

## Caffeine, Its Basicity, Acidity, Hydrolysis & Spectrophotometric Assay

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A specific spectrophotometric assay of caffeine utilizing the large drop in extinction at 273 nm from aqueous medium to 5M alkaline (1/2 hr) is described. The thermodynamic protonation constant has been reported ( $pK_b = 12.98 \pm 0.05$ ). There is no spectrophotometric evidence for ionization even in high (5M) alkaline media ( $pK_a > 16.0$  as against  $> 14.0$ , literature). Rate constants for alkaline hydrolysis of caffeine to caffeidine carboxylate ion (presumably) have been worked out and discussed.

WHILE assay of the stimulant caffeine mostly pertains to pharmaceutical analgesic and antipyretic products, it also comes up in the case of cola drinks in forensic context. The UV determination of caffeine preceded by necessary preliminary separation<sup>1-4</sup>, is now an established procedure.

During the analytical work on caffeine determination in spurious cola drinks, it was found that the data on acidity/basicity of caffeine was scanty or divergent, e.g.  $pK_a$  (neutral<sup>5,6</sup>,  $> 14.0^7$ ),  $pK_b$  ( $< 13.0^6$ ,  $14.15^7$ ,  $13.39^8$ -emf). It was therefore of interest to study the spectra and solution chemistry of caffeine in acid/alkaline media with a view to utilize this data for specific assay of caffeine. A differential spectrophotometric estimation in highly acidic solutions<sup>9,10</sup> has been reported, which worked well for soluble coffee samples though not for APC<sup>9</sup>.

The dispersion of spectra in highly acidic media and hydrolytic changes in alkaline media were therefore utilized to evaluate the  $pK_b$ , arrive at an estimate of the lower limit of  $pK_a$  and have some preliminary data on the hydrolysis kinetics. It was found that the large drop in absorption at 273 nm of caffeine in aqueous medium to that of its hydrolytic product in highly alkaline media could be utilized in its specific assay.

### Materials and Methods

**Protonation/hydrolysis**—Pure BDH caffeine (100  $\mu\text{g/ml}$  aq solution),  $\text{HClO}_4$  (BDH) and NaOH (AR) stock solution (made carbonate-free) diluted freshly to requirements were used to maintain the acidity or alkalinity and Merck  $\text{NaClO}_4$  to maintain ionic strength ( $\mu = 2.0$  for acidic solutions). Spectral measurements against appropriate blank at the given temperature were taken on Zeiss recording UV-VIS spectrophotometer,  $l = 1$  cm; pH measurements were dispensed with (*vide infra*). Caffeine (CfH) is reported to open out to caffeidine carboxylic acid (DH) in alkaline medium<sup>11,12</sup>; the measurements in strong alkaline solutions were made as a function of time. It was found that within  $\sim \frac{1}{2}$  hr the hydrolysis (and ring opening) is complete in 5M  $\text{OH}^-$ . The observation of a sharp

isobestic point ( $E_{\text{CfH}} = E_{\text{D-}}$ ), but not illustrated here, in the spectra of varying alkali strengths, spectra taken in the normal course after the preparation of the mixtures, could give an erroneous impression of the spectral dispersion as a result of ionization and possibly an erroneous estimate of  $pK_a$ . The hydrolysis is alkali-catalysed (*vide infra*) and the spectrum on acidification of the originally highly alkaline medium ( $\sim \frac{1}{2}$  hr) is remarkably different (Fig. 1) from the protonated caffeine spectrum confirming irreversible hydrolytic reaction.

**Preliminary separation for assay of caffeine:** (i) *Cola drink*—Sample (5 ml) with or without added caffeine + liquor ammonia<sup>13</sup> (5 ml) was extracted with  $\text{CHCl}_3$  ( $3 \times 10$  ml). The  $\text{CHCl}_3$  was evaporated and the concentrate taken up in water (10 ml). This solution was diluted with water and alkali (to make 5M  $\text{OH}^-$ ) to the same caffeine concentration.  $\Delta D$  was measured after  $\sim \frac{1}{2}$  hr at 273 nm in the range of 20-25  $\mu\text{g/ml}$ .

(ii) *Aspirin-caffeine*—Powdered tablet material (0.25 g), with or without added caffeine was warmed with 25 ml 0.1N  $\text{NaHCO}_3$ , filtered and the residue washed. The filtrate was extracted with  $\text{CHCl}_3$  ( $3 \times 30$  ml), the solvent evaporated and the residue

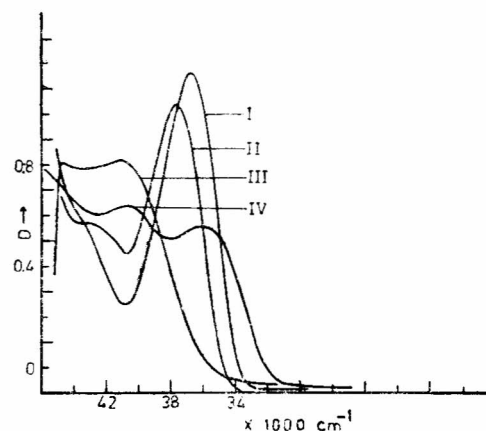


Fig. 1 — UV spectra of aq. caffeine (I), protonated caffeine (II), hydrolysed caffeine alkaline medium (III), hydrolysed caffeine reacidified (IV)

taken up in water (100 ml). It was diluted as in (i) above for  $\Delta D$ .

(iii) *Aspirin-caffeine-quinine* — Powdered tablet material (0.25 g) with or without added caffeine, was warmed with 0.1N NaHCO<sub>3</sub> (25 ml), filtered and the residue washed. The filtrate was extracted with CHCl<sub>3</sub> (3 × 30 ml), the extract washed with dil. HCl (~10<sup>-3</sup>N, 3 × 30 ml). The residue from the CHCl<sub>3</sub> extract was taken up in water (100 ml) and diluted as in (i) for  $\Delta D$ .

(iv) *Aspirin-phenacetin-caffeine* — Powdered tablet material (0.25 g) with or without added caffeine was treated as per IP procedure<sup>14</sup>. The residue was taken in water (100 ml) and diluted as in (i) for  $\Delta D$ .

**Results and Discussion**

*Thermodynamic ionization/protonation constants* — The ionization constant of caffeine (CffH) could be put as

$$K_a = \frac{C_- \cdot f_- \cdot a_{H^+}}{C_0} = \frac{C_-}{C_0} \cdot \frac{K_w}{[OH^-]} \cdot a_{H_2O} \quad \dots(1)$$

where subscripts - and 0 refer to ionized and unionized caffeine respectively, assuming similar activity coefficients of (OH)<sup>-</sup> and (Cff)<sup>-</sup> and neglecting that of uncharged (CffH). The limitations of these assumptions notwithstanding, the pH measurement in these highly alkaline solutions (large Na<sup>+</sup> error) and similar limitations in the estimation of activity coefficient of Cff<sup>-</sup> would leave one with no better alternative than dispensing with the pH measurements and arriving at estimate of such weak ionization constants by suitable procedures under the circumstances<sup>15</sup>.

Even up to 5.0M OH<sup>-</sup>, the zero time readings at  $\lambda_{max}$  and  $\lambda_{min}$  of caffeine extrapolate to the original values as those in aqueous medium (Fig. 2). There is therefore no spectrophotometric evidence of ionization of caffeine in solution even up to 5.0M OH<sup>-</sup>, before hydrolysis had set in. Having a conservative estimate of C<sub>-</sub>/C<sub>0</sub> < 0.1 in 5M OH<sup>-</sup> (presuming that even 10% ionization could not be monitored under the conditions) an estimate of the lower limit of pK<sub>a</sub> can be arrived at

$$pK_a = pK_w + \log [OH^-] - \log a_{H_2O} + \left( \log \frac{C_0}{C_-} \right) \\ = 14.96 + (>1.0), \text{ or } >16.0 \text{ at } 27^\circ$$

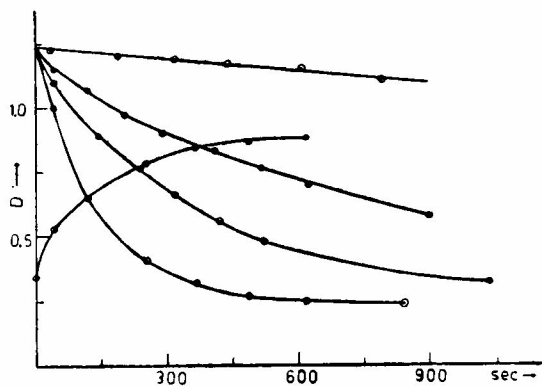


Fig. 2 — Variation of absorption at  $\lambda_{max}$  (0.5, 2.0, 3.0, 5.0M OH<sup>-</sup> respectively) and  $\lambda_{min}$  (5.0M) with time

which may be compared with the value (> 14.0) cited in the literature<sup>7</sup> (details not available). K<sub>w</sub> at the given ionic strength and temperature was estimated from literature data<sup>16</sup> on  $\gamma_{H^+} \cdot \gamma_{OH^-} / a_{H_2O}$  and  $m_{H^+} \cdot m_{OH^-}$ . Estimate of a<sub>H<sub>2</sub>O</sub> was made from literature data on 1:1 electrolyte systems<sup>17</sup> which is in reasonable agreement with the mass-fraction of water in these electrolyte solutions (non-electrolyte solution excluded).

The protonation constant of caffeine to (CffH<sub>2</sub>)<sup>+</sup> similarly can be put as

$$K_b = \frac{C_+ \cdot f_+ \cdot a_{OH^-}}{C_0} = \frac{C_+}{C_0} \cdot \frac{K_w}{[H^+]} \quad \dots(2)$$

where subscripts + and 0 refer to protonated and neutral caffeine respectively, assuming similar activity coefficient for [H<sup>+</sup>] and [CffH<sub>2</sub>]<sup>+</sup> and neglecting that of neutral (CffH)<sup>0</sup>. In view of the limitations of estimating activity coefficients, a similar procedure, dispensing with the pH measurements and using K<sub>w</sub> data was resorted to as described above. The estimate of C<sub>+</sub>/C<sub>0</sub> was made thus: C<sub>+</sub>/C<sub>0</sub> = (D<sub>0</sub> - D)/(D - D<sub>+</sub>).

The data and results of pK<sub>b</sub> are given in Table 1.

The spectra of neutral and protonated caffeine may be represented as due to the species listed in Chart 1 in preference to that postulated<sup>18</sup> earlier.

The results of assay and recovery of added caffeine by the proposed specific method ( $\Delta D$  at 273 nm from aq. to 5M OH<sup>-</sup>, ~½ hr) are given in Table 2.

TABLE 1 — pK<sub>b</sub> OF CAFFEINE

(μ = 2.0, K<sub>w</sub> = 1.20 × 10<sup>-14</sup>)

nm/D <sub>0</sub> /D <sub>+</sub>	[H] <sup>+</sup> (M)	D	pK <sub>b</sub> (27°)
245.5/0.34/0.55	0.10	0.45	12.88
	0.25	0.48 <sub>2</sub>	13.00
273.2/1.26/0.98	0.10	1.14	13.05
	0.25	1.07	13.00
			Av. 12.98 ± 0.05

TABLE 2 — ASSAY AND RECOVERY OF ADDED CAFFEINE

Sample	Amount of caffeine added	Recovery (%)
Aq. caffeine	100 μg/ml	98.1
Cola drink	100 μg/ml	97.0
Aspirin-caffeine	40 mg/g*	100.0
Aspirin-caffeine-quinine sulphate	32 mg/g*	94.0
Aspirin-phenacetin-caffeine	8 mg/g*	95.0

\*Of the tablet weight.

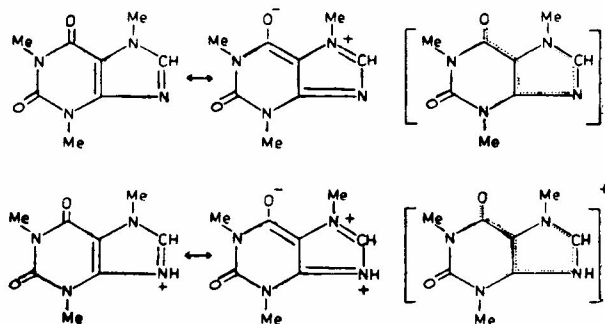
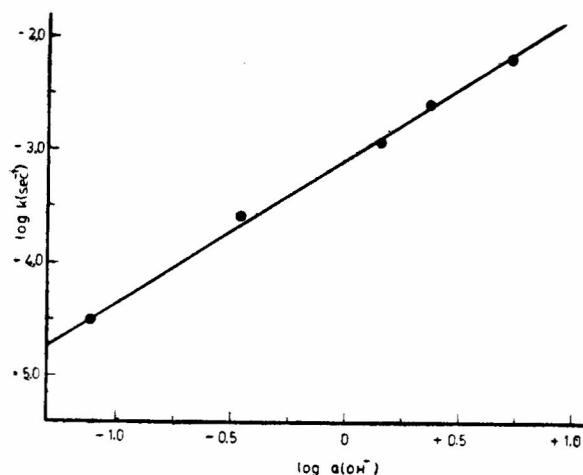
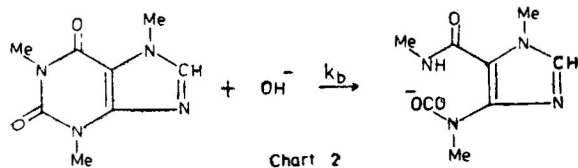


Chart 1

TABLE 3 — HYDROLYTIC RATE CONSTANT AND UNIMOLECULAR RATE CONSTANT OF THE REACTION IN CHART 2

(Temp. = 27°)		
[OH <sup>-</sup> ] (M)	K <sub>b</sub> × 10 <sup>-3</sup> (Eq. 3) (litre mole <sup>-1</sup> sec <sup>-1</sup> )	k <sub>u</sub> × 10 <sup>4</sup> (sec <sup>-1</sup> )
5.0	1.33 ± 0.03	7.06 ± 0.15
3.0	1.22 ± 0.05	2.84 ± 0.12
2.0	0.91 <sub>5</sub> ± 0.04	1.28 ± 0.06
0.5	0.77 ± 0.07 <sub>5</sub>	0.26 ± 0.02 <sub>5</sub>
0.1	0.42 ± 0.05	0.032 ± 0.004


 Fig. 3 — Plot of log  $k_u$  (sec<sup>-1</sup>) versus log  $a_{OH^-}$ 

**Rate constants (hydrolytic reaction)**— Presuming the conversion to caffeidine carboxylic acid in the hydrolytic reaction (Chart 2), the bimolecular rate constant of the reaction could be put as

$$K_b = \frac{2.303}{t \cdot a_{OH^-}} \cdot \log \frac{C}{C-x} = k_u / a_{OH^-} \quad \dots (3)$$

where  $k_u$  is the unimolecular rate constant calculated at different concentrations of OH<sup>-</sup>. Log  $C/(C-x)$  at a given time  $t$  is evaluated as  $\log (D_0 - D_\infty) / (D_t - D_\infty)$  from the spectrophotometric data at 273 nm. The results are given in Table 3.  $k_u$ , as expected with an OH<sup>-</sup>-catalysed hydrolysis, rapidly falls at lower [OH<sup>-</sup>]. The bimolecular rate constant which should in the ideal case be independent

of [OH<sup>-</sup>], gradually decreases at lower [OH<sup>-</sup>]. The ionic strength is not held constant. It would have been necessary to maintain high ionic strength (such as, 5) when estimate of activity coefficients poses a problem. The usual Bronstead-Bjerrum treatment (for example, Katiyar and Sinha<sup>19</sup>) at high ionic strength is not valid and the kinetic activity factor cannot be estimated. Under the circumstances, OH<sup>-</sup> activities and not concentrations have been used in calculating  $K$  (Eq. 3). Activities were taken from literature data<sup>20</sup> on mean activity coefficients of NaOH solutions of various molalities and the same have been utilized.

From Eq. (3),  $\log k_u = \log k_b + \log a_{OH^-}$  showing that for a bimolecular reaction not only  $\log k_u$  vs  $\log a_{OH^-}$  should be linear, but it must have a unit slope too. A plot of  $\log k_u$  vs  $\log a_{OH^-}$  was linear (Fig 3). The value of  $k_b$  read from the graph is  $0.89 \times 10^{-3}$  litre mole<sup>-1</sup> sec<sup>-1</sup> (corresponding to  $\log a_{OH^-} = 0$ ). The slope actually observed is 1.3, showing that the reaction mechanism may not be as simple as assumed above. It may also be stated that  $k_b$  was found to vary even on maintaining constant ionic strength.

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