Preparation of Aldehyde-, Amino-, and Hydrazide-Functionalized Polymer Particles for Direct Immobilization of the Sugars

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ABSTRACT: The reaction of poly(vinyl alcohol) with glutaraldehyde (GA) at different molar ratio was systematically studied. By this reaction, white particles functionalized with aldehyde groups were obtained and their diameters were found to be between 50 and 150 nm. The amount of free —CHO groups on the surface of the particles reached more than 1.6 mmol/g by adjusting the ratio of n(GA)/n(—OH). The free —CHO groups were then converted into alkyl amino, aromatic amino, and hydrazide groups by coupling with hexamethylene diamine, *m*-phenylene diamine, and adipic dihydrazide, respectively, and the length of the spacer was also prolonged. Finally, a series of sugar-particle conjugates were prepared by directly coating the functionalized particles with maltose, D-(+)-glucosamine, and heparin. The anticoagulant experiments show that the heparin immobilized on the aldehyde- and hydrazide-functionalized particles is still biologically active. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 2937–2945, 2009

Key words: PVA; functionalized particles; sugar conjugates; heparin; anticoagulant activity

INTRODUCTION

Carbohydrates are a kind of complex biomolecules, which are as important as DNA and proteins to organisms. During the past 2 decades, researchers have paid more and more attention in this field, but tools and methods for study of the biofunctions of carbohydrates are still very limited.[1,2] Preparation of the sugar-particle conjugates has some significance because analysis of the functions of the glycans on a solid surface is more convenient using the spectral and electrical analytical strategies.[3,4] Sugar-particle conjugates can also be used in many important fields, such as three-dimensional carbohydrate microarray probes,[5,6,7] glycan proofing reagents,[8,9] high-performance supports for chromatograpy columns,[10,11] and therapeutic drugs.[12,13] Furthermore, if necessary, more organic synthesis strategies can be introduced to modify the immobilized carbohydrate molecules on the solid support for enlarging the family of the structure-related glycoligands.[14,15] However, the most challenge work at present is to find a support which has good properties and suitable particle size and an effective method for immobilization of the sugars onto this support but will not destroy the sugars' natural structures.

Methods for immobilization of the sugars onto a certain surface were extensively studied in the field of carbohydrate-microarray technology. They can be generally divided into three directions[5,16–18]: physical adsorption, streptavidin/biotin immobilization, and covalent bonding. Among them, the first direction showed low efficiency and could not bear extensive washing[19–23] without modification of the carbohydrates.[24–28] The second direction proved to be efficient, but in an elaborate and laborious way, that has not widely spread.[29–32] The most commonly researched routes are chemical immobilization of the activated glycoligands onto a functionalized surface[33–47] or, oppositely, direct attachment of the glycoligands onto an activated surface. The former usually needs a tedious modification of the carbohydrate molecules, which would to some extent destroy the original structure of the sugars, especially those which were highly sulfated, phosphated, or those with other sensitive functional groups. The latter is a currently developed method for direct immobilization of glycoligands on a certain surface without prior modification of the carbohydrates. Using aromatic amino groups,[48] aminooxy groups,[49,50] hydrazide groups,[49,51] or phthalimide chromophores[52,53] functionalized

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surfaces were reported as effective routes to achieve this goal. These strategies, though in their infancy, were very attractive and valuable.

We tried to apply the direct covalent immobilization approaches in our research. Herein, poly(vinyl alcohol) (PVA) was selected as an original material because it is a —OH-functionalized polymer and widely applied in various biomedical fields. Good biocompatibility, low toxicity, and good mechanical properties enable this polymer competent for making artificial kidney membranes, contact lenses, wound bandages and dressings, drug delivery systems, etc.[54–59] We first claim using the reaction of PVA with GA to produce the aldehyde-functionalized particles. The —CHO groups were further converted into primary amino, aromatic amino, and hydrazide groups through several reductive amination reactions. Then, modified polymer particles were applied to directly immobilize different sugars for preparation of sugar-particle conjugates.

EXPERIMENTAL

Materials

PVA with degree of polymerization of 1750, 25 wt % glutaraldehyde (GA) solution, hexamethylene diamine (HDA), *m*-phenylene diamine (*m*-PDA), D-(+)-glucosamine (GlcN), maltose (Mal), sodium heparate (Hep) (M_w = 4000–15,000), sodium acetate, and all the organic solvents (analytical grade) were purchased from Sinopharm Chemical Reagent. Sodium cyanoborohydride and adipic dihydrazide (ADH) were obtained from Alfa Aesar Plant. The duck blood was taken out from the carotid of the adult ducks.

Reaction of PVA with GA and preparation of the aldehyde-functionalized particles

The PVA solutions were prepared by dissolving a set amount of the polymer into distilled water at 90°C. Then, the solutions were filtered to remove the impurities. When studying the influence of temperature and the amount of catalyst on the gelation time of PVA, 50 mL of PVA solution with the concentration of 8.80×10^{-2} g/mL was blended with 1.0 g of 25% GA (n(GA) : n(—OH) = 0.05 : 1) and warmed to the required temperature with vigorous stirring. Then, a certain amount of 2.0 mol/L HCl aqueous solution as catalyst was quickly added to the mixture and began to record the time. The gelation point was judged when the reaction system became a plastic solid bulk gel (no flowing liquid was observed). When studying how much free —CHO groups could be generated by the gelation method, a series of 50 mL of 8.80×10^{-2} g/mL PVA solutions were reacted with different amounts of GA at 50°C. Five milliliters of 2.0 mol/L HCl was used as catalyst in each reaction. Later, the obtained gels were washed with large quantities of distilled water (usually 50 mL each time for six times) and dried to a constant weight at 35°C with good ventilation. Trituration of the solid gel to powder and determine the amount of free —CHO groups by hydroxylamine hydrochloride method.

Particles with different density of free —CHO groups were prepared by the following procedure: a set amount of 25% GA aqueous solution was weighed and blended with 5.0 mL of 2 mol/L hydrochloride solution as catalyst. Then, the solution was warmed to 53°C. With vigorous stirring, 220 mL of 2.0×10^{-2} g/mL PVA solution was slowly dropped into the mixture in about 2 h and the reaction continued for about additional 3 h. The resultant mixture was centrifuged and the residue was washed five times with distilled water, and then dried at 30°C with good ventilation. When preparing the aldehyde-functionalized particles (particle **1)** for the followed reactions, the amount of 25 wt % GA aqueous solution used was 50 mL and about 6.97 g of white particles was obtained.

Hydroxylamine hydrochloride method for determining the amount of aldehyde groups

About 0.5000 g of the sample was weighed and added into about 15 mL of distilled water. A total of 0.20 g of hydroxylamine hydrochloride was added into the mixture and reacted for about 30 min with stirring. The amount of formed hydrochloric acid was determined with about 0.05 mol/L sodium hydroxide solution, using a drop of 0.5% bromophenol blue in ethanol as an indicator (pH transition interval 3.0–4.6). The amount of aldehyde groups in the sample was calculated using the following equation:

$$c(\text{mmol/g}) = c(V_1 - V_0)/m,$$

where m is the weight of the sample (in grams), V_1 the tall volume of sodium hydroxide solution used for determination (in milliliters), V_0 the volume of sodium hydroxide solution used for blank (in milliliters), and c the concentration of standard sodium hydroxide solution (given in units of mol/L).

Preparation of alkyl amine-coated particles

Two grams of HDA and 0.5 mL of AcOH were dissolved in 40 mL of DMAc. Then, the solution was heated to 37°C and 1.000 g of particle **1** was slowly added into a mixture with vigorous stirring. After an hour, 0.20 g of $NaBH_3CN$ was added into the reaction mixture and the reaction was kept on in the

same condition for 4 h. The resultant mixture was centrifuged and the residue was washed with 10 mL of 0.1*M* aqueous NaOH twice and 20 mL of distilled water three times and then dried at 35°C to constant weight. A total of 1.15 g of white powder (Particle **2**) was obtained. If $NaBH_3CN$ was not added, the Schiff base intermediate (Particle **2′**) was also separated and characterized by IR spectrum.

Preparation of aromatic amine-coated particles

Two grams of *m*-PDA and 0.5 mL of AcOH were dissolved in 40 mL of DMAc. Then, the solution was heated to 37°C and 1.000 g of compound **1** was slowly added into a mixture with vigorous stirring. After an hour, 0.20 g of $NaBH_3CN$ was added into the reaction mixture and the reaction was kept on in the same condition for 4 h. The resultant mixture was centrifuged and the residue was washed with 10 mL of 0.1*M* aqueous NaOH twice and 20 mL of distilled water three times and then dried at 35°C to constant weight. A total of 1.14 g of hoar powder (Particle **3**) was obtained. If $NaBH_3CN$ was not added, the Schiff base intermediate (Particle **3′**) was also separated and characterized by IR spectrum.

Preparation of hydrazide-coated particles

Two grams of ADH and 0.5 mL of AcOH were dissolved in 30 mL of DMSO and 10 mL of distilled water. Then, the solution was heated to 37°C and 1.000 g of compound **1** was slowly added into a mixture with vigorous stirring. After an hour, 0.20 g of $NaBH_3CN$ was added into the reaction mixture and the reaction was kept on in the same condition for 4 h. The resultant mixture was centrifuged and the residue was washed with 10 mL of 0.1*M* aqueous NaOH twice and 20 mL of distilled water three times and then dried at 35°C to constant weight. A total of 1.19 g of white powder (Particle **4**) was obtained. If $NaBH_3CN$ was not added, the Schiff base intermediate (Particle **4′**) was also separated and characterized by IR spectrum.

Characterization of the synthesized particles

IR spectra were scanned by a Perker Elmer 2000 Fourier transform infrared spectrometer (FTIR) using the KBr disk method. The elementary analysis was carried out on the Axios-Petro X-ray fluorescence spectrometer (XRF). Procedure: (a) the synthesized particles were incubated in 0.1*M* of aqueous HCl for 10 min and then washed thoroughly with distilled water and dried at 35°C to constant weight; (b) 2.00 g of sodium acetate (water free) was dissolved in 20.0 mL of distilled water; (c) blend 2.00 mL of the solution with each gram of the particle sample and then dry the mixture at 35°C with good ventilation; (d) the dried powders were pressed to disks and scanned using the XRF spectrometer. Scanning electron microscope (SEM) images were taken by a Philips-FEI XL30 ESEM spectrometer. Molecular fluorescence (MF) emission backgrounds were scanned by an Edinburg F920 fluorescence spectrometer.

Preparation of sugar-particle conjugates

One gram of carbohydrate and 0.15 g of $NaBH_3CN$ (2.3 mmol) were dissolved in 20 mL of DMAc, and 0.5000 g of particle **1** was added into the solution. The reaction mixture was heated at 37°C in a closed environment with stirring for 12 h and centrifuged. The residue was washed five times with distilled water and dried at 35°C to constant weight. Yield to corresponding carbohydrate: 0.569 g (D-(+)-GlcN), 0.607 g (Hep). One gram of carbohydrate and 0.15 g of $NaBH_3CN$ (2.3 mmol) were dissolved in 20 mL of DMAc and 5 mL of AcOH. A total of 0.5000 g of particle **2** was added into the solution and the reaction mixture was heated at 37°C in a closed environment with stirring for 2 days and centrifuged. The residue was washed five times with distilled water and dried at 35°C to constant weight. Yield to corresponding carbohydrate: 0.508 g (D-(+)-GlcN), 0.517 g (Mal), 0.524 g (Hep). One gram of carbohydrate and 0.15 g of $NaBH_3CN$ (2.3 mmol) were dissolved in 20 mL of DMAc and 5 mL of AcOH. A total of 0.5000 g of particle **3** was added into the solution and the reaction mixture was heated at 37°C in a closed environment with stirring for 2 days and centrifuged. The residue was washed five times with distilled water and dried at 35°C to constant weight. Yield to corresponding carbohydrate: 0.553 g (D-(+)-GlcN), 0.579 g (Mal), 0.585 g (Hep). One gram of carbohydrate was dissolved with 20 mL of DMAc and 0.5 mL of AcOH. A total of 0.5000 g of particle **4** was added into the solution and the reaction mixture was heated at 50°C in a closed environment with stirring for 12 h and centrifuged. The residue was washed five times with distilled water and dried at 35°C to constant weight. Yield to corresponding carbohydrate: 0.557 g (D-(+)-GlcN), 0.591 g (Mal), 0.596 g (Hep). The heparin conjugates of particles **1–4** were called H1–H4, respectively.

Anticoagulant test of the heparin-particle conjugates

Seven tubes were added with 0.2 mL of water, 0.2 mL of water and 10 mg of particle **1**, 2 mg of heparin in 0.2 mL of water, 10 mg of H1/0.2 mL of water, 10 mg of H2/0.2 mL of water, 10 mg of H3/0.2 mL of water, and 10 mg of H4/0.2 mL of water, respectively. Fresh blood was taken out from the

carotid of a duck real time and 2.0 mL of blood was added into each tube. Complete clotting time of the blood was recorded when it came out of the duck's body. Photographs were taken by numerous cameras at different times during the clotting process.

RESULTS AND DISCUSSION

The reaction of PVA with GA has been studied for many decades because it plays such an important role in making hydrogels and films.[54–59] Figure 1 shows that temperature and the amount of acid catalyst, especially the latter, were two main factors influencing the reaction rate. When the temperature rose to 50°C and the amount of hydrochloric acid (2*M*) increased to 5.0 mL, this reaction became so fast that a bulk gel was formed in a few seconds. In fact, there were three types of reaction in this process: intermolecular crosslinking, intramolecular crosslinking, and reacting with one end of GA. The distribution of the three types of reactions was controlled by the n(GA)/n(—OH) ratio.[60] As shown in Figure 2, no bulk gel or a network film was generated until n(GA)/n(—OH) $> 3.13 \times 10^{-3}$, which shows that a network structure could form until the intermolecular crosslinking reached a certain degree. Meanwhile, when n(GA)/n(—OH) < 0.05, the generated product was plastic transparent hydrogel and no free aldehyde groups could be detected from the dried gel. However, when this ratio became much larger the product became harder with serious cracks.[61] This may be why no reports of using this reaction to prepare the aldehyde-functionalized materials have been published up to now.

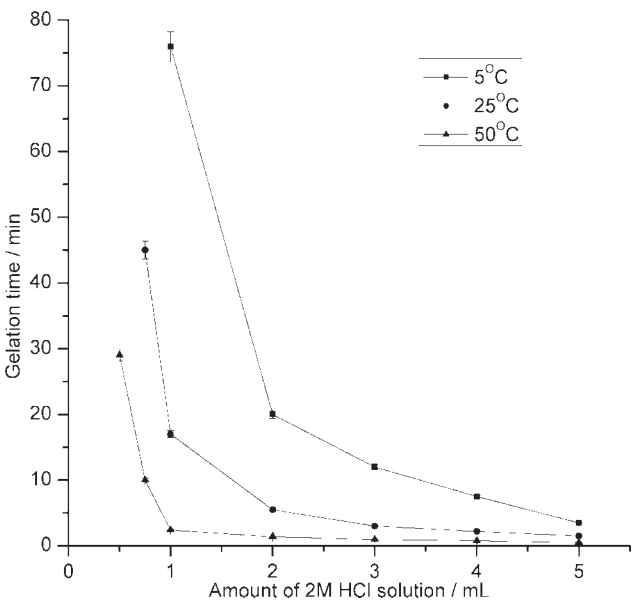


Figure 1 Influence of temperature and the amount of catalyst on the gelation time of PVA. A total of 50 mL of 8.80 $\times 10^{-2}$ g/mL PVA solution was used for each point.

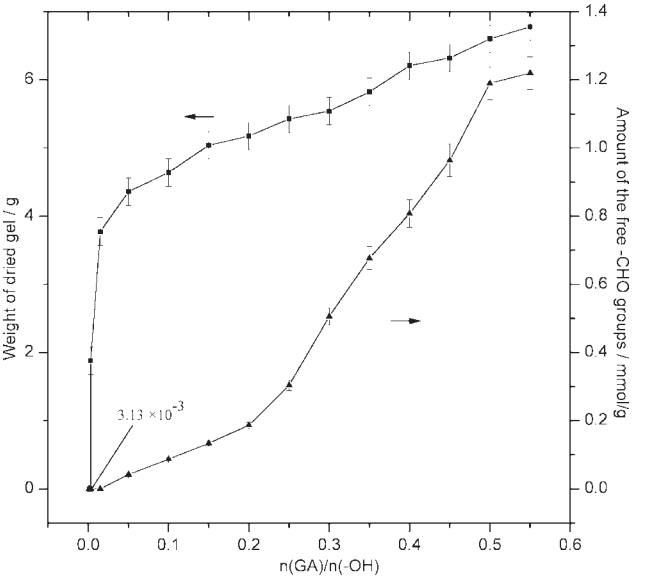
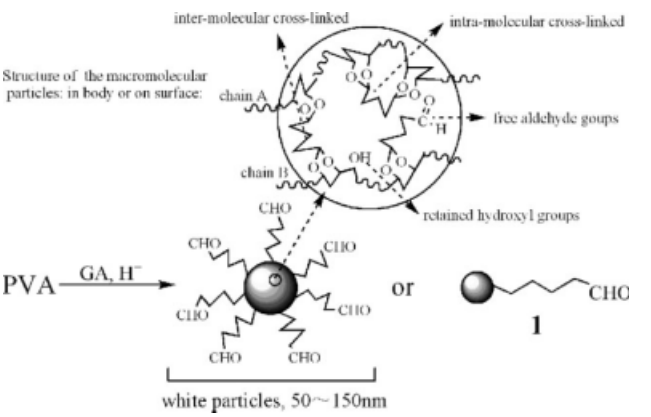


Figure 2 Weight of the obtained dried gel and the amount of free —CHO groups detected. A total of 50 mL of 8.80 $\times 10^{-2}$ g/mL PVA solution was used as material for each point.

We discovered that free —CHO groups appeared when n(GA)/n(—OH) > 0.05, the amount of which can even reach more than 1.2 mmol/g when n(GA)/n(—OH) > 0.55. If a diluted PVA aqueous solution was slowly dropped into excess GA, the intermolecular crosslinking was largely prevented and the obtained product was not bulk gel but uniform white particles. The structure of this particle is described in Scheme 1.

Furthermore, under the used condition for particle preparation, it can be observed that the reaction of —CHO groups with —OH groups on the PVA chain was very fast and randomly selective, and forming six-member rings which were stable and irreversible. Three experimental phenomena supported this



Scheme 1 Route for preparation of the aldehyde-functionalized particles.

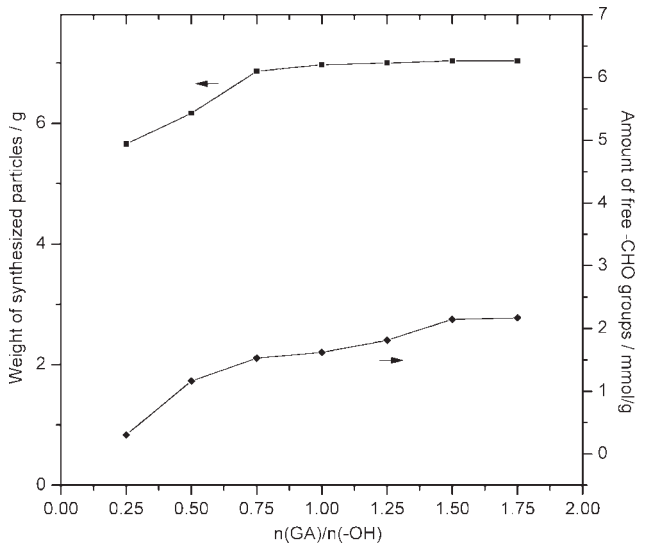


Figure 3 Influence of different n(GA)/n(—OH) ratio on the weight of obtained dried particles and the amount of free —CHO groups determined by hydroxylamine hydrochloride. A total of 220 mL of 2.0×10^{-2} g/mL PVA solution was used as material for each point.

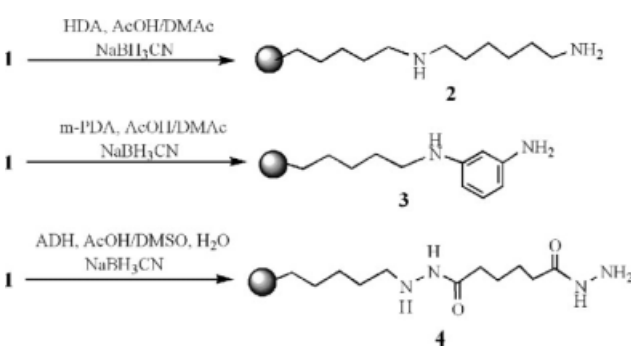
assumption. First, the synthesized particles cannot agglomerate together again because the intermolecule linkages prevented this process. Second, FTIR spectra (see Fig. 4) show that even when GA was far exceeded, there was still a small quantity of —OH groups that did not completely react. This was because the formed rings could not adjust again, with the individual —OH groups sandwiched between two rings or those trapped in the network cages could not be attacked by the —CHO groups again and thus became negative. Third, for the same reason, free —CHO groups were detected even when n(GA)/n(—OH) < 0.25 (see Fig. 2). Therefore, the degree of intermolecule linkages controlled the particle's size, the retained —OH groups and the —O—C—O— moieties endowed the particles with a good hydrophilic property, and the high stability of the six-member rings made the synthesized particles stable enough for doing the followed reactions.

The high amount of free —CHO groups on the synthesized particle's surface can be determined by the hydroxylamine hydrochloride method, as shown in Figure 3. It can be seen that when n(GA)/n(—OH) rose to about 1.25, the amount of free —CHO groups became larger than 1.6 mmol/g and this value reached its maximum when n(GA)/n(—OH) arrived at 1.75.

Aldehyde-functionalized surfaces were very useful in fabrication of biochips or biosensors because the —CHO groups reacted with the amino groups in the biomolecules (e.g., DNAs and proteins) quickly in gentle conditions.[62–64] The —CHO groups can also easily be transformed into other groups by reacting with amino compounds. Hereon, the above synthesized particle **1** was coupled to HDA, *m*-PDA, and ADH, respectively, the length of the linkers was extended, and functional groups were converted into primary amino, aromatic amino, and hydrazide groups, as shown in Scheme 2.

The structures of particles **1–4** and the formed Schiff base intermediates in synthesis of **2–4** were characterized by FTIR spectra. As shown in Figure 4, all the compounds had the framework of R—CH_2, R—CH (v = 2850–2950 cm^{-1} and 1300–1450 cm^{-1}), —O—C—O— (v = 1000–1200 cm^{-1}). Strong absorption at 1722 cm^{-1} and weak Feimi resonance at 2730 cm^{-1} indicated compound **1** contained a large quantity of —CHO groups, and adsorption at 3471 cm^{-1} showed that there were a few —OH which did not react. The peak at 1669 cm^{-1} of compound **2′** was the absorption of the Schiff base structure overlapped with that of the alkyl amino groups. After reduction with $NaBH_3CN$, only the absorption of the alkyl amino groups at 1648 cm^{-1} was left. The spectra of compounds **3′** and **3** were similar to compounds **2′** and **2**, but increased two peaks at 1577 and 1509 cm^{-1}, which were the absorptions of the phenyl rings. Strong absorption between 1550 and 1700 cm^{-1} of compound **4′** matched up to the —CO— groups overlapped with the Schiff base structure. After reduction with $NaBH_3CN$, only strong absorption of the —CO— groups at 1670 cm^{-1} was left. These results were further proved by XRF. It can be calculated from the XRF elementary analysis results (Table I) that the amount of amino moieties contained in particle **2–4** was 1.63, 1.51, and 1.54 mmol/g, respectively.

SEM was used to describe the topography of particles **1–4**. As shown in Figure 5, it can be seen from the images that the diameter of particle **1** was between 50 and 150 nm. After being coupled with *m*-PDA or adipic acid, the diameters increased to about 100–250 nm. HDA induced the particles clustering together to some extent; however, the diameters were still far less than 1 μm. MF emission spectra were used to scan the optical backgrounds of the synthesized



Scheme 2 Prolong the linker length and diversify the functional groups.

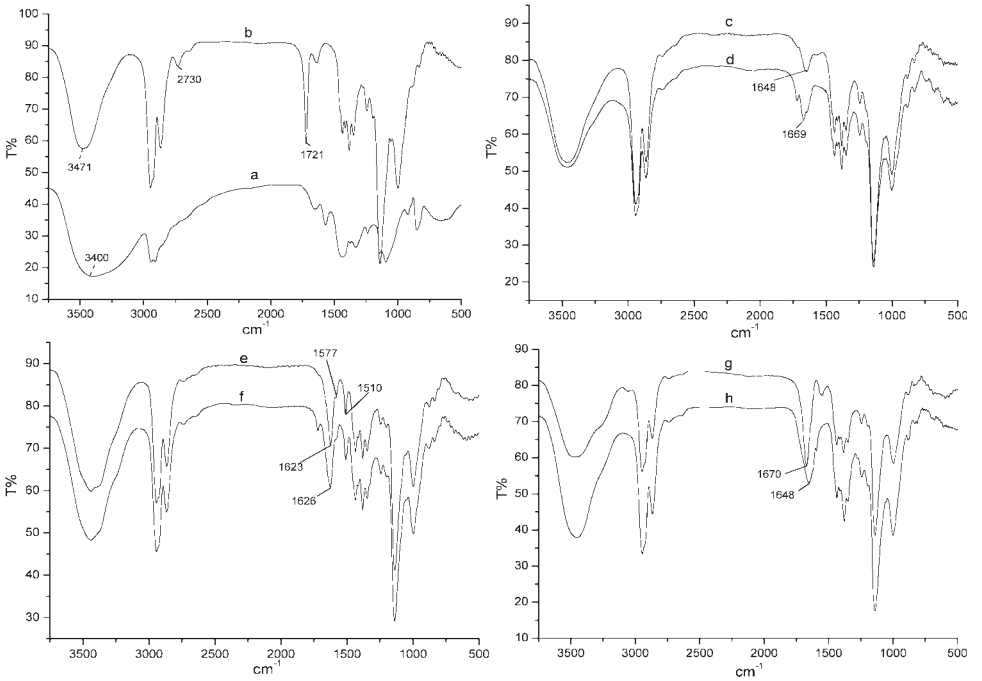


Figure 4 FTIR spectra of synthesized particles: (a) PVA; (b) Compound 1; (c, d) Compounds 2 and 2′; (e, f) Compounds 3 and 3′; (g, h) Compounds 4 and 4′. Compounds 2′–4′ were corresponding to the Schiff base intermediates of Compounds 2–4. These spectra represent the signal averages of 32 scans obtained using a mixture of the particles in potassium bromide.

particles. As shown in Figure 6, apart from compound **2**, particles **1**, **3**, and **4** had very low fluorescence background between 280 and 700 nm. The fluorescence background of particle **2** between 350 and 600 nm may be due to agglomeration of the particles. Therefore, the sizes and optical properties of particles **1**, **3**, and **4** were suitable for making the bioprobes.

Amino and hydrazide groups were widely used for chemical immobilization of carbohydrate molecules and preparation of carbohydrate conjugates. For example, Suda et al.[48] immobilized the carbohydrates through aromatic primary amino groups on synthesized tree molecules. Flinn et al.[65] prepared the Lewis Y tetrasaccharide hydrazide and then immobilized it on BSA for studying its biofunctions. Lee and Shin[49–51] researched the immobilization efficiency of carbohydrates on the hydrazide-coated glass slides and amino-coated slides. In these researches, the mechanisms of the reaction process were also intensively studied. It was proved that both the primary amino groups and the hydrazide groups were reacting with the reductive end moieties of the sugar chain and forming the β-configuration; however, the primary amino group generates acyclic products preferentially[48,49,65–67] and the hydrazide group generates cyclic products predominantly.[49,50] Moreover, these reactions were reversible in aqueous environments, therefore a reduction step is necessary to form the chemically stable end carbohydrate particle conjugates. We did not study the mechanisms any more but use them to prepare the sugar-particle conjugates. As shown in Table II, three different reducing sugars, D-(+)-GlcN, Mal, and Hep were successfully attached onto the synthesized particles. It was found out that the immobilization capacity of the hydrazide-functionalized particles was generally much larger than the amino-functionalized particles, especially to the primary amino-activated particles. This shows that the primary amino moieties lost most of their activity in an acid condition and the sugar-hydrazide conjugates can be formed more easily.[50,65] Moreover, it was necessary to point out that even to the hydrazide-functionalized particles the immobilization capacity of the particles to sugar was always not as large as predicted. This problem had been discussed elaborately by Hage and coworkers,[10,11] it was due to two-point attachment of the diamines, which can not be characterized by XRF. Nevertheless, because the quantity of the aldehyde groups on the material particle **1** was large, hundreds of micromoles of sugars were

TABLE I
Results of XRF Elementary Analysis

Particle	O (wt %)	Cl (wt %)
1	29.10	0.04
2	20.59	5.80
3	20.32	5.37
4	19.76	5.48

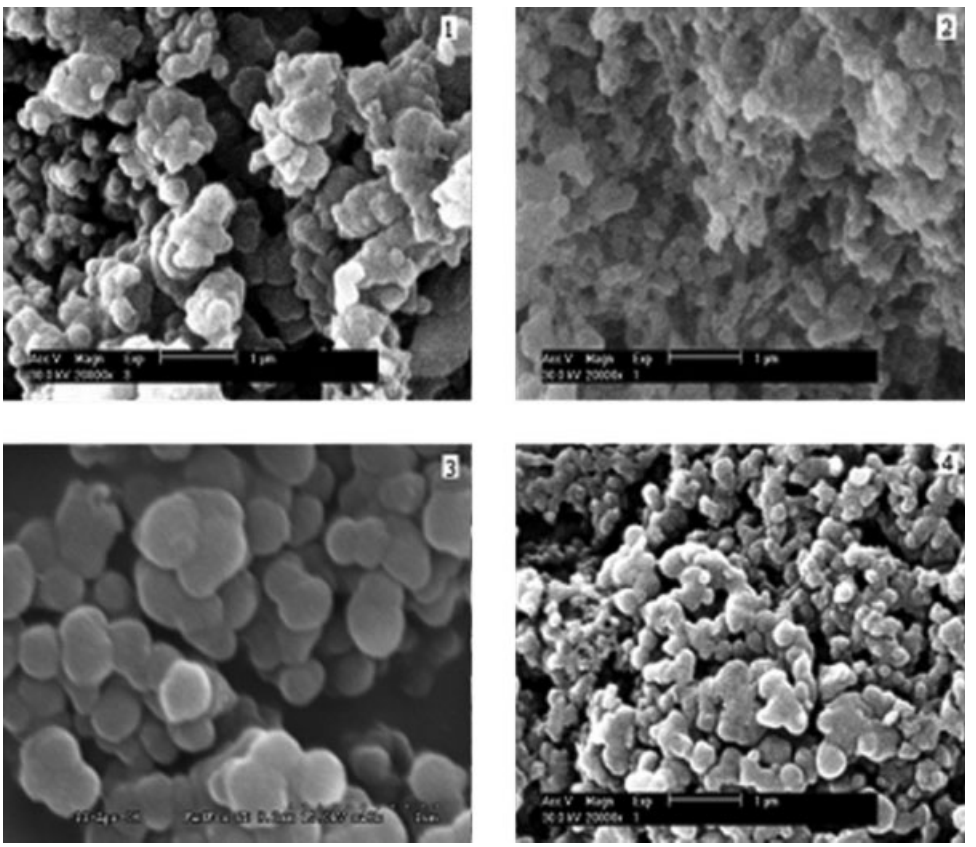


Figure 5 SEM images of particles **1–4**, scale bar = 1 μm.

attached onto each gram of particles, which could be sufficient for expressing their bioactivities.

The anticoagulant test was carried out as a rough method checking the bioactivity of H1–H4. Table III shows the complete clotting time of the fresh duck arterial blood with different anticoagulants. For obtaining the original data, the fresh blood was directly mixed with the anticoagulants without any disposal of it beforehand. In the tests, the blood without anticoagulant clotted in about 3 min and the nonheparinized particle **1** even accelerated this process (in about 90 s). H2 and H3 prolonged the clotting time of blood, but not very effectively (in about 6 h). This may due to two reasons. First, the reaction of heparin with primary amino groups was not easily achieved, and second, this immobilization approach to some extent destroyed the structure of the sugar molecules. Heparin, H1, and H4 were three good anticoagulants, which were able to hold back the blood clotting time generally beyond 20 h. It was proved that the basic anticoagulant active unit in heparin was a pentasaccharide segment, which did not contain free amino groups.[68,69] The aldehyde functional groups on

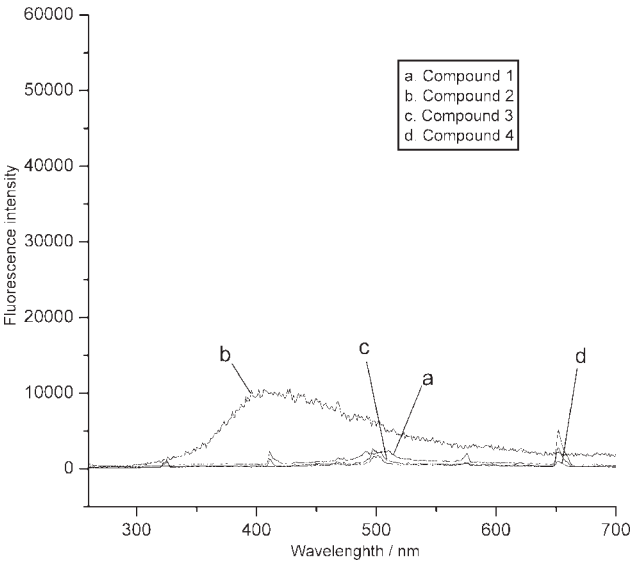


Figure 6 Molecular fluorescence emission intensity of particles **1–4**.

TABLE II
Immobilization Capacity of Different Carbohydrates to the Particles (g/g)

Sugar	Particle **1**	Particle **2**	Particle **3**	Particle **4**
GlcN	0.139 ± 0.01	0.016 ± 0.01	0.106 ± 0.01	0.115 ± 0.01
Mal	–	0.034 ± 0.01	0.157 ± 0.01	0.183 ± 0.01
Hep	0.214 ± 0.01	0.047 ± 0.01	0.170 ± 0.01	0.192 ± 0.01

TABLE III
Complete Clotting Time of Blood with Different Anticoagulant

Compounds	–	Particle **1**	Hep	H1	H2	H3	H4
Clotting time	170 ± 5 s	90 ± 5 s	30 ± 1 h	25 ± 1 h	5 ± 1 h	7 ± 1 h	23 ± 1 h

particle **1** only reacted with free amino groups on the heparin chain. Therefore, this immobilization strategy did not deprive the anticoagulant activity of heparin. The hydrazide functional groups on particle **4** can directly react with the reducing end aldehyde moiety of sugars without other treatment that will also not influence the sugar's structure and function.

More interestingly, there were also some differences between heparin, H1, and H4. Figure 7 gave the photographs taken from the clotting process. It shows that H1 caused the blood's color becoming dark more quickly. It can be explained that because of the steric hindrance, some of the aldehyde groups on particle **1** could not completely reacted, which can rob the oxygen from the red blood cells. However, the complete clotting time of H1 was much longer than H4, which can be explained in the following way; after one more step of reaction, the density of —NH—NH_2 groups becomes less than the original —CHO groups, as well as the immobilized heparin molecules.

CONCLUSIONS

The aldehyde groups'-functionalized white particles with the diameter between 50 and 150 nm were successfully synthesized from the reaction PVA with excess GA. The aldehyde groups were then converted to primary, aromatic amino groups, and hydrazide groups through three reductive amination reactions. All the synthesized compounds were small particles possessing friendly hydrophilic 3D surface, flexible functional linkers on the surface, and a relatively low fluorescence background. A series of sugar-particle conjugates were prepared by coating the synthesized particles with D-(+)-GlcN, Mal, and Hep, which showed that the applied immobilization strategies were efficient. Anticoagulant tests of the immobilized heparin show that the immobilized heparin on the aldehyde- and hydrazide-functionalized particles still had good anticoagulant activity. However, complete application of these particles in the field of biochemistry still requires a lot of further research.

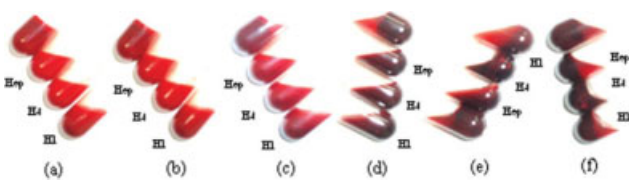


Figure 7 Images of the blood clotting process: (a) 10 min, (b) 30 min, (c) 2 h, (d) 6 h, (e) 24 h, and (f) 30 h. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

References

1. Shriver, Z.; Raguram, S.; Sasisekharan, R. Nat Rev 2004, 3, 863.
2. Raman, R.; Raguram, S.; Venkataraman, G.; Paulson, J. C.; Sasisekharan, R. Nat Methods 2005, 2, 817.
3. Blagoi, G.; Rosenzweig, N.; Rosenzweig, Z. Anal Chem 2005, 77, 393.
4. Delgado, A. D. S.; Leonard, M.; Dellacherie, E. Langmuir 2001, 17, 4386.
5. Dyukova, V. I.; Shilova, N. V.; Galanina, O. E.; Rubina, A. Y.; Bovin, N. V. Biochim Biophys Acta 2006, 1760, 603.
6. Dyukova, V. I.; Dementieva, E. I.; Zubtsov, D. A.; Galanina, O. E.; Bovin, N. V.; Rubina, A. Yu. Anal Biochem 2005, 347, 94.
7. Seo, J. H.; Adachi, K.; Lee, B. K.; Kang, D. G.; Kim, Y. K.; Kim, K. R.; Lee, H. Y.; Kawai, T.; Cha, H. J. Bioconjugate Chem 2007, 18, 2197.
8. Nolting, B.; Yu, J. J.; Liu, G. Y.; Cho, S. J.; Kauzlarich, S.; Gervay-Hague, J. Langmuir 2003, 19, 6465.
9. Kaltgrad, E.; O'Reilly, M. K.; Liao, L.; Han, S.; Paulson, J. C.; Finn, M. G. J Am Chem Soc 2008, 130, 4578.
10. Ruhn, P. F.; Garver, S.; Hage, D. S. J Chromatogr A 1994, 669, 9.
11. Xuan, H.; Hage, D. S. Anal Biochem 2005, 346, 300.
12. Doores, K. J.; Gamblin, D. P.; Davis, B. G. Chem Eur J 2006, 12, 656.
13. Sundgren, A.; Barchi, J. J., Jr. Carbohydr Res 2008, 343, 1594.
14. Baumann, H.; Scheen, H.; Huppertz, B.; Keller, R. Carbohydr Res 1998, 308, 381.
15. Putaux, J. L.; Potocki-Veronese, G.; Remaud-Simeon, M.; Buleon, A. Biomacromolecules 2006, 7, 1720.
16. Dyukova, V. I.; Shilova, N. V.; Galanina, O. E.; Rubina, A. Y.; Bovin, N. V. Biochim Biophys Acta 2006, 1760, 603.
17. Feizi, T.; Fazio, W. F.; Chai, C. H. Curr Opin Struct Biol 2003, 13, 637.
18. de Paz, J. L.; Seeberger, P. H. QSAR Comb Sci 2006, 25, 1027.
19. Willats, W. G.; Rasmussen, S. E.; Kristensen, T.; Mikkelsen, J. D.; Knox, J. P. Proteomics 2002, 2, 1666.
20. Bryan, M. C.; Plettenburg, O.; Sears, P.; Rabuka, D.; Wacowich-Sgarbi, S.; Wong, C. H. Chem Biol 2002, 9, 713.
21. Wang, D.; Liu, S.; Trummer, B. J.; Deng, C.; Wang, A. Nat Biotechnol 2002, 20, 275.
22. Galustian, C.; Park, C. G.; Chai, W.; Kiso, M.; Bruening, S. A.; Kang, Y. S.; Steinman, R. M.; Feizi, T. Int Immunol 2004, 16, 853.
23. Patwa, T. H.; Zhao, J.; Anderson, M. A.; Simeone, D. M.; Lubman, D. M. Anal Chem 2006, 78, 6411.
24. Fukui, S.; Feizi, T.; Galustian, C.; Lawson, A. M.; Chai, W. Nat Biotechnol 2002, 20, 1011.
25. Ko, K. S.; Jaipuri, F. A.; Pohl, N. L. J Am Chem Soc 2005, 127, 13162.
26. Mamidyala, S. K.; Ko, K. S.; Jaipuri, F. A.; Park, G.; Pohl, N. L. J Fluorine Chem 2006, 127, 571.

27. Rhodes, M. A. C.; Childs, R. A.; Kiso, M.; Komba, S.; Narvor, C. L.; Warren, J.; Otto, D.; Crocker, P. R.; Feizi, T. Biochem Biophys Res Commun 2006, 344, 1141.
28. Moller, I.; Marcus, S. E.; Haeger, A.; Verhertbruggen, Y.; Verhoef, R.; Schols, H.; Ulvskov, P.; Mikkelsen, J. D.; Knox, J. P.; Willats, W. Glycoconjugate J 2008, 25, 37.
29. Galanina, O. E.; Mecklenburg, M.; Nifantiev, N. E.; Pazynina, G. V.; Bovin, N. V. Lab Chip 2003, 3, 267.
30. Guo, Y.; Feinberg, H.; Conroy, E.; Mitchell, D. A.; Alvarez, R.; Blixt, O.; Taylor, M. E.; Weis, W. I.; Drickamer, K. Nat Struct Mol Biol 2004, 11, 591.
31. Bochner, B. S.; Alvares, R. A.; Nehta, P.; Bovin, N. V.; Blixt, O.; White, J. R.; Schnaar, R. L. J Biol Chem 2005, 280, 4307.
32. Karamanska, R.; Clarke, J.; Blixt, O.; MacRae, J. I.; Zhang, J. Q.; Crocker, P. R.; Laurent, N.; Wright, A.; Flitsch, S. L.; Russell, D. A.; Field, R. A. Glycoconjugate J 2008, 25, 69.
33. Houseman, B. T.; Mrksich, M. Chem Biol 2002, 9, 443.
34. Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C. H. J Am Chem Soc 2002, 124, 14397.
35. Bryan, M. C.; Lee, L. V.; Wong, C. H. Bioorg Med Chem Lett 2004, 14, 3185.
36. Ratner, D. M.; Adams, E. W.; Disney, M. D.; Seeberger, P. H. ChemBioChem 2004, 5, 1375.
37. Houseman, B. T.; Gawalt, E. S.; Mrksich, M. Langmuir 2003, 19, 1522.
38. Park, S.; Shin, I. Angew Chem Int Ed Engl 2002, 41, 3180.
39. Park, S.; Lee, M.; Pyo, S. J.; Shin, I. J Am Chem Soc 2004, 126, 4812.
40. Park, S.; Shin, I. Org Lett 2007, 9, 1675.
41. Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C. H.; Paulson, J. C. Proc Natl Acad Sci USA 2004, 101, 17033.
42. Blixt, O.; Hoffmann, J.; Svenson, S.; Norberg, T. Glycoconjugate J 2008, 25, 27.
43. Xia, B.; Kawar, Z. S.; Ju, T.; Alvarez, R. A.; Sachdev, G. P.; Cummings, R. D. Nat Methods 2005, 2, 845.
44. Schwarz, M.; Spector, L.; Gargir, A.; Shtevi, A.; Gortler, M.; Alstock, R. T.; Ducler, A. A.; Dotan, N. Glycobiology 2003, 13, 749.
45. Angeloni, S.; Ridet, J. L.; Kusy, N.; Gao, H.; Crevoisier, F.; Guinchard, S.; Kochhar, S.; Sigrist, H.; Sprenger, N. Glycobiology 2005, 15, 31.
46. Yamamoto, K.; Ito, S.; Yasukakawa, F.; Konami, Y.; Matsumoto, N. Anal Biochem 2005, 336, 28.
47. Dubois, M. P.; Gondran, C.; Renaudet, O.; Dumy, P.; Driguez, H.; Fort, S.; Cosnier, S. Chem Commun 2005, 34, 4318.
48. Suda, Y.; Arano, A.; Fukui, Y.; Koshida, S.; Wakao, M.; Nishimura, T.; Kusumoto, S.; Sobel, M. Bioconjugate Chem 2006, 17, 1125.
49. Lee, M. R.; Shin, I. Org Lett 2005, 7, 4267.
50. Zhi, Z. L.; Powell, A. K.; Turnbull, J. E. Anal Chem 2006, 78, 4786.
51. Zhou, X. C.; Zhou, J. Z. Biosens Bioelectron 2006, 21, 1451.
52. Carroll, G. T.; Wang, D. N.; Koberstein, J. T. Langmuir 2006, 22, 2899.
53. Wang, D. N.; Carroll, G. T.; Turro, N. J.; Koberstein, J. T.; Koberstein, J. T.; Kovac, P.; Saksena, R.; Adamo, R.; Herzenberg, L. A.; Steinman, L. Proteomics 2007, 7, 180.
54. Chen, D. H.; Leu, J. C.; Huang, T. C. J Chem Technol Biotechnol 1994, 61, 351.
55. Muhlebach, A.; Muller, B.; Pharisa, C.; Hofmann, M.; Seiferling, B. J Polym Sci Part A: Polym Chem 1997, 35, 3603.
56. Nho, Y. C.; Park, K. R. J Appl Polym Sci 2002, 85, 1787.
57. Wu, K. Y. A.; Wisecarver, K. D. Biotechnol Bioeng 1992, 39, 447.
58. Nuttelman, C. R.; Henry, S. M.; Anseth, K. S. Biomaterials 2002, 23, 3617.
59. Oh, K. S.; Han, S. K.; Choi, Y. W.; Lee, J. H.; Lee, J. Y.; Yuk, S. H. Biomaterials 2004, 25, 2393.
60. Zhao, D.; Liao, G.; Gao, G.; Liu, F. Macromolecules 2006, 39, 1160.
61. Carreras, E. S.; Chabert, F.; Dunstan, D. E.; Franks, G. V. J Colloid Interface Sci 2007, 313, 160.
62. Morozov, V. N.; Gavryushkin, A. V.; Deev, A. A. J Biochem Biophys Methods 2002, 51, 57.
63. Wang, J.; Bai, Y.; Li, T.; Lu, Z. J Biochem Biophys Methods 2003, 55, 215.
64. Alonso, N.; Gallego, F. L.; Betancor, L.; Hidalgo, A.; Mateo, C.; Guisan, J. M.; Lafuente, R. F. J Mol Catal B 2005, 35, 57.
65. Flinn, N. S.; Quibell, M.; Monk, T. P.; Ramjee, M. K.; Urch, C. J. Bioconjugate Chem 2005, 16, 722.
66. Lubineau, A.; Auge, J.; Drouillat, B. Carbohydr Res 1995, 266, 211.
67. Öberg, C. T.; Carlsson, S.; Fillion, E.; Leffler, H.; Nilsson, U. J. Bioconjugate Chem 2003, 14, 1289.
68. Esko, J. D.; Selleck, S. B. Annu Rev Biochem 2002, 71, 435.
69. Whitelock, J. M.; Renato, V. I. Chem Rev 2005, 105, 2745.