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Influence of Gelling Properties on Protein Imprinted Agarose Gel Membrane

Hanbo Feng,[1] Xuan Mao,[2] Bin Chu,[3] Chunlan Xie[2]

[1]Equipment Department, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, People's Republic of China
[2]Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Institute of Biomedical Engineering, Jinan University, Guangzhou 510632, People's Republic of China
[3]Key Laboratory of Biomedical Materials and Implants, Research Institute of Tsinghua University in Shenzhen, Shenzhen 518057, People's Republic of China
Correspondence to: X. Mao (E-mail: tmaox@jnu.edu.cn)

ABSTRACT: A method based on molecular imprinting technique was presented for preparing protein-imprinted agarose gel membrane (AGM) under moderate conditions, and the influencing factors such as molecular weights and modified chemical groups on the adsorption ability and selectivity of AGMs were investigated. The agaroses used for AGMs were prepared through ultrasonic degradation, oxidation degradation, gel-melting method, and sulfation, respectively. Bovine serum albumin (BSA) and hemoglobin were selectively recognized on AGMs. Results showed that the molecular weight was the most crucial influencing factor for the protein recognition ability of AGMs. The lower and upper limit of molecular weight was 100 and 130 kDa, respectively, where the AGMs could maintain both good mechanical strength and high recognition ability, with *K* value around 4.0. The enhancement of ionic strength could make the imprinting effect disappeared even when the concentration of salt was as low as 2 mmol/L. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40323.

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INTRODUCTION

Protein imprinting technology (PIT) is an approach to prepare functional materials with recognition ability for protein molecules, mostly achieved by a prepolymerization process between monomers and template protein molecules, which can theoretically form specific interactions and resultant cavities in molecular degree.[1–6] Hydrogel synthesized by monomer polymerization is found to be preferable for PIT due to its water-carrying ability. This technique has attracted much attention in the last two decades, but few are of practical use. The main drawback is that the ductile protein molecules could not be fixed during imprinting or adsorption process, which results in low selectivity. In addition, the conditions of polymerization (heat, UV radiation, organic initiator, or cross-linking agent, etc.) have great influence on the conformation, the aggregation state, and even the denaturation of proteins.[7,8] To avoid changes in protein conformation, some biocompatible natural polysaccharides, such as chitosan, cellulose, alginate, agarose, and starch, have been chosen as imprinted matrix.[9] However, the relatively weak interactions between tertiary even quarternary structure of both polymer and protein molecules usually results in low recognition specificity, and the unrepeatable physical and chemical property of natural materials is another drawback.

Additionally, some controversies have been raised to doubt the existence of the imprinting effect of proteins as the adsorption amount might be increased through washing treatment because most hydrogels are electrically charged.[10] In our previous work,[11] we chose agarose, a kind of near-neutral gelling agent, as the imprinting matrix for recognizing bovine serum albumin (BSA), and found that it had a selectivity of 3–5 times over hemoglobin (Hb). As the material is neutral, the strongest interaction between the matrix and the imprinted molecules is hydrogen bond, and the matrix would not be influenced by washing treatments. It is easy to identify the existence of the imprinting effect on a neutral matrix. Although, as another natural material, the chemical structure, the substituent, and the molecular weight of agarose will influence the protein imprinting ability of AGMs. The structural unit of agarose is a disaccharide with β-D-galactose and 3, 6-inner ether-L-galactose. The sugar chain usually contains some substituents (such as methoxy, sulfate group, pyruvic acid group, etc.) of which the types and contents are changable with the species and the growing environment of the seaweed. For example, agarose extracted from *Gracilaria* generally contains methoxy on β-D-galactose and 3, 6-anhydro-α-D-galactopyranose; whereas the sulfate group and pyruvic group might bring trace amount of negative charges to

Table I. AGMs Through Various Treatments and Their Intrinsic Viscosity [η] and Viscosity Average Molecular Weight (M_η)

Serial number	Type of agarose/type of treatment	[η] (mL/g)	M_η (kDa)
AGM-1	AG-1, Chemical Regeant Company, Shanghai, China	140.9	13.2
AGM-2	AG-2, Yuanju Biotechnology Company, Shanghai, China	192.9	15.6
AGM-3	AG-3, Promega Biotechnology Company, USA	235.8	17.3
AGM-4	AG-1, formed by heating at 95°C for 120 min, membrane	269.2	18.6
AGM-5	Single-melting AG-1, membrane	-	-
AGM-6	Double-melting AG-1, membrane	-	-
AGM-7	Single-melting AG-3, membrane	219.8	16.7
AGM-8	Double-melting AG-3, membrane	179.4	16.0
AGM-9	AG-1, ultrasonic treatment for 4 h	115.9	11.9
AGM-10	AG-1, ultrasonic treatment for 18 h	112.4	11.7
AGM-11	AG-1, ultrasonic treatment for 146 h	98.2	10.9
AGM-12	Sulfated AG-3, sponge	101.0	11.4
AGM-13	AG-3, chemical degraded for 0.5 h	148.8	13.6
AGM-14	AG-3, chemical degraded for 1 h	130.2	12.7
AGM-15	AG-3, chemical degraded for 2 h	104.7	11.4
AGM-16	AG-3, chemical degraded for 4 h	60.8	8.5
AGM-17	AG-3, chemical degraded for 6 h	55.7	8.1
AGM-18	AG-3, chemical degraded for 8 h	46.8	7.4
AGM-19	AG-4, Low melting point agarose, Promega Biotechnology Company, USA	-	-

agarose molecules if not completely separated.[12] In this article, the effects of molecular weights and chemical components of agaroses from different sources and various physical and chemical treatments were studied, including commercial agarose with different gel strength and gel melting point, agarose treated with thermal, ultrasonic, and chemical degradation, and sulfated agarose. Moreover, the microenvironment of the preparation and protein rebinding of imprints, such as the solvents and reagents used for polymerization, pH value, ionic strength, temperature, washing protocols, etc., also seriously influence the recognition process of protein imprinting.[13] Thus, the effects of the ionic strength on AGMs were also investigated here.

MATERIALS AND METHODS

Materials

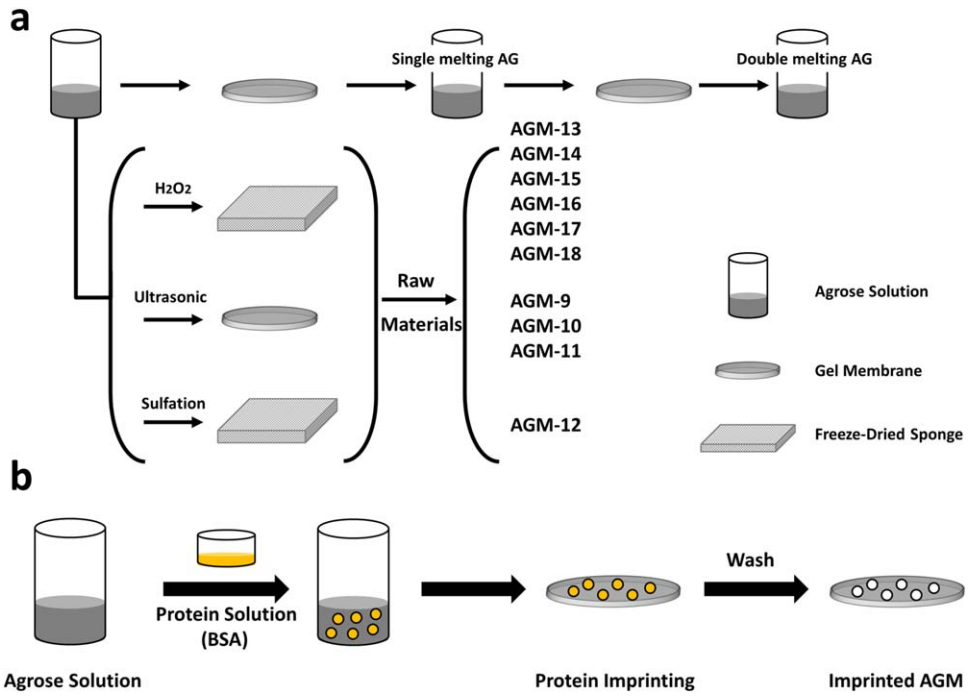
Agarose (electrophoretic grade) was purchased from three companies respectively (see Table I; AG-1 to AG-4). Bovine serum albumin (BSA) was obtained from Sino-America Biotechnology Company (China), bovine hemoglobin (Hb) from Weijia Technology Company (Guangzhou, China), and *Bacillus subtillis* neutral proteinase from Boao Biotechnology Company (Shanghai, China). Coomassie brilliant blue G250 was from Zhanchen Biotechnology Company (Guangzhou, China).

Preparation of BSA Imprinted AGMs from Different Sources

Agarose powders from different companies were dissolved in hot water above 90°C, respectively. The final concentration of the solution was 1% (w/v). The solution was kept at 60°C to prevent from gelling, and then mixed with 5 mg/mL BSA aqueous solution and phosphate buffer solution (PBS) (0.002 mol/L and 0.02 mol/L) by stirring at 50°C in a 5-inch culture dish until being homogeneous. The mass ratio of the two components (agarose/protein) was 4 : 1. The total volume of the mixture was kept at 2.4 mL. Then the mixture was naturally cooled at room temperature, and after the gelation was complete, the gel was further dried at 50°C for 24 h to form gel membrane (see Table I; AGM-1 to AGM-3, AGM-19). The membrane was then immersed in a neutral solution containing neutral *Bacillus subtillis* proteinase (40 μg/mL, in 0.2 mol/L PBS) at 37°C for 8 h to remove the protein specifically adsorbed on the membrane, and further immersed in 10% (v/v) acetic acid solution containing 10% (w/v) sodium dodecyl sulfonate (SDS) solution by shaking for 2−4 h to desorb the nonspecifically adsorbed protein and the remained proteinase. The membrane was finally washed with deionized water for three times until no protein or SDS remained which was detected by Coomassie brilliant blue G250. The resultant membrane was then dried at room temperature for use. A general process of protein imprinting is shown in Scheme 1(b). The nonimprinted AGMs were prepared through the same procedure except that the agarose solution was mixed with protein-free deionized water and PBS solution (0.002 mol/L and 0.02 mol/L).

Preparation of BSA Imprinted AGMs from Thermally Degraded Agarose

The thermally degraded nonimprinted and imprinted AGMs (see Table I; AGM-4 to AGM-8) were prepared through single and double gel-melting treatment toward agarose. The single gel-melting membranes were prepared by using the gels with double-gelation (melting the agarose gel and cooling it again, and then drying it to form a membrane), whereas the double gel-melting membranes with triple-gelation [Scheme 1(a)]. The protein mixing procedures were the same as mentioned in "preparation of BSA imprinted AGMs from different sources" section.



Scheme 1. Diagram of different physical and chemical treatments with agaroses (a) and preparation process of imprinted AGMs (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Preparation of BSA Imprinted AGMs from Low Molecular Weight Agarose

The low molecular weight agarose membranes were prepared from the agarose degraded through oxidative or ultrasonic way, respectively.

Preparation of BSA Imprinted AGMs from Oxidatively Degraded Agarose

The oxidative degradation was achieved using H_2O_2 as oxidant. Agarose solution of 1 wt % was obtained by dissolving agarose completely in water bath at 90–95°C, and then kept at 50°C. After that, a certain volume of H_2O_2 aqueous solution (preheated at 50°C) was added to react with the agarose for a certain period under stirring. The final concentration of H_2O_2 was 0.75% (v/v). The cooled gel was then freeze-dried for 24 h. After that, the sponge-like product was dipped into a certain amount of ethanol over 24 h, then under ultrasonic for a certain period, and finally dried at 50°C for 24h to obtain the degraded sponge-like dried agarose as the raw material for imprinting. The imprinted and nonimprinted oxidatively degraded AGMs (see Table I; AGM-13 to AGM-18) were prepared according to the procedure mentioned in "preparation of BSA imprinted AGMs from different sources" section, by dissolving the degraded agarose in advance.

Preparation of BSA Imprinted AGMs from Ultrasonically Degraded Agarose

Agarose solution of 1% (w/v) was prepared by dissolving agarose completely at 90–95°C, and then kept at 60°C under ultrasonic for a certain interval. The cooled gel was dried at 50°C for 24 h to obtain the membrane-like degraded product. The imprinted and nonimprinted ultrasonically degraded AGMs (see Table I; AGM-9 to AGM-11) were prepared according to the procedure mentioned in "preparation of BSA imprinted AGMs from different sources" section, using the degraded membrane-like dried agarose as the raw material.

Preparation of BSA Imprinted AGMs from Sulfated Agarose

The sulfated agarose was prepared by chlorosulfonic acid-pyridine method. Pyridine of 20 mL was mixed with 4 mL chlorosulfonic acid under ice-salt bath by stirring to form sulfation agent. Agarose of 1 g was dissolved in 70 mL anhydrous formamide, and mixed with the sulfation agent at 65°C by stirring for 4 h. The pH of the mixture was adjusted to neutral by NaOH, and the product was precipitated by ethanol, and then washed, dialyzed, and freeze-dried. The imprinted and nonimprinted sulfated AGMs (see Table I; AGM-12) were prepared according to the procedure mentioned in "preparation of BSA imprinted AGMs from different sources" section, using the sulfated sponge-like dried agarose as the raw material.

The raw materials obtained by the oxidative degradation, ultrasonic degradation, and sulfated agarose, and their corresponding AGMs are shown in Scheme 1(a).

Determination of Protein Adsorption Ability of Imprinted AGMs

AGM of 2.0–4.0 mg was precisely weighed and put into a stoppered glass test tube. Two milliliter (100–150 μg/mL) protein (BSA or Hb) solution was added into the tube with a 1000 μL autopipet. The adsorption process was carried out at room temperature statically for 12 h, and then in an oscillator at 28°C for 6 h. The content of protein in solution was determined by Bradford test. Then the test tube was violently shaken to prevent proteins from adhering to the membrane and the tube wall, 1 mL protein solution was transferred from the

supernatant with a 1000 μL autopipet into another blank stoppered glass test tube, and then mixed with 5 mL Coomassie brilliant blue G250 (0.1 mg/mL) by violently stirring. In the control group, the sample was prepared by mixing 1 mL water with 5 mL G250 solution. The sample was left for 10 min to get stable color, and then its absorbance was recorded by a spectrophotometer at 595 nm (722S, Lengguang Technology Company, Shanghai, China).

The content of protein in supernatant was analyzed according to the slope of a linear standard curve (BSA: $y = 0.0675 + 0.00645x$, $R^2 = 0.98488$; Hb: $y = 0.02804 + 0.00624x$, $R^2 = 0.99648$), and was also determined on a spectrophotometer at 595 nm. The adsorption capacity was calculated by the differences of protein contents before and after adsorption. The adsorption capacity Q (mg/g) was defined as:

$$Q = (C_0 - C_e)V/W \quad (1)$$

where C_0 is the initial protein concentration (mg/mL), C_e is the equilibrium protein concentration (mg/mL), V is the volume of protein solution (mL), and W is the weight of the membrane (g), $n = 2$–5.

The adsorption abilities of the imprinted molecules and nonimprinted ones on the imprinted protein membrane were expressed by factor K_1 and K_2, respectively. Here K_1 stands for the adsorption ability of the BSA imprinted membranes for BSA molecules over the nonimprinted membranes; K_2 for the adsorption ability of the BSA imprinted membranes for Hb molecules over the nonimprinted ones. For AGM-1, the K_1, and K_2 can be expressed as follows:

$$K_1 = Q_{BSA}/Q^0_{BSA}, \quad (2)$$

$$K_2 = Q_{Hb}/Q^0_{Hb}, \quad (3)$$

where Q_{BSA} and Q_{Hb} are the adsorption capacities for BSA and Hb on BSA imprinted AGM-1, respectively; Q^0_{BSA} and Q^0_{Hb} are the corresponding adsorption capacities on nonimprinted AGM-1.

For other AGMs except AGM-1, the K_1, and K_2 can be expressed as follows:

$$K_1 = (Q_{BSA} - Q^0_{BSA}{}')/Q^{NIP-AGM1}_{BSA}, \quad (4)$$

$$K_2 = (Q_{Hb} - Q^0_{Hb}{}')/Q^{NIP-AGM1}_{Hb}, \quad (5)$$

$$Q^0_{Hb}{}' = Q^0_{Hb} - Q^{NIP-AGM1}_{Hb}, \quad (6)$$

$$Q^0_{BSA}{}' = Q^0_{BSA} - Q^{NIP-AGM1}_{BSA}, \quad (7)$$

where $Q^0_{BSA}{}'$ and $Q^0_{Hb}{}'$ are corrections of Q^0_{BSA} and Q^0_{Hb}; $Q^{NIP-AGM1}_{BSA}$ and $Q^{NIP-AGM1}_{Hb}$ are adsorption capacities for BSA and Hb on nonimprinted AGM1, respectively.

The specificity of the membrane for rebinding the imprinting molecule was expressed by factor

$$K = K_1/K_2 \quad (8)$$

Characterization of AGMs

The molecular weights of AGMs were determined according to the method of Vreeman et al.[14] using an Ubbelohde viscometer at 35.0 ± 0.05°C by multipoint method, using 1 mol/L $NaNO_3$ aqueous solution as solvent, $K = 2.79 \times 10^{-8}$, $\alpha = 1.89$. The standard sample was PVA with molecular weight of 77 or 95 kDa. The FTIR spectra of AGMs were recorded on a FTIR spectrophotometer (EQUINOX55, Bruker, Germany). The AGM powder was determined by the KBr tabletting method, and the AGM films were determined directly. The quantitative analysis of the contents of C, H, N, S, and O in AGMs was carried out on an elemental analyzer (EA2400II, Perkin-Elmer, USA).

All results were statistically evaluated by ANOVA and posthoc testing with Bonferroni's correction on SPSS statistic software. Significance was set at the 10% level ($P < 0.10$).

RESULTS AND DISCUSSION

Chemical Structure and Elemental Analysis of Agaroses from Different Commercial Sources

The FT-IR spectra of AG-1 to AG-3 are shown in Figure 1. Agarose has characteristic peaks of sugar, among which the peaks are assigned to[15]: 3420 cm^{-1}, O—H stretching; 2920 cm^{-1}, C—H stretching of the sugar rings; 1640 cm^{-1}, H—O stretching of adsorbed water; 1500–1300 cm^{-1}, C—H bending of the sugar rings; 1370 and 1250 cm^{-1}, S=O stretching of the sulfate ester; 1200–1000 cm^{-1}, C—O—C stretching of the pyranose ring; 1120 cm^{-1}, C—H angular deformation of the pyranose ring; 990 cm^{-1}, C—O stretching of primary alcohol groups with contributions of C—C and/or C—O modes; 970 cm^{-1}, C—O stretching of the glycosydic linkage; 1070 and 932 cm^{-1}, C—O—C stretching of the 3,6-anhydro-L-galactopyranose bridge; 890 and 868 cm^{-1}, C—H angular deformation of β-anomeric carbon in the D-galactose residues; 790 and 718–713 cm^{-1}, characteristic adsorptions in agarose, demonstrating the existence of 3,6-anhydro-L-galactopyranose unit; and 771 and 742 cm^{-1}, skeletal bending of pyranose ring. The content of the adsorption water is various from each kind of agarose, so the intensity of the peak near 1640 cm^{-1} would be different. A certain amount of sulfate esters can be detected in all the three kinds of agaroses, by detecting the peaks around 1250 and 1370 cm^{-1} (indicating the total content of sulfate groups), 852 cm^{-1} (C—O—S stretching of D-galactose-4-sulfate), 837 cm^{-1} (C—O—S stretching of D-galactose-2-sulfate), 818 cm^{-1} (C—O—S stretching of D-galactose-6-sulfate), and 800 cm^{-1} (C—O—S stretching of 3,6-anhydro-L-galactopyranose-2-sulfate). The relative intensity of the peak area at 1370 (standing for the content of sulfate groups) and 930 cm^{-1} (standing for the sugar content) demonstrates that the sulfate content of AG-1 is the most, and the sulfate groups were substituted on almost all possible positions in the galactose ring. Furthermore, the strength of the peak at 1120 cm^{-1} is greater than that in AG-2 and AG-3, which suggests that the induced effect of sulfate groups might increase the polarity of the sugar ring and enhance the angular deformation vibration of C—H.

The elemental contents, the sugar contents of the agaroses, the gel strength and the melting points of the agarose gels from different sources are listed in Table II. Besides the sugar, the rest main constituent of the commercial agaroses might be the adsorptive water. The higher the content of the adsorptive water in the agarose, the lower the sugar content, and the weaker the gel strength of the agarose gel. The contents of sugar and water can be calculated by the change of C% and H%, respectively, and the values are approximate. Results show that the sugar content in AG-1 is the lowest (~85%), and so does its gel strength. Moreover, the contents of

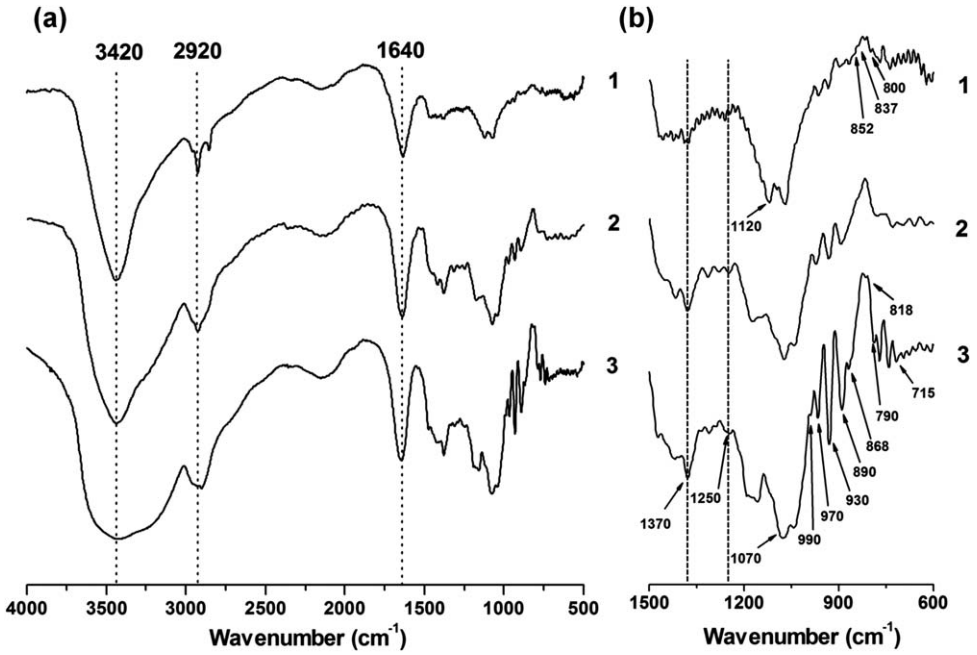


Figure 1. FT-IR spectra (full, a; partial, b) of agaroses from different sources 1: AGM-1; 2: AGM-2; and 3: AGM-3.

N% and S% in AG-1 are higher than the other two samples, which is in accordance with the results from IR spectra. Inspite of that, there is not much difference between elemental contents of agarose samples from various sources, and the value of N% and S% lie within the range of measurement error (0.3%) of the elemental analysis, indicating that the contents of protein and sulfate groups can be treated as trace amounts, so the sulfate group and protein impurities are not the key effecting factors on the adsorption ability and selectivity of AGMs.

Effect of Gel Strength on Protein Recognition Ability of AGMs

The protein adsorption ability and selectivity of AGMs prepared from three kinds of agaroses with similar melting point but different gel strength (named as AGM-1 to AGM-3) are shown in Figure 2. Ideally, agarose gel is a nonfouling material for proteins, but actually most commercial agaroses are slightly charged, and have a small amount of protein adsorption. It is worth noting that the gel strength has an inverse relationship with the imprinting effect. The best imprinting effect is achieved on AGM-1, with the lowest gel strength. The adsorption capability for proteins on unmodified AGMs is mainly determined by the morphology and thickness of the membrane surface. With the increase of the gel strength, the interaction of the agarose chains becomes stronger, and the agarose gel is more easily to shrink when dried. The shrinkage will lead to the increase in surface roughness and thickness of the membrane, which results in the increase of the adsorption of proteins on the AGM. The possible mechanism of the influence of surface roughness on protein adsorption includes the increase in surface area, binding sites for proteins, and the aggregation degree of protein molecules to form multilayered adsorption.[16] The thickness of membrane also influences the protein adsorption. If the membrane was thicker, the protein molecules would transport into the membrane and some would stay in the bulk. From Figure 2(a), it is seen that the AGM-3 gets the highest adsorption for BSA and Hb among the nonimprinted AGMs, which is in accordance with the best gel strength and the highest roughness and thickness of the AGM. After being imprinted, all the adsorptions for BSA and Hb on the imprinted membranes

Table II. Elemental Contents and Gelling Properties of AGMs

					Agarose content			
Agarose	C (%)	H (%)	N (%)	S (%)	Calculated by C%	Calculated by H%	Gel strength (1%, g/cm ^{2})	Melting point (°C)
Theoretical value	47.06	5.88	-	-	100	100		
AG-1	40.44	7.20	0.11	0.53	85.93	88.12	>500	87-89
AG-2	41.79	7.03	0.04	0.44	88.80	89.65	>700	87-89
AG-3	43.72	6.86	−0.08	0.45	92.90	91.98	>1000	87-89
AG-4	-	-	-	-	-	-	>1000	65

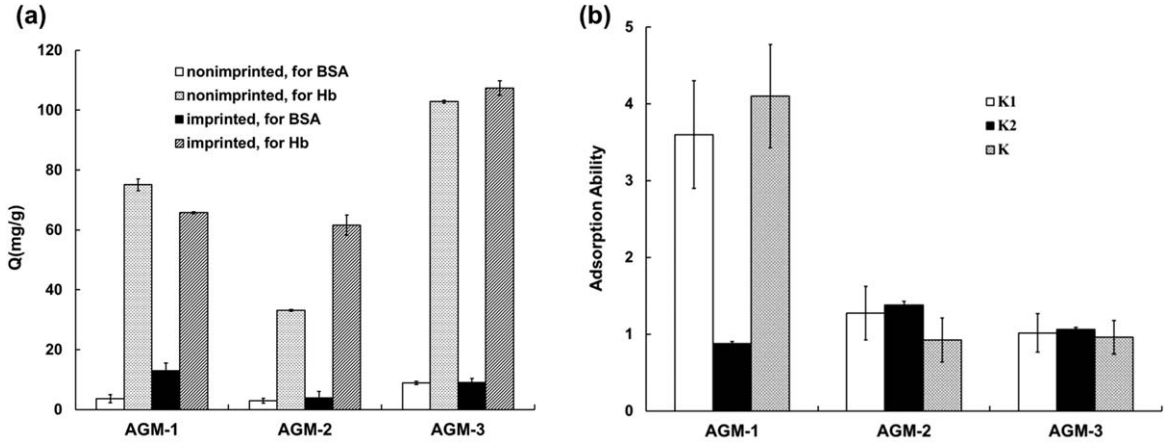


Figure 2. Protein adsorption capacity (a) and selectivity (b) of AGMs with different gel strength.

increase. Among them, the imprinted AGM-3 also gets a relatively high adsorption for BSA and the highest adsorption for Hb, which indicates that the imprinting effect does not work to agarose due to the strong interaction among agarose chains rather than that between protein and agarose. The adsorption of BSA on the nonimprinted AGM-1 is lower but that on the imprinted one is highest, which results in the highest K_1 value. Inspite of the analytical error (5–10%), the K_2 of AGM-1 is close to 1, indicating the repulsion for Hb and the obvious imprinting effect on the imprinted membrane [Figure 2(b)]. The possible reason is that the interaction among the agarose chains is relatively weak to benefit the interaction between the template protein molecules and the agarose during the imprinting process. As for AGM-2, all the adsorptions are the lowest except that K_2 gets the highest value, which may be due to the balance of the imprinting effect and the interactions among agarose chains. It is very clear that the K_1 and K value decreases gradually with the increasing gel strength. The imprinting effect will be enhanced by decreasing the gel strength of agarose, which is mainly determined by the molecular weight, the contents of sugar, and the contents of the substituted groups.

The $[\eta]$ and M_η of the three agaroses are listed in Table I. The three commercial agarose samples show great difference in intrinsic viscosity (around 100 mL/g), and their molecular weights also have some difference (around 40 kDa). As there are small differences in elemental contents and chemical structure, it can be concluded that the crucial factor on the imprinting effect of agarose is the molecular weight and the sugar content.

The $[\eta]$ and M_η of the agaroses through various treatments are also listed in Table I. The samples from AGM-4 to AGM-8 were treated through thermal degradation. It can be seen that, the thermal treatment can only decrease the molecular weight of agarose by around 10 kDa. The samples from AGM-9 to AGM-11 were treated through ultrasonic degradation. The ultrasonic treatment will also decrease the molecular weight of agarose by around 10 to 20 kDa. The samples from AGM-13 to AGM-18 were treated through chemical degradation. The chemical treatment will decrease the molecular weight of agarose dramatically, by around 100 kDa after treated for 6−8 h. However, the gel strength of the resulting membranes through chemical treatment was mostly too low to be used for protein imprinting. It was possibly because the hydroxyl group in agarose chains had partly been oxidized to aldehyde group by H_2O_2, and the hydrogen bonds among the agarose chains were weakened. The sample of AGM-12 is the sulfated agarose, of which the molecular weight is also decreased by 60 kDa after reacting with the chlorosulfonic acid. The strength of the nonimprinted AGMs could be maintained when the molecular weight of the agarose reached 80 kDa, whereas to maintain the strength of the imprinted AGMs, especially during the protein washing process, the molecular weight of agarose should be above 100 kDa.

The FT-IR spectra of the agarose membranes through physical and chemical degradations are shown in Figure 3. The characteristic peaks of the main sugar ring (including peaks at 714, 789, 876, 889, 930, 965, 991, 1000−1150, 1151, and 1182 cm^{-1}) in all the agarose membranes are almost the same, and the peaks for sulfate groups (1370, 1250, 1117, and 844 cm^{-1}) still remain, indicating that the structural unit of agarose membranes remains unchangeable, no matter through physical degradation, chemical degradation, or gel-melting treatment. The agarose chains can be degraded by ultrasonic or other degradation treatments, by some parts of the

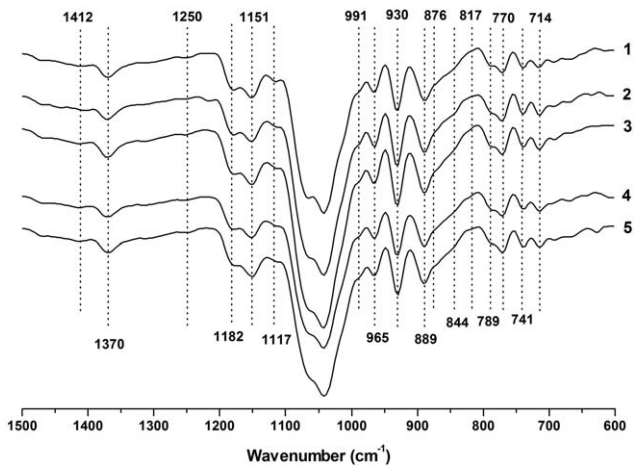


Figure 3. FT-IR spectra of AGMs through physical and chemical treatments 1: AGM-1; 2: AGM-5; 3: AGM-8; 4: AGM-10; and 5: AGM-13.

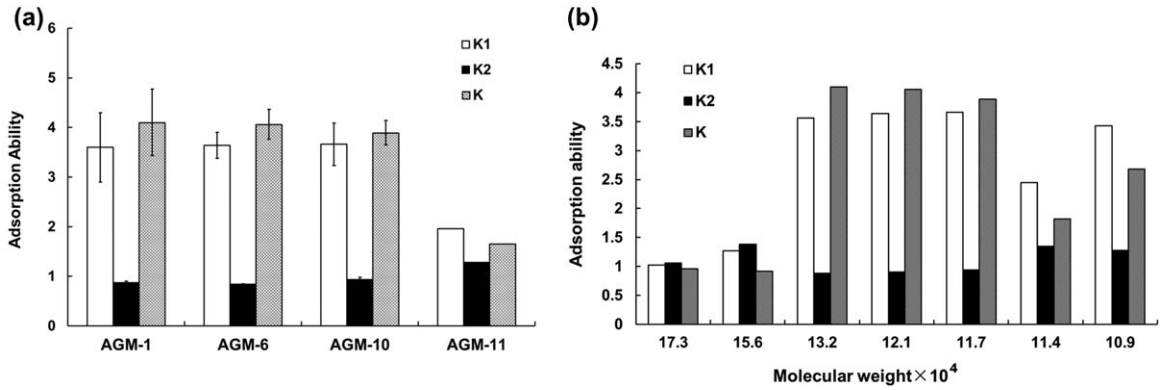


Figure 4. Effect of physical degradation (a) and molecular weight (b) on protein selectivity of AGMs.

glycosydic bridges being oxidized to aldehyde groups and broken. However, this change could not be observed in FT-IR, as the amounts of the aldehyde groups were too small.

The protein selectivity of AGMs through different physical degradation treatments are shown in Figure 4(a). The adsorption ability of the nonimprinted AGMs for BSA dramatically decreases through ultrasonic degradation. The reason might be that the agarose chains are partially oxidized by the synergetic cooperation of cavitation and radical redox under ultrasonic. The oxidized groups such as aldehyde and pyruvic acid groups, would decrease the gel strength of the agarose by decreasing the hydrogen bonds of agarose chains, and also decrease the adsorption ability of the nonimprinted agarose due to the increasing hydrophilicity of the matrix. From Figure 4(a), we can see that the imprinted AGMs with similar molecular weight (AGM-1, AGM-6, and AGM-10) have similar protein adsorption ability and imprinting effect, with an increasing adsorption for BSA and a decreasing adsorption for Hb, compared with the nonimprinted counterpart, no matter what kind of treatment they have been done. There is a distinct decrease in K_1 and K on AGM-11, which might be resulted from the decrease of molecular weight and the increasing amount of oxidized groups.

The effect of molecular weight on protein selectivity of AGMs is shown in Figure 4(b). It is obvious that the best imprinting effect is present in the range of 110–130 kDa, with the highest K value around 4.0. To maintain the imprinting effect of the AGMs, the molecular weight should be kept below 130 kDa. Therefore, the favorite range of molecular weight of agarose for protein imprinting is between 100 and 130 kDa.

Effect of Chemical Components and Structure on Protein Recognition Ability of AGMs

The protein recognition abilities and selectivity of AGMs with different substituted groups are shown in Figure 5. Three groups of agaroses with similar molecular weight are compared, including AGM-1 and AGM-13, AGM-10 and AGM-12, and AGM-3 and AGM-19, with molecular weight around 130 kDa, 115 kDa, and 250 kDa, respectively. It can be seen that, the introducing of substituted groups would weaken the imprinting effect. The oxidized groups in AGM-13 would bring repulsion to protein molecules due to their hydrophilic properties; the sulfate groups in AGM-12 would decrease the adsorption of BSA rather than Hb and lead to decrease of selectivity, due to their negative charges; and the alkoxy groups, such as methoxy and ethoxy in AGM-19, would increase the adsorption of both BSA and Hb seriously due to their hydrophobic properties, and lead to the worst selectivity. The increase in the content of sulfate group leads to the increase in hydrophilic property of the AGM, which also decreases the adsorption ability of both BSA and Hb on nonimprinted AGM. Meanwhile, the imprinted AGM-12 gets a high value for both K_1 and K_2, but the K_2 value is much higher than that on AGM-1, so the value of K is lower than that of AGM-1.

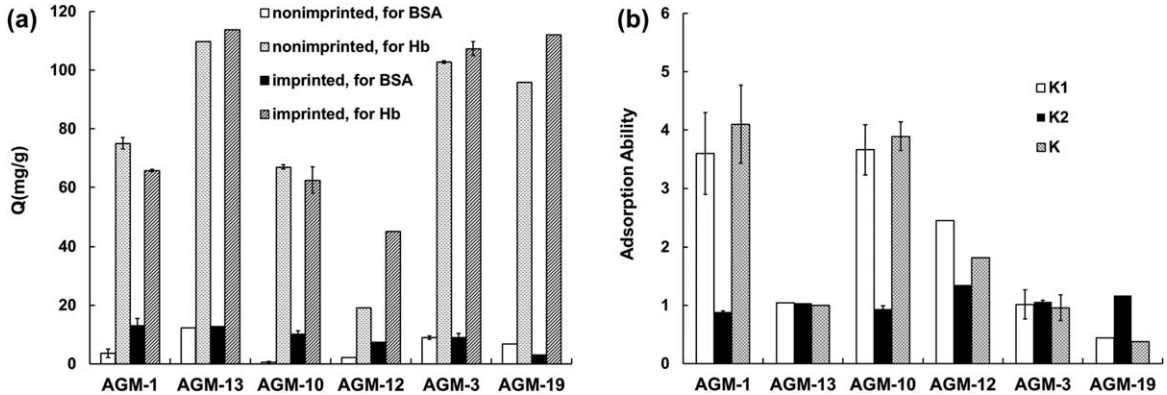


Figure 5. Protein adsorption ability (a) and selectivity (b) of agarose with different substituted groups.

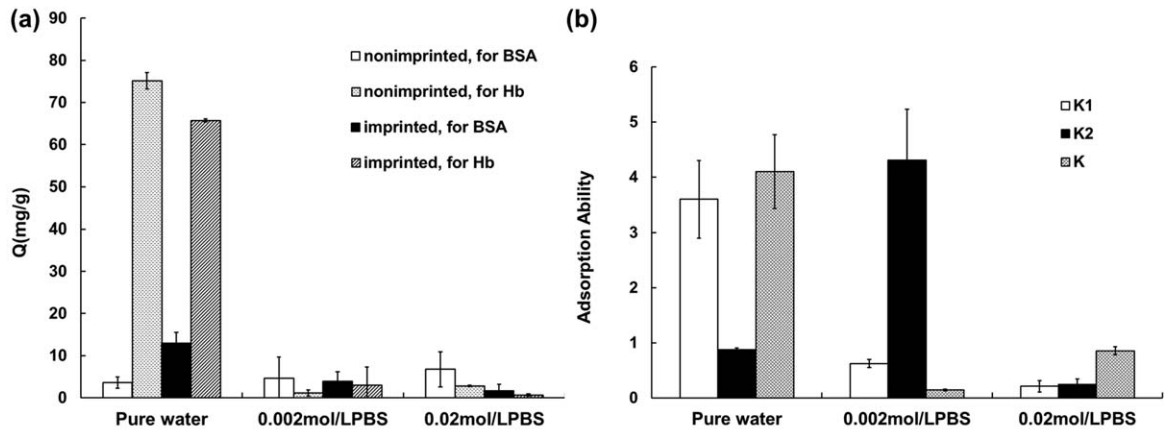


Figure 6. Effect of ionic strength on protein adsorption capability (a) and selectivity (b) of AGMs.

Effect of Salt and Ionic Strength on Protein Recognition Ability of AGMs

The adsorption capacities of BSA imprinted AGM-1 for BSA and Hb in pure water and PBS are listed in Figure 6. It can be seen that the imprinting effect is much better in pure water than in PBS, even the concentration of PBS is as low as 0.002mol/L. The reason that the adsorption for Hb on both nonimprinted and imprinted AGMs is much higher than in PBS is that the pH of pure water (around 6.5) is close to the isoelectric point of Hb (6.5–6.8). In most cases, the maximum protein adsorption reaches near the isoelectric point of protein. In addition, it also enhances the opportunity for the slightly positively charged Hb molecules to react with the slightly negatively charged agarose chain through electrostatic interactions. We also prepared agarose gels using PBS instead of pure water. The resulting gel was translucent and heterogeneous, and showed little imprinting effect both in pure water and in PBS. It can be suggested that PBS will weaken the imprinting effect in the process of both preparation and recognition, as agarose will react with ions prior to proteins.

CONCLUSIONS

The gelling properties have a great influence on the imprinting effect of agarose. Among which, the gelling strength and the molecular weight plays an important role. The effect of protein imprinting of agarose would be weakened when the gel strength increases. The favorable average molecular weight ranges from 100 to 130 kDa. The introducing of some hydrophilic substituted groups, such as carboxyl, sulfate, and pyruvic acid groups, into the agarose molecular structure, would increase the adsorption ability of the nonimprinted AGM but weaken the interaction between agarose and proteins, and decrease the recognition ability of imprinted AGM. The existence of some hydrophobic groups, such as methoxy and ethoxy, would decrease both the adsorption of the template molecule on the nonimprinted and the imprinted AGM. The existence of ions during gelling would seriously weaken the protein recognition ability of agarose. The effect of protein recognition would be severely affected even the ion concentration was 0.002 mol/L, which was probably because that the agarose itself would have interaction with ion in a certain extent.

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