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Abstract

The colloidal precipitate known as tea
cream, which separates when a hot aqueous
infusion of black tea is cooled, is investigated
by electron microscopic (EM) techniques of
shadowing, sectioning, freeze-etching and
scanning and also by optical microscopy. These
indicate tea cream to be an association colloid,
the morphology of which depends on overall solids
concentration. Dilute infusions (0.1% w/w)
produce macromolecular aggregates of about 50 nm,
but at higher tea solids concentrations secondary
aggregation of the initial particles results in
ill-defined clusters of approximately 1 μm in
diameter. At 5% w/w, clear, spherical liquid
droplets, typically 1-2 μm in diameter are
observed. Increasing concentration to 40% w/w
causes an increase in size of the individual
colloidal droplets and an increase in the phase
volume of this disperse phase. The colloidal
phase contains 55-65% solids by weight, the
total solids content appearing to be independent
of overall composition of the solutions from
which it is formed. The colloid may be separated
from cooled tea infusions by centrifugation but
individual particles display strong resistance to
coalescence. At high tea cream phase volumes
phase inversion can occur and dispersions of the
dilute phase in a continuous cream phase are then
observed.

Introduction

Tea cream is a colloidal precipitate formed
by black tea liquors as they cool. The
composition of tea cream and the chemical factors
causing its formation have been the subject of
considerable investigation over the past twenty
years. Previously, attention has been paid to
the chemical causes of the separation of tea
cream and to chemical composition but little
significance has been placed on its physical
nature. This paper reports the cream structures
that are formed from tea infusions with solids
concentrations between 0.1% and 60% w/w,
indicates the phase volumes involved and
describes phase inversion at high tea solids
concentrations (>40%).

Chemical components

Tea cream composition is known to be
complex. It has been reported by Roberts (8) to
consist largely of a mixture of theaflavins,
thearubigins and caffeine. It also contains
other components such as bisflavanols, unoxidised
flavanols, pectin (9), caffeic, gallic and
ellagic acids, and several other compounds
(14,18). In addition, non-caffeine nitrogen and
chlorophyll-derived compounds have been
identified in tea cream (14). Specific
interactions between caffeine and polyphenols
resulting in their incorporation into the tea
cream phase have also received much attention
(9,14).

Roberts (8) has claimed that thearubigins
are by far the most abundant polyphenolic
oxidation products in black tea and that they are
the major constituent of tea cream (approximately
66%). Roberts maintains that thearubigin
fractions of black tea have molecular weights of
the order of 700 and contain no nitrogen (8,10).
Conversely, other authors have suggested them to
be of much higher molecular weight (4). Vuataz
et al. have shown in detailed work (17), that
their preparation of thearubigin contained 0.55%
nitrogen which could not be accounted for by
caffeine or free amino acids and have suggested
that molecular weights of up to 40,000 are
involved. However, acid hydrolysis of the
material produced a wide range of free amino

acids which led them to conclude the thearubigin fraction to be partly composed of substances related to humic acids. Recent work has suggested thearubigins to be pentameric flavon-3-ols and their gallates containing both hydrolysable and non-hydrolysable interflavanoid links. Such structures would be consistent with molecular weight of approximately 1,500 measured for these species (1).

It is clear from the above that the unresolved character of the thearubigins presents difficulties when attempting to rationalise the behaviour of the tea cream phase on a chemical basis. Also it is evident that the behaviour of the "cream phase" depends on the influence of a wide range of components.

Amount of tea cream formed

A colloidal precipitation process involving so many components may be expected to be extremely variable but some clear generalisations can be made. It has been shown that tea cream levels depend on infusion composition; particularly strong tea cream formation occurs in infusions high in polyphenols and caffeine (9). Other compositional factors influencing the quantity of tea cream have been identified as pH (14), concentration of various salts and other solutes (11). At constant composition, however, the principal variables are temperature and tea solids concentration. It seems that the amount of tea cream formed is inversely related to temperature whereas it is directly proportional to total solids concentration.

Nature and properties of tea cream

Despite the many investigations which produced the conclusions outlined above, relatively little attention has been paid to the physical form adopted by the precipitate. Rutter's work (11,12) is a notable exception. Smith (14) had reported that tea cream took the form of small spheres a few micrometers in diameter which formed at approximately 40°C on cooling but which did not disappear until approximately 55°C on reheating.

By optical and electron microscope studies, Rutter confirmed tea cream to be a suspension of polydisperse spheres with diameters in the range 0.3-2.0 μm. He also identified the presence of many smaller particles, the majority being approximately 0.04 μm in diameter in dilute solutions (<3% w/v total solids concentration).

Materials and Methods

So as to minimise the variability inherent in tea samples, this study has exclusively used a bulked sample of dried extract. The extract was prepared by hot water counter-current stripping of black Ceylon tea made initially at approximately 5% tea solids and then concentrated and spray dried to a fine flowing powder. All experimental samples were drawn from the single bulk sample which was stored at room temperature in sealed steel drums. All infusions were prepared with distilled water and are given as % w/w unless otherwise stated.

Cream phase preparation

Rutter (11) showed that the rate of cooling can affect particle size and stability to flocculation of the tea cream phase. Consequently, a routine procedure has been adopted for the tea cream phase preparation in this study. In all cases, reconstituted infusions were prepared by heating preparations of known concentrations at 95°C for 15 minutes in a conical flask fitted with a reflux condenser. The preparations were sealed in glass flasks, cooled against ambient temperature and aged for 4 h. The tea solids used contained 3% moisture and were contaminated with 3.3% of insoluble debris from the original leaf. In addition, a crystalline insoluble material was identified. This has traditionally been reported to be calcium oxalate (16) but shown in this case by powder X-ray diffraction and X-ray emission microanalysis to be principally magnesium oxalate. Where necessary, these figures have been taken into account in quoting soluble tea solids concentrations. The pH of the reconstituted infusions was 4.7.

Phase volume of tea cream

The volume of tea cream produced by a given liquor concentration has been estimated by centrifugal separation (10 cm³ samples 20,000xg, 30 min, 20°C) of the two phases. This approach assumes that, under the centrifugal conditions employed, the tea cream is not compressed in such a way that it is changed from the equilibrium it has reached in free suspension. Secondly, it assumes that the interstitial liquor between droplets does not contribute significantly to the measured amount of the tea cream.

A second series of experiments, in which the relative volumes of the two phases were measured gravimetrically, (by density determinations on the supernatant liquid and the total liquor) supported the data obtained volumetrically. Gravimetric determinations of the solid material dissolved in the supernatant then permitted the determination of the concentration of species in the two phases to be established.

Optical microscopy

This was carried out on a Leitz Ortholux 2 optical microscope. Transmitted phase contrast and plane polarized light were used to examine the prepared infusions. No staining was necessary.

Transmission electron microscopy

Tungsten shadowing

Tea cream from each concentration was fixed in 1% w/v osmium tetroxide solution at room temperature for 30 min. Samples were then

centrifuged, resuspended in distilled water and then sprayed with a nasal atomiser onto a previously carbon covered EM grid. The grids were shadowed with tungsten at an angle of 45° in an Edwards 306 coating unit fitted with an electron beam gun. Finally, grids were examined in a J.E.M. 100C electron microscope at 80 kV.

Sectioning

Samples were prepared by fixing in 1% w/v osmium tetroxide solution at room temperature for 30 min. They were washed with two changes of distilled water, separated by centrifugation and then dispersed in 1% agar solution at just above the gelation temperature (40°C). On cooling, small blocks of the suspending gel were cut, dehydrated in ethanol and embedded in EPON 812 resin by the conventional schedule (2).

Thin sections were cut using a Reichert-Jung Ultracut microtome, stained with lead citrate and examined by transmission electron microscopy in a J.E.M. 100C electron microscope at 80 kV.

Freeze-etching

Tea cream was removed from suspension by gentle centrifugation and a small sample carried on a copper stud was frozen in Freon 22 which had been pre-cooled to its freezing point in liquid nitrogen. The frozen sample was then fractured with a cold knife (-180°C) under vacuum and held at -98°C for 30 seconds in a Polaron freeze-etching module. The etched surface was shadowed with carbon/platinum at 45° and the replica backed with carbon. The replica was cleaned from residual tea cream by floating it on chromic acid overnight and washing it with distilled water.

Scanning electron microscopy (SEM)

Fixed tea cream particles from a 5% infusion were examined in the form of a centrifuge pellet. The pellet was obtained by centrifuging an infusion, fixed with 1% w/v osmium tetroxide solution at room temperature for 30 min, at 1,500xg. The resultant pellet was rinsed with distilled water and dried in a vacuum desiccator. Once dried, the pellet was mounted on a SEM stub, sputter-coated with gold (~20 nm) and examined in a Cambridge Stereoscan 600 scanning electron microscope. The microscope was operated at 25 kV.

Results and Discussion

Optical microscopy

Infusions have been examined over the concentration range of 1-60% solids at 20°C. Tea cream has its most clearly defined form in the concentration range of 5-20% solids where it exists, for the most part, as a dispersion of spheres (Fig. 1). At 5% solids the mean particle diameter is approximately 1-2 μm and this increases to approximately 2-3 μm at a concentration of 10%. The tea cream phase at 5% solids appears homogeneous, but increasing concentration causes the droplets to become
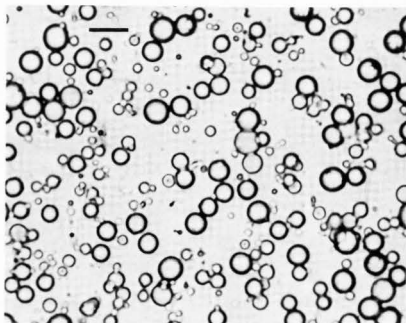

Fig. 1. Optical micrograph of a 20% infusion at 25°C.
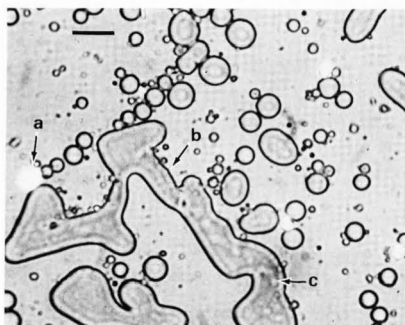Bar = 4 μm

Fig. 2. Optical micrograph of a 31% infusion at 25°C
a - magnesium oxalate crystallite
b - irregular configuration of tea cream
c - inclusions of supernatant phase
Bar = 4 μm

heterogeneous. The heterogeneous nature, or presence of inclusions, is particularly apparent in the largest droplets.

Above 20% solids, the tea cream phase more easily loses its spherical form and can be distorted into rod-like shapes by shearing on the microscope slide. Under these conditions, individual droplets can be made to coalesce. Tea cream phase in infusions of solids concentrations greater than 25% adopts irregular configurations (Fig. 2), whilst on reaching 40% solids, the phase volume of the cream has increased significantly and the tea cream and supernatant phases have roughly similar volumes. It is clear at this concentration that the system is made up
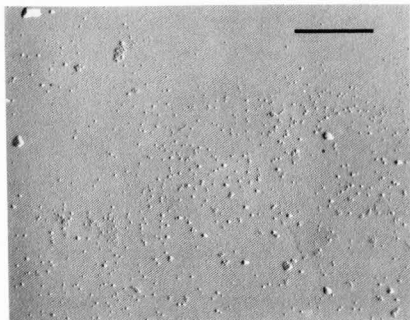
Fig. 3. Tungsten shadowed subunits from a 0.1%
         infusion at 25°C. Bar = 1 μm.
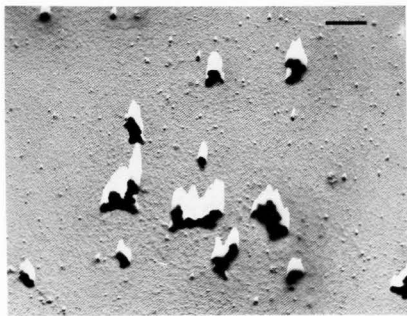

Fig. 4. Tungsten shadowed aggregates from a 1.0%
         infusion at 25°C. Bar = 1 μm.

Fig. 5. Tungsten shadowed tea cream particles
         from a 5% infusion at 25°C.
         Bar = 2 μm.
         Arrow indicates fibrous material
         occluded to cream droplet.

Fig. 6. Tungsten shadowed tea cream particles
         from a 20% infusion at 25°C.
         Bar = 2 μm.

of two liquid phases and observation of the flow
patterns in the two phases indicate the tea cream
phase to be by far the more viscous. The phases
adopt no well defined shape and inclusions of
supernatant phase can be seen in the cream phase
(Fig. 2). In this overall composition,
such droplets coexist with similar sized droplets
of the cream phase. Neither phase is clearly a
continuous phase though, as concentration is
increased, the cream phase volume increases
significantly and the tea cream becomes the
supporting phase. At 40-45% solids only small
amounts of the supernatant phase can be detected
microscopically and at 60% solids the system
appears to be a single phase.

Observations on preparations in the range
43-52% solids did not clearly show at what point
all the supernatant phase was lost. Having shown
that small droplets of the supernatant phase can
exist as inclusions in the cream phase, it seems
likely that these inclusions exist above the
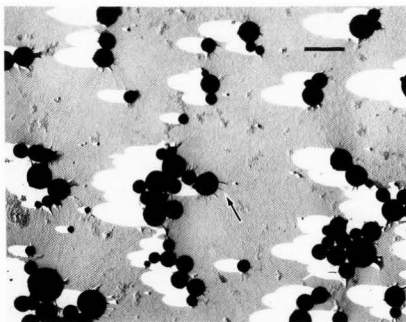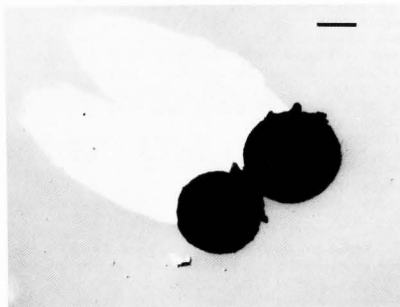concentrations at which they can be observed

microscopically. A composition, above which the
system contains only the cream phase, has been
estimated by electron microscopy to be
approximately 55% solids.

Polarised light microscopy over the whole
concentration range revealed no evidence of
liquid crystallinity in either phase, though a
significant level of the crystallites were
present in the supernatant (Fig. 2).

Tea cream particles from low concentration
infusions (approximately 1%) have less well
defined shapes. At 1% solids, tea cream is
produced as an irregular amorphous precipitate
which gives the appearance of irregular granules
built up from a finely divided precipitate. It
does not adopt the spherical form characteristic
of higher concentrations. The individual
components of the flocculates are difficult to

characterise with the light microscope as they are at (or below) the limit of resolution. Only irregular flocculates (approximately 1-2 μm) are observed. At 2.5% solids, the phase has started to adopt the recognisable globular form.

Electron microscopy

Electron microscope studies have been made on cream phase formed from infusions of 0.1, 1.0, 5.0 and 20.0% solids. Micrographs of osmium-stained and tungsten-shadowed tea cream particles from each concentration are gathered in Figs. 3-6.

At low concentrations (0.1%) (Fig. 3), the small particles appear to be relatively loose aggregates of precipitated macromolecular material. Typically they are 170-200 nm in diameter as measured by the Coulter Nanosizer and have a relatively narrow size distribution. Measurements from electron micrographs of shadowed particles indicate a smaller size, approximately 30-60 nm with only a few particles up to 100 nm in diameter. It is likely that the particle diameter determined by electron microscopy represents the minimum value, considering that a high proportion of the tea cream droplet is water [at higher concentrations of total solids, water occupies approximately 45% by weight (Fig. 7) and this volume may be lost in preparation of the tea cream for electron microscopy]. On the other hand, the Nanosizer quasi-elastic light scattering technique detects an effective hydrodynamic volume. It does this by monitoring the fluctuations in intensity as they are subject to Brownian motion. In this experimental system, the method is likely to overestimate the size of particles which carry extensive polymer adsorbed to the surfaces or are coated with diffuse layers of polymer and which, therefore, may be relatively "anchored" in the aqueous suspension. Analogous discrepancies between these techniques have been noted previously in studies of casein micelles (13) which are similarly heavily hydrated polymeric particles.

Tea cream particles from 1% infusions (Fig. 4) are loose ill-defined aggregates approximately 0.5-1.0 μm in diameter, though some smaller particles characteristic of lower concentrations still remain.

At 5% solids (Fig. 5), particle sizes are of the order of 0.5-1.5 μm whereas larger particles of about 3 μm are typical of 20% infusions. Particles at 5% and 20% solids appear as single isolated spheres as expected from optical microscopy. They are fixed as spheres (probably during staining) and produce elliptical shadows indicating that the particles at the stage of electron microscopy are solid fixed units. On the exterior of tea cream from 20% infusions (Fig. 6) solid material is seen to be occluded, whereas tea cream from 5% infusions clearly has fibrous material projecting from the particles.

Micrographs of stained, embedded and sectioned creams from infusions of 1.0, 5.0 and 20.0% solids are shown in Figs. 8-13.

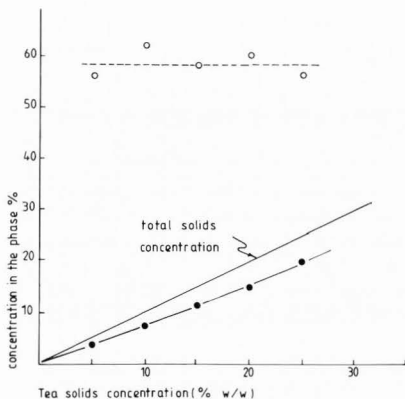The sectioning technique confirms that the

Fig. 7.   Concentration of solids by weight in tea cream phase and supporting medium.

  • supporting medium
  o tea cream phase

loose aggregates formed from 1% infusions are very heterogeneous (Figs. 8 and 9) and that they appear to be built up from the smaller particles which are observed at 0.1% and 1.0% by tungsten shadowing (Figs. 3 and 4). The staining behaviour suggests two types of principal component make up the tea cream particle, one heavily stained and the other more lightly stained. It appears that each particle builds up in a similar way and is a relatively even mixture of heavily and lightly stained components. The fact that aggregates are similar in size may indicate some specificity in their assembly. It is possible therefore, that these particles are the building blocks or subunits of the well-defined structure which is developed by the time the tea solids concentration is increased to 5% (Figs. 10 and 11).

With the sectioning technique, still more detail of the heterogeneity can be resolved. The two magnifications shown in Figs. 8 and 9 indicate clearly that under the conditions occurring in 1% infusions, the precipitate is made up of two components one of which stains only lightly. In all cases, material is present which stains only weakly on the surface leaving the interior unstained. It is unlikely that this material can be forming as hollow vesicles and it is thus suspected to be aggregated precipitate (probably polymeric) itself not susceptible to staining but which has adsorbed on it some stained material.

All micrographs at this concentration emphasise the heterogeneity of the precipitate, particularly with respect to its staining behaviour. Even the components taking stain strongly can be seen to be extremely heterogeneous with respect to staining sites.
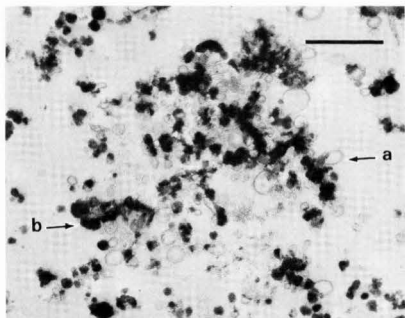
Fig. 8. Sectioned tea cream particles from a 1%
         infusion.
         Bar = 1 μm.
         a = surface stained component
         b = heavily stained component

Fig. 10. Sectioned tea cream particles from a 5%
          infusion. Arrow indicates poorly
          stained, hollow structure.
          Bar = 800 nm.
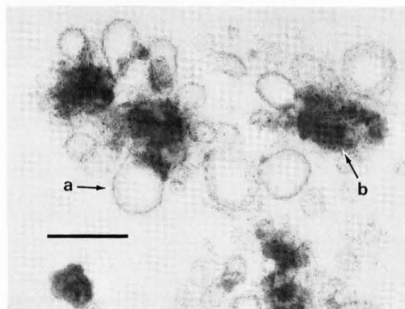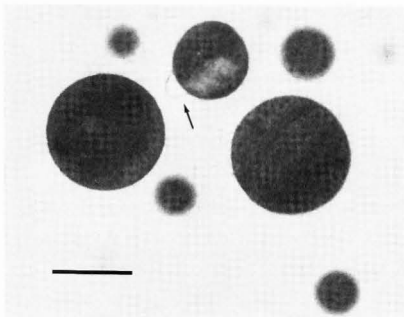

Fig. 9. Sectioned tea cream particles from a 1%
         infusion.
         Bar = 400 nm.
         a = surface stained component
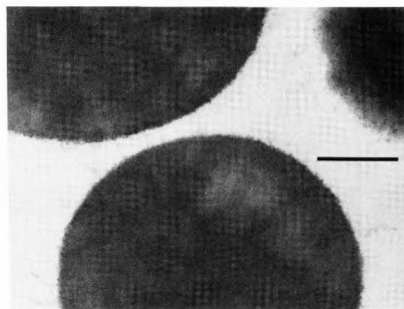   b = heavily stained component

Fig. 11. Sectioned tea cream particles from a 5%
          infusion.
          Bar = 200 nm.

The fixation and staining chemistry of
osmium tetroxide have been described in some
detail (7), but the actual staining mechanism of
tea cream by osmium tetroxide is not understood.
It is known to covalently bind at sites of
unsaturation but is also reported to interact
with species such as proteins, amino acids and
sugars. It is likely that in addition to
producing the differential staining shown in the
present micrographs, the osmium tetroxide acts as
a fixative for these particular structures.

As with the shadowing technique, tea cream
from 5% infusions provides the simplest picture.
Again, the particles appear dense and
homogeneously stained, but now the diffuse and
ill-defined nature of the surface of the sphere

is more apparent. Each cream droplet has
occluded to its surface an amorphous layer of
precipitate which frequently extends into the
supporting phase. This is shown in Fig. 11. In
addition to cream droplets, some diffuse,
suspended material is observed. This sometimes
adheres to the cream and occasionally it is
freely suspended. Frequently small, poorly
stained, apparently hollow structures can be seen
(Fig. 10).

The discrete spherical nature of tea cream
from 5% infusions is clearly seen in scanning
micrographs Figs. 14 and 15. That the individual
cream droplets survive centrifugation and
dehydration can probably be attributed, in some
degree, to the fixing action of the osmium stain.
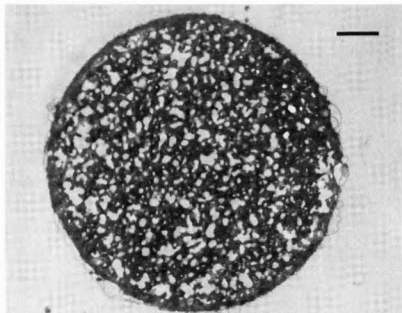
Fig. 12.   Sectioned tea cream particle from a 20%
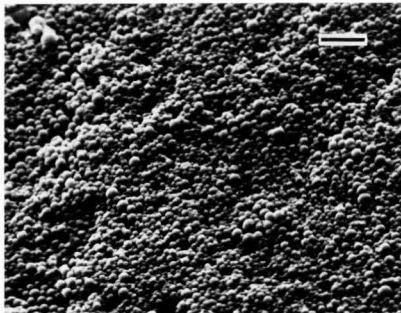   infusion.
           Bar = 500 nm.

Fig. 14.   Scanning electron micrograph of tea
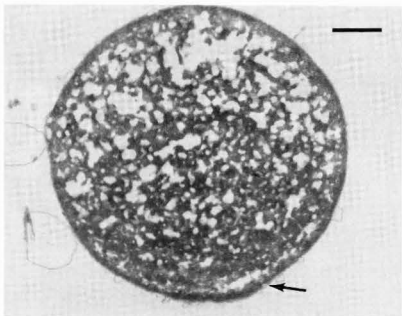   cream particles from a 5% infusion.
           Bar = 10 μm.

Fig. 13.   Sectioned tea cream particle from a 20%
           infusion. Arrow shows heavily stained,
           detached surface.
           Bar = 1 μm.
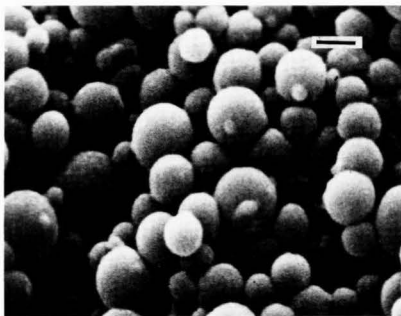

Fig. 15.   Scanning electron micrograph of tea
           cream particles from a 5% infusion.
           Bar = 2 μm.

On increasing total solids concentration from 5% to 20%, two major changes are observed. Most obviously, the particle loses the homogeneous internal structure (Fig. 11) and becomes heterogeneous, the internal material segregating into fractions, one heavily stained with osmium and the other unstained or, at most, weakly stained (Figs. 12 and 13). Furthermore, the particles now appear more densely consolidated than at 5% solids concentration. There are few cases of precipitated material extending long distances into the supporting phase and the particle surfaces are more distinct. It is noticeable that the more strongly stained material forms a continuous surface round the tea cream droplet. This surface, or skin, is occasionally pulled away from the remainder of the particle in some micrographs (Fig. 13). As with 5% preparations,

apparently hollow structures can be seen and in many cases these are strongly occluded to the cream particle surface.

On increasing to 50-60% solids concentration, only the cream phase can be detected by optical microscopy. It may be assumed, however, that the type of internal segregation noted on the 20% cream particles also takes place at the higher solids levels.

Freeze-etching has been found to be the most reliable technique for estimating the total solids concentration at which the dilute phase disappears (Fig. 16). No supernatant phase has been distinguished in preparations at 60% solids and so we conclude that only the cream phase exists at this concentration. Micrographs of freeze-etched samples taken from the top and bottom of a pellet produced by centrifugation of unstained tea cream are shown in Fig. 17 and 18
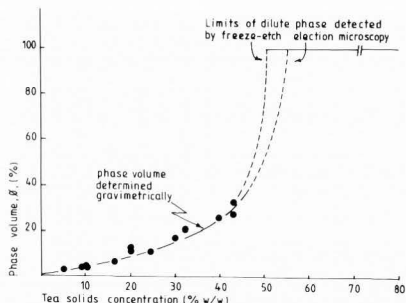
Fig. 16. Phase volume of tea cream as a function of tea solids concentration at 25°C.
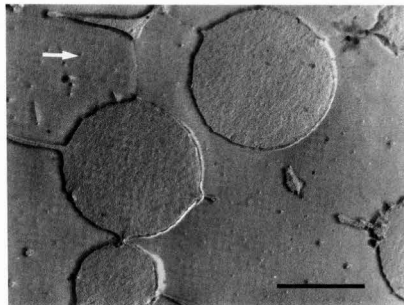

Fig. 17. Freeze-etched tea cream from a 5% infusion.
Arrow indicates direction of shadowing.
Bar = 1 μm.

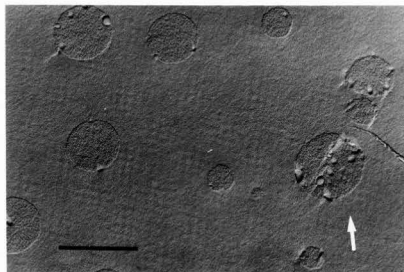
Fig. 18. Supernatant inclusions in tea cream pellet formed by centrifuging a 5% infusion.
Arrow indicates direction of shadowing.
Bar = 1 μm.

to illustrate how clearly the phases can be distinguished. At the top, tea cream droplets can still be clearly identified whereas at the bottom of the pellet, the cream phase is now continuous and spherical inclusions of the dilute phase are seen.

In Fig. 17 the cream droplets project above the background material (shadowed from left-to-right), as expected from the relative solids concentrations in the two phases (Fig. 7). Fig. 18 illustrates the reverse situation where the dilute phase is discontinuous and is etched below the level of the surrounding cream phase (shadowed from the bottom).

The sequence of events occurring with increasing tea solids is laid out schematically in Table 1. Taking the significant concentrations in order, in the region of 0.1%, (at least) two types of subunit are present. These presumably are composed of material which is inherently insoluble at this concentration or which is intrinsically soluble but has been rendered insoluble through formation of a complex with another soluble component. Whichever is the case, on increasing the concentration (above 1.0% solids), these subunits aggregate and finally knit closely together, the compaction to a sphere of heavily hydrated precipitate being completed between 1-5% solids. The progression is presumably accompanied by internal rearrangement within the tea cream droplet so that these polymeric materials carrying the highest percentages of hydrophilic groups are located towards the exterior with the least hydrophilic ones innermost.

Table 1

Tea solids concentration (% w/w)	Comments
Infinitely dilute	molecules in solution
0.1	subunits + solution
1	tea cream phase + solution
20	tea cream phase with internal segregation + solution
50-60	liquid tea cream phase only
100	tea glass

The processes in the above sequences could be explained by considering the primary polymeric solutes to have hydrophilic (ionic or polar) and hydrophobic regions. As solute levels are increased, the competition for the available water would be increased and the most hydrophobic parts of the molecules would be increasingly excluded from solution. This explanation of tea cream formation implies that the phase results from hydrophobic interactions (5,15) (cf. surfactant micelles). Alternatively, the cream

phase may separate as a result of complex coacervation - this arises from the mutual attraction of oppositely charged species (3, 6).

At concentrations greater than 20% solids, two separate structural elements can be clearly identified within the tea cream. Such separation may result from a negative heat of mixing two polymers and, whilst only detectable microscopically at these concentrations, similar effects may be contributing at much lower total solids to the overall phase behaviour.

As the chemistry of the components described is not yet resolved, these mechanisms cannot be progressed beyond speculation. However, it seems unlikely that tea cream formation takes place solely by hydrophobic bonding but that the process results from a combination of hydrophobic bonding and simple and complex coacervation of material onto a nucleus of the inherently insoluble species of tea.

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References

1. Cattell DJ, Nursten HE. (1976). Fractionation and Chemistry of Ethyl Acetate Soluble Thearubigins from Black Tea. Phytochemistry. 15, 1967-1970.

2. Glauert AM. (1974). Practical Methods in Electron Microscopy. North-Holland. Amsterdam 3 (1), 144-148.

3. Kruyt HR. (1949). Colloid Science - Irreversible Systems. Elsevier, Amsterdam. II, 230-258 and 433-482.

4. Millin DJ, Rustidge DW. (1967). Tea Manufacture. Process Biochem. 2, 9-13.

5. Mittal KL. (1976). Symposium on Micellisation, Solubilisation and Microemulsions. Albany, N.Y., Plenum Press, New York.

6. Overbeek JTG, Voorn MJ. (1957). Phase Separation of Polyelectrolyte Solutions. Theory of Complex Coacervation. J. Cell. Comp. Phys. 49, (Suppl. 1), 7-26.

7. Pearse AGE. (1980). Histochemistry : Theoretical and Applied. Churchill Livingstone. Edinburgh. 4th ed. 1, 114-119.

8. Roberts EAH. (1962). In Chemistry of Flavonoid Compounds. Pergamon, London. Geissman TA, (Ed.) 468-512.

9. Roberts EAH. (1963). The Phenolic Substances of Manufactured Tea X. The Creaming Down of Tea Liquors. J. Sci. Fd. Agric. 14, 700-705.

10. Roberts EAH, Cartwright RA, Oldschool M. (1957). The Phenolic Substances of Manufactured Tea. Fractionation and Paper Chromatography of Water Soluble Substances. J. Sci. Fd. Agric. 8, 72-80.

11. Rutter P. (1971). A Physicochemical Study of Tea Cream. PhD. Thesis. Leeds. UK.

12. Rutter P, Stainsby G. (1975). The Solubility of Tea Cream. J. Sci. Fd. Agric. 26, 455-463.

13. Schmidt DG, Walstra P, Buchheim W. (1973). The Size Distribution of Casein Micelles in Cow's Milk. Neth. Milk Dairy J. 27, 128-142.

14. Smith RF. (1968). Studies on the Formation and Composition of Cream in Tea Infusions. J. Sci. Fd. Agric. 19, 530-534.

15. Tanford C. (1973). The Hydrophobic Effect : Formation of Micelles and Biological Membranes. Wiley-Interscience, New York.

16. Thorpe's Dictionary of Applied Chemistry. Longmans. London. 4th ed. XI, 451.

17. Vuataz L, Brandenburger H. (1961). Plant Polyphenols III. Separation of Fermented and Black Tea Polyphenols by Cellulose Column Chromatography. J. Chromatog. 5, 17-31.

18. Wickremasinghe RL, Perera KPWC. (1966). Analysis of Cream of Tea. Tea Quarterly. 37, 131-133.

Discussion with Reviewers

R.J. Carroll: What is a fine thread-like material observed in the background of Figure 5?
Authors: Figure 7 shows there to be a significant concentration of tea solids dissolved or suspended, which is not incorporated into the tea cream phase. This can also be seen in Figure 11, where loosely aggregated material extends outside the tea cream phase. We take the fibrous material seen in Figure 5 to result from such polymeric material originally present in the dilute phase. Its chemical nature is not known.

W. Buchheim: How do the authors explain the fibrous material on Figure 5 and its absence in Figures 3, 4 and 6?
Authors: Figures 3 and 4 show tea cream particles from infusions of low concentrations (0.1 and 1.0 respectively). It is assumed that these concentrations are below that required for the necessary level of polymeric material to be

present in the dilute phase. At the higher concentration (20%), shown in Figure 6, it is possible that the material which produces the fibrous structure in Figure 5 has been precipitated from solution by build-up of the very soluble solutes and ions present in black tea. Indeed, such further precipitation could be responsible for the occluded solid material noted in Figure 6.

P. Walstra: Do the authors have any idea of the magnitude of the interfacial tensions between tea cream and the supporting medium as a function of concentration and temperature?
Authors: No, these data have not been measured. At present we have no idea of how clearly the interface is defined. As the tea cream contains a substantial proportion of water (~40%) the idea of a classical interfacial tension cannot be followed too rigorously. However, the droplets are naturally spherical and it is recognised that a study of the apparent interfacial tension may be useful in understanding the cohesive forces between the components of the tea cream phase.

P. Walstra: Do the authors agree that besides hydrophobic bonding and electrostatic interaction the conformational entropy of either phase and the interfacial free entry between them should be considered to explain the association?
Authors: Yes, the formation of tea cream is a highly complex process which is certainly controlled by many factors. The morphology we have described here is that which forms spontaneously on slow cooling and it should also be remembered that more rapid formation rates result, for example, in smaller tea cream droplets and particle flocculation (11).

D.P.Dylewski: How does time affect the morphology and chemistry of black tea cream?
Authors: Although tea cream particles settle out with time, provided there is no microbiological contamination, the infusions remain stable for several months and the suspensions can be redispersed by gentle shaking. The effect of time on the chemistry of black tea cream has not been examined, though it is assumed that further polymerisation of the polypherolic components is possible. Some increase in particle size seems to take place but the bulk of the increase has occurred in the first four days.

W. Buchheim: The osmium tetroxide stained cream droplets shown in Figure 10 and 11 differ decisively in their fine structure from those in Figures 12 and 13. Is this structural difference unequivocally related to compositional differences or could preparatory artifacts have occurred? What did the freeze-etched preparation of a 20% infusion look like?
Authors: We are confident that the internal structure of cream droplets from 5 and 20% infusions are different and that the areas of unstained and stained material in particles from 20% infusion represent segregation of material within the cream droplet. It can be speculated

that these respective regions are built up from the strongly staining and poorly staining material noticed at lower concentrations (Figures 8 and 9). We have no freeze-etched preparations from 20% infusions.