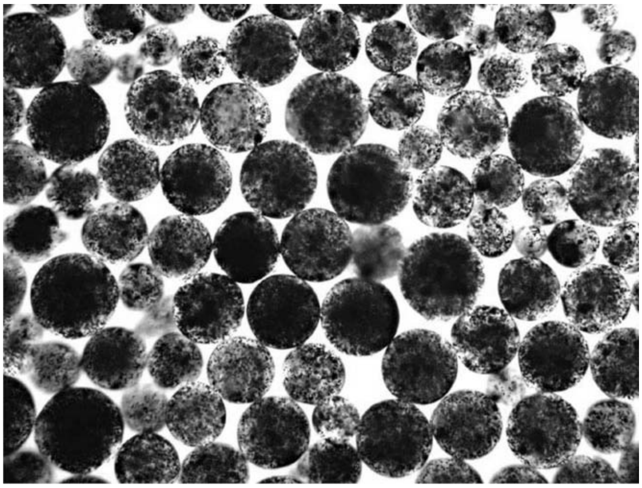

Fig. 2. Photograph of particle appearance under the optical microscope (100×).

Typically, the yield of composite beads for one batch is about 80% (by volume) at the optimum preparation conditions mentioned above. The beads prepared are named as CroCD–TuC 1–CroCD–TuC 7 according to the mass ratio TuC/CD of 0, 1.0, 1.75, 2.5, 3.25, 4.0 and 5.0, respectively. The photograph of bead appearance is showed in Fig. 2. It can be seen that the particles have regularly spherical shape without conglutinated beads, and TuC powder is embedded well in the CD crosslinked polymer.

3.2. Size distribution

The particle size distribution of CroCD–TuC 1–CroCD–TuC 7 composite beads is shown in Fig. 3. It can be seen that all the matrices prepared follow good logarithmic normal distribution and are similar to each other. The mean diameters of varying CroCD–TuC beads just slightly change within the range of 143–168 μm, indicating that the addition of TuC powder hardly influenced the size of composite beads.

3.3. Density, water content and shrinkage

As shown in Fig. 4, the wet density of matrix increases almost linearly from 1.11 to 2.00 g cm^{-3} as the ratio TuC/CD increases from 0 to 4.0. The relationship between them is expressed as following equation Eq. (4) with a decision coefficient (R^2) above 0.99:

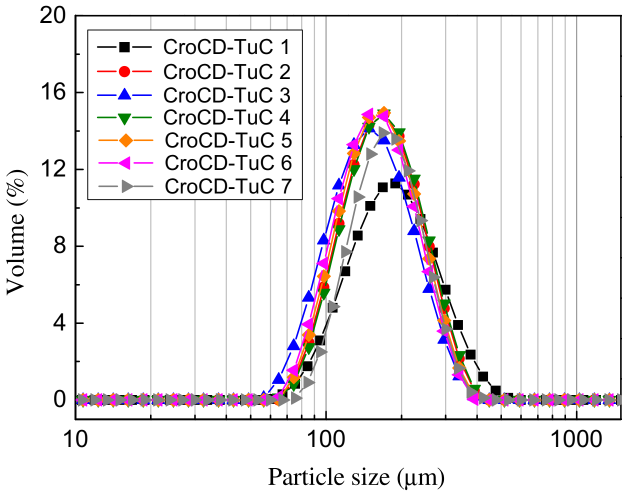

Fig. 3. Size distribution of CroCD–TuC beads.

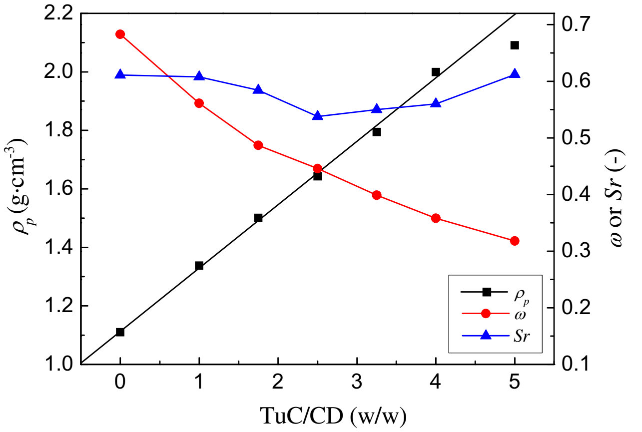

Fig. 4. Wet density, water content and shrinkage of CroCD–TuC beads as the function of TuC/CD (w/w).

$$\rho_p = 1.113 + 0.217 \cdot R_{\mathrm{TuC/CD}} \tag{4}$$

where $R_{\mathrm{TuC/CD}}$ represents the mass ratio of TuC/CD. It means the matrix density can be accurately controlled by the amount of TuC added in the preparation process. However, it is a failure to calculate the density of CroCD–TuC 7 (2.09 g cm^{-3}), which is much lower than the calculated value (2.20 g cm^{-3}), due to the leakage of TuC powder in the bead-forming procedure.

Fig. 4 also shows water content and shrinkage of the CroCD–TuC matrices. Water content decreases remarkably as increasing the ratio of TuC/CD, but the shrinkage of the matrices just slightly changes in the range of 0.53–0.61. The reason is that water content is calculated based on the weight of whole particles, but shrinkage is calculated on the basis of the sedimented bed volume. When the ratio of TuC/CD increases, TuC powder gradually occupies the interspace inside the CD polymer skeleton, resulting in the sharp increasing on the weight of whole particles. However, the volume percentage of TuC in the matrix is just *ca.* 8.5% even at the TuC/CD ratio of 5.0 (the calculation method refers to Miao, Lin, and Yao (2005)). That means TuC powder just occupies a small volume of matrix interspace. Since the interspace volume inside the matrix just changes slightly, the shrinkage is somewhat independent of the amount of TuC addition.

3.4. Pore properties

Pore properties including porosity, specific surface area and mean pore diameter of CroCD–TuC beads are determined and the results are described in Fig. 5. Generally, the porosity just slightly

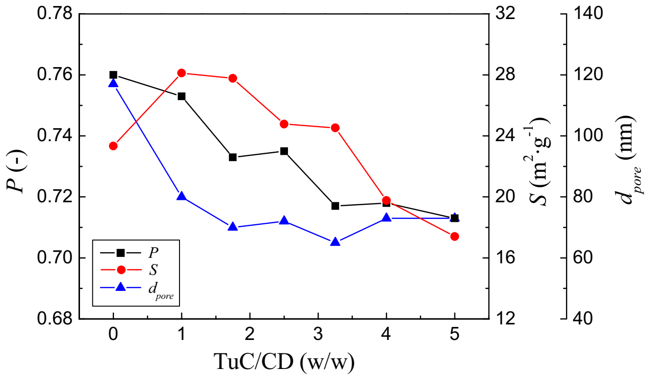

Fig. 5. Porosity, specific surface area and mean pore diameter of CroCD–TuC beads as the function of TuC/CD (w/w).

varies in the range of 0.71–0.76, although it decreases gradually as the ratio of TuC/CD increases. The results demonstrate the effect of TuC/CD ratio on the porosity is not remarkable.

Specific surface area of CroCD–TuC beads is determined by MB adsorption in aqueous solution. The planar molecule MB can easily replace the water molecules adsorbed on the sites of CD skeleton surface, and then associates with the skeleton by a hydrogen-bonding interaction between the atoms of N and S in MB and the hydroxyl groups on the skeleton (Kipling & Wilson, 1960; Kaewprasit, Hequet, Abidi, & Gourlot, 1998). Based on the adsorption isotherm of MB at 25 °C (Fig. 6), the experimental data are following Langmuir isothermal equation, indicating the monolayer adsorption assumption of MB on the pore surface of the matrices. The saturated adsorption capacity of CroCD–TuC matrices generally decreases with TuC/CD ratio increases, as a result the specific surface area of the matrices gradually decreases with TuC/CD ratio increases as described in Fig. 5, probably due to the decline of CD polymer content in the matrices.

As seen in Fig. 5, the addition of TuC in the matrix may cause a decrease of the pore diameter. However, most of matrices have the mean pore diameter of 65–80 nm, when the TuC/CD ratio is higher than 1.0.

3.5. Mechanical strength

Fig. 7 is the results of pressure drop *vs*. flow rate in a packed bed. Here $\Delta P/H$ is used in order to eliminate the influence of different sedimented bed height. It is obvious that the pressure drop of the packed bed shows continuous rise with increasing flow rate up to 22 cm min^{-1}. It shows a mainly decreasing tendency of pressure drop at the same flow rate with the increase of TuC/CD ratio, but no remarkable changes from CroCD–TuC 2 to 7. An abnormally high pressure drop is found for CroCD–TuC 1 compared with other matrices tested. CroCD–TuC 1 is produced without TuC added, and it shows notable compressibility, as revealed in Fig. 7, the plot of bed compression factor (F_{compr}) *vs*. flow rate. As flow rate increases to 16 cm min^{-1}, F_{compr} reduces to 0.8 for CroCD–TuC 1 and 0.91 for CroCD–TuC 2, but it shows just slight decrease on F_{compr} for CroCD–TuC 3 to 7. The results indicate that adding proper amount of TuC can greatly improve the rigidity of the matrices.

3.6. Chemical stability in acid or alkali

As mentioned in Section 2.6, the chemical stability of matrix CroCD–TuC 4 in acid or alkali is evaluated by mass decrease of the matrices. Fig. 8 reveals the relative mass change in HCl and NaOH solutions of different concentration. It is found that the mass of matrix in HCl nearly remains unchanged even after 30 h at 30 °C. Moreover, there is no evidence that TuC powder leaks out from the beads, indicating the matrices are stable in HCl in this condition. However, there is a surprising mass increase for the matrix soaked in NaOH solutions. It is considered that the hydroxyl groups at the C_2-position are the most acidic ($pK_a = 12.2$ (Li & Purdy, 1992)) of the three types (C_6-, C_2-, and C_3-position) of hydroxyl groups present in CDs, thus they will be deprotonated in strong basic conditions as a result (Khan, Forgo, Stine, & D'Souza, 1998; Rong & D'Souza, 1990). The increased mass of matrix derives from the ionization of CD skeleton and the formation of sodium salts in the solid phase. Also described in Fig. 8, larger amount of sodium salts formed as the NaOH concentration increases, and the maximum increment can reach *ca.* 15% (w/w) for CroCD–TuC 4 in 1 mol L^{-1} NaOH solution, which can be explained by the inhibition of hydrolysis of ionized CD in concentrated NaOH solution. The ionized CD-based matrix will probably provide amazing applications in ion-exchange adsorption.

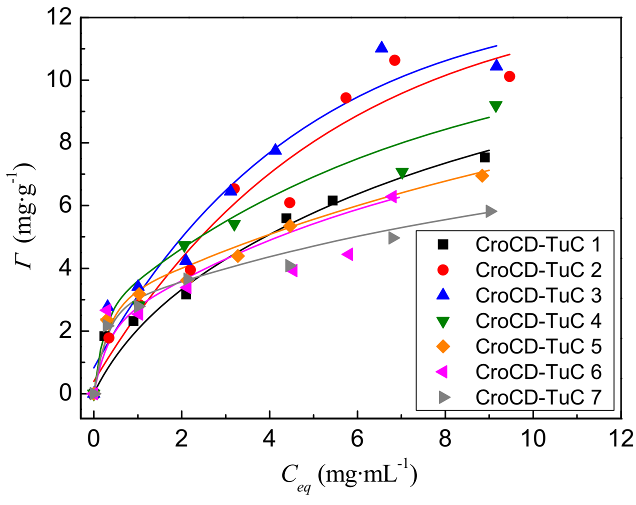

Fig. 6. Adsorption isotherm of CroCD–TuC beads adsorbing MB in aqueous solution.

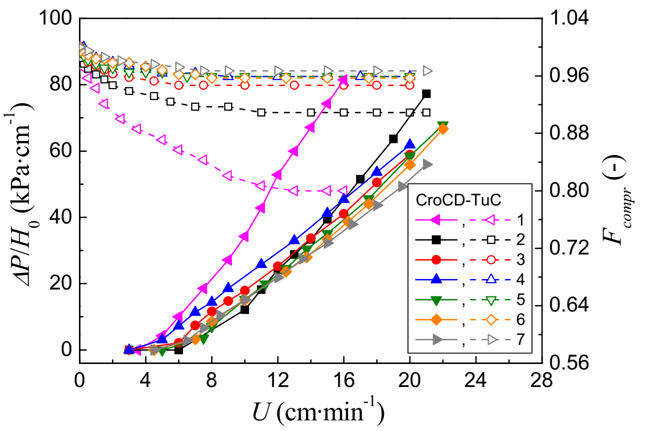

Fig. 7. Pressure drop (solid symbols) and bed compression factor (open symbols) as the function of flow rate for CroCD–TuC beads.

4. Conclusions

New series of β-CD polymer/TuC composite matrices, CroCD–TuC, are prepared by reversed-phase suspension crosslinking

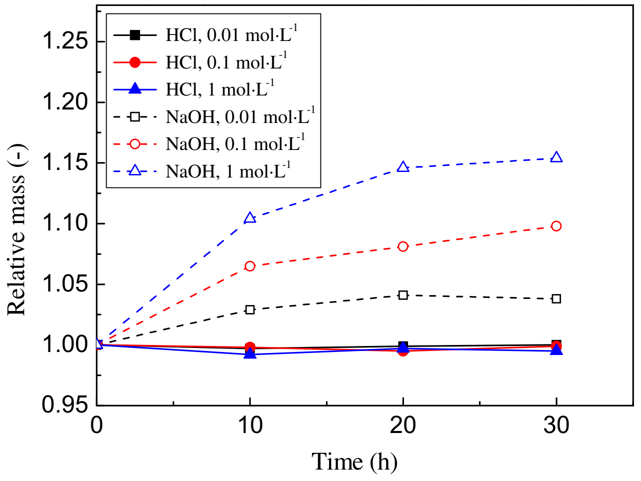

Fig. 8. The relative mass change of CroCD-TuC beads in HCl or NaOH solutions at 30 °C.

technique. The preparation process is investigated and optimized, and CroCD–TuC matrices are obtained with regular spherical shape and a proper diameter of 80–320 μm. With the control of TuC addition, CroCD–TuC matrices with varying density are prepared. The main physical properties are characterized and evaluated in detail. The size distribution of the matrices follows the logarithmic normal distribution, and the mean diameter slightly changes within the range of 143–168 μm. The matrices have maximum wet density of 2.09 g cm^{-3}, which is beneficial for the operation in expanded bed at high flow rate. The matrices also have suitable water content and pore properties for the use as the chromatographic medium. It is found that the rigidity of the matrices can be greatly improved by the addition of TuC, which means the CroCD–TuC composite matrices can tolerate high pressure and high flow rate in the column. Furthermore, the matrices can be ionized in NaOH solution, which may behave as an ion-exchange resin and show amazing applications. More beneficial information for developing the EBA adsorbents will be proposed, and the expansion and hydrodynamic properties in expanded bed will be studied in detail in the coming work.

Acknowledgments

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References

Anspach, F. B., Curbelo, D., Hartmann, R., Garke, G., & Deckwer, W.-D. (1999). Expanded-bed chromatography in primary protein purification. *Journal of Chromatography A, 865*, 129–144.

Carbonnier, B., Janus, L., Lekchiri, Y., & Morcellet, M. (2004). Coating of porous silica beads by in situ polymerization/crosslinking of 2-hydroxypropyl β-cyclodextrin for reversed-phase high performance liquid chromatography applications. *Journal of Applied Polymer Science, 91*, 1419–1426.

Chase, H. A. (1994). Purification of proteins by adsorption chromatography in expanded beds. *Trends in Biotechnology, 12*, 296–303.

Dowding, P. J., & Vincent, B. (2000). Suspension polymerisation to form polymer beads. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 161*, 259–269.

Fenyvesi, E., Szilasi, M., Zsadon, B., Szejtli, J., & Tudos, F. (1981). Water-soluble cyclodextrin polymers and their complexing properties. In J. Szejtli (Ed.), *Proceedings of the 1st International Symposium on Cyclodextrins* (pp. 345–356). Dordrecht: D. Reidel.

Harada, A. (2001). Cyclodextrin-based molecular machines. *Accounts of Chemical Research, 34*, 456–464.

Hjorth, R. (1997). Expanded-bed adsorption in industrial bioprocessing: Recent developments. *Trends in Biotechnology, 15*, 230–235.

Hubbuch, J., Thömmes, J., & Kula, M. R. (2005). Biochemical engineering aspects of expanded bed adsorption. *Advances in Biochemical Engineering/Biotechnology, 92*, 101–123.

Kaewprasit, C., Hequet, E., Abidi, N., & Gourlot, J. P. (1998). Quality measurements-application of methylene blue adsorption to cotton fiber specific surface area measurement: Part I. Methodology. *Journal of Cotton Science, 2*, 164–173.

Khan, A. R., Forgo, P., Stine, K. J., & D'Souza, V. T. (1998). Methods for selective modifications of cyclodextrins. *Chemical Reviews, 98*, 1977–1996.

Kim, I. W., Choi, H. M., Yoon, H. J., & Park, J. H. (2006). β-Cyclodextrin-hexamethylene diisocyanate copolymer-coated zirconia for separation of racemic 2, 4-dinitrophenyl amino acids in reversed-phase liquid chromatography. *Analytica Chimica Acta, 569*, 151–156.

Kipling, J. J., & Wilson, R. B. (1960). Adsorption of methylene blue in the determination of surface areas. *Journal of Applied Chemistry, 10*, 109–113.

Li, S., & Purdy, W. C. (1992). Cyclodextrins and their applications in analytical chemistry. *Chemical Reviews, 92*, 1457–1470.

Mannen, T., Yamaguchi, S., Honda, J., Sugimoto, S., & Nagamune, T. (2001). Expanded-bed protein refolding using a solid-phase artificial chaperone. *Journal of Bioscience and Bioengineering, 91*, 403–408.

Miao, Z.-J., Lin, D.-Q., & Yao, S.-J. (2005). Preparation and characterization of cellulose-stainless steel powder composite particles customized for expanded bed application. *Industrial and Engineering Chemistry Research, 44*, 8218–8224.

Nussstein, P., Staudinger, G., & Kreuzer, F.-H. (1994). Cyclodextrin polymers and process for their preparation. US Patent Office, Pat. No. 5 360 899.

Pai, A., Gondkar, S., & Lali, A. (2000). Enhanced performance of expanded bed chromatography on rigid superporous adsorbent matrix. *Journal of Chromatography A, 867*, 113–130.

Pålsson, E., Gustavsson, P.-E., & Larsson, P.-O. (2000). Pellicular expanded bed matrix suitable for high flow rates. *Journal of Chromatography A, 878*, 17–25.

Rong, D., & D'Souza, V. T. (1990). A convenient method for functionalization of the 2-position of cyclodextrins. *Tetrahedron Letters, 31*, 4275–4278.

Smolková-Keulemansová, E. (1982). Cyclodextrins as stationary phases in chromatography. *Journal of Chromatography, 251*, 17–34.

Solms, J., & Egli, R. H. (1965). Harze mit Einschlusshohlräumen von Cyclodextrin-Strucktur. *Helvetica Chimica Acta, 48*, 1225–1228.

Szejtli, J., Fenyvesi, E., Zoltan, S., Zsadon, B., & Tudos, F. (1980). Cyclodextrin–poly (vinyl alcohol) polymeric compositions in film, fiber, bead, and block polymers. DE Patent Office, Pat. No. 2 927 733.

Thömmes, J. (1997). Fluidized bed adsorption as a primary recovery step in protein purification. *Advances in Biochemical Engineering/Biotechnology, 58*, 185–230.

Thuaud, N., Sebille, B., Deratani, A., & Lelievre, G. (1991). Retention behavior and chiral recognition of β-cyclodextrin-derivative polymer adsorbed on silica for warfarin, structurally related compounds and Dns-amino acids. *Journal of Chromatography, 555*, 53–64.

Wiedenhof, N., Lammers, J. N. J. J., & van Eck, C. L. (1969a). Properties of cyclodextrins. III. Cyclodextrin–epichlorohydrin resins. Preparation and analysis. *Stärke, 21*, 119–123.

Wiedenhof, N. (1969b). Properties of cyclodextrins. IV. Features and use of insoluble cyclodextrin–epichlorohydrin resins. *Stärke, 21*, 163–166.

Xia, H.-F., Lin, D.-Q., & Yao, S.-J. (2007). Preparation and characterization of macroporous cellulose–tungsten carbide composite beads for expanded bed applications. *Journal of Chromatography A, 1175*, 55–62.

Zhao, J., Yao, S.-J., & Lin, D.-Q. (2009). Adsorbents for expanded bed adsorption: Preparation and functionalization. *Chinese Journal of Chemical Engineering, 17*, 678–687.