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Characterisation of colloidal gas aphrons for subsequent use for protein recovery

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Abstract

Colloidal gas aphrons (CGAs) were first reported by Sebba (*J. Colloid Interface Sci., 35* (4) (1971) 643) as micro bubbles (10–100 μm), composed of a gaseous inner core surrounded by a thin surfactant film, which are created by intense stirring of a surfactant solution. Since then, these colloidal dispersions have been used for diverse applications (clarification of suspensions, removal of sulphur crystals, separation of organic dyes from wastewater, etc.). However, there have been no reports, as yet, of their direct application for protein recovery. In this study, CGAs are created from an anionic surfactant (AOT) and are characterised in terms of stability and gas hold-up for a range of process parameters relevant to their proposed use for protein recovery, at a later stage. A statistical experimental design was developed in order to study the effect of different factors (surfactant concentration, salt concentration, pH, time of stirring and temperature) on the stability and gas hold-up of CGAs. The analysis of results from the experimental design provides predictive statistical models. Stability was found to depend mainly on salt and surfactant concentration. Several interactions are shown to be significant including the time–temperature interaction. Gas hold-up was found to depend mainly on salt and surfactant concentration and time of stirring. Also, results from power measurements are presented and the minimum energy for the formation of CGAs, for one set of solution properties, is determined.

Keywords: Colloidal gas aphrons; Protein recovery; Micro bubbles; Anionic surfactant

1. Introduction

Felix Sebba [1,2] reported, that when surfactant solutions are mixed at high speeds, micro bubbles (10–100 μm in diameter) are formed. He postulated that these are composed of a gaseous inner core surrounded by a thin soapy film (see Fig. 1) and named these micro bubbles colloidal gas aphrons (CGAs). The structure of CGAs is often questioned and there have been no conclusive reports presented in the literature which consider this issue. The main interaction forces present in dispersions of CGAs are surface forces and electrostatic interactions. The direct application of CGAs for protein recovery has not previously been reported. However, several properties of the structure proposed by Sebba, indicate that they may be attractive for this purpose. These properties include: high stability due to the thin soapy shell surrounding the gaseous core (which delays their coalescence); large interfacial area due to the small size of CGAs; and the possibility of different mechanisms for protein adsorption, for example hydrophobic or electrostatic interactions.

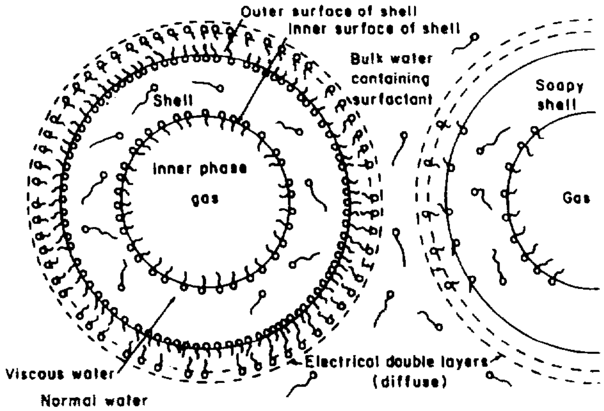

Fig. 1. Structure of CGAs proposed by Sebba [2].

There have been a number of reported applications for CGAs including: intensification of mass transfer in aqueous two-phase systems for enzyme extraction [3]; clarification of suspensions [4]; predispersed solvent extraction of dilute products [5]; removal of sulphur crystals [6]; removal of heavy metals from aqueous solutions [7]; coflotation and solvent sublation processes [8]; separation of organic dyes from wastewater [9]. Table 1 gives a summary of these applications.

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Table 1
Literature review for CGAs in chronological order, up to 1995

Reference	Surfactant type	Parameters and conditions	Bubble size distribution	Stability	Gas hold-up	Application
Sebba, 1971 [1]	—	—	—	half-life	—	Qualitative description
Ciriello et al., 1982 [7]	EHDA (c)SDBS (a)	C_{sf} (300 ppm)pH (3–12)	—	—	√	Removal of heavy metals
Wallis et al., 1995 [29]	Arquad C-50 (c)	Flotation time (3, 6, 10 min)C_{sf} (0.5 g l^{-1})	—	—	√	Flotation of unicellular algae
Caballero et al., 1989 [8]	HTAB (c)	C_{sf} (0.1, 0.2, 0.5 mg ml^{-1})pH (7.5–12)CGA flow rate (10–30 ml min^{-1})	—	—	—	Coflotation and solvent sublation
Subramaniam et al., 1990 [4]	AOT (a)SDBS (a)Lux$^{\mathrm{TM}}$ flakes (ni)BDHA (c)	C_{sf} (5.1 g l^{-1} Lux flakes; 2 g l^{-1} AOT; 1.2 g l^{-1} SDBS; 1.3 g l^{-1} BDHA)pH (3–10, 12)Volume of CGA (120–540 ml)T (30–70 °C)	—	half-life	√	Clarification of suspensions
Amiri and Woodburn, 1990 [6]	TTAB (c)	C_{sf} (0.15, 0.47, 1.2, 3.5, 5.6, 13.5 mM)pH (2.14, 5.8, 12.05, 13)	—	creaming rate	√	Removal of sulphur crystals
Matsushita et al., 1992 [5]	HTAB (c)DTAB (c)CPB (c)SDS (a)SDBS (a)Polyoxy ethene triglyceride (ni)Tergital (ni)	C_{sf} (0.25, 0.50, 1, 2.5 mg ml^{-1})Speed (5 000–8 500 rpm)Stirring time (0.5, 1, 2, 5 min)	—	half-life	√	Predispersed solvent extraction of dilute products
Roy et al., 1992 [9]	HTAB (c)SDBS (a)	C_{sf} (350 ppm HTAB, 500 ppm SDBS)Volume of CGAs (0–500 ml HTAB and 0–1 000 ml SDBS)	√	"stability" (DV fraction)	—	Separation of organic dyes from waste water
Chapalkar et al., 1994 [10]	Tergitol 15-S-12 (ni)SDBS (a)HTAB (c)	C_{sf} (200, 500, 1 000 mg l^{-1} DDBS; 50, 100, 500, 1 000 mg l^{-1} Tergitol; 200, 328, 500 mg l^{-1} HTAB)C_{sl} (200 mg l^{-1} NaCl)	√	"stability"	√	Waste treatment
Save et al., 1993 [3]	HTAB (c)SDBS (a)LAEO (ni)	C_{sf} (0.12, 0.33 mg ml^{-1})pH (4.3)PEG and salt phases composition	—	half-life	√	Aqueous two-phase extraction of an enzyme
Chapalkar et al., 1994 [30]	Tergitol 15-S-12 (ni)SDBS (a)HTAB (c)	C_{sf} (50, 100 mg l^{-1} Tergitol; 200, 328 mg l^{-1} HTAB; 200, 500 mg l^{-1} SDBS)pH (10.1, 7.4, 3)Volume of CGAs (0–250 ml)	√	"stability" (DV fraction)	—	Removal of pentachlorophenol from aqueous solutions
Save and Pangarkar, 1993 [11]	SDBS (a)HTAC (c)CPC (c)DTAC (c)SLS (a)DMDSAC (c)Triton X-100 (ni)LA-EO3 (ni)	pH (2, 4.3, 7)Stirring time (60–520 s)C_{sl} (2.87, 0.86 mM SDBS; 0.346, 1.03 mM SLS; 0.282, 0.85 mM CTAC; 0.294 mM CPC; 0.344, 1.03 mM DTAC)Impellerclearance(0.2–0.8 C/D)Enzyme addition (0–0.25 mg ml^{-1})Polymer addition (50, 100, 200 ppm polyacrylamide)Solvent addition (0–6 ml ether and TBP)Non-ionic surfactant addition (1, 3, 5 ml l^{-1})Addition of salt (0.25, 1, 2 M NaCl)	√	half-life	√	Characterisation of CGAs
Save and Pangarkar, 1995 [31]	SDBS (a)SDS (a)HTAC (c)DTAC (c)CPC (c)	pH (2, 4.3, 7)Bivalent salts ($BaCl_2$ 0.1 M, $CaCl_2$ 0.1 M)Cell loading (2, 4, 7 Kg m^{-3})V_{d} (0.5–2.5 m s^{-1})	√	—	√	Harvesting of microorganisms

For notation see Nomenclature.

In previous reports (mentioned above), the focus has mainly been on applications for CGAs, although limited fundamental aspects have been described for some systems. The effect of pH and concentration of cationic (BDHA) and anionic (AOT, SDBS and LUX flakes) surfactants on stability of CGAs was studied by Subramaniam et al. [4]. The optimum concentrations were selected for each surfactant and pH was reported not to have a significant effect on the stability. The effect of type of surfactant (SDS, SDBS, CTAB, DTAB and CPB), surfactant concentration, stirring speed and time of stirring on the stability and air contents of CGAs was reported by Matsushita et al. [5]. The results indicated a significant effect of speed on stability when speed was increased from 5000 to 5500 rpm, but further increases up to 8500 rpm did not have a significant effect. The length of the alkyl chain of cationic (CTAB and DTAB) and anionic (SDS and SDBS) surfactants affected the stability of CGAs created from cationic surfactants; the longest chain surfactant was reported to give the highest stability. Matsushita et al. [5] also reported a significant effect of stirring time on gas content, but not on stability. Bubble size and stability were measured by Chapalkar et al. [10] for different types of surfactants (anionic, SDS; cationic, HTAB; non-ionic, Tergitol), surfactant concentration and ionic strength. They found that the bubbles generated by the non-ionic surfactant were of smaller diameter than those generated by the ionic surfactants. Increasing surfactant concentration led to smaller diameter bubbles for all three surfactants. The effect of concentration on the bubble size was significant only above the critical micelle concentration (cmc) of the surfactant. An increase of ionic strength (by adding 3.4 mM NaCl) resulted in a reduction of bubble size, but this effect was found to apply only to the ionic surfactants. The distribution of bubble sizes was found to be fairly constant with time. Save and Pangarkar [11] presented a generalised characterisation of CGAs, for a number of surfactants, with respect to life time, gas content and bubble size and made an attempt at explaining and modelling the coalescence of colloidal gas aphrons.

For CGAs to be used directly for protein recovery, the principle of operation would be as follows: CGAs would be generated as described above and then mixed with a protein solution. It is expected that proteins will interact with the surfactant in the aphrons due to electrostatic and/or hydrophobic forces (selective adsorption of one protein from a mixture of proteins may be possible; depending on the surfactant and protein properties). Once mixing ceases, the aphron phase should separate easily from the solution due to its buoyancy. This aphron phase should be enriched in the protein and effective recovery therefore achieved. For this protein recovery process to be successful, aphrons should be relatively stable: stability should be high enough to allow protein adsorption, but since protein–surfactant interaction may cause protein denaturation [12–14], aphrons need to collapse in a relatively short time period to minimise deleterious effects. Small bubbles and a uniform bubble size distribution will be advantageous if the interfacial area available for the protein adsorption is to be maximised.

In the present study, power consumption during formation of colloidal gas aphrons was initially determined for one particular surfactant. Power input depends on the fluid characteristics and impeller performance and determines the energy input for creation of aphrons. In previous reports [3–9], the variation of CGA stability and gas hold-up with impeller speed has been presented. It is, perhaps, more useful to attempt to correlate output parameters such as stability, gas hold-up and bubble size with power input rather than impeller speed; this will also allow comparison between mixing systems. The anionic surfactant AOT (sodium bis-(2-ethyl hexyl) sulfosuccinate) was chosen for this study. This surfactant has been widely used for bioseparations, mainly in reverse micelles separations for protein recovery [15–18]. AOT has been considered for CGAs by Subramaniam et al. [4], but under only a very limited conditions (see Table 1).

Also, described here is a study which characterises and optimises the stability of colloidal gas aphrons generated from the anionic surfactant AOT, for a range of process variables. The process variables were chosen on the basis of their likely importance for subsequent use of CGAs for protein recovery, i.e. pH, salt concentration (which affects ionic strength), surfactant concentration, temperature and time of stirring. Empirical models giving the dependence of stability and gas hold-up as a function of the main process variables are presented.

2. Experimental

2.1. Material

AOT (sodium bis-(2-ethyl hexyl) sulfosuccinate) was obtained from Fisons plc. The aphronic solutions were made from buffered solutions of AOT (acetate buffer, CH_3COOH/CH_3COONa 0.1 M, for pH = 4; phosphate buffer, H_2PO_4Na/HPO_4Na_2 0.025 M and 0.01 M, for pH = 6 and 8 respectively). Acetic acid, sodium acetate (anhydrous), sodium dihydrogen orthophosphate monohydrated, di-sodium hydrogen orthophosphate (anhydrous) and sodium chloride, were supplied by BDH (AnalaR grade). The 0.1 N HCl standard solution was prepared by dilution of a 1 N HCl "ConvoL" (AnalaR grade) standard solution supplied by BDH. The laboratory mixer (SL2T model) fitted with a four bladed impeller (D = 30 mm), surrounded by a high shear screen and with a speed digital read out, was supplied by Silverson Ltd.

2.2. Methods

2.2.1. Formation of aphrons

The formation of CGAs requires, as Sebba described [19], a horizontal disc that rotates at very high speeds. Baffles are also necessary in order to achieve the required mixing regime.

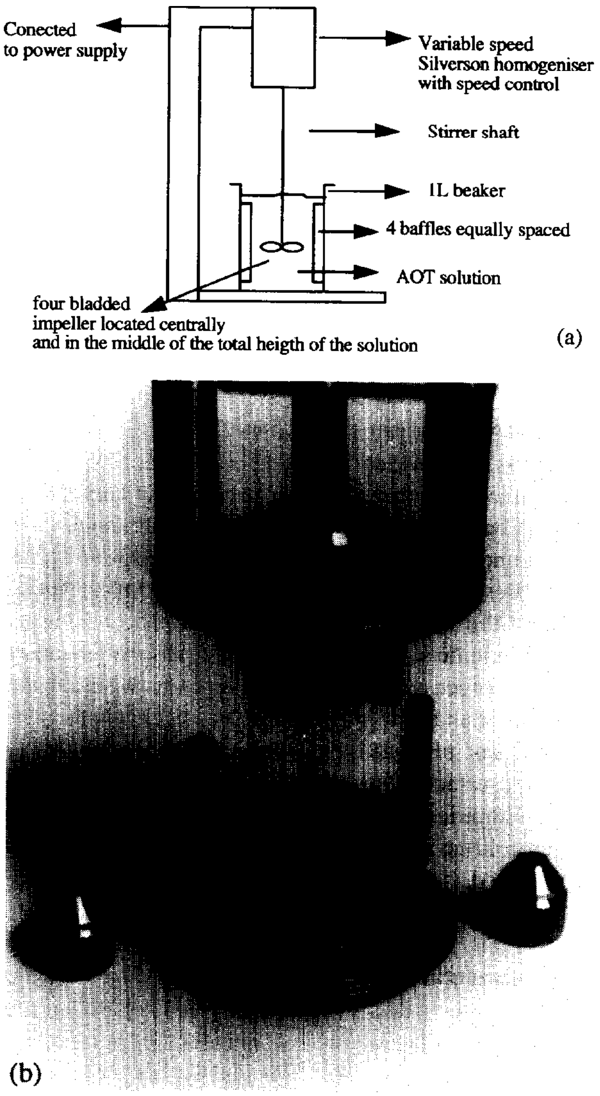

Fig. 2. (a) Stirring system used in the formation of CGAs. (b) Four-bladed impeller and high shear screen (underneath the impeller) used for the formation of CGAs.

The experimental apparatus was based on Sebba's original design [19], with variations in type and dimensions of impeller and speed. A fully baffled beaker (volume, 1 l mm^3; diameter, 105 mm) containing a buffered solution (0.4 l) of AOT was stirred at very high speeds (from 5000 to 10 000 rpm) using a laboratory mixer (see Fig. 2). For each experiment, the stability of the aphrons was measured in terms of τ, which is defined here as the time required for half the amount of original liquid to drain. The gas hold-up (ϵ) which is the gas volumetric ratio, i.e. the ratio between the gas volume and the dispersion final volume (after stopping stirring), was also measured for each of the experiments.

2.2.2. *Determination of cmc*

The cmc of AOT was determined by measuring the change of surface tension as a function of surfactant concentration. Five different solutions of AOT were prepared and the surface tension of each was measured using a Krüss surface tensiometer at 29 °C. The Wilhelmy plate method was used for these measurements. The cmc value for an aqueous solution of AOT at 29 °C was found to be 2.4 mM.

2.2.3. *Determination of pK_a*

The pK_a of AOT was determined following the method described by Ramette [20]. For the determination of pK_a of AOT, a known amount of AOT was weighed and mixed with a standard solution of HCl (0.1 N). The pH of the resulting solution was measured and with these data the equilibrium constant of the sodium salt of AOT was determined (pK_b). From the pK_b data the pK_a was determined to be 2.94. Subramaniam et al. [4] reported the pK_a of several surfactants and for AOT they established a value of 5.75. From the information given in their report, it is not possible to determine the reason for the differences between their value and that reported here.

2.2.4. *Power measurement*

The power requirement for non-gassed Newtonian fluids is characterised by a dimensionless power number (N_p) which, by definition, is the ratio of the external force to the inertial force exerted by the fluid and is determined from the equation below [21]:

$$N_p = P/N^3D^5\rho \qquad (1)$$

where P is the power supplied to the agitator, N is the rotational speed of the impeller, D is the diameter of the impeller and ρ is the density of the fluid. So for a given fluid system, the power number will depend on the geometry and power of the agitator. From this equation, the power consumption in a non-gassed system can be expressed as follows:

$$P = N_p\rho N^3D^5 \qquad (2)$$

In an agitated system, the fluid flow is characterised by the Reynolds number (Re):

$$\mathrm{Re} = ND^2\rho/\mu \qquad (3)$$

where μ is the fluid viscosity. Three different fluid flow regions can be identified: turbulent ($\mathrm{Re} > 10^3$), transition ($10 < \mathrm{Re} < 10^3$) and laminar ($\mathrm{Re} < 10$).

Studies carried out for non-gassed systems for different geometry impellers at different Reynolds number, show that the power number for each geometry of impeller remains constant in the turbulent region [22]. Therefore, when working at very high speeds (turbulent region), the power number should depend only on the impeller geometry.

Studies for gassed systems show that the presence of gas has an effect on power consumption. The presence of gas bubbles reduces the density of the system, resulting in a reduction in power consumption. Many different correlations have been reported for agitated vessels into which gas has been introduced [23,24].

The system under consideration is a gas–liquid dispersion where the gas hold-up varies with impeller speed and surfactant concentration. In order to measure power consumption, Eq. (1) was modified to take account of the gas hold-up (ϵ) as follows [25]:

$$P = N_p \rho N^3 D^5 (1-\epsilon) \qquad (4)$$

To determine the power consumption in gassed systems, the power number of the impeller was first determined from Eq. (1) and measurements of power for each impeller speed studied (from 4 100 to 10 000 rpm). Measurements were carried out in a beaker containing deionised water (note: there was no gas entrainment). In a vessel stirred at very high speed the change of temperature with time is significant. By assuming that all the energy transmitted by the impeller is dissipated as heat, the following equation can also be written for power number:

$$N_p = \frac{(mC_p)_i}{D^5 \rho} \times \frac{(\mathrm{d}T/\mathrm{d}t)}{N^3} \qquad (5)$$

where $(mC_p)_i$ is the sum of the products of mass and specific heat factor of the different parts of the mixing system (water, glass, baffles and shaft) and $(\mathrm{d}T/\mathrm{d}t)$ is the change of temperature with the time. The temperature change with time was monitored for each of the speeds tested (the mixer system was totally insulated in order to avoid any heat losses). The power numbers obtained for each speed were then used in Eq. (4) to calculate the power consumption during formation of CGAs.

2.2.5. *Stability and gas hold-up measurements*

In order to determine the effect of different process parameters and physical conditions on the stability and gas hold-up of the aphrons, a statistical experimental design, (a modified central composite design [26]) was developed (see Table 3). Five factors were chosen: surfactant concentration; pH; temperature; salt concentration and time of stirring. Each factor was studied at three different levels (low, medium and high), except for the temperature factor which was studied at only two levels (see below). This experimental design involved running 38 experiments. The factors studied and the levels chosen are given below.

- *Concentration.* Low, medium and high levels were 0.1 mM, 2.5 mM and 61 mM respectively. The low value corresponds to a value below the critical micelle concentration (cmc) for most solution conditions, the medium to a value approx equal to the cmc and the high value to a value higher than the cmc. It is important to note that the cmc is dependent on solution conditions: the lowest value being for the solution with the highest salt concentration, for which the cmc is 0.1 mM.
- *pH.* Low, medium and high levels were 4, 6 and 8 respectively. The values of pH chosen were above the pK_a value so that the AOT was ionized and therefore charged.
- *Temperature.* Two temperature levels were included to allow for non-controlled (1) and controlled (2) temperatures. In the model these levels were assigned a numerical value of 1 and 2 respectively. Experiments carried out at controlled temperatures were performed by placing the beaker in a vessel full of water, where water was flowing in and out continuously, thus maintaining the temperature almost constant (the increment of temperature was <4 °C). For the experiments carried out under non-controlled temperatures, the final temperature varied depending, mainly, on the time of stirring and concentration of the surfactant.
- *Salt concentration (NaCl).* Low, medium and high levels were 0 M, 0.07 M and 0.14 M respectively. Salt concentration was chosen as a variable as it will effect electrostatic interactions between surfactant molecules.
- *Time of stirring.* Low, medium and high levels were 4, 10 and 16 min, respectively. These values were chosen according to results obtained in preliminary experiments which showed that if the time of stirring was less than 4 min, the stability of the aphrons was difficult to determine for low concentrations of surfactant.

Speed was fixed to 8000 rpm as explained in Section 3 for measurement of power.

3. Results and discussion

3.1. *Power measurements*

In order to determine the power consumption during mixing for a gas liquid dispersion by the method described above, several assumptions were made.

- The physical properties (density and viscosity) of water and the aphronic solution were assumed to have the same values.
- The system was totally insulated and no heat losses occurred.
- All the energy transmitted by the stirrer was dissipated as heat into the fluid and other parts of the system (baffles, beaker and shaft).

It was observed that the measured power number for five different speeds was constant (see Table 2), as expected for the turbulent region.

From the power measurement study (Table 2), it can be seen that power decreases as the gas hold-up (ϵ) increases. Aphrons created at stirrer speeds of 4100 rpm are very unstable. Indeed, for the lowest and medium concentrations of surfactant no aphrons were detected. Conversely, when the highest concentration of surfactant studied was used to create aphrons at 4100 rpm, formation of aphrons was observed. Therefore, the critical speed for aphron formation depends significantly on the surfactant concentration, which in turn affects the gas hold-up (see gas hold-up measurements later in this section) and hence the power consumption (see Eq. (4)). The energy needed to form aphrons can be calculated from a knowledge of the gas hold-up of the dispersion. From the results in Table 2, it can be concluded that the minimum energy needed to create aphrons when using a low and

Table 2
Results from the power measurement for three different solutions of surfactant. (i) High: AOT 34 mM in an acetate buffer 0.1 M, pH = 4. (ii) Medium: AOT 2.5 mM in phosphate buffer 0.025 M, pH = 6. (iii) Low: AOT 0.1 mM in acetate buffer 0.025 M, pH = 4

N (rpm)	dT/dt ($K\ s^{-1}$)	N_p	C_{sf}	ϵ	P (W)	τ (s)
4100	0.002	1.24	high	0.018	9.25	30
			medium	[a]	9.41	[a]
			low	[a]	9.41	[a]
5100	0.006	1.28	high	0.085	17.38	180
			medium	0.09	17.29	54
			low	0.06	17.86	40
6000	0.009	1.2	high	0.31	25.29	240
			medium	0.1	33.35	80
			low	0.09	33.35	45
7700	0.014	1.2	high	0.58	25.69	758
			medium	0.37	38.53	200
			low	0.25	45.87	60
10 000	0.019	1.26	high	0.65	49.38	930
			medium	0.63	55.4	405
			low	0.4	64.85	66

[a] Not detectable.

medium concentration of surfactant and solution conditions described above is 45 kW m^{-3}.

As described above, power input is a useful characterisation variable, however, it would be very difficult to design experiments with power held constant. Therefore in subsequent experiments, speed rather than power input was the measured variable (power can be calculated from Eq. (4) for each of the cases studied). In order to select the speeds at which experiments were to be performed, stability for five speeds was plotted at three different concentrations (see Fig. 3). For two of the conditions, i.e. "low" and "medium" concentrations of surfactant, the resulting stability of CGAs reaches a maximum at 8000 rpm, therefore this speed was chosen for the subsequent set of statistically designed experiments (in the case of "high" concentration of surfactant, stability was levelling off but had not yet reached a maximum). Power follows a similar trend; this is shown in Fig. 4 for the high concentration of surfactant.

3.2. Stability and gas hold-up measurements

Data for τ and ϵ obtained in these experiments were analysed using a linear regression procedure within the statistical computer package SAS [27]. Polynomial models which relate τ and ϵ to the process variables through linear quantitative and interaction coefficients were fitted. The resulting equations predict the variation of stability of the aphrons (τ) and gas hold-up (ϵ), as functions of the selected factors and their interactions. The full set of experimental data for τ and ϵ are shown in Table 3.

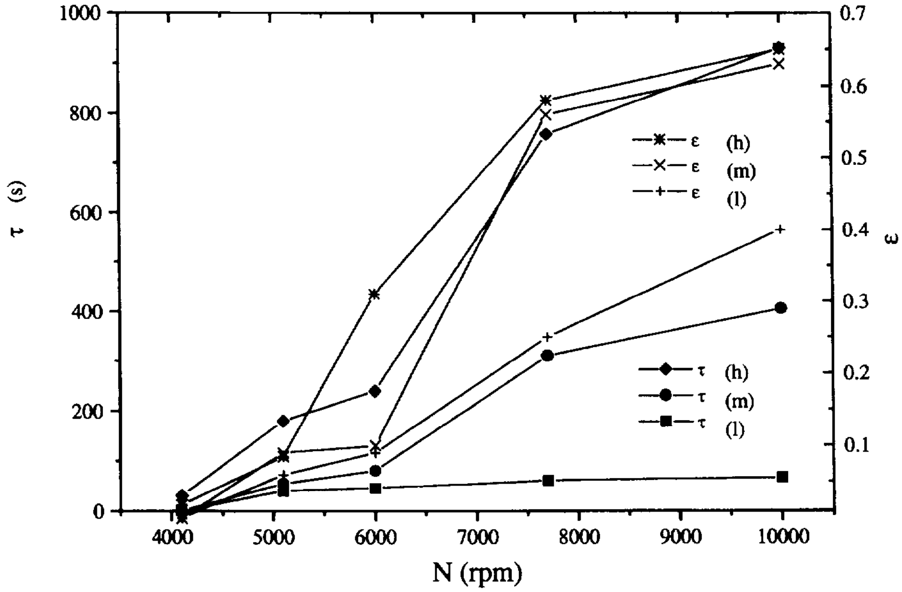

Fig. 3. Stability (τ) and gas hold-up (ϵ) measurements for the three different concentrations of surfactant (l, m and h, corresponding to low, medium and high respectively) at the five stirrer speeds studied.

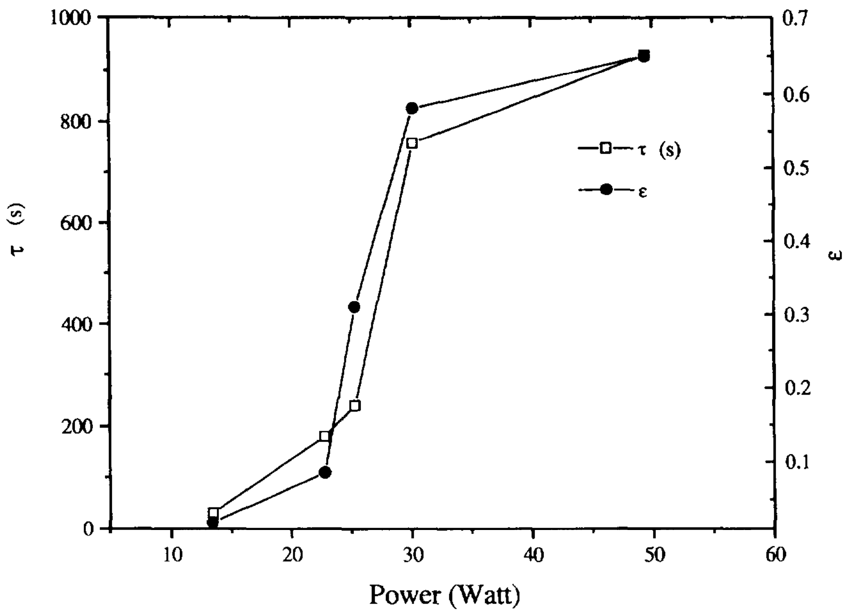

Fig. 4. Stability (τ) and gas hold-up (ε) measurements for the corresponding power values of the five speeds studied for the "high" concentration surfactant solution.

3.2.1. *Stability (τ)*

The statistical analysis of the data using analysis of variance (see, for example, Ref. [28]) showed that the concentrations of surfactant and salt are the main factors affecting the stability of CGAs (i.e. significant at 0.01% level). Time, pH and temperature had lesser effects, but the following interactions were significant $\ln(C_{sf})\times\text{pH}$, $\ln(C_{sf})\times C_{sl}$, $\ln(C_{sf})\times T$ and $t\times T$. The analysis of variance is given in Table 4. Having determined the most significant parameters, a statistical model was determined for the data. In order to select the best fitting model, several transformations on the response (stability) were assessed. The transformations used were: square root, reciprocal, reciprocal square root and natural logarithm. The natural logarithm of the response gave the best fit (R-square = 0.95 and F value = 48.23 which is significant at the 0.1% level). The best fitting model was:

$$\begin{aligned}\ln(\tau) &= 5.9443 + 0.0004\text{pH} - 10.9275C_{sl} + 0.4744\ \ln C_{sf}\\ &\quad - 0.0766t - 0.4850T + 35.7518C_{sl}^2\\ &\quad - 0.0164(\ln C_{sf})\text{pH} - 0.4946(\ln C_{sf})C_{sl}\\ &\quad - 0.0579(\ln C_{sf})T + 0.0554tT\end{aligned}$$

The numerical coefficients have dimensions such that the overall equation is dimensionally consistent, i.e. if one of the process factors is measured on a different scale, the corresponding coefficient would change so that the overall equation is not affected.

The above model is obviously empirical, but it does highlight the relative importance of individual and interactive terms. The effect of each of the different parameters is considered separately below.

- *Concentration of surfactant* (C_{sf}). Higher concentrations of surfactant give higher stability. The repulsive forces between aphrons are likely to increase as the concentration of surfactant increases either in the surfactant shell or in the bulk liquid phase; this will lead to more stable dispersions.
- *Concentration of salt* (C_{sl}). The decreasing stability of the aphrons with increasing concentration of salt can be explained on the basis of electrostatic interactions which will play an important role in the stability of this type of dispersion. There will be repulsive electrostatic interactions between the negatively charged aphrons and these interactions will stabilise the system. The addition of salts or electrolytes will have an effect on the electrostatic interactions. Increasing the concentration of salt (NaCl) will cause these interactions to be suppressed leading to the formation of a less stable dispersion.
- *Interaction between salt concentration and surfactant concentration* (see Fig. 5). At low concentrations of surfactant, salt concentration has little effect on the stability, but as surfactant concentration increases, the effect of the salt concentration also increases. Therefore the maximum stability is obtained at the highest concentration of surfactant and lowest concentration of salt.
- *pH*. pH does not show a significant effect on CGA stability. A possible reason for this is that for the pH range in which the experiments were performed, the surfactant in solution was present mainly in the ionic form ($\text{p}K_a = 2.9$). The electrostatic interactions resulting from the presence of the ionic form of the surfactant will contribute to the stabilisation of the aphrons. The degree of ionisation, which changes with pH does not appear to affect the stability of the system. This is consistent with observations reported by Subramaniam et al. [4] and Save and Pangarkar [11]. Amiri and Woodburn [6] reported a significant effect of pH on the stability but this was due to changes in ionic

Table 3
Statistical design for the characterisation of CGAs

Exp.	C_{sf} (mM)	pH	C_{sl} (M)	t (min)	T	τ (s)	ϵ	T_f (°C)
1	0.1	4	0	4	1	90	0.17	29
2	0.1	4	0	16	2	107	0.32	20
3	0.1	4	0.14	4	2	45	0.17	22
4	0.1	4	0.14	16	1	30	0.12	43
5	0.1	8	0	4	2	90	0.26	23
6	0.1	8	0	16	1	68	0.32	41
7	0.1	8	0.14	4	1	60	0.2	27
8	0.1	8	0.14	16	2	75	0.28	20
9	61	4	0	4	2	420	0.5	23
10	61	4	0	16	1	840	0.57	35.5
11	61	4	0.14	4	1	290	0.44	25.5
12	61	4	0.14	16	2	306	0.46	19
13	61	8	0	4	1	594	0.53	25.4
14	61	8	0	16	2	710	0.57	18
15	61	8	0.14	4	2	180	0.4	21
16	61	8	0.14	16	1	163	0.5	34
17	0.1	6	0.07	10	2	60	0.27	36
18	61	6	0.07	10	1	249	0.52	30
19	0.1	6	0.07	10	2	80	0.23	24
20	61	6	0.07	10	2	220	0.51	24
21	2.5	4	0.07	10	1	160	0.49	32
22	2.5	8	0.07	10	1	152	0.51	32
23	2.5	4	0.07	10	2	170	0.5	19
24	2.5	8	0.07	10	2	120	0.49	21
25	2.5	6	0	10	1	372	0.61	32
26	2.5	6	0.14	10	1	115	0.36	36
27	2.5	6	0	10	2	375	0.59	23.4
28	2.5	6	0.14	10	2	90	0.3	21
29	2.5	6	0.07	4	1	150	0.36	27
30	2.5	6	0.07	16	1	140	0.53	38
31	2.5	6	0.07	4	2	110	0.25	24
32	2.5	6	0.07	16	2	150	0.51	25
33	2.5	6	0.07	10	1	128	0.47	34
34	2.5	6	0.07	10	1	140	0.46	34
35	2.5	6	0.07	10	1	145	0.49	33
36	2.5	6	0.07	10	2	133	0.48	24
37	2.5	6	0.07	10	2	140	0.46	19
38	2.5	6	0.07	10	2	150	0.47	25

C_{sf}, C_{sl}, T, t and ϵ correspond to concentration of surfactant, concentration of salt (NaCl), temperature, time and gas hold-up (T_f is the temperature measured once stopped stirring).

Table 4
Analysis of variance for the parameters within the stability model

Source	MSE [a]	F value	Pr > F
$\ln C_{sf}$	13.332	341.85	0.0001
t	0.0302	0.77	0.3856
pH	0.0167	0.43	0.5182
C_{sl}	3.977	101.97	0.0001
T	0.002	0.05	0.8055
$(C_{sl})^2$	0.291	7.46	0.0109
$\ln C_{sf} \times$ pH	0.176	4.51	0.424
$\ln C_{sf} \times C_{sl}$	0.197	5.05	0.0326
$\ln C_{sf} \times T$	0.173	4.44	0.0445
$t \times T$	0.552	14.15	0.0008
Error	0.039		

[a] The mean square error (MSE) corresponding to this model is 0.039.

strength. Thus stability of CGAs does not depend significantly on pH but it does depend on ionic strength.

- *Interaction between pH and surfactant concentrations.* pH interacts with concentration (see Fig. 6), so that pH only has an important effect at high concentrations of surfactant (maximum stability is obtained at pH = 4 and 61 mM AOT).
- *Temperature and time.* Temperature is included in the model, having a value of either 1 or 2, corresponding to controlled and non-controlled temperature (see Section 2.2). When the temperature is not controlled it will vary depending on the levels of the other parameters. Neither temperature or time have a significant effect on stability.
- *Interaction between time and temperature.* There is a significant interactive effect of time and temperature on sta-

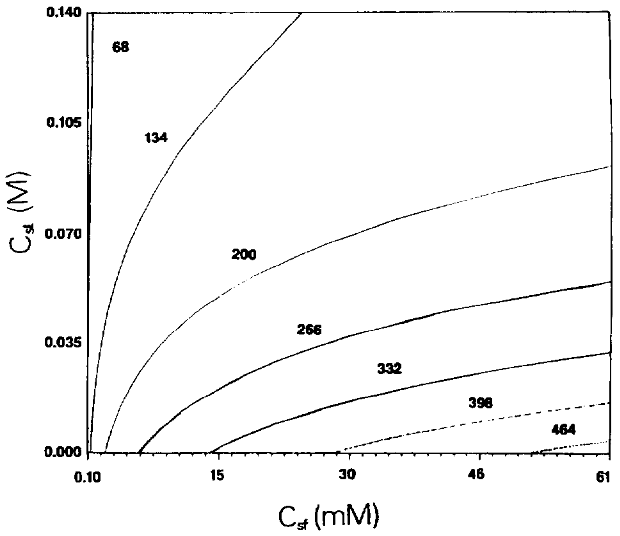

Fig. 5. Contour plot of the predicted values of stability (τ) (values on top of curves) for concentration of surfactant (C_{sf}) and concentration of salt (C_{sl}) as independent variables at pH = 6, $t=4$ min and $T=2$ (controlled temperature).

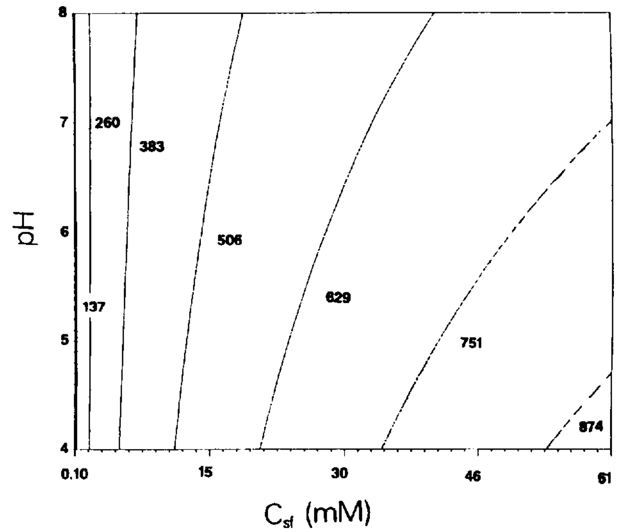

Fig. 6. Contour plot of the predicted values of stability (τ) (values on top of curves) for pH and concentration of surfactant (C_{sf}) as independent variables at $C_{sl}=0$ M, $t=4$ min and $T=1$ (non-controlled temperature).

Table 5
Analysis of variance for the parameters within the gas hold-up model

Source	MSE [a]	F value	Pr > F
$\ln C_{sf}$	0.355	118.33	0.0001
t	0.0405	13.50	0.0008
C_{sl}	0.073	24.33	0.0001
$(\ln C_{sf})^2$	0.086	28.67	0.0001
Error	0.003		

[a] The mean square error (MSE) corresponding to this model is 0.003.

bility. The highest stability is obtained at longer time of stirring under controlled temperature (below or equal to 25 °C) or at shorter time of stirring under non-controlled temperatures. Thus the effect of temperature is negative at long times of stirring so it needs to be controlled, but positive at short times of stirring when it does not need to be controlled.

- *Interaction between temperature and concentration of surfactant.* It was observed that the rate of temperature rise was greater for low concentration of surfactant, thus low concentrations of surfactant appear to be more sensitive to the temperature effect.

3.2.2. Gas hold-up (ϵ)

The statistical analysis of the data showed that surfactant concentration, salt concentration and time of stirring are the main factors affecting the gas hold-up. The analysis of variance is given in Table 5. ϵ shows no significant dependence on pH and temperature in the range of studied values. The best fitting model was (R-square = 0.85, $F=46.82$ which is significant at a 0.1% level):

$$\epsilon = 0.403 + 0.058\ln C_{sf} + 0.008t - 0.864C_{sl} - 0.009(\ln C_{sf})^2$$

As surfactant concentration increases, gas hold-up increases in a quadratic manner; gas hold-up tends to a maximum at about 25 mM surfactant concentration (see Fig. 7) (the equation predicts a maximum at about 25 although it is already very close to the maximum by about 12 mM). The higher the concentration of salt, the lower the gas hold-up (see Fig. 7). Finally, as time of stirring increases, gas hold-up also increases (see Fig. 7).

Fig. 8 shows a plot of stability versus gas hold-up. As stability increases, gas hold-up can be seen to increase, tailing of to a value between 0.5 and 0.6 for conditions giving high aphron stability (see Fig. 8). There are a cluster of points representing high gas hold-ups at relatively low stabilities, these correspond to points with medium or high surfactant concentration and medium or high salt concentration. As surfactant concentration increases, the volume of aphrons increases, but as the salt concentration increases this causes a shielding of electrostatic repulsions between the surfactant molecules and results in a less stable dispersion.

4. Conclusions

Power consumption is an important parameter of aphron stability. For the aphrons to be formed a certain amount of energy needs to be transmitted to the fluid. In this study, the minimum energy required to create colloidal gas aphrons for one set of solution conditions was determined. Also, stability and gas hold-up were measured for a range of impeller speeds for CGAs created from one ionic surfactant, AOT; this allowed selection of the optimum speed for subsequent characterisation studies.

The characterisation of CGAs was carried out by studying the effect of process parameters (pH, salt and surfactant concentrations, temperature and time) on stability and gas hold-up of CGA dispersions. Empirical statistical models for the dependence of τ and ϵ on process parameters are presented.

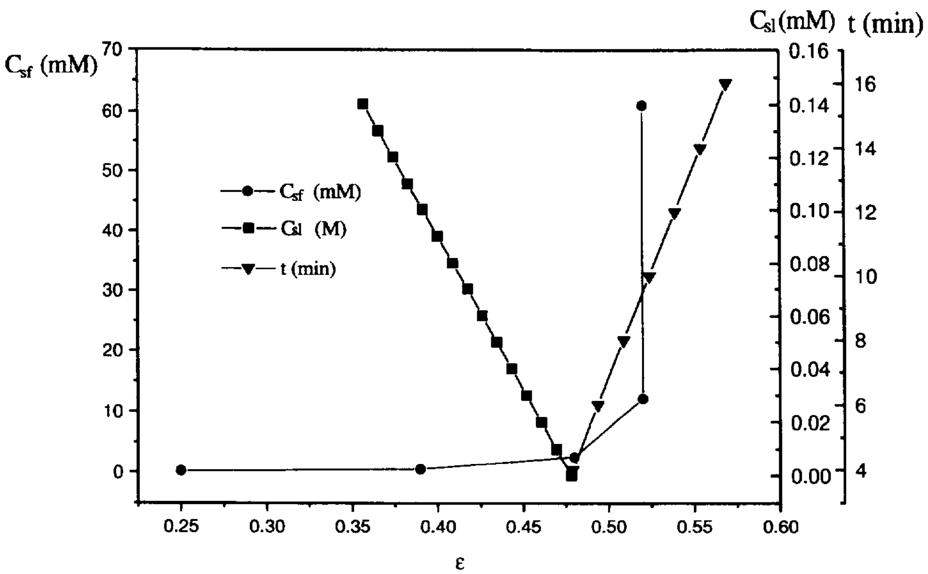

Fig. 7. Combined plot to show effect on the predicted gas hold-up (ε) of: concentration of surfactant (C_{sf}) at $C_{sl}=0$ M and $t=4$ min; time (t) at $C_{sf}=2.5$ mM and $C_{sl}=0$ M; concentration of salt (C_{sl}), for $C_{sf}=2.5$ mM and $t=4$ min.

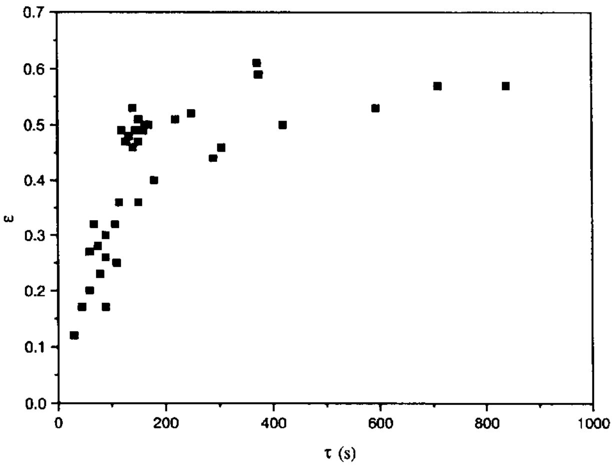

Fig. 8. Plot of measured stability values (τ) against measured gas hold-up values (ε).

Maximum stabilities are obtained at high concentration of surfactant (above the cmc value) at any of the pH values. Addition of salt, i.e. high ionic strength, causes a decrease of stability of CGAs, which implies, as would be expected, that electrostatic forces play an important role in the formation and stability of CGAs. Time, temperature and pH did not affect stability independently, however, as significant interactive effects between (i) surfactant concentration and salt concentration, (ii) pH and surfactant concentration, (iii) temperature and time, and (iv) temperature and concentration of surfactant, were identified and discussed.

Gas hold-up was found to depend on time, concentration of surfactant and concentration of salt.

On the basis of the results of this study, further research is now under way to assess the potential of using CGAs created from AOT for protein recovery.

5. Nomenclature

a	anionic surfactant
AOT	sodium bis-(2-ethyl hexyl)sulfosuccinate
BDHA	benzyldimethyl-n-hexadecylammonium chloride
c	cationic surfactant
C/D	impeller diameter to impeller clearance ratio
cmc	critical micellar concentration
CPB	cetylpyridinium bromide
CPC	cetylpyridium chloride
C_{sf}	concentration of surfactant (mM)
C_{sl}	concentration of salt (M)
D	diameter of the impeller (m)
DMDSAC	dimethyl distearyl ammonium chloride
dT/dt	change of temperature with the time (°C s^{-1})
DTAB	dodecyltrimethyl ammonium bromide
DTAC	dodecyltrimethyl ammonium chloride
EHDA	ethylhexadecyldimethylammonium bromide
F value	F value from analysis of variance of the data
HTAB-CTAB	hexadecyltrimethyammonium bromide
HTAC	hexadecyltrimethyammonium chloride
LAEO	lauryl alcohol-ethylene oxide
Lux™ flakes	[4]
$(mC_p)_i$	sum of products of mass and specific heat factor of the different parts of the mixing system (J °C^{-1})
MS	mean square
MSE	mean square error
N	rotational speed of the impeller (s^{-1})

ni	non-ionic surfactant
N_p	power number
P	power supplied to the agitator (W)
$\mathrm{p}K_\mathrm{a}$	negative logarithm of the acidic dissociation constant
$\mathrm{p}K_\mathrm{b}$	negative logarithm of the basic dissociation constant
Pr	probability from analysis of variance of the data
R-square	regression constant from analysis of variance of the data
Re	Reynolds number
rpm	revolution per minute
SDBS	sodium dodecylbenzene sulfonate
SDS	sodium dodecyl sulphate
SLS	sodium lauryl sulphase
T	temperature factor in the statistical design
t	time of stirring (min)
TBP	trybutyl phosphate
Tergital	polyoxyethene tri-glyceride alcohol
T_f	measured temperature after stopping stirring (°C)
TTAB	tetradecyltrimethyl ammonium bromide
V_D	dispersed phase velocity ($\mathrm{m\ s^{-1}}$)

5.1. Greek letters

ρ	density of the fluid ($\mathrm{kg\ m^{-3}}$)
μ	viscosity of the fluid ($\mathrm{kg\ m^{-1}\ s^{-1}}$)
τ	time for half the liquid to collapse (s)
ϵ	gas hold-up

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References

[1] F. Sebba, *J. Colloid Interface Sci.*, *35* (4) (1971) 643.
[2] F. Sebba, *Foams and biliquid foams–aphrons*, Wiley, Chichester, 1987, Chap. 5.
[3] S.V. Save, V.G. Pangarkar and S.V. Kumar, *Biotechnol. Bioeng.*, *41* (1993) 72.
[4] M.B. Subramaniam, N. Blakebrough and M.A. Hashim, *J. Chem. Technol. Biotechnol.*, *48* (1990) 41.
[5] K. Matsushita, A.H. Mollah, D.C. Stuckey, C. Del Cerro and A.I. Bailey, *Colloids Surf.*, *69* (1992) 65.
[6] M.C. Amiri and E.T. Woodburn, *Trans. Ins. Chem. Eng.*, *68* (A) (1990) 154.
[7] S. Ciriello, S.M. Barnett and F.J. DeLuise, *Sep. Sci. Technol.*, *17* (1982) 521.
[8] M. Caballero, R. Cela and J.A. Perez-Bustamante, *Sep. Sci. Technol.*, *24* (9&10) (1989) 629.
[9] D. Roy, K.T. Valsaraj and S.A. Kottai, *Sep. Sci. Technol.*, *25* (5) (1992) 573.
[10] P.G. Chapalkar, K.T. Valsaraj and D. Roy, *Sep. Sci. Technol.*, *28* (6) (1994) 1287.
[11] S.V. Save and V.G. Pangarkar, *Chem. Eng. Commun.*, *127* (1993) 35.
[12] D.A. Attwood and A.T. Florence, *Surfactant Systems: Their Chemistry, Pharmacy and Biology*, Chapman and Hall, London, 1983, p. 629.
[13] A. Helenius and K. Simons, *Biochim. Biophis. Acta*, *415* (1975) 29.
[14] S. Lapanje, *Physicochemical Aspects of Protein Denaturation*, Wiley, New York, 1978, pp. 156, 304.
[15] Q. Chang, H. Liu and J. Chen, *Enzyme Microbiol. Technol.*, *16* (1994) 970.
[16] S.R. Dungan, T. Bausch, T.A. Hatton, P. Plucinski and W. Nitsch, *Colloid Interface Sci.*, *145* (1991) 33.
[17] R.B. Gupta, C.J. Han and K.P. Johnston, *Biotechnol. Bioeng.*, *44* (1994) 830.
[18] K. Takeda, K. Harada, K. Yamaguchi and Y. Moriyama, *J. Colloid Interface Sci.*, *164* (1994) 382.
[19] F. Sebba, *Chem. Ind.*, *3* (1985) 91.
[20] R.W. Ramette, *Chemical Equilibrium and Analysis*, Addison–Wesley, Reading, MA, 1981, p. 363.
[21] J.H. Rushton, E.W. Costich and H.J. Everett, *Chem. Eng. Progr.*, *46* (1950) 467.
[22] B. Atkinson and F. Marituna, *Biochemical Engineering and Biotechnology Handbook*, 2nd edn., Stockton Press, Basingstoke, 1991, Chap. 11 & 12.
[23] G.A. Hughmark, *Ind. Eng. Chem, Process. Des. Dev.*, *19* (1980) 638.
[24] B.J. Michel and S.A. Miller, *Am. Inst.Chem. Eng.*, *8* (1962) 262.
[25] K. Niranjan, personal communication, 1995.
[26] R.H. Myers and P.C. Montgomery, *Response Surface Methodology: Process and Product Optimisation Using Designed Experiments*, Wiley, New York, 1995.
[27] SAS Institute Inc., *SAS/STAT™ Users guide*, Release 6.04, SAS Institute Inc., Cary, NC, 1987.
[28] R. Mead, R.N. Curnow and A.M. Hasted, *Statistical Methods in Agriculture and Experimental Biology*, 2nd edn., Chapman and Hall, London, 1993.
[29] D.A. Wallis, D.L. Michelsel, F. Sebba, J.K. Carpenter, *Biotechnol. Bioeng. Symp.*, *15* (1995) 401.
[30] P.G. Chapalkar, K.T. Valsaraj and D. Roy, *Sep. Sci. Technol.*, *29* (7) (1994).
[31] S.V. Save and V.G. Pangarkar, *Chem. Technol. Biotech.*, *62* (2) (1995) 192.