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Comparative study of the reversed-phase high-performance liquid chromatography of black tea liquors with special reference to the thearubigins

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ABSTRACT

New high-performance liquid chromatographic methods for the analysis of black tea phenolic pigments, using Hypersil ODS, Hypersil octyl wide-pore, and Hamilton PRP-1 columns, and several new results arising from them are presented. Very good resolution of a wide range of phenolic tea pigments, was obtained using a Hypersil ODS column with a citrate buffer, and, for the first time, eight theaflavins were observed in a single chromatogram. Unresolved brown phenolic pigments (thearubigins), ran as a convex broad band on the Hypersil wide-pore and Hamilton PRP-1 columns, and the Hamilton PRP-1 column showed an anthocyanidin to be a significant contributor to liquor colour. The pigments were classified into three groups by chromatographic behaviour: group I, pigments running close to the void volume of the columns; group II, resolved pigments; and group III, unresolved pigments. Group II pigments were divided into four Sub-Groups by their photodiode-array UV–VIS spectra, as follows: sub-group II.1, theaflavins; sub-group II.2, theaflavic acids; sub-group II.3, type I resolved pigments; and sub-group II.4, type II resolved pigments. Pigments in sub-groups II.3 and II.4 were designated as resolved thearubigins, and those in group III as unresolved thearubigins.

INTRODUCTION

The work in this paper continues our earlier studies of the high-performance liquid chromatography (HPLC) of black tea liquors, in which the main classes of polyphenolic compound were identified, using a photodiode-array detector and associated data system as a combined chromatographic–spectroscopic technique [1]. Although many polyphenolics were separated and identified in this way, the chromatography of the thearubigins has proved to be a more difficult problem.

Two main classes of pigment are formed in black tea manufacture, the theaflavins and the thearubigins [2–5]. The theaflavins are compounds of known structure, but the thearubigins are an inhomogeneous group of phenolic pigments of unknown structure [2–5]. Roberts [2] suggested that the thearubigins were acidic brown pig-

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ments, formed by the oxidative degradation of the theaflavins. However, Berkowitz *et al.* [6] thought that they derived from theaflavic acids, and Brown *et al.* [7] suggested that they were polymeric proanthocyanidins (the chromatograms described in this paper suggested that the thearubigins studied were a mixture of phenolic pigments with different chemical structures).

Robertson and Bendall [8] used a Hypersil ODS column with isocratic elution to analyse thearubigins produced in a model system, whereas Roberts *et al.* [9] used gradient elution, to study the degradation of thearubigins on storage, but only limited separation was achieved in either case. Opie *et al.* [10] obtained better resolved chromatograms of thearubigins formed in a model system, using a Hypersil ODS 3-μm column with a non-linear gradient. They claimed, without providing supporting evidence, that decaffeination was an essential sample pretreatment step, required for the successful chromatography of the thearubigins.

The chromatography of the thearubigins is likely to be difficult for two principal reasons, firstly, they are considered to be compounds with a wide range of molecular masses (700 to 40 000 daltons) [2,3], and, secondly, they adsorb strongly onto active surfaces. Generally, chromatographic conditions optimal for compounds of lower molecular mass are not optimal for compounds of higher molecular mass [11]; therefore, one set of conditions is unlikely to be suitable for the chromatography of the full range of thearubigins. In addition to the molecular-mass problem, secondary retention [12–15], due to the interaction of the thearubigins with hydroxyl groups or metals on the surface of silica-based reversed-phase packings, is a likely cause of the peak broadening and peak tailing observed on tea chromatograms.

Three different types of reversed-phase columns were used in this study, Hypersil ODS, Hypersil octyl wide-pore, and Hamilton PRP-1. Hypersil ODS, a narrow-pore, high surface area packing, was used for its proven ability to resolve lower molecular mass polyphenolics [1]. Hypersil octyl wide-pore phase was used with the aim of obtaining improved chromatograms of higher molecular mass thearubigins. Generally, wide-pore phases are useful for the chromatography of polymers; they have a lower surface-area and are less retentive than narrow-pore phases [11,16–19], potentially valuable properties for the chromatography of the thearubigins. Hamilton PRP-1 styrene-divinylbenzene copolymer [20–22] has no surface hydroxyl groups or surface metals, and was used to study the chromatography of tea liquors in the absence of secondary retention.

Although the secondary retention encountered with silica-based phases can be eliminated by using polymer phases, these phases have a lower resolving power and different selectivity than the silica-based ones, and are not a direct replacement for them [20–22]. The secondary retention caused by the surface metals of silica-based phases has been removed by adding EDTA [23,24] or phytic acid [24] to the aqueous solvent. Therefore, the Hypersil ODS column was used with solvents containing, first, EDTA and then sodium citrate, duly resulting in greatly improved chromatograms. Photodiode-array UV–VIS spectra were extracted from 33 peaks in the citrate chromatogram, and the spectra were used to classify the resolved peaks into four groups

MATERIALS AND METHODS

Equipment

The Hewlett-Packard diode array system and standard samples were as described previously [1]. A Gilson gradient system, consisting of Model 302 pumps, Data Master, and Apple computer, with a Bryans BS600 chart recorder and ABI Spectroflow 757 UV–VIS detector, was used for off-line HPLC work. Sources for the columns are given below.

HPLC analysis methods

The columns and gradients used were as follows:

Hypersil ODS. Packing: Hypersil C_{18}, 5-μm, pore size 12 nm; column size: 25 × 0.49 cm; column source: Hichrom Excel grade: a linear gradient of 8% to 31% organic over 50 min, at a flow-rate of 1.5 ml/min, was used.

Hypersil octyl wide-pore. Packing: Hypersil C_8, 5-μm, pore size 30 nm; column size: 25 × 0.49 cm (a 15 × 0.49 cm column gave similar results); column source: HPLC Technology Ltd: a linear gradient of 0% to 26% organic over 50 min, at a flow-rate of 1.4 ml/min, was used.

Hamilton PRP-1. Packing: Hamilton PRP-1 styrene-divinylbenzene copolymer, 5-μm, pore size 7.5 nm (a 10-μm column proved less useful); column size: 15 × 0.49 cm; column source: Anachem: a linear gradient of 5% to 33% organic over 50 min, at a flow-rate of 1.0 ml/min, was used.

Two different aqueous phases were adopted for the HPLC methods, 2% acetic acid and 1% citric acid (the latter adjusted to pH 2.8 with solid sodium hydroxide). In addition, 2% acetic acid, made 2% in EDTA disodium salt, was used for method development, but, as an aqueous phase, it held no advantage over citrate. The organic phase was acetonitrile.

The tea, Clone SFS 204, was obtained from the Tea Research Foundation of Central Africa, Malawi. Tea liquors were prepared as described previously [1] and volumes of 20 μl were injected.

In our work, chromatograms were monitored at 280, 380 and 460 nm —280 nm was used to detect all polyphenolics, 380 nm to detect flavonol glycosides, theaflavins and thearubigins, and 460 nm to detect theaflavins and thearubigins without interference from flavonol glycosides [25]. In this paper, emphasis is given to chromatograms monitored at 460 nm, a wavelength used in the spectrophotometric method for the determination of thearubigins by Roberts and Smith [26,27].

RESULTS AND DISCUSSION

Hypersil ODS column

The 280 nm chromatogram of a Malawi tea, on the Hypersil ODS column with an acetic acid gradient, has been discussed previously [1]. The chromatogram of Clone SFS 204 was similar (Fig. 1), and showed peaks close to the void volume of the column, a convex broad band, and resolved and partially resolved peaks with the broad band as baseline. The 460 nm chromatogram of this tea showed few sharp well defined peaks other than the theaflavins (Fig. 2). There were minor peaks close to the void volume of the column, an unresolved broad-band spreading throughout the

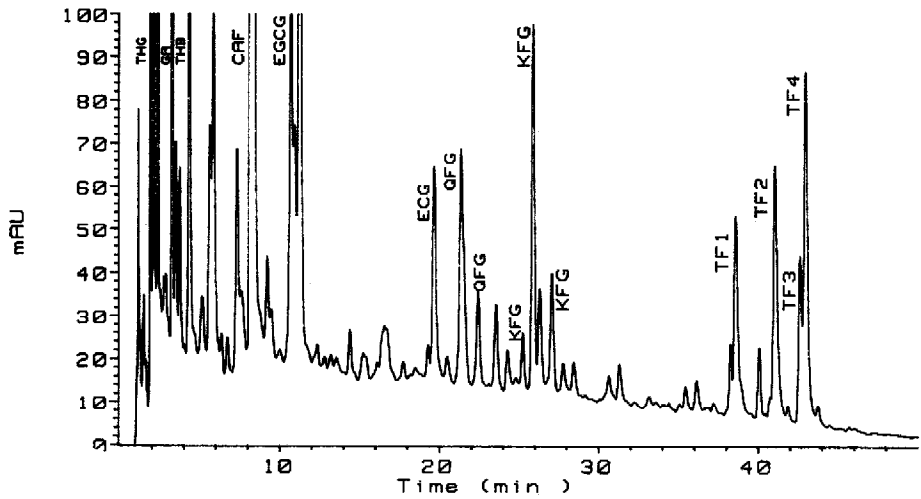

Fig. 1. Tea chromatogram (280 nm) on the Hypersil ODS column. Aqueous solvent 2% (v/v) acetic acid. Organic solvent acetonitrile. Linear gradient, 8 to 31% organic over 50 min. THG = Theogallin (5-galloylquinic acid); GA = gallic acid; THB = theobromine; CAF = caffeine; EGCG = (−)-epigallocatechin gallate; ECG = (−)-epicatechin gallate; QFG = quercetin flavonol glycoside; KFG = kaempferol flavonol glycoside; TF1 = theaflavin; TF2 = theaflavin-3-gallate; TF3 = theaflavin-3′-gallate; TF4 = theaflavin-3,3′-digallate.

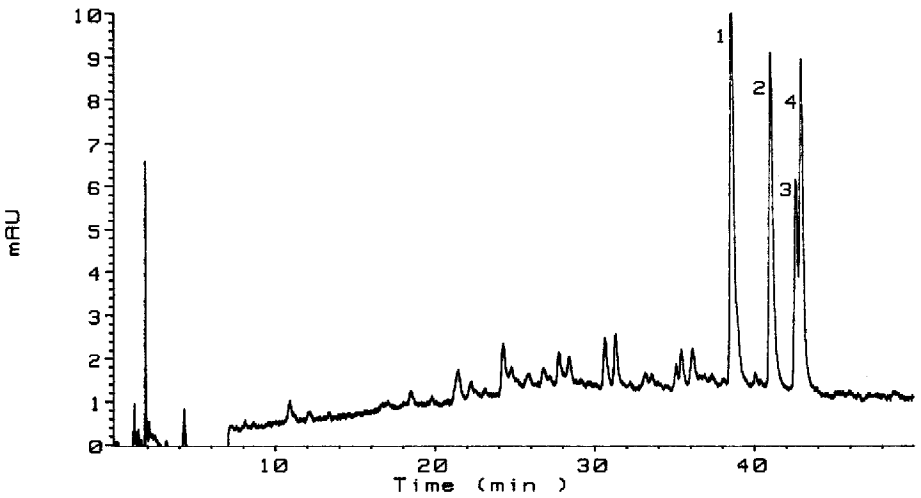

Fig. 2. Tea chromatogram (460 nm) on the Hypersil ODS column. Aqueous solvent 2% (v/v) acetic acid. Organic solvent acetonitrile. Linear gradient, 8 to 31% organic over 50 min. Peaks: 1 = theaflavin; 2 = theaflavin-3-gallate; 3 = theaflavin-3′-gallate; 4 = theaflavin-3,3′-digallate.

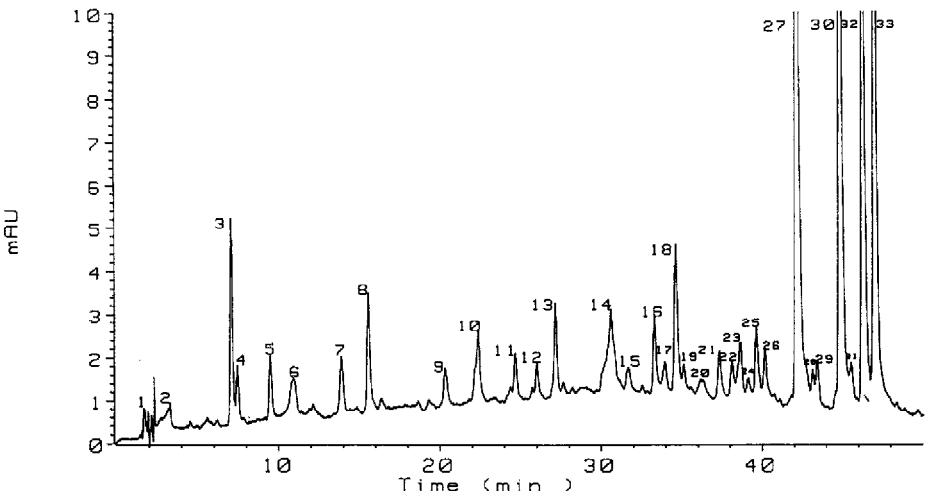

Fig. 3. Tea chromatogram (460 nm) on the Hypersil ODS column. Aqueous solvent 1% (w/v) citric acid, adjusted to pH 2.8 with solid sodium hydroxide. Organic solvent acetonitrile. Linear gradient, 8 to 31% organic over 50 min. For key see Table I.

whole chromatogram, and resolved peaks superimposed on the broad band. Peaks 1–4 in Fig. 2 were assigned to theaflavins [1], the remaining resolved peaks and the broad band remained as unassigned phenolic pigments.

In order to test the hypothesis that the chromatography of these pigments was degraded by secondary retention due to surface metals, a chromatogram was run on the Hypersil ODS column, using 2% acetic acid made 2% in EDTA. Chromatograms obtained in this way showed improvements in resolution and peak shape. However, EDTA was not very soluble in acetic acid and crystallised on standing, so citric acid, a more soluble chelating agent, was used for further work. Chromatograms, run with a citrate aqueous phase, showed further improvements in resolution and peak shape, the 460 nm chromatogram being reproduced in Fig. 3.

Irreversible and reversible improvements were observed with citrate, and, when acetic acid was used again after the use of citrate, much, but not all, of the improvement was maintained. However, the most highly resolved chromatograms were obtained with citrate. This suggested that the irreversible improvements were the result of a reduction of secondary retention, caused by the removal or masking of surface metals by the citrate. The reversible effect was probably caused by prevention of polyphenol–metal interactions by citrate. Tea liquors contain metals [28], and a wide range of polyphenol–metal interactions, some involving thearubigins [29], are theoretically possible. The prevention or removal of these interactions would require the presence of citrate. The use of the Hypersil ODS column in conjunction with citrate, was adopted as a new method for the analysis of black tea pigments.

Hypersil octyl wide-pore column

The previous acetic acid gradient did not work with the Hypersil wide-pore column, most material being eluted very rapidly in an unresolved band, reminiscent

of the polymers discussed by Snyder *et al.* [11]. However, a chromatogram with a useful level of resolution was obtained with a modified acetic acid gradient. The 280 nm tea chromatogram, using an acetic acid gradient, showed peaks close to the void volume of the column, a convex broad band, and resolved and partially resolved peaks with the broad band as baseline. The 460 nm tea chromatogram, using an acetic acid gradient (Fig. 4), showed minor peaks close to the void volume of the column, a convex broad band, and resolved and partially resolved peaks with the broad band as baseline. The baseline of a solvent blank was flat, so the broad band was not caused by refractive index changes during the gradient. The maximum of the broad band in the 460 nm chromatogram occurred at a longer retention time than in the 280 nm chromatogram. This suggested that there were two types of unresolved material in the liquor, only one of which was coloured.

The resolved peaks were less well resolved than on the Hypersil ODS column, an intelligible result of the change from a narrow-pore to a wide-pore column [11]. The broad band had a convex shape, the detector output rising to a maximum and then falling to the baseline. This type of chromatogram is characteristic of an unresolved complex mixture of compounds of lower molecular mass [30] or a polydisperse polymer [11], and the broad band in Fig. 4 could have been due to either of these. A wide-pore phase allows faster solute diffusion and favours the chromatography of polymeric material [11], but it also has a low surface area and is less likely to cause secondary retention of strongly adsorbing material. Therefore, the possibility, that the convex broad band was an unresolved complex mixture of strongly adsorbing compounds of lower molecular mass, cannot be ruled out. However, the broad band was tentatively designated as a strongly adsorbing polydisperse polymer.

When the column was used with citrate, small irreversible and reversible im-

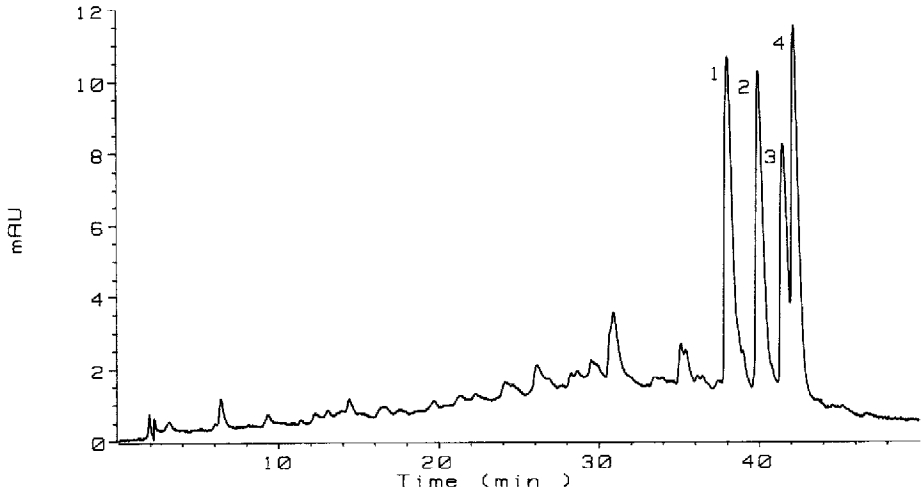

Fig. 4. Tea chromatogram (460 nm) on the Hypersil octyl wide pore column. Aqueous solvent 2% (v/v) acetic acid. Organic solvent acetonitrile. Linear gradient, 0 to 26% organic over 50 min, flow-rate 1.4 ml/min. Peaks: 1 = theaflavin; 2 = theaflavin-3-gallate; 3 = theaflavin-3′-gallate; 4 = theaflavin-3,3′-digallate.

provements were observed. The fact, that the wide-pore column gave smaller irreversible improvements than the Hypersil ODS column, probably reflected the lower surface area of its wide-pore stationary phase.

Hamilton PRP-1 column

Styrene–divinylbenzene stationary phases, such as Hamilton PRP-1, operate most efficiently with acetonitrile [21], so a linear gradient using acetonitrile and acetic acid was developed. The 280 nm tea chromatogram showed peaks near the void volume of the column, a convex broad band, and resolved and partially resolved peaks with the broad band as baseline. The 460 nm chromatogram (Fig. 5) showed minor peaks near the void volume of the column, a convex broad band, and resolved and partially resolved peaks with the broad band as baseline. As with the Hypersil wide-pore column, the maximum of the broad band occurred at a longer retention time in the 460 nm chromatogram, than in the 280 nm chromatogram.

The chromatograms obtained with this column were consistent with the properties of the Hamilton PRP-1 stationary phase, which, being narrow-pore, resolved lower-molecular-mass material better than the Hypersil wide-pore column, and, being inert, allowed strongly adsorbing material to elute more successfully than the Hypersil ODS column. Although the Hamilton PRP-1 column resolved lower molecular mass polyphenolics well, its different selectivity made it a less useful column for these compounds than the Hypersil ODS column.

Peak 1 in Fig. 5 was a very prominent peak which did not have a counterpart in the other chromatograms. A photodiode-array three-dimensional plot of the chromatogram suggested that the peak was an anthocyanidin, and the photodiode-array UV–VIS spectrum extracted from the peak (Fig. 6a) confirmed this. The position of

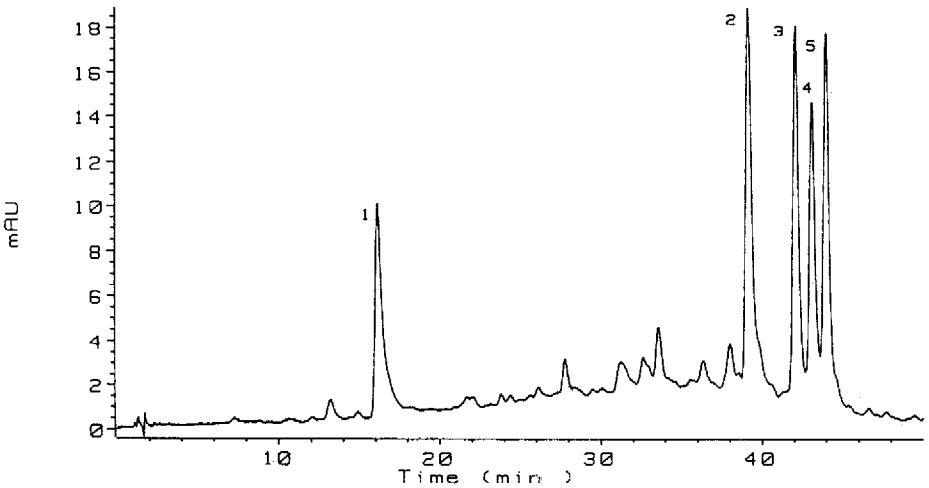

Fig. 5. Tea chromatogram (460 nm) on the Hamilton PRP-1 column. Aqueous solvent 2% (v/v) acetic acid. Organic solvent acetonitrile. Linear gradient, 5 to 33% organic over 50 min, flow-rate 1.0 ml/min. Peaks: 1 = anthocyanidin; 2 = theaflavin; 3 = theaflavin-3-gallate; 4 = theaflavin-3′-gallate; 5 = theaflavin-3,3′-digallate.

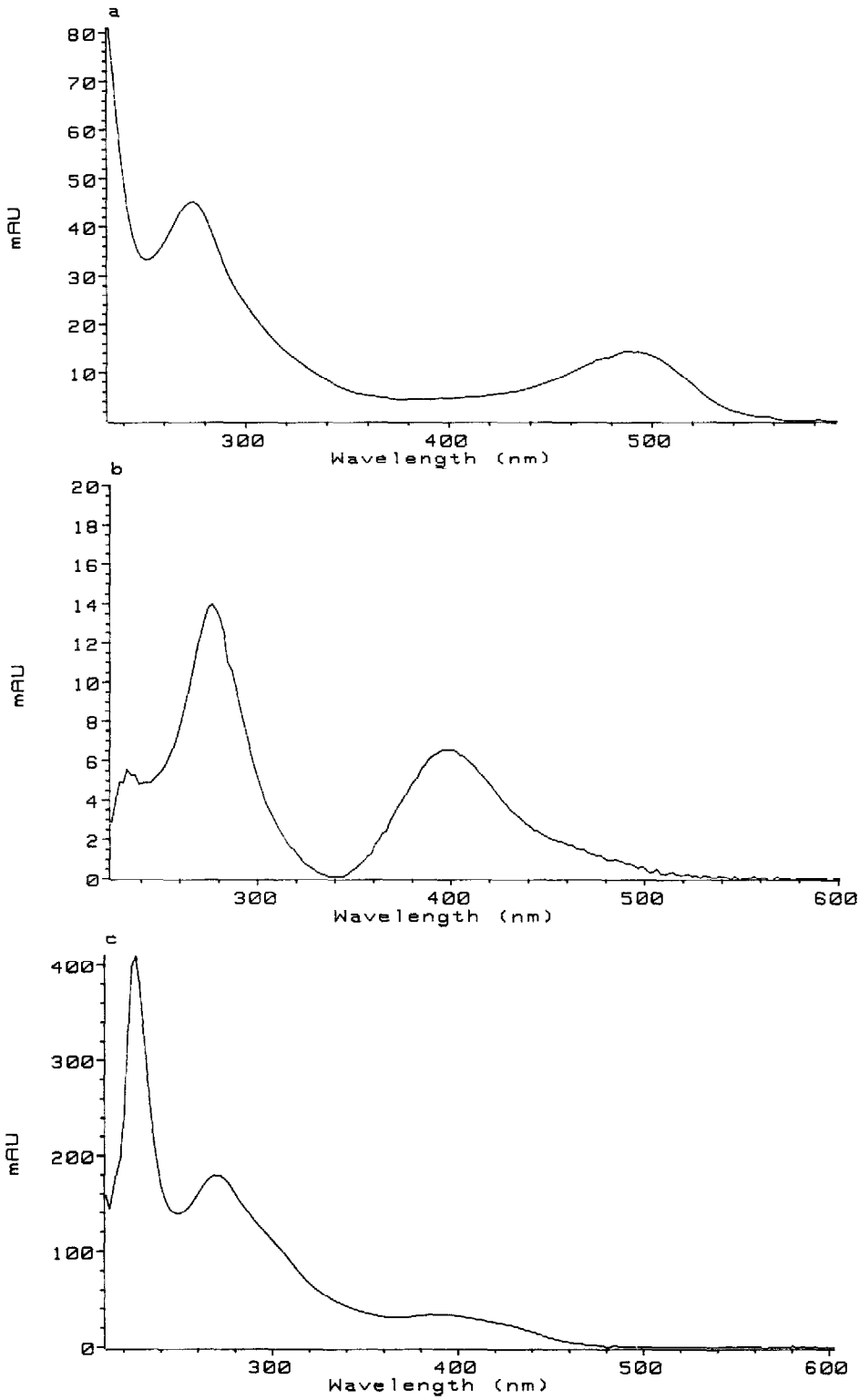
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Fig. 6.

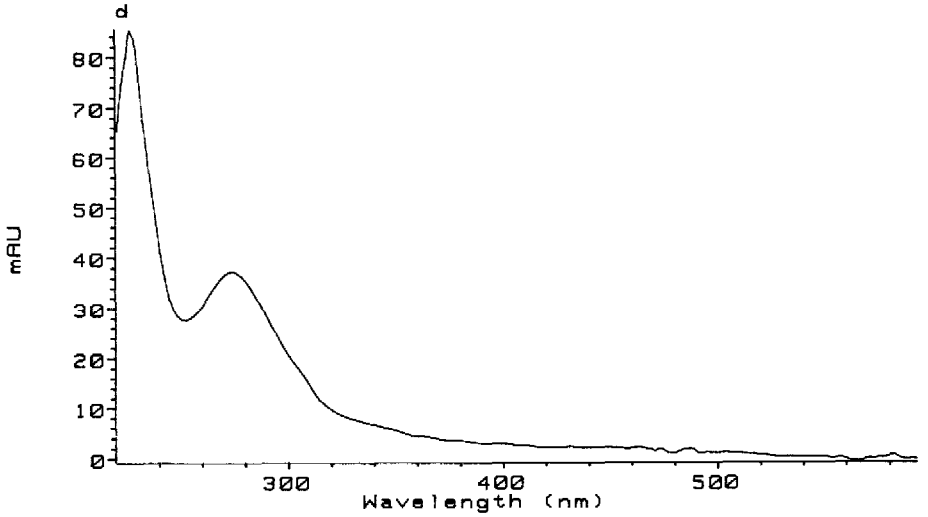

Fig. 6. Photodiode-array UV–VIS spectra. (a) Peak 1 of Fig. 5, an anthocyanidin; (b) peak 13 of Fig. 3, a theaflavic acid; (c) peak 3 of Fig. 3, a type I resolved pigment; (d) peak 7 of Fig. 3, a type II resolved pigment.

the absorption maximum (486.5 nm, in about 15% acetonitrile/85% acetic acid) was different to that of tricetinidin (520 nm, in ethanol), an anthocyanidin isolated from black tea liquors by Roberts and Williams [2,31]. However, an anthocyanidin band measured in aqueous solution is blue-shifted by 25 to 35 nm with respect to the band position measured in ethanol [32]. The difference in the absorption maxima of peak 1 and tricetinidin (33.5 nm) was within this range, so peak 1 in Fig. 5 was tentatively assigned to tricetinidin.

Small reversible improvements were observed with citrate, but no irreversible improvements. The fact, that no irreversible improvements were observed with this column, was probably due to the absence of surface metal contamination of its polymer stationary phase.

Classification of the phenolic tea pigments

From the different chromatograms on the different columns, a classification of the phenolic tea pigments was devised. The pigments were classified by their chromatographic behaviour into three groups, as follows: group I pigments, pigments which ran close to the void volume of the columns; group II pigments, resolved pigments; and group III pigments, unresolved pigments. The photodiode-array UV–VIS spectra extracted from the 33 largest peaks in the chromatogram shown in Fig. 3 (Hypersil ODS column with citrate) allowed the group II pigments to be classified further into four sub-groups (sub-groups II.1, II.2, II.3, and II.4), consisting of peaks with similar photodiode-array UV–VIS spectra (Fig. 3; Table I).

Group I pigments appeared to be totally excluded from all the columns, and ran close to the void volumes of the columns. This material may have been polymeric, hydrogen-bonded, associated, charged, or any combination of these, exclusion being

TABLE I

CLASSIFICATION OF THE GROUP II PIGMENT PEAKS FROM THE HYPERSIL ODS COLUMN BY PHOTODIODE-ARRAY UV–VIS SPECTRA

Peak	Wavelength (nm)	Classification: assignment
1	510	Anthocyanidin polymer
2	271, tailing into visible	II.4: type II resolved pigment
3	271, 387	II.3: type I resolved pigment
4	277, tailing into visible	II.4: type II resolved pigment
5	271, 379	II.3: type I resolved pigment
6	273, tailing into visible	II.4: type II resolved pigment
7	273, tailing into visible	II.4: type II resolved pigment
8	273, 387	II.3: type I resolved pigment
9	271, tailing into visible	II.4: type II resolved pigment
10	273, tailing into visible	II.4: type II resolved pigment
11	271, tailing into visible	II.4: type II resolved pigment
12	263, tailing into visible	II.4: type II resolved pigment
13	275, 395	II.2: a theaflavic acid
14	275, 395	II.2: a theaflavic acid
15	271, tailing into visible	II.4: type II resolved pigment
16	275, 389	II.2: a theaflavic acid
17	267, tailing into visible	II.4: type II resolved pigment
18	267, 371, 461	II.1: a theaflavin
19	267, tailing into visible	II.4: type II resolved pigment
20	271, 382	II.3: type I resolved pigment
21	271, 373, 445	II.1: a theaflavin
22	267, 389	II.3: type I resolved pigment
23	271, 387	II.3: type I resolved pigment
24	275, tailing into visible	II.4: type II resolved pigment
25	271, 371, 450	II.1: a theaflavin
26	271, 370, 450	II.1: a theaflavin
27	271, 371, 460	II.1: theaflavin
28	273, 415	II.3: type I resolved pigment
29	275, 301, 405	II.3: type I resolved pigment
30	269, 371, 451	II.1: theaflavin-3-gallate
31	274, tailing into visible	II.4: type II resolved pigment
32	273, 371, 451	II.1: theaflavin-3′-gallate
33	273, 373, 447	II.1: theaflavin-3,3′-digallate

by molecular size, strong electrostatic repulsion, or both. Photodiode-array three-dimensional plots of the early peaks of the Hypersil ODS tea chromatogram showed three peaks with absorption which extended across the entire spectral region (200–600 nm), which, for this reason, were thought to be brown pigments, and a peak with an absorption maximum at 510 nm, which was thought to be a anthocyanidin pigment.

Although work on the identification of these substances is continuing, some comments on their nature may be made at this point. A spectrum consisting of low-intensity absorption extending across the visible region, is characteristic of substances which are brown [33]. The non-dialysable thearubigins, isolated by Millin and co-workers [34,35] using thin-film counter-current dialysis, were brown protein-containing substances of high molecular mass, probably in the form of a protein–polyphenol complex [36]. Substances of this type could be of sufficient size to be excluded

from the HPLC columns. Somers [37] has shown that anthocyanidin pigments, charged flavanol polymers with units oxidised to the anthocyanidin level, occur in various degrees of association as pigments in red wine. If a pigment of this type were present in the tea liquor, its size and charge would cause it to elute very quickly, and this could account for the peak with the absorption band at 510 nm.

Sub-group II.1 pigments consisted of eight peaks, with spectra which showed three bands in the ranges 267–273, 371–373 and 445–451 nm. Literature theaflavin spectra [38,39] have three bands in the ranges 268–278, 372–378 and 456–461 nm (Table II). The photodiode-array UV–VIS spectra were blue-shifted compared with the literature spectra, but had retained their characteristic shape, so these peaks were assigned to theaflavins. Peaks 27, 30, 32 and 33 in Fig. 3 were assigned as before [1], but the other peaks remain to be assigned to individual theaflavins.

Bailey *et al.* [1] pointed out that nine theaflavins can be formed by the coupling of the appropriate flavanol *o*-quinones, so the assignment of eight peaks to group II.1 is not unreasonable. Some peaks were too small to give reliable spectra, and were not included in the classification; the ninth theaflavin may have been one of these peaks. Other theaflavins can be formed by the oxidation of (−)-epigallocatechin, in the presence of catechol (categallin) or pyrogallol (pyrogallin) [38,39], but the data at present are insufficient for any of the peaks in the chromatogram to be assigned to these compounds.

Sub-group II.2 pigments consisted of three peaks with spectra which showed two bands, at 275 and 389–395 nm (Fig. 6b). Literature theaflavic acid spectra [38] show two bands in the ranges 278–280 and 398–400 nm (Table II), so the three peaks were assigned to theaflavic acids. Three theaflavic acids can be formed by the reaction of the appropriate flavanol *o*-quinones with gallic acid [6,38], and this accounts for the three peaks in our chromatogram.

Sub-group II.3 pigments consisted of eight peaks, the spectra of which showed bands in the ranges 267–275 and 379–405 (Fig. 6c), and with a different absorption maxima ratio to the group II.2 pigments. Peaks with a spectrum of this type, were assigned to type I resolved pigments. They may have been thearubigins of lower

TABLE II

LITERATURE UV–VIS ABSORPTION DATA OF THE THEAFLAVINS AND RELATED COMPOUNDS

All spectra in methanol.

Compound	Wavelength (nm)	Ref.
Theaflavin	268, 378, 461	38
Theaflavin-3-gallate	272, 376, 455	38
Theaflavin-3′-gallate	278, 376, 452	38
Theaflavin-3,3′-digallate	278, 378, 455	38
Categallin	274, 373, 453	39
Pyrogallin	281, 307, 356, 425	39
Epitheaflavic acid	280, 400	38
Epitheaflavic acid-3′-gallate	279, 398	38
Theaflavic acid	278, 398	38

molecular mass, being oxidation products of theaflavins [2], of theaflavic acids [6], and/or of catechins [40].

Sub-group II.4 pigments consisted of thirteen peaks with spectra which showed an absorption maximum in the range 271–277 nm, and absorption which tailed across the entire spectral region (Fig. 6d). The spectra from peaks 12, 17, and 19 each had a band outside this range, but these were not pure peaks. Peaks with a spectrum of this type were assigned to type II resolved pigments. This spectrum is characteristic of substances which are brown [33], so type II resolved pigments were brown phenolic substances, possibly thearubigins, of lower molecular mass. They may have been oxidation products of theaflavins [2], of theaflavic acids [6], and/or of catechins [40]. However, the possibility that some of these peaks were the products of non-enzymic browning reactions, cannot be ruled out completely.

Group III pigments were unresolved pigments, which ran as a convex broad band on the Hypersil wide-pore and Hamilton PRP-1 columns. The photodiode-array UV–VIS spectra of group III pigments showed a band at about 280 nm, and absorption which tailed across the entire spectral region, indicating that they were brown phenolic pigments, possibly polymeric thearubigins. Brown *et al.* [7] suggested that some thearubigins were proanthocyanidin polymers, and indeed McMurrough [41] and Putman and Butler [24] have shown that proanthocyanidin polymers run as unresolved broad bands on an ODS column. However, tea manufacture involves a complex set of enzymic and chemical reactions capable of producing highly polydisperse polymers [2–5]. Therefore, the group III pigments may be polydisperse flavanol polymers incorporating chromophoric monomer units, rather than proanthocyanidin polymers.

This classification thus highlights that a range of pigments were present in the black tea liquor studied, some resolvable by HPLC and some, at this time, unresolvable.

CONCLUSIONS

New HPLC methods for the analysis of black tea liquor have been developed. Very good separation of phenolic tea pigments has been achieved using a Hypersil ODS column in conjuction with a citrate buffer. The suggestion was put forward, that the improved performance of this column on changing from acetic acid to citrate was mainly caused by the removal or masking of surface metals from the stationary phase by the citrate. Unresolved, probably polymeric, pigments formed a convex broad band on the Hypersil octyl wide-pore (Fig. 4) and Hamilton PRP-1 (Fig. 5) columns. The Hamilton PRP-1 column provided a method for the analysis of an anthocyanidin, and showed it to be an important contributor to liquor colour (Fig. 5).

The phenolic pigments in black tea liquor were classified by their chromatographic behaviour, as follows: group I pigments, pigments which ran close to the void volume of the HPLC columns; group II pigments, resolved pigments; and Group III pigments, unresolved pigments. Group II pigments were then further classified by photodiode-array UV–VIS spectra, into four sub-groups, as follows: sub-group II.1, three bands in the ranges 267–273, 371–373 and 445–451 nm (theaflavins); sub-group II. 2, two bands at 275 and 389–395 nm (theaflavic acids); sub-group II.3, bands in the ranges 267–275 and 379–405 nm, of different shape to sub-group II.2 spectra (type I

resolved pigments); and sub-group II.4, a band in the range 271–277 nm and absorption which tailed into the visible region (type II resolved pigments). Pigments in sub-groups II.3 and II.4 were designated as resolved thearubigins, and those in group III as unresolved thearubigins.

This work is part of a larger project, the aim of which is to obtain instrumental measures of tea quality. In parallel with the qualitative work reported here, quantitative statistical studies are in progress, the aim of which is to discover what relation, if any, the classes of pigments discussed in this paper have to tea quality. Further qualitative work will also be carried out on the identification of these pigments, and the further improvement of the analytical methods.

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