

Origin and Thermodynamic Properties of the Instability of Synthetic Azo Colorants in Gum Arabic Solutions

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The instability of some industrially important synthetic azo colorants, including sunset yellow, azorubine, and allura red, toward gum arabic in aqueous solution has been a long-standing problem for the beverage and confectionery industries. Precipitation of these colorants causes the deterioration of product appearance and properties. This work examines the origin and nature of the problem by analysis of the precipitate and thermodynamic studies of gum arabic–colorant interactions using isothermal titration calorimetry (ITC). The presence of divalent alkaline earth metals in gum arabic samples, that is, calcium and magnesium, is shown to be responsible for the precipitation of the azo colorants. There is no direct interaction between gum arabic and the colorant molecules, and the precipitate is formed likely due to the mediation/bridging by the divalent cations. The thermodynamic knowledge gained from the ITC studies, for example, binding affinity, stoichiometry, and enthalpy, enables interpretation of many industrial observations.

KEYWORDS: Gum arabic; synthetic azo colorants; precipitation; thermodynamics; ITC

INTRODUCTION

Gum arabic is an exudate obtained from the stems and branches of *Acacia* trees. It has been widely used in food industries such as confectionery, beverages, and flavor encapsulation (1). Structural studies reveal that gum arabic is a complex branched polysaccharide containing also some proteinaceous materials (2). The carbohydrate part consists of a β -1,3-linked D-galactose core with extensive branching through 3- and 6-linked galactose and 3-linked arabinose. Rhamnose and glucuronic acid are located at the periphery of the molecules, where they terminate some of the branches (1). By using hydrophobic chromatography, gum arabic can be separated into three fractions: a high molecular mass arabinogalactan-protein (AGP), a glycoprotein (GP), and an arabinogalactan (AG) (3, 4). The AGP fraction is believed to be responsible for the good emulsifying ability of gum arabic, because it is constituted of hydrophilic and hydrophobic units as demonstrated by the WATTLE BLOSSOM model (Scheme 1a) (1). Gum arabic is diverse not only in species and chemical structure but also in ionic composition. It can contain many types of metal ions,

among which calcium, magnesium, and potassium are the most abundant cations (5).

Although gum arabic is a well-recognized emulsifier in the beverage industry, problems can occur when it is used in combination with certain synthetic azo colorants. For example, the beverage industry often needs to formulate colored emulsion concentrate as a precursor of beverage production, using both gum arabic and synthetic azo colorants. During the storage of the emulsion concentrate (particularly at lower temperatures), precipitations of colorants can occur, which result in the instability of emulsion and deterioration of product. The most common problematic colorants include sunset yellow, azorubine, and allura red (Scheme 1b–d). Industrial experience pointed to cations, such as calcium and magnesium, as causing the problem, but direct scientific study of this problem is not available. There are currently different approaches to reducing the problems associated with the precipitation of azo colorants with gum arabic. However, none of them fully addresses the problem, due to the limitations imposed either by food regulations or by cost.

This study investigates this problem and aims to gain further understanding about the fundamental aspects of the precipitations between gum arabic and azo colorants. The fundamental knowledge acquired in the work would shed light on the search of new methods to prevent/inhibit the precipitation between gum arabic and synthetic azo colorants.

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Scheme 1. (a) WATTLE BLOSSOM model of arabinogalactan-protein (AGP) complex of gum arabic (1) and chemical structures of some synthetic azo colorants that would precipitate in the emulsion concentrate stabilized by gum arabic: (b) sunset yellow; (c) azorubine; (d) allura red

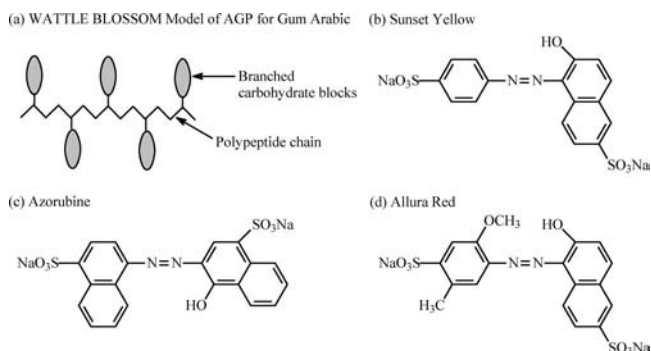


Table 1. Molecular Characteristics of Gum Arabic^a

	peak 1	peak 2	peak 3
M_w ($\times 10^5$)	5.4	27.2	4.0
M_n ($\times 10^5$)	2.5	22.1	2.4
M_w/M_n	2.2	1.2	1.7

^a The molecular characteristics were determined by a DAWN EOS multiangle laser light scattering system (Wyatt Technology Corp.) after fractionation by a Superose 6 10/300GL GPC column at 25 °C; 0.2 M NaCl aqueous solution was used both as a solvent and as eluent. Peak 1 refers to the whole gum; peak 2 refers to AGP fraction; peak 3 refers to the aggregate of AG and GP fractions. The definition of the peaks follows ref 6.

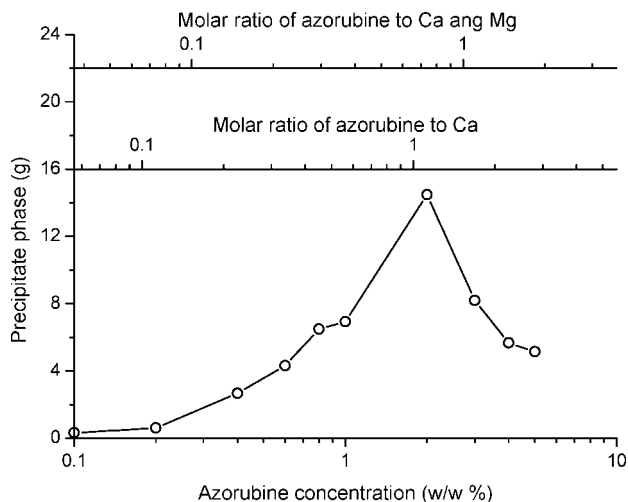


Figure 1. Plot of the weight of precipitate phase as a function of azorubine concentration in 20% (w/w) gum arabic solutions. The weight of mixture solution is 20 g. Another two x-axes, the molar ratios of azorubine/Ca and azorubine/(Ca + Mg), are also included, providing the concentrations of intrinsic Ca and Mg in 20% gum arabic (1417 and 453 ppm, respectively).

EXPERIMENTAL PROCEDURES

Materials. Gum arabic (lot 040309) was supplied by San-Ei Gen F.F.I. Inc., which has a moisture content of 9.36%. Its molecular characteristics, determined by GPC-MALS, are listed in **Table 1**. The cationic composition of the gum arabic determined by an inductively coupled plasma optical emission spectrometry (ICP-OES) system (Barian Inc.) is 7080 ppm of Ca, 2264 ppm of Mg, 8760 ppm of K, and 189 ppm of Na. Synthetic azo colorants, sunset yellow and azorubine, were provided by Givaudan, and allura red was obtained from Sigma-Aldrich. All other chemical reagents used in

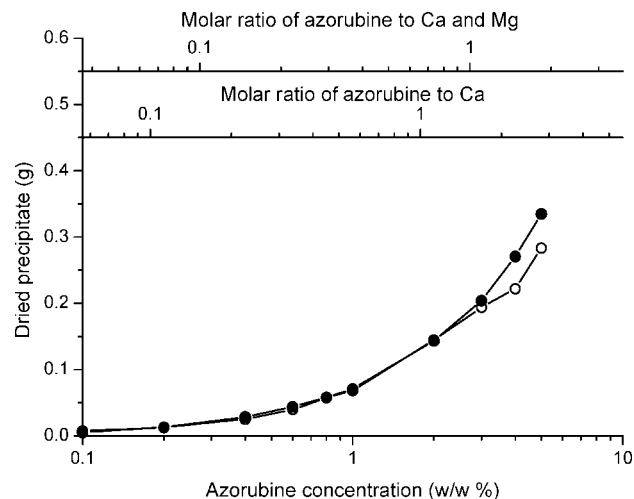


Figure 2. Plot of the amount of dried precipitate as a function of azorubine concentration in two different saline solutions: one containing 1417 ppm of Ca (○) and the other containing 1417 ppm of Ca + 453 ppm of Mg (●). The weight of mixture solution is 10 g.

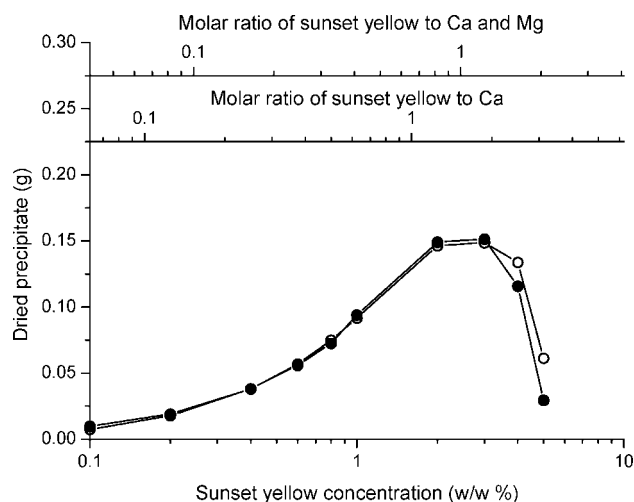


Figure 3. Plot of the amount of dried precipitate as a function of sunset yellow concentration in two different saline solutions: one containing 1417 ppm of Ca (○) and the other containing 1417 ppm of Ca + 453 ppm of Mg (●). The weight of mixture solution is 10 g. Similar to **Figure 2**, the y-axis refers to the weight of dried residue of precipitate phases.

this work were purchased from Fisher Scientific and were of analytical grade.

Precipitate Analysis. Gum arabic solutions (20% w/w) were prepared by dispersing gum arabic powder into distilled water and left overnight under gentle shaking to ensure complete hydration. Azorubine was added to the gum solutions to achieve different colorant concentrations. After vigorous mixing, the mixtures were stored at 5 °C overnight. To fully sediment the precipitate phase, all of the mixtures were centrifuged at a relative centrifugal force of 1160g for 30 min. The precipitate phases were separated out by decanting the supernatants and then weighed. The precipitate phases contain a large amount of water. The concentration of gum arabic present in the precipitate phases was analyzed using a spectrophotometric method. A certain amount of each precipitate phase was redissolved by dilution with distilled water. Absorptions at wavelengths of 217 and 516 nm were measured on a Perkin-Elmer Lambda 5 UV-vis spectrophotometer. Because the absorption at 516 nm purely arises from azorubine, the concentration of azorubine in the precipitate phases can be known from this absorption by establishing an azorubine calibration curve. Subsequently, the absorptions of azorubine and gum arabic at 217 nm can be separated,

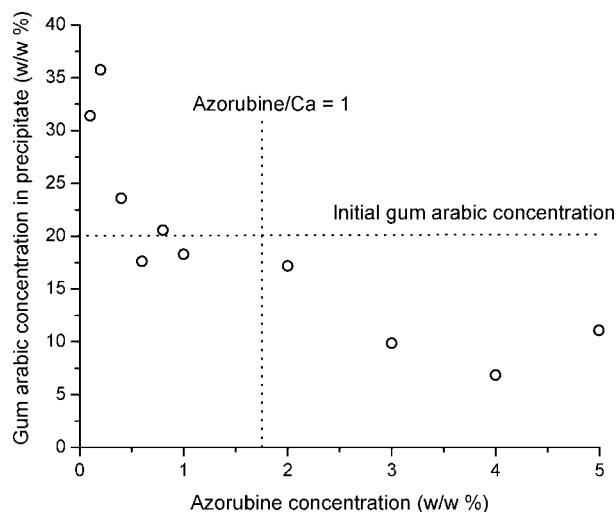


Figure 4. Gum arabic concentration in precipitate phases as a function of azorubine concentration. The horizontal dotted line represents the initial concentration of gum arabic in the mixtures (20% w/w), and the vertical dotted line represents the position of azorubine/Ca = 1.

assuming that they are of linear addition. After subtraction of the contribution of azorubine, the absorption at 217 nm due to gum arabic can be calculated, and the gum arabic concentration was determined from a gum arabic calibration curve.

The precipitation of azorubine and sunset yellow in different saline solutions that replicate the ionic condition of 20% (w/w) gum arabic solutions was also examined. The precipitate phases were separated by centrifugation and dried at 105 °C for 12 h. The amount of precipitate was plotted as a function of the colorant concentrations. Azorubine samples were stored at 5 °C overnight before analysis,

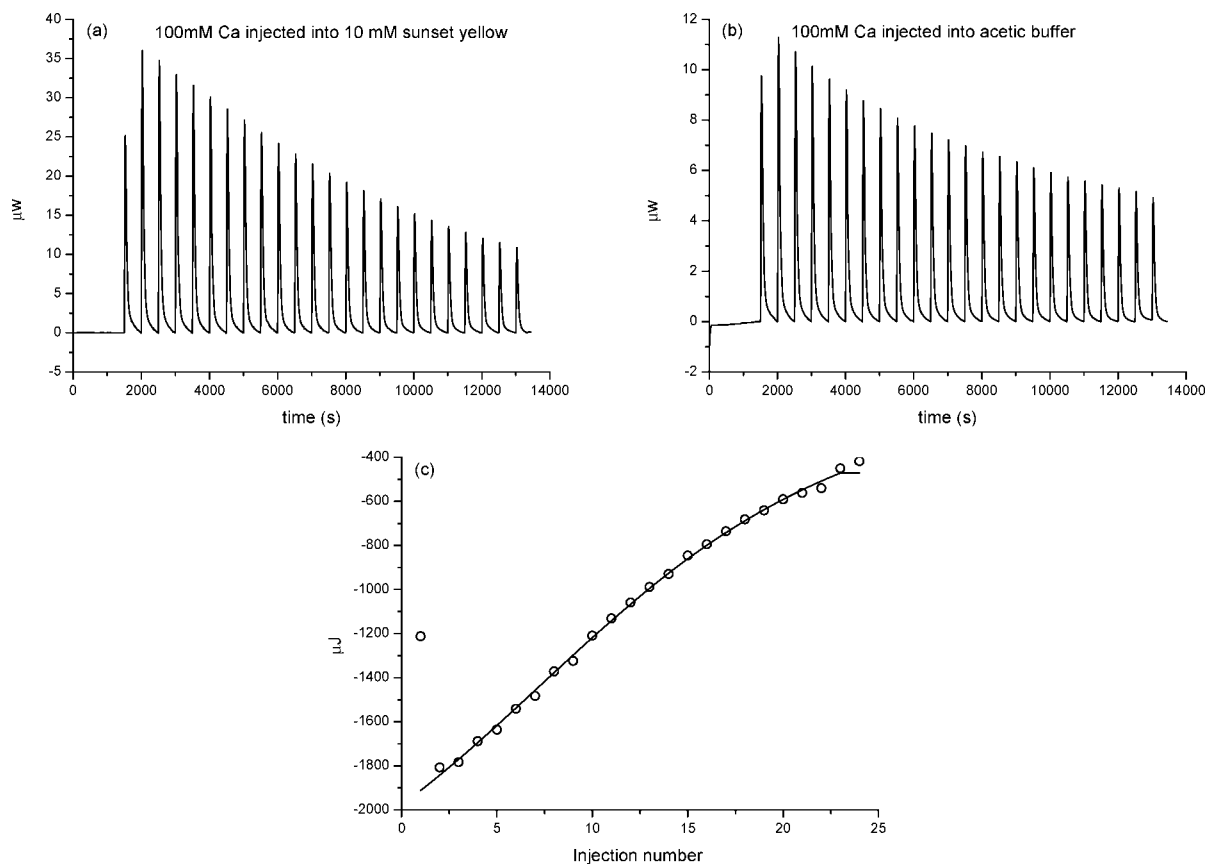


Figure 5. ITC thermograms recorded during the titration of 100 mM CaCl_2 into (a) 10 mM sunset yellow and (b) acetate buffer. The corresponding interaction/binding isotherm is plotted in (c) where the solid line represents the curve-fitting result by a model of independent binding sites (eq 1).

Table 2. Thermodynamic Parameters of the Binding of Ca to Azo Colorants and Gum Arabic^a

	<i>n</i>	<i>K</i> (M)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol · K)
Ca/sunset yellow	1.14	188.9	-2.83	-12.99	34.09
Ca/allura red	0.99	342.8	-6.01	-14.46	28.37
Ca/azorubine	1.26	2734.6	-11.35	-19.61	27.70
Ca/gum arabic	0.40	3422.3	-2.38	-20.16	59.69

^a $\Delta G = -RT \ln K$; $\Delta S = (\Delta H - \Delta G)/T$, where *R* is the gas constant and *T* is the absolute temperature. The thermodynamic parameters for the binding of Ca to gum arabic are calculated on the basis of the number of glucuronic acid units, assuming a glucuronic acid content of 15% (w/w) in the total gum.

whereas the sunset yellow samples need to be left for 1 week to allow complete precipitation.

Isothermal Titration Calorimetry (ITC). The titration of 100 mM CaCl_2 into 10 mM azo colorant, 10 mM CaCl_2 into 0.2% gum arabic, or 10 mM azo colorant into 0.2% gum arabic was made using a CSC 4200 isothermal titration calorimeter (Calorimetry Sciences Corp.) to investigate the interactions of Ca–colorant, Ca–gum arabic, and colorant–gum arabic, respectively. Deionized gum arabic (via H^+ exchange resin) was used to avoid the effects of intrinsic divalent metal ions that are present in normal gum arabic samples. The mineral composition of the deionized gum arabic determined by ICP-OES is 12 ppm of Ca, 32 ppm of Mg, 48 ppm of Na, and 136 ppm of K. The content of divalent cations is negligible. All of the ITC samples were prepared in 20 mM acetic buffer (pH 4). Acetic buffer was used to eliminate the experimental errors due to pH mismatch. The injection volume, number of injections, interval between two successive injections, and stirring speed were set at 10 μL , 24, 500 s, and 297 rpm, respectively. The ITC cell has a fixed volume of 1300 μL . Blank experiments were also done to calibrate the heat effect of the dilution of titrants. The binding isotherm obtained by

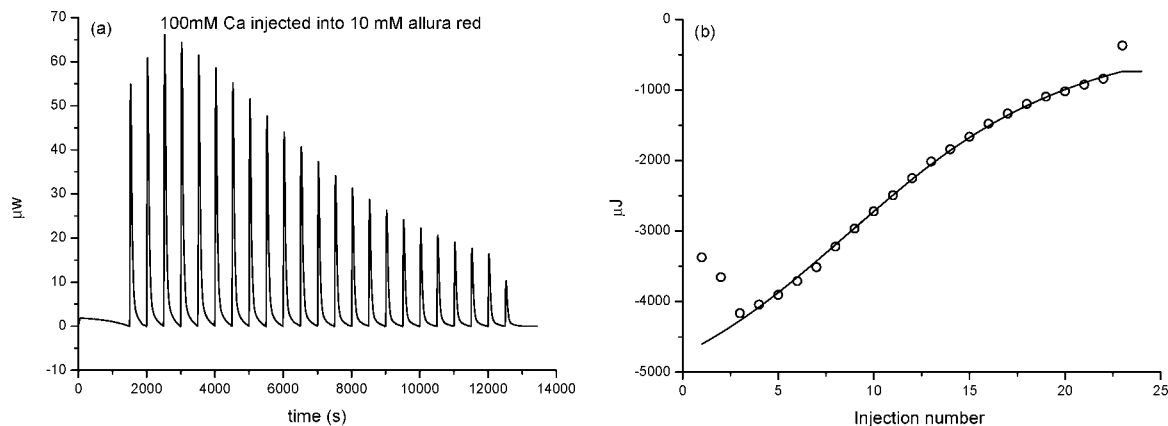


Figure 6. ITC thermogram recorded during the titration of 100 mM CaCl_2 into 10 mM allura red (a). The corresponding interaction/binding isotherm is plotted in (b), where the solid line represents the curve-fitting result by a model of independent binding sites (eq 1). Note that the dilution heat of CaCl_2 during titration is shown in **Figure 5b**.

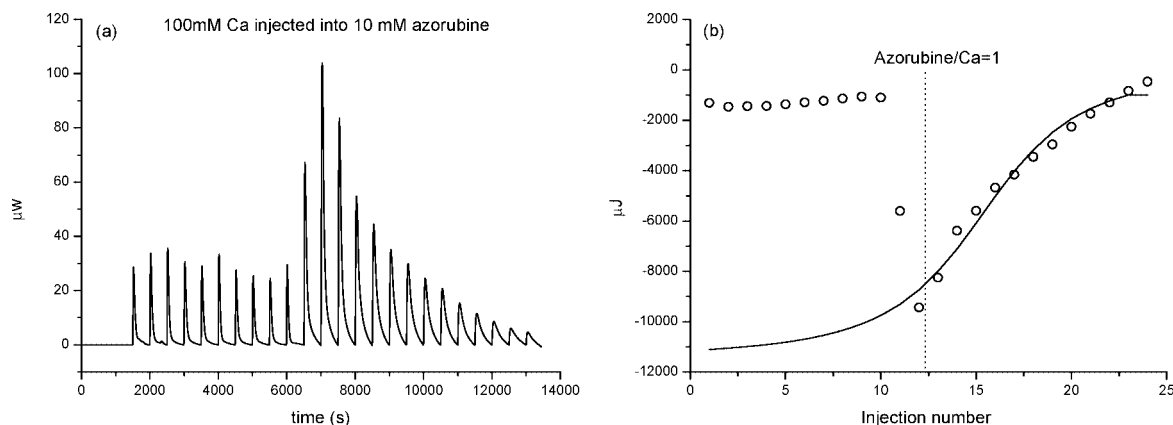


Figure 7. ITC thermogram recorded during the titration of 100 mM CaCl_2 into 10 mM azorubine (a). The corresponding interaction/binding isotherm is plotted in (b), where the solid line represents the curve-fitting result by a model of independent binding sites (eq 1). The vertical dotted line in (b) marks the position of azorubine/Ca = 1. Note that the dilution heat of CaCl_2 during titration is shown in **Figure 5b**.

integrating injection peaks could be fitted by a model of independent binding sites (7):

$$Q = V \cdot \Delta H \cdot \left[[L] + \frac{1 + [M] \cdot n \cdot K - \sqrt{(1 + [M] \cdot n \cdot K - [L] \cdot K)^2 + 4 \cdot K \cdot [L]}}{2K} \right] \quad (1)$$

where Q is accumulative heat and V , $[L]$, and $[M]$ are the volume of cell, the concentration of the substance loaded in the syringe, and the concentration of the substance put in the cell, respectively. Iterative curve fitting yielded thermodynamic parameters including the binding constant K , binding enthalpy ΔH , and stoichiometry n .

Note that all of the precipitate analyses were conducted in duplicate, and average values were used in this paper. All of the ITC experiments were repeated to ensure a reproducibility within $\pm 5\%$.

RESULTS AND DISCUSSION

Precipitate Analysis of Gum Arabic with Azo Colorants.

First, the precipitation behaviors of azorubine and sunset yellow were investigated in 20% (w/w) gum arabic solutions, which is a typical concentration encountered in the beverage industry. **Figure 1** plots the amount of precipitate phase as a function of the concentration of added azorubine. At the fixed concentration of gum arabic, the amount of precipitate phase increases with increasing azorubine concentration and then reaches a maximum. After this maximum, the amount of precipitate phase starts to reduce. The maximum appears at an azorubine concentration of 2%. The concentrations of intrinsic calcium and magnesium

in 20% gum arabic solution are 1417 and 453 ppm, respectively. On this basis it is possible to show that the maximum is located at an azorubine/Ca ratio of ~ 1 , or an azorubine/(Ca + Mg) ratio of ~ 0.75 . The former value actually coincides with the 1:1 stoichiometry of a charge-neutralized Ca–azorubine complex, because Ca is a divalent cation and azorubine is a divalent anion (bearing two sulfate groups, as in **Scheme 1c**). This indirectly indicates that the occurrence of precipitate between azorubine and gum arabic is attributed to calcium ions present in gum arabic. The position of the maximum is not related to charge neutralization by the total concentration of Ca and Mg, which means that the precipitation of azorubine is controlled by Ca rather than Mg, and the interaction between Mg and azorubine is not as strong as that between Ca and azorubine. This will be further supported by ITC studies.

The precipitation of azorubine in two different saline solutions, one containing 1417 ppm of Ca and the other containing 1417 ppm of Ca and 453 ppm of Mg, was also investigated. These two saline solutions simulate the divalent cation compositions of 20% (w/w) gum arabic solutions, but without gum arabic being present. The experimental protocol adopted here is to highlight the role of divalent cations alone. The result is displayed in **Figure 2**. Note that different from **Figure 1**, the y-axis in **Figure 2** is the weight of dried residue of precipitate phases, because no gum arabic is present.

In **Figure 2**, the amount of dried precipitate increases regularly with increasing azorubine concentration in either of the two saline solutions. At azorubine/Ca < 1, no difference in

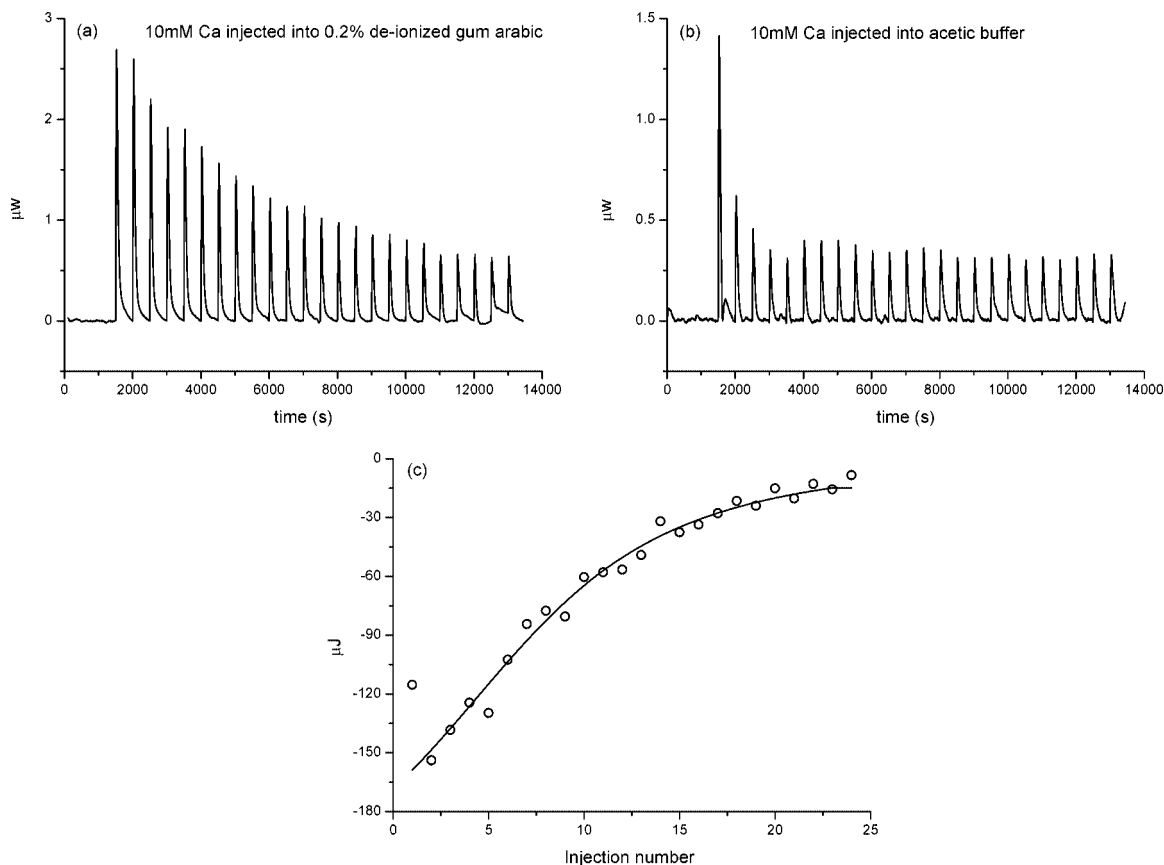


Figure 8. ITC thermograms recorded during the titration of 10 mM CaCl_2 into (a) 0.2% deionized gum arabic and (b) acetate buffer. The corresponding interaction/binding isotherm is plotted in (c), where the solid line represents the curve-fitting result by a model of independent binding sites (eq 1).

the amount of precipitate is observed between the two saline solutions. However, when azorubine/ $\text{Ca} > 1$, the amount of precipitate in the saline solution of 1417 ppm of $\text{Ca} + 453$ ppm of Mg starts to exceed that in the 1417 ppm of Ca solution. This is additional evidence that azorubine/ Ca has a stronger binding/interaction affinity than azorubine/ Mg and that azorubine is bound to Ca preferentially.

In comparison with azorubine, the precipitation behavior of sunset yellow in the two saline solutions is illustrated in **Figure 3**. It can be seen that sunset yellow shows significantly different precipitation behavior. The increase in the amount of precipitate is not monotonic and has a maximum. The maximum seems to be located at a sunset yellow/ $(\text{Ca} + \text{Mg})$ ratio of ~ 1 , rather than at sunset yellow/ $\text{Ca} = 1$. This implies that both Ca and Mg control the precipitation of sunset yellow, and there is no remarkable difference in the binding affinity of sunset yellow with Ca and Mg . This is consistent also with the results of the ITC studies. Why the amount of precipitate decreases when sunset yellow/ $(\text{Ca} + \text{Mg}) > 1$ remains unclear at this stage. It could possibly be due to the oversaturation of sunset yellow relative to the amount of divalent cations, which makes the precipitate deviate from the charge-neutralized point and thus increases the solubility of the precipitate.

Ca and Mg ions have thus been shown to be related to the precipitation of synthetic azo colorants in gum arabic solutions. A question then arises: is gum arabic itself involved in the precipitation of the colorants? To clarify this, we chose to analyze the precipitate phases of azorubine with 20% gum arabic by the spectrophotometric method described to determine the concentration of gum arabic. **Figure 4** displays the variation of the gum arabic concentration in the precipitate phases as a function of azorubine concentration.

As a general trend, the concentration of gum arabic in the precipitate phases decreases upon increasing concentration of azorubine. Roughly, when azorubine/ $\text{Ca} < 1$, the amount of gum in the precipitate is greater than the initial concentration of gum arabic (20%), indicating enrichment of gum arabic in the precipitate phases. In contrast, when azorubine/ $\text{Ca} > 1$, the amount of gum in the precipitate is lower than the initial gum concentration, indicating the depletion of gum arabic from the precipitate phases. Gum arabic contains a number of carboxylic groups (I), which can be also bound to Ca . At azorubine/ $\text{Ca} < 1$, the excess of Ca over azorubine needs additional gum arabic to participate in the precipitation to neutralize the overall charge, which brings more gum arabic into the precipitate phases. On the other hand, when azorubine/ $\text{Ca} > 1$, azorubine is in excess. When the precipitate would have an overall negative charge, the gum arabic would be charge repelled from the precipitate phases, resulting in the depletion of gum arabic. The results demonstrate that, depending on the conditions, gum arabic can also be involved in the precipitation of synthetic azo colorants.

Thermodynamics of Interactions between Gum Arabic and Azo Colorants. The analysis of precipitates reveals that most likely gum arabic, divalent cations, and azo colorant are involved in the formation of precipitates. The three components interplay with each other. It is therefore desirable to single out two of them and examine their interactions. To do so, a deionized gum arabic is produced in which the ion exchange with H^+ resin removes almost all of the Ca and Mg ions from the original gum arabic samples. The interactions between two of the three components were then studied using the ITC technique.

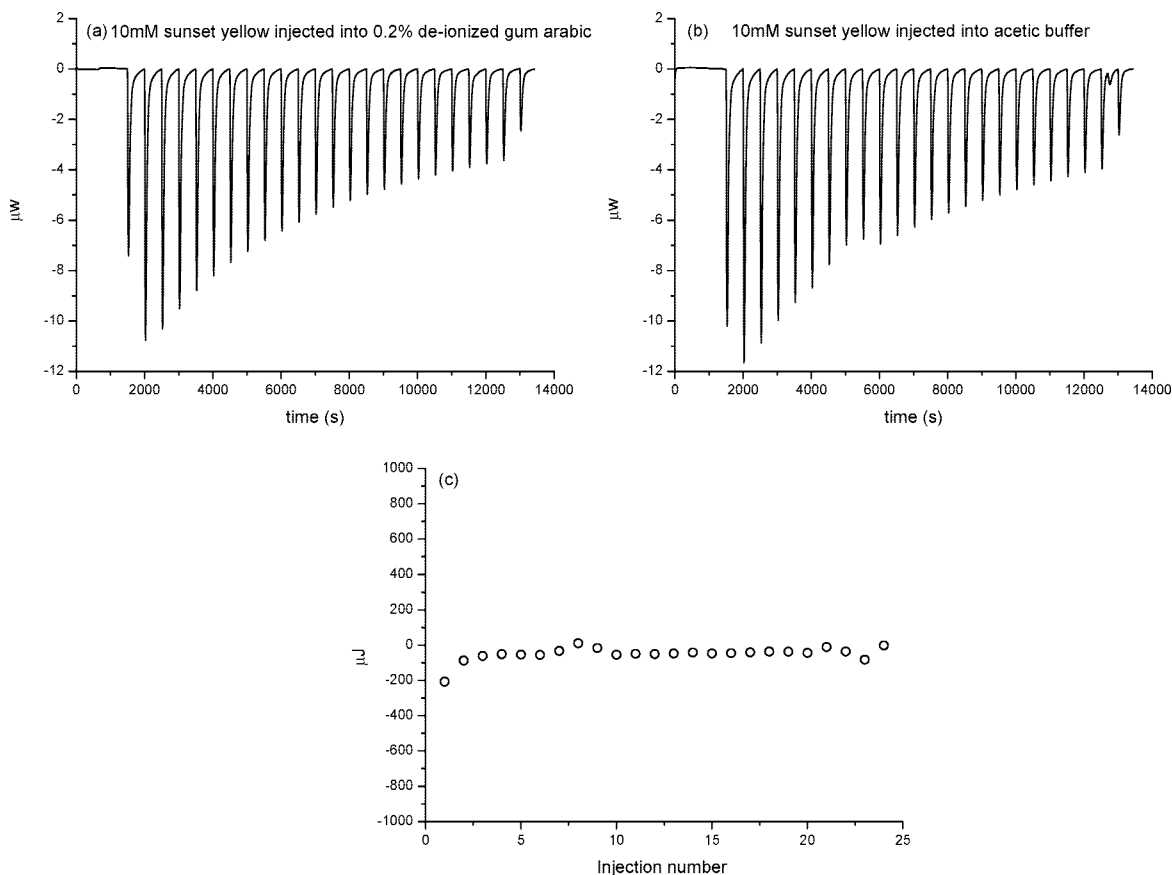


Figure 9. ITC thermograms recorded during the titration of 10 mM sunset yellow into (a) 0.2% deionized gum arabic and (b) acetate buffer. The corresponding interaction/binding isotherm is plotted in (c).

Calcium/Colorant Interactions. **Figure 5a** shows the ITC thermogram recorded during the sequential titration of 100 mM CaCl_2 into 10 mM sunset yellow. The height of the peaks decreases steadily with increasing number of injections. The result of a blank experiment is displayed in **Figure 5b**, where 100 mM CaCl_2 is injected into acetic buffer solvent. The blank experiment is used to calibrate the effect of dilution heat of CaCl_2 during titration. The integration of ITC peaks after removal of the dilution heat of CaCl_2 yields the interaction/binding isotherm of CaCl_2 to sunset yellow (**Figure 5c**). The isotherm fits well into a model of independent binding sites (eq 1), indicating the absence of cooperativity between the interactions of Ca with sunset yellow. The resultant thermodynamic parameters from curve fitting are listed in **Table 2**.

Figure 6a shows the ITC thermogram when 100 mM CaCl_2 is injected into 10 mM allura red. The interaction/binding behavior of CaCl_2 with allura red is in general similar to that of CaCl_2 with sunset yellow, with the height of injection peaks reducing regularly with increasing number of injections. The binding isotherm is shown in **Figure 6b** after the effect of dilution heat of CaCl_2 was taken into consideration. The binding isotherm can similarly be described by a model of independent binding sites. The associated thermodynamic parameters are given in **Table 2**.

The interaction/binding behavior of Ca with azorubine is distinctly different from those with sunset yellow and allura red (**Figure 7**). At the beginning, the injection peak remains almost constant with increasing number of injections. Around the 12th injection, the injection peak grows abruptly and reaches a maximum. Thereafter, the injection peak reduces steadily. From the binding isotherm shown in **Figure 7b**, it is evident

that the first stage of the binding has a relatively small interaction heat, implying that no strong interaction occurs between Ca and azorubine. Interestingly, the maximum is actually located at the molar ratio of azorubine to Ca of ~ 1 (the dotted line in **Figure 7b**). This means that the interaction between Ca and azorubine is triggered only when charge neutrality is reached between Ca and azorubine. The second stage of the binding also can be approximated to a model of independent binding sites, and the results are listed in **Table 2**.

The thermodynamic parameters shown in **Table 2** reveal that all three colorants have almost the same stoichiometry n of around 1, with azorubine deviating relatively more from 1. This stoichiometry can be interpreted in terms of the charge neutralization between Ca and the azo colorants, because all of the colorants bear two sulfate groups (-2 charge, see **Scheme 1**). The values of K (and also ΔG) lead to the conclusion that the interaction/binding affinity of the colorants with Ca increases in the order sunset yellow < allura red \ll azorubine. This is consistent with the experience accumulated in the beverage industry that azorubine has the most serious precipitation with calcium, followed by allura red and sunset yellow; the precipitate between azorubine and Ca is formed instantaneously once their solutions are mixed. In contrast, the precipitations of allura red and sunset yellow with Ca only occur within a period of weeks. The interactions of Ca with the three colorants have negative values of binding enthalpy ΔH , which means that lower temperature is favorable for the interactions. This then explains why it was observed that the storage of beverage emulsion concentrates at lower temperatures facilitates/accelerates the formation of precipitates. The positive values of entropy ΔS

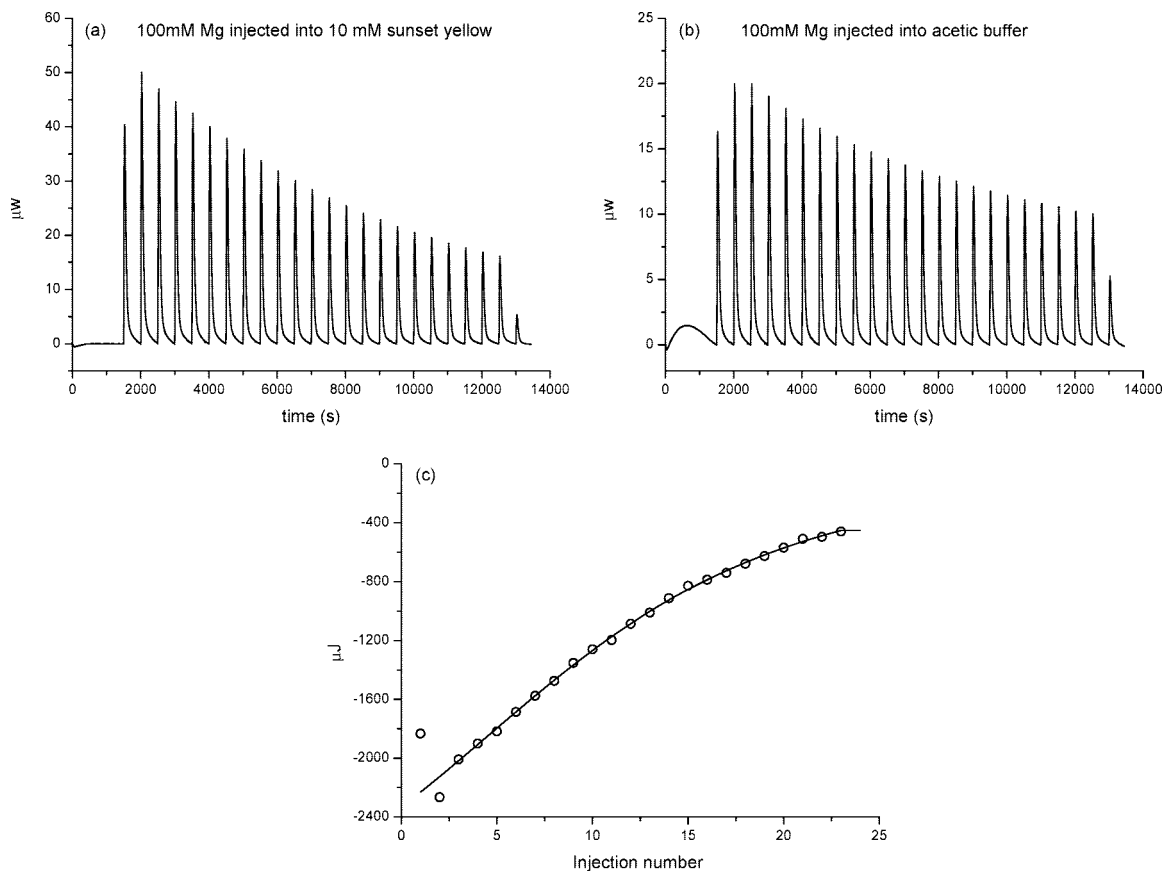


Figure 10. ITC thermograms recorded during the titration of 100 mM MgCl_2 into (a) 10 mM sunset yellow and (b) acetate buffer. The corresponding interaction/binding isotherm is plotted in (c), where the solid line represents the curve-fitting result by a model of independent binding sites (eq 1).

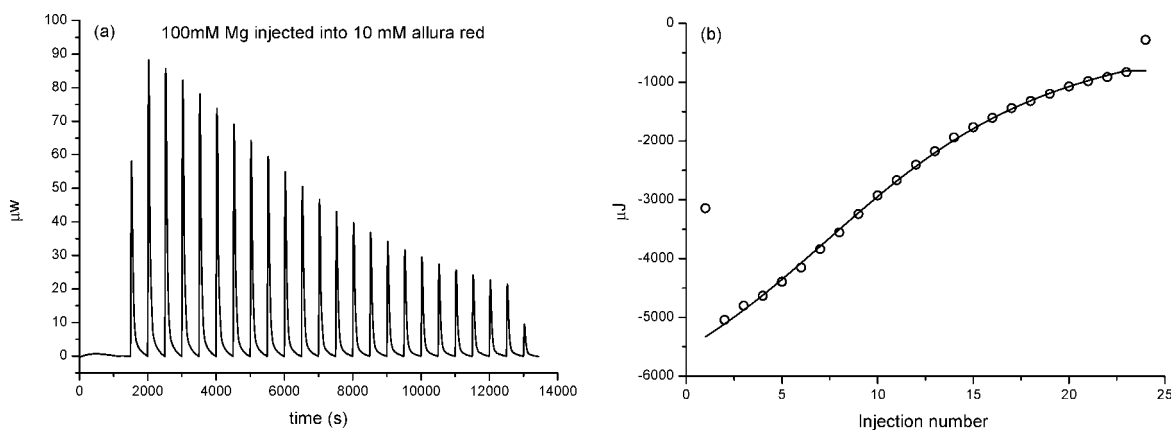


Figure 11. ITC thermogram recorded during the titration of 100 mM MgCl_2 into 10 mM allura red (a). The corresponding interaction/binding isotherm is plotted in (b), where the solid line represents the curve-fitting result by a model of independent binding sites (eq 1). Note that the dilution heat of MgCl_2 during titration is shown in Figure 10b.

indicate an increase in the system freedom during the interaction of Ca with the colorants. This might be due to the release and subsequent mixing of the counterions of Ca and the colorants.

Calcium/Gum Arabic Interaction. The interaction of Ca with gum arabic can be studied by titrating CaCl_2 into a solution of gum arabic sample that is free of intrinsic Ca^{2+} (i.e., deionized gum arabic). Figure 8a shows the recorded ITC thermogram for injecting 10 mM CaCl_2 into 0.2% deionized gum arabic. The corresponding blank run is shown in Figure 8b, which can be used to take account of the effect of the dilution heat of CaCl_2 . The binding isotherm of Ca to gum arabic fits well into a model of independent binding sites (eq 1) (Figure 8c).

Table 3. Thermodynamic Parameters of the Binding of Mg to Azo Colorants^a

	<i>n</i>	<i>K</i> (M)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol · K)
Mg/sunset yellow	0.93	180.3	-3.62	-12.87	31.03
Mg/allura red	0.91	306.6	-7.34	-14.18	22.98
Mg/azorubine					

^a $\Delta G = -RT \ln K$; $\Delta S = (\Delta H - \Delta G)/T$, where *R* is the gas constant and *T* is the absolute temperature. The thermodynamic parameters for Mg/azorubine failed to be determined by curve fitting.

Gum arabic samples of different origins have a glucuronic acid content of ~15% w/w (8). Assuming that the carboxylic groups of the glucuronic acid units are the main interaction/

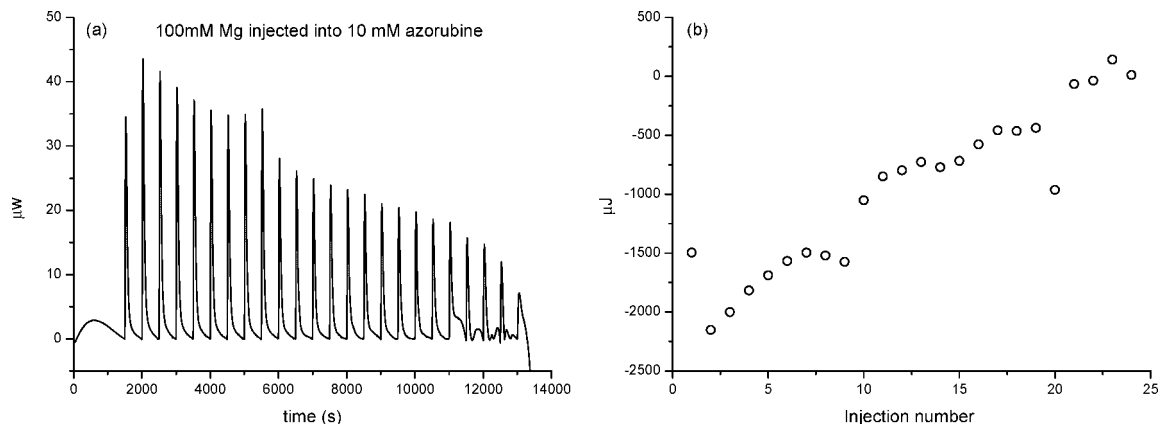


Figure 12. ITC thermogram recorded during the titration of 100 mM MgCl_2 into 10 mM azorubine (a). The corresponding interaction/binding isotherm is shown in (b). Note that the dilution heat of MgCl_2 during titration is shown in **Figure 10b**.

binding sites for Ca and a glucuronic acid unit content of 15% w/w, the curve fitting of the binding isotherm yields the thermodynamic parameters shown in **Table 2**. The resulting stoichiometry n is 0.40, which agrees reasonably well with the value of 0.5 predicted on the basis of charge neutralization. The intriguing point is that the affinity constant K for Ca binding to gum arabic is even larger than any of those between calcium and the colorants. This at first sight could be considered contradictory with the observations that gum arabic forms precipitates with azo colorants. The larger affinity constant of Ca with gum arabic would imply a preferential binding of Ca to gum arabic rather than to the colorants. However, it should also be noted that a 20% gum arabic solution has a total Ca concentration of 1417 ppm, of which ~ 800 ppm of Ca is in free form by Ca^{2+} -selective electrode measurement, and the remainder is in bound form. It is the free calcium ions that mainly contribute to the formation of precipitates with azo colorants. Actually, the larger affinity constant of Ca with gum arabic can explain the involvement of gum arabic in the precipitations as already shown, because Ca might at one end bind to a carboxylic group of gum arabic and at the other end to a sulfate group of the colorants.

Colorants/Gum Arabic Interactions. Deionized gum arabic is also used to investigate if there are direct interactions between gum arabic and azo colorants. **Figure 9a** shows the ITC thermogram for titrating 10 mM sunset yellow into 0.2% deionized gum arabic. The corresponding blank experiment is displayed in **Figure 9b**. The injection of sunset yellow into either deionized gum arabic or acetate buffer is an endothermic process. The endothermic peaks are reduced with increasing number of injections. The two experiments have almost the same heat generation at each injection. This is reflected in **Figure 9c**, where the binding isotherm shows nearly zero heat. This indicates the lack of direct interactions between sunset yellow and deionized gum arabic that is free of divalent cations. The interactions between deionized gum arabic and allura red and azorubine were also investigated by ITC (data not shown). Neither of these two colorants has direct interactions with deionized gum arabic.

The absence of direct interactions between deionized gum arabic and the synthetic azo colorants can be understood because both are similarly negatively charged, so that no strong binding/interaction can occur due to electrostatic repulsions. These findings are confirmed by the observations that no precipitate was formed between deionized gum arabic and azo colorants.

However, gum arabic and azo colorants can interact indirectly under the mediation of Ca, for example, in the case of commercial gum arabic, where a large amount of Ca is present.

Comparison of Ca with Mg Ions. Apart from Ca, commercial gum arabic also contains a significant amount of Mg, which might also be responsible for the precipitations of azo colorants. It is thus also important to investigate the interaction/binding behavior of Mg with the azo colorants and to compare with Ca. From **Figures 10** and **11**, it can be seen that the interaction/binding behaviors of Mg with sunset yellow and allura red are basically similar with those of Ca (**Figures 5** and **6**). For example, the injection heat smoothly decreases with increasing number of injections. The binding isotherms also can be well described by a model of independent binding sites. The thermodynamic parameters obtained from curve fitting for Mg are given in **Table 3**. As expected, the binding of Mg to sunset yellow and allura red has a binding stoichiometry approximate to 1. Moreover, the values of K , ΔH , ΔG , and ΔS are very close to those for Ca binding (**Table 2**), implying that Mg is almost identical with Ca in its interaction/binding behavior with sunset yellow and allura red. These results are consistent with **Figure 3**, where the precipitation of sunset yellow reaches the maximum at sunset yellow/(Ca + Mg) = 1 rather than at sunset yellow/Ca = 1. Both of these two divalent cations control the precipitations of sunset yellow and allura red and have almost equal roles in inducing precipitation.

The interaction/binding behavior of Mg with azorubine is quite different from that of Ca with azorubine. **Figure 12a** represents the ITC thermogram for injecting 100 mM MgCl_2 into 10 mM azorubine. In general, the injection peak decreases with increasing number of injections, except that there is a small discontinuity around the ninth injection, which is reminiscent of the maximum appearing during the titration of Ca to azorubine (**Figure 7a**), but much smaller in magnitude. This behavior indicates that the interaction/binding between Mg and azorubine is significantly weaker than that between Ca and azorubine. This complies with the findings in **Figure 2**, which show that azorubine preferentially interacts with Ca and then Mg. It is Ca rather than Mg that controls the precipitation of azorubine. Because of the discontinuity in the curve and relative scattering binding isotherm of Mg with azorubine, it is not possible to fit the curve to determine the thermodynamic parameters.

Conclusion. Calcium and magnesium ions contained in commercial gum arabic samples are the origin that causes the

precipitation of synthetic azo colorants in gum arabic solutions. Depending on conditions, gum arabic can also be involved in the precipitates.

Among the colorants investigated, azorubine has the strongest interaction/binding affinity with divalent cations and therefore presents the most serious problem of precipitation. Moreover, it shows a preferential interaction/binding toward Ca rather than Mg. Sunset yellow and allura red have a much weaker affinity with divalent cations, and they show no selectivity between Ca and Mg. No direct interaction is found between gum arabic and the azo colorants. The participation of gum arabic in the precipitation might be possible only via the mediation of the divalent cations.

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