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Capillary electrophoretic separation of metal ions using complex forming equilibria of different stabilities

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Abstract

Capillary electrophoresis was investigated for the determination of metal cations in the presence of various complexing agents. Separation of most metal ions is complicated by their similar electrophoretic mobilities and their weak UV absorption. In this study, the use of ligands forming metal complexes of different stabilities was examined. These complexing agents can selectively modulate the mobility of cations by forming metal complexes with varying degrees of stability. To cover a wide range of complex stability, different agents were studied such as α-hydroxyisobutyric acid (lg β 2...5), 8-hydroxyquinoline-5-sulfonic acid (lg β 7...12) and ethylenediaminetetraacetic acid (lg β 14...20). In the experiments, a model mixture of five metal ions (Cu, Zn, Pb, Ni and Fe) was used for all ligands, although in practice complexing agents and separation parameters will be chosen according to the properties of the analyzed cations. Using computer simulation, the average complexation degree for each metal ion with the different ligands under the chosen separation conditions was calculated. Detection was carried out by direct or indirect UV absorption due to the properties of the complexing agent or the formed complexes.

Keywords: Complex formation; Metal ions; Hydroxyisobutyric acid; Hydroxyquinolinesulfonic acid; Ethylenediaminetetraacetic acid

1. Introduction

Capillary electrophoresis (CE) has rapidly developed into a reliable microanalytical separation technique for a variety of applications. Metal ion analysis [1], however, covers a relatively small part of CE applications compared with separations in the areas of pharmaceuticals, oligonucleotides, proteins and peptides. Considering metal ion analysis, problems occur due to the similar electrophoretic mobilities and the weak UV absorption of most metal ions.

The latter problem has been solved by the introduction of indirect UV absorption to CE [2,3]. Furthermore, detection of metal ions can be achieved by the addition of an UV-absorbing ligand or by the formation of UV-active complexes of the sample ions [4].

Separation in CE is based on differences in electrophoretic mobilities. Most transition metal ions have similar mobilities due to their similar size and identical charge. This fact is demonstrated in Fig. 1 for five transition metal ions. The larger radius of the Pb^{2+} ion and therefore the resulting lower degree of hydration of the cation causes the higher value for the electrophoretic mobility of lead.

To achieve an electrophoretic separation, mobilities have to be modified by introduction of a complex-forming equilibrium. If different degrees of complexation result for the ions during the inter-

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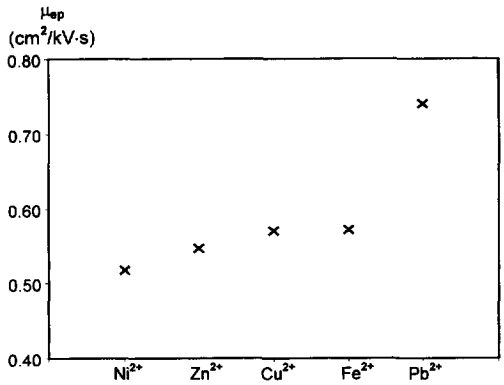


Fig. 1. Electrophoretic mobilities of the metal ions [5].

action with a ligand, their apparent electrophoretic mobilities should be different also [6].

We compared different ligands for the separation of transition metals by complex formation. Separation is achieved by differences of the complexation degree resulting in different charged species or by diverse complex structures. In an earlier publication [7] we described the complex formation theoretically by calculation of the average number, $\bar{n}$, of ligands of the complex $ML_{\bar{n}}$ in order to estimate suitable ligand concentrations for the electrophoretic separation. Due to the acidic properties of the ligands, complex formation is also affected by the pH of the electrophoretic buffer. The computer simulation program, MARTIN, was used to calculate the concentration of the several species under separation conditions.

Table 2
Stability constants of the metal complexes

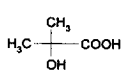
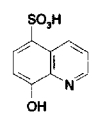
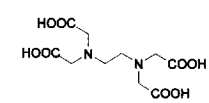
	α-HIBA			HQS		EDTA
	lg β_1	lg β_2	lg β_3	lg β_1	lg β_2	lg β_1
Cu^{2+}	2.74[a]	4.34[a]	4.38[a]	12.50[b]	23.10[b]	18.80[d]
Zn^{2+}	1.72[a]	3.01[a]	3.40[a]	8.40[b]	15.10[b]	16.50[d]
Pb^{2+}				8.53[b]	16.13[b]	18.04[d]
Ni^{2+}	1.67[a]	2.80[a]	2.84[a]	10.00[b]	18.10[b]	18.62[d]
Fe^{2+}				8.40[b]	15.10[b]	14.32[d]
Fe^{3+}				11.6[c]	22.8[c]	25.10[d]

[a] [8], [b] [10], [c] [11], [d] [13].

Table 1 shows the investigated complexing agents. α-hydroxyisobutyric acid (α-HIBA), very often used for the separation of metal ions [8,9], is a monovalent ligand that forms weak complexes. Stepwise complex formation occurs leading to a mutual equilibrium of the complexes ML_n (see Table 2). In contrast to α-HIBA, the divalent 8-hydroxyquinoline-5-sulfonic acid (HQS) forms metal complexes of intermediate strength. Relatively stable complexes, ML and ML_2, are formed due to the significantly high value of lg β_1 (Table 2). Ethylenediaminetetraacetic acid (EDTA) was used to form strong complexes. Complexation takes place as a one-step reaction forming stable chelates encapsulating the metal ion.

The calculated data included only the complex formation with the investigated ligand. In the case of iron speciation, competing complexation with buffer components has been investigated in more detail. An attempt was made to predetermine the separation

Table 1
Structures and pK_a values of the ligands [13]

α-Hydroxyisobutyric acid	8-Hydroxyquinoline-5-sulfonic acid	Ethylenediaminetetraacetic acid
		
HL	H_2L	H_4L
$pK_{a1} = 3.97$	$pK_{a1} = 4.112$ $pK_{a2} = 8.757$	$pK_{a1} = 2.00$ $pK_{a2} = 2.69$ $pK_{a3} = 6.13$ $pK_{a4} = 10.19$

conditions and the obtained data were compared with the results of electrophoretic separation.

2. Experimental

All measurements were performed using the P/ACE 2100 capillary electrophoresis system of Beckman (Palo Alto, CA, USA) with an UV detector. Untreated fused-silica capillaries of different lengths and internal diameters, from Polymicro Technologies (Photonetics, Stuttgart, Germany), were used.

Standard solutions of $FeCl_2$, $FeCl_3$, $NiCl_2$, $ZnCl_2$, $CuCl_2$ and $Pb(NO_3)_2$ were prepared from the salts (Aldrich, Steinheim, Germany). α-HIBA, 8-HQS and EDTA were used as complexing agents; creatinine, disodium hydrogen phosphate, disodium tetraborate, boric acid, salicylic acid, polyethylene glycol and hexadecyltrimethylammonium bromide (HTAB) were used to prepare the buffers. All chemicals were of analytical-reagent grade (Fluka, Neu-Ulm, Germany). Buffers and standard solutions were prepared with triply distilled water and the pH was adjusted with HCl or NaOH.

3. Results and discussion

3.1. Theoretical considerations

By addition of a complexing agent to the separation buffer an equilibrium is formed between the free metal cation and the various complexes:

$$M^{2+} + L^- \rightleftarrows ML^+ \quad \beta_1 = \frac{[ML^+]}{[M^{2+}][L^-]} \tag{1}$$

$$M^{2+} + 2L^- \rightleftarrows ML_2 \quad \beta_2 = \frac{[ML_2]}{[M^{2+}][L^-]^2} \tag{2}$$

$$M^{2+} + mL^- \rightleftarrows ML_m^{(m-2)-} \quad \beta_m = \frac{[ML_m^{(m-2)-}]}{[M^{2+}][L^-]^m} \tag{3}$$

In solution, the complexes exist in mutual equilibrium. The apparent electrophoretic mobility is then a combination of the more mobile free metal ion and the slower, complexed forms of the cation. Due to the fast equilibration between the different complexes, it is possible to describe the system by a brutto complex $ML_{\bar{n}}^{(2-\bar{n})+}$ with the average number, $\bar{n}$, of ligands [7], that can be calculated as follows:

$$ML_{\bar{n}}^{(2-\bar{n})+}$$

$$\bar{n} = \frac{\beta_1[L^-] + 2\beta_2[L^-]^2 + \cdots + m\beta_m[L^-]^m}{1 + \beta_1[L^-] + \beta_2[L^-]^2 + \cdots + \beta_m[L^-]^m} \tag{4}$$

From Eq. 4, it follows that $\bar{n}$ depends only on the concentration of free ligand $[L^-]$, which is variable depending on the total concentration of complexing agent added to the buffer or by variation of the pH and therefore the degree of dissociation of the ligand. Both parameters (pK_a, pH) were considered in the applied computer simulation. The simulations were performed with the program MARTIN, developed at our institute, and taking into account the dissociation of the ligand, the stepwise complex formation, the concentration of the metal ions and of the ligand as well as the pH of the separation buffer. As a result, we obtained the concentration of all species present in the buffer.

3.2. Separation of the α-HIBA complexes

α-HIBA is a monovalent ligand that forms complexes with different degrees of complexation. Due to the low differences of the lg β values and the fast equilibration, the different complexes coexist, but can be described as $ML_{\bar{n}}$ with the average number $\bar{n}$ of ligands. Fig. 2A shows the calculated values of $\bar{n}$, highest mobility differences should be expected in the range from 10 m*M* to 5 *M* α-HIBA. As a result of our computer simulation, the metal ions are migrating as a mixture of M^{2+} and ML^+, with small amounts of ML_2 and only traces of the fully complexed ML_3^-, under separation conditions. So, the apparent charge of $ML_{\bar{n}}$ should be in the range between +1 and +2, the species are separated as cations.

Fig. 2B shows the electrophoretic separation of the α-HIBA complexes. Due to the weak complex formation, a large excess of complexing agent has to be added to the separation buffer to modify the mobilities of the cations effectively. Copper, by reason of the highest lg β values, migrates the slowest, corresponding with the curves of $\bar{n}$ (Fig.

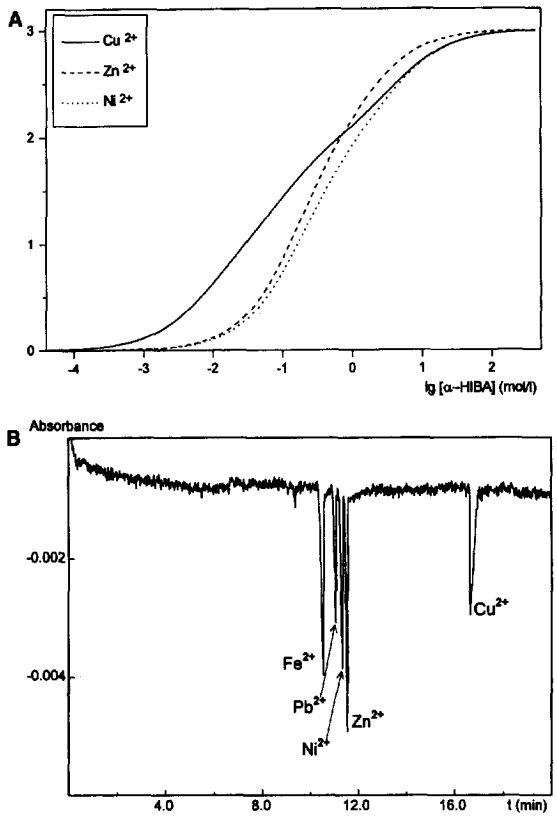


Fig. 2. α-HIBA complexes. (A) Calculation of the average number, $\bar{n}$, of ligands. (B) Separation of the α-HIBA complexes. Sample, 0.05 m*M* Cu^{2+}, Ni^{2+} and Zn^{2+}; 0.1 m*M* Pb^{2+} and Fe^{2+}. Buffer, 5 m*M* creatinine–15 m*M* α-HIBA, pH 3.45. 5 s (5 kV) injection; U=8 kV; wavelength, 200 nm; column, 50/57 cm, 50 μm I.D.

2A). To achieve an acceptable peak shape for Cu^{2+}, the concentration of the ligand in the separation buffer has to be in the range of 10 to 15 m*M*, otherwise the peak becomes broader and the tailing increases dramatically due to higher differences in the mobilities of Cu^{2+} and the background electrolyte. On the other hand, the differences of complexation and therefore of resolution were poor for the other metal ions at a α-HIBA concentration of 10 m*M*. Resolution of these analytes should be better at higher ligand concentrations, but above 50 m*M* α-HIBA the separation time increases and such conditions should therefore be avoided. Nevertheless, the calculated migration order, as well as the predicted resolution of the five cations, was confirmed by practical separation. Therefore, no further reaction besides those considered in the computer simulation play a role in the separation of these complexes with low stability.

3.3. Separation of the HQS complexes

Similar to α-HIBA, a stepwise complexation is assumed for the HQS complexes. The calculation of the average degree of complexation $\bar{n}$ is shown in Fig. 3A. Unfortunately, separation of the metals at ligand concentrations below the stoichiometric value was impossible and no peak resolution was achieved. Only at stoichiometric or excessive ligand concentrations were well separated peaks for all five

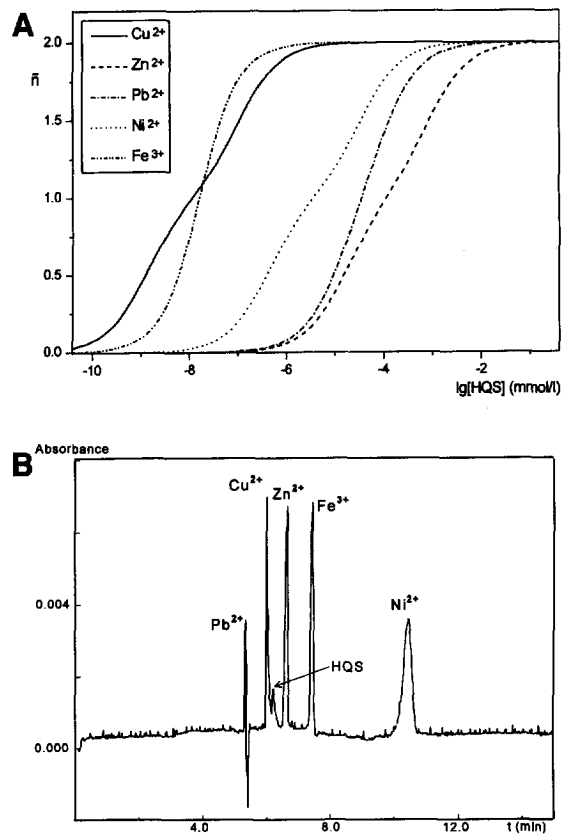


Fig. 3. HQS complexes. (A) Calculation of the average number $\bar{n}$ of ligands. (B) Separation of the HQS complexes. Sample, 0.02 m*M* Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+} and Fe^{3+}, each in 0.2 m*M* HQS. Buffer, 10 m*M* phosphate–6 m*M* borate–0.1 m*M* HQS–0.1% polyethylene glycol pH 8.00. 5 s pressure injection; U=25 kV; wavelength, 254 nm; column, 50/57 cm, 75 μm I.D.

complexes observed. Therefore, electrophoresis was carried out with the fully or nearly fully complexed metal ions and structural differences had to be used for the separation.

As the simulation shows, all complexes should exist predominantly in the ML_2^{2-} form (conditions as in Fig. 3B). However, for the metals with lower lg β values, considerable amounts of ML are present under equilibrium conditions (1.5% for Zn^{2+} and 0.5% for Pb^{2+} and Ni^{2+}), so these ions should migrate as a mixture of 1:2 and 1:1 complexes.

Using alkaline buffers, the HQS complexes were separated at normal polarity without any pretreatment of the capillary. Under these conditions the complexes are carried to the detector by electroosmosis.

Fig. 3B shows the electropherogram of the separation of the HQS complexes. It must be pointed out that Pb^{2+} migrates fastest, although it has an intermediate stability constant. This may be caused by the higher mobility of the Pb^{2+} ion or by differences in the complex structure due to the larger ion radius. It should not be an effect of less complexation compared with the other metal ions. In general, the HQS complexes are not separated due to adjusted differences in the average number $\bar{n}$ of ligands. The formation of mixed-ligand complexes, differences in the complex structure or kinetic effects should be responsible for the electrophoretic separation. The existence of such multiple species within a sample zone may be a source of peak broadening [12], as can be seen in Fig. 3B for the Ni^{2+} ion. Due to the extreme complexity of this system, further experiments have to be performed to explain the separation behavior in more detail.

3.4. Separation of the EDTA complexes

In contrast to α-HIBA, EDTA forms stable complexes with the investigated metal ions, due to the higher stability constants (see Table 2) caused by the chelating effect. The calculation of $\bar{n}$ (Fig. 4A) predicts that differences in complexation occur only at very low values of L^{4-}. Although the concentration of free L^{4-} is very low under separation conditions (pH 4, [EDTA]=0.7 m*M*) the dissociation equilibrium of EDTA is shifted to L^{4-}, caused by the complex formation with the metal ions. So all

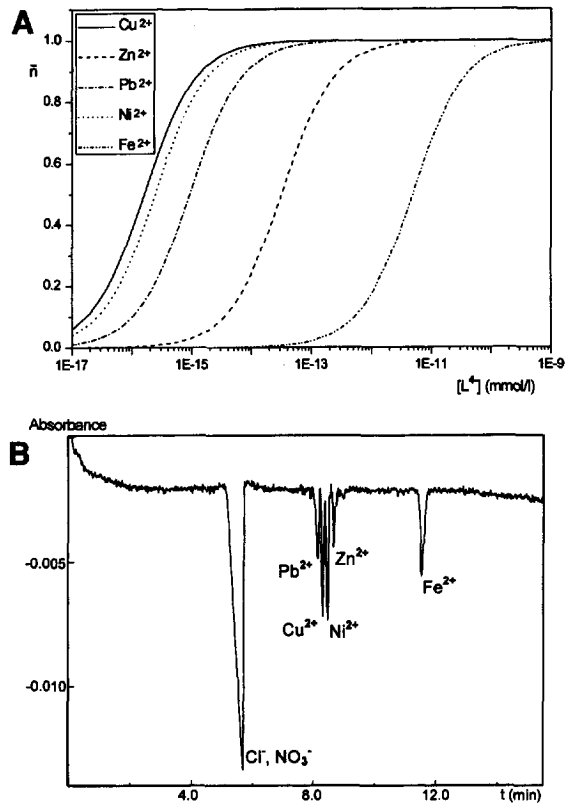


Fig. 4. EDTA complexes. (A) Calculation of the average number $\bar{n}$ of ligands. (B) Separation of the EDTA complexes. Sample, 0.1 m*M* Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, 0.2 m*M* Fe^{2+} in 0.6 m*M* EDTA. Buffer, 7.5 m*M* salicylic acid–0.1 m*M* EDTA–0.02 m*M* HTAB, pH 4.01. 8 s (5 kV) injection; U = −12 kV; wavelength, 200 nm, column, 50/57 cm, 50 μm I.D.

metal ions form ML^{2-} complexes to a great extent [14,15]. This fact is confirmed by our computer simulation. Nearly all metal ions are completely complexed by L^{4-}, with the exception of Fe^{2+}, due to the smallest lg β value (compare with the curve for $\bar{n}$ in Fig. 4A). The mobility is determined mainly by the complex ML^{2-}, and the species are migrating as anions. Therefore, the separation was carried out with reversed polarity (an electropherogram showing the separation of the EDTA complexes is shown in Fig. 4B). HTAB was used to reduce the electroosmotic flow.

Assuming that the migration order depends only on the degree of complexation, it should be Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Fe^{2+} (due to the applied buffer concentration of the ligand all metal ions are com-

plexed by more than 99.5%). In contrast, we observed that Pb^{2+} migrates fastest and we have found concurring results in the literature [14]. The higher mobility of the Pb^{2+} complex may be caused by differences in the complex structure due to different ion radii of Pb^{2+} and the other metal ions.

Using the same buffer with a higher content of EDTA, a separation of the iron species Fe^{2+} and Fe^{3+} becomes possible (Fig. 5). It must be noted, that in contrast to the expected migration order, the $[Fe(III)EDTA]^-$ complex migrates faster than $[Fe(II)EDTA]^{2-}$ (proved by single injection and standard addition). This could possibly be caused by competitive or mixed-complex formation of the iron species with the separation buffer or by the different coordination behaviours of both ions.

Due to the high stability constants for salicylate complexes (Fe^{3+} lg $\beta_3 = 35.31$, Fe^{2+} lg $\beta_2 = 11.2$) strong interaction with this buffer component was expected. Therefore, the mixed EDTA–salicylate–Fe^{2+}–Fe^{3+}-system was calculated with the computer program using the real concentrations in the separation capillary. Nevertheless, 100% of the Fe^{3+} and more than 99.5% of Fe^{2+} are complexed with EDTA under separation conditions. Despite the fact that the formation of competing salicylate complexes is unlikely, mixed-ligand complexes could also be the reason for the unexpected behaviour of the iron species. Until now, we had not carried out such calculations for iron complexes, because stability constants for mixed-ligand complexes are seldom available.

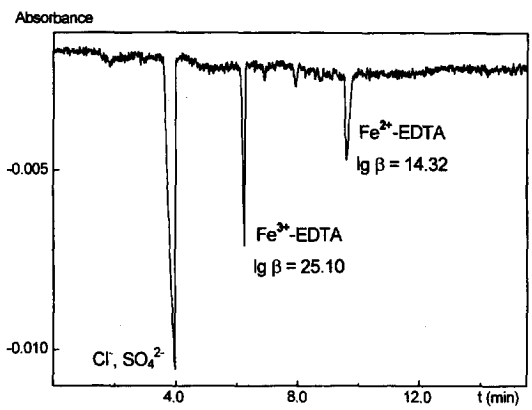


Fig. 5. Separation of iron species. Sample, 0.2 m*M* Fe^{2+} and Fe^{3+} in 0.4 m*M* EDTA. Buffer, 7.5 m*M* salicylic acid–0.5 m*M* EDTA–0.02 m*M* HTAB, pH 4.01. 8 s (5 kV) injection; U = −20 kV; wavelength, 200 nm, column, 50/57 cm, 50 μm I.D.

Differences in coordination chemistry should have an influence on migration too. For Fe^{3+}, a pentagonal bipyramidal coordination (coordination number 7) is suggested [16] in comparison to Fe^{2+}, which prefers an octahedral coordination in the EDTA complex (coordination number 6). However, the water molecule, which is bound in the Fe^{3+}–EDTA complex, does not influence charge and structure of the molecule as much as would be necessary for the observed fast migration. Further investigations have to be performed to explain the unexpected electrophoretic behavior of these complexes.

Electrophoretic separation of the EDTA complexes is more difficult than that of the α-HIBA complexes, due to very small differences in the degree of complexation and fewer possibilities of influencing the complexation by pH or ligand concentration or by the formation of mixed-ligand complexes using additional buffer components.

4. Conclusions

In principle, the separation of metal ions with nearly the same electrophoretic mobilities is possible using formation of complexes with different stabilities.

The application of weak complex equilibria (e.g. with α-HIBA) could be used for the generation of different complexation degrees. This leads to different electrophoretic mobilities due to the various charges on the complexes depending on the average number of ligands $\bar{n}$. Resolution of the analytes could be influenced by the pH of the buffer or by the concentration of the complexing ligand. If no competing reaction takes place, calculation of migration order and maximum resolution is possible using simple computer simulation.

More stable complexes (e.g. with HQS or EDTA) could be separated by differences in structure or charge. Variation of the average number of ligands $\bar{n}$ could not be utilized to improve the separation. Due to the high stability of the complexes, the optimization had to be performed in a concentration range, which is at (HQS) or orders of magnitude below (EDTA) the detection limit of the complexes. There

are possibilities of influencing the resolution by the use of additional buffer components that are able to occupy the remaining coordination sites of the complexed metal ion. The introduction of competing complexing ligands, which could change the structure of the complex, could be more effective. Calculation of the migration order and of the expected resolution does not give sufficient results using simple computer simulation.

If the buffer contains more than one complexing ligand (in some cases even phosphate or borate), all ligands have to be included in the calculation and even mixed-ligand complexes must be taken into consideration, in order to predict the separation precisely.

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