Plants, colloids and tinctures – nature's pharmaceutics

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For many years, modern pharmacy has been unravelling the complexities of colloid science in an attempt to improve the absorption by the body of water insoluble drugs. Here, for the first time, evidence is produced which shows that a fundamental evolutionary characteristic of most, if not all, plant molecular biology provides just such a universal mechanism for the solubilisation of natural biologically active compounds.

This realisation leads to the hypothesis that liquid structures called 'microemulsions' may be at least partly responsible, not only for optimisation of plant secondary metabolites in nature, but also for unique aspects of herbal medicine, especially of tinctures. It is claimed this characteristic may be the physical basis of the quality of 'vitality' that many herbalists apply to their medicine.

The result of almost ten years research and practical experience growing, manufacturing and analysing commercial extracts at Rutland Biodynamics, this relatively simple breakthrough offers important new understanding to herbal pharmaceutics at a time when quality issues are under the spotlight by regulators.

Plants, colloids and tinctures – nature's pharmaceutics
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Preface

DESPITE many years of systematic study, tinctures and their use continue to pose many perplexing problems to science. Anyone coming for the first time from the outside world to traditional western herbal medicine might wonder why we prepare such an enormous breadth of plant medicines (mainly the flowering plants or *Angiospermae*) in such a basic and similar way. Is the use of the tincture merely a convenience, and is it just laziness that stops us finding better ways of preserving the biological activity of plants? What is the function of alcohol in tinctures? Does alcohol affect the biological quality of herbal medicines and if so, how? Why, after centuries, has no serious replacement for the use of ethanol been found? Why do batches of tincture made in apparently identical ways have different keeping qualities? What determines the 'shelf-life' of a tincture? Why do sediments appear in some batches and not in others? Why does sediment sometimes appear immediately and at other times many months after making? Do these factors affect therapeutic quality? Given so many imponderables, is it possible to distinguish a good batch from a poor one? If so, how?

The difficulties are perhaps compounded by the idiosyncrasies of herbal science, which may derive to an extent from the fact that the main *principles* of herbal science pre-date modern scientific enquiry. Many, if not most herbalists think of herbal quality in terms of 'vitality', a notion that modern science finds hard to define. More controversial is what to some scientists is an apparent misunderstanding of the thermodynamic concept of 'energetics', perhaps as a result of the current differences between the concept of 'energy' (*qi*) in Traditional Chinese Medicine and that of post-Cartesian Western science.

Over a narrow dose range amongst the stronger acting drugs, response is sometimes proportional to the concentration of a physiologically active compound. When herbalists dispense from a catalogue of thousands of products, in which the whole plant part used is defined by the European Pharmacopoeia as the 'active ingredient', the adjustment of *homeostasis* depends on very much more than the concentration of 'active principals' in the pharmaceutical dosage form. Therefore the suggestion often implied that physiological response to multi-factorial and complex biological agents is proportional to some ill-defined 'active principal' is an elementary logical confusion between sufficient and necessary pharmacological causes. Nevertheless, the inexactitude of our knowledge of the relationship between dosage characteristics and plasma concentrations does give rise to hindrance of the advancement of herbal medicine by pharmacy-dominated regulation.

The following pamphlet shows through scientific experiment, for the first time, that some of these and similar fundamental questions may one day be largely resolved by the application of a "microemulsion theory of herbal tinctures". The

corollary of this theory may be that the 'vitality' of herbal tinctures may indeed be some function of the surface energy of dynamic molecular components. Although this is a modern scientific pamphlet, it's conclusions appear to lend support to the ancient concept of vitality in herbal medicine. Perhaps this may help to bring together disparate, and sometimes warring, ideas and ideals.

Future work may include testing the hypothesis over wider ranges of natural products and of using such science to unlock the controversial secrets of the *steigbildemethode*, a form of paper chromatography that depends on molecular surface activity.

This pamphlet is being privately published and distributed, under the banner of *Herbal Research Notes*. This is because of the unfortunate lack of herbal scientist peer-reviewed journals published in English at the present time. This has allowed, however, an opportunity to present exactly what the author intends, rather than having to write for an editor – an unusual luxury. This has been indulged by way of a rather longer than usual, albeit still brief, introduction to the background science of surface phenomena and partially ordered systems, and why this is important to biological activity of orally ingested plant extracts. As not all herbalists have science backgrounds, Sections 1–4 are aimed at outlining relevant areas of physical chemistry that a professional journal of pharmaceutics or phytochemistry would consider superfluous, yet most herbal publications may deem too technical. The experimental data are presented in a 'stand-alone' scientific format in Section 5 and so scientifically trained readers may wish to omit Sections 1–4 and move directly to Section 5. Informed criticism is welcomed by the publisher/author, at the address above. In this way, the publication is designed to be accessible to all, whilst covering the new ground in a scientific way. It is hoped that this may generate sufficient interest from those involved in the field to develop into a web blog, or even to blossom into a further publication. We hope that further editions of *Herbal Research Notes* may issue in the future. Potential contributors are encouraged to contact the publisher.

This work is dedicated to all the many colleagues in the herbal profession who have asked difficult and probing questions over the last decade. To give due credit to all those who have helped this work along the way would be quite impossible. The author is particularly indebted to Professor Jack Woolley, who has been most supportive of efforts to put practical science in the way of herbalists and for writing the Foreword to the first edition; to Krystyna Krzyzak MNIMH for copy editing the text; to Dr Midge Whitelegg for her enthusiastic support and to Dr Dave Greenway (who provided the ultra-centrifuge results), both of the University of Central Lancashire. To Dr Haijo Knipgenger of the Natural Science Department, Goetheanum, Dornach, Switzerland, for his suggestion of a colloidal basis for the *steigbildemethode*, Dr Jens Otto, Steiner Project, Arhuus, Denmark who demonstrated that method to me and to Dr Hans Strüh, WALA, Stuttgart,

Germany, my thanks, not only for putting up with all my questions about quality and its determination by 'sensitive' anthroposophical methods but also for going out of their way to try to explain some of the problems, thus giving stimulus to this work. Lastly, but by no means least my thanks to all my colleagues at Rutland Biodynamics Ltd, in particular to Kate Stokes MSc, chemist and forensic scientist, for her tireless help with experiments and data collection.

The author alone bears all responsibility for all the opinions expressed and, of course for errors in the text.

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Brooke, Rutland, UK
2008

Note to Second Edition

This second edition has been prompted by a perceived need to modify the emphasis on the ubiquitous occurrence and function of microemulsions throughout Nature and particularly as a factor in the xenobiological activity of plants, of which herbal medicine is one area. A technical error in the first edition, kindly pointed out by Dr Alan Lakin, has been corrected in the second edition. The title of this edition has been amended.

Foreword to the first edition

HERBAL practitioners treat their patients as individuals since responses differ from patient to patient. Similarly they recognise that plant preparations, being natural materials, vary and they are skilled in adjusting doses (and in some cases mixtures) accordingly. Mainstream medicine now recognises that, what we might call "pharmaceutical" drugs, are metabolised differently in different individuals and there has been much discussion that tailor made drugs will be available in the future designed according to the patients' genetic patterns as seen through single nucleotide polymorphisms (SNPs) in their DNA profiles. So it appears that the aims of both schools are the same. However, there is much distrust between the two parties and this is unfortunate. Physicians often forget that many "pharmaceutical" drugs that they prescribe are natural materials. It is unlikely that physicians tell patients suffering from leukaemia that their medicine comes from the leaves of a Madagascan plant (*Catharanthus rosea*), or that lung cancer cases are treated with a medicine derived from the underground parts of a Himalayan bush (*Podophyllum hexandrum*). Can you imagine a consultant saying to a woman suffering from ovarian cancer: "I've got just the thing for you – it's from the leaves of poisonous yew (*Taxus baccata*) – it will have you right in no time." Not very likely is it? Indeed a consultant would probably think that it undermined his professional credibility to say such and the patient might think he was acting like some sort of modern day witch doctor. Trust and truth aren't always the same thing. Critics from the "authentic" medical profession often dismiss the use of herbal remedies because there is no **evidence** that they work. This is not true. For example, the fruit of saw palmetto (*Seranoa serrulata)* has been shown to be as effective as the "authentic" treatment, finasteride, in the treatment of benign prostatic hyperplasia (Sokeland, 2000; Van Coppenolle et al, 2000). There are many other examples (Barnes, Anderson and Phillipson, 2002).

Manufacturers of herbal remedies have a special responsibility to ensure that their products are made to the highest standards. Quality control, therefore, is a critical issue. The product has to have what you want in it, but exclude what you don't. As far as potentially toxic components (for example herbicides or pesticides) are concerned then the organic grower has a significant advantage. Monitoring what you want in a product is difficult. The manufacturer cannot test it on a patient each time to ensure that it is satisfactory and to ascertain the correct dose. Most resort to some form of chromatography to match the profile of their product with an authentic standard. Or perhaps they will monitor the product for the content of a single component.

Herbal practitioners have always insisted that whole plant extracts, not single

components therefrom, provide the "correct" medicine; the whole being more efficacious than the sum of the parts. There is now scientific evidence to support the hypothesis of synergy between components of natural medicines (Williamson, 2001; Adams, 2006).

In mainstream medicine, it is now well understood that having the correct dose of a particular drug in a medicament does not necessarily lead to the correct therapeutic blood levels required for efficacy. The correct formulation of the drug is a vital consideration when preparing liquid or solid final dosage forms. Bioavailability is the term used to describe the factors that govern the transfer of the medicament from the medicine into the blood stream and thence to the site of action. Formulation of the medicament crucially influences bioavailability (Ashford, 2002).

In the following article Paul Chenery describes some interesting and original work on the physical nature of tinctures and tries to explain why there is variability and variable bioavailability in herbal products that are prepared in apparently identical ways.

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1 Introduction

ALTHOUGH it is apparent that many of history's famous herbalists preferred to use plants fresh from the fields, garden or hedgerow whenever they could, for the sake of convenience and in the absence of technology in earlier days, herbal medicines have always been traded in their dried form. As for thousands of years herbs were the main source of medicine, modern scientific medicine thus grew up around dried herbs and their extracts. Western pharmacy became a profession and an industry in the nineteenth century through the systemisation of the vast array of dried plant materials provided by the growth of imperial trade. Modern plant chemistry (phytochemistry[1]) has subsequently unravelled a great deal of the chemistry of dried plants and has discovered and isolated many plant molecules with medical benefits. Yet the 'active substance' of herbal medicine as defined by the European (and other) Pharmacopoeia is the whole herb material, not just one or more compounds. Modern pharmacy thus became predominantly a 'single molecule' phenomenon at the same time as the molecular understanding of herbal medicine has become increasingly complex.

Once a plant cell is dehydrated, the delicate balance of forces that previously preserved its homeostasis during life, forces that Erwin Shroedinger (1943) called *negentropic* processes, are destroyed. These cannot be re-hydrated. It is not possible to bring a dead plant back to life by putting it into water (with a few exceptions, such as plant seeds). On other hand, some molecules, such as 'menthol', for example, can be recovered.

One difficulty perceived by science with the use of conventional dried herbs in modern medicine is that the apparently active complexes often consist of both water-soluble and water insoluble (fat or lipid soluble) components. Modern pharmacy expends considerable effort into the formulation of similar molecules or complexes into special physical structures, generally termed 'microemulsions', such as liposomes and other nanoparticle fluid structures, so that they can be easily transported through the patient's physiology in order to target very specific molecular ligands.

As is shown in this pamphlet, however, the natural world has been using these methods throughout evolution. Not only are most biological fluids microemulsions, but this fact must have been pivotal in the evolutionary development of interactions between living species (known also as *xenobiological*[2] systems). Although humans have been using dried herbs for many tens and perhaps even hundreds of thousands of years, we tend to forget that herbs have been used by people and animals in the natural fresh state for many millions of years before that.

[1] Phytochemistry: chemistry of plants.

[2] Xenobiological activity: Causing biological activity (physiological effects) in other species. We might thus say that medicinal herbs exhibit 'xenobiopharmaceutical' activity.

The biological activity of plants is not accidental but the result of extremely ancient and well developed *biologically inter-active systems;* this is the case despite the subsequent adaptive redundancy of many of these systems (Rosenthal and Janzen, 1975). Such systems are absolutely fundamental to the survival strategies of plant species throughout evolution and are often referred to as *plant defensive systems*, although the term *competitive systems* might be considered more appropriate for the modern age. Nevertheless, plant medicinal activity is a special case of biological activity, because the evolution of specifically medicinal actions may have been incidental to the selection pressures on plants.

As the herbalist is generally more interested in the transference of the entire biological activity of a medicinal plant to the patient than in the purification of single active compounds, an essential task for the herbalist is to discover more about the xenobiological mechanisms utilised by plants. This paper first briefly discusses one such type of mechanism that arises from the physical chemistry of ubiquitous cellular components (microemulsions), and then reports scientific experiments demonstrating the transference of these properties into herbal tinctures. The broad conclusion is that the ultimate quality of plant medicines may to an extent be dependant upon the maintenance of fully hydrated systems, throughout the process from living plant all the way to the patient's target cells.

Plant biological activity has been widely investigated and studies in plant ecology during the twentieth century underpin the Darwinian proposal that species only exist(ed) because their ancestors successfully took competitive advantage of every conceivable mechanism available in Nature. Continued advances in phytochemical techniques and the growth of data have allowed us to cloak this bare statement with very many exemplars of the rule. Plants are to the herbalist as to the pharmacist, however, much more than mixtures of 'active' compounds; they are dynamic, biologically active complexes. In this pamphlet, ideas from various branches of science, such as plant ecology, phytochemistry and basic physics, are combined into a unified theory that is proposed to explain many of the well-observed effects of herbal medicines.

When a plant suffers disruption to its cellular matrix, fundamental principles of plant chemical defence against predation apply. Although biological competition originated amongst plants before the evolution of animals, predation by herbivores over evolutionary time-scales generally, although not invariably, involves the ingestion and to a greater or lesser extent digestion, of plant material. Most herbal medicine, likewise, is administered by the oral route. Although plant parts are sometimes used for their very specific physical properties (such as the bulk laxatives, for example), it appears that the cells of the thousands of medicinal plant parts identified in the world's pharmacopoeia and in the *materia medica* of herbalists often contain only very small fractions of essentially biopharmaceutically active chemical compounds and complexes. These are compounds that exert physiological effects, usually when absorbed

by various means into the circulatory system, which in the case of higher animals is the blood stream.

Compounds designed to promote biological fitness have, by their very nature, had an unpredictable and often complex evolution and the concept of a chemical 'arms-race' has been much used in the discussion of the co-evolution of optimal defences between plants and herbivores (Rhoades, 1973). During biological evolution, this often resulted in complex and energetically 'expensive' biosynthesis of pivotal compounds. Consequently, systems would be expected to have evolved to optimise the *efficiency* of these compounds. For example, sometimes metabolites are produced only or mainly in response to various 'stressors'. Plant physiologists such as Janzen (1973), who proposed a secondary metabolites 'community structure', have recognised for many years that plants have evolved whole systems in order to optimise the value, or effectiveness, of these compounds in biological situations, even including co-operative activity with the other species with which they cohabit. Although many active compounds may have evolved a long time prior to mammals, nevertheless many of the most basic biochemical routes are conserved amongst the metabolism of species, especially in the endocrine system (Baker, 1995; Stoka, 1999). Thus compounds produced by plants as, say, insect endocrine modifiers (phytoecdysones), may still have an effect, albeit a quite different effect, in the human endocrine system.

The form of presentation of these valuable compounds has thus always been a very critical control point in the evolution of defences by plants. As whole plant cells are also disrupted during modern extraction processes such as tincture production, it may be useful to look for evidence of natural *biochemical* mechanisms unlocked when this occurs, in order to ensure that such mechanisms are maintained in the extraction process.

It therefore seems natural for the herbalist to ask what mechanisms plants might use to *optimise* the effectiveness of the complex and biologically 'expensive' compounds that were designed by long time-scale evolutionary pressures to have activity in other species, when orally ingested.

Plant physiologists have for a long time recognised that such processes generally extend to the whole plant tissue, as it is this, rather than chemical compounds or complexes, which makes up the fundamental living unit. For this reason, the active ingredient is generally regarded as the whole plant tissue. Tinctures consist of water, from 25% to 90% alcohol and from about 2% to about 20% dissolved plant components (the exact proportion varying widely, depending on the plant species, the nature of the starting materials and the formulation). Simple experiments, reported below, show that tinctures are in fact colloidal dispersions. Traditionally in phytochemistry, extracts for analysis are taken from freshly harvested samples (Harborne, 1998). The dissolved solids of whole plant tinctures made from such high quality material contain significant proportions of macromolecules, such as lipids, sugars and proteins. In fact the total percentages of such

molecules in the freshly extracted plant are far greater than that of biopharmacological, active compounds.

Colloidal mixtures of biologically detergent amphiphilic compounds, such as the phospholipids or glycolipoproteins which are abundant in plant cells, would be expected under basic physical theory to spontaneously form partially ordered systems known as emulsions. If this is true, this is a significant finding, because at certain critical concentrations such dynamic systems form aggregates called *micelles*. When ingested orally, micelles can be responsible for 'smuggling' otherwise insoluble active compounds into the blood-stream in remarkably efficient ways that may completely or nearly completely avoid the body's defence mechanisms, especially in a digestive 'first pass'. This enhanced adsorption of active compounds may thus be a very important way in which the biosynthetically 'expensive' molecules are optimally conserved in xenobiosis. If so the mechanism may be critical to the way in which herbal medicines work and so it was decided first to investigate whether there is evidence for micelle formation in tinctures.

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2 Elementary theory: Phases and mixing properties of molecules

THE physical world largely results from electrostatic interactions (or 'weak' forces) amongst molecular compounds. Attraction and repulsion amongst biological molecules allows the performance of the basic molecular functions of life within the boundaries of cells. These same forces create the membranes that give form to organelles and even the organs themselves.

For convenience, matter tends to be thought of as existing in a number of different phases, such as solid, liquid, vapour, gas. Much of our understanding of these phases depends upon characteristic behaviour. We think of solids as unflowable and of liquids as flowable. Often phase transformation occurs with changes of temperature or pressure, for example ice turning to water and thence to steam and so on. On closer study, we find that in fact many solids (such as glaciers and landmasses) do indeed flow and also that the behaviour of liquids depends very much on their components. Because we are here interested in herbal medicine and because pharmacological activity (and almost all plant–animal interactions) occurs in the liquid domain, we shall concentrate our study on certain characteristics of liquids. Depending on their components, liquids may separate into two or more further phases, such as an oil phase and a water phase and so on. At the interfaces between different liquid phases, special conditions exist which are of great significance to the way in which the natural world is constructed.

Liquids may be chemically 'pure' substances, such as pure water or pure

ethanol, or they may be *mixtures* or *solutions*. Those that are both mixtures and solutions are referred to as mixtures. A further system, which is very widespread in Nature, is the *colloid*[3], in which compounds are dispersed within a dispersal (or continuous) medium, in a specially structured way.

Mixtures are combinations of two or more substances that do not react chemically together (by chemical bond breaking and formation). In mixtures, each molecular compound retains its own identity and its individual characteristics within the mixture. Although they do not react together chemically, nevertheless, because they mix, components of mixtures do have physical inter-relationships resulting from the weak forces that exist between them. Mixtures may be said to be either *homogeneous* (that is, uniformly mixed throughout) or *inhomogeneous*, or *heterogeneous*, when the mixture in not uniform. The different parts of a heterogeneous mixture will, at any given point in time, contain varying proportions of the components. An example of a homogeneous mixture is that of ethanol and water at room temperature, which mix completely in any proportions. The molecular structure of water and ethanol permit this complete mixing. We therefore say that ethanol is *hydrophilic*, because its molecules 'like' water molecules and mix freely with them.

An example of a heterogeneous mixture is when sand and water are put together, or when oil and water are shaken together. In these cases, the *immiscible* components will settle out, with sand falling to the bottom or oil rising to the top of a water mixture. The fundamental molecular structure of sand or oil does not permit them to mix at all with water. We therefore say that sand and oil are *insoluble* in water but also say that oil, being a fluid like water, is *hydrophobic*, because it is incompatible with water at the molecular level. The term *lipophilic* (meaning 'oil liking') is also sometimes used. The further terms *lyophilic* (and *lyophobic*) mean 'solvent liking' (or 'hating') and thus further broaden the concept into liquid media other than water-based. For simplicity, most modern authors dealing with water-based liquids just use the terms *hydrophobic* and *hydrophilic* and this convention will be followed here.

Solutions, which are uniform at the very smallest level of nature (ions, atoms or molecules), are special types of practically homogeneous mixtures, because some, usually reversible, chemical change may occur to some of the components on their dissolution. In *ionic* solutions, such as solutions of salts in water (called *aqueous solutions*), redistribution of chemical charge amongst components causes a shift in the reactivity of components.

Sometimes, the distinction between the terms 'homogeneous mixture' and 'solution' is not useful at all and may lead to confusion.

The concept of hydrophilicity, which is used very widely in scientific discussion, often appears to depend upon the suggestion of 'black and white' idea that

[3] In simple colloids, the dispersed medium may be gas, liquid or solid and (with the exception of a gas in gas), the dispersal (or continuous) medium may also be gas, liquid or solid.

molecules are either clearly hydrophobic or hydrophilic. It is the case, however, that molecules exhibit widely ranging *relative* hydrophilicity. Furthermore, the hydrophilicity of molecules may vary at different sites *within* the molecule and, critically for this discussion, within large molecules. Molecules with varying hydrophilicity at different sites are called *amphiphilic*[4] (or sometimes *amphipathic*). Large amphiphilic molecules (such as proteins that have water soluble as well as water insoluble sites within their structure) contort themselves in liquids and 'fold' in ways to satisfy the requirement for some areas of the molecule to mix with the solvent whereas other areas of the same molecule are unable to mix with the solvent. An interesting consequence of this ability is that the larger the molecule, the greater are the possibilities for varying hydrophilicity at different sites within that molecule. We know that most molecules made by living organisms are indeed very large and complex in comparison to those in the inanimate natural world and might speculate that a main reason for the widespread evolution of such large molecules is the special characteristics that come from having varying relative hydrophilicity at specific sites along the molecule. A well-known example of this effect is when enzymatic proteins fold in very special ways in the presence of water, giving themselves a molecular conformation that is often essential to living processes. Correct folding is essential for health and if the molecule has a small fault this may lead to one of a number of metabolic disorders known as 'misfolding diseases', for example cystic fibrosis or Marfan syndrome. The ability to 'code' relative hydrophilicity in different ways along the peptide backbone of proteins has given living organisms an almost limitless store of potential reactivity within the proteins.

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3 Interfacial and surface phenomena

WHEN immiscible liquids are placed together and they separate into different phases, an *interface* between the two phases exists. At this interface between these phases certain special conditions exist. This a vast and interesting subject, more about which may be found in texts such as *Bentley's Textbook of Pharmaceutics* (Rawlins, 1977). The presence of amphiphilic molecules in such mixtures may radically alter the characteristics of the interface. This is because amphiphilic molecules accumulate at the interface, tending to both mix with, and also not mix with, each or either of the two immiscible phases. This property is called *amphiphilic surfactance*. Under certain circumstances, the presence of a surfactant can cause the *dispersal* of immiscible compounds intimately with one other. Such in-

[4] Amphiphile: a chemical compound possessing both hydrophilic and hydrophobic properties at different sites within its molecular structure.

timate dispersals are known as *emulsions*. The stability of some emulsions can be increased by the presence of a *co-surfactant*. Ethanol is possibly the co-surfactant best known to science (Everett, 1988).

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4 Dynamic, partially ordered systems

EXAMPLES of immiscible molecules are water and most lipids, and ethanol and most proteins. Convention dictates that if immiscible components remain solubilised after centrifugation at say, 12,000 × g for one hour, then the mixture falls under the broad definition of the term 'emulsion'[5]. Emulsions combine properties associated with colloidal sols[6] and amphiphilic aggregates (Evans and Wennerström 1999). Because emulsion droplets can transform in shape, size and number, these systems are very challenging to understand. Micelles spontaneously form in solvent from liquid amphiphilic molecules when they reach sufficient concentration to be able to cluster in a thermodynamically efficient way, with their hydrophobic tails together, in order to shield from the solvent, and their hydrophilic heads freely interacting in a solvated fashion with the solvent molecules. This accounts for the observations of breaks in the linearity of physical characteristics at certain concentrations. Some simple models of such clusters are reproduced in Figures 4.1 and 4.2.

Biological cells are a special type of micellar structure called a *vesicle* (itself containing compartments called *organelles*) held together within semi-rigid, relatively fluid walls built out of a bi-layer of amphiphilic molecules. These phospholipid molecules present an insoluble surface to both the inner and outer cell surfaces, yet retain a semi-liquid character that allows the water-based passage of many components such as nutrients and metabolites. Living cells, including all plant cells, contain a great preponderance of amphiphilic molecules, for example, those lipid bilayers preserving the integrity of membranes of organelles within the cell, as well as enzymatic proteins. Theory suggests that when such molecules as those found in herbal tinctures are dispersed in an aqueous medium, the 'oily' (or lyophobic) components are not mixed at the molecular scale, but remain separated by well-defined interfaces, onto which amphiphilic, surfactant molecules (such as proteins and lipids) are adsorbed in dense layers (Hoar and Schulman, 1943). There is a wide diversity of these structures, which are known under the general heading of *microemulsions*[7].

Microemulsions have been classified into various types based on their charac-

[5] Emulsions are mixtures of immiscible liquids, usually stabilised by an 'emulsifier'.

[6] Colloidal sol is the dispersion of a solid in a liquid medium.

[7] Microemulsions are clear, stable, isotropic liquid mixtures of oil, water and surfactant. Often they are found in combination with a 'co-surfactant', of which ethanol is the best known.

Figure 4.1 **Some simple models of types of micellar structure: (a) spherical, (b) disc-like, (c) cylindrical, (d) lamellar, (e) spherical vesicle (biological cells are such vesicles). The hydrophilic head groups of amphiphilic molecules are shown as filled circles and the hydrocarbon (or hydrophobic) groups are shown as tails.** (*after Everett, 1988*)

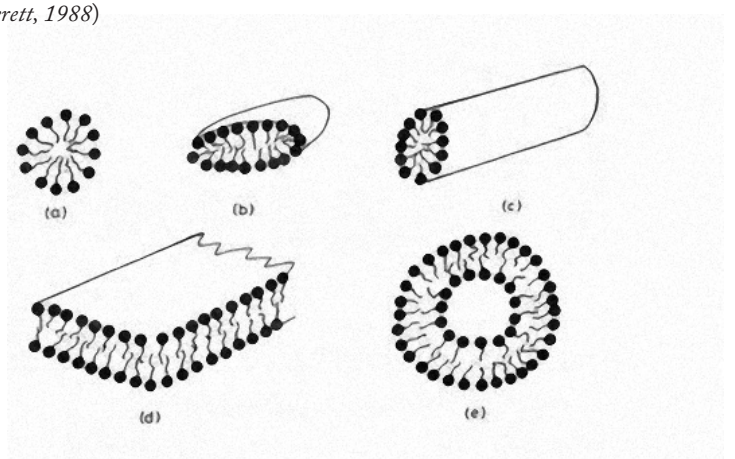
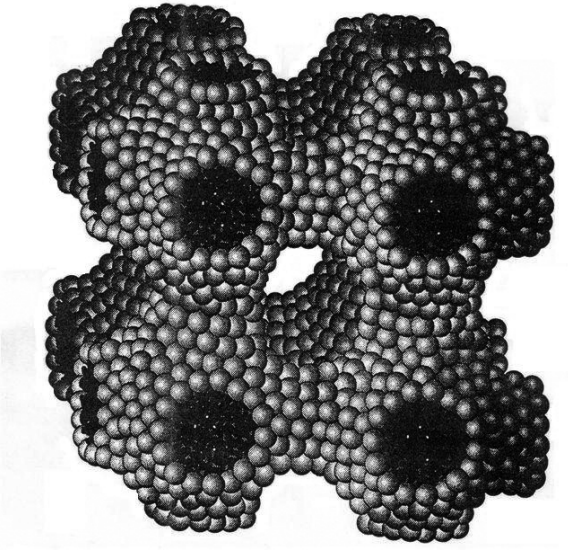


Figure 4.2 **Bicontinuous structures, in which two radii of curvature are equal but of opposite sign, leading to a small mean curvature, are also thought to exist in some emulsions** (*after Evans and Wennerström, 1999*)



teristics. A dilute microemulsion (containing only a few percent of dispersed phase, such as we find in tinctures) is usually observed as micellar (Auvray, 1994). Furthermore, in these dilute microemulsions, the mutual interactions between micelles are small, although not negligible. As the surfactant film of the droplets is flexible, then droplet deformation must be considered. This gives rise to the theory of 'polydispersity': droplet radii are distributed around an average value, and the shapes of droplets may vary, especially due to thermal fluctuations. The polydispersity and shape of the droplets are related to the curvature energy of the film, and possibly to the interaction between the droplets (depending on the concentration). The shape of droplets has been deformed experimentally by external perturbations (observation of magnetic birefringence of a strongly diamagnetic surfactant by Meyer et al, 1982).

More can be found in specialist texts, such as Gelbert et al (1994).

Simple structures can be characterised by a number of methods. If a molecule, or ion, is part of a larger aggregate for a period of time, its mobility, and thus conductivity and reactivity, is more limited compared with that of the free molecule, which is barely hindered at all if part of the continuous phase. Therefore methods can be devised to measure changes that are related to the mobility of a molecule when part of a partially ordered system. Some of these methods are shown in Figure 4.3.

In particular, surface tension measurements are considered to be one of the most direct ways of measuring changing interfacial characteristics. In Figure 4.3

Figure 4.3 **Effect of concentration of a typical surfactant on various physical properties** (*after Lindman and Wennerström, 1980*)

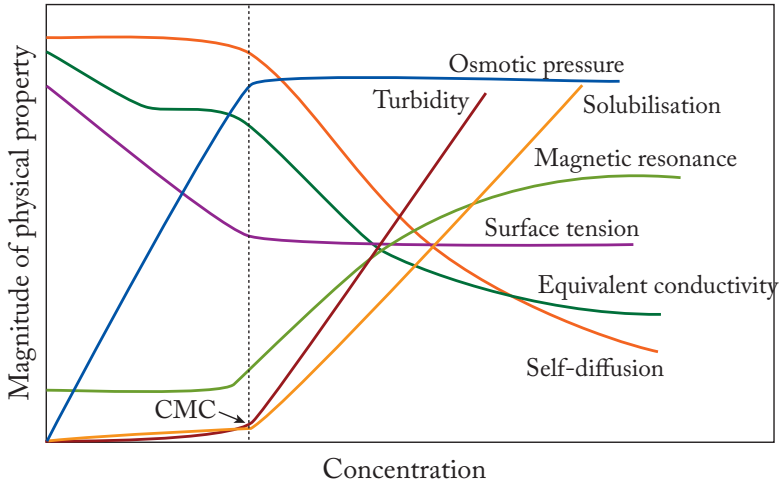
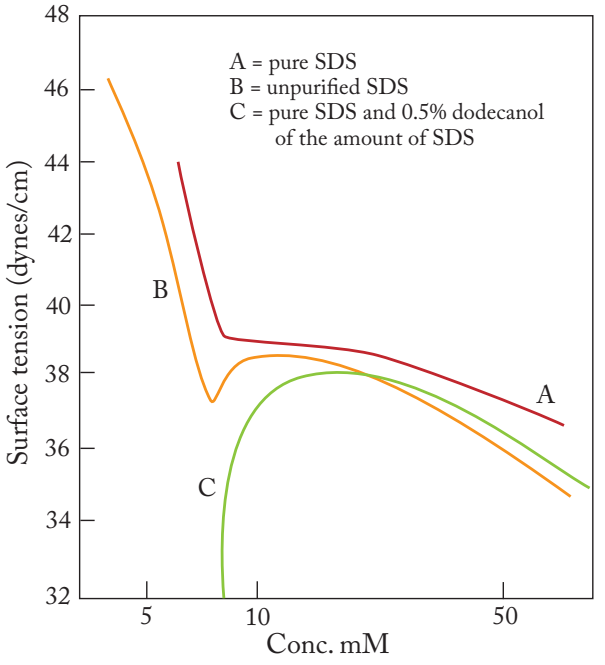


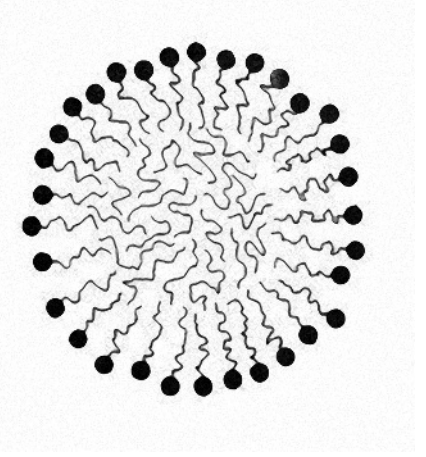
Figure 4.4 **Effect of concentration of dodecanol on surface tension of solutions of SDS** (*after Elworthy and Mysels, 1966*)



several physical properties show an abrupt change at a critical concentration, as opposed to the more gradual changes observed on dilution. It has been shown that this property is due to the spontaneous organisation into micelles at a critical concentration and is thus called the critical micelle concentration (CMC) (Hoar and Schulman, 1943). Figure 4.3 shows a typical simple model system, familiar to science, of a pure solvent, pure dispersed compound and pure surfactant. Figure 4.4, on the other hand, shows how the system is affected when more components are involved. By adding a further component to the simple model system, the shape and even direction of the graph in Figure 4.4 becomes extremely dependent upon the relative concentrations of the components. This is of interest to the study of herbal extracts because of the high number of inter-acting components involved.

Although microemulsions seem to consist of coherent regions of oil and water separated by sheets of surfactant, there is no consensus as to the overall total structure, which is most likely a dynamic one, especially in the case of mixtures as complex as tinctures. The '*efficiency*' of the solubilisation of the hydrophobic and

Figure 4.5 **Solubilisation may involve the incorporation of non-polar organic material into the core of a micelle. Many pharmaceutically active molecules are hydrocarbons that may be secreted into micellar cores** (*Illustration after Everett 1988*)



hydrophilic media is defined as increasing when the ratio of surfactant to these media needed to accomplish solubilisation, is lowered. This factor is assumed to correlate with some, still unknown, parameters of the structural relationships (Gompper and Schick, 1994).

The characteristics of macroemulsions, on the other hand, are even less well defined. These are thermodynamically unstable dispersions of at least two immiscible liquids and an emulsifier resulting from the adsorption of amphiphilic particles onto the interface between the lyophobic liquid emulsion droplets and the dispersion medium which, in the case of tinctures, is alcohol in water solution. Whereas stable microemulsions are always surfactant-stabilised, metastable macroemulsion mixtures may be stabilised by a surfactant, or by a polymer, or by small particles, with amphiphilic properties. The rate of development of macroemulsions depends on a number of factors, such as the solubility of the amphiphile in the dispersion medium. Macroemulsions usually require mechanical or chemical energy for preparation.

Microemulsions generally and amphiphilic micelles in particular are of interest in pharmacy because the micellar core consists of the thermodynamically ideal conditions in which to solubilise lyophobic components (Figure 4.5). General examples of such lyophobic components in herbal tinctures might be hydrocarbon fractions, chlorophylls, aglycones, essential oils, terpenoids, steroids, fat soluble vitamins, lipids, alkanes, polyacetylenes, some amino acids and alkaloids.

5 The microemulsion theory of herbal tinctures

5.1 Introduction

UNDER current regulatory regimes, the 'active ingredient' of a 'traditional herbal medicine' is defined as the whole plant tissue (the 'herbal drug') and not one or more purified molecules (Europe, 2002). Recently there has been a rapid revival in the use of such herbal medicines in the developed world (Mintel, 2003-2007). In the UK this contributed to a House of Lords Enquiry, which recommended further research into complementary medicine (UK, 2000). Because whole plant tissues are chemically complex, one of the biggest problems facing herbal medicine generally is controlling for the quality of efficacy. As a result of these difficulties, recent European law accepts proof of efficacy of botanically pure 'traditional' plant medicines merely on the basis of empirical report in the literature over 30 years (Europe, 2004). Although it is generally accepted that frequently the *in vivo* physiological activity of such traditional herbal medicines is due to complex synergy (or antagonism) amongst bio-active molecular components (Williamson, 2001; Gilbert and Alves, 2003), few studies have looked at the overall presentation of whole-plant extracts for any ubiquitous or underlying common bio-pharmaceutical factors. Plant extracts in water and ethanol (called 'tinctures') made up a large part of the Western Pharmacopoeia until recent times and these are still the most popular form of herbal presentation amongst Western practitioners. There are concerns, however, about unexplained variability found amongst tinctures. The project described therefore sets out to discover the cause of variations amongst batches of tinctures made in apparently identical ways.

Biological cells share many common characteristics, such as a preponderance of amphiphilic molecules, for example lipids and proteins. In life these afford a degree of physical stability to cells and allow control of their internal mechanisms. On preparation of thermodynamically stable aqueous whole-plant extracts, the hydrophobic components are not mixed at the molecular scale, but remain separated by well-defined interfaces, onto which amphiphilic, surfactant molecules (such as proteins and biologically detergent lipids) may be adsorbed in dense layers. These structures are known as 'microemulsions' (Hoar and Schulman, 1943). The simplest case is the spherical micelle but a wide diversity of structures is known to occur, such as bi-continuous worm-like threaded structures (Figures 4.1 and 4.2). The structural nature of these is dependent upon thermodynamic considerations, such as chemical potential, concentration and temperature. Microemulsions are always thermodynamically stabilised by surfactant molecules, sometimes co-effected by co-surfactants such as short-chain alcohols (Auvray, 1994). Less stable and poorly understood macroemulsions may also occur, resulting from surface adsorption, not only by surfactants, but also by polymers or particles (Evans and Wennerström, 1979).

The spontaneous formation of micelles within microemulsions at critical con-

centrations of surfactant amphiphilic molecules has long been a topic of great interest as a delivery system in conventional pharmacy. A critical quality issue in herbal medicine, therefore, is whether such structures spontaneously arise in plant products. Whole plant extracts are anything but pure model systems and it is well known that 'impurities' have dramatic effects on classical textbook critical micelle concentrations (CMCs) (see Figure 4.4).In a review, Eder and Mehnhert (1998) sub-divided compounds found in plant extracts into what they called 'main active substances' and 'concomitant', or 'co-effector compounds'. Schöpke and Bartlakowski (1997) investigated the influence of naturally occurring plant saponins of different molecular structure on the aqueous solubility of quercetin. These saponins had CMCs of between 20μg/cm^3 and 400μg/cm^3. The investigation concluded that solubilisation of quercetin is enhanced, not only by some pure saponins above their CMC, but also by some mixtures of saponins below their CMC, indicating an unknown mechanism of complex micelle formation. In the experiments described below, it was observed that most, or perhaps all, traditional herbal tinctures show one of the classical signs of colloidal dispersion, the Tyndall effect or scattering of light by particles in suspension. In the chosen model system, experiments with *Hypericum perforatum* show apparently complex CMCs, which may be due, in part at least, to the presence of proteins and lipids.

5.2 **Materials and Methods**

5.2.1 Materials

Tinctures of 137 different herbal drugs manufactured in the UK and commercially available to British herbalists were obtained from Rutland Biodynamics Ltd (UK). *Hypericum perforatum* plants were produced from seed supplied by Oregon Herbs, USA, 2002 to 2006 on registered biodynamic, Fladbury series III plots (deep, poorly drained medium gley loam alluvial clay soils over Marlstone rock and Jurassic river gravels), in the English Midlands at 130 m above sea level. Randomised samples from the top 15 cm of *Hypericum perforatum* plants were taken for analysis weekly throughout each of the years 2002 to 2006. The fresh plant samples were taken to the laboratory immediately after harvesting, shredded and macerated within an hour of harvest. Fresh homogenised herb (100 g) was placed in a 500 ml wide-neck container and covered with 200 ml of 96.5% (v/v) commercial partially rectified ethanol (distilled from fermented organic Paraguan sugar cane) and the container sealed. The resulting mixture was thus macerated for a minimum of two months at ambient room temperature (about 22°C). The crude tincture sample was then obtained by expression of the macerated product between two perforated stainless steel plates and decanted into glass containers, which were sealed and stored at a temperature not exceeding 25°C whilst awaiting analysis, during which some sediment was noted to occur.

It is considered one of the characteristics of microemulsions that they cannot be separated by centrifuge at 12,000 × g for one hour at 25°C (Aboofazelli et al, 2000). The samples were therefore prepared by centrifuge at 17,000 × g (average) for one hour at 25°C. The supernatant was transferred to a clean vessel for further investigations (described below) and any precipitate discarded. A small additional sample was subject to an average ultracentrifuge force of 171,500 × g for one hour and reserved for the second light scattering experiment described in paragraph 5.2.4 below.

5.2.2. Lipid analysis

The pH of the samples varied from 4.8 to 5.3. This was very gradually increased, initially by the addition, to the samples, of water and thereafter dilute ammonium hydroxide, until the pH reached 8. This produced precipitation across the whole range of pH from 6–8, which could be centrifuged into a pellet. When aspirated, the entire pelleted sediment was readily taken up into solution by a Bligh-Dyer (1959) mixture. A mixture consisting of methanol: chloroform: water (2:1:0.8 v/v) was added to the aspirated sediment and agitated by vortex for one minute. The resulting mixture was then gradually brought to methanol: chloroform: water in equal proportions (v/v/v). This was then subjected to two-dimensional thin layer chromatography (TLC) on silica gel 60 plates according to the method given for the analysis of lipids by Harborne (1998). The solvent in the first dimension is chloroform-methanol-acetic acid-water (170:25:25:4) and in the second dimension chloroform-methanol-7M ammonium hydroxide (65:30:4). Detection was with 25 % (v/v) sulphuric acid followed by heating the plate to 230°C and observing under white light. Sterols give a red-purple colour, glycolipids a red-brown colour, sulpholipids a bright red colour and other lipids pale brown colours (Harborne, 1998).

5.2.3. Protein analyses

These were carried out using the Bradford (1976) method. This method had the apparent advantage of a low pH and samples remained translucent during the experiment, which is essential for accurate spectrophotometry.

5.2.4. Tyndall effect

Using a lecture theatre laser pointer, a beam of laser light was passed through two cylindrical glass 30 cm^3 universal containers (VWR) in series. The first container held 20 cm^3 of an aqueous copper sulphate solution and the second container held the tincture sample that had been first subject to centrifugation at 17,000 × g for 1 hour. Observation of the passage of the light beam was arranged from above and perpendicular to the light beam, and was recorded photographically. Scattering of laser light beams was similarly photographically recorded in a sample before and after ultracentrifuging the extract for 1 hour at 171,500 × g (average).

5.2.5. Surface tension of the extracts at different concentrations
Various dilutions of the tincture sample were prepared (100%, 85%, 70%, 55%, 40% and 25% [v/v]) of a tincture sample in diluent (45% ethanol in water [v/v]). In order to ensure constant temperature during these experiments, the samples were stored in a water bath at 18 °C for 15 min before measurements were taken in the same water bath. Surface tension was measured according the method described by Fender (1956). Density (q) was calculated as one tenth of the mass in g of a 10 cm^3 volume at constant 18°C. The rise of each sample in a clean capillary tube was then measured. The surface tension (S) was calculated according to the formula given by Fender (1956):

$$S = RhqG/2$$

Where R = radius of capillary tube
q = density
G is a constant = 980 cm/sec/sec
h = the height of the column supported by the surface tension at the circumference of the meniscus.

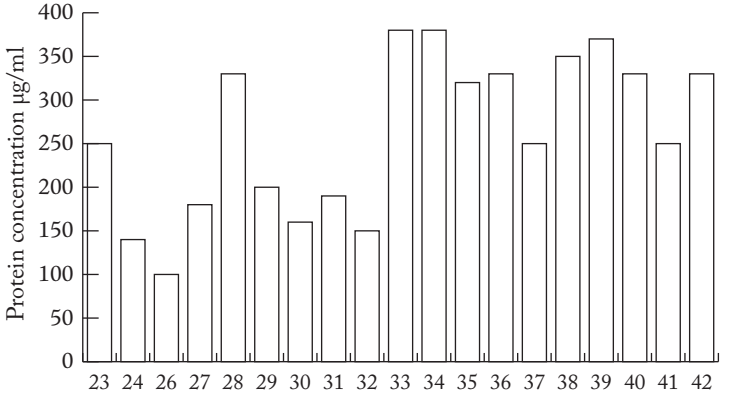
The average radius (R) was calculated from the volume of a cylinder, which was found by measuring the mass of a column of unit height of liquid drawn into the capillary and dividing the mass into the density to give the volume. Measurements were analysed using Sun Microsystems OpenOffice.org Calc. vs.2.0

5.3 **Results**

5.3.1 Lipids
The tinctures were found to be acidic (pH 4.8–5.3). Upon gradually increasing pH from 5.3 to 8 components precipitated, which after separation could be dissolved in a Bligh-Dyer (1959) solution. After two-dimensional chromatographic separation, treating with dilute sulphuric acid and heating to 230°C, glycolipids were identified as giving red-brown coloured spots, sulpholipids as a bright red and others as lipids giving pale brown coloured spots (Harborne, 1998). Biological lipids are difficult to purify and no attempt was made to quantify the lipid fractions identified in the samples.

5.3.2 Protein
Protein as measured by the Bradford (1976) method varied between 100 μg/cm^3 and 375 μg/cm^3, with an average approximately of 200 μg/cm^3 (figure 5.3.1). Generally, a greater mass of protein was found in plant material harvested in weeks 33–42 than in weeks 24–32.

Figure 5.3.1 **Protein content (µg/ml) of weekly harvested (2005) *Hypericum perforatum* tinctures as determined by the Bradford method (1976)**



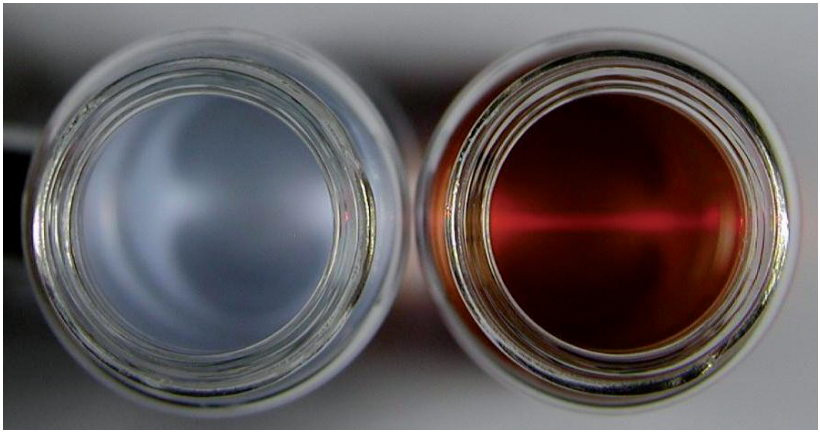
5.3.3 The Tyndall effect

Figure 5.3.2 shows that a focussed laser light beam can be clearly observed passing through the tincture sample (B) after centrifuging at 17,000 × g for one hour, whilst the beam is not visible when passing through the salt solution (A). This is explained by the fact that the salt solute particles (ions) are very much smaller than the wavelength of the light, and so their resulting surface area is too small to scatter the light. The particles in the tincture sample, on the other hand, have a sufficiently large surface area to scatter the light in all directions, permitting the observation of the beam passing through the tincture sample (rather like a projector beam in smoke). This is called the Tyndall effect and is characteristic of fluid colloidal dispersions. Although still a subjective test at this stage, all 68 *Hypericum perforatum* tincture samples obtained from 2002 to 2006 gave similar positive results. Subsequently, tincture samples of 137 different herbal drugs commercially available were subjectively examined in the same way. In all cases the Tyndall effect was observed. When laser light beams were similarly photographically recorded in a sample before and after ultracentrifuging the extract for 1 hour at 171,500 × g (average), no significant change in the intensity of the reflected light was observed (Figure 5.3.3).

5.3.4 Surface tension

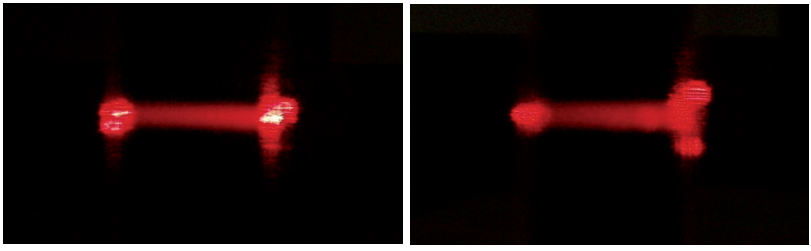
The surface tension (ST) of the tincture sample was measured over a range of dilutions representing concentration of the original tincture sample from 25% to 100% (Figure 5.3.4). There appear to be three distinct phases in the response of surface tension to dilution. In the phase 25–40% concentration of the orig-

Figure 5.3.2 **The Tyndall effect.** A laser light source is shone through two solutions, A and B. A is a 'true' solution of a copper salt. B is a sample of *Hypericum perforatum* tincture after centrifuging at 17,000 × g for one hour.



A B

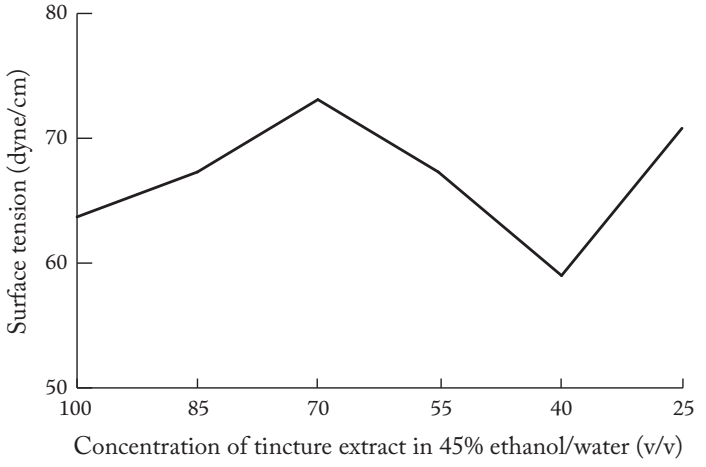
Figure 5.3.3 **Comparison of scattered light before and after centrifuging sample at 171,500 x g for one hour. Image (i) was taken in a 1-cm cuvette before centrifugation and image (ii) was taken after centrifugation, again in a 1-cm cuvette**



(i) (ii)

inal tincture sample, ST decreases with concentration. In the phase 40–70%, ST increases with concentration. Between 70% and 100%, ST is found to decrease again with the ST of the mixture tending towards that of water, which is 72.8 dyn cm^{-1} or mNm^{-1} at 20°C. Thus there appears to be a distinct sharp break in the relationship at the dilution representing a 40–70% concentration of the tincture sample in diluent. Between the points 40% and 70%, significant changes occur in the ST of the mixture. Below 40%, ensuing dilution of the mixture results in an abrupt reversion to the tendency followed between 100% and 70%.

Figure 5.3.4 **Variation of surface tension (dyn cm -1) of the tincture sample with increasing dilution of the tincture sample.** The tincture sample was diluted with 45% (v/v) ethanol/H_2O. A percentage original extract of 100% refers to the undiluted tincture sample; a 70% original extract refers to 70% tincture sample, 30% diluent (v/v) etc. The figure shows five readings (ST water @ 20°C ~ 72.8 dyn cm^{-1})



5.4 **Discussion**

After being centrifuged at 17,000 × g for one hour, all of the tincture samples show the Tyndall effect and so it seems likely that they are classical colloidal systems. The sample subjected to ultracentrifuge for 1 hour at 171,500 × g (average) showed no apparent change of the Tyndall effect and so the extracts would appear to be quite stable colloidal systems. This is consistent with the presence of a microemulsion.

Simple solutions follow 'Newtonian' rules and surface tension (and other colligative properties) change proportionately with concentration. In the tincture sample tinctures examined, on the other hand, sharp breaks in the surface tension slope occurs over a range of concentrations of the tincture sample selected.

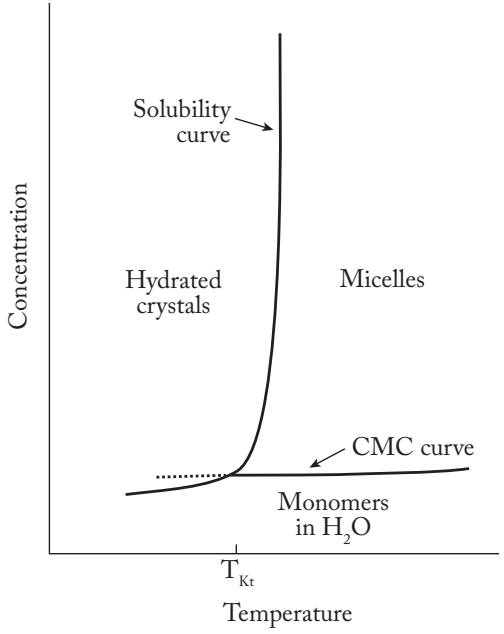
The above data point to self-assembly of structures in the range 40–70% concentration of the original tincture studied. There are, of course, some handicaps in the use of complex tincture samples for classical microemulsion study. Because of the nature of the system, the only parameter that can be altered with certainty is the solvent. Both the amphiphiles and also the hydrophobic components being diluted during the experiment are unknowns. Furthermore, any complex structures are possibly in dynamic equilibria with the other components of the system. With the exception of the water-ethanol medium, therefore, it is not possible to exper-

imentally alter one phase independently of any other. Although not as sharply delineated as the CMC elicited from experiments with simple 'text-book' model systems, significant changes of slope direction did occur over a band of concentration in the experiments described. Such breadth of band, or repeated directional reversal of the slope is characteristic of 'impure' model systems (see Figure 4.4).

The situation is further complicated by the *Kraft point*, the temperature below which hydrated crystals, rather than micelles, form (Figure 5.4.1). In complex, or 'impure' systems, various interacting components may give rise to systems with various Kraft points, to the extent that, at ambient temperatures, some components might form crystals, and others micelles. Such crystals might form particles adsorbed onto surfaces in poorly defined macroemulsion structures. Complex or 'impure' systems may construct a range of micellar structures from different components, or micelles of varying complexity at different concentrations making stability hard to predict. There are thus several reasons why tinctures might not be expected to show sharp or predictable CMCs. With tinctures, any of these effects may account for the breadth of the band observed at the experimental CMC.

A number of lipids were identified in the samples. Many biological lipids are

Figure 5.4.1 **Effect of Kraft point on determination of aggregates to crystallise or form micelles at the CMC** (*from Lindman and Wennerström, 1980*)



amphiphilic (and usually biologically detergent) molecules. These precipitated out when the pH was raised, suggesting that their solubilisation mechanism may be pH dependent. Unlike animal proteins, plant proteins have high isoelectric points and raising the pH may be expected to precipitate plant proteins, as well as precipitating any protein-associated lipids. Lipids may thus be dependent upon protein solubility for their own solubilisation. Proteins, which have been found in surprisingly high concentrations in the tincture (probably up to 5% of the total tincture sample) are also examples of amphiphilic molecules. The protein range of samples apparently varies across the 2005 harvest season, from 100–375 $\mu g/cm^3$. The CMC for the samples in which surface tension was measured appears in the range 40–80 % of the concentration of the tincture sample, at which concentrations of 300 $\mu g/cm^3$ (or less) of protein occur (as measured by the Bradford [1977] method). This concentration is in the same order of the CMC of many typical model surfactants (Evans and Wennerström, 1979). Most plant proteins (except some seed proteins) are normally insoluble in alcohol, yet they are found in all the tinctures analysed. Proteins also remained in the tincture bulk phase even after centrifugation at 17,000 × g for one hour. Both these findings point to the involvement of proteins in some form of thermodynamically stable, partially ordered system. Microemulsions are examples of such systems. A simplistic view of the native characteristic of proteins is their ability to fold in the presence of water as a result of varying hydrophobic and hydrophilic molecular moieties. The reality must be somewhat more complex, because the 'water' of the living cell is in fact a dynamic, rich milieu of solutions, surfactants, monolayers, bilayers, emulsions and association colloids, many of which may appear in the tincture sample. Proteins, whose charges are occupied with lipid, sugar or other ligands, may however, be the amphiphilic 'backbone' giving these molecules their liquid-like mechanical properties (Everett, 1988). Complex molecules, such as phospholipids, lipoproteins, glycolipids, glycolipoproteins or phosphoglycolipoproteins, therefore extend the possibilities for amphiphilic behaviour almost endlessly. Thermodynamically labile, thermally fluctuating flexible lyotrophic lamellar sheets (as described by Roux et al, 1994) typically arise from the dynamic binding specificities due to spontaneous self-assembly of such complexes. Although there are many phospholipids found in plants, very few glycolipids have been identified, although the most important are the ubiquitous monogalactosyl and digalactosyl diglycerides, which are indeed notably surfactant molecules (Harborne, 1988), involved in chloroplast metabolism.

Following disruption of plant cellular organisation during extraction processes, thermodynamic considerations require that ubiquitous amphiphilic molecules attempt to regain the lowest energy structural conformation in the resulting liquid extract. These molecules are not broken down by normal, traditional cold extraction processes. Short-chain alcohols, such as the ethanol normally used to make

tinctures, are very important co-surfactants in many classical model CMC experiments and practical applications (Evans and Wennerström, 1979). Thus it seems possible that, if tinctures are dilute microemulsions, ethanol might act as a co-surfactant, helping stabilise aggregates in the colloidal domain, in which small but highly critical amounts of active compounds may be solubilised. Cells are broken down into micelles by the 'tincturing' process. The kinetics of self-assembly is, however, not well characterised, despite more than 30 years' research (Chandler 2007). Therefore the precise mechanism of solubilisation remains in the realms of speculation, although this is speculation supported by a growing body of evidence. Mechanical energy is a characteristic of both traditional tincture manufacture (at the pressing stage) and also a requirement of metastable macroemulsions. In summary, the analytical evidence presented favours the widespread existence of dilute emulsions and micellar organisation in tinctures. Nevertheless, the co-existence of less stable macroemulsions is not excluded by the evidence. Indeed, within the overall dynamics of the system, the precipitation of sediments, over indeterminate timescales, may be the most direct evidence yet available of macroemulsion structures. According to conventional theory, macroemulsion surfactance may be due to the properties of various different classes of component (amphiphile, polymer or particle). In such complex systems as that studied, such structures may be secondary to the dynamics of the more stable microemulsion system indicated by the experimental results.

If it is indeed the case that spontaneously self-assembling structures and consequent solubilisation of hydrophobic components are widespread characteristics of herbal preparations, as the evidence suggests, then it seems possible that the features and properties of these systems may also play an important part in herbal biopharmaceutical synergy (and antagonism). Self-assembly may also be a critical, but hitherto overlooked, synergistic system in the stability, or 'lasting quality' of tincture samples. If this is so, then shelf-life and efficacy could be qualities linked by stability of self-assembling structures. In this case, it seems likely that the ethanol present in tinctures acts as a co-surfactant. Because it is, above all, the concentration of amphiphiles that is critical to self-assembly, then the 'quality' of a herbal medicine may depend in part on the concentration of surfactant amphiphilic molecules of tincture samples. Too low a concentration, and there may be insufficient amphiphilic surface energy to create the micelles on which bioavailability (and so herbal medicinal efficacy) may to some extent depend. Too high a concentration and insoluble sediments may appear, as macroemulsion components disintegrate over time.

In the past, macromolecules such as phospho- and other lipids, proteins and sugars and their complexes, such as phosphoglycolipoproteins, have been regarded generally as little more than impurities in herbal medicines. The critical micellar

concentration of these amphiphilic surfactants may, however, prove to be a 'missing link' in the quest to understand whole-plant herbal quality. It is thus possible to speculate that variations of the molecular composition and relative concentration of biological amphiphiles within herbal drugs may account for some variability of the biological efficacy, or vitality, of herbal preparations.

Fresh extracts, rather than extracts from dried materials are traditionally used in the modern phytochemistry laboratory (Harborne, 1998). That herbal biopharmaceutics might be dependent upon the partially ordered systems found above in fresh extracts, is consistent with current theory of the evolution of plant secondary defences (Rosenthal and Janzen, 1979). Such biological systems are, however, critically dependent on the presence of intra-cellular water. Although it is commonly believed that drying *Angiospermae* tissues has little effect on many of the chemical components previously identified in the laboratory, drying tissues prior to extraction may denature proteins or other macromolecules or complexes critical to spontaneous self-ordering of biological solubilisation systems. Further work therefore needs to done to establish the extent to which the denaturing that may occur when drying, affects the re-establishment of, or changes within, such natural systems, upon re-hydration and the subsequent commercial production of herbal tinctures. We also need to know whether it is possible to devise simple control techniques to indicate the quality of molecular surface energy of dynamic plant complexes.

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6 Statistics

Using Sun Microsystems OpenOffice.org Calc. vs.2.0, the following statistical values were obtained:

(i) Figure 5.3.1 Protein measurements: n =6; median SD 2.58 (range 2.04–3.76).
(ii) Figure 5.3.4 Surface tension graph: n = 6; median SD 0.35 (range 0.26–0.75). In the formula deriving surface tension, for density (q) readings were averaged, n = 6; median SD 1.49.

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