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EXPERT REVIEW

# Physical Approaches to Masking Bitter Taste: Lessons from Food and Pharmaceuticals

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**ABSTRACT** Many drugs and desirable phytochemicals are bitter, and bitter tastes are aversive. Food and pharmaceutical manufacturers share a common need for bitterness-masking strategies that allow them to deliver useful quantities of the active compounds in an acceptable form and in this review we compare and contrast the challenges and approaches by researchers in both fields. We focus on physical approaches, i.e., micro- or nano-structures to bind bitter compounds in the mouth, yet break down to allow release after they are swallowed. In all of these methods, the assumption is the degree of bitterness suppression depends on the concentration of bitterant in the saliva and hence the proportion that is bound. Surprisingly, this hypothesis has only rarely been fully tested using a combination of adequate human sensory trials and measurements of binding. This is especially true in pharmaceutical systems, perhaps due to the greater experimental challenges in sensory analysis of drugs.

**KEY WORDS** bitter · emulsion · encapsulation · food · taste

## INTRODUCTION

The development of bitter taste can be understood from the perspective of the eater or the eaten. Animals have evolved a bitter sense to screen out potentially toxic compounds before they are swallowed, while plants have evolved bitter-tasting compounds to discourage consumption. Perhaps unsurprisingly then, the compounds recognized as bitter are both numerous and structurally diverse. Similarly, the receptor system for bitter tastes must be highly complex to respond to such a wide range of potential stimuli at very low levels. Humans have ~25 intact bitter receptor genes, and ligands have been identified for 21 of these (1, 2). However, emerging evidence suggests bitterness may not be a singular unitary percept (e.g. (3)) and indeed the view that bitterness is merely a sign of harmful toxins to be avoided may be an oversimplification (see (4)).

Bitter tastes are desirable in only a small set of foods at only moderate levels (e.g., tea, beer, grapefruit) and typically preference for these foods has to be learnt with infants and children being particularly averse towards bitter tastes (5). Plant breeding has selected for less-bitter varieties and food processing often involves peeling and chopping to remove the bitterest parts of the plant or more advanced processing methods to further reduce bitterness (e.g., treatment of orange juice with naringinase). However, there are cases when, rather than simply avoiding them, we want to encourage the consumption of bitter substances by increasing their palatability.

Many drugs and phytochemicals are bitter, and the unpleasant taste reduces compliance with a treatment regiment (6), or the selection of certain "healthy" foods in a diet (7) respectively. Again, these factors are particularly important in infants and children who may be less willing to weigh the long-term benefit over the short term cost of eating something that does not taste good, as well as possibly having a higher sensitivity to bitter tastes (8). As the food or pharmaceutical would not be functional without the bitter ingredient or drug, it is necessary to find ways to suppress its bitterness within a formulation, rather than simply removing the source of bitterness.

Bitter drugs can often be delivered intravenously or swallowed as a coated tablet, preventing stimulation of oral chemoreceptors so that the bitterness is not perceived. However pre-school children are usually seen as being incapable of swallowing tablets, so liquid formulations and orodispersible tablets are usually the preferred model of delivery (9). In these cases the liquid preparation can be in the mouth for an extended period of time, resulting in the perception of bitterness. There is a clear parallel here between the

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challenges faced in the pharmaceutical and food industries. The need for bitter-masking formulations for pediatric medications is particularly pressing as European regulation requires a pediatric development plan in which control of bitter and unpleasant tastes is an important issue (10, 11).

There are three main approaches to taste masking (12): (i) peripheral interactions where a compound antagonizes a particular taste receptor, (ii) central cognitive interactions where one strong taste or aroma reduces perception of the other in the brain, and (iii) encapsulation where the compound is physically prevented from interaction with the active sites either by modifying solubility or by introducing a barrier (13, 14). The most successful bitter-blocking strategies will involve aspects of all of these approaches, but the focus of this review is only on physical encapsulation. An excellent recent review by Gaudette and Pickering [15] considers the full range of bitterness masking strategies with a focus on functional foods.

Furthermore as we are concerned with the overlap between pharmaceuticals and foods, we will not consider tablets that are intended to be swallowed intact.

While there are useful similarities in the approaches used for taste masking in foods and in pharmaceuticals, there are also important differences and we will start our review by considering these. Next we briefly review the mechanisms of bitter taste perception. We will then look at binding of the bitter compounds by polymers, cyclodextrins, lipids and surfactants. Finally we consider the future scope for bitter taste encapsulation in different applications.

## TASTE MASKING IN FOODS VERSUS PHARMACEUTICALS

It is a common goal in both food and pharmaceutical science to formulate products with a bitter but healthful ingredient while reducing perceived bitterness. There are, however, important differences in the objectives and constraints of the two fields. Crucially, while drugs are designed to be given in measured doses to a specific individual under specific circumstances and under the control of a medical professional, foods are selected by the consumer based on their sensory properties, cost, and apparent healthfulness. The taste of pharmaceuticals should be acceptable, while foods should taste delicious. A pediatric drug formulation that tastes too good could pose a risk for overdose, while the better-tasting food is usually the one purchased. While phytochemicals are usually regarded by the consumer as "healthy", for any compound "the dose makes the poison" and we should be cautious that there may be consequences in making it too easy to consume bitter foods.

Having made the decision to reduce bitterness, there are different formulation challenges for foods and pharmaceuticals. Pharmaceuticals usually contain a very small set of active ingredients, while foods contain many taste and aroma-active compounds. Selectively suppressing the taste of one compound is likely to be easier in pharmaceuticals than foods. On the other hand, the quantity of bitter compounds in foods is likely to be small, while some medications may require hundreds of milligrams for a single dose.

Sensory evaluation of bitter foods by children is challenging but possible whereas sensory evaluation of drugs by children, particularly sick children, is likely to be limited or even prohibited by ethics boards (10, 16, 17). Indirect testing methodologies including use of adults with child-like palates, animal studies or even chemical analyses may be the only choices available (11).

Finally the economics and marketing concerns of foods and drugs are very different. Foods are typically manufactured at a larger scale than pharmaceuticals and the finished product is much cheaper so the additional processing/ingredient costs of encapsulation are more likely to be prohibitive (18). Food manufacturers often seek to communicate "healthfulness" to consumers with a clean label that limits the ingredients available for encapsulation. On the other hand pharmaceutical manufacturers rely on clinical data for their active ingredient to market their product to medical professionals and the inactive ingredients carry less stigma.

## MECHANISM OF BITTER TASTE

Bitterness is one of five prototypical tastes carried centrally by Cranial Nerves seven, nine and ten (CN VII, IX, and X) (19). In addition to sweet, sour, salty, savory (umami) and bitter sensations carried by these nerves, foods and medications also evoke other oral sensations (e.g. burn, astringency, tingling), but they are not classically considered tastes. For tastes, we abstract singular unitary percepts from a broad range of stimuli (e.g. sugars, alcohols, and some peptides are sweet, acids are sour, etc.). Regarding bitterness, secondary metabolites from plants like alkaloids, terpenoids and flavonoids are commonly described as bitter, as are some salts and peptides (see 'Bitter Tasting Molecules' below). For some stimuli, as concentration increases, new side tastes/sensations can appear or in a few cases the dominant percept may change. In these cases, the stimuli are perceived as mixtures of multiple sensations. For example, sucralose has bitter and metallic side tastes that increase with concentration, but over a vast majority of the stimulus range, sucralose is perceived as a mixture of both sweetness and bitterness, with sweetness predominating at all but the highest concentrations.

In the last decade, substantial advances in molecular genetics have elucidated many of the genes that encode taste receptors, providing improved understanding of taste transduction (20). To be perceived, a non-volatile tastant must first dissolve in saliva before diffusing across a mucous layer to

reach taste receptors expressed on microvilli on the apical end of taste receptor cells (TRCs); indeed, individuals with diminished salivary production can show impaired taste perception (21). These specialized TRCs are located within taste buds, and the taste buds are found within taste papillae located throughout the oral cavity. The taste bud is an onion/pear like structure containing ~75 cells of four different types (Types I, II, III, and basal cells). At the top of the taste bud is a small opening, the taste pore, into which the receptor coated microvilli project. Type II (receptor) cells express G-protein-coupled receptors (GPCRs) that detect stimuli described as bitter, sweet or savory (umami). Critically, a single Type II cell only expresses GPCRs for a single taste quality (i.e., bitter or sweet, but not both). Less is known about Type I (glial-like) cells, but they have been implicated in salty taste, and Type III (presynaptic) cells appear to play a role in sour taste. Interactions and crosstalk between the various classes of taste cells are still being elucidated, but current understanding suggests presynaptic (Type III) cells integrate input from receptor (Type II) cells. Through mechanisms that are not entirely understood, Type II and Type III cells excite sensory afferent neurons in Cranial Nerves VII, IX, and X, which project to the nucleus of the solitary tract (NTS) of the brainstem. From the NTS, signals travel to the ventroposteromedial (VPM) nucleus of the thalamus, and finally the orbitofrontal cortex (OFC), where taste signals are integrated with other flavor inputs including olfaction and somatosensation. For further information, see excellent reviews by Chaudhauri and Roper (22) and Verhagen (23).

In humans, bitter receptors (hT2Rs) are encoded by 25 different bitter receptor genes (*TAS2R*s) located on three different chromosomes. This contrasts with individual heterodimeric receptors for sweet (hT1R2/hT1R3) and savory (hT1R1/hT1R3), explaining in part how we are able to detect thousands of structurally diverse compounds as being bitter. Notably, there are also substantial species differences for bitterness: the number of putatively functional bitter receptor genes varies dramatically across mammals, ranging from 12 in cows, to 35 and 37 in mice and rats, respectively. Humans have ~25 intact bitter receptor genes and 11 pseudogenes; this high rate of pseudogenetization compared to other primates may reflect relaxation of selective pressure due to detoxification via cooking (24).

Of the 25 hT2Rs that are functional in humans, some are specialists detecting only a few compounds, while others are generalists that are broadly tuned. For example, quinine activates nine different receptors, while phenylthiocarbamide activates just one (1). Also, very small structural changes can dramatically alter the ability of a compound to activate the receptor (e.g. chloramphenicol versus thiamphenicol (2)). Some *TAS2R* genes also contain functional polymorphisms, resulting in differential perception in humans (e.g. (25–27)).

Critically, not all bitter receptors are expressed on all bitter responsive Type II cells. Rather, different patterns of hT2Rs are expressed across cells, providing a means to discriminate between compounds described as bitter (28, 29). Likewise, this ability appears to translate to differential neural coding for various bitterants, at least in mice (3). While the existence of perceptually distinct bitters in humans remains to be demonstrated, the existence of differential neural coding in mammals provides a potential mechanism to explain why acquired liking for one bitter food doesn't always generalize to liking for other bitter foods (e.g. hoppy beers and black coffee).

Finally, no discussion of bitter taste would be complete without mentioning the 'enduring myth' (30) of the tongue map. As noted above, taste buds containing taste receptor cells are located throughout the oral cavity on the tongue, soft palate, epiglottis, larynx and pharynx. Accordingly, all taste qualities can be sensed over the entire tongue, wherever there are taste receptors. That is, the so-called tongue map showing sweet is sensed on the tip and bitter on the back is wrong, as shown by (31). The absence of a tongue map can be easily disproven by painting various taste solutions on different regions of the tongue.

## BITTER TASTING MOLECULES

Humans have the capacity to identify a wide range of materials as bitter, including simple ions to complex polyphenolic compounds and small peptides. Yet, within a class even apparently small changes in molecular structure (e.g., D- vs. L-isomers of amino acids) can profoundly modify the taste. It would be extremely helpful in drug development or in the design of bitter receptor antagonists to have a robust prediction of bitter taste from molecular structure, but this remains elusive. However, within limited chemical classes, there are some good predictions of bitterness; for example by excluding bitter ions and peptides, Rogers et al. (32) were able to classify a large database of molecules as either bitter or not with 72.1% accuracy. From the point of view of predicting or designing physical binding strategies for bitterants, a cruder understanding of structure may still be helpful. Recently Wiener et al. (33), published a database of over 500 molecules reported in the literature as bitter-tasting. While a survey of such a database only reveals trends amongst the molecules reported, it is interesting to note that most of the molecules were moderately hydrophobic (Fig. 1a) and with molecular weights in the low hundreds (Fig. 1b).

Beyond this gross categorization of bitter molecules, any attempt to decide on which are the "important" examples is

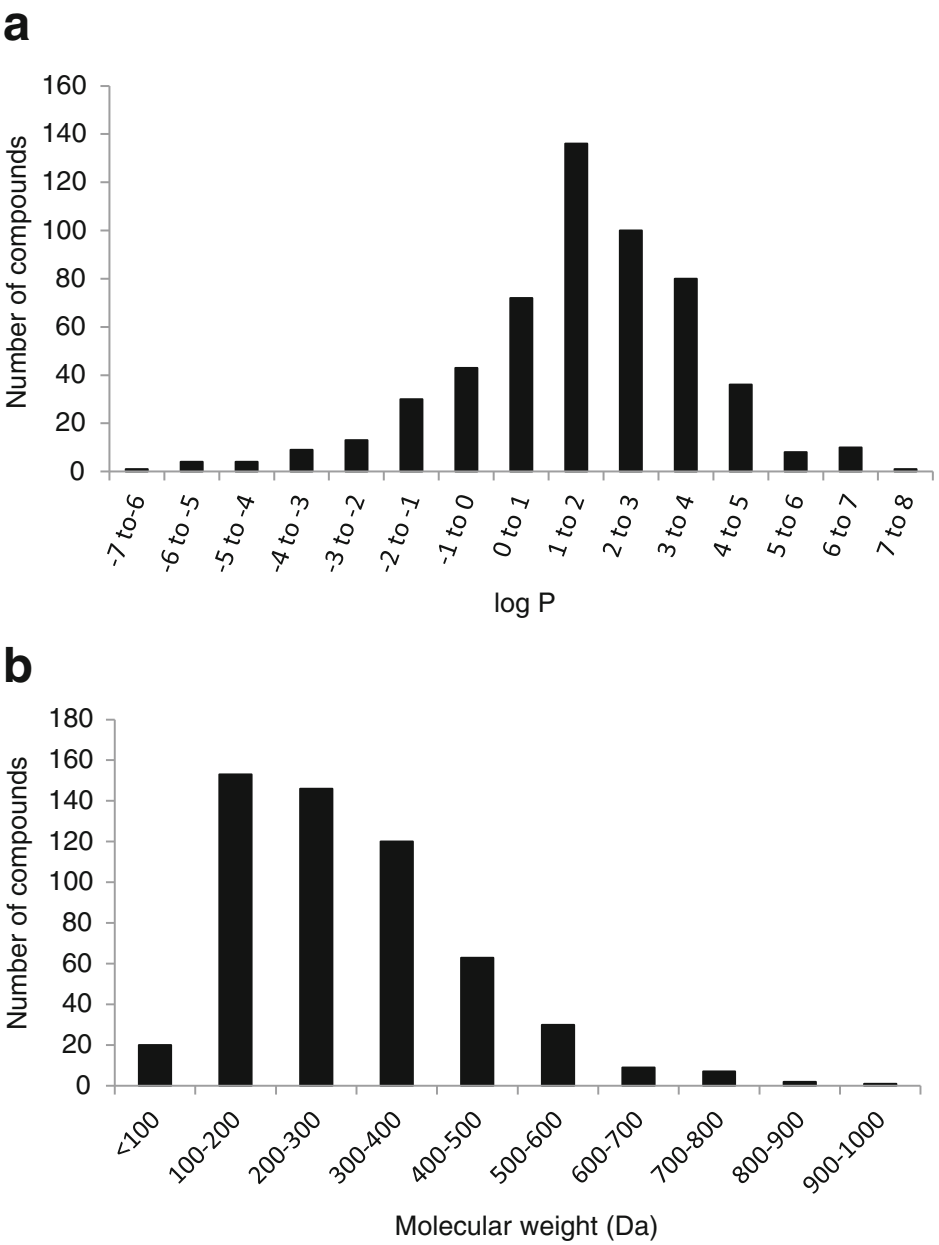


**Fig. 1** Number of compounds in the Bitter DB database as a function of (**a**) log P value, (**b**) molecular weight. Database was queried Oct 8th 2012.

probably fruitless but it may be helpful to show some example structures. In a review of the 1997–2007 patent literature (34), the authors note that the majority of the bitter-masking work on drugs is focused on "aggressively bitter tasting drugs like the macrolide antibiotics, non-steroidal anti-inflammatory drugs and penicillins". In a survey of the research articles in Web of Science since 2007 with the keywords "bitter" and "food" (conducted Oct. 8th 2012), 85 studies were primarily about a bitter taste in a specific food matrix. Within this set, the most widely studied materials included: olives and olive oil, beer, protein hydrosylates, cheese, wine, tea, andcoffee. See in particular (7) for a description of the role of bitter compounds in plant foods.

## DELIVERY SYSTEMS TO MASK BITTER TASTE

A recent review article (35) suggested five design principles to guide the design of delivery systems for nutraceutical compounds in foods. These principles, slightly modified, can be applied to the delivery of bitter-tasting compounds in foods and drugs as follows:

1. "[*T*]*he delivery system should efficiently encapsulate an appreciable amount of the functional component in a form that is easily incorporated into food systems*." Here "appreciable" is defined by the clinical dose of the bitter drug or an amount appropriate for a marketing claim for a bitter ingredient.

2. "[*T*]*he delivery system may have to protect the functional component from chemical degradation* ….". Rather than chemical degradation we are primarily concerned with preventing the interaction of the bitter molecules with taste receptors in the mouth. However, protection for labile ingredients during processing and storage may also be important.
3. "[*T*]*he delivery system may have to release the functional component at a particular site of action*…" . The structure protecting the bitter ingredient in the mouth must breakdown during later digestion to release the active compound so that it becomes bioavailable. Some control of the digestion process may be appropriate, for example selecting an alkali-labile encapsulation matrix to favor release in the small intestine.
4. "[*T*]*he delivery system should be compatible with the specific food matrix that surrounds it.*" It should not itself be recognizable within the food, for example by contributing an unpleasant taste or a grainy texture. Depending on the texture of the particle and the surrounding foods, solids less than a few tens of micrometers are not typically perceived in the mouth. In pharmaceutical applications, there is not such a strict need that the delivery system be imperceptible and a preparation that is not too unpleasant and readily ingestible should be acceptable.
5. "[*T*]*he delivery system should be resistant to the various kinds of environmental stresses that a food experiences during its production, storage, transport, and consumption.*" While the stresses of manufacture and storage are well understood in food and pharmaceutical manufacture, the stresses during consumption are only recently appreciated. Solid foods are macerated for some time during chewing and diluted with saliva before swallowing while liquids have a much lower residence time in the mouth but still interact with saliva (36).

Various types of materials have been used, alone or in combination, to encapsulate bitter molecules according to these principles. We will consider these materials in turn, first in terms of the nature of the structures formed and their potential for interacting with bitter molecules, second by reviewing some examples of how bitter molecules have been shown to be bound, especially where this is shown to be related to a loss of bitterness. Very often the approaches used span multiple modes of interaction (e.g., a lipid emulsion that also binds bitter compounds to interfacial protein) and wherever possible we will describe these systems after first considering the component interactions.

## Cyclodextrins

### *Structures and Interactions*

Cyclodextrins are cyclic oligosaccharides derived enzymatically from starch hydrosylates; they are a widely accepted food ingredient and have good water solubility and only a mild sweet taste (37–39). The ring structure can have 6 (α-cyclodextrins), 7 (β-cyclodextrins, the most widely used), 8 (γ-cyclodextrins) or more glucopyranose units connected by α-glycosidic bonds. Chemically modified cyclodextrins are available with modified binding properties although they are not yet permitted as food additives (40). Cyclodextrins can be derivatized to form amphiphilic molecules that can spontaneously self-assemble into nanoparticles and associate better with biological membranes (41).

Cyclodextrin molecules form a tapered cone structure with an inner diameter of 5–10 A and a hydrophobic inner face and a hydrophilic outer face (42). In solution, the cone is hydrated, but hydrophobic small molecules or hydrophobic groups on larger molecules readily displace the interior water to form a thermodynamically stable complex. Amphiphilic molecules can be bound by cyclodextrins with the hydrophobic group inside the ring and the hydrophilic group excluded. Cyclodextrins can also be used to solubilize hydrophobic drugs for liquid preparations (42). If the part of the bitter molecule that interacts with the bitter receptor is included in the ring, then it cannot contribute to perceived bitterness.

The binding affinity for a cyclodextrin for a given compound is given by an association constant, $K_{ass}$:

$$K_{ass} = \frac{[complex]}{[cyclodextrin][guest]} \quad (1)$$

The proportion of free guest molecules is shown as a function of guest:cyclodextrin molar ratio and $K_{ass}$ in Fig. 2. The larger the value of $K_{ass}$, the more cyclodextrin must be present to reduce the bitterness of the product. However, if the affinity is too low or the bitterant:cyclodextrin ratio is too low there will be some remaining free bitter molecules to produce a bitter taste.

Typical values for $K_{ass}$ were suggested by Szejtli and Szente (43) to be in the range 0.01–10,000 $M^{-1}$ while Brewster and others (42) proposed mean values of 130, 490 and 350 $M^{-1}$ for α-, β-, γ-cyclodextrins respectively. In a study of aroma

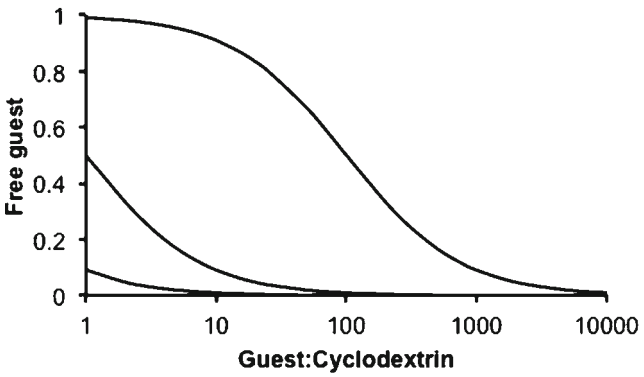


**Fig. 2** Proportion of free guest molecules as a function of cyclodextrin concentration for different values of $K_{ass}$.

compounds,Astray et al. (44) showed $K_{ass}$ was lower for α-cyclodextrins than for β-cyclodextrins (i.e., the larger ring could bind more efficiently) and increased linearly with log P (i.e., more hydrophobic compounds were bound more readily). Selecting a cyclodextrin with higher binding efficiency may be a net economic advantage if less is required for a given degree of bitterness reduction (45). Cyclodextrins usually bind bitter molecules at a fixed stoichiometry (usually 1:1). Therefore even if $K_{ass}$ is low (i.e., strong binding affinity), there must be an excess of cyclodextrin present to assure they are not saturated. However, this constraint should rarely be a problem considering the low cost, low taste, and high solubility of cyclodextrins as well as the relatively low levels of bitter molecules often present.

The rate of complex formation approaches the diffusion limit, so it is often possible to get a good level of bitterness suppression by merely having an excess of cyclodextrin present in the food or pharmaceutical product before it is eaten (43). Alternatively cyclodextrin complexes can be prepared as ingredient delivery systems. The complexes will also dissociate quickly so the dilution of a powder in water or a food in saliva will also immediately change the fraction of bound bitter molecules, particularly, if $K_{ass}$ is low.

### *Examples*

The bitterness of drugs and foods are readily masked by cyclodextrins. For example, adding about 0.5% of cyclodextrin halved the bitterness of naringin and limonin (46). Szejtli and Szente (43) provide an extensive tabulation of studies of bitterness reduction in drugs and foods by cyclodextrins so we will confine our discussion to a few more recent examples.

Ono and others (47) measured the effects of α-, β-, and γ-cyclodextrins as well as a derivatized β-cyclodextrin on the bitterness of a range of antihistaminic drugs. As would be expected, they showed that the level of bitterness suppression was correlated with the binding coefficient $K_{ass}$. A higher $K_{ass}$ would mean a lower free bitterant concentration and hence less bitter taste. However there have been some exceptions reported to the expected relationship between binding and bitterness reduction. For example, while Rescifina and others (48) demonstrated the formation of a cyclodextrin-caffeine complex, Gaudette and Pickering (49) did not observe any suppression of caffeine bitterness. In another example, the bitterness of propanthaline and oxyphenonium bromide is suppressed more effectively by α- and γ-cyclodextrins than by β-cyclodextrin (50) yet the affinity of oxyphenonium bromide for cyclodextrins decreases in the sequence $\beta < \gamma < \alpha$ (51). (Interestingly, in this work, results from the ion selective electrodes correlated with sensory measurements of bitterness, which may offer a pathway to avoiding sensory trials of pharmaceutical preparations.)

Cyclodextrins can bind bitter-tasting amino acids depending on their chemical structures. For the α-cyclodextrins the binding efficiency decreases in the sequence: phenylalanine ~ tryptophan > proline > isoleucine ~ tyrosine ~ histidine (52) while for the β-cyclodextrins the binding efficiency decreases: tryptophan > tyrosine > phenylalanine > proline > histidine > iso-leucine (53). Both cyclodextrins changed the taste of the amino acids but there was no consistent suppression of bitterness. Cyclodextrins were capable of partially suppressing the bitterness of soy protein, soy protein hydrosylates (52, 53) and whey protein hydrosylates (54).

In other recent food studies, Gaudette and Pickering [55] showed β-cyclodextrin suppressed, but did not eliminate the bitter taste of catechin, and only in the presence of sucrose or a bitter-blocking compound. (45) showed γ-cyclodextrin was about ten times more effective than β-cyclodextrin in suppressing the bitterness of ginseng in energy drinks. β-cyclodextrin forms 1:1 complexes with biphenols from olive oil with binding constants about 40 times greater than the complexes with caffeine, although the effects on sensory bitterness were not measured (48). Of course, cyclodextrins are not oil-soluble so they could not be used to suppress olive oil bitterness directly but only in a food preparation containing some water.

Cetrazine forms a 1:1 complex in solution with at α-, β-and γ-cyclodextrins (56). The smaller (α-, β-) cyclodextrins form a more stable complex with part of the cetrazine, while the larger γ-cyclodextrin forms a less stable complex with the entire molecule. Both α- and β-cyclodextrin suppressed the bitter taste of cetrazine while γ-cyclodextrin was ineffective, perhaps because of its lower $K_{ass}$. Stojanov et al.(57) formulated chewing gum containing cetrazine and cyclodextrins and showed the cyclodextrin facilitated the release of the drug from the gum base during chewing. However, because of the speed of interaction, merely having the cyclodextrin in the formulation had the same effects as pre-forming the complex. In a similar approach, cyclodextrins were used to reduce the bitterness of diltiazem hydrochloride (58) and cetirizine (59) in orodispersible tablets.

In some cases, a third component is needed to promote guest-cyclodextrin interactions. For example, Famotidine has a relatively low $K_{ass}$ with β-cyclodextrin, but adding hydroxypropyl methyl cellulose increased the binding and further reduced the bitterness (60).

## Polymers

### *Structures and Interactions*

Synthetic and natural polymers have been used to bind small molecules in foods and drug delivery systems. In food applications only natural polymers of plant, animal or microbial origin are accepted as ingredients while in

pharmaceuticals a range of synthetic polymers are used. We will first consider important polymers used and their potential for direct binding of small molecules before looking at the types of polymer structures that can be built as delivery systems.

*Proteins*. Proteins are linear polymers with a primary structure defined in the genetic code of the organisms where they were synthesized. The protein primary structure is usually quite hydrophobic and in many cases the native protein spontaneously folds into a globule with the more hydrophobic residues in the core and the more hydrophilic residues at the surface. Protein conformation is defined under physiological conditions but frequently changes as a result of denaturation and aggregation during processing and extraction. Proteins are polyelectrolytes so the charge on the molecule changes gradually from positive to negative as pH increases. Different proteins have very different solubility in water depending on their interactions with one another, with other components in the system and with water. In many cases, rather than dissolving the protein forms a more-or-less stable sol of proteins or protein aggregates. Proteins are typically not oil soluble (although it may be possible to disperse them in oils) but will often associate with membranes or adsorb to oil-water interfaces.

Proteins are naturally present in foods, and are commonly purified and added as ingredients in foods and pharmaceuticals (e.g., casein, whey proteins, gelatin and soy proteins). Although these are non-toxic and biodegradable there may be allergenicity concerns or specific protein sensitivities (e.g., celiac disease). In foods proteins are used to stabilize dispersions and form gels.

While most proteins have little taste and are not effective bitter blockers, there are some exceptions. For example, while several proteins did not suppress bitterness (e.g., β-lactoglobulin), their phospholipid conjugates were effective against a wide array of bitter stimulants (61). Similarly a riboflavin binding protein isolated from egg could only bind certain bitterants yet it suppressed the bitterness of a much wider range of compounds (62). In both of these cases, the authors argued that bitterness suppression is due to an interaction with bitterness receptors on the tongue rather than the binding of bitter molecules. There are few reports of the use of proteins to modulate bitterness in pharmaceutical formulations; presumably largely due to concerns about allergenicity or with difficulties in making stable formulations.

*Polysaccharides*. Polysaccharides are linear or branched polymers of simple sugars usually synthesized by living organisms. Their primary structure is often heterogeneous with respect to molecular weight and sequence but most polysaccharides can be generally described as homopolymers (e.g., amylose), repeating heteropolymers (e.g., xanthan gum) or block copolymers (e.g., alginate). Polysaccharide conformation typically varies between some sort of helical conformation and a disordered coil conformation depending on temperature and molecular interactions. Polysaccharides may have anionic (e.g., sulfate or carboxylic acid) or cationic (e.g., amine) functional groups. As there is typically only one type of ionizable group per polymer, the titration curve from negative to neutral or neutral to positive occurs over a narrower range than for proteins. Polysaccharides have variable water solubility, are insoluble in oils and only rarely have significant surface activity.

Polysaccharides are present naturally in many foods, and are added as ingredients in foods and pharmaceuticals. Commonly used polysaccharides include alginate (anionic, seaweed derived), starch (neutral, plant derived), chitosan (cationic, animal derived) and xanthan gum (cationic, microbially derived). They are used in foods to increase viscosity and to form gels.

*Other Polymers*. Various synthetic polymers are available for use in pharmaceutical formulations. For example, various types of polyethylene glycol are used in drug delivery systems as an excipient, a lubricant, and to slow the biological clearing of an active ingredient. Other synthetic polymers that can change their conformation in response to the solution conditions are useful in delivery systems. For example, Eudragit® is a variety of polymethacrylate copolymers with acid and basic functional groups (63). The ionizable groups make Eudragit a very pH-responsive polymer, dissolving and precipitating in response to added acid or base.

Polymers can bind small molecules via a number of non-covalent interactions. Hydrophobic domains on the surface of a globular protein or synthetic copolymer or the interior of some polysaccharide helices (notably amylose) can accommodate hydrophobic guest molecules. Polar guest molecules can interact with hydrophilic segments of polymers by hydrogen bonding or electrostatic interactions.

*Polymer Association Structures*. Polymer association structures often give better small molecule binding properties. Polymers in solution can aggregate to form nanoparticles - sometimes classified as nanocapsules and nanospheres (64, 65). Nanocapsules are vesicles with the active compound incorporated into the core while in nanospheres the active compound is uniformly dispersed within the polymer. Common methods of nanoparticle formation include changes in solution conditions (i.e., simple coacervation, for example when ethanol is added to an aqueous gelatin solution or when water is added to a solution of a hydrophobic polymer in a water-soluble organic solvent) and addition of another polymer to form a complex (i.e., complex coacervation, for example when oppositely charged whey protein and gum Arabic solutions are mixed). Other methods to make polymer nanoparticles

include dissolving the drug and hydrophobic polymer in an organic solvent, emulsifying into water then evaporating off the organic solvent or by emulsion polymerization. The drug-polymer particles can be separated, perhaps with the assistance of filtration or centrifugation, and the particles harvested. Small molecules can bind to coacervates either by adding them after the particles have formed or, more typically, to the initial solution phase.

A simpler, and more economical, method to make polymer-small molecule particles without the need for a solvent is hot melt extrusion (HME) (66, 67). In HME the drug is dissolved in a polymer melt then extruded to form fine fibers that can be ground to form a powder. It is important the polymer is soluble, yet chemically stable in the melt. If the goal is to suppress bitterness then the polymer selected should not be soluble at mouth pH yet dissolve in the stomach to release the active agent.

More complex polymer structures can form by controlled self-assembly. Denatured and partially denatured globular proteins can aggregate under certain conditions to form fibrils (68). Di- and tri-block copolymers with hydrophobic and a hydrophilic segments (e.g., Poloxamers) can self -assemble to form polymer micelles (69) and polymersomes (70) similar to the liposomes formed from emulsifiers described below. Like liposomes, polymersomes can incorporate drugs either in the internal phase, in the hydrophobic membrane or attached to the membrane surface. One important special case is the dairy protein casein. Casein is a mixture of different proteins found naturally in milk as self-assembled micelles. The formation of casein micelles can also be seen as the association of the components as block copolymers but a wider range of intermolecular forces including calcium binding by phosphatidyl serine must be invoked to explain their stability (71). Casein micelles have been used to solubilize hydrophobic small molecules (72).

If the solution conditions change, the protein nanoparticle may dissolve, e.g., a complex coacervate where changes in pH mean the two components no longer have opposite charges. In other cases some residual bonding (e.g., chemical crosslinking) between the components mean the particle merely takes on water to swell and form hydrogel nanoparticles (73). The diffusion coefficient of solute molecules in a swollen polymer particles is much higher than that in an unswollen particle and in some cases the kinetics of swelling can be rate limiting in the release process.

### *Examples*

Ion exchange resins are insoluble polymer beads that have acidic or basic functional groups that can readily bind an oppositely charged compound and prevent its release into the aqueous phase until the pH is changed or until it is displaced by another ionic group. They have been used as food processing aids to extract bitter compounds from fruit juices or directly consumed as drug delivery systems. Drug-resin conjugates have been formed into orodispersible tablets that have been shown to eliminate the bitterness of a number of drugs including chloroquine phosphate (74) donepezil hydrochloride (75), Risperidon (76), dextromethorphan hydrobromide (77), and tramadol (78). However, it should be noted that in many of these studies the bitterness suppression is claimed on the basis of reduced release into simulated saliva rather than direct sensory analysis.

Polymers in solution can be precipitated by changes in solution conditions and trap suspended material in the coacervate. Hydroxypropyl methyl cellulose (HPMC) for example is hydrated and soluble at low temperatures but tends to dehydrate and either precipitate or gel at higher temperatures. Weiβ and others (79) used HPMC derivatized with phthalate groups to form a polymer microparticles with ibuprofen in response to added salt and increased temperature. As ibuprofen is effectively insoluble under the experimental conditions (pH 5.45) the phase behavior of the polymer was unaffected by the presence of the drug and the coacervate formed in layers around the ibuprofen crystals. However this polymer had good solubility at pH 7.2 so, while sensory properties were not measured, the particles would likely dissolve rapidly in the mouth and not mask the taste of the drug. Better taste masking could likely be achieved with polymer complexes insoluble at mouth pH.

Various polymers can be used to form electrostatic complexes with charged bitter molecules. Lu and others (80) formed an insoluble electrostatic complex between the amide groups of erythromycin (pK = 10.2) with the acid groups of a polyacrylic acid (Carbopol®). The complex was formed by mixing the drug and polymer in ethanol then precipitated by slowly decanting into cold water. The insoluble particles were harvested and coated with hydroxypropyl methylcellulose phthalate in a fluidized bed coater. The particles were suspended in a sweetened xanthan solution and, although quantitative sensory data are not reported, the authors claim that the bitterness is reduced and could be further reduced by increasing the thickness of the coating polymer.

A range of pH-responsive synthetic polymers is available with the potential for either entrapping or binding bitter drugs. In particular, basic butylated methacrylate copolymer (Eudragit EPO®, Evonik Industries, Germany) is permeable at pH > 5 and soluble at pH > 5 so can be used to make particles that are insoluble in the mouth yet dissolve in the stomach. In one example, clarithromycin was mixed into a hot melt of polymer and glycerol monostearate then spray chilled to form a powder ($d \sim 80\ \mu m$) (81). The antibiotic was released slowly into pH 6.5 buffer (i.e., low mouth pH) but almost immediately at pH 4 (i.e., high stomach pH). Clarithromycin has an amine functional group (pK = 9) so is positively charged at mouth pH and would not form an electrostatic complex

with the polymer so the difference in release is probably due to the increase in polymer solubility at lower pH; at mouth pH the drug cannot diffuse through the polymer particle and is entrapped. A suspension of the powder in sweetened, thickened water did not taste bitter (although quantitative data were not reported). In related work, a basic bitterant (Trimebutine) was complexed with a basic pH-responsive polymer (polyvinylacetal diethylaminoacetate) (82). In this case, the drug was suspended in water and was emulsified into an organic solution of the polymer (i.e., a w/o emulsion) that was in turn emulsified into water to form a w/o/w emulsion. The emulsion was dried to form powdered polymer microspheres insoluble at pH 6 that dissolved almost instantaneously at pH 5 and bitterness of Trimebutine was reduced. In a final example, the bitterness of paracetamol (i.e. Tylenol/acetominophen) was suppressed by forming a HME with two synthetic polymers but the extent of the reduction decreased with drug loading (83).

For acidic bitterants, electrostatic complexes with basic pH-responsive polymers can be used to enhance the suppression of bitterness. Ibuprofen was dissolved in a synthetic polymer melt (Eudragit EPO) and extruded into fibers which were ground to form fine particles which could then be formed into orodispersable tablets (84). Eudragit EPO has an amine group (pK = 10) which form electrostatic complexes with the acid group of the ibuprofen (pK = 5.2) at mouth pH (6.5–7.0) and in all formulations the drug bitterness was eliminated. This approach would be expected to be applicable for other thermally stable acidic bitterants.

Poorly soluble protein complexes have also been used to reduce bitterness. For example, Hoang Thi and others (14) showed the dissolution kinetics and (instrumentally measured) "taste" of paracetamol (acetominophen) was reduced by spray drying with caseinate. In a similar study the sensory bitterness of casein hydrosylates was reduced by spray drying with soy proteins (85). Interestingly though these workers assessed the taste of the powder directly and it seems likely in a liquid medium the particles would dissolve and the benefits would be lost. However, in an intermediate or low moisture food this may be a helpful approach, for example a protein bar formulated with a bitter casein hydrosylate was less bitter when the bitterant was spray dried with maltodextrins (86). Presumably the spray-dried powder did not have time to hydrate and dissolve before the product was swallowed. Indeed, casein hydroslylates spray dried with pectin and gelatin dissolved much more slowly (5 min vs. 1 min) than in the absence of the added encapsulating polymers (87). These workers also spray dried casein hydrosylates with soy protein-gelatin mixtures.

Polyphenols, particularly higher molecular weight tannins, are readily bound by proteins. Indeed gelatin or caseinate are used to remove tannins from wine, and milk serves to reduce the astringency of tea. The low molecular weight polyphenols in olive oil only interact relatively weakly with proteins but this was enough for 1-4% sodium caseinate to reduce the bitterness of aqueous-extracted olive oil polyphenolic compounds (88). Interestingly dietary polyphenols have been shown to inhibit the formation of the protein fibrils associated with human neurodegenerative disorders (89) and EGCG even to remodel them after formation (90). This suggests some unusual protein-polyphenol interaction that may have value in taste-masking.

Shpigelman and co-workers [91] produced a clear suspension of nanoparticles ($d$ = 1–20 nm) by heating β- lactoglobulin with EGCG. About 70% of the EGCG was bound by a 1% solution of the denatured proteins via hydrogen bonds leading to a moderate reduction in bitterness. The protein nanoparticles also protected the EGCG from oxidation and from digestion in the stomach.

## Surfactants and Microemulsions

### *Structures and Interactions*

Surfactants are small molecules[1] which tend to adsorb at surfaces and lower the interfacial tension. Typically they have one or more hydrocarbon tail groups that "try" to partition out of an aqueous phase, and a charged or polar headgroup that prefers contact with water (92, 93). Surfactants are widely used as food and pharmaceutical ingredients to stabilize dispersions, as wetting agents, and to solubilize lipophilic compounds. Surfactants are anecdotally described as having off-tastes, especially soapiness, and indeed in our experience many are unpleasant. However we are unaware of literature either comparing the taste of different surfactants or the dose-response relationship between concentration and aversive taste. The fact that surfactants are widely used in foods without causing taste problems suggests there is scope to use them as bitterness masking agents.

Surfactants are often characterized by the nature of the head group—uncharged or charged. Uncharged surfactants are usually preferred in food and pharmaceutical applications as they are less-affected by changes in pH and ionic strength and are less toxic [93]. Examples include sorbitan esters and polysorbates. Sorbitan esters (Spans) have a sorbitol residue as a hydrophobic headgroup and a fatty acid tail group. They are typically more lipid-soluble. Polysorbates (Tweens) are sorbitan esters rendered more hydrophilic by ethylene oxide groups. Ionic surfactants are widely used for cleaning purposes and for their antimicrobial action, as well as occasionally in foods and pharmaceuticals. Examples include, sodium dodecyl sulfate (SDS), an anionic surfactant with a sulfate

[1] Block copolymers (e.g., Poloxamers) are sometimes described as surfactants as many of their functional properties are similar (e.g., micelle formation, solubilization of hydrophobic molecules).

headgroup and a lauric acid tail group, and cetrimonium bromide, a cationic surfactant with a quaternary ammonium headgroup and a palmitic acid tail group.

Lecithin deserves special attention as a surfactant because of its wide application and somewhat unusual properties. Lecithins are commonly purified from egg and soy as well as sometimes from dairy sources. They are sold as either the crude extract, which commonly contains some fatty acids as well as fatty acid glyceride esters, or as increasingly purified forms. In all cases the active components are phospholipids; largely diglycerides which act as the non-polar tails and a phosphate group and simple organic molecule acting as the polar head. The most common phospholipid is phosphatidyl choline which is a zwitterionic molecule with a negatively charged phosphate and positively charged choline as the head group. As well as acting as surfactants, components of lecithin have unusual taste properties in their own right.

Katsuragi and co-workers first showed that lipoproteins effectively suppress the bitterness of a wide range of stimuli apparently by binding directly with gustatory cell membranes (61) then went on to investigate the use of the phospholipid portion of the lipoprotein as a simpler and more economical ingredient (94). They showed that phosphatidic acid (1%) lowered the bitterness of quinine, berberine, a wheat protein, hydrosylates, propranolol, and brucine, and to a lesser extent thiamine and strychnine but had no effect on the bitterness of a peptide from whey. There was a lesser degree of bitterness suppression by phosphatidyl inositol and while other lecithin fractions were not effective, their presence in crude extracts did not interfere with the performance of phosphatidic acid. These workers argued that, similarly to the lipoprotein, the bitterness suppression is by direct interaction of the lecithin fractions with gustatory cell membranes rather than any form of encapsulation although they did not test for the presence of association complexes. The fact that only a few of the lecithin fractions were effective however, suggests that complexation is unlikely to be important however the fact that bitter blocking was not universal to all stimuli suggests the mechanism is not fully understood.

As well as its potential to block bitter tastes directly, lecithin, along with other surfactants, can contribute taste to food. Lecithins are prone to oxidation giving rise to volatile, aroma-active molecules and hydrolyzed lecithin is described as having a strawy, nutty odor as well as a bitter taste (95). The bitterness of the hydrolyzed lecithins is believed to be due to the presence of free fatty acids, with linoleic and linolenic acid having the lowest taste thresholds (67 and 11 mg respectively per 100 g of an emulsion) (95). Any study of bitterness suppression by lecithin should consider direct taste-blocking, the inherent tastes of lecithin components as well as any potential molecular binding by association structures.

Surfactants are soluble in water up to a certain point (the critical micelle concentration, CMC) and beyond that will tend to self-assemble to minimize the contact between the hydrophobic parts of the molecules and water. Initially micelles form but as concentration is increased further a variety of liquid crystalline forms can be seen including elongated micelles packing in a hexagonal or cubic geometries or lamellar phases (92). Which structure forms depends on the concentration of surfactant, the preferred packing angle of the surfactant molecules, temperature, solution composition and the presence of other molecules that can pack alongside the surfactant molecules as co-surfactants (e.g., long-chain alcohols).

Surfactant self assembled structures can accommodate a range of guest molecules. More polar molecules will tend to partition amongst the hydrocarbon tails while amphiphilic molecules will partition in the palisade layer with parts of their structure close to the polar head groups. Finally hydrophilic molecules may associate with the surface of the surfactant structure while remaining in the aqueous phase. The amount of solute that can be accommodated by the surfactant structure is finite and usually expressed as the phase boundary in a three-component phase diagram. For example SDS micelles can accommodate 63 molecules of caffeine but only 17 of theophylline (96). In addition, the solute can act as a co-surfactant and affect the properties of the micelles.

The thermodynamically stable structures formed from lipophilic molecules (i.e., co-surfactants), surfactant and water are called microemulsions. While they are formed from similar ingredients as the conventional emulsions considered below, there are important differences (97). First, microemulsions are thermodynamically stable while emulsions are kinetically stabilized but thermodynamically unstable structures. The structure of a microemulsion depends on the phase diagram and may quickly change upon dilution, addition of other ingredients or changes in temperature. For example, micelles diluted below the CMC will dissociate to monomers while emulsions can usually be diluted without dissolution. Second, microemulsions tend to be formed with a higher surfactant: lipid ratio than emulsions. Finally, in microemulsions, all of the hydrophobic molecules are intimately associated with the tail groups of the surfactant while in emulsions there is also a distinct population of effectively pure lipid.

Some surfactant phases can be broken up (usually mechanically using a homogenizer or sonicator) to form dispersions of liposomes in the aqueous phase. The most common of these liposomes are vesicles (formed from lamellar phases) but cubic and hexagonal phases can be broken up to form cubasomes and hexasomes respectively. While the self-assembly of the surfactant is thermodynamically stable, the colloidal suspension formed from their fragmentation is not. Vesicles deserve special attention because they can accommodate hydrophilic guest molecules in the internal aqueous phase as well as within the surfactant bilayers. They are typically between 50 and

500 nm in diameter and can consist of single bilayer or multilayers. Liposomes are used for the solubilization, protection and delivery of drugs (98) and food ingredients (99). Liposomes are sometimes stabilized by physically adsorbing polymers at the surface or, for intravenous applications, to reduce their interaction with blood components and increase their circulation time.

### *Examples*

Appropriate amounts of polyphenolic compounds in the diet are widely believed to have a positive effect on human health and, while there are several possible mechanisms, some of these include interaction with membranes. Sirk et al.(100) used molecular dynamics to investigate the interactions of seven green tea polyphenolic compounds with membranes. They showed that the polyphenols interacted with the surface of the membrane via hydrogen bonds and some of the smaller compounds could also penetrate beneath the membrane surface. The amount of polyphenolic compounds bound to the surface decreased with increasing negative charge on the membrane and with increases in aqueous salt concentration (101). Many of the liposomes described above provide membrane-like environments which can bind polyphenolic compounds preventing them frominteracting with the tongue.

Suzuki and others (102) investigated the properties of a commercial bitter-masking compound based on lecithin (Benecoat BMI-40, Kao Corporation, Japan). The bitterness of an acetaminophen or quinine solution was significantly reduced in the presence of 1% Benecoat. Similarly the bitterness of olive oil is reduced by the addition of granular lecithin (up to 0.3%) (103). These workers suggested the phenolic compounds of the olive oil are entrapped in lecithin reverse micelles or liposomes but their presence was not determined analytically and the other potential effects of lecithin on bitter taste were not considered. Nevertheless, there were also significant differences in other taste and aroma parameters suggesting there is some level of small molecule binding by the phospholipids.

Gülseren and others (104) showed green tea polyphenols could be incorporated in liposomes from milk phospholipids (at 4 mg $ml^{-1}$) without changing the particle size ($d \sim 200$ nm). However other workers have shown that even modest concentrations of polyphenolic compounds ($\sim > 30\ \mu M$) can cause liposomes to leak and burst (105).

## Lipids and Emulsions

### *Structures and Interactions*

Many bitter molecules are hydrophobic, so if they partition into a lipid phase, the aqueous concentration is reduced, which should reduce perceived bitterness. The distribution of molecules between a lipid and aqueous phase is given by the oil water partition coefficient:

$$K_{ow} = \frac{c_{oil}}{c_{water}} \tag{2}$$

where c is the concentration (or more strictly activity) in the subscripted phase (106). The concentration of solute molecules in the aqueous phase of an oil-water mixture is shown as a function of oil concentration and $K_{ow}$ in Fig. 3. For hydrophobic molecules (large $K_{ow}$) increasing the amount of oil increases the concentration in the aqueous phase while the reverse is true for hydrophilic molecules. For very hydrophobic compounds the presence of even a small amount of oil can have a large effect on the aqueous concentration.

Any factor that changes the molecular interactions between the solute molecule and either the aqueous or the lipid phase will change the value of $K_{ow}$ For example, if the bitter molecule has any ionizable groups, changes in the pH that favor the presence of the ionized form of the molecule will reduce $K_{ow}$ because there are stronger intermolecular attractions between ions and water than between polar groups and water and because charged groups have effectively no solubility in oil. Thus while lowering the pH of caffeic acid (from 7 to 3) allows some to partition into a lipid phase (107), similar pH changes have no effect on the partitioning behavior of catechin (108). Similarly, compounds that increase the solubility of the compound in one phase (e.g., aqueous micelles of cyclodextrins that solubilize hydrophobic molecules in the aqueous phase) will decrease the concentration in the other.

Unfortunately there are few values of $K_{ow}$ published for bitter molecules in the lipid and aqueous phases of interest (Table I). In many cases octanol-water partition coefficients (P) are often used as a proxy for $K_{ow}$. Values for log P are more widely available (109) and can be estimated from structure (e.g., (110)). However octanol is a more polar phase than the triacylglycerol mixtures usually of interest and so log P values are more useful in comparing the properties of different bitterants than in giving a quantitatively satisfactory prediction.

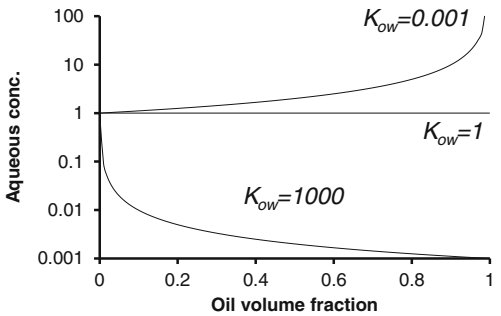


**Fig. 3** Effect of oil- volume fraction on the aqueous concentration of a hydrophilic ($K_{ow} = 0.001$), neutral ($K_{ow} = 1$) and hydrophobic ($K_{ow} = 1{,}000$) compound.

**Table I** Triglyceride-Water Partition Coefficients for Bitter Molecules

| Molecule | Lipid phase | Aqueous phase | $K_{ow}$ | Reference |
|---|---|---|---|---|
| Caffeic acid | Bulk stripped corn oil | pH 7 phosphate buffer | All aqueous | (108) |
| Catechin | Bulk stripped corn oil | pH 7 phosphate buffer | 0.008 | (108) |
| Catechin | Bulk stripped corn oil | Distilled water | 0.033 | (111) |
| Hydroxytyrosol | Bulk Stripped olive oil | pH 3 citrate buffer | All aqueous | (112) |
| Hydroxytyrosol acetate | Bulk Stripped olive oil | pH 3 citrate buffer | 1.63 | (112) |
| Olive oil phenolics | | | ~1 | (88) |
| Efavirenz | Medium chain triglycerides | Water | 4.65 | (113) |

Lipids are much more typically consumed as oil-in-water emulsions rather than as bulk fats. In an oil-in-water emulsion, the oil is dispersed as fine droplets (diameter typically 100 nm to 10 μm) in a continuous aqueous phase. Submicron emulsions are sometimes described as nanoemulsions, although the term would be better reserved for diameters less than 100 nm where the dispersion starts to become optically clear. An emulsion is a thermodynamically unstable structure, due to the surface excess free energy, and will tend to phase-separate over time via droplet flocculation and coalescence, and creaming (114). However, emulsions can be kinetically stabilized using appropriate emulsifiers, usually small molecule surfactants or proteins, to reduce the interfacial tension and produce repulsive interdroplet interactions. If the lipid molecules are relatively hydrophilic (e.g., flavor oils) then Ostwald ripening may also be important as a destabilization mechanism. Ostwald ripening is the diffusion of lipid molecules from one smaller to larger droplets driven by a difference in surface curvature. Ostwald ripening can be slowed by adding a highly water-insoluble oil to the formulation.

Emulsions can be modified to produce a range of emulsion-based delivery systems (EBDS) (35, 115–117).

- The internal lipid phase can be crystallized to form solid lipid nanoparticles (SLN, a term usually reserved for submicron particles) (115). SLN are typically plate-like rather than spherical and the dispersions are frequently physically unstable. SLN were first developed in an effort to slow the release of hydrophobic drugs from emulsion droplets. However, in most cases crystallization of the lipid forces the drug out of the crystal core to the surface of the particle or immediately expelled into the aqueous phase.
- Nanolipid composites (NLC) have also been used where a small fraction of liquid oil is present alongside the solid fat of the SLN. Again the liquid oil is most commonly expelled to the particle surface where it acts as a reservoir for the hydrophobic solute molecule (118).
- A fine water-in-oil emulsion can be used as the dispersed phase of an oil-in-water emulsion resulting in a water-in-oil-in-water (w/o/w) multiple emulsion. Multiple emulsion offers a way of segregating a water soluble compound from saliva as the food is consumed but they are frequently physically unstable due to osmotic pressure gradients between phases,
- Multilayered droplets can be generated by adsorbing successive layers of material on top of one another at the interface (119). The multilayers can be designed to dissociate in response to an external stimulus (e.g., changes in pH) so solutes trapped in the interfacial layer will be released.

The interfacial region can usefully be regarded as a third phase in the system. The "interphase" has a composition different from either of the bulk phase and contains very high concentrations of emulsifiers. Because the interface is asymmetric with a polar side and a non-polar side there is scope for amphiphilic molecules to adsorb. Most bitter-tasting molecules have both hydrophobic and polar functional groups so even if the log P value is high (Fig. 1), they may have low triglyceride solubility and will instead preferentially adsorb at an interface. The binding capacity of an interface can be expressed in terms of a surface partition coefficient, $K^*_{iw}$ :

$$K^*_{iw} = \frac{\Gamma A}{c_{water}} \tag{3}$$

where the concentration of the compound of interest at the interface is expressed as a product of surface excess concentration (Γ) and total surface area (A) (120). It is helpful to combine Eqs. 1 and 2 to give an effective partition coefficient between the droplets (i.e., interface plus oil phase) and water:

$$K_{dw} = \frac{c_{droplets}}{c_{water}} = \frac{K_{ow}}{1 + K^*_{iw}} \tag{4}$$

Equation 3, unlike Eq. 1, depends on the particle size of the dispersion as $A = 6\phi/d_{32}$ ($\phi$ is the oil volume fraction and $d_{32}$ is the Sauter mean diameter). If surface binding is important, decreasing the particle size of an emulsion should reduce the aqueous concentration but if it is not the lipid:water phase volume ratio is the only important factor. The value of $K^*_{iw}$ is a measure of the interactions of the bound molecule with the interphase. The interphase is likely highly concentrated with protein and/or surfactant molecules so the types of interactions are probably similar to those described for polymers and

surfactants above, and the value of $K^*_{iw}$ will depend on the composition of the interface.

Values for $K_{ow}$ can be measured relatively easily by gently shaking the two phases together until the bitter molecule has reached equilibrium between them, then separating the oil and water and measuring the concentration in each. However, in an emulsion, the interface cannot be separated so measuring $K^*_{iw}$ is challenging. Stockmann and Schwartz (121) developed a method to determine the distribution of small molecules based on separation of the phases by ultrafiltration and dialysis. In general when the amount of lipid was held constant and the amount of surfactant increased, a lipophilic solute moves from the oil and water phases to the interface. For example, almost half of 3,4-dihydroxybenzoic acid ethyl ester is present in the aqueous phase of a 20% o/w emulsion but if 5% SDS is added almost none remains (Fig. 4a). Rather than separating and analyzing the phases, (122) obtained similar results by electrochemically measuring the rate of reaction of 16-$ArN_2BF_4$ with gallic acid in a opaque, coarse mixture of corn oil, water and surfactant. The 16-$ArN_2BF_4$ was selected as an initiator as it has no water or oil solubility so the reaction with gallic acid occurs exclusively at the interface. By measuring the apparent rate constant as a function of surfactant content at two lipid concentrations they were able to calculate oil-water and interface-water partition coefficients for tocopherol and calculate the concentration in each phase. Gallic acid did not partition into the lipid phase but the amount in the aqueous phase decreased dramatically with even small amounts of added Tween 20 (Fig. 4b).

Alternatively some spectroscopic techniques can be used to distinguish the distribution of small quantities of small molecules in different environments in an emulsion. Electron paramagnetic resonance (EPR) spectroscopy is sensitive to the amount and polarity of an unpaired free radical. (124) measured the EPR spectra of PTMIO in an emulsion system (Fig. 5a). PTMIO is a phenolic compound with a nitrone group, a stable free radical attached. The characteristic EPR spectrum of PTMIO in oil is three distinct peaks, but in an emulsion the spectra is more complex as the molecule is partitioned between three different environments each with different polarities. The complex spectra can be deconvoluted to calculate the amount of PTMIO in the oil, water and interface (Fig. 5b). This method allows direct measurement of the distribution of a probe molecule in an intact emulsion but as a spin-labeled probe must be used it is hard to be certain if the results are relevant to real bitter molecules.

The use of a three-phase binding model (i.e., lipid, aqueous and interface) to describe interactions with small molecules with an emulsion is complicated as there is very often free protein or surfactant in the aqueous phase. For example, Watrobska-Swietilowska et al. (125) used a series of

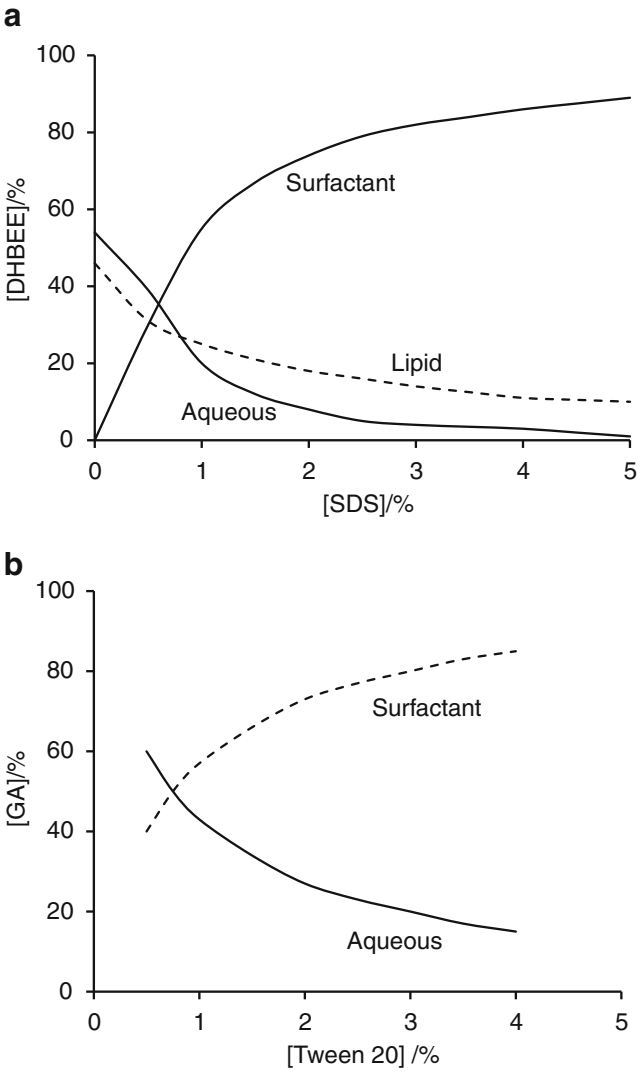


**Fig. 4** (**a**) Proportion of 3,4-dihydroxybenzoic acid ethyl ester solubilized by the lipid phase and its constituents (SDS and oil) at different surfactant concentrations (SDS) at a constant oil level of 20%. Reproduced from (121). (**b**) Distribution of GA between the aqueous (*circles*) and interfacial (*triangles*) regions of a 10% corn oil in water emulsion prepared with different amount of Tween 20. Reproduced with minor modifications from (123).

phase separation and partitioning experiments to show propyl paraben (log P~3) partitioned between the aqueous phase (4.6%), aqueous micelles (3%), interface (58.2%) and lipid phase (33.9%) of a lecithin-stabilized oil-in-water emulsion. However these findings should be treated with caution as the method to separate the "interfacial" lecithin may not be representative of the surface of the intact emulsion. It is an open question as to how small molecules partitioned into each of these phases might differently contribute to taste.

### *Examples*

The bitterness of quinine is less readily detected in oil than in water or a viscosity-matched aqueous methylcellulose

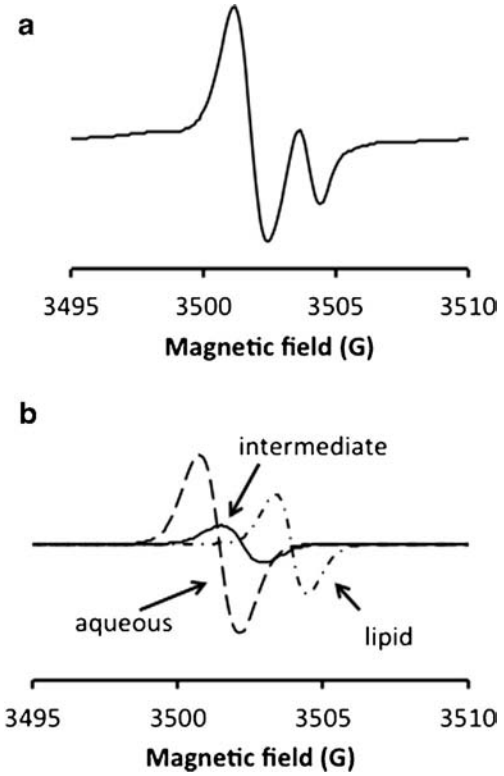


**Fig. 5** EPR spectra of PTMIO in a 10% tetradecane in water emulsions ($d$ = 200 nm) stabilized with sodium caseinate (1%). The experimental spectra (**a**) can be deconvoluted into contributions from the lipid phase, aqueous phase and an intermediate population associated with the phospholipids (**b**).

solution (126). Quinine is relatively hydrophobic (log $P$=3.1) so would be expected to remain in a lipid matrix rather than partition into the saliva. Indeed, even if quinine is ingested in a fat-free matrix the presence of a oily mouth coating can suppress its bitterness (127). The "mouth burning" qualities of efavirenz, an antiretroviral drug, were reportedly reduced when consumed in solution in a liquid oil than when consumed as a suspension of the powder in viscosified water (113). It should be noted that this conclusion (i.e., dissolution in lipid suppresses taste) is confounded by differences in viscosity, appearance and in the presence of other tastants.

However, bulk oils are rarely consumed directly, and in any case the type of partitioning likely responsible for any effects are easier to study if the oil was first emulsified. This was achieved elegantly by Metcalf and Vickers (128) who showed increasing the fat content of an o/w emulsion depressed the bitterness of quinine. By diluting a stock quinine solution in two levels of either water or oil they could demonstrate the concentration in the aqueous phase was responsible for the bitter taste.

The suppression of bitterness by fat is not universal and depends on the properties of the molecules responsible. For example milk fat content in an emulsion had a significant effect on suppressing the bitterness of only one of two polyphenolic plant extracts considered (129). In other cases the results are apparently contradictory. For example, while Mattes (130) showed the bitter taste threshold of caffeine was increased in the presence of 1% linoleic acid sonicated with 5% acacia gum solution (pH 4.57), Keast (131) found the opposite trend and caffeine in full fat milk (4% milkfat) was more bitter than in reduced fat or skim milk (2% or 0% fat respectively). Caffeine is more hydrophilic than quinine, so the partitioning into a fat phase would be expected to be less important than in the quinine studies and, as Keast also suggested in his study, strong interactions between caffeine and milk proteins might swamp any lipid effects. Similarly while aqueous caseinate reduced the bitterness of phenolic compounds extracted from olive oil, the presence of oil droplets did not (88) and in another study the bitterness of hydrophilic ibuprofen was similar in skim milk, full fat milk and half-and-half (132) (although interestingly the throat-burning sensation associated with ibuprofen was suppressed).

The type of liquid oil has been shown to affect the bitterness; for example phenolic compounds from olives are more bitter in emulsions formed from monounsaturated oils than in polyunsaturated oils (133). Similarly, Koriyama et al.(134) showed the bitterness evoked by quinine sulfate in tuna oil emulsions (containing highly unsaturated fatty acids) was less than the bitterness in similar emulsions formulated from vegetable oils. This may be a physical effect, due to the different polarities of the oils affecting the distribution of the bitter molecules, or a cognitive effect due to the different affinities of different fatty acids for the CD-36 "fatty taste receptor" (135), the aroma of fatty acid oxidation products, or the bitterness of unsaturated free fatty acids (95, 136). Other workers have seen no effect of lipid type on bitter taste in emulsions (137).

For hydrophilic bitterants, the presence of a lipid phase would serve only to increase the aqueous phase concentration and the bitter taste. One approach to reducing the bitterness of aqueous molecules is to segregate them in the internal phase of a water/oil/water (w/o/w) multiple emulsion. Mendanha and co-workers (138) formed a water-in-oil emulsion containing soy hydrosylate solution and then emulsified that onto soy protein solution to form a w/o/w emulsion with the bitter component in the internal phase. Pectin was added to form a coacervate, which was separated and freeze dried. Encapsulation reduced the solubility of the protein hydrosylates, and suppressed the bitterness. It is not clear in this work if the protein hydrosylates remained in the internal phase of the multiple emulsion or instead adsorbed to the interfaces or perhaps complexed with the pectin.

Higher molecular weight saturated fats often have melting points approaching or exceeding body temperature, so it is possible to formulate structures that will remain solid during mastication. In a few cases the fat crystal matrix has been used to entrap bitterants. For example, Suzuki et al. (102) showed

chewable acetaminophen tablets formed from a hard fat reduced the bitterness in a dose-dependent manner (although it was not possible to formulate a liquid-oil control). In subsequent experiments, they showed fat blends with higher melting points, greater than body temperature, tended to have lower rates of drug release as well as lower bitterness (139). It seems likely that, as the fat did not completely melt during chewing, the residual solid structure helped retain the bitter compound. However the high melting fats were rated as unpleasant and it seems unlikely this principle could be applied outside pharmaceuticals. It is possible to formulate fine suspensions of crystalline fats (i.e., solid lipid nanoparticles) and the rheology of these suspensions is often similar to the corresponding liquid-droplet nanoemulsion so this might be a more acceptable way to deliver a solid fat. In most cases it appears that any hydrophobic solute is excluded from the droplet upon crystallization and will adsorb to the surface (115). While solid lipid nanoparticles have not been evaluated as bitterness suppressors, it seems unlikely they will perform better than the corresponding liquid oil nanoemulsion.

While most studies in this work cite a two-phase partitioning model to explain their work there has been little consideration of the potential for emulsion microstructure to affect bitter tastes and the results are inconclusive. The bitterness of quinine in fish oil-in-water emulsions was only significantly dependent on droplet size for one of the fish oils studied, suggesting limited interaction with the droplet surface (140). On the other hand, in the same study rats significantly preferred the quinine-containing emulsions with smaller droplet sizes for all the three fish oils in a two-bottle preference test. Quinine is aversive to rats so presumably they tasted it less in the emulsions with larger surface areas. In other work, Barylko-Pikielna and others (141) showed the bitterness of caffeine in w/o and o/w emulsions of the same composition was similar despite the drastically different microstructure. However, as noted above, lipids probably affect caffeine less than other bitter compounds and different results might be expected for other bitterants.

The role of the surface as a domain for amphiphilic bitter molecules leading to taste suppression deserves more consideration. There is considerable evidence that many bitter compounds have some surface activity. For example, catechin was shown to lower the oil-water and air-water interfacial tension (142) while [143] showed several flavonoids (including naringin) will adsorb at an oil-water interface and can even serve to stabilize an emulsion in the absence of another emulsifier. The alpha-iso-acids in beer are reported to increase foam stability which suggests either a level of surface activity for these compounds or, more likely, an interaction with polymers adsorbed at the surface (144, 145). A systematic investigation of the effects of particle size (i.e., interfacial area) on different bitterants using the dilution methodology developed by Metcalf and Vickers (128) would be valuable.

## CONCLUSIONS

There is a need to increase compliance with medical or nutritional advice to consume bitter compounds that are typically aversive. The nature of these compounds varies widely and while it is not possible to build a universal predictor of bitterness, we can see most of the molecules surveyed share some gross features; they tend to be somewhat hydrophobic but with some polar or charged functional groups and have molecular weights in the hundreds. The bitterness of these compounds depends on the concentration in the saliva, which can be reduced by either binding them to an excipient or by entrapping them in a particle they cannot diffuse out of.

Binding could involve interaction with hydrophobic or polar parts of the structure. However as most bitter molecules are at least somewhat amphiphilic, a pure lipid phase may not be the most effective here. Small lipid droplets with more interfacial area would allow more scope for bitter molecules on the surface, particularly if that surface could be covered with a protein or polymer that favors binding. The amount of interfacial area in an emulsion increases with oil content and decreases with the square of particle size, so unless the bitterant has a particularly high affinity for the surface it may not be practical to generate enough surface area by this method for effective bitter masking. Self-assembled surfactant structure, particularly those formulated from bitter-masking fractions of lecithin, or cyclodextrins allow much more amphiphilic binding per unit mass and may be more effective at lower levels.

Alternatively, the bitter molecules could be bound via their polar group. This may be particularly useful if it is possible to make a strong electrostatic complex with a charged bitterant. Polyelectrolytes provide a useful material in this case, particularly co-polymers with hydrophobic groups that can also provide a domain for the hydrophobic group on the bitterants. To be effective the polyelectrolyte must have an opposite charge to the bitterant at mouth pH (6.5–7.0). In pharmaceutical applications the basic butylated methacrylate copolymers (e.g., Eudragit®) offer a useful way to bind acidic bitterants and poly-acidic ion exchange resins can bind basic compounds. Several food polysaccharides are negatively charged at mouth pH (e.g., alginate, pectin) and a smaller set is positively charged (e.g., chitosan).

If binding alone cannot provide adequate protection, it may be possible to entrap the bitterness in a particle it cannot diffuse out of. This is likely to be more successful in products that are stored and consumed in a dry state and have a limited time for the bitter compound to diffuse out before they are swallowed (e.g., orodispersible tablets, intermediate and low moisture foods). In high moisture foods and liquid pharmaceutical preparations, even slow diffusion is likely to allow significant release over weeks or months. Again the basic butylated methacrylate copolymers show promise here as they

dissolve quickly at pH found in the stomach but not the mouth. In foods, spray drying with an appropriate slow-dissolving polymer seems a promising approach, particularly if some fat can be incorporated into the blend to slow the access of water. Drying a bitterant-cyclodextrin or bitterant-lecithin complex into a poorly soluble powder would provide even higher levels of bitterness suppression.

If physical structures to mask bitterness are to be intelligently designed according to these principles, they require proper physicochemical (i.e., where is the bitterant, how is it bound, how quickly is it released?) and sensory characterization (i.e., what does it taste like to people intended to eat it under conditions close to real consumption). Too often in the literature one of these is missing: a sensory-led study will report one material tastes different to another without any attempt to characterize structure, or a chemistry-led study will merely report ratings of bitterness without any controls or measures of statistical significance or, worse yet, statistical power. A particular challenge to the meaningful physical characterization of bitter-masking structures will be to understand the changes occurring in the mouth. Recent work on food emulsions showed a profound and unexpected destabilization as a result of interaction with salivary mucins and shear between the tongue and the roof of the mouth (146, 147). Structures characterized in vitro are likely not the same as those present in vivo. Testing in the vulnerable populations of interest will be a particular challenge for the meaningful sensory characterization of bitter-masking structures for drugs.

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