Determination of Interfacial Areas in Emulsions Using Turbidimetric and Droplet Size Data: Correction of the Formula for Emulsifying Activity Index

David R. Cameron,[†,‡] Martin E. Weber,[†] Edmund S. Idziak,[‡] Ron J. Neufeld,[†] and David G. Cooper[*,†]

Departments of Microbiology and Chemical Engineering, McGill University, Montreal, Quebec, Canada H3A 2A7

Derivation of the emulsifying activity index (EAI) (Pearce, K. N.; Kinsella, J. E. *J. Agric. Food Chem.* 1978, 26, 716–723) from first principles demonstrates that a simple correction must be made. The original EAI does not provide units of interfacial area per gram of emulsifier as claimed: its value varies with the oil volume fraction. The correction provides the index with the desired units of square meters of interfacial area per gram of emulsifier. The interfacial areas of three suspensions of monodisperse latex beads were determined in four different ways: by turbidimetry using the original and corrected formulas for EAI; by calculation from the droplet size distribution measured by laser diffraction; and by calculation using bead diameters supplied by the manufacturer. For three bead sizes covering the range of droplet sizes in typical emulsions the interfacial area value based on the distribution of particle sizes was closest to the actual value. The interfacial area calculated by original formula for EAI was least accurate. The correction to the EAI formula improved correspondence of interfacial area measured turbidimetrically to the actual value and eliminated its dependence on the oil volume fraction in the emulsion.

INTRODUCTION

Emulsifiers are essential components of foods that contain two immiscible phases, one of which is dispersed as droplets within the other. The emulsifying agent reduces the rate of separation of the two phases to an acceptable level. The emulsifier properties of protein ingredients in foods are some of their most important functional properties. In practice, synthetic emulsifiers (e.g., polysorbates) are frequently incorporated in foods since these can be custom designed to provide particular characteristics and consequently are more effective emulsifiers than most of the protein preparations available.

Viscosifying agents (e.g., xanthan gum) are also common additives that improve emulsion stability in foods. These compounds have little surface activity but stabilize emulsions by increasing the viscosity of the continuous phase such that collisions between droplets of the dispersed phase are less frequent and phase separation takes longer to occur.

Emulsions are thermodynamically unstable due to the high free energy of the interface between the two phases. Emulsions can break by a variety of processes which include creaming, flocculation, coalescence, and oiling-off (Becher, 1965). Equilibrium is reached when the area of contact between the two phases is at a minimum. Emulsifiers slow the rate of phase separation. Emulsifiers often have an amphiphilic molecular structure and concentrate at the interface between two phases. Intuitively, an attractive method of evaluating emulsifiers would directly measure the area of contact between the two phases. Such a method was devised by Pearce and Kinsella (1978) and has been widely used.

According to the Mie theory of light scattering, the turbidity of a dilute suspension of spherical particles is related to its interfacial area (Kerker, 1969; Van de Hulst,

1957). Pearce and Kinsella (1978) used this relationship as the basis of the emulsifying activity index (EAI). They defined EAI = $2T/\phi c$, where turbidity, T, is 2.303 (absorbance at 500 nm)/(optical path length), ϕ is the oil volume fraction in the emulsion, and c is the mass of emulsifier per unit volume of the aqueous phase before the emulsion is formed. They claimed the EAI has units of square meters of interfacial area per gram of emulsifier, while in fact this is not so: the EAI values depend on ϕ. Their derivation of the formula was not presented.

We determined interfacial area in a dilute emulsion by a second method, by laser diffraction using a Malvern particle sizer. The interfacial area was computed from the droplet size distribution and phase concentration, and an index with the units claimed to be provided by the EAI was calculated. Wide divergence in the values obtained by the two methods led us to derive the formula for EAI from first principles. A simple correction to the formula of Pearce and Kinsella (1978) provides a corrected EAI that has units of square meters of interfacial area per gram of emulsifier and thereby makes the index theoretically sound. Data illustrating the effect of the correction are provided for model oil-in-water emulsions and for suspensions of monodisperse latex beads covering the range of droplet sizes in typical food emulsions.

THEORY

For a polydisperse suspension the following definitions are used: τ, optical transmittance; D, particle diameter; τ, $\exp[-(3/2)\phi l(\bar{K}/D_{32})]$ (Dobbins and Jizmagian, 1965); ϕ, volume fraction of the dispersed phase; l, optical path length; $\bar{K}$, mean scattering coefficient; D_{32}, volume-surface mean diameter.

The terms surface area and interfacial area of particles are equivalent.

Both $\bar{K}$ and D_{32} are defined in terms of the particle size distribution function $N(D)$

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† Department of Chemical Engineering.
‡ Department of Microbiology.

$$D_{32} = \frac{\int_0^{D_{max}} N(D)D^3 \, dD}{\int_0^{D_{max}} N(D)D^2 \, dD} = \left(\frac{\text{volume of particles}}{\text{area of particles}}\right) \times 6$$

$$\bar{K} = \frac{\int_0^{D_{max}} K(D,m)N(D)D^3 \, dD}{\int_0^{D_{max}} N(D)D^2 \, dD}$$

where D_{max} is the largest particle in the suspension.

For calculation of the total surface area of a given mass of particulate material the D_{32} is a useful quantity. If the polydispersion is replaced by a monodispersion possessing the same volume-to-surface ratio, then the diameter of the particles composing the monodispersion equals the D_{32} of the polydispersion.

The scattering coefficient K of a dielectric sphere is defined as the ratio of the scattering cross section to the geometrical cross section. $\bar{K}$ is a function of m and D, where m is the ratio of the refractive index of the suspended phase to that of the suspending medium. If $\bar{K}$ is plotted against the phase shift parameter

$$\rho_{32} = 2(m - 1)\pi(D_{32}/\lambda)$$

where λ is the wavelength of light in the suspending medium, the value of $\bar{K}$ reaches a maximum at $\rho_{32} \approx 4$ and $\bar{K}$ approaches 2 at ρ_{32} values greater than approximately 20 (Dobbins and Jizmagian, 1965; Kerker, 1969). In the present study where $m = 1.20$ for poly(vinyltoluene) or styrene/divinylbenzene latex beads in water or $m = 1.10$ for corn oil in water emulsions, the maximum value of $\bar{K}$ is approximately 3.1 (Van de Hulst, 1957, pp 177–178). Specific turbidity

$$T = 1/l \ln (1/\tau)$$

substituting for τ

$$T = \frac{3}{2}\phi\frac{\bar{K}}{D_{32}}$$

but

$$\frac{\text{volume of particles}}{\text{surface area of particles}} = \frac{D_{32}}{6}$$

Hence

$$T = \frac{3}{2}\phi\bar{K}\frac{\text{surface area of particles}}{6 \times \text{volume of particles}}$$

$$\frac{4T}{\bar{K}} = \phi \times \frac{\text{surface area of particles}}{\text{volume of particles}}$$

If

$$c = \frac{\text{mass emulsifier}}{\text{aqueous phase volume}}$$

as in Pearce and Kinsella (1978)

$$\frac{4T}{\bar{K}\phi c} = \frac{\text{surface area of particles}}{\text{mass emulsifier}} \times \frac{\text{aqueous phase volume}}{\text{volume of particles}}$$

Note that

$$\frac{\text{aqueous phase volume}}{\text{volume of particles}} = \frac{1 - \phi}{\phi}$$

Hence

$$\frac{4T}{\bar{K}\phi c} = \frac{\text{surface area of particles}}{\text{mass emulsifier}} \times \frac{1 - \phi}{\phi}$$

Let EAI = $4T/\bar{K}\phi c$; note that if $\bar{K} = 2$, then there is

agreement with Pearce and Kinsella (1978). Then

$$\frac{\text{surface area of particles}}{\text{mass emulsifier}} = \text{EAI} \times \frac{\phi}{1 - \phi}$$

Hence the units of the EAI as originally defined by Pearce and Kinsella (1978) are actually

$$\frac{\text{interfacial area of particles}}{\text{mass emulsifier}} \times \frac{\text{aqueous volume}}{\text{volume of particles}}$$

and not

$$\frac{\text{interfacial area of particles}}{\text{mass emulsifier}}$$

as claimed.

Going back to

$$\frac{4T}{\bar{K}} = \phi \times \frac{\text{surface area of particles}}{\text{volume of particles}}$$

since

$$\phi = \frac{\text{volume of particles}}{\text{total volume}}$$

then

$$\frac{4T}{\bar{K}} = \frac{\text{surface area of particles}}{\text{total volume}}$$

Let

$$c' = \frac{\text{mass emulsifier}}{\text{total volume}} [=c(1 - \phi)]$$

then

$$\frac{4T}{\bar{K}c'} = \frac{\text{surface area of particles}}{\text{mass emulsifier}}$$

Now assuming that $\bar{K} = 2$, a theoretically more sound index would be

$$\frac{2T}{c(1 - \phi)} = \frac{\text{interfacial area of particles}}{\text{mass emulsifier}}$$

This index will be referred to subsequently as the corrected EAI. It has the units square meters of interfacial area per gram of emulsifier.

The following experiments demonstrate the effect of the correction when interfacial area of a model emulsion is calculated. The values obtained by the original and corrected EAI are compared to that determined from a distribution of particle sizes obtained by laser diffraction using a Malvern particle sizer. The accuracy of the three measures for evaluating interfacial area was determined on a defined system, i.e., a monodisperse suspension of latex beads of known size.

MATERIALS AND METHODS

Reagents. Tween 60 (polyoxyethylene 20 sorbitan monostearate) from Atkemix (Montreal) was used as the test emulsifier. A commercial brand of corn oil free of additives was used. Reagent grade dextran (average M_r 487 000) and NP-40 were from Sigma (St. Louis, MO). Latex beads were from Seradyn (Indianapolis, IN). Distilled water (Milli-Q; Millipore, Mississauga, ON) was used throughout.

Preparation of Emulsions. Emulsifying agent was dissolved in water. This solution was adjusted to pH 7 with NaOH or HCl and was made up to the desired volume. This was added to a measured volume of corn oil in an Oster blender, and the mixture was homogenized for 2 min at the highest speed setting.

Measurement of Turbidity and Droplet Size Distribution. Immediately after blending, 0.1-mL samples of the emulsions were removed and added to 39.9 mL of diluent that contained 1% (w/v) dextran and 0.1% (v/v) NP-40, pH 7.0. Dextran was

included in the diluent to increase viscosity and reduce the rate of flotation of oil droplets, and the detergent was used to prevent flocculation and coalescence of oil droplets. Unlike sodium dodecyl sulfate, the nonionic detergent NP-40 produced a minimal degree of low-angle scattering of laser light in the particle sizer. The turbidity and particle size distributions of the diluted samples did not change over 1 h.

Stock solutions of latex beads were diluted in distilled water and mixed vigorously before turbidity and particle size distributions were measured.

Optical density readings were taken by using an LKB Biochrom Ultrospec II spectrophotometer set at 500 nm, using quartz cuvettes with a light path of 1 cm. Samples were diluted (with diluent described earlier) to yield ODs of less than 0.4. Readings were taken on triplicate samples. Turbidity was calculated by the formula $T = 2.303 \text{ OD}_{500} \times \text{dilution}/0.01$ m light path.

The droplet size distribution was measured on the same samples by using a Malvern 2600C droplet and particle sizer (Malvern, England), using a cuvette with a 14.3 mm light path and the 63-mm lens. Illumination is provided by an HeNe laser (633 nm). The sample was diluted to the appropriate concentration as indicated by the instrument software.

Measurement of Dispersed Phase Volume. The oil volume fractions (ϕ) of these oil-in-water emulsions were measured by drying triplicate emulsion samples (usually 1 mL) to constant weight at 110 °C. The concentration of latex beads in stock solutions provided by the manufacturer (nominally 10% solids) was measured by drying triplicate 10-mL samples after the stock solution was diluted 1:100 with distilled water. Weight concentrations were converted to volumetric concentrations by dividing by density: 0.92 g/mL for corn oil (Merck Index); 1.027 g/mL for poly(vinyltoluene); and 1.05 g/mL for 95% styrene/5% divinylbenzene (Seradyn product data sheet).

Emulsion Stability. Stability of emulsions over time was evaluated by using a method similar to that of Pearce and Kinsella (1978) and Jackman et al. (1989). The emulsion tested consisted of 37.5 mL of 0.133% (w/v) Tween 60 mixed with 12.5 mL of corn oil. The emulsion was poured into a buret and samples were taken at timed intervals from the bottom by opening the valve at the base.

RESULTS

In the presence of 1% dextran and 0.1% NP-40, oil droplets did not adhere to surfaces of the cuvette or sample containers. Turbidity was directly proportional to the concentration of the dispersed phase up to an optical density of 0.4. Scanning over the range from 320 to 900 nm showed that the optical density of the diluted emulsion was independent of wavelength (data not shown).

An emulsion was made with an oil volume fraction of 0.4 and was then serially diluted to provide ϕ = 0.2, 0.1, and 0.05. The value of interfacial area per gram of emulsifier, which was expected to be identical for all dilutions, was calculated by the EAI and the corrected EAI and by computation from the droplet size distribution. The values are plotted in Figure 1. Values obtained with the corrected EAI and the particle sizer correspond, although there is a constant difference. Values obtained with the original EAI diverge from the other two increasingly at low oil volume fractions.

To compare the accuracy of the three methods, suspensions of monodisperse latex beads of known size were used. The data are presented in Table I. For each of the three bead sizes the mean diameter measured with the particle sizer was larger than specified by the manufacturer. The measured standard deviations of the mean diameters were also larger than those provided by the supplier.

The surface areas of latex beads per milliliter of suspension measured by the particle sizer were 87%, 94%, and 93% of the values calculated for suspensions of beads 2.02, 10.2, and 19.6 μm in diameter, respectively. The corresponding surface areas per milliliter of suspension
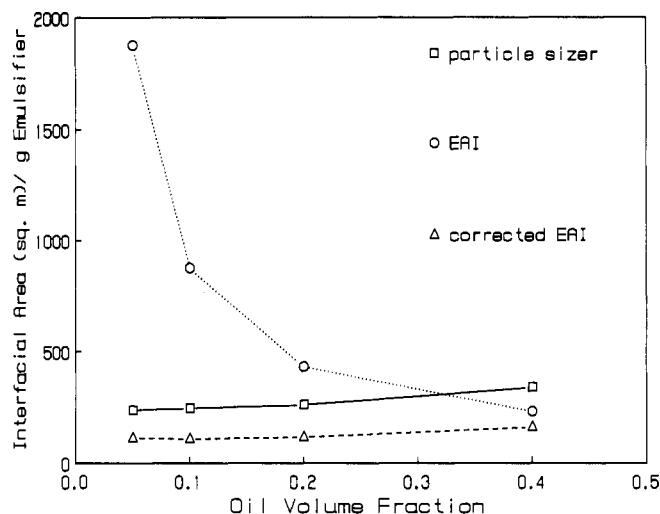

Figure 1. Comparison of values of interfacial area per gram of emulsifier calculated by using the original and corrected emulsifying activity index and data from the particle sizer. One emulsion was serially diluted with water to provide various oil volume fractions. Values were calculated as, for the particle sizer, (specific surface area $\times \phi$)/$c(1 - \phi)$, EAI = $2T/\phi c$, and the corrected EAI = $2T/c(1 - \phi)$. For calculation purposes c was expressed in grams per cubic meter of the original aqueous phase.

calculated by the corrected EAI were 71%, 45%, and 48% of those obtained from the known bead sizes and concentrations. The surface areas per milliliter calculated from the original EAI were far from correct, i.e., 628%, 383%, and 383% larger than the predicted values.

Stability of a model emulsion was monitored over time. Samples were taken from the lower part of the emulsion at timed intervals, and OD_{500}, ϕ, and particle size distribution were determined. The data are shown in Table II. In this emulsion, the OD_{500} of the lower phase remained roughly constant even though the oil volume fraction decreased from 25.3% to 10.3%. Values for interfacial area per milliliter calculated by the corrected EAI remained approximately constant. According to the EAI in its original form, the interfacial area per milliliter increased approximately 3-fold during 2 h. Data from the particle sizer demonstrate that the volume-surface mean diameter decreased from 6.5 to 3.9 μm and that the interfacial area per milliliter also decreased. Changes in the volumetric distribution of droplet sizes for this emulsion are shown in Figure 2.

DISCUSSION

The development of the emulsifying activity index by Pearce and Kinsella (1978) was a useful advance in the science of comparing and evaluating emulsifiers and has been widely used. The index has physical units that are easily interpreted in terms of the dynamics of emulsions. As these authors noted, the values obtained are strongly dependent on the apparatus used to make and measure the emulsion; nevertheless, comparison of different emulsifiers in any given laboratory is possible.

As shown by this work, the correction made to the original formula increases the accuracy of the EAI for determining interfacial area in emulsions on the basis of turbidimetric measurements. The improvement will be particularly noticeable when emulsions that contain different oil volume fractions are compared.

As noted by Pearce and Kinsella (1978) and Dickinson and Stainsby (1988), and as demonstrated here with latex beads, the absolute values for interfacial area calculated from turbidity as measured with typical unmodified

Table I. Comparison of Nominal and Experimentally Determined Diameters and Surface Areas of Three Sizes of Latex Beads

nominal D,[a] μm (SD)	measd D (SD), using droplet sizer	oil vol fraction ϕ	OD_{500}[b]	surface area, m^2/mL of suspension			
					measd by using		
				nominal[c]	droplet sizer[d]	EAI[e]	corrected EAI[f]
2.02 (0.014)	2.29 (0.24)	0.1024	0.118	0.304	0.265	1.91	0.217
10.2 (0.31)	10.73 (1.25)	0.1048	0.150	0.0616	0.0578	0.2360	0.0276
19.6 (0.59)	19.77 (5.3)	0.1127	0.182	0.0345	0.0321	0.1320	0.0167

[a] Values for D (SD) from the supplier. The standard deviation for 10.2-μm diameter beads was determined microscopically. [b] Dilution factors for measuring OD_{500} were 4000, 400, and 200 for beads 2.02, 10.2, and 19.6 μm, respectively. [c] Nominal surface area/mL = no. beads/mL $\times$ πD^2. [d] Surface area/mL = specific surface area (m^2/mL of dispersed phase) $\times$ ϕ. [e] Surface area/mL = $2T(1 - \phi)/\phi$ = EAI $\times$ $c(1 - \phi)$, where T = (2.303 OD_{500} $\times$ dilution)/0.01 m light path. [f] Surface area/mL = 2T.

Table II. Stability over Time of an Emulsion Made with 0.133% Tween 60 and Initial Oil Volume Fraction ϕ = 0.25

time, min	oil vol fraction	OD_{500}[a]	D_{32},[b] μm	interfacial area,[c] m^2/mL, calcd by using		
				EAI	corrected EAI	droplet sizer
0	0.253	0.309	6.5	0.336	0.114	0.238
1	0.246	0.336	6.5	0.379	0.124	0.230
3	0.213	0.335	6.2	0.456	0.123	0.210
10	0.168	0.334	5.6	0.610	0.123	0.185
20	0.142	0.353	4.9	0.786	0.130	0.178
40	0.122	0.359	4.2	0.952	0.132	0.182
60	0.115	0.344	4.2	0.975	0.127	0.168
90	0.111	0.341	3.9	1.01	0.126	0.175
120	0.103	0.341	3.9	1.09	0.126	0.166

[a] All samples diluted 1:800. [b] Volume-surface mean diameter. [c] Calculated as in Table I.
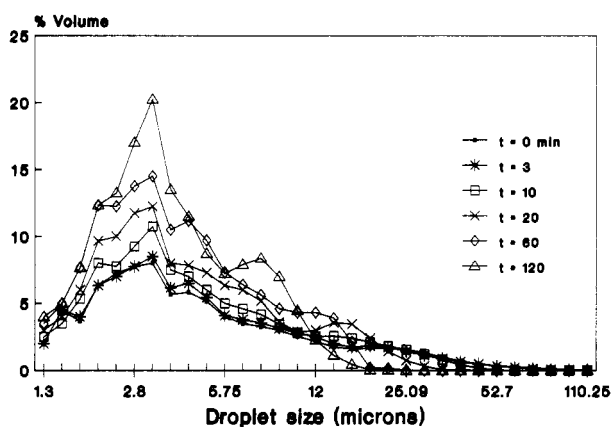

Figure 2. Change in volumetric distribution of droplet sizes over time in samples from the lower part of an emulsion made with 0.133% Tween 60 and 0.25 oil volume fraction.

laboratory spectrophotometers are not highly accurate. Ways to improve their precision have been described in detail elsewhere (Walstra, 1965a,b; Heller and Tabibian, 1957).

The principal sources of error may be ascribed to two factors: (a) the design of the spectrophotometer and (b) the dependence of the value of the scattering coefficient on particle size. Typical laboratory spectrophotometers are more suitable for measuring absorbance than for light scattering. Due to the short distance between sample and photodetector, light scattered at low angles may be received by the detector as transmitted light. For droplets large relative to the wavelength of light a large proportion of the total light scattered is diffracted into a small cone in the forward direction (Lothian and Chappel, 1951). Although the theoretical value of the scattering coefficient is based on an acceptance angle of 0°, even after modification most spectrophotometers provide a solid angle of 1° or more, and a correction factor must be used to provide a realistic value of $\bar{K}$ (Walstra, 1965a).

In addition, the value of the scattering coefficient varies with droplet diameter. For a polydispersion the value of $\bar{K}$ depends primarily on the volume-surface mean diameter and is only weakly dependent on the shape of the droplet size distribution function (Dobbins and Jizmagian, 1965). For corn oil in water emulsions (m = 1.1) and for optical densities measured at 500 nm (in water λ = 0.374 μm), $\bar{K}$ has a maximum theoretical value of approximately 3.1 for oil droplets 2.4 μm in diameter (at ρ = 4). Its value approaches 2 for particles 11.9 μm in diameter (at ρ = 20) (Van de Hulst, 1957). However, with a short distance between the sample and photodetector and consequently a large acceptance angle, some of the scattered light reaches the detector and the value of the apparent $\bar{K}$ will be less than 2 (Sinclair, 1947; Walstra, 1965a).

Because the value of the scattering coefficient varies with droplet diameter and may differ from the assumed value of 2, the values for interfacial area calculated from turbidity measurements will not necessarily be correct. When two or more emulsifiers are compared by using a given system, the difference between the actual and the assumed values of $\bar{K}$ will tend to underestimate interfacial areas. Any detected differences in the corrected EAI values will therefore be genuine.

Despite its high initial cost, the droplet sizer provides certain advantages for comparing emulsifying agents and emulsions. This machine measures droplet sizes on the basis of Fraunhofer (low angle) diffraction of laser light by particles. The lower size limit for particles to be accurately measured is 1.2 μm. In a typical determination laser light is diffracted by several hundred thousand individual particles. The intensity of light scattered at low angles is measured by 32 concentric photodetector elements. The expected pattern of light diffracted by a theoretical distribution of particles is compared to the observed intensities, and the best fit is chosen by computer. The primary data are based on a volumetric distribution, but this may be readily converted to surface, diameter, or numerical distributions by using the software accompanying the instrument.

Experiments with suspensions of monodisperse latex beads (Table I) show good correspondence to actual bead sizes measured by electron microscopy by the manufacturer. The three sizes of beads were chosen to cover the range of droplet sizes in the model emulsions produced here. The somewhat higher values for mean diameters according to the particle sizer may have been due to occasional clumping of two or more beads. This would also broaden the particle size distribution, as was observed in the standard deviation of particle diameters from the mean. Accordingly, the specific surface area of the dispersed phase was slightly smaller than that predicted.

For measurement of emulsion stability over time, the particle sizer presents distinct advantages over turbidimetry. As creaming occurs, the mean droplet size decreases

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to particles that have a negligible upward velocity (described by the Stokes equation), and the theoretical value of the scattering coefficient changes. Hence the accuracy of values of interfacial area derived by turbidimetry will change likewise. The particle sizer is highly accurate throughout its specified range.

In conclusion, the original formula for the emulsifying activity index is incorrect. Interfacial areas are greatly overestimated in emulsions with a low oil volume fraction. The correction made here removes the false dependence of the EAI on the oil volume fraction and provides a corrected EAI that is theoretically sound. Interfacial areas in suspensions of latex beads and model emulsions measured by turbidimetry and particle sizing support the correction. Particle sizing was most accurate, but use of the corrected EAI nevertheless permits valid comparisons of emulsifiers to be made with standard laboratory equipment.

ACKNOWLEDGMENT

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