An improved procedure for estimating size distribution parameters of spherical and non-spherical particles from their planar cross-sections.

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1. Introduction

The quantitative characterization of spherical particles or structures in multicomponent disperse systems like colloidal solutions, emulsions (liquid or solid), suspensions or foams requires either the determination of frequency distributions (e.g., number, surface or volume) or physically meaningful average size parameters like the volume-surface average diameter, the volume-moment average diameter or the surface area per unit volume. Besides advanced optical procedures (laser diffraction, photon correlation spectroscopy) or methods based on electrical conductivity measurements (Coulter Counter principle) direct measurements by light microscopy and especially electron microscopy are possible and frequently used. Depending, e.g., on the type of disperse system, the structure and composition of individual components, the presence of various types of particles and a different behaviour during necessary pretreatments, the various methods of size determination have their limitations and may even result in unrealistic size data.

Direct size measurements, especially those by electron microscopical methods, are generally time-consuming and, therefore, not widely applied. On the other hand, they offer an advantage, if, e.g., multicomponent systems or particle types composed of different materials (e.g., protein-fat complexes in evaporated milk) are to be analyzed. Furthermore, there are special systems, like, e.g., dried topping emulsions which show a complete structural transformation of their globular lipid phase when water interacts with the powdered material (5). For this special disperse system the only possible method for characterizing, e.g., the surface area per unit volume of fat, S_v, is freeze-fracturing (7). Freeze fracturing mostly results in planar cleavages through individual particles, i. e., only size and shape of cross-sections can be analyzed instead of the corresponding three-dimensional parameters.

It is known since long that, e.g., the meaningful parameter S_v of a spherical or non-spherical disperse phase is related in a simple way to the observed cross-sectional areas and their circumferences (10). Furthermore the mathematical procedures are known to convert the apparent size distributions of thin sections or planar cleavages through spherical particles, i.e., circular cross-sections, to the true three-dimensional size distributions (6, 8). For spherical particles Bach (1, 3) has derived the direct relationships between the moments of distribution of the sphere radii and those of corresponding circular cross-sections. Vreeman et al. (9) have discussed in detail various problems

related to the conversion of apparent two-dimensional size distributions to the true three-dimensional distributions and the resulting calculation of certain average size parameters when comparing the calculation methods of Goldsmith (6) and Bach (1, 2).

Irrespective of these more fundamental or mathematical aspects it is necessary to limit the time necessary for deriving reliable size distribution data from direct measurements. For many applications of particle size analysis it may not be necessary to derive reliable frequency distributions as histograms with a sufficiently high number of size classes but only reliable values of meaningful average size parameters as discussed above. This paper describes a procedure to minimize the measuring efforts for obtaining these average size parameters.

2. Mathematical relationships

It is a long known fact in quantitative stereology that three-dimensional objects lead to certain profiles when sectioned into thin slices or when cleaved in a plane (10). Certain parameters of these two-dimensional profiles are related to parameters of the corresponding three-dimensional objects as, e.g., the surface area per unit volume (surface to volume ratio), S_v, which is related to the lengths of the two-dimensional profiles (circumferences), C_i, and the areas, A_i, within these profiles by $S_v = {}^4/\pi\ (\Sigma C_i/\Sigma A_i)$. This basic relationship is valid for particles irrespective of their shape. For determining S_v it is necessary to measure a sufficiently large number of profiles, i.e. C_i and A_i.

Bach (1, 3) has derived general relationships between the moments of distribution of spheres, M_k, and those of their circular profiles, m_k, seen on thin sections or planar cleavages. For a finite number of spherical particles ,N, with diameters, $D_1 \ldots D_N$, or of corresponding circular cross-sections, $d_1 \ldots d_N$, the k^{th} moment of distribution is defined as follows:

$$M_k = \sum_{i=1}^{N} D_i^k \quad \text{or} \quad m_k = \sum_{i=1}^{N} d_i^k$$

According to Bach (1, 3) the general relationship is:

$$m_k = \sqrt{\pi} \cdot \frac{\Gamma\left(\frac{k+2}{2}\right)}{\Gamma\left(\frac{k+3}{2}\right)} \cdot M_{k+1} + \delta \cdot M_k$$

where δ represents the section thickness and Γ (1+x) the gamma function. In case the freeze fracture technique is applied, the δ-value is zero, otherwise, i.e., in case the thin section technique is applied, the thickness of sections has to be known and included in the equation system.

The values of the gamma function are:

$\Gamma(1 + n) = n! = 1 \cdot 2 \cdot 3 \ldots \cdot n$ (for n = 1, 2, 3, ...), $\Gamma(1) = 1$, $\Gamma(\frac{1}{2}) = \sqrt{\pi}$, and

$$\Gamma(1 + \tfrac{1}{2} + n) = \sqrt{\pi} \cdot \frac{1 \cdot 3 \cdot 5 \ldots (2n+1)}{2^{n+1}} \quad \text{(for n = 0, 1, 2, 3, ...)}$$

In case δ is zero, the following relationships between M_{k+1} und m_k are valid:

$$M_0 = \frac{1}{\pi} m_{-1}; M_1 = \frac{1}{2} m_0; M_2 = \frac{2}{\pi} m_1; M_3 = \frac{3}{4} m_2; M_4 = \frac{8}{3\pi} m_3$$

The moments of distribution M_k are directly related to the most commonly used average size parameters like, e.g.,

the number average diameter $d_n = d_{10} = \frac{M_1}{M_0}$

the volume-surface average diameter $d_{vs} = d_{32} = \frac{M_3}{M_2}$

the volume-moment average diameter $d_{vm} = d_{43} = \frac{M_4}{M_3}$

the surface area per unit volume $S_v = \frac{6}{d_{32}} = \frac{6M_2}{M_3}$

or the distribution width $c_S = \left(\left(M_2 M_4 / M_3^2\right) - 1\right)^{0.5}$.

The values for the moments of distribution M_k (or m_k) steadily increase due to the number of particles (or cross sections) included, whereas the average size parameters like d_{10}, d_{32}, d_{43} or S_v represent characteristic values for the system or the component under study. This means that these average parameters represent a function of the particle number which must approximate a constant value. When analyzing a limited but representative number of micrographs it is, therefore, advantageous to follow the course of this function with increasing number of particles measured in order to obtain satisfactory estimations of the average parameters from a minimum of particles measured.

3. Image Analysis and Calculation Procedure

The parameters of the objects (particle cross-sections) are obtained manually via a digitizer by following the contours with a digitizer pen and by subsequently calculating the circumference (C_i) and area (A_i). In case of globular objects, i.e., circular cross-sections, the diameters (d_i) are derived from the A_i values. Thereafter, the moments of distribution m_k are determined and according to Bach's equations the moments M_k and all size distribution parameters which are directly related to the M_k, as, e.g., d_{32} or d_{43} parameters. This approach allows the dependence of these parameters to be followed as a function of the number of cross-sections measured.

The user of this system has, thus, a criterion which allows him to decide which amount of data is sufficient to derive reliable accuracy for the parameters of interest and to limit the number of measurements. In case of full visibility of individual particles, like, e.g., during light microscopical observation, the moments M_k are directly calculated from the true particle diameters D_i and the course of the functions for size distribution parameters is evaluated accordingly.

4. Results and Discussion

For demonstrating the suitibility of this procedure four examples of disperse systems are given, i.e. (I) an o/w-type emulsion (sunflower oil stabilized by a plant protein isolate), (II) a dried o/w-type emulsion (topping powder), (III) a dispersion of liposomes (from soya lecithin) and (IV) a dispersion of colloidal protein aggregates (casein micelles from mare milk). Electron micrographs of freeze-fracture preparations of these four types of samples were analyzed as described above and the various size distribution parameters calculated in relation to the number of cross-sections measured. Fig. 1 shows representative views of the appearance of the particulate constituents of the four samples under study. It should be mentioned that Fig. 1 b shows the internal structure of a spray-dried topping powder particle and not a hydrous dispersion in which the globular lipid particles undergo a rapid structural and shape transition due to crystallization processes (4). Fig. 2 shows the

course of two selected size distribution parameters, i.e., d_{32} and d_{43}, as a function of the number of cross-sections measured. It can be seen that not more than 100-200 measurements are needed in these cases to reach an approximation to an equilibrium value which should represent a satisfactory estimation for the corresponding parameter. Similar approximations have been found for determinations of S_v values with particles of more irregular shape like, e.g., larger protein aggregates in certain fresh cheeses. Such systems are normally very difficult to characterize quantitatively as to particle size. The advantages of the procedure described here may be seen in the reduction of measurements in case only certain size distribution parameters are needed which are directly related to the moments of distribution M_k. Alternatively the true size distributions (of globular particles only!) have to be determined from the apparent size distributions of their circular cross-sections, e.g., by the methods of Goldsmith (6) or Rose (8), which normally requires a ten-fold of particles to be considered. Then the moments of distributions have to be calculated. It should, of course, not be neglected that the SEM and TEM techniques are rather time-consuming and require adequate equipment and experience. Nevertheless there are numerous types of dispersions with particle sizes in the micron and sub-micron range, especially multicomponent systems, which are rather difficult to be reliably characterized by indirect methods, as, e.g., laser diffraction.

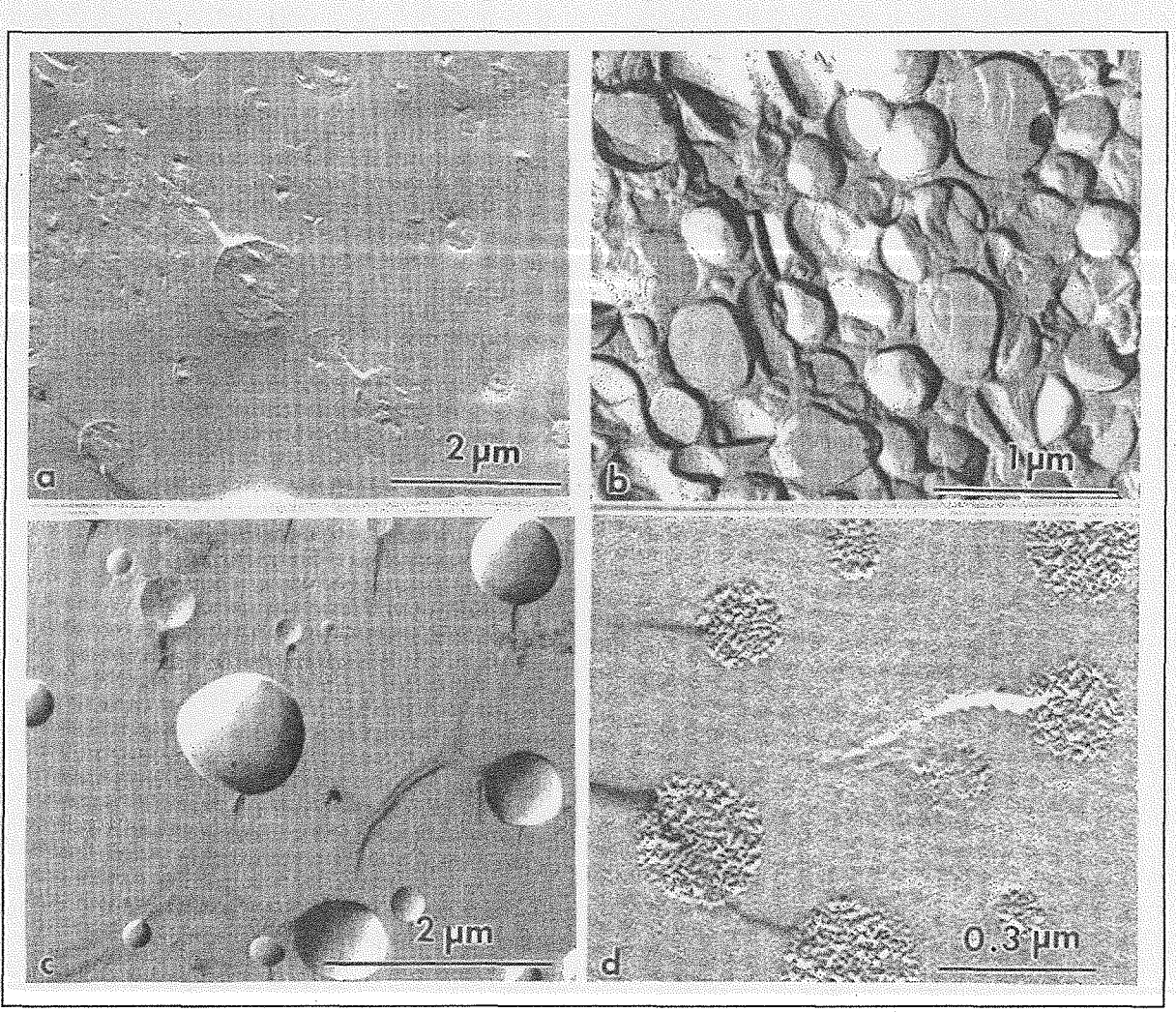

Fig. 1: Freeze-fracture electron micrographs (TEM) of the disperse systems used for particle size analysis; a: o/w-type emulsion (sunflower oil stabilized by a plant protein isolate), b: dried o/w-type emulsion (topping powder), c: dispersion of liposomes (from soy lecithin), d: dispersion of colloidal protein aggregates (casein micelles from mare milk)

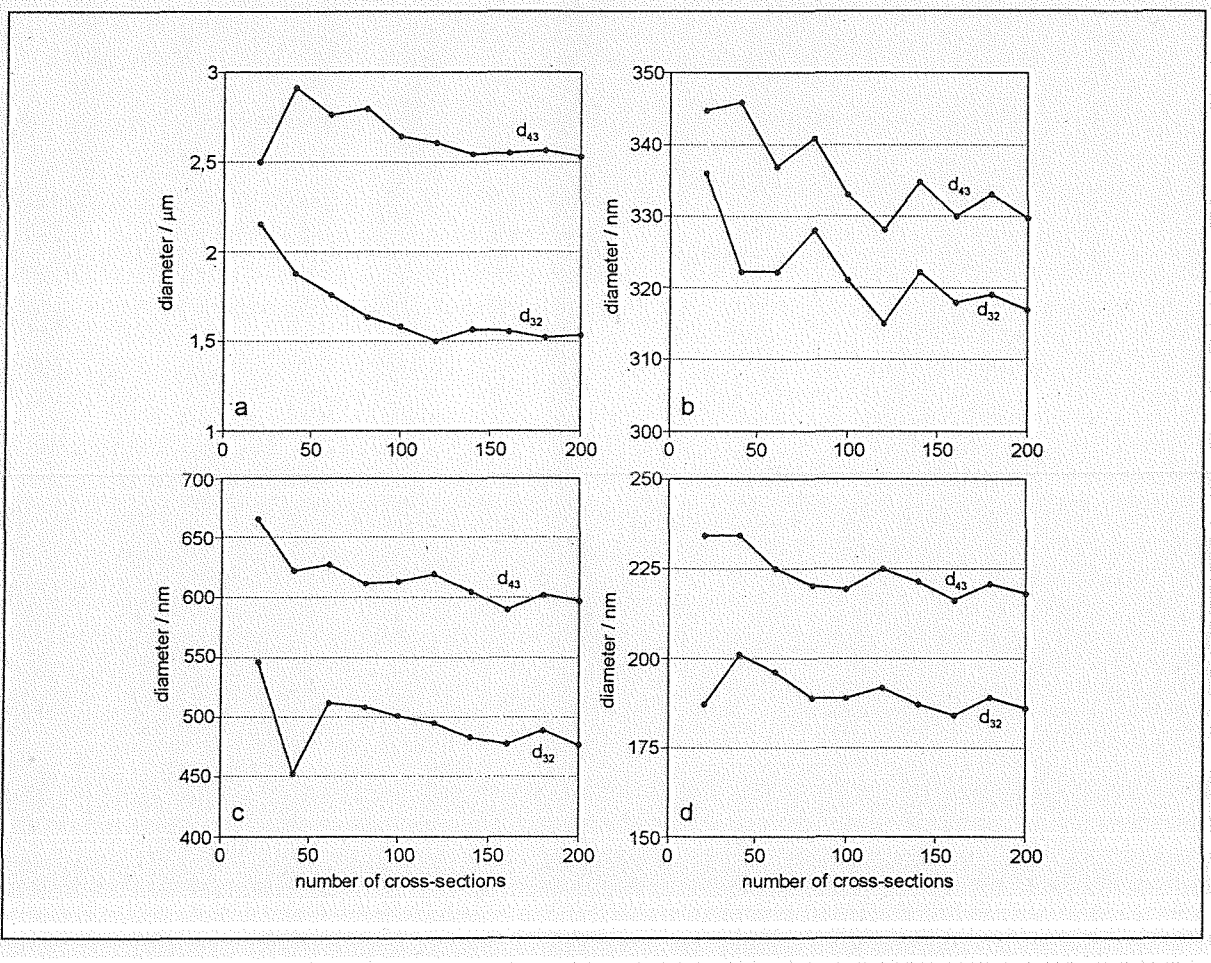

Fig. 2: Size distribution parameters d_{32} and d_{43} as function of the number of cross-sections measured on freeze-fracture electron micrographs (a-d correspond to the system a-d of Fig. 1).

5. References

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6. Summary

Buchheim, W., Krause, J.-P., Rathjen, G: **An improved procedure for estimating size distribution parameters of spherical and non-spherical particles from their planar cross-sections.** Kieler Milchwirtschaftliche Forschungsberichte **47** (2) 177-183 (1995)

25 Particlesize analysis (electron microscopy, calculation procedure)

Direct determination of size distributions or average size parameters of particles in disperse systems on the basis of thin planar sections or cleavages, as e.g. from TEM micrographs of thin-sectioned or freeze-fractured specimens, requires adequate mathematical procedures for converting the apparent, i.e. two-dimensional, to the true, i.e. three-dimensional, data. In case only certain average size parameters are needed to characterize the disperse phase it is relevant to limit the amount of measurements, especially if automated image analysis is not applicable or available. This paper describes a procedure to simplify the efforts required for measurement and calculation. Four examples of disperse systems are given, i.e. (I) an O/W-type emulsion, (II) a spray-dried O/W emulsion (topping powder), (III) phospholipid liposomes and (IV) colloidal protein aggregates in milk.

Zusammenfassung

Buchheim, W., Krause, J.-P., Rathjen, G.: **Ein verbessertes Verfahren zur Abschätzung von Größenverteilungsparametern bei kugelförmigen und nicht-kugelförmigen Teilchen aus der Verteilung planarer Teilchenquerschnitte.** Kieler Milchwirtschaftliche Forschungsberichte **47** (2) 177-183 (1995)

25 Teilchengrößenanalyse (Elektronenmikroskopie, Auswertungsverfahren)

Bei der direkten Bestimmung von Größenverteilungen bzw. Größenverteilungsparametern (z. B. Durchmessermittelwerten) von Teilchen in dispersen Systemen auf der Basis dünner planarer Schnitte oder Brüche, wie z. B. im Fall elektronenmikroskopischer Aufnahmen (TEM) von Dünnschnitt- oder Gefrierbruchpräparaten, ist die Anwendung geeigneter mathematischer Verfahren zur Umrechnung der scheinbaren (zweidimensionalen) in die wahren (dreidimensionalen) Verteilungsdaten notwendig. Für den Fall, daß nur gewisse Größenverteilungsparameter zur Charakterisierung der dispersen Phase zu ermitteln sind, ist insbesondere dann eine Minimierung des Aufwandes anzustreben, wenn keine automatische Bildanalyse anwendbar bzw. verfügbar ist. Dieser Beitrag beschreibt ein vereinfachtes Meß- und Berechnungsverfahren, dessen Eignung an vier Systemen, d.h. einer o/w-Emulsion, einer sprühgetrockneten Emulsion (Toppingpulver), einer Phospholipid-Liposomendispersion und einer kolloidalen Milchproteindispersion demonstriert wird.

Résumé

Buchheim, W., Krause, J.-P., Rathjen, G.: **Un procédé amélioré pour estimer des paramètres de la distribution des tailles des particules sphériques et non-sphériques à partir de leurs sections transversales planes.**.Kieler Milchwirtschaftliche Forschungsberichte **47** (2) 177-183 (1995)

Pour la détermination directe des distributions des tailles ou bien des paramètres de la taille moyenne des particules dans des systèmes dispersés basée sur des sections planes minces ou des clivages, par exemple à l'aide de "TEM" micrographies de spécimens "thin-sectioned" ou "freeze-fractured" des procédés mathématiques appropriés sont nécessaires pour convertir les données apparentes, i.e. bidimensionnelles, en des données vraies, i.e. tridimensionnelles. Si l'on exige seulement certains paramètres de la taille moyenne pour caractériser la phase dispersée il est important de limiter le nombre de mesurages, particulièrement dans le cas où des analyses d'image automatisées ne sont pas applicables ou disponibles. On décrit un procédé pour simplifier les efforts requis pour le mesurage et le comptage. On donne 4 exemples de systèmes dispersés, i.e. (I) une émulsion du type "O/W", (II) une émulsion "O/W" séchée (topping en poudre), (III) des liposomes phospholipidiques et (IV) des agrégats de protéines colloïdaux dans le lait.