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A simple method for determination of surface volume mean diameters of oil in water submicron emulsions

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Plots of the droplet size parameter, $\alpha = \frac{\pi n D}{\lambda}$ versus the total scattering coefficient or specific turbidity, K, are sinusoidal curves. It was noticed that, along one branch of the K-α curve (up to 1.1 μm for relative refractive indices (rr_I) <1.2), the wavelength dependence on turbidity; i.e. the extent to which light at different wavelengths is scattered showed a progressive decrease with increase in droplet diameter. It was in fact found that when the logarithm of the relative standard deviations (RSD's) in specific turbidities at four different wavelengths (for a particular droplet diameter and relative refractive index) was plotted as a function of that diameter, a straight line resulted. This relationship provides a valuable new method for estimating the surface volume mean droplet size, D_{vs}, for submicron emulsions by means of simple spectro-turbidimetry.

1. Introduction

There exist several methods to determine particle size by transmission measurements most of these methods being strictly applicable to monodisperse systems. Walstra (1968) obtained the specific turbidity of highly diluted emulsions as a function of the incident wavelength but he matched the results with spectra theoretically computed for various assumed globule size distributions. Gledhill's method (Gledhill 1962) assumed a log normal distribution evaluated in part from a log-log plot of specific turbidity versus wavelength utilizing a graphical calibration grid for a system of known optical constants and distributional form. It does not appear to have been recognized however, that the logarithmic progression of the spread of scatter among visible wavelengths, is a linear function of the particle size.

2. Investigations and results

Fig. 1 and 2 are representative transmittance plots for two different standard diameters which encompass the particle size range studied (0.096 and 1.112 μm) at an rr_I of 1.078. Each plot represents four dilutions whose transmittances are measured at the four wavelengths. It is seen that they are all straight lines ($r^2 > 0.99$) although the regression coefficients become marginally smaller ($r^2 > 0.97$) for the larger size of 1.112 μm. For a given concentration, the smaller diameter particles transmit more light than the larger diameter particles. For a given particle size, the transmittance decreases as the concentration increases. For the size range studied; i.e 0.096 μm to 1.112 μm the transmitted light intensity decreases as the incident light wavelength decreases. This is an indication that the particle size range lies on a unique branch of the K-α plot.

The transmittances from Fig. 1 and 2 were converted into turbidity values and plotted in Fig. 3 and 4. The lines are linear passing through the origin. It is again seen that particles encompassing this size range scatter light at 430 nm more strongly than at 650 nm as evident from the slopes (specific turbidities) of the lines (Table 1). Analogous to the transmittance *versus* concentration plots, the turbidity of the suspension consisting of the smaller diameter spheres is less at a given concentration. For a given particle size, the turbidity increases with concentration. For both the particle sizes, the turbidity increases as the incident wavelength decreases.

Table 1 shows the values of the specific turbidities at four wavelengths for different droplet diameters for different rr_i. It is readily discernible that specific turbidities

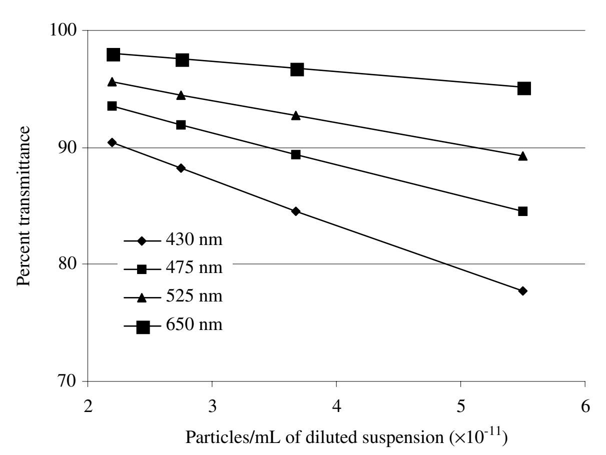

Fig. 1: Transmittance of 96 nm spheres at different concentrations and wavelengths

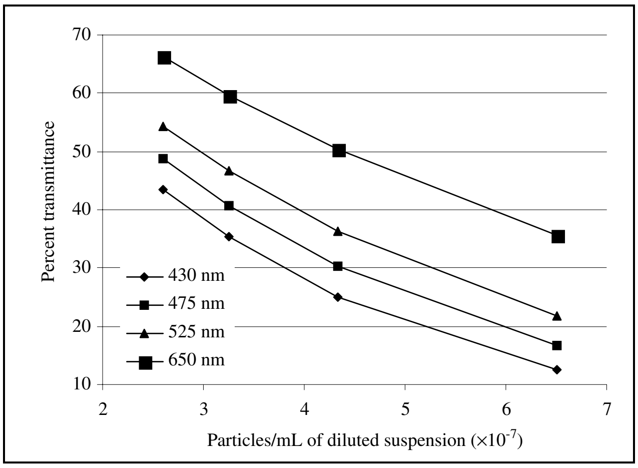

Fig. 2: Transmittance of 1.112 μm spheres at different concentrations and wavelengths

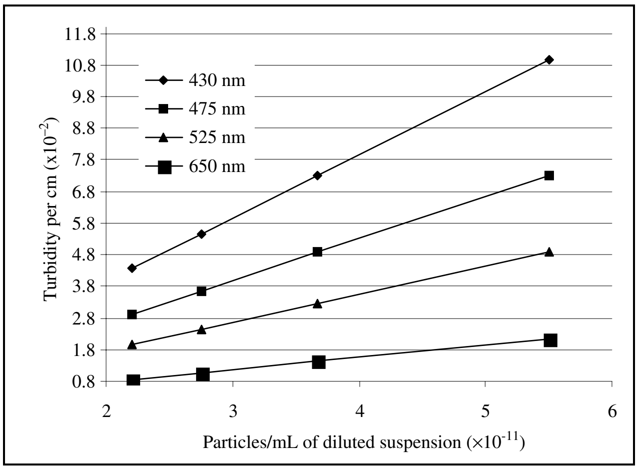

Fig. 3: Turbidity of 0.096 μm spheres at different concentrations and wavelengths

decrease for all diameters with increasing wavelength for rr_I <1.195. This indicates that one is indeed operating along one (ascending) branch of the K-α plot for diameters <1.112 μm and rr_I <1.195.

Fig. 5 is a plot of the logarithm of the RSD's of the specific

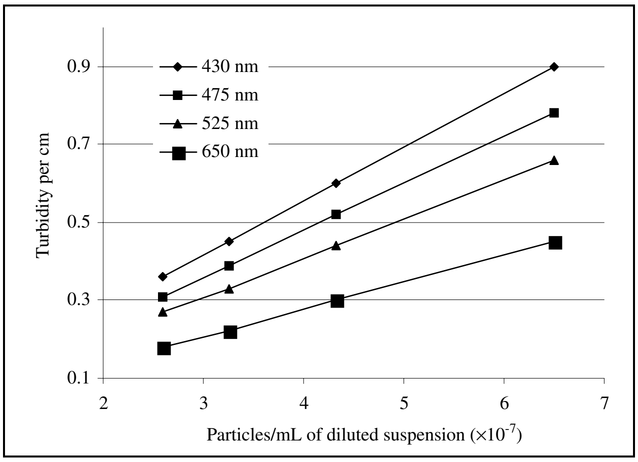

Fig. 4: Turbidity of 1.112 μm spheres at different concentrations and wavelengths

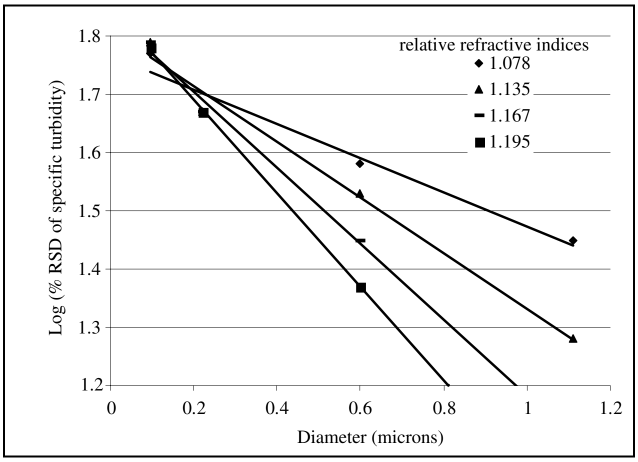

Fig. 5: Spread of wavelength scatter as a function of particle size

turbidities (last column of Table 1) against the corresponding diameter. Even though the correlation coefficients here are marginally lower (> 0.95), it is evident that a linear relationship does exist between the droplet diameter and the measure of variation of scatter with wavelength. When calculating an unknown size, the logarithm of the RSDs' of

Table 1: Specific turbidities

Relative refractive index*	Diameter** (μm)	Specific turbidity***				% RSD of specific turbidities
		430 nm	475 nm	525 nm	650 nm	
1.078	0.096	1.99×10^{-13}	1.33×10^{-13}	0.89×10^{-13}	0.39×10^{-13}	59.1
	0.22	2.5×10^{-11}	1.8×10^{-11}	1.34×10^{-11}	0.73×10^{-11}	47
	0.6	1.38×10^{-9}	1.1×10^{-9}	0.86×10^{-9}	0.52×10^{-9}	37.8
	1.112	1.39×10^{-8}	1.2×10^{-8}	1.02×10^{-8}	0.69×10^{-8}	27.9
1.135	0.096	4.7×10^{-13}	3.1×10^{-13}	2.0×10^{-13}	0.8×10^{-13}	61.8
	0.22	6.78×10^{-11}	4.88×10^{-11}	3.63×10^{-11}	1.97×10^{-11}	47
	0.6	3.03×10^{-9}	2.49×10^{-9}	2.01×10^{-9}	1.26×10^{-9}	34.2
	1.112	2.45×10^{-8}	2.28×10^{-8}	2.06×10^{-8}	1.53×10^{-8}	19.1
1.167	0.096	6.3×10^{-13}	4.1×10^{-13}	2.7×10^{-13}	1.1×10^{-13}	62.2
	0.22	9.4×10^{-11}	6.79×10^{-11}	5.06×10^{-11}	2.73×10^{-11}	47
	0.6	4.4×10^{-9}	3.89×10^{-9}	3.28×10^{-9}	2.16×10^{-9}	28.2
1.195	0.096	7.0×10^{-13}	4.7×10^{-13}	3.0×10^{-13}	1.3×10^{-13}	60.9
	0.22	1.08×10^{-10}	0.78×10^{-10}	0.58×10^{-10}	0.32×10^{-10}	46.6
	0.6	4.97×10^{-9}	4.56×10^{-9}	4.02×10^{-9}	2.78×10^{-9}	23.3
	0.9	1.25×10^{-8}	1.21×10^{-8}	1.14×10^{-8}	0.91×10^{-8}	13.6

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[*] Solvents (medium) used to obtain relative refractive indices 1.078, 1.135, 1.167 and 1.195 were dimethyl sulfoxide (DMSO), 52% w/v glycerol, 24% w/v glycerol and Purified water, USP respectively.

[**] Represent diameters of NIST certified standard latex polystyrene spheres with a refractive index of 1.59.

[***] Units are cm^{-1}/(# polystyrene particles per mL of diluted suspension)

Table 2: **Correlation between theoretically calculated and experimentally determined D_{vs}**

Latex standards used (microns)	Volume ratio mixed	Theory D_{vs}	Experimentally obtained D_{vs}
0.096 : 0.22	1 : 1	0.1165	0.13
0.22 : 0.6	1 : 1	0.324	0.37
0.6 : 1.112	1 : 5	0.891	0.84
0.6 : 1.112	1 : 1	0.707	0.68
0.096 : 0.22: 0.6: 1.112	1 : 5 : 2 : 1	0.187	0.38

specific turbidities can be substituted in the relevant equation to obtain the D_{vs} provided the refractive index of the dispersed phase is known.

Table 2 represents theoretical surface volume diameters calculated by mixing known quantities of the standard latex suspensions and the experimentally determined surface volume diameters as obtained using this method. There is a reasonably good correlation between theory and experiment (± 50 nm) for diameters upto 512 nm apart. At large polydispersities however, the deviation becomes too large to accurately estimate the D_{vs}.

Finally, the surface volume mean diameter of three Intravenous emulsions possessing a predominantly submicron size distribution were measured using the method described above and compared to the diameter obtained by photon correlation spectroscopy (PCS). Even though these two methods measure droplet size differently; i.e. hydrodynamic diameter *versus* surface volume mean diameter, there was reasonably good agreement between the two methods (Table 3).

3. Discussion

It is well documented in the literature that plots of the droplet size parameter versus the total scattering coefficient yield sinusoidal curves (Lothian 1951). This means that for some droplet diameters, the amount of light scattered decreases as the incident wavelength increases (an ascending branch of the K-α curve), while for other droplet diameters, the amount of light scattered increases as the incident wavelength increases (a descending branch of the K-α curve). It was noticed that, along one branch of the K-α curve from 0.096 μm to 1.112 μm, the wavelength dependence on turbidity i.e the extent to which light at different wavelengths is scattered showed a progressive decrease with an increase in droplet diameter for relative refractive indices less than 1.2. It was in fact found that when the logarithm of the relative standard deviations in specific turbidities for the four different wavelengths for a particular droplet diameter was plotted as a function of that diameter, a straight line resulted (Fig. 5).

It can be readily ascertained that, below a refractive index of 1.2, one is operating along the ascending branch of the K-α plot which reaches a maximum at $\alpha = 9$ and then starts to decline (Goulden 1958; Kerker 1969). This size parameter corresponds to a maximum mean droplet diameter of approximately 1 μm at $\lambda = 430$ nm. Hence, as long as the rr_I <1.2, the validity of this method is established for submicron size distributions due to operating along one branch of the K-α plot. For relative refractive indices >1.2, the maximum in the K-α plot shifts to a lower size parameter and hence a lower droplet diameter. This then provides a valuable method for estimating the mean droplet diameter for submicron dispersions by means of simple spectro-turbidimetry. It is possible that the same relation exists for particular size ranges along other ascending or descending branches of the K-α plot at higher K values, although the authors have no experimental evidence for the same.

Table 3: **Measurement of D_{vs} of I.V emulsions**

Emulsion type	Mean by PCS (nm)	D_{vs} by this method (nm)
Liposyn II 20%	317	320
Diprivan	207	260
Fluosol DA 20%	350	290

Liposyn II 20% Intravenous fat emulsion, Abbott laboratories, North Chicago, IL, Lot # 70–491-DE

Diprivan (Propofol), 10 mg/mL, 50 mL single patient infusion vial, Stuart Pharmaceuticals, Wilmington, DE, Lot # 5284N

Fluosol DA 20% Intravascular Perfluorocarbon emulsion, Green Cross Corporation, Osaka, Japan, Lot # SO132H1

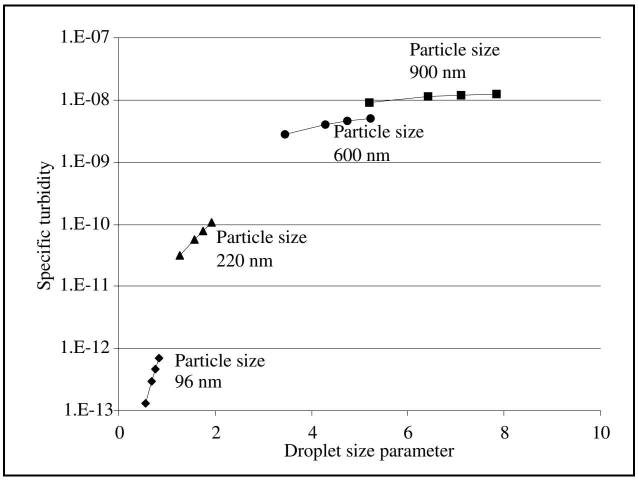

Fig. 6: Droplet size parameter versus specific turbidity, relative refractive index = 1.2

Since the method relies only on the relative standard deviations in specific turbidities at different wavelengths, it has the advantage that the weight (or number) fraction of spheres in suspension need not be known, mere knowledge of the dilution factor is enough. The method however, does not permit deduction of a size distribution; only the mean surface volume mean diameter can be found.

It is important that multiple scattering be absent in calculating turbidity from transmittance measurements. The absence of multiple scattering is demonstrated by the fact that, at the dilutions used, the plots of turbidity versus dilution are all straight lines. It must be borne in mind that the relative refractive index varies with wavelength. This has not been taken into account here. Wallach (1961) has shown that for submicron systems, such a correction is not important enough to be applied.

Any method using dilution to measure particle size assumes that the particle size does not change with dilution. This is a reasonable assumption provided dilutions are made in one region of the surfactant/water phase diagram and lie within a fairly narrow domain in the same. Fortunately, the CBC (critical bilayer concentration – analogous to the CMC of surfactants containing one hydrocarbon chain) of phospholipids is of the order of 10^{-7} g/mL (Farinato 1983). This low CBC allows dilutions of considerable magnitude before packing order or geometry (and hence particle size) become compromised. Suspensions or emulsions utilizing phospholipids as primary emulsifierss whose mean diameters fall in the submicron range and which possess a narrow size distribution or polydispersity (such as iv emulsions and liposomal preparations) therefore are eminently suitable for sizing with this method.

Table 4: Polystyrene latex microsphere specifications

Diameter (μm)	Uncertainty (nm)	Standard deviation (nm)	Concentration (particles/mL)
0.096	3.9	Not determined	1.1×10^{14}
0.22	6.0	3.5	3.4×10^{12}
0.6	5.0	6.6	1.7×10^{11}
1.112	22	11.0	1.3×10^{10}

Standards are manufactured by Duke Scientific, Palo Alto, CA and these specifications are listed on the manufacturer's certificate of calibration and traceability.
Refractive index of the particles is 1.59 at $\lambda = 589$ nm and specific gravity is 1.05 g/mL

The authors acknowledge that the applicability of such a method would be limited, given that the size and distribution of submicron colloidal systems can be measured highly accurately by instruments such as the Microtrac® or Malvern® by application of the Mie theory or calculation of diffusion coefficient. However, it must be pointed out that the solutions obtained by PCS are ill defined functions and measure the so called 'hydrodynamic' diameter. In contrast, the specific turbidity has been shown to be inversely proportional to the surface-volume mean diameter (Mysels 1959).

4. Experimental

NIST traceable polystyrene latex microspheres from Duke Scientific, Palo Alto, CA, stored under refrigeration, were used as standards. These are supplied as aqueous suspensions containing an anionic surfactant to prevent aggregation. A uniformly dispersed suspension of the latex microspheres was assured by gentle inversion and mixing of the container as per the manufacturer's instructions prior to sampling the suspension. Their certified mean diameters and standard deviations are specified in Table 4. Water used was purified water, USP. Dimethyl sulfoxide (DMSO) and glycerol were analytical reagent grade obtained from Mallinckrodt. The refractive index of purified water was taken to be 1.33 at ambient room temperature (22 ± 2 °C).

Usually, 50 to 100 μL of the standard suspension was diluted to 10, 15, 20 and 25 mL with different solvents so as to obtain the desired relative refractive index (rr_I). Solvents (medium) used to obtain rr_I of 1.078, 1.135, 1.167 and 1.195 were DMSO, 52% w/v glycerol, 24% w/v glycerol and purified water, USP respectively (Handbook of chemistry and physics, 66th ed.). The percent transmittance of the diluted suspensions was measured at four wavelengths, using the corresponding solvent as the reference, at 650 nm, 525 nm, 475 nm and 430 nm, corresponding to red, green, blue and violet light respectively. A Shimadzu UV-160A spectrophotometer and quartz cells of path length 1 cm were used to perform the transmittance experiments. The turbidity of the suspensions was calculated from the natural logarithm of the ratio of the incident light intensity (100%) to the transmitted intensity (Schott 1983). Specific turbidities were the slopes of the linearly regressed lines for the four different concentrations at each wavelength. The droplet size parameter was calculated from eq. (1)

$$\alpha = \frac{\pi n D}{\lambda} \tag{1}$$

where D = diameter of the suspended particles (nm), λ = wavelength of the incident light (nm) and $n = rr_I$ (the ratio of the refractive indicex of the particles to the refractive index of the medium; the relative refractive index).

All experiments were repeated. In no instance was the % RSD of two transmittance measurements >0.2%. The results in the graphs represent the average of two values.

A Malvern Zetasizer® IV with PCS version v1.23 software was used for the photon correlation spectroscopy measurements. The samples were diluted with water until the photon counts were found to be within range by the software. A He-Ar laser at a wavelength of 633 nm was used as the incident light source. Scattering was measured at an angle of 90° to the incident radiation. A linear series of 64 channels was used to calculate the time dependency of light intensity fluctuations to generate a diffusion coefficient function. The mean particle size was derived using the Stokes-Einstein equation.

The total scattering coefficient, K, is formally analogous to the specific turbidity which measures the loss of incident light intensity due to scattering and absorption.

References

Farinato RH, Rowell RL (1983) In: Becher P (ed.), "Encyclopedia of emulsion technology" Dekker, NY, NY, p. 449

Gledhill RJ (1962) Particle size distribution determination by turbidimetry. J Phys Chem 66: 458.

Goulden JDS (1958) Light transmission by dilute emulsions. Trans Faraday Soc 54: 941.

Handbook of Chemistry and Physics, 66th ed., CRC press, Boca Raton, FL, 1985.

Kerker (1969) Analysis of particle size. In: The scattering of light and other electromagnetic radiation. Academic press, San Diego, CA

Lothian GF (1951) The transmission of light through suspensions. J Appl Chem 1: 475–482.

Mysels KJ (1959) In: "Introduction to colloid chemistry". Interscience, NY, NY.

Schott H (1983) Effect of inorganic additives on solutions of nonionic surfactants V: Emulsion stability. J Pharm Sci 72: 1427.

Van de Huls HC (1957) In: "Light scattering by small particles", Wiley, NY, NY.

Wallach ML (1961) Theoretical investigations on the light scattering of Colloidal spheres. XII. The determination of size distribution curves from turbidity spectra. J Chem Phys 34: 1796–1802.

Walstra P (1968) Estimating globule size distribution of oil in water emulsions by spectroturbidimetry. J Coll Interfac Sci 27: 493–500.