Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Interaction of catechins with aluminum in vitro

TANG De-song (唐德松)[1], SHEN Sheng-rong (沈生荣)[†1], CHEN Xun (陈 勋)[1],
ZHANG Yu-yan (张玉艳)[1], XU Chong-yang (许重阳)[2]
([1]*Department of Tea Sciences, Zhejiang University, Hangzhou 310029, China*)
([2]*Zhejiang Hzsun Pharmaceutical Co. Ltd, Taizhou 318000, China*)
[†]E-mail: shrshen@zju.edu.cn
Received Nov. 16, 2003; revision accepted Jan. 16, 2004

Abstract: Tea is one of the most popular beverages, consumed by over two thirds of the world's population; but the aluminum accumulation property of tea plant is becoming the focus of many researches because of aluminum's known adverse effect on human health. Investigation of the interactions of catechins with Al^{3+} showed that during the interaction of catechins with Al^{3+}, the UV-vis spectrum of catechins was changed. Absorption of EGCG at 274 nm decreased and increased at 322 nm; EC and C's at 278 nm changed little. The ratio of Al^{3+} to EGCG was 1:1 in pH 5.0 buffer solution; in pH 6.2 buffer solution, the ratio in the Al-EGCG complex was 1:1. Interestingly, while the ratio reached to over 2, after the complex of Al-EGCG started polymerization, the ratio in the polymer was 2:1. In pH 6.2 buffer solution, the complex behavior of C with Al^{3+} was the same as that of EGCG, with a little difference for EC. When the ratio of Al^{3+} to EC was <1, the complex in ratio was 1:2, but, the complex polymerized when the ratio of Al^{3+} to EC was >1. It was found that the ratio of Al^{3+} to EC in the polymer was 1:1. Polymerization of Al-catechin complexes might reduce aluminum absorption in the intestine. *Kow* value was also employed to study the properties of aluminum species in tea infusion (at gastric and intestine pH condition) and the effect of catechins and tea polyphenols on *Kow* in buffer solution. Results showed that *Kow* value rose much higher at the intestine pH than at the gastric pH. Tea polyphenols and catechins could greatly reduce aluminum *Kow* value in acetic buffer, indicating that these compounds may reduce aluminum absorption during tea intake.

Key words: Aluminum, Tea polyphenols, Catechins, Complex, *Kow*
Document code: A **CLC number:** R151.3

INTRODUCTION

Because of the aluminum accumulation in tea plant, the safety hazard of aluminum absorption while drinking tea became the subject of more and more researches (Flaten, 2002). Aluminum species in environmental and physical condition and its physio-chemical properties are often the determinants of its bio-availability and toxicity.

Polyphenols are the most important among organic compounds in tea infusion, and can strongly complex with metal ion to form stable complexes (Hynes and Coinceanainn, 2001). Researchers found that catechin-metal complexes had strong bioactivities (Midori *et al.*, 2001). But, it is not clear what the mechanism of combination of catechins with aluminum ion is (Inoue *et al.*, 2002), and also very few reports on the properties of aluminum in tea infusion and catechins-aluminum complex. Interaction of catechins with aluminum might affect the bioactivity of aluminum (Yokel, 2002).

In the equation of organic compound partial coefficient, *Kow*=Co/Ca, Co and Ca are concentrations in *n*-octanol and aqueous phase, are important parameters for predicting the distribution of compounds in environment and living body, and are also linked with bio-availabilities (He *et al.*, 1994; Yu and Xu, 1993; Huang *et al.*, 2002). *Kow* was used to probe the property of tea infusion in this study on the interactions between catechins, tea polyphenols showing that the *Kow* value was affected by catechins and fluoride in acetic buffer; and may affect bioactivities and the safety of aluminum in tea infusion.

MATERIALS AND METHODS

Chemicals

(+)-catechin(C), (−)-epicatechin(EC), EGCG (−)-epigallate catechin gallate (EGCG) were from Sigma (St. Louis, Mo). Tea polyphenols were obtained from Tea Science Research Institute, Chinese Academy of Agriculture (purity>98%). Green tea, black tea and Oolong tea were purchased from the market in Hangzhou, China. All other chemicals were standard reagent grade.

Methods

(1) UV-vis spectrophotometric assay (Emiko *et al.*, 1999)

Precisely weighed EGCG (11.5 mg), (+)-C and (−)-EC (14.5 mg) dissolved in 25 ml distilled water were used to make up the stock solution, 0.5 ml of which was put into three tubes to which were added pH 5.0 distilled water, and pH 6.2 acetate buffer solutions respectively, to make up a total volume of 10 ml. Three ml of the solution was put into the micro cell and measured on spectrophotometer.

Microlitre pipette was used to add 0.01 mol/L NaOH solution in small steps of 5 μl into the catechins distilled water solution, whose concentration was measured each time.

Microlitre pipette was used to add 0.01 mol/L $AlCl_3$ solution in small steps of 5 μl into the catechins buffer solution, whose concentration was measured each time.

Thirty μl 0.01 mol/L $AlCl_3$ was added into the catechins solution, and then 0.01 mol/L NaF was added in small steps of 5 μl using microlitre pipette into the solution. The UV-vis absorbance was measured each time.

(2) Samples treatment

Carefully weighed 0.5 g black tea, 0.5 g green tea, 1.25 g Oolong tea, and acid mixture (nitric acid/perchloric acid=4/1, v/v) were put into a digester, where the 3 kinds of tea were digested until white crystal appeared. After 0.1 mol/L NaOH solution was added to neutralize the surplus acid, and distilled water was added to make up total volume of 25 ml, the aluminum concentration was determined.

Boiling purified water was added into three beakers which containing 3 g black tea, 3 g green tea and 5 g Oolong tea respectively, making up total volume of 150 ml for both black tea and green tea, 110 ml for Oolong tea. The beakers with contents were finally immersed in 100 °C water for 30 min and then filtrated to get tea infusion.

After 20 ml tea infusion and 1 ml acid mixture were put into the digester, the solution was digested until the colors disappeared. Then 0.1 mol/L NaOH was added to neutralize the surplus acid and then distilled water was added to fix the final volume to 20 ml.

Twenty ml tea infusion was put into each of the three tubes, one was adjusted to gastric pH 1.3; one was adjusted to intestine pH 7.6 (Zhong, 1998); the other tube's content was used as control. Twenty ml *n*-octanol was added into each tube, shaken for 3 min and then allowed to equilibrate overnight. The aqueous phase and 1 ml acid mixture were put into the digester, and digested until the solution colors disappeared. Then 0.1 mol/L NaOH was added into the solution to neutralize the surplus acid and distilled water was added to make up a final solution volume of 20 ml.

(3) Effects of tea polyphenols (TP), and catechins on the aluminum partial coefficient

TP and catechins solution (1−6 ml) were put into six tubes, into each of which was added 10 ml aluminum stock solution, 4 ml pH 6.2 acetic buffer, and distilled water to make up a total volume of 20 ml. After 20 ml *n*-octanol was added into the solution, it was shaken and then kept still until the two phases were separated into two layers; then 10 ml of the aqueous phase was digested. After addition of distilled water to the digestion product to adjust the volume to 10 ml, the aluminum concentration was determined.

(4) Aluminum concentration determination (Deng, 2002)

After proper volume of treated samples were put into a 25 ml flask, 1 ml bromopyrogallol red (BPR), 1.5 ml cetyltrimethyl ammonium bromide (CTMAB), 1 ml Triton X-100, and 1 ml acetic buffer (pH 6.2) were put into the flask, it was shaken, and then after the flask's content had settled, the content was detected using spectrophotometer at 610 nm wavelength 30 min later. The corresponding regent was used as control.

Standard curves: One to five ml of 20 μg/ml aluminum stock solution was put into 25 ml volume flasks，the absorbance values were used to plot the curve of UV-visible absorbance versus aluminum concentration. The fitting line was obtained using Origin software.

Detection was repeated three times and then average values were taken.

RESULTS AND DISCUSSION

EGCG complex with aluminum

The spectrum changed differently at various pH values when aluminum ion was added into the EGCG solution. In pH 5.0 buffer solution, the absorbance of the solution decreased at 274 nm and increased at 322 nm, the spectrum changed little when added aluminum ion was 2.5 times EGCG in molars (Fig.1a$_1$&a$_2$). But in pH 6.2 buffer solution, when aluminum molars was 2.5 times EGCG in molars, the spectrum rose and then dropped in parallel

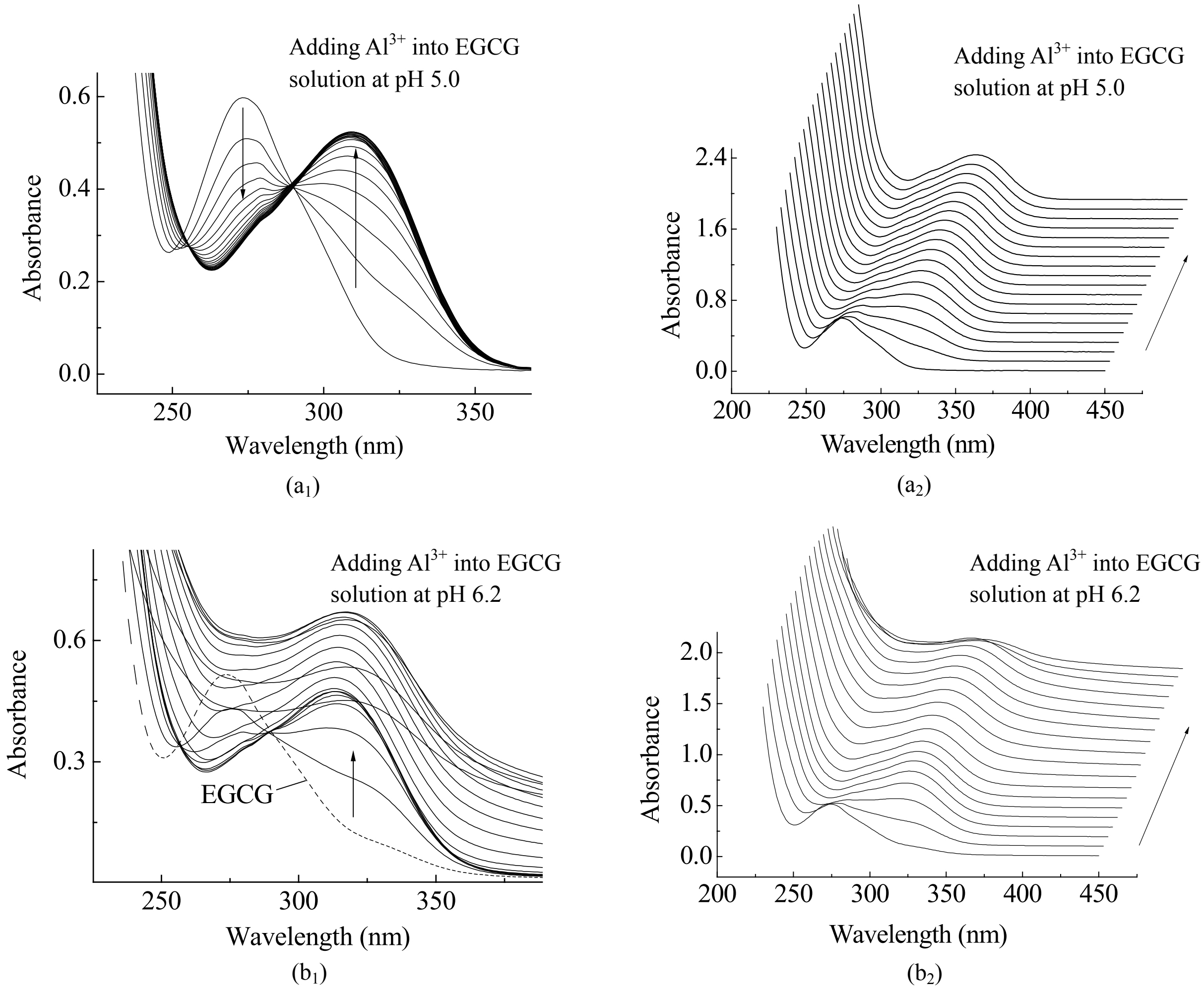

Fig.1 UV-vis spectra changes of EGCG at different pH (a$_1$&a$_2$: pH 5.0; b$_1$&b$_2$: pH 6.2)

(Fig.1b_1&b_2) and precipitation occurred at the end.

In the plot of UV-visible absorbance at 274 nm and 322 nm versus ratios of aluminum ion to EGCG at different pH values (Fig.2), lines were drawn to find the inflexion point. At pH 5.0, the inflexion point was at 1; which meant that the ratio of aluminum to EGCG in the complex was 1:1; but at pH 6.2, the absorbance changed at the 2 inflexion points of 1 and 2. Because of the UV-vis spectrum changes and precipitation occurrence, we could infer that, when the ratio of aluminum to EGCG was higher than 2, the complex of aluminum-EGCG began polymerization, and that the ratio of aluminum to EGCG in the polymer was 2. When the molecular size of the polymer became large enough, precipitation occurred.

C and EC complex with aluminum

The spectra changes of C and EC were similar; absorbance at 278 nm changed little while the spectrum shape changed at the beginning, then the spectrum rose in parallel (Fig.3).

In the plot of absorbance at 257 nm, 278 nm and 298 nm versus ratio of aluminum to C or EC, for the case of C, there were two inflexions at the ratio of 1 and 2, but for EC, the two inflexions were at 0.5 and 1 (Fig.4). We infer that the ratios of aluminum to C or EC were 1 and 0.5 respectively; and that the complexes began polymerization when the ratios of aluminum ion to catechin exceeded 2 and 1 for C and EC respectively. The difference between C and EC complex with aluminum ion meant that the hydroxyl group at site 3 on c-cycle took part in the complex interaction (Fig.5).

Effect of fluoride on the interactions of catechins and aluminum

The spectra changes of catechins solution with aluminum ion were studied in order to determine the effects of fluoride on interactions between cate-

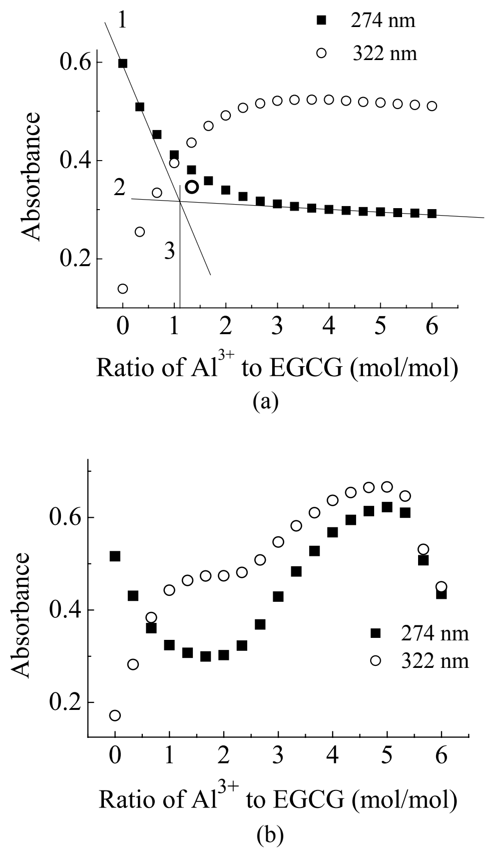

Fig.2 Spectrum changes of EGCG at different pH at 274 nm and 322 nm (a) pH 5.0; (b) pH 6.2

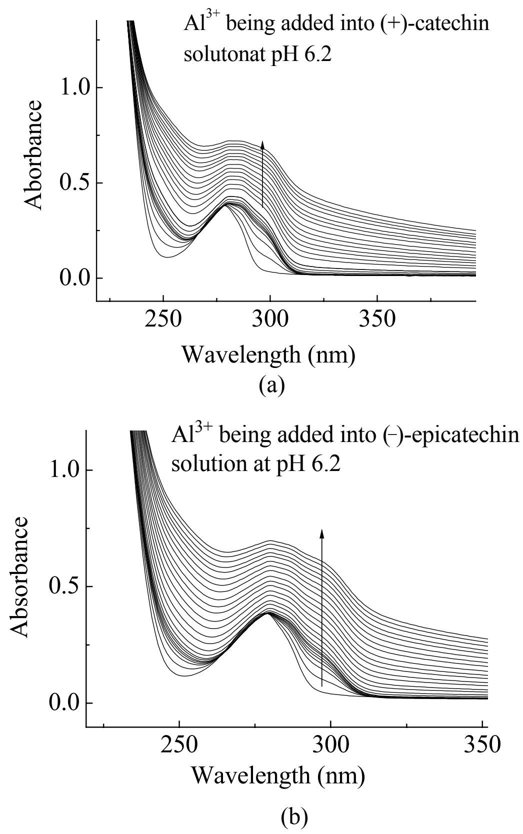

Fig.3 Spectrum changes of C (a) and EC (b) solution when Al^{3+} was added

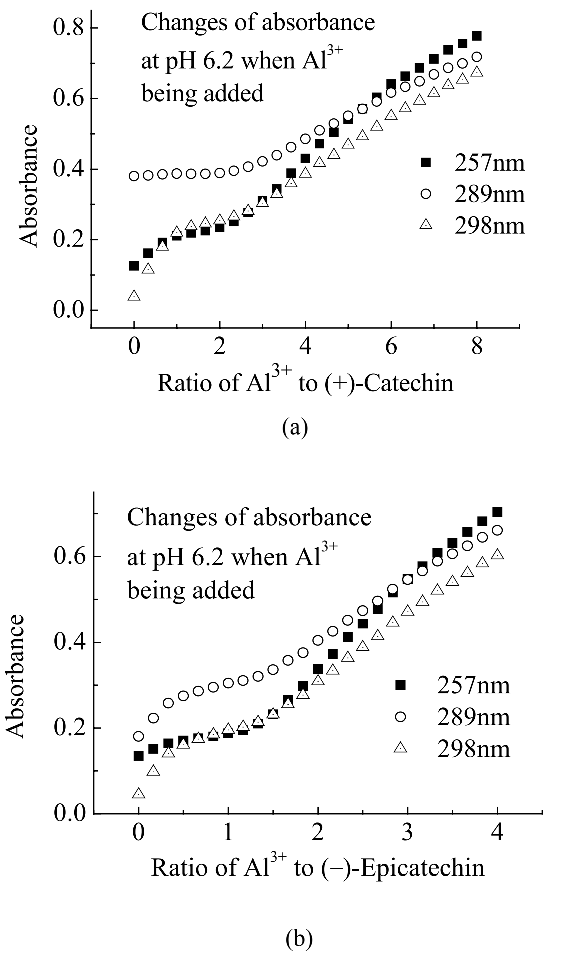

Fig.4 Spectrum comparison of C (a) and EC (b) at 257, 289 and 298 nm when Al^{3+} was added

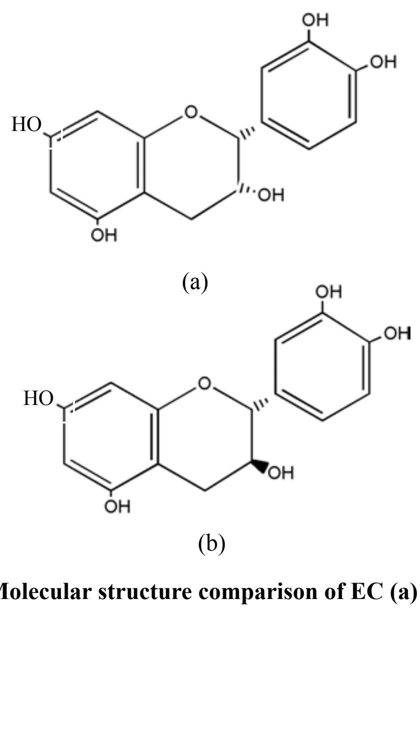

Fig.5 Molecular structure comparison of EC (a) and C (b)

chins and aluminum.

The results showed that F^- changed the spectrum apparently, and the changes vary at different pH value. In EGCG solution with the same concentration of Al^{3+} in molars at pH 6.2 condition, the spectrum decreased in parallel (Fig.6a). But at pH

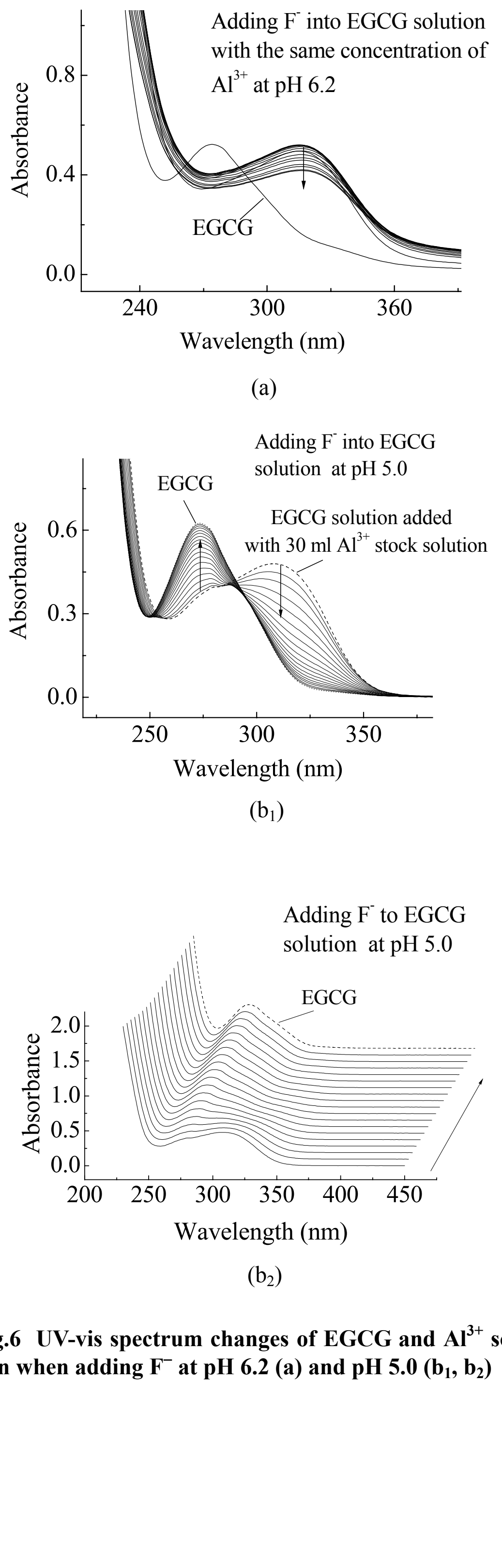

Fig.6 UV-vis spectrum changes of EGCG and Al^{3+} solution when adding F^- at pH 6.2 (a) and pH 5.0 (b_1, b_2)

5.0 solution, the spectra shape changed step by step to that of free EGCG without Al^{3+}, until at last the spectrum shape became nearly the same as that of free EGCG solution (Fig.6b_1&b_2).

For C and EC, the spectrum changed similarly at pH 5.0 and pH 6.2 conditions, the curve moved to the spectrum of catechin without aluminum ion, but it was difficult to close up to the spectrum of free catechin9 (Fig.7).

Because Al^{3+} and F^- complex easily, F^- showed competiveness to combine Al^{3+} in catechin solution, and the competing ability was stronger at lower pH solution.

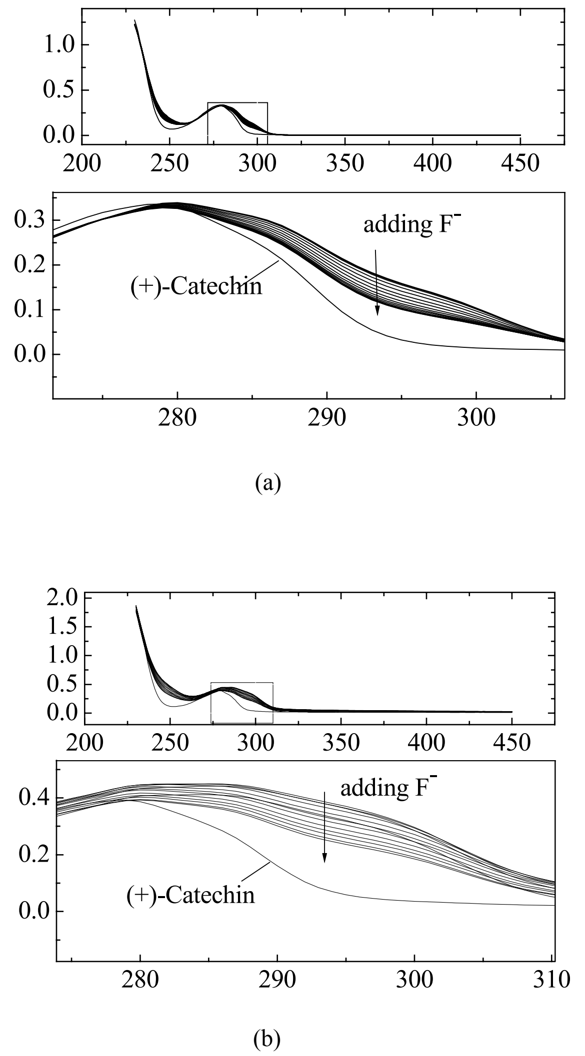

Fig.7 UV-vis spectrum changes of C solutions when F^- being added at pH 5.0 (a) and pH 6.2 (b)

Aluminum content in dry tea leaf

Detected by the method above, the aluminum contents of the dry tea leaves employed in the experiments were 325 μg/g, 483 μg/g and 637 μg/g for green tea, black tea and oolong tea, respectively.

The concentrations of aluminum in the tea infusion brewed as described above were 4.9 μg/ml for green tea, 6.7 μg/g for black tea, and 9.5 μg/g for Oolong tea, and the extraction rates were 75.5%, 69.4% and 32.7%, respectively.

The partial coefficient (*Kow*) of aluminum in tea infusion

Table 1 and Fig.8 show the results at gastric acid condition, *Kow* values were very low; log-*Kow*<0, under this condition, the aluminum species could be sorted as polar compounds. At intestine pH condition, the *Kow* value of green tea and black tea were higher than 1, log*Kow*>0; the *Kow* value of green tea was much higher than that of the other two teas; and the polarity of the aluminum species in the green tea and black tea decreased, and the possibility of being absorbed increased. Aluminum *Kow* values in Oolong tea were low in the three conditions, so we inferred that aluminum absorption of Oolong tea infusion was very low. It was reported that in healthy male volunteers, the average amounts of Al excreted were 144 μg and 86 μg resulted from their drinking green tea and Oolong tea every day (Flaten, 2002). Regardless of the concentration difference of aluminum in the infusion of the two kinds of tea, the difference of aluminum absorption between two kinds of tea may play an important role in.

Efects of tea polyphenols and catechins on *Kow* value of aluminum in acetic buffer

Adding TP and catechins into aluminum buffer solution, *Kow* values are shown in Table 2 and Fig.9.

These data showed that catechins and tea polyphenols could affect *Kow* value greatly; and that the value became lower with increase of tea polyphenols or catechins concentration. The function of EGCG and tea polyphenols was much stronger than EC and C.

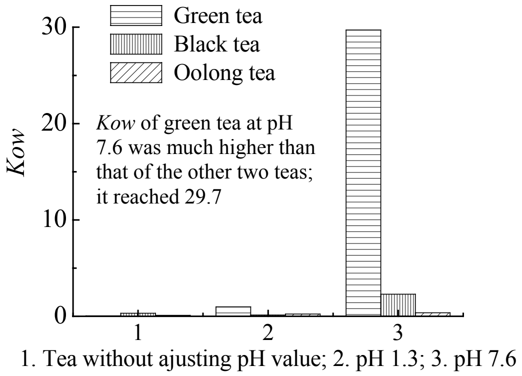

Fig.8 Comparison of *Kow* values of Al in three kinds of tea infusion

CONCLUSION

From the changes of *Kow* value at different pH conditions, we concluded that aluminum absorption mainly occurred in the intestine, as similarly reported (Berthon, 2002). Tea polyphenols and catechins can decrease the aluminum *Kow* value in acetic buffer, among them EGCG and TP had much strong function. At high pH value condition, the complex of Al-catechin could polymerize with aluminum concentration increasing. Large moleculars were more difficult to be absorbed. These two factors may affect aluminum absorption during tea intake.

Table 1 Concentrations and *Kow* of Al in tea infusions after different treatments*

Green tea	C_{Al} in aqueous phase	*Kow*	Black tea	C_{Al} in aqueous phase	*Kow*	Oolong tea	C_{Al} in aqueous phase	*Kow*
Tea infusion	4.91		Tea infusion	6.7		Tea infusion	9.47	
Not adjust pH	4.67	0.0514	Not adjust pH	5.11	0.311	Not adjust pH	8.62	0.0986
pH 1.3	2.48	0.98	pH 1.3	5.87	0.141	pH 1.3	7.61	0.244
pH 7.6	0.16	29.7	pH 7.6	2.03	2.3	pH 7.6	6.96	0.361

**Kow* = aluminum concentration in *n*-octanol phase/aluminum concentration in aqueous phase, aluminum concentration in *n*-octanol phase = aluminum concentration in tea infusion − aluminum concentration in aqueous phase

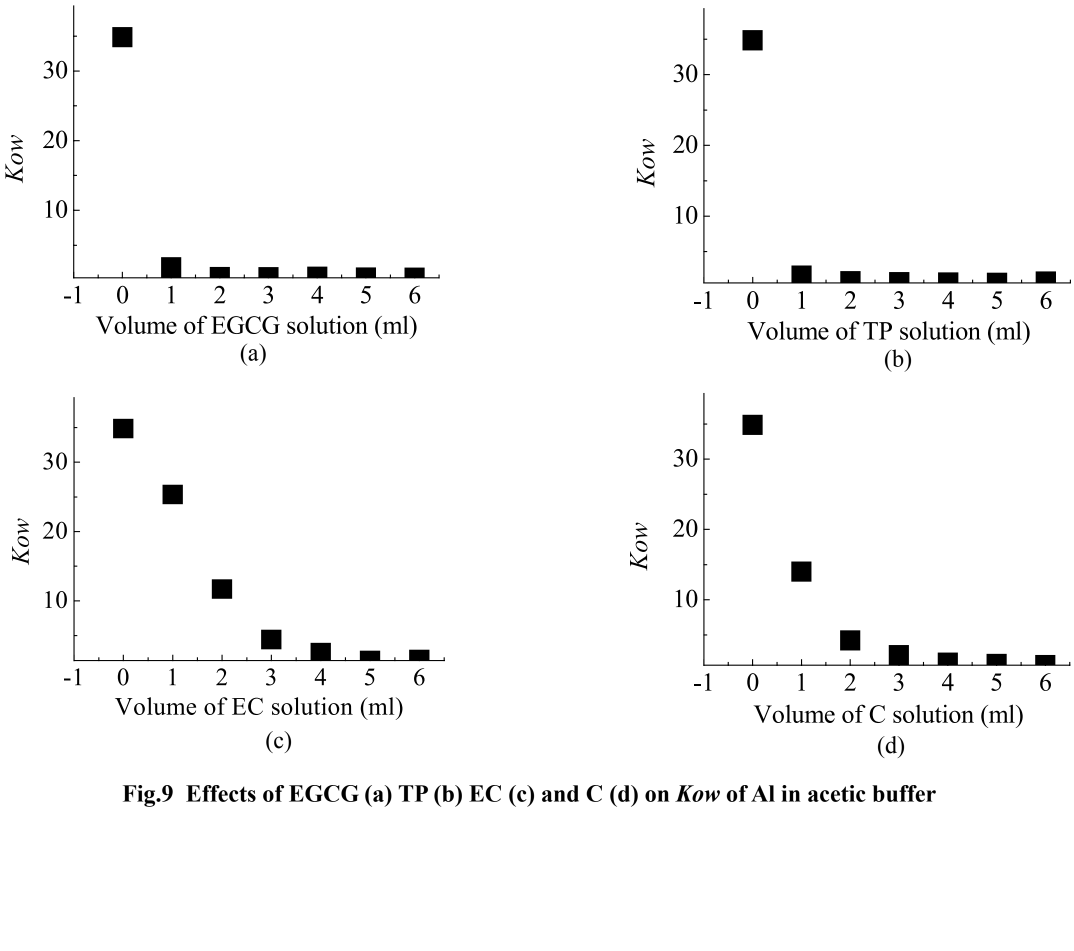

Fig.9 Effects of EGCG (a) TP (b) EC (c) and C (d) on *Kow* of Al in acetic buffer

Table 2 Effects of tea polyphenols and catechin on *Kow* of aluminum in acetic buffer*

EGCG (ml)	*Kow*	EC (ml)	*Kow*	C (ml)	*Kow*	TP (ml)	*Kow*
1	1.82	1	25.36	1	14.00	1	1.58
2	0.43	2	11.70	2	4.21	2	0.78
3	0.41	3	4.42	3	2.14	3	0.66
4	0.47	4	2.54	4	1.04	4	0.60
5	0.36	5	1.40	5	0.87	5	0.56
6	0.33	6	1.53	6	0.71	6	0.74

*aluminum *Kow* value for potassium aluminum sulfate in distilled water was 0.072, in pH 6.2 acetic buffer is 34.84

References

Berthon, G., 2002. Aluminum speciation in relation to aluminun bioavailability, metabolism and toxity. *Coordination Chemistry Reviews*, **228**:319-341.

Deng, M.E., 2002. Spectrophotometric determination of aluminum with bromopyroga11o1red. *Metallurgical Analysis*, **22**(3):60-61.

Emiko, O., Tomoko, S., Masahiko, K., 1999. Complexation of aluminum with (–)-epigallocatechin gallate studied by spectrophotometry. *Journal of Inorganic Biochemisty*, **73**:31-34.

Flaten, T.P., 2002. Aluminium in tea – concentrations, speciation and bioavailability. *Coordination Chemistry Reviews*, **228**:385-395.

He, Y.B., Zhao, Y.H., Wang L.S., 1994. Determination of *n*-octanol/water partition coefficient of organic compound. *Environmental Chemistry*, **131**:95-197.

Huang, J., Yu, G., Zhang, P.Y., 2002. Predicting the lg*Kow* of PCDFs using single-phenyl-ring cl-substitution pattern index. *Environmental Science Research*, **15**:1-5.

Hynes, M.J., Coinceanainn, M.Ó., 2001. The kinetics and mechanisms of the reaction of iron(III) with gallic acid, gallic acid methyl ester and catechin. *Journal of Biochemistry*, **85**:131-142.

Inoue, M.B., Inoue, M., Fernando, Q., Valcic, S., Timmermann, B. N., 2002. Potentiometric and ^{1}H NMR studies of complexation of Al^{3+} with (−)-epigallocatechin gallate, a major active constituent of green tea. *Journal of Inorganic Biochemistry*, **88**:7-13.

Midori, K., Tamiyoshi, S., Kinuyo, N., Masaaki, T., 2001. Effects of pH and metal ions on antioxidative actives of catechins. *Biosci. Biotechnol. Biochem.*, **65**:126-132.

Yokel, R.A., 2002. Aluminum chelation principles and recent advances. *Coordination Chemistry Reviews*, **228**:97-113.

Yu, G., Xu, X.B., 1993. Determination of the 1-octanol/water partition coefficients of nitro-pan. *Environmental Chemistry*, **12**:299-303.

Zhong, G.F., 1998, Physiology, the Third Edition. People's Medical Publishing House, Beijing, p.110, 117.