Two-Step Imprinting Procedure of Inter-Penetrating Polymer Network-Type Stimuli-Responsive Hydrogel-Adsorbents

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ABSTRACT: We prepared an imprinted hydrogel-adsorbent, which consists of poly(*N*-isopropylacrylamide (NIPAm)) and poly(sodium acrylate (NaAAc)) gel. We used an inter-penetrating polymer network (IPN) system in the preparation of thermosensitive hydrogel in order to overcome the fundamental defect that the PNIPAm hydrogel loses the thermosensitive volume-phase transition behavior in the presence of polyelectrolyte. Our imprinting strategy of the hydrogel-adsorbent is the following two-step procedure; (1) Firstly, we prepared a soft PNaAAc gel that can bind a guest molecule and change its conformation in accordance with the guest molecule, and then the hydrogel was incubated in heavy metal ion solution to bind the template molecules. (2) Secondly, we tightly fasten the metal bound hydrogel with a secondary gel-network of NIPAm by means of IPN system in order to memorize the coordination structure for the metal ions on the PNaAAc gel. The imprinted hydrogel-adsorbent selectively bound the template ion above a lower critical solution temperature (LCST) of PNIPAm. The memory was fixed by shrinking above the LCST, and was deleted by swelling below the LCST.

KEY WORDS Hydrogel / Inter-Penetrating Networks / Metal Ion Adsorbent / Molecular Imprinting / Stimuli-Responsive Polymers /

Imprinted polymers are attracting much attention in fields of analytical chemistry and functional polymer chemistry because of their ability to bind specific molecules. The characteristics of imprinted polymers, however, are limited to the recognition of their own substrates, since they have very rigid structures containing over 80 mol% of cross-linking reagents in order to memorize a shape of the template.[1] If we can prepare the imprinted hydrogels having soft structures and can control their recognition ability by external stimuli like pH, temperature, and chemicals, it is expected to endow the hydrogels with further biomimetic function that an enzyme and antibody have. The ordinary hydrogel is, however, too soft to memorize a shape of the template molecule. Therefore, there have been only a few attempts to prepare the imprinted hydrogels because of the difficulties in supplying the molecular recognition site to the soft hydrogel.[2–6]

Tanaka *et al.*[2] reported the theoretical presumption that even the hydrogels can exhibit molecular-specific swelling change and can recognize the template molecules. Peppas *et al.*[3] forecast the advantage and the potential of an imprinting hydrogel in their review. Watanabe *et al.*[4] prepared an imprinted hydrogel which exhibited a thermosensitive molecular specificity by using a poly[acrylic acid (PAAc)-*co*-*N*-isopropylacrylamide (NIPAm)] hydrogel. Their imprinted hydrogel could recognize a guest molecule, adrenaline, by using a hydrogen bond under the shrunken state of the PNIPAm units. The PNIPAm hydrogel is a well-known thermosensitive hydrogel, which exhibits a volume-phase transition in response to a temperature change.[7]

We attempted to prepare an imprinted hydrogel-adsorbent for heavy metal ions, which is a poly[sodium acrylate (PNaAAc)-*co*-PNIPAm]. It is expected that their binding ability and selectivity for metal ions can be controlled by external stimuli. However, the NIPAm hydrogel-adsorbent did not exhibit a thermosensitive volume-phase transition behavior, because a PNIPAm copolymer has much polyelectrolyte units like PNaAAc that play a role of a binding site for metal ions. The volume-phase transition of PNIPAm hydrogel is very sensitive to a balance of hydrophobicity and electrostatic repulsion around the polymer main chain.[8, 9] In fact, Tanaka *et al.*[7] have reported that the NIPAm copolymer hydrogels possessing 18 mol% of sodium acrylate units no longer shrunk even up to 80°C due to high ionic osmotic pressure. We could not incorporate the sufficient amount of binding site of a metal ion into the NIPAm copolymer adsorbent system. We, therefore, attempted to use an inter-penetrating polymer network (IPN) system consisting of PNIPAm hydrogel and PNaAAc hydrogel in this imprinting system. The interaction between both of the gels may decrease, because they can individually behave in the IPN copolymer.[10]

The goal of the present study is to prepare the imprinted hydrogel-adsorbent that selectively adsorbed a

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High Selectivity

Low Selectivity

Shrinking State at 50 °C   Swelling State at 25 °C
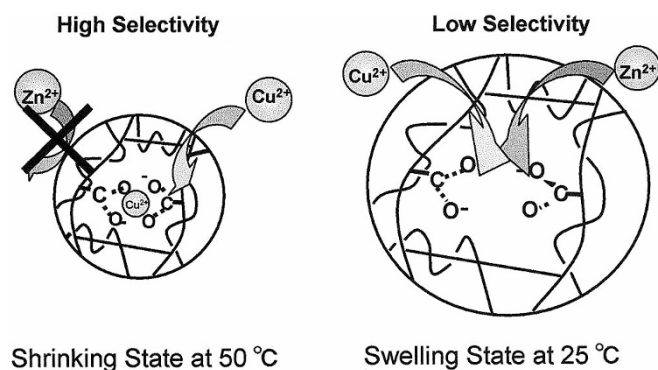
Scheme 1. Cu(II) recognition by using imprinted hydrogel adsorbent.

target metal ion according to our order. That is, we expected the IPN-type imprinted hydrogel to shrink above a lower critical solution temperature (LCST; 32 °C) of PNIPAm and to selectively bind the metal ions under shrunken state (Scheme 1). In this study, we report the two-step preparation method of the stimuli-responsive imprinting hydrogel and its application as a hydrogel-adsorbent for heavy metal ions. We can control the recognition ability of the imprinted hydrogel-adsorbent by using the external thermal stimuli.

EXPERIMENTAL

Materials

Acrylic acid (AAc) was obtained from a commercial source and purified by distillation just before polymerization. N-Isopropylacrylamide (NIPAm) and N, N'-methylenebisacrylamide (MBA) were obtained from a commercial source and purified by recrystallization from ethanol (EtOH) just before polymerization. Azobisisobutyronitrile (AIBN) was recrystallized from methanol solution. Solvents used were purified by the usual method. Metal salts and other reagents were obtained from commercial sources and used without further purification. We used a semipermeable membrane (Viskase Companies UC-36-32-100 seamless Cellulose Tubing, pore size 50 Å, permeable molecular weight is 14000) for metal ion adsorption test. A testing sieve (250 μm pore size) was used for aliening the size of the hydrogel adsorbents.

Preparation of Imprinting Hydrogels

The soft hydrogel network of PNaAAc (G-1) was prepared by ordinary radical polymerization of AAc (3 mol L^{-1}) with 1 mol% of N, N'-methylenebisacrylamide (MBA) in the presence of 1 mol% of AIBN in EtOH at 50 °C for 24 h. The polymerization yield of G-1 hydrogel is 78.9%. The resulting hydrogel was neutralized with 0.1 mol L^{-1} of aqueous

NaOH for 18 h to introduce binding sites for heavy metal ions. Then, 300 mg of G-1 (3 mmol unit) was imprinted with Cu(II) ion by incubating in 200 mL of CuSO$_4$ (5 mmol L^{-1})aqueous solution for 24 h. The blue hydrogel was filtered off from the CuSO$_4$ solution, and was washed several times with deionized water to remove the physically bound metal ions. The amount of bound metal ion was about 3 mmol g^{-1} dried resin. The resulting Cu(II) bound G-1 (100 mg) was sufficiently swollen with an minimum DMSO (25%) aqueous solution consisting of NIPAm (1.5 mol L^{-1}, [NIPAm]/[NaAAc]:4/1), MBA (0.3 mol L^{-1}), with ammonium persulfate (1 mol%) (G-2 and G-3). The MBA contents of G-2 and G-3 were 16.7 mol% and 9.1 mol%, respectively. The swollen hydrogel was then incubated at 50 °C for 24 h to carry out the secondary polymerization. The resulting imprinted hydrogel were washed with deionized water several times to remove the remaining NIPAm monomer and initiator. The template Cu(II) ions were removed from the imprinted hydrogel by washing with 1 mol L^{-1} of HCl aqueous solution for 3 h at 25 °C. The imprinted hydrogel was washed with deionized water several times, and then neutralized by 0.1 M of NaOH solution, and then washed with deionized water to remove excess of alkaline. We used the imprinted hydrogels at metal ion adsorption tests after lyophilization. We also prepared the IPN-type non-imprinted hydrogel (G-4) that did not have a specific binding site for Cu(II) ions. G-4 was prepared by the same procedure of the imprinted hydrogels (G-2 and G-3) except for using G-1 that did not bind Cu(II) ions. Their copolymer compositions are shown in Table I.

Measurement of Water Absorption

A quantity of 50 mg of the imprinted hydrogel was stored in a tea-pack. Then, the pack was incubated in a 50 mL of aqueous solutions (5 mmol L^{-1} of CuSO$_4$ aqueous solution and an DMSO (25%) aqueous solution consisting of NIPAm (1.5 mol L^{-1}), MBA (0.3 mol L^{-1})) without stirring at given temperature. After a given time the pack was picked up from the solution and was wiped by Kimpiwes® to remove the excess aqueous solution on the pack, and then it was weighed to estimate the amount of absorbed aqueous solution (g/g dried resin).

Metal Ions Adsorption

The metal ion adsorption test by the imprinted hydrogel was carried out batchwise. A quantity of 20 mg of the imprinted hydrogel (unshifted powder type) was stored in a pack (50 mm square) made by semipermeable membrane (permeable molecular weight is < 14000). Then, the pack was incubated in a 20 mL

Table I. Preparation of IPN-type imprinted hydrogel-adsorbents[a]

| | Imprint[b] | Composition of IPN Copolymers | | Conv.(%) |
		NIPAm/NaAAc (M/M)[c]	MBA (mol%)[d]	
G-2	Cu imprint	3.01	16.7	65.4
G-3	Cu imprint	2.65	9.1	60.6
G-4	Non imprint	2.35	16.7	73.4

[a] $[NIPAm] = 1.5 \, mol \, L^{-1}$ ($[NIPAm]/[AAc] = 4/1$) and $[MBA] = 0.3 \, mol \, L^{-1}$ were copolymerized by APS (1 mol%) in plesence of 0.1 g of G-1 gel containing of 3 mmol g^{-1} dried resin Cu(II) in 25 vol% of DMSO aq. at 50 °C for 24 h. [b] Cu(II) content is 3 mmol g^{-1} resin. [c] Determined by elemental analysis. [d] MBA contents in comonomers.
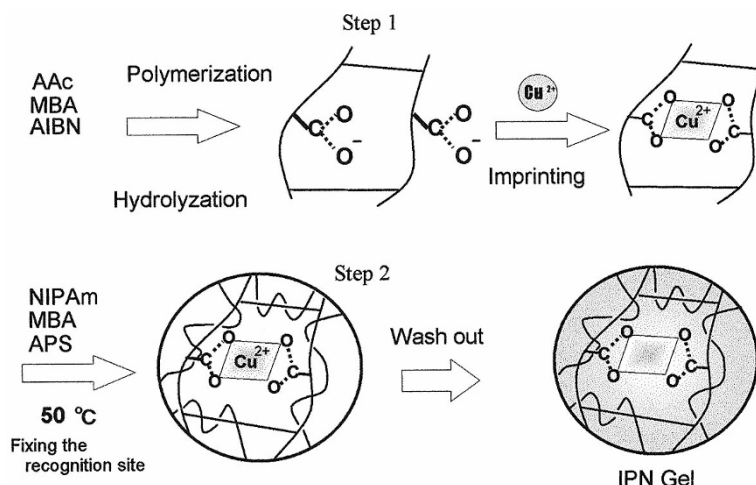

Scheme 2. Two-step imprinting procedure of IPN-type stimuli-responsive hydrogel adsorbent.

of CuSO$_4$ (or ZnSO$_4$) aqueous solution (5 mmol L^{-1}) without stirring. The amount of Cu(II) or Zn(II) ion adsorbed was estimated from the decrease of the metal ion concentration in the aliquot of the solution separated from the reaction mixture. The amounts of the adsorbed and desorbed metal ions were quantified by a chelatometry.[11] We carried out the metal adsorption test more than twice for the reproducibility.

RESULTS AND DISCUSSION

Preparation Strategy of the Imprinted Hydrogel-Adsorbent

We attempted to prepare the IPN-type imprinted hydrogel that shrinks above the LCST of PNIPAm and that selectively binds the target metal ion under shrunken state. Our preparation strategy of the IPN-type imprinted hydrogel-adsorbent is the following two-step procedure (Scheme 2). Firstly, we prepared a soft PNaAAc hydrogel (G-1) possessing the small amounts of cross-linking reagents (1 mol%), which freely change the conformation in accordance with template ions, and then G-1 was incubated in heavy metal ion solution to bind the template ions. Secondly, the Cu(II) bound G-1 gel tightly fastened with a secondary gel-network of PNIPAm by means of the IPN system
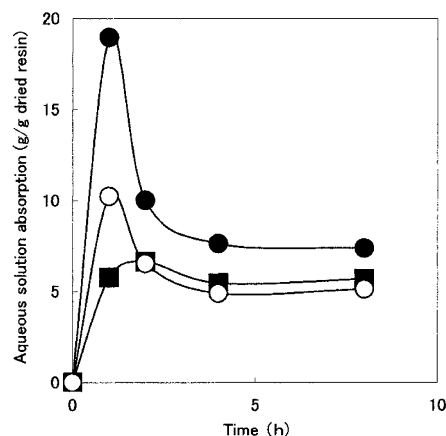
Figure 1. Aqueous solution absorption by Cu(II) imprinted hydrogel (G-2), (●); in CuSO$_4$ solution at 20 °C, (○); in CuSO$_4$ solution at 50 °C, (■); in NIPAm/MBA comonomer solution (25% of DMSO) at 50 °C.

in order to memorize the coordination structure of the template ions on the G-1 gel.

We estimated their swelling ratio by means of the amount of water absorption in order to study their thermosensitive volume-phase transition behavior. The swelling ratios of the imprinted hydrogel (G-2) in the CuSO$_4$ solution are shown in Figure 1. The IPN-type hydrogels shrunk at 50 °C and swelled at 25 °C even in CuSO$_4$ aqueous solution. The IPN-type hydrogel
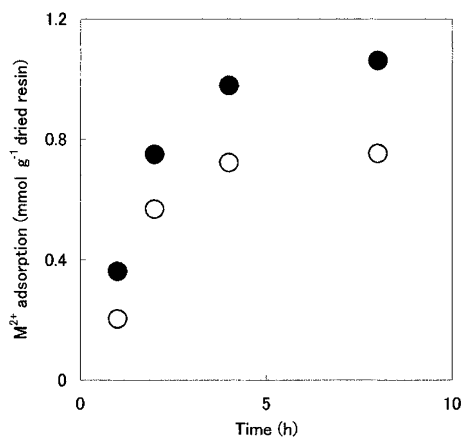
Figure 2. Metal ion adsorption by Cu(II) imprinted hydrogel (G-2) at 50 °C. [MSO_4] = 5 mmol L^{-1}, [G-2] = 20 mg/20 mL, (●); Cu(II) ion, (○); Zn(II) ion.

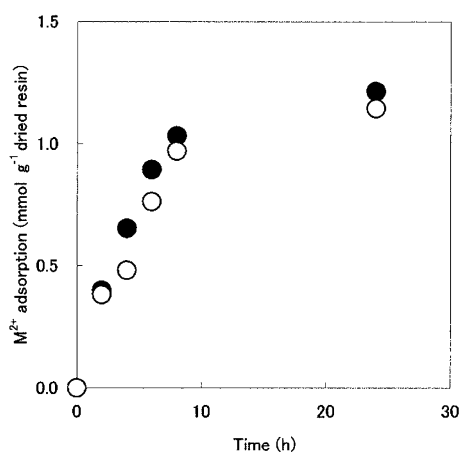**Figure 3.** Metal ion adsorption by non-imprinted hydrogel (G-4) at 50 °C. [MSO_4] = 5 mmol L^{-1}, [G-4] = 20 mg/20 mL, (●); Cu(II) ion, (○); Zn(II) ion.

exhibited the thermosensitive volume-phase transition behavior in spite of the 20 mol% of NaAAc contents. Naturally, G-1 that did not contain NIPAm units did not exhibit any thermosensitive volume-phase transition behavior. We have also detected that NIPAm-*co*-NaAAc random copolymer containing 20 mol% of NaAAc units did not exhibit the volume-phase transition behavior.[12] The IPN system prevented the polyelectrolyte units from interfering with the thermosensitive volume-phase transition of the PNIPAm hydrogel.

The Effect of Imprinting in Metal-Adsorption Test

The results of the Cu(II) and Zn(II) ion adsorption test by using the Cu(II)-imprinted hydrogel (G-2) and the non-imprinted hydrogel (G-4) at 50°C are shown in Figures 2 and 3, respectively. Both of the hydrogels shrunk at 50°C, because they have inter-penetrated PNIPAm gel unit. G-2 adsorbed much Cu(II) ions than Zn(II) ions under this condition. G-4 did not have any metal ion selectivity in this adsorption-test.[13] We as-
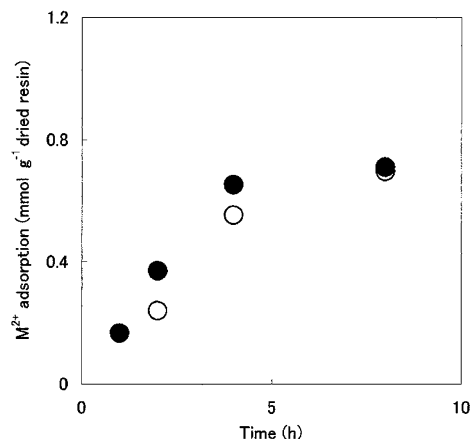

Figure 4. Metal ion adsorption by Cu(II) imprinted hydrogel (G-2) at 20 °C. [MSO_4] = 5 mmol L^{-1}, [G-2] = 20 mg/20 mL, (●); Cu(II) ion, (○); Zn(II) ion.

sume that the swelling ratio of the imprinted hydrogel under the metal-adsorption condition is very important to recognize their substrates. The water swelling ratios of G-2 in $CuSO_4$ solution at 20 °C and at 50 °C were compared with that in NIPAm/MBA comonomer solution (25 vol% of DMSO) at 50 °C (Figure 1). The swelling ratio of G-2 in $CuSO_4$ solution at 50 °C is low as same as that in the DMSO comonomer solution, in which the hydrogel was imprinted with Cu(II) ions. The shrunken G-2 would recall the memory that was imprinted with Cu(II) ion. That is, the shrunken G-2 has a suitable binding site for Cu(II) ion that prefers a square planar coordinate structure. The imprinted hydrogel can discriminate between the coordinate structure of Cu(II) ion (square planar structure) and that of Zn(II) ion (tetrahedral structure).[14]

The Effect of the Gel Morphology

We expected the IPN-type imprinted hydrogel to shrink above the LCST of PNIPAm (32 °C) and to selectively bind the metal ions under shrunken state. That is, the IPN-type imprinted hydrogel does not have any selectivity for metal ions below their LCST (under swollen state). The results of the metal-adsorption test of G-2 at 20 °C are shown in Figure 4. This temperature (20 °C) is lower than the LCST of NIPAm polymer. It is well known that a NIPAm hydrogel swells below the LCST and shrinks above the LCST. We detected that G-2 swelled well by absorbing large amounts of water at 20 °C than that at 50 °C (Figure 1). G-2 no longer had any binding selectivity under swollen state. It was clarified that even the imprinted hydrogel could not recognize their target metal ions under swollen state. Because the specific binding site for Cu(II) ions on the IPN-type imprinted hydrogel collapsed under a swollen state at 20 °C, the binding selectivity for Cu(II) ion at 20 °C is lower than that at 50 °C. We could erase
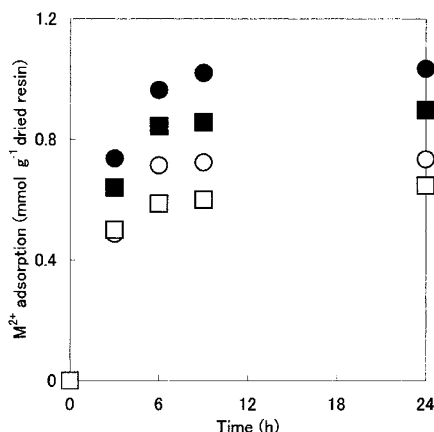
Figure 5. The effect of the gel mesh size on metal ion adsorption by Cu(II) imprinted hydrogel (G-2) at 50 °C. Adsorption by fine grain hydrogel (Cu(II) imprinted G-2; mesh size is < 250 μm). (●); Cu(II) ion, (○); Zn(II) ion, and by coarse grain hydrogel (mesh size is > 250 μm), (■); Cu(II) ion, (□); Zn(II) ion. [MSO$_4$] = 5 mmol L^{-1}, [G-2] = 20 mg/20 mL.

the memory of G-2 that was imprinted with Cu(II). We could control a molecular selectivity of the IPN-type imprinted hydrogel-adsorbent by means of external stimuli.

We sieve the Cu(II) imprinted hydrogel, G-2, by using a testing sieve (250 μm pore size), in order to check the effect of particle size of hydrogel on the adsorption test (Figure 5). We expected that the particle size will not affect the amount of adsorbed metal ion, and will not affect their metal ion selectivities at equilibrium state. Contrary to our expectation, a small pore size hydrogel (< 250 μm) easily adsorbed metal ions as compared to a large pore size hydorgel (> 250 μm). However, the pore size of the hydrogel did not affect their metal ion selectivity. We presumed the hard skin phase was generated on the face of the coarse particle hydrogel when the hydrogel adsorbed Cu(II) ions in the aqueous solution. The hard skin phase will prevent that the inner metal binding site freely adsorb the metal ion. Therefore, the adsorption ability of the coarse particle hydrogel is less than that of fine particle hydrogel. The hard skin phase did not affect on the essential metal binding selectivity of the imprinted hydrogel adsorbents.

The Effect of Concentration of Cross-Linking Reagent

In our preparation strategy, there is two important points. Firstly, the original PNaAAc hydrogel has to be soft to freely change its conformation in accordance with a target metal ion. Secondly, the PNaAAc hydrogel that bound the metal ions has to be tightly fixed by using a secondary gel-network of PNIPAm. Therefore, the amount of crosslinking reagent (MBA) in the secondary gel network is very important for the recog-
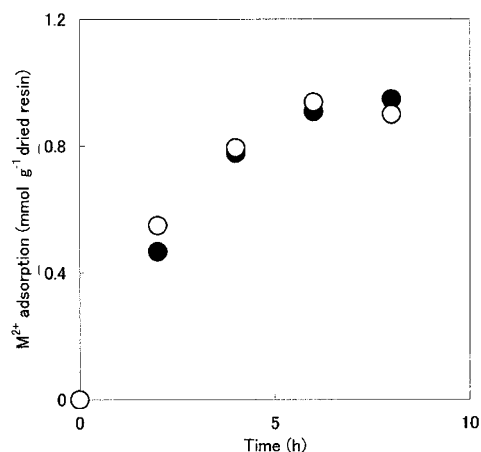

Figure 6. Metal ion adsorption by Cu(II) imprinted hydrogel possessing lower cross-linking points (G-3) at 50 °C. [MSO$_4$] = 5 mmol L^{-1}, [G-3] = 20 mg/20 mL, (●); Cu(II) ion, (○); Zn(II) ion.

nition ability that responds to the external stimuli. A small amount of MBA will cause the imprinted hydrogel to lose its recognition ability. On the other hand, a large amount of MBA will cause the imprinted hydrogel to lose its response to the external stimuli. The results of the adsorption test by using G-3 are shown in Figure 6. This imprinted hydrogel-adsorbent that has low cross-linking points (9.1 mol% of MBA) did not have any binding selectivity for metal ions even under shrunken state (at 50 °C). These results suggested that the tightly fixation of the imprinted hydrogel by a secondary gel-network of PNIPAm is very important to recognize the template selectively.[15]

CONCLUSION

This IPN-type imprinted hydrogel-adsorbent has the following specificities; (1) The imprinted hydrogel could recognize the template molecules and could change their binding selectivity in response to the thermal stimuli. It is because the PNaAAc gel and the PNIPAm gel individually behaved in this IPN system. That is, the PNaAAc gel plays a role of a binding site for metal ions, and the PNIPAm gel plays a role of thermosensitive fixed rope for the recognition site in this IPN system. (2) We could write in the memory of template directly on the PNaAAc hydrogel by means of the two-step imprinting method. (3) We could delete the memory on the imprinted hydrogel by using the external thermal stimuli.

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