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Intrinsic Significance of Molecular Aggregation in Sucrose–Surfactant Separation by Dialysis

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ABSTRACT

Aqueous sucrose–surfactant mixtures were dialysed and the diffusates examined quantitatively for sucrose at intervals by spectrophotometric analysis. The effect of surfactants (lecithin and glycerol monostearate) on the dialysability of sucrose from these solutions was also investigated and the amount of sucrose dialysed was found to increase steadily only up to about 2 h with lecithin slowing down the rate of sucrose dialysis much more than GMS. These variations in the dialysability of sucrose from the mixtures are explained in terms of molecular aggregation and these are confirmed by freezing point depression measurements. The results are discussed in terms of the significance of molecular aggregation on the gustatory qualities of sapid molecules.

INTRODUCTION

Dialysis is a membrane transport process in which solute molecules are exchanged between two liquids separated by a membrane. It is an indispensable technique in the recovery and purification of materials in food, chemical, biological and pharmaceutical preparations (Dean, 1969). Since non-diffusion through a membrane is generally a criterion of

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the colloidal state (Jacobs, 1951), it forms the basis of separation of colloidal materials from crystalloids.

In the taste chemoreception process, enhanced lipophilicity has been viewed as a function which can facilitate the accession of stimuli to receptors (Dziedzic, 1980), thus resulting in elevated sweetness. Clearly the lipophilicity conferred on the sugar molecule by the formation of a sucrose–surfactant aggregate would not constitute a steric factor, as originally envisaged for enhanced sweetness (Kier, 1972), but a facilitation of the approach and accession of stimuli to fresh receptor sites. This might, for example, be more related to temporal characteristics of taste response.

In the dialysis of sucrose from binary sucrose–surfactant solutions, the sucrose would be expected to diffuse out, leaving the colloidal surfactants as well as, possibly, sucrose–surfactant aggregates. The freezing point depression effect produced can be applied as an indication of such molecular aggregation as it is a physical property depending on the number of molecules in solution and is characteristic of undissociated molecules (Kearsley, 1976). The transfer of material across the dialysing membrane is normally governed by certain factors, viz., the inherent mobility or dialysability of the crystalloid, which is enhanced by the capacity of the membrane for swelling in the solvent, and the adsorption of colloidal material on the membrane surface, which blocks the pores and exerts a restrictive effect on both solute and solvent species (Perry & Chilton, 1973). Thus the rate of sucrose transfer across the membrane, as affected by surfactant concentration, should provide an indication of the relative importance of molecular aggregation and possibly viscosity effects.

If similar considerations are extended to the diffusion of stimulus molecules in the vicinity of taste receptor membranes, then the significance of the molecular aggregation in terms of overall gustatory response can be evaluated.

MATERIALS AND METHODS

Sucrose (AR grade obtained from May and Baker Ltd, UK) and food grade surfactants, glycerol monostearate (GMS, obtained from BDH Poole, UK) and Lecithin 'Wylfo' A (LEC, obtained from Wynmouth Lehr and Fatoils Ltd, London, UK) were used in this work.

Solutions containing sucrose and graded concentrations of the surfactants were prepared and complete mixing was achieved by using the Ultra-turrax tissue macerator for 60 s. These solutions were subjected to dialysis and freezing point measurements. Two replicate measurements were taken in each case and the mean values obtained are presented throughout.

Dialysis

Precut and open-ended dialysis 'sacks' which are membranes of 24 Å pore diameter obtained from Sigma Chemical Co. were used. These were expanded by soaking in distilled water at room temperature overnight and patted damp-dry before use. These membranes were found suitable for the requirements of high permeability, pore size and mechanical stability (Leonard, 1965). Each was knotted at one end while 10 ml aliquots of 5 % w/v sucrose only, or 5 % w/v sucrose solutions containing 0·5 or 1·0 % w/v added surfactants GMS or LEC were pipetted in through the open end which was also subsequently knotted. Each membrane was then totally immersed in 100 ml distilled water, in order to ensure as large a dialysing surface as possible. The beakers were placed in a water bath maintained at 50 °C and fitted with a shaker, since mass transfer effectiveness in fluids is greatly enhanced by agitation. The diffusate containing only sucrose was examined quantitatively at intervals over a 4 h period by taking 2 ml aliquots for spectrophotometric analysis using the phenol–sulphuric acid method for total carbohydrate (Dubois *et al.*, 1956). Sucrose content of diffusate was obtained from the calibration curve prepared with every batch of samples using standard solutions containing 10, 20, 40, 60, 80, 100, 150 and 200 μg 2 ml^{-1} sucrose. Optical density was measured at 490 nm on a Unicam SP 600 spectrophotometer.

Freezing point measurements

The Hortvet apparatus for the determination of freezing points of solutions (Pearson, 1976) was used. The calibration of the Hortvet thermometer was carried out with a series of sucrose solutions (1–10 % w/v) at 20 °C. Aqueous surfactant solutions containing 0·10–1·0 % w/v GMS or LEC were prepared and the freezing points of the solutions determined. Freezing points of sugar–surfactant mixtures containing 2 % w/v sucrose and 0·10, 0·20, 0·40, 0·60, 0·80, and 1·00 % w/v of surfactants

GMS or LEC, respectively, were also determined. The observed freezing point depression was taken as the difference between the thermometer readings for water and the solution of dissolved solutes, while the calculated freezing point depression was taken as the average of the sum of the individual freezing point depressions of dissolved solutes.

RESULTS AND DISCUSSION

Figure 1 shows the plot of sucrose dialysed at different time intervals. Only the crystalloidal sucrose would be expected in the diffusate since the hydrocolloidal surfactants would not diffuse through the membrane. This was confirmed by placing 0·5 and 1·0 % w/v solutions of surfactants GMS or LEC only, in dialysis membranes under the same conditions as the dialysing mixtures. After 2 h, no change in the freezing points of the dialysing solutions (surfactants) as well as the diffusates (distilled water)

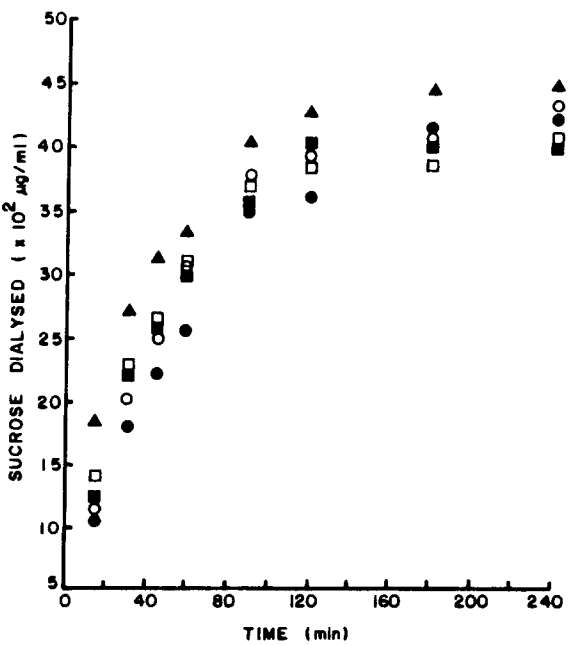

Fig. 1. Amount of sucrose present in diffusate at different time intervals. ▲ sucrose standard (5 % w/v) only, ○ sucrose + 0·5 % w/v GMS, ● sucrose + 1·0 % w/v GMS, □ sucrose + 0·5 % w/v LEC, ■ sucrose + 1·0 % w/v LEC. Mean values of two replicate measurements are plotted.

was found. The quantity from the standard solution is consistently greater than that of the sucrose–surfactant mixtures especially up to 2 h. This discrepancy reflects the probable effects of sugar–surfactant associations reducing the overall dialysability of crystalloidal sucrose. If the dialysis from such a mixture takes place by diffusion then the rate of transport of sucrose molecules through the membrane must be proportional to the sucrose concentration gradient in the direction of diffusion, i.e. Fick's 1st law of diffusion is obeyed. At equilibrium, the concentration of sucrose in dialysing solution will be equal to the concentration in the diffusate.

Thus if the equilibrium concentration is C_∞ and initial volumes of dialysing solution and diffusate are 10 ml and 100 ml respectively, then the total volume = 110 ml, and

$$C_\infty = 5/100 \times 10/110 \times 10^6 \,\mu g\, ml^{-1},$$
$$= 45{\cdot}45 \times 10^2 \,\mu g\, ml^{-1}.$$

Let the volume of dialysing solution = V_0 ml, containing C_0 $\mu g/ml^{-1}$ sucrose initially. At time t, the concentration of sucrose in dialysing solution = $C\,\mu g\,ml^{-1}$. If volume of diffusate = V ml and concentration in diffusate at time $t = C'\,\mu g\,ml^{-1}$, then assuming that (i) no volume changes occur on either side of the membrane, and (ii) mass transfer coefficients are constant along the diffusion path, then the mass of sucrose is conserved, and $VC' + V_0C = V_0C_0$

or
$$VC' = V_0(C_0 - C)$$
$$C' = V_0/V)/(C_0 - C) \tag{1}$$

Since rate of sucrose transport is proportional to the sucrose concentration difference

$$\frac{dm}{dt} = A(C - C')$$

where A is a constant. If dm is transported as concentration C falls by $-dC$, then $dm = -V_0\,dC$. Therefore

$$dC/dt = -(A/V_0)(C - C') \tag{2}$$

Combining equations gives

$$\frac{dC}{dt} = -A\left\{\frac{C}{V_0} - \frac{C_0}{V} + \frac{C}{V}\right\} \tag{3}$$

After an infinite period of dialysis

$$C = C' = C_\infty$$

$$VC' + V_0C = V_0C_0$$

or

$$VC_\infty + V_0C_\infty = V_0C_0$$

i.e.

$$C_\infty = \frac{V_0}{V + V_0} \cdot C_0 \tag{4}$$

Combining eqns (3) and (4) gives

$$\frac{dC}{dt} = -A\left(\frac{1}{V_0} + \frac{1}{V}\right)(C - C_\infty)$$

$$dC/dt = -B(C - C_\infty)$$

where

$$B = A\left(\frac{1}{V_0} + \frac{1}{V}\right)$$

or

$$\frac{dC}{C - C_\infty} = -B\,dt \tag{5}$$

On integration

$$\int_{C_0}^{C} \frac{dC}{C} - C_\infty = -B\int_0^t dt$$

$$[\ln(C - C_\infty)]_{C_0}^{C} = -B(t)_0^t$$

$$\ln\frac{C - C_\infty}{C_0 - C_\infty} = -Bt \tag{6}$$

Since

$$C' = \frac{V_0}{V}(C_0 - C)$$

$$C = C_0 - (V/V_0)C'$$

Thus, substituting C in eqn (6) gives

$$\ln \frac{C_0 - (V/V_0)\,C' - C_\infty}{C_0 - C_\infty} = -Bt$$

$$\ln\left(1 - \frac{VC'}{V_0(C_0 - C_\infty)}\right) = -Bt \tag{7}$$

As equilibrium is reached, $C' \rightarrow C_\infty$ and

$$\ln \frac{C_\infty - C'}{C_0 - C_\infty} \cdot \frac{V}{V_0} = -Bt$$

$$\ln (C_\infty - C') = -Bt - \ln\left(\frac{V}{V_0(C_0 - C_\infty)}\right) \tag{8}$$

Thus a plot of $\ln (C_\infty - C')$ against t should be linear. When values of $C_\infty - C'$ against time presented in Table 1 are plotted on a log-linear scale, deviations from linearity are observed. These might be due to adsorption effects as a result of viscosity changes or molecular aggregation either resulting in the blocking of membrane pores or generally reducing the amount of sucrose passing through the membrane into the diffusate. If the quantity dialysed $(C_\infty - C')$ at time t is taken as a

TABLE 1

Values of $(C_\infty - C') \times 10^2\,\mu g\,ml^{-1}$ of Sucrose Dialysed at Various Time Intervals

Time (min)	*Concentration difference of sucrose dialysed,* $(C_\infty - C') \times 10^2\,\mu g\,ml^{-1}$*, from 5% w/v sucrose solutions containing added surfactant*				
	0% w/v surfactant	*0·5% w/v GMS*	*1·0% w/v GMS*	*0·5% w/v LEC*	*1·0% w/v LEC*
15	26·7	34·2	35·2	31·2	33·2
30	18·2	25·2	27·2	22·6	23·2
45	14·0	20·2	23·0	18·5	20·0
60	12·0	15·0	19·7	14·5	15·2
90	4·70	7·70	10·5	8·70	10·2
120	2·70	6·20	9·20	5·20	7·20
180	0·70	4·45	3·95	4·70	6·70
240	0·20	1·70	2·95	4·45	5·25

C_∞ = Equilibrium concentration of sucrose in diffusate = $45{\cdot}45 \times 10^2\,\mu g\,ml^{-1}$.
C' = Actual concentration of sucrose in diffusate at time t, obtained from Fig. 1.

measure of the efficiency of sucrose–surfactant associations, then Table 1 shows that as the concentration of surfactant is increased, the efficiency of interaction is significantly increased ($P < 0{\cdot}001$) and the difference between the surfactants GMS and LEC is probably significant in this respect ($P < 0{\cdot}05$) with LEC slowing down the rate of sucrose dialysis much more than GMS. However, from Table 2, on the basis of their respective molecular weights, LEC is equivalent to about 3·5 times weight for weight of GMS. Thus, if efficiency of surfactant in slowing down the rate of sucrose dialysis is related to molecular size, then the difference between the surfactants GMS and LEC should be greater than is indicated from Table 1.

TABLE 2
Average Molecular Weights of 5% w/v Surfactant Solutions Compared with Reference Values

Compound	*Average molecular weight (determined by freezing point method)*	*Average molecular weight*
Glycerol monostearate	358	359[a]
Soya lecithin (Wylfo 'A')	1261	—
α-lecithin	677	678[b]

[a] BHD Chemicals, UK.
[b] CRC *Handbook of Chemistry and Physics*, 1979.

Generally the medium in which a sapid molecule like sucrose is transported to the environment of the taste receptor affects its subsequent penetration or diffusion into tastebud pores. In sugar–surfactant solutions, the viscosity effect of the hydrocolloid may thus significantly affect perception. However, it has been shown that while GMS increases the viscosity of aqueous sucrose solutions, LEC has little or no effect (Ogunmoyela & Birch, 1982) and the viscosity effect is probably insignificant. Table 1 shows that LEC, which has no appreciable viscosity effect, slows down the initial rate of sucrose dialysis much more than GMS. This confirms that the viscosity effect is indeed insignificant which is not surprising at the low viscosities (< 50 cps) considered here.

Since the log concentration difference has been shown to be a negatively accelerating function of time, investigations of slope and intercept values of log-linear plots of $C_\infty - C'$ versus time should confirm the significance

of molecular aggregation effects and in addition the insignificance of pore-blocking effects. Table 3 lists the slope and intercept values obtained from log-linear data in Table 1. If the intercept is assumed to be a measure of the factors affecting the dialysability of sucrose from the solution, it is obvious that the intercept values are comparable with or without surfactant addition and the dialysability is therefore not likely to be affected by adsorption effects arising from viscosity and/or pore-blocking

TABLE 3

Slope and Intercept Values of Log-linear Plots of $(C_x - C') \times 10^2\ \mu g\,ml^{-1}$ Versus Various Time Intervals

Statistic[a]	*Concentration of added surfactant in 5% w/v sucrose solutions*				
	0% surfactant	*0·5% w/v GMS*	*1·0% w/v GMS*	*0·5% w/v LEC*	*1·0% w/v LEC*
Slope (m)	−0·022	−0·012	−0·011	−0·009	−0·008
Intercept (c)	+3·58	+3·36	+3·61	+3·25	+3·29

[a] $m = -B$; $C = \ln V/V_0.(C_0 - C_x)$.

of membrane by surfactant or sugar–surfactant aggregates. However, the slope values may be assumed to reflect a more profound effect, presumably the significance of sucrose–surfactant aggregates and their efficiency in slowing down the rate of sucrose dialysis. The negative slope value obtained for the solution containing no surfactant ($m = -0{\cdot}22$) is much higher ($P < 0{\cdot}001$) than the values obtained for other solutions containing surfactant and the values appear to decrease with increasing concentration of surfactant GMS or LEC, reflecting the importance of complexation effects in the sucrose–surfactant mixtures in slowing down the rate of sucrose dialysis. The slope values for the LEC solutions are slightly lower ($P < 0{\cdot}05$) than those for GMS solutions and confirm the previous suggestion that LEC is more efficient than GMS in complexing sucrose. But the efficiency of LEC relative to GMS observed from the slope differences does not parallel the efficiency predicted on the basis of their molecular weight differences (Table 2) since LEC has a molecular size about 3·5 times greater than GMS. It is therefore apparent from the foregoing that GMS may cause relatively greater effects than LEC on the gustatory response to the sugar.

According to Junk & Pancoast (1973), any soluble salts or other substances associated with sugar in a food product may materially change the freezing point of the mixture. Thus the freezing point depression effect might be expected to be a reliable index of such sugar–surfactant associations observed here as it is a physical property depending on the number of molecules in the mixture and is characteristic of undissociated molecules in such solutions. Table 4 shows the depression effect produced

TABLE 4
Change in Freezing Point Depression (Δ°C) with Concentration of Sucrose Solutions

Concentration of sucrose (% *w/v*)	*Freezing point depression*
1·0	0·0505
2·0	0·1100
2·5	0·1650
5·0	0·3085
7·5	0·4540
10·0	0·6150

with increasing sucrose concentration while Table 5 shows the change in freezing point depression of water and 2% sucrose solutions produced with increasing concentrations of surfactants GMS or LEC. The observed freezing point depression values for the mixtures (Table 5) are consistently greater than the corresponding average calculated values and the differences are statistically significant ($P < 0{\cdot}001$). Differences between the surfactants in terms of these values with increasing concentrations are also statistically significant ($P < 0{\cdot}001$). This strongly suggests that molecular aggregation or complexation between sugar and surfactant occurs and the greater activity of the solutes in the mixtures probably reflects the strong competition between sugar–surfactant associations and sugar–water or sugar–sugar associations. The slightly higher freezing point depression values produced by LEC solutions relative to GMS parallel the difference between LEC and GMS in terms of the incremental change produced in the optical rotation of sucrose solutions containing increasing concentrations of the surfactants (Birch & Ogunmoyela, 1980) but does not reflect the differences predicted on the

TABLE 5

Freezing Point Depression (Δ°C) of Aqueous Solutions Containing Increasing Concentrations of Surfactants Only, and 2% w/v Sucrose[a]

Concentration of surfactant (% *w/v*)	*GMS*			*LEC*		
	Observed Δ°C for surfactant solution only	*Observed Δ°C for sugar surfactant mixture*	*Calculated average Δ°C for sugar surfactant mixture*	*Observed Δ°C for surfactant solution only*	*Observed Δ°C for sugar surfactant mixture*	*Calculated average Δ°C for sugar surfactant mixture*
0·10	0·041	0·111	0·076	0·043	0·113	0·077
0·20	0·042	0·113	0·076	0·047	0·118	0·078
0·40	0·045	0·114	0·077	0·054	0·120	0·082
0·60	0·047	0·117	0·079	0·060	0·123	0·085
0·80	0·050	0·122	0·080	0·067	0·129	0·089
1·00	0·052	0·128	0·081	0·074	0·134	0·092

[a] Δ°C for 2% w/v sucrose = 0·110°C; see Table 4.

basis of their molecular weight differences (Table 2). It is therefore conceivable that GMS should cause greater effects than LEC on the gustatory response to the sugar, as has been previously reported (Ogunmoyela & Birch, 1982).

From the foregoing considerations, it is evident that observation from physicochemical techniques (e.g. dialysis and freezing) may enable valid predictions to be made of intrinsic sensory properties of foods and other materials and may therefore be invaluable supplements to current objective–subjective correlations in taste and olfaction.

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