Observations on Ion Interaction Chromatographic System: Reversed Phase Column—H₃BO₃/TBAOH Mobile Phase and the Effect of Temperature

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Abstract
New ion interaction chromatographic (IIC) system with RP column and boric acid plus tetra- n-butylammonium hydroxide (TBAOH) mobile phase was investigated. In the system: CPhenylHexyl—10 mM H₃BO₃/4 mM TBAOH, a large group of anions viz. F⁻, IO₃⁻, Cl⁻, ClO₂⁻, BrO₃⁻, NO₂⁻, Br⁻, NO₃⁻, ClO₃⁻, I⁻, HPO₄²⁻, SO₄²⁻, CrO₄²⁻, S₂O₃²⁻, benzoate⁻, SCN⁻, ClO₄⁻ and phthalate²⁻, could be isocratically resolved. A study on the effect of temperature revealed that some ions added to ion exchange are also held in the stationary phase by the hydrophobic adsorption. The retention loss for all anions with time was observed. This effect however, was quite slow and good separations could be obtained even after the column stayed a few hundred hours in the mobile phase.

Keywords Anions · Ion interaction chromatography (IIC) · Boric acid · Tetra-n-butylammonium hydroxide (TBAOH) · Thermodynamics of ion exchange/sorption reaction · Retention loss with time

Introduction
With the advent of a new generation of reversed phase (RP) columns which are sufficiently stable at alkaline solutions (at least up to pH = 11.5) and withstand temperatures up to at least 60 °C [1, 2], new perspectives appeared to the use of ion interaction chromatography (IIC) for the separation of anions. IIC is considered as an alternative to ion chromatography [3–5]. Temperature seems to be relatively underestimated and underexplored factor in the chromatography although it can influence retention of the analytes, column performance, affect the peak shapes etc. [6, 7]. A change of retention factor (at constant mobile phase composition) with temperature has been used as a criterion for qualitative identification of species in complex mixtures [8]. In ion chromatography where bicarbonate/carbonate or hydroxyl eluents are commonly used for the separation of anions with stationary phases containing quaternary ammonium functional groups, the use of elevated temperature is severely limited due to thermal instability of the columns.

In the previous paper we have demonstrated that interesting separations of anions could be achieved with the use of the system consisting of a RP column (C₁₈ or CPhenyHexyl) and 2.8 mM NaHCO₃/0.7 mM TBAOH (tetra-n-butylammonium hydroxide), pH = 9.90 purely aqueous mobile phase. It was also shown that in this system the temperature could be used as a factor regulating the selectivity and changing the elution order of individual anions without irreversible damaging the column [9]. It was also found that the apparent enthalpy changes, ΔH for hydrophobic ions like I⁻, SCN⁻ and ClO₄⁻ largely exceeded 3 kcal/mole suggesting that added to ion exchange they are retained by hydrophobic adsorption [9]. Therefore it seemed interesting to investigate whether similar phenomena would be observed in another IIC system, i.e. RP column—H₃BO₃/TBAOH mobile phase which has not been examined so far.
Experimental

Chemicals

The stock solution of 0.2 M $\text{H}_3\text{BO}_3$ was prepared by weighing 12.36 g $\text{H}_3\text{BO}_3$ (Merck, Germany) and then dissolving it in water in a 1000 mL volumetric flask. The solution of 400 g L$^{-1}$ TBAOH (Sigma-Aldrich, Germany) in water was employed as a stock solution of TBAOH. Both reagents were of analytical grade. Methanol, ethanol and $n$-propanol used as an addition to the mobile phase were of HPLC Gradient Grade (JT Baker, Netherlands). Solutions for column regeneration were prepared from Ultra Gradient HPLC Grade reagents (acetonitrile $\text{CH}_3\text{CN}$—JT Baker, Netherlands; $\text{iso}$-propanol and hexane—Merck, Germany). All solutions used in the investigations were prepared in 18 MΩ cm water produced by a Milli-Q Direct System (Millipore) and then deoxygenated with nitrogen.

Apparatus and Procedure

The experiments were performed using a Dionex 2000i/SP ion chromatograph (Dionex Corporation, Sunnyvale CA, USA), equipped with two detectors; a CDM-II Dionex conductivity detector connected to an anion self-regenerating suppressor (Dionex ASRS-300, 4 mm) and a Dionex VDM-II, UV–Vis variable wavelength detector. Separations of anions were made by isocratic elution with the use of a Dionex, AGP gradient pump. Flow rate in all elutions was 1 mL min$^{-1}$. Two RP-HPLC columns: Kromasil Eternity XT5-C$_{18}$ and Kromasil Eternity XT5-Phenyl Hexyl (Akzo Nobel, 250 mm—length and 4.6 mm I.D.), both equipped with 13 mm guard columns were used in this study. The samples were injected to the chromatograph manually with a 50 µL Dionex Tefzel sample loop. The column was immersed in a water bath in which the temperature was controlled within ± 1 ºC by a UTU-4 ultrathermostat (Tarnów, Poland). Data collection as well as calculation of chromatographic parameters were performed employing the Dionex Peak Net chromatography software resident in an IBM PC. In all experiments the chromatograms were recorded by means of two detection modes: conductometric and UV at $\lambda=210$ nm to be sure of the proper identification of chromatographic peaks of anions.

Results and Discussion

Retention Behavior of Anions in the System: Kromasil Eternity C$_{18}$—$\text{H}_3\text{BO}_3$/TBAOH Aqueous Solution at Changing Reagents Ratio

Preliminary investigation was performed with Kromasil Eternity C$_{18}$ column, a set of 8 anions viz. $\text{Cl}^−$, $\text{BrO}_3^−$, $\text{Br}^−$, $\Gamma^−$, $\text{HPO}_4^{2−}$, $\text{SO}_4^{2−}$, $\text{ClO}_4^−$, $\text{SCN}^−$ and $\text{H}_3\text{BO}_3$/TBAOH solutions at changing reagent ratio. In each series of experiments the concentration of $\text{H}_3\text{BO}_3$ was fixed at: 0.7, 2, 5, 7 and 10 mM, respectively, and TBAOH concentration was consecutively set at 0.7, 2, 5, 7 and 10 mM, respectively. The composition of the mobile phase i.e. the $\text{H}_3\text{BO}_3$/TBAOH ratio, affects both the magnitude of retention factors of individual ions as well as the order of affinity (cf. Fig. 1). The system: with 10 mM $\text{H}_3\text{BO}_3$ vs. × mM TBAOH is shown in Fig. 1a and the eluent composition: 10 mM $\text{H}_3\text{BO}_3$/4 mM TBAOH which offered the best conditions for the separation of anions studied and indicated the possibility for accommodating larger number of ions in isocratic separation was chosen for further studies. From among many other $\text{H}_3\text{BO}_3$/TBAOH ratio combinations the system with 2 mM $\text{H}_3\text{BO}_3$ vs. × mM TBAOH is also shown in Fig. 1b just for comparison how profoundly the reagent ratio may affect the selectivity. The peculiar feature of this system (including all other mobile phase compositions) was that retention drift with time occurred for all anions.

Separation of Anions in the System: Kromasil Eternity XT-5 PhenylHexyl, $\text{C}_\text{PhenylHexyl}$—10 mM $\text{H}_3\text{BO}_3$/4 mM TBAOH Aqueous Solution

It was shown in the previous work [9] that the change from C$_{18}$ to $\text{C}_\text{PhenylHexyl}$ stationary phase in the case of NaHCO$_3$/TBAOH solution, had a positive influence on the separation of anions (at the same composition of the mobile phase). Therefore further investigations were done employing $\text{C}_\text{PhenylHexyl}$ column. Very good separation of several anions could be achieved in the system: Kromasil Eternity $\text{C}_\text{PhenylHexyl}$—10 mM $\text{H}_3\text{BO}_3$/4 mM TBAOH as shown in Fig. 2.

Retention parameters i.e. retention factors ($k$) separation factors ($α^2_1$), and resolutions ($R^{1−2}$) were calculated from known formulae [6]:

$$k = (t_R − t_M)/t_M$$

(1)

$$α^2_1 = k_2/k_1$$

(2)

$$R^{1−2} = \frac{(t_2 − t_1)}{2(σ_1 + σ_2)}$$

(3)
where $t_M$ is dead time and $\sigma_1$, $\sigma_2$ are standard deviations of respective chromatographic peaks.

When column was in contact with the mobile phase, retention factors of all anions decreased with time. E.g. $k_{\text{ClO}_3}$ values were 13.7, 12.6, and 6.40 after the column was 167.5 h, 189 h and 552 h, respectively, in contact with 10 mM H$_3$BO$_3$, 2 mM TBAOH mobile phase. The analogous values for $k_{\text{NO}_3}$ amounted to 9.95, 9.16, and 4.78, respectively. For iodides, $k_i$ was 27.2 and 13.9 after 189 h and 552 h, respectively (cf. Fig. 2). As can be seen from Fig. 2 despite gradual diminishing of retention factors the separation of all investigated ions remained very good for a long time, including the ClO$_2^-$–BrO$_3^-$ pair which still could be partially resolved after the column stayed 550 h in the eluent. Only after ca. 600 h the ClO$_2^-$–BrO$_3^-$ appeared in the eluate as a single peak. One should note that separation factors of anions changed very little with time. E.g. the values for Br$^-$–NO$_3^-$ separation, $\alpha_{\text{Br}^-\text{NO}_3}$ were: 1.38, 1.37 and 1.38 after 167.5 h, 189 h and 552 h the column was in contact with the eluent, with the corresponding resolutions, $R_{\text{Br}^-\text{NO}_3}$ of 9.83, 9.38 and 1.20, respectively. The analogous data for NO$_3^-$–ClO$_3^-$ were: $\alpha_{\text{ClO}_3^-\text{NO}_3}$: 1.38, 1.37 and 1.34 with the corresponding resolutions, $R_{\text{NO}_3^-\text{ClO}_3^-}$: 10.3, 10.2 and 5.35, respectively. So,
the decrease in resolution was mainly due to the gradual decrease of column performance.

**Effect of temperature and thermodynamics**

The retention factors of individual ions in the system: C<sub>PhenylHexyl</sub>—10 mM H<sub>3</sub>BO<sub>3</sub>/4 mM TBAOH tended to decrease with time. Therefore when studying the effect of temperature, the column was regenerated before each run by passing consecutively: 80 mL (95% H<sub>2</sub>O + 5% CH<sub>3</sub>CN), 80 mL (100% CH<sub>3</sub>CN), 20 mL isopropanol, 80 mL hexane, 20 mL isopropanol, 80 mL CH<sub>3</sub>CN, 80 mL (95% H<sub>2</sub>O + 5% CH<sub>3</sub>CN) [10], followed by equilibration with eluent solution. This procedure should have assured keeping the column in the quasi-steady state before each run at the temperatures 10°, 25°, 40°, 50° and 65 °C, respectively. The apparent column exchange capacity was controlled during this cycle of experiments by determining the break through curve of iodide ions. The solution 10 mM H<sub>3</sub>BO<sub>3</sub>/4 mM TBAOH containing iodides (10 ppm) was passed through the column.

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**Fig. 2** Chromatograms of several anions as a function of time. Stationary phase: Kromasil Eternity C<sub>PhenylHexyl</sub>; mobile phase: 10 mM H<sub>3</sub>BO<sub>3</sub>/4 mM TBAOH; pH 8.97. Flow rate 1 mL/min; temp. 25 °C, conductometric detection. a After 167.5 h in this eluent; b After 189 h in this eluent; c After 552 h in this eluent.
recording the conductance of the effluent. The retention time at 0.5 height of the breakthrough curve was taken as a measure of the apparent column capacity, in a manner analogous to that used in the previous paper [9]. The apparent exchange capacity was found to maintain approximately constant value (x ± s): 11.3 ± 6% μequiv./column. Considering inherent problems associated with measurement of retention parameters in the system studied, this accuracy was assumed to be satisfactory for the purpose of comparison of the C<sub>PhanylHexyl</sub>—H<sub>3</sub>BO<sub>3</sub>/TBAOH system with the other IIC system: RP column—NaHCO<sub>3</sub>/TBAOH studied earlier [9].

Effect of temperature on the separation of several ions in the investigated system is demonstrated in Fig. 3. Several selectivity reversals with the change of temperature like e.g.: iodides versus chromates, and benzoate versus thiocyanates should be noted. It was shown in the previous study that the adsorption of TBAOH on the RP column in the temperature range 25–60 °C remained practically constant (relative standard deviation < 2%) [9].

The retention factor k can be treated as a conditional constant, the change of free energy of an ion exchange/sorption reaction (ΔG) is then given as:

![Fig. 3 Effect of temperature on the chromatograms of several anions. Stationary phase: Kromasil Eternity C<sub>PhanylHexyl</sub>; mobile phase: 10 mM H<sub>3</sub>BO<sub>3</sub>/4 mM TBAOH; pH 8.97. Flow rate 1 mL/min.; spectrophotometric detection. a 10 °C; b 40 °C; c 65 °C](image-url)
\[ \Delta G = -RT \ln k \]  
\[ \Delta H = -R \frac{d \ln k}{d(1/T)} \]  
\[ \Delta S = \frac{\Delta H - \Delta G}{T} \]

where \( R \)—gas constant (1.987 cal°K\(^{-1}\) mol\(^{-1}\)) and \( T \) is absolute temperature.

The other thermodynamic functions i.e. change of enthalpy, \( \Delta H \):

\[ \Delta H = -R \frac{d \ln k}{d(1/T)} \]  

and change of entropy, \( \Delta S \):

\[ \Delta S = \frac{\Delta H - \Delta G}{T} \]

were calculated as described in Ref. [11].

Selected values are shown in Table 1. One should note that for all investigated ions \( \Delta H \) is not constant in the temperature range investigated what is equivalent to the statement: \( \Delta C_p \neq 0 \) (\( \Delta C_p \) = heat capacity). Van’t Hoff’s plots (not shown in this paper) are curvilinear. Several Van’t Hoff’s plots have an extremum (\( \Delta H = 0 \)) in the temperature range accessible for investigation. \( T_{\text{extrem.}} \) (minimum or maximum) as well as \( \Delta C_p \) values (cal/mol,°C) are also quoted in Table 1.

It is known from several works on classical ion exchange chromatography (IEC) as well as ion chromatography (IC), that the changes of enthalpy values for the exchange of inorganic anions and cations on common (non chelating) ion exchange resins usually do not exceed 3.0 kcal/mol. As follows from Table 1, at \( t = 25 \) °C the enthalpy changes for large hydrophobic ions as \( \text{ClO}_4^- \), \( \text{SCN}^- \), \( \text{I}^- \) and benzoate\(^-\) are considerably greater than this limiting value. At higher temperatures high negative \( \Delta H \) values were observed for these and still several other ions. It is logical to assume that here these ions are held by the stationary phase not only by ion exchange mechanism but also by hydrophobic adsorption [3, 9]. One should note that those ion exchange/sorption reactions which are highly exothermic, are characterized also by rather high negative changes of entropy. This may be interpreted as the partial loss of the ability of free movement by those hydrocarbon chains which are aggregated around the big hydrophobic ion. At \( t > 40 \) °C all ion exchange/sorption reactions are exothermic and this results in significant decrease of retention factors as well as in some selectivity reversals with the rise in temperature (cf. Fig. 3). The values from Table 1 can be compared with analogous IIC system: Gemini C\(_{18}\) column—2.8 mM/0.7 mM TBAOH (pH 9.90), where \( \text{ClO}_4^- \), \( \text{SCN}^- \), \( \text{I}^- \) ions also showed values of enthalpy change distinctly exceeding 3.0 kcal/mol what was interpreted as a contribution from hydrophobic adsorption [9].

### Concluding Remarks

The new IIC system with C\(_{18}\) and C\(_{\text{PhenylHexyl}}\) stationary phases and \( \text{H}_3\text{BO}_3/\text{TBAOH} \) mobile phase offers interesting possibilities for the separation of many inorganic and organic ions in one run. Halogen containing ions like \( \text{Cl}^- \), \( \text{ClO}_2^- \), \( \text{ClO}_3^- \), \( \text{ClO}_4^- \), \( \text{Br}^- \), \( \text{BrO}_3^- \), \( \text{I}^- \), \( \text{IO}_3^- \) i.e. including those which are important from the health protection point of view, together with several other ions can be simultaneously separated. The unexpected decrease of retention volume of all ions with time, when using this system was observed, which however was relatively slow, so that good separation of many ions could be still be achieved even after the column was in contact with the mobile phase for a few hundred hours. Our study on the effect of temperature contributes to the understanding of mechanism of retention of ions in IIC systems, emphasizing the role of hydrophobic adsorption. The work at higher temperatures does not cause irreversible damage to the column which can be regenerated and used again. This is in contrast to the behavior of anion exchange columns with quaternary ammonium functional groups which undergo fast and irreversible degradation under the action of higher temperatures [12].

The mechanism of gradual retention loss observed in this work differs from that referred to in literature as “retention time loss” or “phase collapse” [13–15]. In RP column—\( \text{H}_3\text{BO}_3/\text{TBAOH} \) mobile phase system, the stop of flow and restart does not result in sudden retention drop. Gradual retention loss was also observed in non 100% aqueous solutions i.e. in solutions containing 3–5% of methanol or higher alcohols as well as acetonitrile, although the process was slower, and the retention factors were much lower. Boric acid is a very weak acid and probably the low ionic strength of the \( \text{H}_3\text{BO}_3/\text{TBAOH} \) solution is the reason that in the long run the tendency of hydrocarbon chains of the stationary phase to aggregate in contact with aqueous phase, may prevail over the repulsive forces of ions present in the solution and the mobile phase is gradually squeezed out of the pores. In the similar IIC systems with \( \text{NaHCO}_3/\text{TBAOH} \) mobile phase the retention drop with time did not occur [9].
Table 1 Thermodynamic functions: free energy ($\Delta G$, kcal/mol), enthalpy ($\Delta H$, kcal/mol) and entropy ($\Delta S$, cal/K·mol) changes for the ion interaction chromatographic separation of anions in the system: Kromasil C-PhenylHexyl—10 mM $\text{H}_2\text{BO}_3$/4 mM TBAOH (pH 8.97)

| Anion | Function | Temperature (°C) | $\Delta C_p$ (cal/K·mol) | $T_{\text{extrem}}$ (°K) |
|-------|----------|-----------------|--------------------------|--------------------------|
|       |          | 5 | 25 | 40 | 50 | 65 |        |                     |
| F$^{-}$ | $\Delta G$ | 0.339 | 0.265 | 0.302 | 0.370 | 0.531 | −105.0 | 300.9 |
|        | $\Delta H$ | 2.402 | 2.302 | −1.273 | −2.323 | −3.898 |                     |                     |
|        | $\Delta S$ | −0.004 | 0.123 | −5.034 | −8.334 | −13.10 |                     |                     |
| Cl$^{-}$ | $\Delta G$ | −0.084 | −0.181 | −0.111 | 0.000 | 0.261 | −161.5 | 295.7 |
|        | $\Delta H$ | 2.859 | −0.371 | −2.793 | −4.408 | −6.830 |                     |                     |
|        | $\Delta S$ | 0.001 | −6.636 | −8.568 | −13.647 | −20.98 |                     |                     |
| $\text{ClO}_2^-$ | $\Delta G$ | −0.366 | −0.437 | −0.337 | −0.200 | 0.104 | −173.4 | 291.4 |
|        | $\Delta H$ | 2.238 | −1.139 | −3.740 | −5.474 | −8.075 |                     |                     |
|        | $\Delta S$ | 0.004 | −2.357 | −10.873 | −16.33 | −24.20 |                     |                     |
| $\text{NO}_2^-$ | $\Delta G$ | −0.455 | −0.540 | −0.449 | −0.319 | −0.024 | −173.9 | 292.0 |
|        | $\Delta H$ | 2.435 | −1.043 | −3.652 | −5.391 | −8.000 |                     |                     |
|        | $\Delta S$ | 0.005 | −3.842 | −11.92 | −17.09 | −23.60 |                     |                     |
| Br$^{-}$ | $\Delta G$ | −0.666 | −0.701 | −0.582 | −0.437 | −0.123 | −164.4 | 286.8 |
|        | $\Delta H$ | 1.441 | −1.846 | −4.311 | −5.955 | −8.421 |                     |                     |
|        | $\Delta S$ | 0.004 | −3.842 | −11.92 | −17.09 | −23.60 |                     |                     |
| $\text{NO}_3^-$ | $\Delta G$ | −0.903 | −0.956 | −0.843 | −0.699 | −0.383 | −171.9 | 286.8 |
|        | $\Delta H$ | 1.516 | −1.922 | −4.500 | −6.219 | −8.797 |                     |                     |
|        | $\Delta S$ | 0.006 | −3.241 | −11.68 | −17.09 | −24.89 |                     |                     |
| $\text{ClO}_3^-$ | $\Delta G$ | −1.201 | −1.197 | −1.063 | −0.914 | −0.604 | −148.2 | 279.3 |
|        | $\Delta H$ | 0.198 | −2.765 | −4.989 | −6.670 | −8.892 |                     |                     |
|        | $\Delta S$ | 0.001 | −5.263 | −12.54 | −17.20 | −23.93 |                     |                     |
| $\text{HPO}_4^{2-}$ | $\Delta G$ | −1.272 | −1.610 | −1.613 | −1.501 | −1.170 | −283.0 | 299.9 |
|        | $\Delta H$ | 6.208 | 0.548 | −3.698 | −6.528 | −10.77 |                     |                     |
|        | $\Delta S$ | 0.036 | 7.242 | −6.659 | −15.56 | −28.41 |                     |                     |
| $\text{SO}_4^{2-}$ | $\Delta G$ | −1.315 | −1.712 | −1.732 | −1.620 | −1.270 | −312.9 | 301.2 |
|        | $\Delta H$ | 7.272 | 1.013 | −3.682 | −6.812 | −11.51 |                     |                     |
|        | $\Delta S$ | 0.044 | 9.143 | −6.230 | −16.08 | −30.28 |                     |                     |
| $\text{CrO}_4^{2-}$ | $\Delta G$ | −1.595 | −1.964 | −1.958 | −1.826 | −1.444 | −318.9 | 298.9 |
|        | $\Delta H$ | 6.662 | 0.284 | −4.500 | −7.689 | −12.47 |                     |                     |
|        | $\Delta S$ | 0.049 | 7.543 | −8.121 | −18.15 | −32.63 |                     |                     |
| I$^{-}$ | $\Delta G$ | −1.834 | −1.735 | −1.544 | −1.368 | −1.035 | −121.2 | 259.9 |
|        | $\Delta H$ | 2.804 | −4.622 | −6.440 | −7.653 | −9.471 |                     |                     |
|        | $\Delta S$ | −3.429 | 9.690 | −15.64 | −19.46 | −24.96 |                     |                     |
| Benzoate | $\Delta G$ | −2.239 | −2.302 | −2.206 | −2.076 | −1.786 | −163.4 | 279.6 |
|        | $\Delta H$ | 0.256 | −3.012 | −5.462 | −7.096 | −9.546 |                     |                     |
|        | $\Delta S$ | 0.003 | −2.379 | −10.40 | −15.54 | −22.96 |                     |                     |
| SCN$^{-}$ | $\Delta G$ | −2.610 | −2.462 | −2.240 | −2.041 | −1.671 | −125.2 | 250.5 |
|        | $\Delta H$ | 3.442 | −5.946 | −7.824 | −9.076 | −10.95 |                     |                     |
|        | $\Delta S$ | −0.041 | 11.69 | −17.84 | −21.78 | −27.47 |                     |                     |
| $\text{ClO}_4^-$ | $\Delta G$ | −2.716 | −2.633 | −2.443 | −2.259 | −1.900 | −143.4 | 260.8 |
|        | $\Delta H$ | 2.465 | −5.334 | −7.485 | −8.920 | −11.07 |                     |                     |
|        | $\Delta S$ | −0.031 | 9.064 | −16.11 | −20.62 | −27.13 |                     |                     |
| Phthalate | $\Delta G$ | −2.715 | −2.931 | −2.873 | −2.733 | −2.380 | −249.2 | 288.9 |
|        | $\Delta H$ | 2.736 | −2.249 | −5.987 | −8.480 | −12.22 |                     |                     |
|        | $\Delta S$ | 0.034 | 2.291 | −9.952 | −17.79 | −29.11 |                     |                     |

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights Statement This article does not contain any studies with human participants or animals performed by any of the authors.

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