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A Simple Approach To Detect Caffeine in Tea Beverages

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Supporting Information

ABSTRACT: The photophysical properties of commercially available dye Acridine Orange (AO) describe an excellent probe for selective and sensitive detection of caffeine in aqueous solution. AO exists in monomer−dimer equilibrium in water. AO monomer is a fluorophore, but the dimer is not. Addition of caffeine to the AO leads to a shift in the monomer−dimer equilibrium toward the direction of AO monomer (fluorophore) and results in enhancement of AO fluorescence intensity. Enhancement of AO fluorescence intensity in the presence of caffeine has been treated as a signal for caffeine sensor. Furthermore, the caffeine-induced shift in AO monomer−dimer equilibrium is attributed to the binding of caffeine with AO monomer, and the binding constant was higher at a low pH range (pH ∼2) compared to pH ∼7, which results in superior caffeine sensitivity at pH ∼2. Finally, caffeine content in commercial tea beverages has been evaluated and compared with the value obtained with a standard HPLC method.

KEYWORDS: *Acridine Orange, caffeine, tea, fluorescence*

INTRODUCTION

Caffeine is an alkaloid, present in many natural products and known to aggregate with polyphenols.[1−3] This association also can alter the mouthfeel of beverages. Moreover, caffeine has been used as an ingredient in beverages for many years. Consumption of caffeine is regarded to be harmless for adults (<100 mg/day), but it is a matter of concern to young children and pregnant women as several health-related disorders are attributed to it.[4−7] Therefore, caffeine content is essential information for quality (taste and health benefit of the product) control of the product. Chromatographic techniques are well established for the quantitation of such an analyte in different natural sources or marketed products. Demand for easy and shorter analysis time has initiated research on alternative spectroscopic methods for the quantitation of such molecules. Recent progress in the field of designing new fluorescent chemosensors for different analytes has gained intense popularity due to their high selectivity, sensitivity, and shorter analysis time.[8] Despite this, the availability of a completely aqueous-based highly sensitive fluorescent chemosensor for caffeine detection is a gap in the recent advancement.

In general, dye molecules play an important role in understanding the mechanism of molecular complexation of many supramolecular aggregates. This phenomenon has been widely used to explore new chemosensors or to probe chemical properties at interfaces, especially in biochemistry and molecular biology.[8−14] Similarly, in the present context, few laboratories have used the same strategy to detect caffeine.[15−17] Siering et al. has synthesized triphenylene ketal derivatives with three urea groups, which bind with caffeine through noncovalent interactions.[16] Authors have exploited this phenomenon to sense caffeine in organic solvent. In continuation of this work, the same group has also established that use of a competitive guest molecule with the triphenylene ketal derivative increased the sensitivity (0.25 mM caffeine) of the sensor system.[17] Use of complicated synthesis steps and organic solvent limits the easy handling of such techniques in practical applications.[8,9] Therefore, despite these contributions, a completely aqueous-based fluorescent chemosensor of caffeine for practical application is still a major gap to fill.

In the present contribution we have used the noncovalent interaction between caffeine and Acridine Orange (AO, Figure 1) for the fluorometric determination of caffeine in water

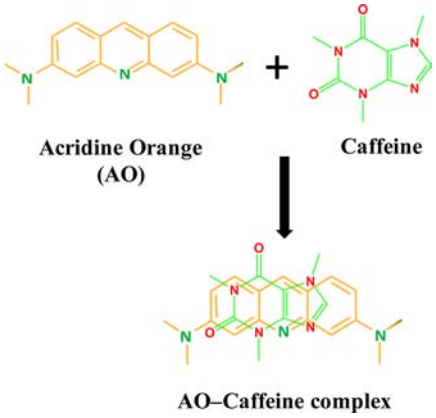

Figure 1. Molecular structures of Acridine Orange (AO), caffeine, and AO −caffeine complex.

without using any complicated instrumentation or organic solvent. Cationic AO (pH <10) displays monomer−dimer equilibrium; AO monomer is fluorescent, but AO dimer is not.[18] Cationic AO monomer is also known to form aggregates with other aromatic compounds, which alter the AO monomer−dimer equilibrium and hence the fluorescence pattern of AO.[19] On the other hand, caffeine is also known

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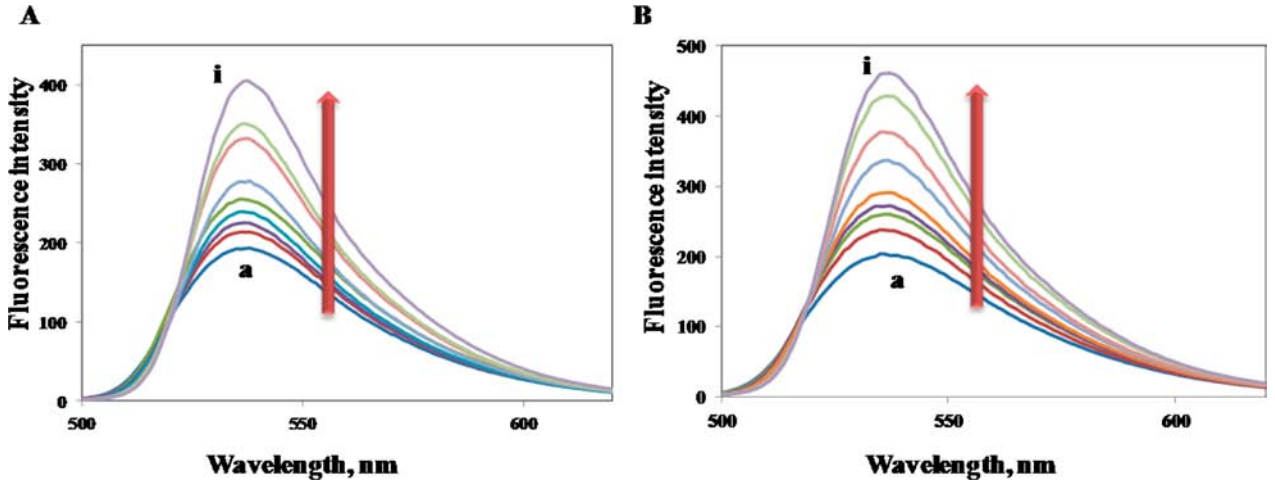

Figure 2. Fluorescence emission of AO ([AO] = 1×10^{-4} M) with an increase in caffeine concentration, at pH ~7 (A) and pH ~2 (B), where [caffeine] varied from 0 mM (a) to 5 mM (i).

to form π-complexes with aromatic compounds.[15−17] This set of interesting properties of AO and caffeine prompted us to study AO−caffeine interactions with the objective of developing a caffeine sensor that could be useful in practical application. In this study caffeine-induced enhancement of AO fluorescence intensity has been used for fluorometric determination of caffeine in an aqueous medium. Finally, the usefulness of this method has been demonstrated by measurement of the caffeine content in commercial tea samples and comparison with the value obtained with a standard HPLC method.

EXPERIMENTAL PROCEDURES

Materials. AO, caffeine, catechin, gallic acid, epigallocatechin-3-gallate (EGCG), and polyvinylpolypyrrolidone (PVP) were procured from Sigma-Aldrich Chemicals Co. HCl was purchased from Merck-India. Red Label black tea (sample S1), Lipton Yellow label black tea (sample S2), Taj Mahal black tea (sample S3), and Lipton clear green tea (sample S4) were obtained from the local market for the analysis of caffeine content in commercial tea products. Water was obtained from a Milli-Q purification system. All experiments were performed with freshly prepared solutions.

Methods. *Fluorescence.* All fluorescence studies were performed using a Shimadzu 5301 PC spectrophotometer at room temperature (25 °C). The excitation wavelength for AO was set at 491 nm, and emission spectra were measured from 500 to 650 nm. Slit widths for both excitation and emission were set at 5 nm. All fluorescence studies were done with an AO concentration of 1×10^{-4} M. All solutions were prepared in ultrapure water (pH 7.2, conductance = 2×10^{-6} S cm^{-1}, turbidity < 0.1 NTU). To ensure equilibrium, measurements were conducted after 24 h. All of the experiments were done in triplicates, and the average of those values is reported.

UV−Vis. The UV−vis absorbance measurements were performed using a Perkin-Elmer Lambda 35 UV−vis spectrophotometer. All studies were done with a dye (AO) concentration of 1×10^{-4} M. Absorbance values of the sample solutions were measured in the wavelength range of 200−800 nm. All solutions were prepared in ultrapure water and mixed well before measurement. Measurements were taken in absorbance mode. All of the experiments were done in triplicates, and the average of those values is reported.

Preparation of Tea Infusion. Commercial tea samples were taken for the detection of caffeine in the tea beverages. First, tea infusions were made. A commercial tea sample [2 g for black tea (S1, S2, S3) and 1 g for green tea (S4)] was placed in a beaker, then 100 mL of boiling water boiling water was added, and the mixture was stirred (once for black tea and five times for green tea) and kept for 1 min (including the stirring time). Finally, the mixture was filtered through Whatman no. 1 filter paper to get the tea infusion. Green tea and black tea infusions were made separately. These infusions were taken forward for HPLC and fluorescence study.

Separation of Caffeine from Tea Infusion by PVP. Stock solution of PVP was made by dissolving 2 g of PVP into 100 mL of water at room temperature (25 °C). Addition of PVP to the tea infusion led to the formation of colored (brown for black tea and yellow for green tea) insoluble aggregates and supernatant that became colorless. The color of the tea infusion is attributed to the presence of polyphenols.

First, the amount of PVP required for the precipitation of most of the polyphenols (from tea infusion) was optimized. PVP solution was added to the tea infusion (separately to the green and black tea infusion) in portions, and the pH of the infusion was maintained at ~2. Each time 50 μL of PVP stock solution (in 10 mL of infusion) was added to the supernatant, obtained after 1 h of mixing (in a shaker bath at 200 rpm) and centrifugation (for 10 min at 10000 rpm or relative centrifugal force of 7826*g*) at 25 °C. Addition of more PVP leads to the reprecipitation of the supernatant liquid. Moreover, after reprecipitation, the color of the supernatant became clearer. The optimized values of the PVP stock solution were 500 and 450 μL for 10 mL of black tea and green tea infusions, respectively. Incorporation of PVP at greater than this amount does not lead to any precipitation of the clear supernatant.

After that, the optimized amount of PVP solution was added to the tea infusion (at pH ~2), and the whole mixture was kept in a shaker bath (at 200 rpm and 25 °C) for 5 h. Then supernatant and colored residue were separated by centrifugation for 10 min at 10000 rpm (or relative centrifugal force of 7826*g*). This clear supernatant liquid was taken forward for the HPLC and fluorescence measurements.

HPLC. Catechins, caffeine, and gallic acid were analyzed according to the ISO 14502-2:2005(E) method using HPLC (Agilent HPLC 1100 series). HPLC conditions were as follows: injection volume, 20 μL; column, Phenomenex Luna Phenyl hexyl 5, 250 × 4.60 mm, fitted with a C18 security guard cartridge from Phenomenex; mobile phase A, 9% (volume fraction) acetonitrile, 2% (volume fraction) acetic acid with 20 μg/mL ethylenediaminetetraacetate (EDTA); mobile phase B, 80% (volume fraction) acetonitrile, 2% (volume fraction) acetic acid with 20 μg/mL EDTA; flow rate, 1 mL/min; detector, diode array detector; detection wavelength, 278 nm; oven temperature, 35 °C; analysis time, 55 min. For this analysis, caffeine was used as a standard as described in the ISO-catechin method.

RESULTS AND DISCUSSION

Commercially available water-soluble fluorophore AO (Figure 1) displays many interesting fluorescent properties. AO monomer exists in monocationic form (protonation of ring

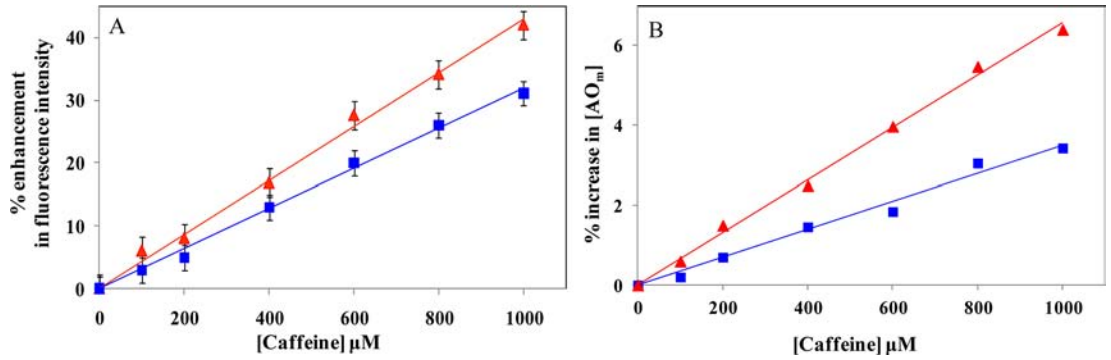

Figure 3. (A) Percent enhancement of fluorescence intensity and (B) percent increase in fraction of AO monomer with caffeine concentration at pH ~2 (red line) and pH ~7 (blue line).

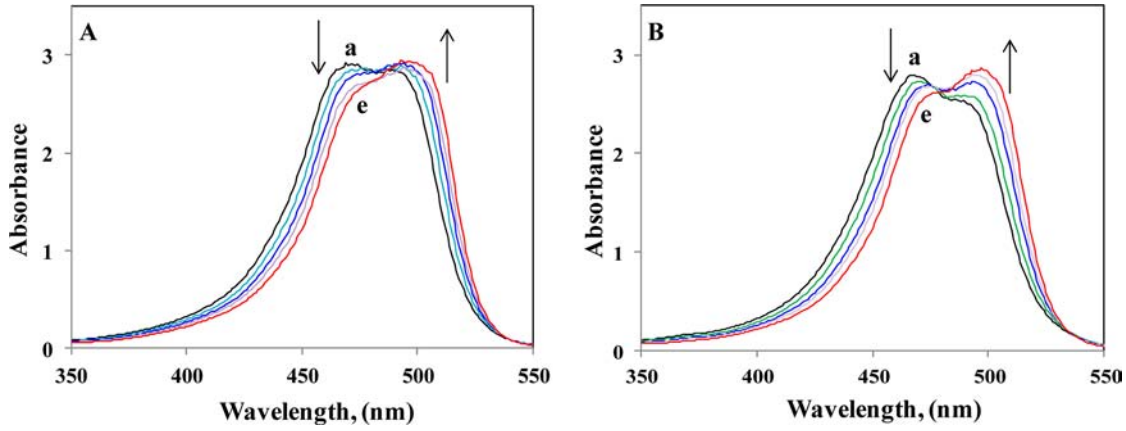

Figure 4. Absorbance spectra of AO with increase of caffeine concentration at pH ~7 (A) and pH ~2 (B) at 25 °C. Caffeine concentration varied from 0 mM (a) to 5 mM (e). The direction of the arrow indicates the decrease or increase in the respective peak.

nitrogen) at pH >2 (pK_a ~ 1.6), in tricationic (protonation of two more $-NMe_2$ groups) at pH <2, and in neutral form at pH >10 (pK_a ~ 9.5).[20] Cationic AO polymerizes into dimer form because of π-stacking between two AO monomers and displays monomer−dimer equilibrium.[21] The propensity of AO dimer formation increases with the increase in cationicity of the AO monomer.[22] As a result, the percentage of AO monomer and dimer changes with the variation in pH of the aqueous medium.[23] Moreover, it is well documented in the literature that cationic AO displays monomer−dimer equilibrium in the concentration range of 1×10^{-6}−1×10^{-4} M.[23] Considering these solution properties of AO, steady state fluorescence emission of AO (1×10^{-4} M) in water was performed in the presence and absence of caffeine at pH ~7 and ~2 (Figure 2).

The fluorescence intensity of AO increased with an increase of caffeine concentration at both pH ranges (Figure 3A). However, percentage enhancement of fluorescence intensity as a function of caffeine concentration reveals that the degree of enhancement in fluorescence intensity was higher at pH ~2 compared to pH ~7 (Figure 3A). This result indicates the possibilities of various noncovalent interactions between AO and caffeine. These have been validated by the absorbance spectra of AO in the presence and absence of caffeine. In a pure aqueous system, AO shows two absorption maxima (λ_{max}), one at ~490 nm for AO monomer and another at ~465 nm for AO dimer.[24] Addition of caffeine to AO leads to a red shift of λ_{max} for AO monomer by ~4 nm (at ~494 nm) and ~7 nm (at ~497 nm) at pH ~7 and ~2, respectively (see the Supporting Information, Figure S1). This shift in absorption maxima supports the possibility of interaction between Acridine Orange and caffeine,[25,26] but the intensity of this interaction could be different at various pH values. To gain mechanistic understanding, absorption spectra of AO in water were recorded (at 25 °C) at different concentrations of caffeine (0−5 mM), at pH ~7 and ~2 (Figure 4). The absorption spectrum clearly indicates a boost (at ~490 nm) in AO monomer concentration and a lowering (at ~465 nm) of AO dimer concentration with increasing amount of caffeine. This observation suggests that addition of caffeine to the AO (mixture of dimer and monomer) leads to an increase in the concentration of AO monomer, at the expense of AO dimer. This in turn suggests a shift in AO monomer−dimer equilibrium toward the direction of AO monomer. Percentage increase in the concentration of AO monomer (AO_M) with caffeine concentration was plotted (Figure 3B) by considering the fact that the amount of monomer or dimer present in the aqueous medium will be proportional to the absorbance value of respective species. The concentration of AO monomer increased with an increase of caffeine concentration at both pH values, but the rate of increment was higher at pH ~2 compared to pH ~7. The same study was also performed at different temperatures (6 and 50 °C), and a similar trend (increase in concentration of AO monomer with increase of caffeine concentration) was observed (see the Supporting Information, Figures S2 and S3).

$$[AO^{O}{}_{M}] \xrightarrow{\text{caffeine}} [AO^{CF}{}_{M}] \quad (1)$$

The binding constant for the AO monomer with caffeine is

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$$K_{AO-CF} = ([AO_M] - [AO^O{}_M])/[AO^O{}_M][\text{caffeine}] \quad (2)$$

where $[AO^O{}_M]$ is the concentration of free AO monomer, $[AO^{CF}{}_M]$ is the concentration of AO monomer complexed with caffeine, and $[AO_M]$ is the concentration of total AO monomer (free and complexed with caffeine).

Equation 2 can be rewritten as follows:

$$[AO_M] = ([AO^O{}_M]K_{AO-CF}) \times [\text{caffeine}] + [AO^O{}_M] \quad (3)$$

Therefore, the binding constant for the association of AO monomer with caffeine (K_{AO-CF}) can be calculated from the ratio of the slope/intercept of the graphical plot of $[AO_M]$ versus [caffeine] (see the Supporting Information, Figure S4). Again

$$\ln K_{AO-CF} = (-\Delta H/R) \times 1/T + (\Delta S/R) \quad (4)$$

$$\Delta G = -RT \ln K_{AO-CF} \quad (5)$$

$$\Delta G = \Delta H - T\Delta S \quad (6)$$

where R is the universal gas constant, T is the absolute temperature, ΔH is the change in enthalpy, ΔS is the change in entropy, and ΔG is the change in free energy.

Therefore, the value of ΔH can be calculated from the intercept of graphical plot of $\ln K_{AO-CF}$ versus $1/T$ (Supporting Information, Figure S5). Similarly, the values of ΔG and ΔS can be calculated from eqs 5 and 6.

We have calculated the AO−caffeine association constant (Table 1) to probe the extent of AO−caffeine association at different pH values and temperatures. At 25 °C, AO−caffeine association constant was found to be higher at low pH (~2, 48.19 M^{-1}) compared to pH ~7 (36.13 M^{-1}). Moreover, changes in thermodynamic parameters (free energy, enthalpy, and entropy) for the AO−caffeine association were calculated at different temperatures and pH values (Table 1). At a fixed temperature, the binding constant was found to be maximum at low pH range. Apart from the binding constant, changes in free energy (ΔG) and enthalpy (ΔH) for the AO−caffeine association were more negative at pH ~2 (compared to pH ~7), which indicates more favorable association at low pH range.

Table 1. Association Constant (K) and Change in Thermodynamic Parameters [Free Energy (ΔG), Enthalpy (ΔH), and Entropy (ΔS)] of Cationic AO Monomer with Caffeine at Different pH Values and Temperatures

		AO−caffeine	
	temp (K)	pH ~2	pH ~7
K (M^{-1})	279	64.45	50.00
	298	48.19	36.13
	323	23.21	19.96
ΔG ($kJmol^{-1}$)	279	−9.66	−9.07
	298	−9.60	−8.88
	323	−8.44	−8.03
ΔH ($kJ\ mol^{-1}$)		−17.52	−15.71
ΔS ($J\ mol^{-1}K^{-1}$)		−27.63	−23.50

UV−vis absorption study and binding constant values, described in the above text, suggest that binding of AO monomer with caffeine causes shifts in the AO monomer−dimer equilibrium toward the direction of AO monomer, and the magnitude of the shift is higher at pH ~2 (compared to pH ~7), because of the stronger AO−caffeine association at low pH range. On the basis of this collective information, it is possible to conclude that at pH ~2, addition of caffeine results in an increase in the concentration of AO monomer (fluorophore), which could be the possible reason for the higher percentage of enhancement in fluorescence intensity of AO at pH ~2 (compared to pH ~7, Figure 3A).

The caffeine-induced fluorescence enhancement feature of AO allowed us to detect caffeine at very low levels (100 μM, most sensitive fluorescence sensing of caffeine in water reported to date[15−17]). The caffeine-induced enhancement in fluorescence intensity of AO was higher at pH ~2. Hence, for better sensitivity, this system was taken forward (instead of pH ~7) for further study related to the utility of the sensor in practical applications. The percentage enhancement of fluorescence intensity varies linearly with caffeine concentration and is considered to be a calibration curve for caffeine estimation (Figure 3A). To the best of our knowledge, this is the first report for a pH-dependent caffeine-induced AO fluorescence enhancement, based on the change in AO dimer−monomer equilibrium. Moreover, incorporation of caffeine to the aqueous solution of AO also changes the solution color from orange to yellow. Here, enhancement of AO fluorescence intensity in the presence of caffeine was treated as a signal for caffeine sensing.

For the usefulness of the sensor system, evaluation of the utility of the sensor in practical applications is an important measurement. In this study we have evaluated the potential of the present system in terms of its ability to detect caffeine in commercial tea samples. Earlier studies on organic solvent based caffeine sensors were focused on caffeine measurement in coffee beverages,[15−17] whereas tea is one of the most popular beverages across the world, and consumption of tea is placed second after water.[27] This wide popularity of tea draws our attention toward the evaluation of the usefulness of the present sensor system for caffeine measurement in commercial tea samples.

The major class of chemical components present in tea is flavonoids (polyphenols).[27] For that reason, first we have evaluated the selectivity (or interference) of the present sensor system in the presence of tea polyphenols. On the basis of processing, tea is classified under two broad headings, green tea and black tea. Green tea mainly contains gallic acid and galloylated and nongalloylated catechin derivatives such as catechin (C), epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin-3-gallate (EGCG).[27] However, in addition to gallic acid and catechins, black tea also contains small amounts of theaflavins and thearubigins.[27] Thearubigins are high molecular weight polyphenols, and determination of their molecular structure is still under intense investigation.[28,29] Therefore, we have chosen gallic acid, catechin (nongalloylated derivative), and EGCG (galloylated derivative) as analytes for the selectivity test (see the Supporting Information, Figure S6). Analyte concentration was kept fixed at 20 mg/100 mL, because the average amount of these individual ingredients in tea beverages ranges from ~1 to 10 mg/100 mL (measure of an average size cup). Interference of the polyphenols toward the present sensor system is shown in Figure 5. Addition of catechin and gallic acid shows an insignificant effect (<1% enhancement in fluorescence intensity) on the fluorescence pattern of AO. However, EGCG enhances the fluorescence intensity by ~7.5% (compared to ~48% enhancement by caffeine). Galloylated derivatives of catechin (such as EGCG)

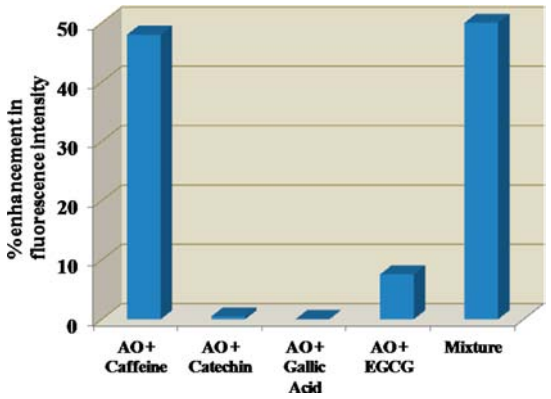

Figure 5. Analyte (caffeine, catechin, gallic acid, and EGCG) induced fluorescence enhancement of AO at pH ~2. Analyte concentration was fixed at 20 mg/100 mL.

are known to aggregate with aromatic compounds due to the formation of π-complexes driven by hydrophobic interactions.[1] This could be the reason for EGCG-induced small enhancement in the AO fluorescence intensity, whereas caffeine induces a significant increment (~48%) of AO fluorescence intensity (compared to any other analytes, gallic acid, catechin, and EGCG), suggesting the good selectivity of the present caffeine sensor system in the presence of tea polyphenols.

Finally, to validate the usefulness of the present research, we have determined the caffeine content of tea beverages. Commercially available tea samples [Red Label black tea (S1), Lipton Yellow label black tea (S2), Taj Mahal black tea (S3), and Lipton green tea (S4)] were evaluated. First, tea infusion was made by brewing the tea samples in hot water (~100 °C). Tea infusion is associated with the problem of creaming (extensive agglomeration between polyphenols) under prolonged storage at room temperature.[30] Creaming leads to an unstable colloidal system, which would be difficult to handle in fluorescence measurements. To avoid this problem, it is important to separate polyphenols and caffeine present in the tea infusion. In the earlier studies, the authors have done this by solvent extraction of caffeine from marketed beverages.[15−17] This is also applicable in the case of caffeine separation from tea infusion. However, solvent extraction and further purification of caffeine from solvent are time-consuming and complicated. To avoid the use of organic solvent, more specifically to make our system easier and shorter, we have separated polyphenols from the tea infusion by an aqueous route. We have used the water-soluble polymer PVP, which leads to precipitation upon selective adsorption of polyphenols from the tea infusion and caffeine remains soluble in the supernatant.[31] Results obtained from HPLC (see the Supporting Information, Figures S7−S14) indicate that the supernatant contains caffeine along with small amount of catechins, whereas tea infusion contains caffeine (which is similar to the supernatant) along with a large amount of catechins (Table 2). Finally, caffeine content in the supernatant liquid was measured by fluorescence (see the Supporting Information, Section 6) using the calibration curve (Figure 3A) and compared with the caffeine content of the corresponding tea infusion obtained from HPLC measurements (Figure 6).

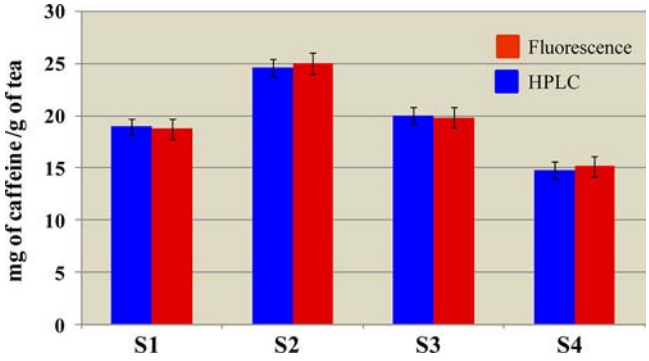

Figure 6. Caffeine content in green tea and black tea infusions as determined by HPLC (blue) and fluorescence spectroscopy (red) using AO at pH ~2.

The amount of caffeine measured by fluorescence technique (18.75 mg/g for S1, 25.03 mg/g for S2, 19.85 mg/g for S3, and 15.16 mg/g for S4) showed good agreement with the value obtained from standard HPLC measurement (18.94 mg/g for S1, 24.62 mg/g for S2, 20.02 mg/g for S3, and 14.77 mg/g for S4). Accuracy and precision of the proposed method were calculated by considering the fact that the value obtained from the HPLC method is the actual or true value (see the Supporting Information, Table S1). Precision was expressed by

Table 2. Amount of Different Catechins and Caffeine Present in the Different Tea Infusions and Their Supernatants[a]

	sample	gallic acid (mg/g)	theobromine (mg/g)	EGC (mg/g)	catechin (mg/g)	caffeine (mg/g)	EC (mg/g)	EGCG (mg/g)	ECG (mg/g)
S1	tea infusion	0.85	0.85	1.5	3.37	18.94	1.535	0.885	1.28
	supernatant after adsorption by PVP	0.73	0.77	0	0	17.98	0.68	0	0
S2	tea infusion	2.68	1.8	0.53	3.33	24.62	3.03	0.84	0.44
	supernatant after adsorption by PVP	1.74	1.35	0	0	24.05	0	0	0
S3	tea infusion	2.52	1.25	0.56	2.52	20.02	2.46	0.88	0.4
	supernatant after adsorption by PVP	1.72	0.93	0	1.36	19.82	1.68	0	0
S4	tea infusion	1.76	1.22	21.61	0.96	14.77	5.64	21.57	5.2
	supernatant after adsorption by PVP	1.6	1.14	16.71	0.72	14.47	4.74	1.73	0

[a]EGC, epigallocatechin; EC, epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate.

standard deviation (SD) and relative standard deviation (RSD, %), whereas accuracy was expressed by error (%). The relative standard deviation of the proposed method was < ∼4%, and the error (%) was in the range of −1.00 to 2.64%. A possible source of error in this proposed method could be the interference of different classes of chemical components present in tea (other than caffeine). The major class of chemical constituent in tea is polyphenols. In the context of interference by tea polyphenols, we have already discussed that only higher amounts (20 mg/100 mL) of EGCG interfere with the detection of caffeine to a small extent, whereas the results shown in Table 2 indicate that even after separation of polyphenols from caffeine (by PVP), a very small amount of EGCG was present in the supernatant liquid (0.00 mg/g in S1, 0.00 mg/g in S2, 0.00 mg/g in S3, and 1.73 mg/g in S4). This could be one possible source of error in the proposed method. Other than polyphenols, tea also contains three methylxanthines (caffeine, theobromine, and theophylline). Generally, in dry tea leaf caffeine content ranges from 20 to 60 mg/g, theobromine ranges from 0.6 to 1.7 mg/g, and theophylline exists in very low concentration (not detectable by HPLC method).[32,33] HPLC of the commercial tea samples (Table 2) showed that the concentration of caffeine was in the range of 15−25 mg/g and theobromine was in the range of 0.85−1.8 mg/g. Theophylline was not detected in these tea samples. Now, to investigate the possibility of interference by theobromine, we have recorded the fluorescence emission of AO in the presence and absence of theobromine at pH ∼7 and ∼2. The fluorescence intensity of AO was found to increase upon addition of theobromine (see the Supporting Information, Figures S15 and S16). The minimum concentration of theobromine required to increase the AO fluorescence intensity was 200 μM or 3.6 mg/100 mL (minimum limit of detection of theobromine), compared to 100 μM or 1.9 mg/100 mL for caffeine. However, the concentrations of caffeine and theobromine (with tea supernatant) were in the ranges of 5−17 and 0.2−0.6 mg/100 mL, respectively, in the solutions subjected to fluorescence measurement (with tea supernatant). This concentration of theobromine was significantly lower than its detection limit (200 μM or 3.6 mg/100 mL). Therefore, a higher amount of theobromine can affect caffeine detection by this fluorescence method, but a very low concentration of this compound in tea infusion ignores the possibility of interference by theobromine toward the caffeine detection in commercial tea samples. In addition to that, the results shown in Table 2 also indicate that very small amounts of caffeine have been lost (0.96, 0.57, 0.20, and 0.30 mg/g in S1, S2, S3, and S4, respectively) during the separation of polyphenols from caffeine by PVP, which could be another possible source of error in the proposed method. However, the contribution of these individual factors toward the overall error (−1.00 to 2.64%) of the proposed method is expected to be very low because the amount of EGCG in the tea supernatant (after separation), the concentration of theobromine, and the amount of caffeine lost (during the separation) were significantly low. The overall error of the proposed method (−1.00 to 2.64%) could be a combined effect of these factors.

In summary, we have shown that the association of caffeine and AO monomer results in a shift of the AO dimer−monomer equilibrium toward the direction of AO monomer (fluorophore). The binding constant between caffeine and AO is higher at low pH (∼2) compared to pH ∼7. The caffeine-induced shift in equilibrium (due to AO−caffeine association) toward the direction of fluorophore (AO monomer) leads to enhancement in fluorescence intensity, which was utilized as a signal for caffeine sensing. Finally, this strategy was capitalized to develop a novel aqueous-based fluorescent chemosensor for caffeine detection in water, which has been used to quantitate caffeine in commercial tea beverages and compared with the standared HPLC method. Therefore, designing novel fluorescent chemosensors for different classes of analytes (caffeine, polyphenols, etc.) could be a potential alternative to the traditional chromatographic techniques, which involve complicated instrumentation, longer analysis time, and also organic solvents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

UV−vis spectroscopy of AO in the absence and presence of caffeine at different pH values (∼2 and ∼7) and temperatures; calculation of binding constant for the association of caffeine with AO monomer at different pH values (∼2 and ∼7) and temperatures; HPLC of green tea and black tea infusion, before and after separation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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