

Kinetics and Equilibria of Tea Infusion: Part 8—The Effects of Salts and of pH on the Rate of Extraction of Theaflavins from Black Tea Leaf

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ABSTRACT

The rates of extraction of theaflavins from sieved Kapchorua Pekoe Fannings (600–710 μm) and from a Ceylon Broken Orange Pekoe Fine Leaf Blend have been measured at 80°C with a set of aqueous salt and buffer solutions spanning the pH range 3.1 to 8.1. There was little effect on adding 1:1 electrolytes but considerable decreases in rate occurred in the presence of calcium chloride. The rate constants in acid buffers were the same as in a normal unbuffered infusion but in alkaline buffers of pH 8 they were over 50% larger. This phenomenon was quantitatively accounted for by the partial dissociation of theaflavins at higher pH and the greater diffusion coefficient of the resulting ionic salt. The relevance of these findings to tea brewing and to industrial theaflavins extraction is discussed.

INTRODUCTION

We reported in the preceding paper (Spiro & Price, 1987*b*) how the rate of extraction of caffeine from a black Kenyan CTC tea, Kapchorua Pekoe Fannings, was affected by the addition of various salts to the water. The rate was not, however, dependent on pH. We now present corresponding data for the extraction of all the theaflavins (ungallated, monogallated and digallated, together abbreviated as TF). The same sieved 600–710 μm Kapchorua leaf was used (experiments by W.E.P.) as well as Ceylon Broken

Orange Pekoe fine leaf blend (experiments by W.M.M.). All runs were again performed at 80°C.

EXPERIMENTAL

The kinetic experiments were carried out as described in the preceding paper (Spiro & Price, 1987*b*), with 200 cm³ solution at 80°C containing either 4 g Kapchorua PF leaf or 4.66 g Ceylon BOP leaf. The compositions of the buffers have been specified previously (Spiro & Price, 1987*a*). The pHs of samples taken from buffer infusions were adjusted to 4.8 before analysis, due allowance being made for the small additions of alkali or acid by correcting the results with appropriate dilution factors. Samples from the Kapchorua PF infusions were analysed for theaflavins by the Flavognost method (Spiro & Price, 1986) and those from the Ceylon BOP infusions by the method of Roberts & Smith (1963) as modified by Spiro & Siddique (1981). From two to four independent kinetic runs were carried out with each system.

The dissociation constant of theaflavins was determined (by M.A.) by first infusing 8 g Kapchorua PF leaf in 400 cm³ water at 80°C for 30 min. The liquor was filtered through glass wool and allowed to cool to room temperature (24 ± 1°C). Successive 10 cm³ samples were then diluted to 25 cm³ with various combinations of 0.1 mol dm⁻³ solutions of KH₂PO₄, KOH (Bates, 1973) and H₂SO₄ to produce phosphate buffer solutions with pH ranging from 4.0 to 9.2. Aliquots of 2 cm³ of each buffered tea liquor were analysed for TF by the Flavognost method.

TABLE 1

Kinetic Data for Theaflavins Infusion from Kapchorua PF into Different Aqueous Media of Ionic Strength 0.11 mol dm⁻³ at 80°C

<i>Solution</i>	<i>pH</i> ^a	<i>k</i> _{obs} (min ⁻¹)	<i>Intercept</i>	<i>t</i> _{1/2} (s)
Water	4.8	0.97	0.03	41
NaCl		0.98	0.04	40
KCl		1.13	0.06	34
CaCl ₂		0.75	0.07	50
CaCl ₂ ^b		0.60	0.07	63
NaO ₃ SPh		1.11	0.01	37
Bu ₄ NCl		1.07	0.06	35
Citrate buffer	3.0	0.97	0.09	37
Acetate buffer	4.7	1.03	0.06	37
Phosphate buffer	6.8	1.32	0.11	26
CHES ^c buffer	8.0	1.53	-0.14	33

^a Final pH of equilibrium tea infusion.

^b 0.11 mol dm⁻³ CaCl₂ solution of ionic strength 0.33 mol dm⁻³.

^c 2-(cyclohexylamino)ethanesulphonic acid.

TABLE 2

Kinetic Data for Theaflavins Infusion from Ceylon BOP Fine Leaf Blend into Three Aqueous Solutions at 80.4°C

<i>Solution</i>	<i>pH</i>	<i>k_{obs}</i> (<i>min</i> ⁻¹)	<i>Intercept</i>	<i>t</i> _{1/2} (<i>s</i>)
Phthalate buffer	3.1	0.34	0.32	65
Water	4.8	0.31	0.40	57
Bicarbonate buffer	8.1	0.54	0.22	53

RESULTS

In the kinetic runs the concentration of TF, c , at first rose rapidly with time, t , and then levelled off to a plateau value, c_∞ . The results were fitted by a least-squares programme to a first-order rate equation with an intercept:

$$\ln\left(\frac{c_\infty}{c_\infty - c}\right) = k_{\text{obs}}t + a \quad (1)$$

Values of $t_{1/2}$, the half-time of infusion, were calculated for $c = \frac{1}{2}c_\infty$. The uncertainties in these parameters for Kapchorua leaf, calculated as standard deviations of their means, are $\pm 0.06 \text{ min}^{-1}$ for k_{obs} , ± 0.04 for a and $\pm 3 \text{ s}$ for $t_{1/2}$. The results are summarised in Tables 1 and 2.

DISCUSSION

Examination of the results

From Table 1 it can be seen that the addition of 1:1 salts slightly increases the rate constant of TF extraction. Unlike the situation with caffeine (Spiro & Price, 1987*b*), there appears to be no difference between the effects of 'normal' salts like NaCl and KCl and those containing a large ion (PhSO₃Na and Bu₄NCl). On the other hand, CaCl₂ exerts a sizeable depressing effect on the rate. When the CaCl₂ concentration is increased threefold, from 0.037 to 0.11 mol dm⁻³, the decrease in k_{obs} is still greater but not by as much as a factor of three. The rate constant thus changes progressively less with further increments of the salt. This means that a small addition of calcium ions to water will exert a disproportionately large effect on the rate and so tea brewing will take slightly longer with hard water: the calcium concentration of good quality drinking water may be up to 3.5 mmol dm⁻³ and can be much higher in certain mineral waters (Rodier, 1975). With caffeine, too,

CaCl_2 produced an unusually large decrease in rate. A possible explanation, kindly suggested to us by Dr B. L. Wedzicha, is that calcium ions could complex with, and so stiffen, the products of hydrolysed pectin contained in the cell walls of the leaf (Selvendran & Perera, 1971). This is analogous to the formation of gel when Ca^{2+} ions act on alginates (Coulter, 1984). Such a structural change would increase the viscosity of the system and thereby lower the diffusion coefficients of solubles like caffeine and theaflavins.

The kinetic behaviour in two of the buffer mixtures in Table 1, citrate and acetate, resembles that in the salt solutions. This contrasts with our finding that the *extents* of extraction in these buffers are greater than in water (Spiro & Price, 1987a). In phosphate buffer, however, the rate constant is significantly higher and it is higher still in CHES buffer. A similar rise of k_{obs} with increasing pH in the alkaline region is seen in Table 2 where both a different tea leaf and a different method of analysis were employed. No pH variation was encountered with caffeine. Theaflavin differs from caffeine in being a weak acid which dissociates in alkaline media, and we shall show below that it is this property of TF that explains its extraction behaviour in solutions of higher pH.

Although the discussion so far has centred on the rate constants, the data in Table 1 demonstrate that for the CTC Kapchorua leaf the $t_{1/2}$ values exhibit trends complementary to those of k_{obs} . For most solutions $t_{1/2}$ lies in the range 38 ± 3 s. Much longer half-times result on the addition of CaCl_2 and shorter ones in the alkaline buffers. This is a consequence of the rather similar intercept values for all the runs except that in the CHES buffer. With Ceylon BOP leaf the intercepts are much larger and the rate constants much smaller, as would be expected for leaf subject to the less severe orthodox manufacturing method (Price & Spiro, 1985).

Dissociation Constant of TF

Samples of a large batch of filtered Kapchorua tea liquor were buffered to different pH values by addition of KH_2PO_4 , KOH and H_2SO_4 . Then a 2 cm^3 aliquot of each solution was analysed by extracting the TF with 4 cm^3 isobutylmethylketone (IBMK). The organic layer was mixed with excess of ethanolic Flavognost and the absorbance of the resulting green complex measured at 625 nm (Spiro & Price, 1986). These absorbances are plotted in Fig. 1 as a function of the pH. The absorbance is seen to decline from about pH 4.7 onwards, at first gently and then more steeply, falling to almost zero just beyond pH 9. McDowell *et al.* (1985) have independently discovered and already published the same effect. They measured the TF concentrations by the Flavognost method for three different commercial African teas at seven pH values ranging from 4 to 9.5. Their plots of TF concentration against pH

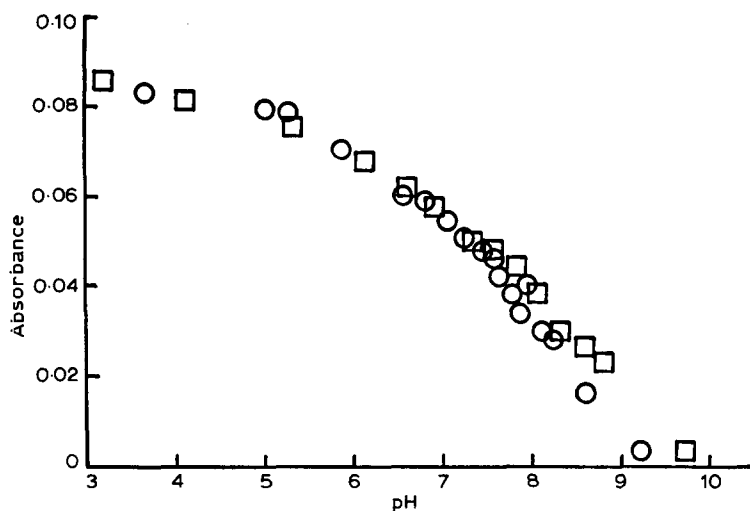


Fig. 1. Absorbances of the TF-Flavonost complex at 625 nm as a function of the pH of the sample. The different symbols refer to experiments carried out with two separate *Kapchorua* PF infusions.

exhibit shapes very similar to that in Fig. 1, their minimal values being reached at pH 8.5. We shall now essay a quantitative analysis of these curves.

The absorbances fall virtually to zero in quite alkaline solutions where one would expect TF to be largely dissociated. Thus the anion is either not extracted into IBMK or it does not complex with Flavonost; spectroscopic experiments indicate that the former is the case. The absorbances in Fig. 1 are therefore proportional to the concentrations of *undissociated* theaflavins in the aqueous buffer mixtures.

The system may now be represented by Fig. 2 in which HA stands for the undissociated theaflavins. Suppose that the volume of IBMK used in each analysis is n times the volume, V , of the aqueous aliquot (in the present work $n = 2$, in that of McDowell *et al.* (1985) $n = 1$). The total number of moles of theaflavins in the system, N , is given by:

$$N/V = n[\text{HA}]_{\text{IBMK}} + [\text{HA}] + [\text{A}^-] \quad (2)$$

where the square brackets denote concentration and lack of subscript refers to the aqueous phase. If the partition constant of HA between water and IBMK is K_p , and the concentration dissociation constant of HA in the aqueous solution is K_{HA} , eqn (2) becomes:

$$\begin{aligned} N/V &= nK_p[\text{HA}] + [\text{HA}] + K_{\text{HA}}[\text{HA}]/[\text{H}^+] \\ &= [\text{HA}](nK_p + 1 + K_{\text{HA}}/[\text{H}^+]) \end{aligned} \quad (3)$$

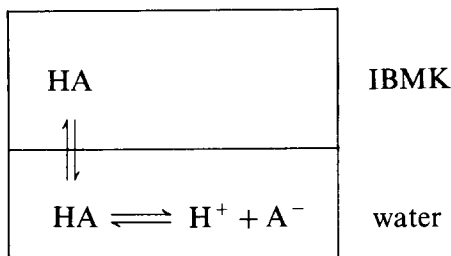


Fig. 2. Schematic representation of the dissociation of theaflavins (HA) in aqueous solution and their partition into isobutyl methyl ketone.

The absorbance produced with Flavognost depends upon the HA concentration in the IBMK layer which is given by:

$$[\text{HA}]_{\text{IBMK}} = K_p[\text{HA}] = K_p N/V(nK_p + 1 + K_{\text{HA}}/[\text{H}^+]) \quad (4)$$

The maximum absorbance occurs in sufficiently acid solutions:

$$[\text{HA}]_{\text{IBMK}}^{\text{max}} = K_p[\text{HA}]_{\text{max}} = K_p N/V(nK_p + 1) \quad (5)$$

At any given pH the absorbance will thus be a fraction β of the maximum absorbance observed at low pH, where:

$$\beta = \frac{[\text{HA}]_{\text{IBMK}}}{[\text{HA}]_{\text{IBMK}}^{\text{max}}} = \frac{nK_p + 1}{nK_p + 1 + K_{\text{HA}}/[\text{H}^+]} \quad (6)$$

It follows that:

$$K_{\text{HA}} = \left(\frac{1 - \beta}{\beta}\right)(nK_p + 1)\left(\frac{\{\text{H}^+\}}{\gamma_{\text{H}^+}}\right) \quad (7)$$

where the curly brackets denote activity (as calculated from pH) and γ_{H^+} is the activity coefficient of the hydrogen ions.

Equation (7) allows us to calculate K_{HA} from the plot in Fig. 1. However, an analysis along these lines soon shows that this curve is much too widely spread out to be fitted by any single value of K_{HA} . If γ_{H^+} is taken as 0.80 and K_p as 4.15 (Spiro & Price, 1986), the points at pH 6, 7 and 8 lead to values of $10^7 K_{\text{HA}}/\text{mol dm}^{-3}$ of 22, 6.0 and 1.6, respectively. At the same pHs, the three plots of McDowell *et al.* (1985) for different teas yield mean values of $10^7 K_{\text{HA}}/\text{mol dm}^{-3}$ of 12, 3.6 and 2.1. This apparent pH dependence of K_{HA} found by both groups must be attributed to the fact that all these tea infusions contain four different theaflavin species: one ungalated, two monogalated and one digalated. The proportions of these in black tea extracts were found by Collier & Mallows (1971) to be 11%, 46% and 43%, respectively. P. D. Collier (private communication) has spectrophotometrically determined the K_{HA} value of ungalated theaflavin as $1 \times 10^{-8} \text{ mol dm}^{-3}$. No values appear

to be available for the gallated forms, but substitution by gallate should render the theaflavin increasingly acid. This accounts for the monotone increase in the calculated K_{HA} value as the pH falls. Although we cannot assign specific K_{HA} values to the various theaflavin species without knowing their proportions in the tea infusions in question, eqn (7) does enable us to calculate the *effective* K_{HA} value for TF at any selected pH. This is the parameter that is required in the next section.

Interpretation of the results at high pH

As the pH rises, the weakly acidic theaflavins progressively dissociate. Both undissociated TF molecules and their anions then diffuse through the leaf. The diffusion coefficients, D , of a variety of smaller acid molecules, HA, have been measured in the literature while the limiting trace diffusion coefficients of their anions can be calculated from the Nernst equation (Robinson & Stokes, 1959):

$$D_{A^-} = (RT/F^2)\lambda_{A^-}^0 \quad (8)$$

where R is the gas constant, F the Faraday constant, T the absolute temperature and $\lambda_{A^-}^0$ the limiting ionic conductance of the anion A^- . The resulting ratio D_{HA}/D_{A^-} for the monocarboxylic acids from formic to hexanoic ranges from 0.92 to 1.11 (Dunn & Stokes, 1965; Albery *et al.*, 1967), for the dicarboxylic acids from butane-1,4-dioic acid to heptane-1,7-dioic acid it spans 0.89 to 1.00 (Albery *et al.*, 1967; Spiro & Selvaratnam, 1970), for orthophosphoric acid it is 1.01 (Leaist, 1984; Selvaratnam & Spiro, 1965), and only the ratio for citric acid (0.81, Müller & Stokes, 1957) differs from unity by more than 11%. For the theaflavins one can therefore predict that:

$$D_{HA} = (1 \pm 0.11)D_{A^-} \quad (9)$$

However, the TF anions will not diffuse alone but in company with positively charged co-ions, M^+ . These are likely to be potassium ions, the most abundant mineral cation in tea (M. Spiro and A. J. Pierce, unpublished work). The limiting mutual diffusion coefficient for the electrolyte M^+A^- is given by the equation (Robinson & Stokes, 1959)

$$D_{M^+A^-} = \frac{2RT\lambda_{M^+}^0\lambda_{A^-}^0}{F^2(\lambda_{M^+}^0 + \lambda_{A^-}^0)} \quad (10)$$

Combination of eqns (8), (9) and (10) shows that:

$$\frac{D_{M^+A^-}}{D_{HA}} = \frac{(2 \pm 0.22)\lambda_{M^+}^0}{\lambda_{M^+}^0 + \lambda_{A^-}^0} \quad (11)$$

The M^+ (mainly K^+) cation will possess a much higher conductance than the big TF anion. Molecular models give effective radii for the anions of

theaflavin, theaflavin monogallate and theaflavin digallate of approximately 6.0, 6.8 and 7.6 Å, respectively. However, these anions are not perfectly spherical and there can be free rotation in many of the bonds attached to the rings in the molecules. We shall neglect solvation for these large species and take the effective radius of the TF anion as 6.8 ± 1 Å. The hydrodynamic or Stokes radius of the K^+ cation at 80°C is estimated as 1.4₄ Å from data in Robinson & Stokes (1959). The conductance of the K^+ ion will therefore be higher than that of a TF anion by a factor lying between 4.0 and 5.4. It follows that the ratio of the diffusion coefficients in eqn (11) will lie between 1.42 and 1.88. In infusion experiments with a buffer containing a sodium salt, the main cation in the leaf may become Na^+ whose Stokes radius at 80°C is *ca.* 1.9 Å. In such cases the diffusion coefficient ratio in eqn (11) will lie between 1.34 and 1.79.

In a medium in which a fraction α of the TF molecules is dissociated, the effective diffusion coefficient for theaflavins is:

$$D = (1 - \alpha)D_{HA} + \alpha D_{M^+A^-} \quad (12)$$

where α is given by the equilibrium equation:

$$K_{HA} = \left(\frac{\{H^+\}}{\gamma_{H^+}} \right) \left(\frac{\alpha}{1 - \alpha} \right) \quad (13)$$

The diffusion coefficient in eqn (12) is therefore higher than that in acid media by a factor:

$$D/D_{HA} = (1 - \alpha) + \alpha D_{M^+A^-}/D_{HA} \quad (14)$$

in which the diffusion coefficient ratio in the last term is given by eqn (11).

We now compare the experimental results in the more basic buffers with those predicted from eqn (14). To do so we need to assume that the value of K_{HA} of theaflavins in the leaf at 80°C is the same as that derived at the same pH from the room temperature plot in Fig. 1. This may seem a drastic assumption but dissociation constants of many weak acids do not vary greatly with temperature (Bates, 1973). The buffers and pH values of interest are listed in Table 3 together with the values of K_{HA} calculated from eqn (7), α from eqn (13), and the D/D_{HA} range from eqn (14) assuming K^+ to be the co-ion. The last column gives the enhancements in the observed rate constant from the data in Tables 1 and 2. It can be seen that the increases in k_{obs} are comfortably accounted for by the predicted increases in the TF diffusion coefficient. Even in the case of the sodium bicarbonate buffer, where Na^+ could become the main co-ion and lower the D/D_{HA} range to 1.32–1.74, the theory explains the increase in the experimental rate constant.

The success in applying the diffusion equation means that we can make further predictions with some confidence. The results in Fig. 1 and the

TABLE 3

Comparisons Between Predicted and Experimental Rate Enhancements in the Three Most Basic Buffers

Buffer	pH	$10^7 K_{HA}$ (mol/dm ³)	α	D/D_{HA} Range	$\frac{k_{obs}(buffer)}{k_{obs}(normal)}$
Phosphate	6.8	8.0	0.80	1.34–1.70	1.3 ₆
CHES	8.0	1.6	0.93	1.39–1.82	1.5 ₈
Bicarbonate ^a	8.1	1.36	0.93	1.39–1.82	1.7 ₄

^a With a different black tea whose effective K_{HA} may differ.

diffusion theory show that the rate constant of TF extraction will begin to rise at about pH 5 and will reach a plateau value around pH 9. Here k_{obs} is at least 42%, and possibly up to 88%, greater than in an unbuffered infusion. The greater extraction rate at high pH, or the greater extent of extraction at low pH (Spiro & Price, 1987a), could be made use of in any industrial process designed to extract theaflavins selectively from black tea leaf. The pH factor is also relevant to the brewing of tea in homes and restaurants. As long ago as 1861, Mrs Beeton wrote, 'Economists say that a few grains of carbonate of soda, added before the boiling water is poured on the tea, assist to draw out its goodness'. Indeed, a far darker infusion does then result. Its appearance of greater strength is partly illusory inasmuch as it arises from the spectral changes produced by the dissociation of TF and possibly of SII thearubigin, and partly real, for the experiments reported in this paper have shown that TF does infuse more rapidly at higher pH. Although, for these reasons, some catering establishments may indulge in the practice of adding sodium bicarbonate to the teapot, they should also heed Mrs Beeton's warning: 'care must be taken to use this ingredient sparingly, as it is liable to give the tea a soapy taste'.

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