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Phase separation and drop size distributions in "homogeneous" Na-alginate/Na-caseinate mixtures

A.W. Pacek[a,*], P. Ding[a], A.W. Nienow[a], M. Wedd[b]

[a]*School of Chemical Engineering, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*
[b]*Malvern Instruments Ltd., Malvern WR14 1XZ, UK*

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Abstract

Phase separation and drop size distributions in dilute Na-caseinate/Na-alginate mixtures has been investigated using simultaneously two different measuring techniques: light scattering and image analysis. It has been found that even at very low concentrations of either polymer, where according to literature data the mixture should be homogenous, two phases can be observed. This phase separation was detected by both techniques and in each case, the drop size distributions measured by each of them were in good agreement. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Aqueous two phase system; Na-alginate; Na-caseinate; Phase separation; Light scattering; Image analysis

1. Introduction

Aqueous–aqueous two phase systems (ATPS) are used in the food industry (Kasapis, Morris & Norton, 1991; Tolstoguzov, 1996) and in the separation of biological materials ranging from proteins to cells (Albertsson 1985; Huddlestone & Lyddiatt, 1990). Recently, it has been reported that aqueous–aqueous two phase systems can also be used in the extraction of metal ions and other inorganic components (Graber, Andrews & Asenjo, 1999). In each of the above applications, knowledge of the phase diagram of the polymer solutions, e.g. the composition of the mixture at which separation in two phases starts, is essential. Despite considerable research and the growing popularity of ATPS, currently there is no theory capable of predicting phase separation from the chemical structure of the polymers or from their physical properties. Attempts of an theoretical description of phase separation are based on one of two contradictory assumptions: (i) that the macromolecular structure of polymers plays the major role in phase separation; or (ii) that it is the character of water as a solvent which plays the essential role (Zaslavsky, 1995). Whichever assumption is adopted, a reliable experimental technique to determine the composition and the structure of the system at phase separation is necessary to verify such theoretical models.

Several methods of measuring the binodal for the phase diagram are reported in the literature. The composition of the separated phases can be measured by direct chemical and/or physical analysis. This approach usually requires several independent analytical methods such as refractive index differences, polarimetry and dry weights have to be combined to measure the concentrations in mixture of polymers (Bamberger, Brooks, Sharp, Van Alstaine & Webber, 1985). Alternatively, the cloud point method is used. Here, one starts with a relatively concentrated solution of one polymer to which the solution of other polymer is added in very small quantities. After each addition, the polymers are carefully mixed and the first appearance of turbidity, i.e. the cloud point, indicates that the system is about to move from the single phase part of the phase diagram to the two phase part (Bamberger et al., 1985; Merchuk, Andrews & Asenjo, 1988). There are two problems with this method: (i) the change from clear to turbid solution often occurs gradually; and (ii) for polymers with similar refractive indices, the cloud point can be seen only at a high volume fraction of dispersed phase, e.g. when the system is well within the two phase area. Therefore, the position of the binodal cannot be determined accurately using the cloud point method.

Recently Blonk, van Endenburg, Koning, Weisenborn and Winkel (1995) reported a new approach, namely the use of Confocal Scanning Laser Microscope to measure the phase diagram for the Na-alginate/Na-caseinate system.

* Corresponding author. Tel.: +44-121-414-5308; fax: +44-121-414-5324.

E-mail address: a.w.pacek@bham.ac.uk (A.W. Pacek).

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Nomenclature

d	drop size (μm)
$n(d)$	number density function
$d_{10} = \frac{\int_0^\infty dn(d)\mathrm{d}d}{\int_0^\infty n(d)\mathrm{d}d}$	number mean diameter (μm)
$d_{32} = \frac{\int_0^\infty d^3n(d)\mathrm{d}d}{\int_0^\infty d^2n(d)\mathrm{d}d}$	Sauter mean diameter (μm)
$d_{43} = \frac{\int_0^\infty d^4n(d)\,\mathrm{d}d}{\int_0^\infty d^3n(d)\mathrm{d}d}$	mass mean diameter (μm)
x_{al}	average concentration of Na-alginate (%(w/w))
x_{cs}	average concentration of Na-caseinate (%(w/w))
ϕ_d	volume fraction of dispersed phase (%v/v)
μ	viscosity (Pa s)

This method not only requires rather expensive equipment but also complex, system specific labeling of each polymer is necessary. Its accuracy is further limited by the lack of precise correlation between the fluorescence intensity and concentration of each polymer. The literature results for the measurements of the binodal and phase diagram with different methods for Na-alginate/Na-caseinate are summarised in Fig. 1.

In Fig. 1, the coordinates of a point represent the average concentration of each polymer (mass of biopolymer per the total mass of the mixture). If such a point is between the binodal (the solid line in Fig. 1) and the axes, the mixture is expected to be homogenous and phase separation does not occur. If the point is above the binodal, the system separates into two phases, each of a composition given by the binodal and their amounts given by a tie-line and the lever rule. Despite some scatter, the literature data are fairly consistent and they imply that phase separation in Na-alginate/Na-caseinate mixture occurs only if the concentration of polymers exceeds certain relatively high values.

The required accuracy of the binodal depends to same extent on the application of such mixtures. One might argue that when ATPS is applied to bioseparation, the accuracy is not very important, because in such cases, what really matters is the efficiency of such a system in partitioning, e.g. the partitioning coefficient. However, if ATPS are used in manufacturing of food substitutes, the exact concentrations at which separation occurs (the precise position of binodal) might be essential for determining the structure of the mixture.

In this paper, we report measurements related to the structure of Na-alginate/Na-caseinate mixtures. The system was selected because: (a) binodal literature data are already available; and (b) similar systems have direct application in the food industry as the texture of water–casein–sodium alginate mixture at high concentration resembles muscle tissue so such a system can form the basis of meat analogues (Antonow, Grinberg, Zhuravskaya, & Tolstoguzov, 1980; Suchkov, Grinberg, & Tolstoguzov, 1981). The measurements were carried out simultaneously by two different techniques: a light scattering technique (Malvern Mastersizer) and by image analysis (Pacek, Moore, Calabrese & Nienow, 1994). The measurements covered concentrations

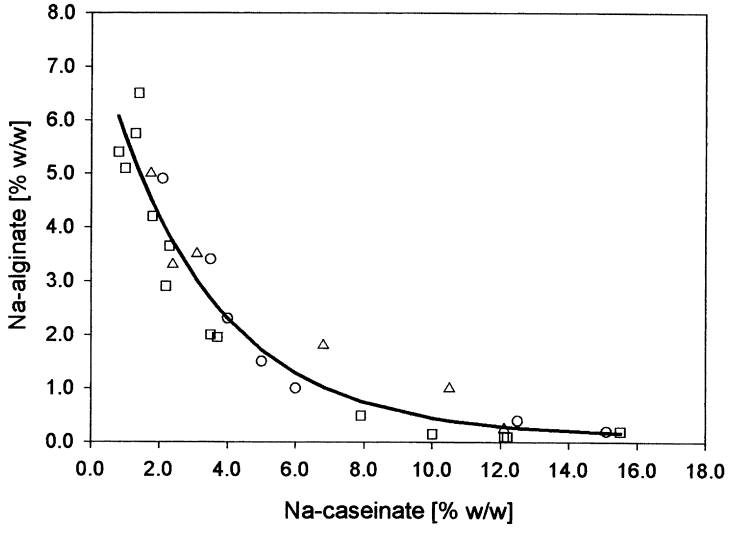

Fig. 1. Phase diagram of Na-alginate/Na-caseinate mixture: (○)—Antonow et al. (1980); (□)—Blonk et al. (1995), chemical analysis; (△)—Blonk et al. (1995), Confocal Scanning Laser Microscope, binodal—the best fit to all experimental data calculated by nonlinear regression — solid line.

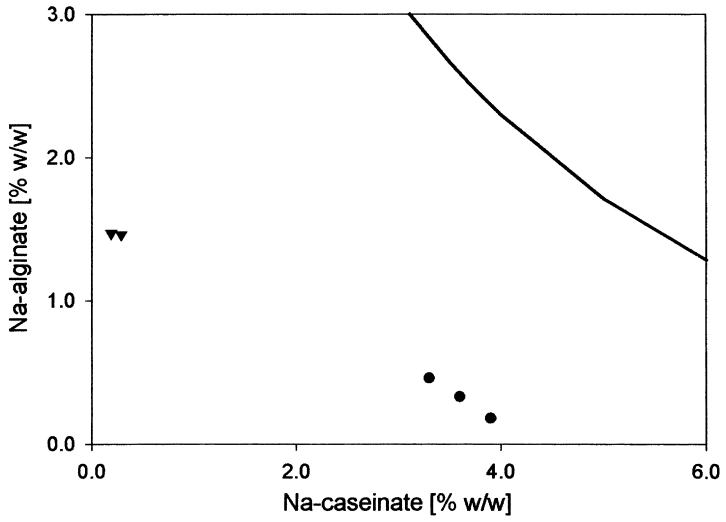

Fig. 2. The concentrations of Na-alginate and Na-caseinate employed in reported investigations: (▼) small amount of 12% Na-caseinate added to initially 1.5% Na-alginate; (●) small amount of 2% Na-alginate added to initially 4% Na-caseinate; solid line—binodal from Fig. 1.

in the "homogeneous" region and led to some surprising results, which are reported here. Work in the two phase region will be reported later.

2. Experimental

Measurements were made at very low concentrations, well away from the binodal (Fig. 2). Surprisingly, though these systems appeared quite homogeneous to the naked eye (often used to detect cloud point), both measuring techniques detected phase separation, i.e. drops were found. Therefore, two cases were systematically studied. In the first case, the concentration of Na-caseinate was gradually increased from zero, which should lead to a Na-caseinate rich phase being dispersed after phase separation (▼ in Fig. 2). In the second case, the concentration of a Na-alginate was gradually increased, also from zero, which should give Na-alginate rich phase being dispersed after phase separation (● in Fig. 2).

For each case stock solutions were used to give the concentrations indicated in Fig. 2: in the first case, 1.5% Na-alginate solution to which 12%(w/w) Na-caseinate solution was gradually added and in the second series of experiments, 4% Na-caseinate solution to which 2%(w/w) Na-alginate solution was added. These stock solutions were prepared following the procedure outlined by Blonk et al. (1995). Na-caseinate (molecular weight ~25 000 Da, supplied by Sigma, lot 36H0408) gives solutions of relatively low viscosity (see below), so the powder could be gradually added to deionized, distilled water at room temperature and be well mixed by a magnetic stirrer. The pH of the solution was continuously monitored and adjusted to pH = 7 using 0.1 N NaOH. The final solution was centrifuged at 10 000 rpm for 2.5 h to separate undissolved particles and the concentration of Na-caseinate was measured by evaporation. Na-alginate (Manucol DM, molecular weight ~500 000 Da, supplied by Kelco lot No 590961) gives much more viscous solutions (see Fig. 3) and therefore it was dissolved in a jacketed stirred vessel fitted with baffles and a helical screw impeller and connected to a water bath. Na-alginate was gradually added to deionized, distilled water at 50°C continuously stirred at $N = 300$ rpm. pH was continuously monitored and adjusted with 0.1 N NaOH to pH = 7. The final solution was heated to 70°C and "conditioned" at this temperature for 30 min and after that time cooled to 22°C. The concentration of Na-alginate was measured by evaporation. Sodium azide at concentration of 0.03%(w/w) was added to both solutions to avoid biological degradation. The density and viscosity of all solutions were measured and the results are summarised in Table 1 and Fig. 3. Both Na-caseinate solutions are of constant relatively low viscosity whereas Na-alginate solutions are weakly shear thinning and more viscous (Fig. 3).

2.1. Experimental rig and procedure

The experimental rig for investigation of phase separation and for the measurement of drop size distributions in the Na-alginate/Na-caseinate mixtures is shown schematically in Fig. 4. Initially, a precise amount of the stock solution of one polymer was charged into a glass stirred vessel (1) of diameter of $T = 0.1$ m, fitted with baffles, and a Rushton turbine impeller of diameter $D = 0.05$ m and stirred at an impeller speed of $N = 200$ rpm. The solution was continuously circulated through the measuring cell (4) of the Mastersizer type S (3) by peristaltic pump (2). To minimize the possible change of the structure of the solution (or more precisely the structure of the mixture after phase separation) caused by the action of the pump and by shear during flow through the connecting tubes, the solution was sucked by the

Table 1
Physical properties of stock solutions at 22°C

	Na-alginate		Na-caseinate	
Concentration (%(w/w))	1.5	2.0	4.0	12.0
Density[a] (kg/m^3)	1006	1010	1008	1032
Viscosity [b] (Pa s)	See Fig. 3		0.003	0.200

[a] Measured by density bottle.
[b] Measured by Carri-Med rheometer.

pump and passed through the measuring cell before it passed through the pump (see direction of the arrows in Fig. 4a) and the distances between the vessel, the Mastersizer and the pump were made as short as possible. Great precautions were also undertaken to avoid air entrainment.

With the stock solution of one polymer in the vessel, the optics were carefully aligned and the background level was measured. The Mastersizer was set in automatic measuring mode and small aliquots of the stock solution of the second polymer were added into the vessel to give the composition shown in Fig. 2. Small samples were also withdrawn from the vessel and their structure was examined under the microscope and drop size distributions were measured by image analysis. Such a procedure allowed practically simultaneous monitoring of the structure of the mixture and the measurements of drop size distributions by two independent techniques: in situ by light scattering (Mastersizer) and in the sample (image analysis).

Two sets of experiments were performed. In the first set, the vessel was initially filled with Na-alginate solution and Na-caseinate solution was added in small aliquots. In this case, after phase separation, Na-caseinate rich phase was dispersed. In the second set of experiments, the vessel was initially filled with Na-caseinate solution and Na-alginate solution was added leading to a Na-alginate rich phase being dispersed after phase separation. In each experiment the structure of the mixture was monitored and drop size distributions were measured by Mastersizer and by image analysis at regular time intervals.

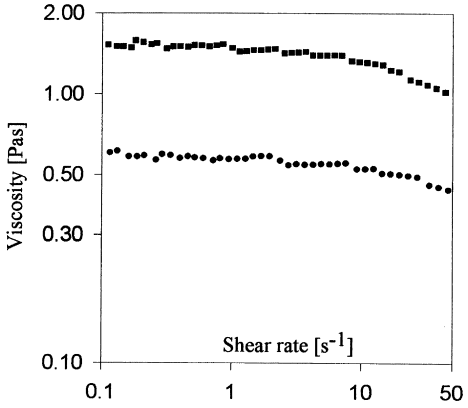

Fig. 3. Viscosity of Na-alginate solutions at 22°C: (■) 2%(w/w) Na-alginate; (●) 1.5%(w/w) Na-alginate.

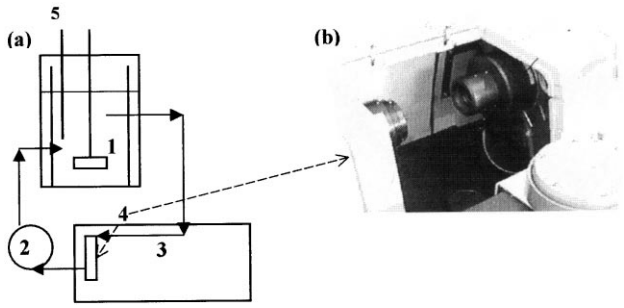

Fig. 4. Experimental rig for investigation of phase separation and measurements of drop size distribution: (a) general lay out (1) stirred vessel, (2) peristaltic pump, (3) Mastersizer, (4) measuring cell, (5) temperature and pH probe; and (b) details of measuring cell.

3. Results and discussion

3.1. Na-caseinate (initially[1] Newtonian,$\mu = 0.2$ Pa s) added to Na-alginate (shear thinning, $\mu \sim 0.6$ Pa s)

The vessel was initially filled with 620 ml of 1.5%(w/w) Na-alginate solution, all air bubbles were expelled and the background noise was measured. The Mastersizer was set in the automatic measuring mode and the measurements were carried out every 2 min and the results are summarised in Figs. 5 and 6.

The first measurements, from time $t = 0$ to $t = 2$ min, were done with pure Na-alginate solution and as one would expect, no drops were detected. At time $t = 2$ min, 10 ml of 12%(w/w) Na-caseinate solution was added giving the concentration of Na-alginate and Na-caseinate in the mixture of $x_{al} = 1.47\%$ and $x_{cs} = 0.19\%$, respectively. Immediately after the addition, the Mastersizer detected the presence of second phase (drops) at a volume fraction of $\phi_d = 0.04\%$ (see Fig. 6) and measured drop size distributions (see Fig. 5, time 3 min). Drops were also clearly seen in the sample withdrawn from the vessel as shown in Fig. 7. (It should be noted that in Fig. 7, the depth of field is about 300 μm. The mean size of the drops is of the order of 10 μm. Thus as the drops are evenly distributed throughout the sample, many layers are seen so that the concentration of drops appears much bigger than 0.1%).

Initially, the drop size distribution was slightly bimodal with the largest drops of the order of 300 μm, but after 2 min of stirring and circulating of the mixture, those drops disappeared and the number of small drops (of the order of 5 μm) increased and the distribution became single-modal (Fig. 5). The large drops clearly resulted from the initial addition of Na-caseinate and it then took time to disperse Na- caseinate uniformly throughout the volume of the vessel. The dispersion was stirred for

[1] It is important to recognize that the drops detected by Mastersizer or seen by image analysis (see later) and discussed here did not have the same composition as that of the second phase initially added. Thus the actual physical properties of the dispersed and continuous phase are not known.

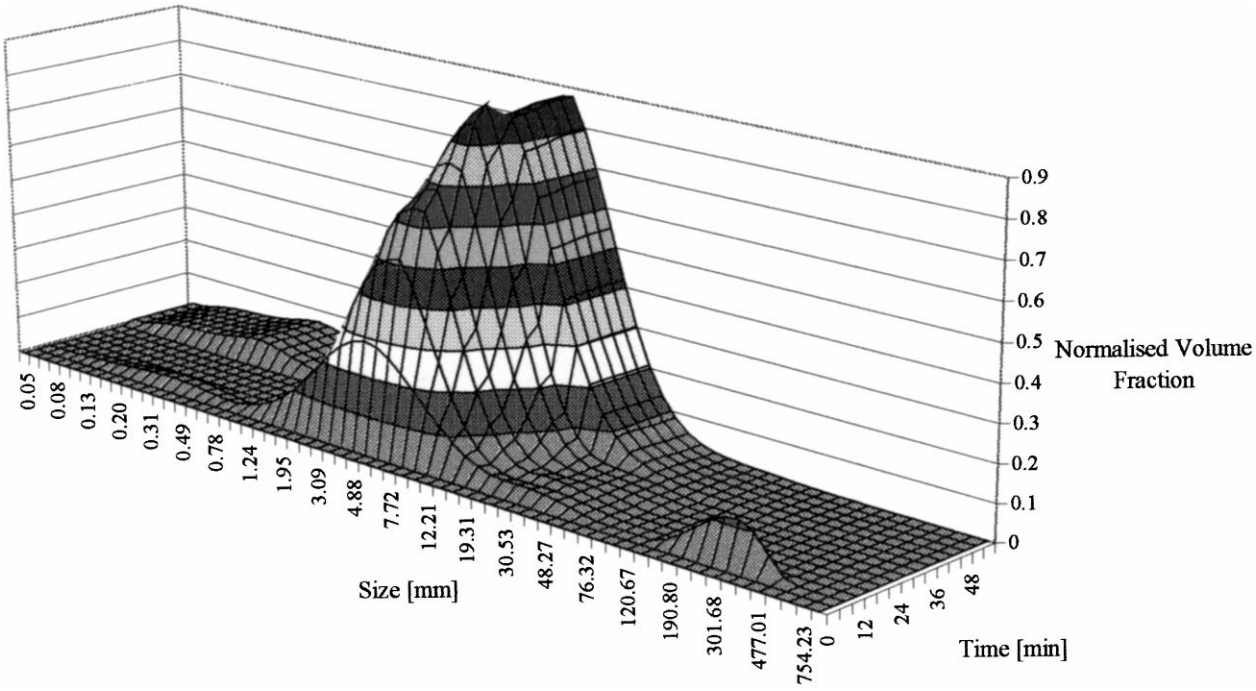

Fig. 5. Drop size distributions measured by Mastersizer in Na-caseinate/Na-alginate mixture after the addition of a series of aliquots of Na-caseinate to Na-alginate solution. Na-caseinate rich phase dispersed.

30 min and during that time both measured volume fraction of dispersed phase (Fig. 6) and number of small drops (Fig. 5) gradually increased. These increases can be explained by the fact that the pure Na-alginate solution was rather viscous and shear thinning and therefore the longer time was necessary to achieve uniform dispersion of the Na-caseinate on the micro level.

After 30 min, another 5 ml of Na-caseinate solution was added giving the concentrations $x_{al} = 1.46\%$ and $x_{cs} = 0.29\%$ and dispersion was stirred for next 30 min. In this case, the behaviour of the mixture was different from that found after the first addition. Firstly, the large drops were not detected (drop size distributions stayed single modal) and secondly, within the first 5 min of stirring, the volume fraction of dispersed phase increased to $\phi_d = 0.10\%$ and remained unchanged after that time (see Fig. 6). This

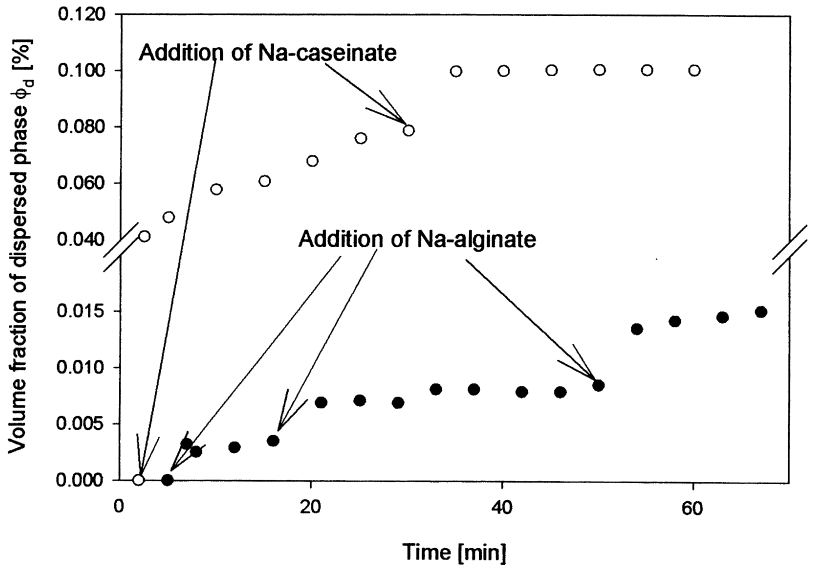

Fig. 6. The volume fraction of dispersed phase after the addition of: (○) 10 and 5 ml of 12% Na-caseinate to 620 ml of 1.5% Na-alginate; (●) after the addition of a series of 50 ml aliquotes of 2% Na-alginate to 500 ml of 4% Na-caseinate.

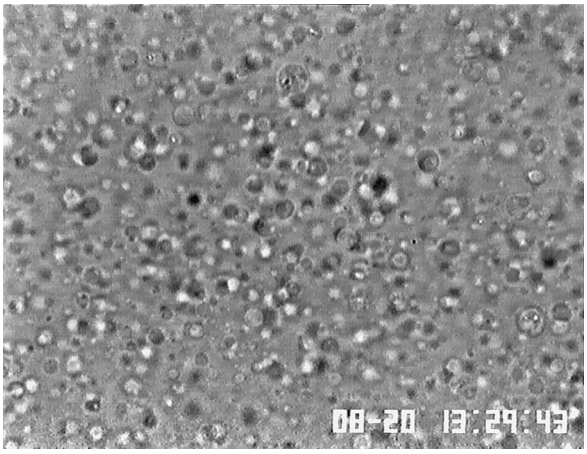

Fig. 7. The image of the Na-caseinate/Na-alginate mixture at $x_{cs} = 0.19\%$, and $x_{al} = 1.47\%$. Na-caseinate rich phase dispersed. Height of the picture is equal to 240 μm.

difference in the behaviour of the mixture after the first and the second addition can be attributed to: (a) the fact that the amount of Na-caseinate added was much smaller, so it was easier to disperse it uniformly; and (b) after the first addition the mixture became less shear thinning and the apparent viscosity decreased again leading to easier mixing. Both those factors caused that it was much easier to achieve the uniform concentrations in the system. The image of the sample withdrawn from the vessel shortly after the second addition was very similar to the image shown in Fig. 7 and again clearly confirmed the presence of the large number of small drops.

The mean drop size and drop size distributions were also measured by image analysis. The measurements were carried out on the samples withdrawn from the vessel (example of the typical image is shown in Fig. 7) at the time when the drop sizes were measured in-situ by the Mastersizer and the results are summarised in Fig. 8 and Table 2. The results obtained by the video technique are in excellent agreement with the corresponding results (at 10 and 45 min) obtained by Mastersizer. Both techniques clearly show that even at very low concentrations of Na-caseinate, the mixture is already phase separated, whereas according to the literature, the mixture should have been homogenous (see Fig. 2).

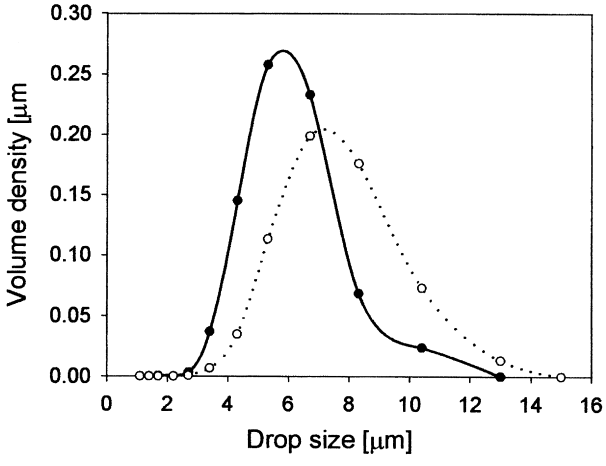

Fig. 8. Volume probability density functions measured by image analysis in the samples withdrawn from the vessel: (●) at time $t = 10$ min; $\phi_d = 0.06\%$ (○) at time 45 min; $\phi_d = 0.1\%$ (see also Fig. 5 and Table 2). Na-caseinate rich phase dispersed.

3.2. Na-alginate (initially[2] shear thinning $\mu \sim 1.5$ Pa s) added to Na-caseinate (Newtonian, $\mu \sim 0.003$ Pa s)

The vessel was initially filled with 500 ml of 4%(w/w) Na-caseinate solution, all air bubbles were expelled, the solution was stirred and circulated through the measuring cell and the background noise was measured. The Mastersizer was set in the automatic measuring mode and the measurements were taken every 2 min. The results, addition sequence and volume fraction are summarised in Fig. 6 and the drop size in Fig. 9.

The first measurements, from $t = 0$ to $t = 5$ min, were taken with pure Na-caseinate solution so that drops were not detected. At time $t = 5$ min, 50 ml of 2%(w/w) Na-alginate solution was added giving the average concentrations of biopolymers in the mixture of $x_{al} = 0.18\%$ and $x_{cs} = 3.9\%$. After several seconds of stirring, the Mastersizer detected the presence of a dispersed phase at a volume fraction of $\phi_d = 0.0025\%$ (see Fig. 6) and measured the drop size distributions (see Fig. 9). In this case, the time elapsed between the addition of Na-alginate and the appearance of first drops was longer than in the previous experiment, but this can be explained by the much higher viscosity of the added phase (see Fig. 3) which took longer to disperse and equilibrate. The measured drop size distributions shown in Fig. 9 are clearly bimodal with drops in two size ranges: 0.2–2.0 and 5–250 μm and they did not change during the next 15 min of stirring. At 15 min, the next 50 ml of Na-alginate solution was added giving average concentrations of $x_{al} = 0.33\%$ and $x_{cs} = 3.6\%$, and causing a step increase in volume fraction of dispersed phase to $\phi_d = 0.0075\%$. The drop size distributions remained bimodal but the lower and the upper range of size distribution became wider, with the smaller drops in the range 0.2–3.5 μm and the large drops in the range 5–300 μm. Neither the volume fraction of dispersed phase nor the drop size distributions changed during stirring for nearly 30 min. At 50 min, the next 50 ml of Na-alginate was added, to give $x_{al} = 0.46\%$ and $x_{cs} = 3.3\%$, causing a further increase of volume fraction of dispersed phase to $\phi_d = 0.015\%$ and the drop size distribution stayed bimodal. The increase of the number of the smaller small drops (0.2–5 μm) was much stronger than that of large drops (10–150 μm) and again the continued stirring did not change the shape and span of the drop size distribution.

[2] See earlier footnote.

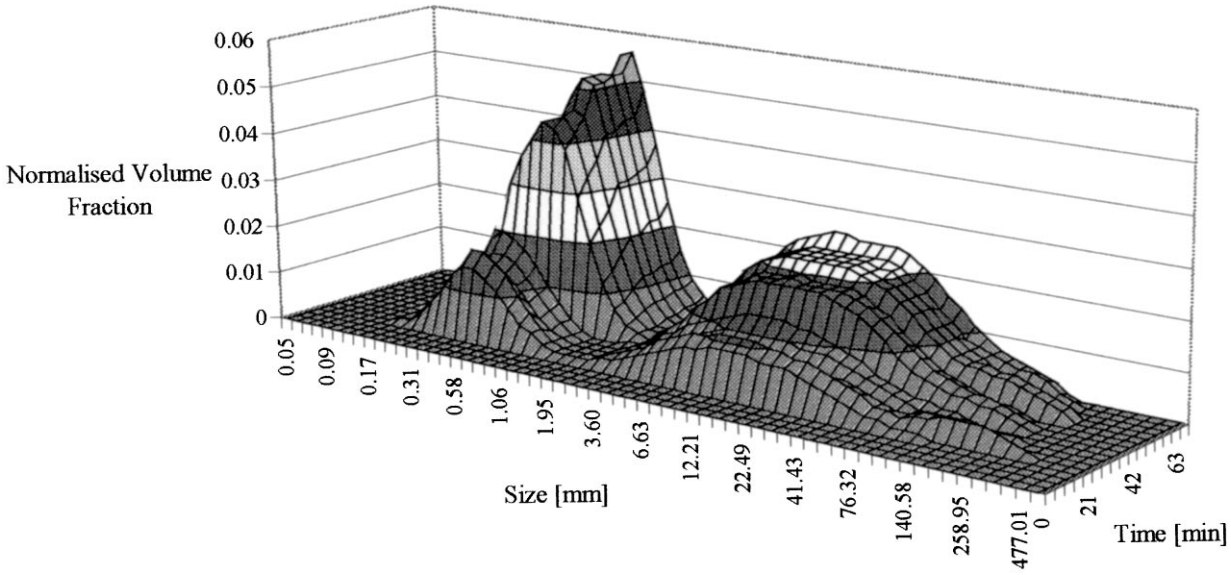

Fig. 9. The drop size distributions measured with the Mastersizer after the addition of a series of 50 ml aliquots of 2% Na-alginate to 500 ml of 4% Na-caseinate. Na-alginate rich phase dispersed.

After each addition of Na-caseinate, a small sample of the mixture was withdrawn from the vessel and the structure was analysed, to give drop size distributions from digitised images and compared with the results obtained with Mastersizer. A typical image of the separated mixture is shown in Fig. 10 and the measured drop size distributions and mean drop size are summarised in Fig. 11 and Table 3, respectively. After the first addition of Na-alginate, drops were not detected. However, this can be explained by the fact that the spatial resolution of the microscope was of the order of 1 μm so that the majority of the drops which were below 1 μm (see Fig. 9) could not have been seen. As the volume fraction of dispersed phase increased, with the second addition, small drops of a Na-alginate rich phase dispersed in a Na-caseinate rich phase can clearly be seen in Fig. 10. However, the large drops (above 10 μm), which were detected in-situ by the Mastersizer (Fig. 9) were not seen in the samples (see Fig. 11). Perhaps, the very low number of such drops in the dispersion can explain their absence in the sample. It is also possible that the large entities detected by Mastersizer were in fact aggregates of loosely attached small drops that were broken during sampling. Aggregation could have been caused by the very low interfacial tension between continuous and dispersed phase (of the order of 10^{-6} N/m, (Pacek, Ding & Nienow, in preparation)) and relatively high viscosity of dispersed phase. A similar situation was observed after the third addition, when again only small drops were seen in the sample. The volume density functions measured by the video technique in the sample are

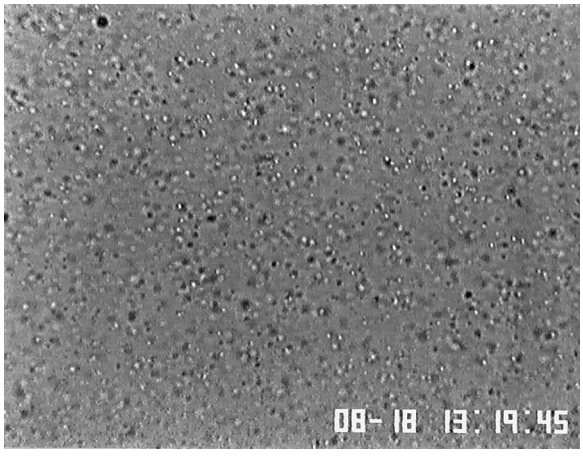

Fig. 10. The image of the Na-alginate/Na-caseinate mixture at $x_{al} = 0.33\%$ and $x_{cs} = 3.6\%$. Na-alginate rich phase dispersed. Height of the picture is equal to 240 μm.

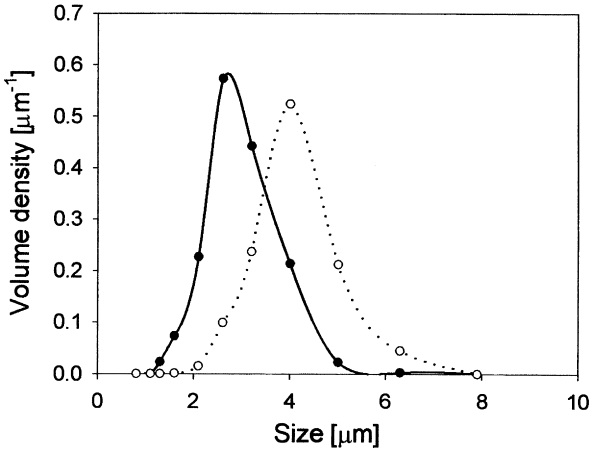

Fig. 11. Volume probability density functions measured by image analysis in the samples withdrawn from the vessel: (●) at time $t = 35$ min; $\phi_d = 0.0075$ (○) at time 65 min; $\phi_d = 0.015\%$ (see also Fig. 9 and Table 3). Na-alginate rich phase dispersed.

Table 2
Mean drop sizes in Na-alginate/Na-caseinate dispersion measured by image analysis at the same times as shown in Fig. 5. Na-caseinate rich phase dispersed

Time (min)	Na-caseinate (%)	Na-alginate (%)	d_{10} (μm)	d_{32} (μm)	d_{43} (μm)
10	0.19	1.47	5.2	5.9	6.3
45	0.29	1.46	6.3	7.3	7.8

Table 3
Mean drop sizes in Na-alginate/Na-caseinate mixture measured by image analysis at the same times as shown in Fig. 9. Na-alginate rich phase dispersed

Time (min)	Na-caseinate (%)	Na-alginate (%)	d_{10} (μm)	d_{32} (μm)	d_{43} (μm)
10	3.9	0.18	No drops		
35	3.6	0.33	3.0	3.4	3.6
65	3.3	0.46	3.6	4.0	4.2

fairly close to these for the smaller sizes in the distributions measured by Mastersizer in-situ.

Most importantly, though there is some discrepancy in detail between the two techniques, again, even at very low concentrations of one polymer two phases were detected in the mixture whereas, according to the phase diagram (Fig. 1), the system should be homogenous.

4. Conclusions

The experiments discussed in detail in this paper involving researchers from two independent groups, stemmed from earlier, preliminary experiments carried out by each group independently. In all those earlier experiments, phase separation in Na-alginate/Na-caseinate mixture was observed over a wide range of very low concentrations of either polymer (data not shown). Here, a coherent series of experiments has been carried out for simultaneous analysis of the structure of Na-alginate/Na-caseinate mixtures by two independent techniques. The two techniques, light scattering (Mastersizer) and image acquisition/analysis (Video Technique) clearly show that even at very low concentrations well below binodal as given in the literature, the system is phase-separated. Two phases can be detected whether a Na-caseinate rich phase is dispersed or a Na-alginate rich phase is dispersed. In both cases, the mean drop size and drop size distributions do not change significantly with the stirring time (i.e. at constant hydrodynamic condition).

The use of image analysis and the Mastersizer both proved to be very powerful as a mean of revealing the structure of aqueous–aqueous dispersions that to the naked eye appear to be homogeneous. Despite the fact that the difference in refractive indices between a Na-alginate rich phase and Na-caseinate rich phase is very low, the presence of drops of either phase can be quickly detected by both techniques. As the drops resulting from phase separation are of the order of a few microns and below, the Mastersizer seems to be an ideal tool for the measurements of drop size as long as the system is very dilute. The image analysis/video technique, however, though it misses the smallest sizes, also allows measurements to be taken in more concentrated mixtures as well a detailed analysis of the complex structure occurring at higher concentration of polymers, including the detection of phase inversion (Pacek et al., 1994; Pacek et al., in preparation).

These results question the phase diagrams published in the literature and to some extent the current understanding of the phenomenon of phase separation in two phase aqueous–aqueous systems.

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