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Preparation and Sugar Binding Property of Microspheres Having Surface-anchored Phenylboronic Acid Groups

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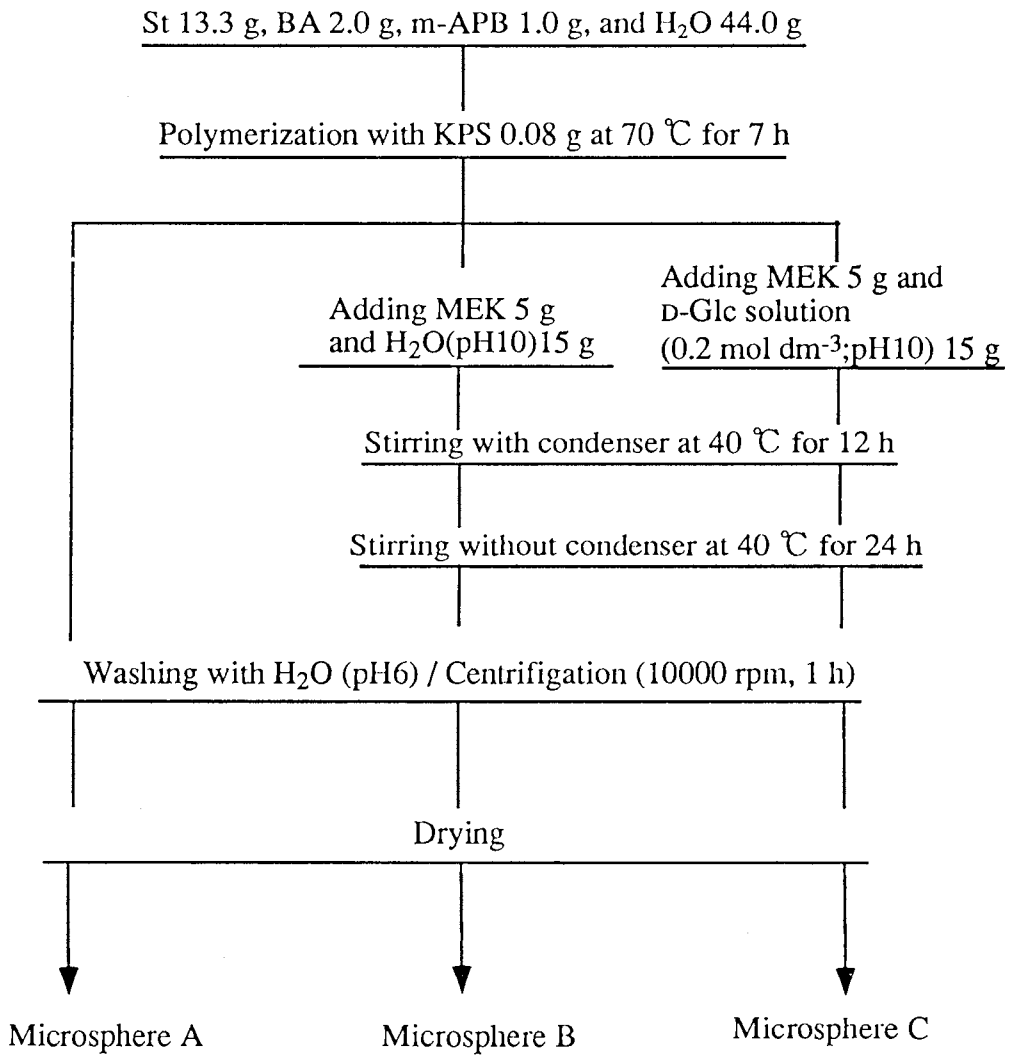
Microspheres having phenylboronic acid groups on the resin surface were successfully prepared by emulsion polymerization of styrene, butyl acrylate, and m-acrylamidophenylboronic acid. The microspheres were swollen in an alkaline aqueous suspension by adding methylethylketone so that phenylboronic acid groups in the resin migrate out to the aqueous-organic interface in their ionized forms. D-Glucose was bound onto the microsphere by complex (ester) formation between phenylboronic acid moiety and *cis*-diol group of D-Glucose.

Microspheres from synthetic polymers have attracted much attention for functional materials development.[1] They have a large specific surface which is not due to the porosity of the resin but to their small physical size. This enables one to carry out reactions such as complexation, catalysis and so on at the surface of the microsphere in a substantial mass scale. Such reactions can proceed rapidly and show better reversibility as compared to the similar reactions that take place in the resin matrix. In addition, the microspheres can be handled by column or centrifugation separation technique. These features make microspheres especially attractive to analytical and separation purposes. Okubo *et al.* reported a preparation of surface-carboxylated microspheres by seeded emulsion polymerization.[2] We took advantage of this procedure and developed a new technique of surface-imprinting on the microspheres by metal ions.[3]

On the other hand, recognition and separation of saccharides have been recently studied in several research groups.[4-10] Boronic acid is a powerful tool for this purpose since it can form a stable complex (ester) with *cis*-diol in basic conditions.[8-10] In the present work, we have successfully prepared a microsphere having phenylboronic acid structure on its surface. The obtained microspheres are expected to be useful for separation of biological constituents comprising a *cis*-diol group.

Three methods were used for the preparation of microspheres as seen in Scheme 1. In a flask (200 cm^3) fitted with a condenser were placed styrene (St, 13.3 g), butyl acrylate (BA, 2.0 g), m-acrylamidophenylboronic acid[11] (m-APB, 1.0 g), and water (H_2O, 44.0 g). The mixture was stirred vigorously with a magnet. An emulsion polymerization was carried out under N_2 atmosphere at 70 ℃ and pH6 for 7 h by use of potassium peroxodisulfate (KPS, 0.08 g) as an initiator. The obtained emulsion solution was divided into three portions and each portion (20 cm^3) was subjected to the following treatments to give Microsphere A, B, and C, respectively.

The first portion of emulsion was centrifuged (10000 rpm, 1 h) and the supernatant solution was replaced



Scheme 1. Procedures of syntheses for Microsphere A, B, and C.

with pure water. The re-suspended emulsion was again centrifuged and the microsphere precipitated was dried to give Microsphere A. To the second portion of emulsion methylethylketone (MEK, 5 g) and H_2O (15 g) were added. The pH was adjusted to 10 by adding aqueous potassium hydroxide. The emulsion was stirred at 40 ℃ for 12 h in a flask fitted with a condenser and then further stirred for 24 h without a condenser to remove MEK. The emulsion was subjected to centrifugation - washing - centrifugation procedure and the precipitate was dried to give Microsphere B. To the third portion of emulsion were added MEK (5 g) and D-glucose (D-Glc) (Merck) solution (2.0×10^{-1} mol dm^{-3}, 15 g). After the pH was adjusted to 10, the mixture was treated similarly to that for Microsphere B to give Microsphere C.

These microspheres were confirmed to have average diameters of about 0.5 μm as determined by particle grading analysis. Figure 1 shows a typical view of Microsphere B by scanning electron microscopy. The microspheres were neatly spherical and uniform. Similar particle form and distribution pattern were observed for Microsphere A and C.

The binding of saccharides on microspheres was examined by using D-Glc and 1-methyl- α -D-glucoside (Tokyo Kasei Kogyo Co., Ltd.) as probes. Microspheres (0.2 g) were added to a saccharide solution (2.5 x 10^{-4} mol dm^{-3}, 1.0 cm^3; pH 5.8 - 10.8, Na_2HPO_4 / NaH_2PO_4 or Na_2CO_3 / $NaHCO_3$ buffer solutions) and the mixture was left at 20 ℃ for 1.0 h. After centrifugation, the supernatant solution was analyzed for saccharide concentration by phenol-sulfuric acid method.[12] The saccharide binding ability of microspheres was expressed as percentage of bound saccharide to the total saccharide added.

The binding of D-Glc onto Microsphere A, B, and C at pH10.8 were 51, 73, and 61%, respectively. The improved binding ability of Microsphere B over Microsphere A should be ascribed to the MEK-alkali treatment.[13] With this treatment, St-BA-APB emulsion particles become swollen by the added MEK so that arylboronic acid groups should migrate effectively to the aqueous-organic interface to turn in its anionic form (pKa of phenylboronic acid is reported to be 8.7.[14]).

In the preparation of Microsphere C, the obtained polymer emulsion was thoroughly washed with H_2O (pH6) until D-Glc was not detected in the wash solution. The D-Glc binding ability of Microsphere C became smaller than that of Microsphere B. The origin of this effect is not yet clear. It is possible that the D-Glc boronate ester species are partially burried in the polymer matrix, and after their hydrolysis some arylboronic acid sites become not readily accessible by D-Glc in solution.

The pH dependence of saccharide binding was studied with Microsphere B (Fig. 2). D-Glc was adsorbed above pH8 and the binding increased with increasing pH. On the other hand, 1-methyl- α -D-glucoside was not adsorbed over the entire pH range. This clearly indicates that D-Glc is bound to the microsphere surface with the aid of ester formation between arylboronic acid sites and *cis*-diol group. The amount of boronic acid groups on the surface of Microsphere B was about 1.5 x 10^{-5} mol g-resin^{-1} estimated from the saturation study of D-Glc binding.

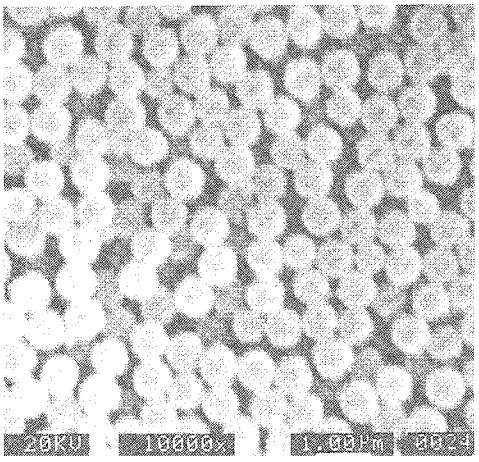


Fig.1. Scanning electron micrograph of Microsphere B.

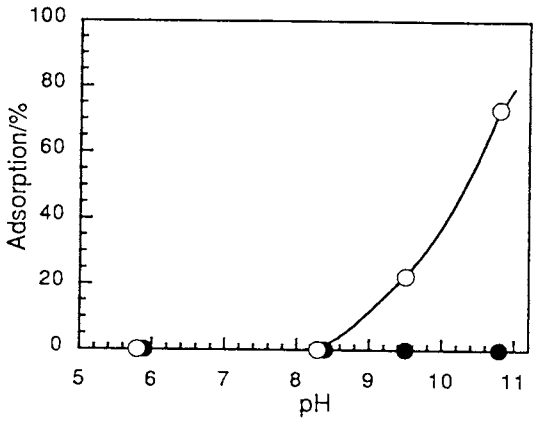


Fig. 2. Effect of pH on saccharide adsorption. ○, D-Glucose; ●, 1-methyl- α -D-glucoside. Conditions: Microsphere B, 0.2 g; saccharide solution(2.5x10^{-4} mol dm^{-3}), 1.0 cm^3; shaking time, 1 h; buffer, Na_2HPO_4/ NaH_2PO_4(pH5.8, 8.3), Na_2CO_3/ $NaHCO_3$(pH9.5, 10.8).

The results of this study encourages one to use such microspheres for separation and concentration of biological constituents comprising *cis*-diol groups, e. g., glyco- and nucleo-proteins, nucleic acids as well as

saccharides. Cellulose-,[15] polyacrylamide-,[16] and glass bead-[17] supported arylboronic acids have been prepared for affinity chromatographic separation of *cis*-diols. However, these supports have problems in mechanical and/or chemical stability. Hydrogels are rather weak under pressure, while glass beads exhibit poor stability under basic conditions. The microsphere obtained in this study might hopefully give some resolution to such uses as in high pressure chromatography and centrifugal separations, due to its mechanically hard characteristic of synthetic polymer.

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