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Journal of Chromatography A, 668 (1994) 85–94

JOURNAL OF CHROMATOGRAPHY A

Continuous separation of whey proteins with aqueous two-phase systems in a Graesser contactor

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Abstract

The isolation and purification of α-lactalbumin and β-lactoglobulin from whey can be conducted continuously in a Graesser contactor using an aqueous two-phase system based on polyethylene glycol and potassium phosphate. Processing of more than 600 g of whey proteins per day was performed in the apparatus described. β-Lactoglobulin partitioned almost quantitatively into the salt-rich bottom phase, and α-lactalbumin was enriched in the top-phase of the system applied. The residence time distribution and the fractional hold-up as a hydrodynamic parameter were determined as the basis for a qualitative prediction of the extraction efficiency of the process described.

1. Introduction

The application of aqueous two-phase systems to the large-scale purification of proteins implies the consideration of continuous counter-current operation in the design of such a process [1]. This mode of operation may reduce fixed and variable costs, increase space–time yield, maintain high yields of labile proteins and allow process automatization and the continuous recycling of process chemicals [2,3]. Single or multi-stage equipment of conventional liquid–liquid extraction can be applied for extraction using aqueous two-phase systems (ATPS). Mixer–settler designs have been successfully operated continuously, offering one theoretical stage for protein purification [4]. Multi-stage installations were described by use of spray, York–Scheibel, packed bed or pulsed sieve plate columns [1,5,6].

Another tool for liquid–liquid extraction is the Graesser raining bucket contactor (see Fig. 1). Being patented in 1962 [7] it offered a very promising technology for the treatment of phase systems with low interfacial tension, low density difference and a high tendency of emulsification. The contactor has been used previously in many

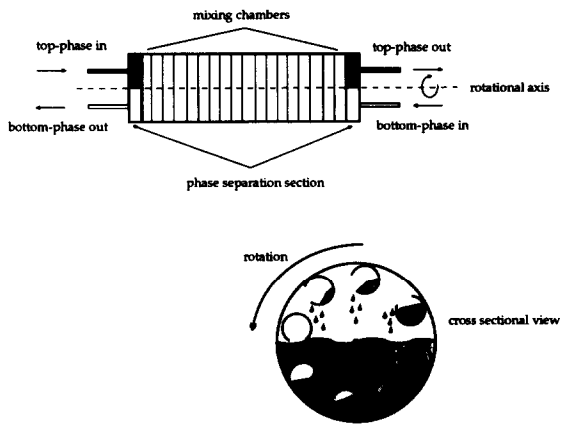

Fig. 1. Schematic view of the Graesser contactor (after ref. 8, compare also ref. 10 and 11).

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0021-9673/94/$07.00
SSDI 0021-9673(94)00004-S

industrial processes, *e.g.* pyrethrum refining, purification of herbicides or dearomatization of naphtas [8].

The Graesser contactor consists of a cylindrical glass vessel and is operated horizontally, two immiscible phases being introduced in counter-current mode from both ends of the cylinder. On the horizontal axis a single rotor performs mixing by a series of cylindrical buckets, mounted around the axis and partly open in the direction of mixing, that are separated by circular baffles. Counter-current flow occurs by the annular gap between the baffles and the contactor walls. Under operation the interface level of the immiscible liquids is controlled at the centre line of the vessel. Gentle mixing is provided by the movement of the buckets, transporting portions of each phase to the other phase, releasing them at the top or bottom of its circular movement and causing the liquid to cascade through the opposing phase back to the interface. Phase separation occurs in the end zones on the right and left of the cylindrical vessel. Fig. 1 schematically shows the operating principle of the Graesser contactor. For details of construction and operational principles see also ref. [8].

This special design leads to some specific hydrodynamic characteristics of the Graesser contactor, which makes it especially suitable for aqueous two-phase systems:

(i) The gentle mixing avoids emulsification, as the mean drop size is kept large compared to other continuously operated extractors.

(ii) The drop rise and fall velocities are high, thus a fast interface renewal is achieved leading to good mass transfer characteristics of the extraction system.

(iii) Maintaining the interface level at the equatorial centre of the glass vessel and keeping the two phases thoroughly separated by the horizontal mode of operation, helps processing systems with low density difference.

(iv) The separation of the vessel into different compartments reduces axial mixing, a problem in vertically operated column extractors [1].

(v) Good mass transfer in spite of very gentle mixing makes this extractor suitable for sensible products, *e.g.* proteins.

In conclusion, the Graesser raining bucket contactor seems to fit the demands very well of continuously operated purification of proteins using ATPS. This was predicted already by Hustedt *et al.* [9], who used a Graesser contactor for continuous operation with ATPS, simulating protein purification by separation of two dyes.

Fundamental research of the special features of the Graesser contactor has not been performed as intensively as with other extraction systems. Sheikh *et al.* [10] and Wang *et al.* [11] studied axial mixing as well as back mixing in a bench-scale Graesser contactor (75 cm length, 15 cm diameter) with a kerosene–butylamine–water system. They found, that axial mixing behaviour was considerably influenced by the phase properties. The authors proposed slow agitation rates and a high ratio of solvent feed flow for successful extraction in this type of contactor. Al-Hemiri and Kareem [12] described the drop size distribution in a Graesser contactor. By photographic techniques they determined a decreasing mean drop size along the horizontal axis reaching a constant drop size distribution towards the outlet of the contactor using several water–organic solvent systems. To our knowledge, these are the only reports on the basic principles of the Graesser contactor.

Studies on the applications of the Graesser contactor to protein purification using ATPS calls for a real "bulk product" as a model. First, to keep the process close to reality, and secondly to minimise the cost of raw material as much as possible, as the high capacity of even bench-scale contactors demands large amounts of process chemicals and proteins to obtain steady-state data in a continuous mode of operation. A group of proteins, that has steadily gained interest, are the whey proteins. The world production in the 80's was nearly 90 million tons of whey per year [13], a considerable amount of which is presently discarded, while the rest is used in lactose production or in animal feed. The average protein content in bovine whey is 6–9 g/l, so there is a large potential of unused protein in whey. Formulations for infant nutrition are designed on the basis of whey proteins. A fact, that should be considered when using whey directly for infant

formulae is the elevated content of β-lactoglobulin (β-Lg) in bovine milk compared to mammalian milk [14]. β-Lg is known as the major allergenic milk protein [15], therefore in processing whey proteins for infant nutrition the reduction of the β-Lg content, while retaining the concentrations of the other milk proteins, is important. Up to now several procedures for the isolation of β-Lg from the rest of the whey proteins have been described, with the major aim to separate α-lactalbumin (α-La) from β-Lg. Kaneko *et al.* [14] described the importance of retaining immunologic properties of whey, *e.g.* keeping the immunoglobulin and lactoferrin concentration in the formulations close to the original values. The methods proposed are precipitation of β-Lg with $FeCl_3$ [14], precipitation of β-Lg with polyphosphate [16], ion-exchange chromatography [13], sub-unit exchange chromatography [17], salting out [18] and thermal methods [19]. Recently, a polyethylene glycol (PEG) –phosphate aqueous two-phase system was described, which separated the two important whey proteins by the partitioning of β-Lg predominantly into the salt-rich bottom-phase and by the enrichment of α-La in the top phase [20].

Based on these literature results, we studied the separation of α-La and β-Lg from bovine whey as an example of a bulk product, that can be processed in a Graesser contactor using ATPS. On the one hand whey is an easy accessible and cheap source of proteins, and on the other hand there is a need for large-scale processes in whey protein purification that do not require intense pretreatment of the protein source. Whey contains considerable amounts of salts, so many purification methods cannot be applied directly. ATPS are salt-tolerant and should be well suited for direct processing of whey proteins.

The first purpose of our examinations was to evaluate the basic hydrodynamic features of the Graesser contactor with ATPS. As Wang *et al.* [11] found the contactor hydrodynamics to be dependent on the phase composition, their results with the kerosene–dibutylamine–water system cannot be transferred to ATPS; therefore experimental effort was necessary in this respect. Based on the results of Chen [20], a phase system was chosen, that allowed separation of α-La and β-Lg from whey in batch experiments, and this system was utilized for the continuous experiments with the Graesser contactor. The results of these experiments were compared to the qualitative predictions made from the hydrodynamic characterization of the contactor.

2. Experimental

2.1. Materials

Pure α-Lactalbumin and β-Lactoglobulin were obtained from Sigma (Deisenhofen, Germany), PEG 400, 600, 1550, 4000 and 12 000 from Hüls AG (Marl, Germany), potassium phosphate from B.K. Landenburg (Landenburg, Germany), cibacron blue from Sigma and cheese whey powder (whey protein index, Nx6.38, 26%) was a generous gift from Meggle Milchindustrie GmbH (Wasserburg, Germany).

2.2. Partition experiments

Aqueous two-phase systems of PEG–salt (potassium phosphate) were prepared from stock solutions of PEG (50%, w/w) and salt (pH 7.0, 30% w/w) for the experiments with pure α-La and β-Lg. The required mass of the phase system was obtained by weighing of the solutions in centrifuge tubes on a 10-g scale. Amounts of 5 mg of protein were added followed by adjustment with water until the total mass was 10 g. The systems were mixed by inversion (5 min) and separated by centrifugation for 5 min at 4000 *g*. Aliquots of the top and bottom phase were withdrawn for protein determination.

The influence of the salt content and different PEG molecular masses on the phase volume relation was observed. By this criterion the use of PEG 400, 600, 4000, 12 000 were considered unfavourable.

For the large-scale experiments with whey different quantities of PEG (1550), potassium phosphate (predetermined amounts of mono- and dihydrogenphosphate were adjusted to give

Table 1
Buffer systems investigated

	Buffer A (pH = 7)	Buffer B
1	0.02 *M* Piperazine	1 *M* NaCl in A
2	0.02 *M* Bis-Tris	1 *M* NaCl in A
3	0.02 *M* Bis-Tris	1 *M* NaAc in A
4	0.01 *M* Potassium phosphate	1 *M* NaCl in A

pH 7.0) and water were weighed. The compounds were gently stirred for about 2 h and the system was allowed to settle overnight in a 300-l recipient. Whey was dissolved in the bottom phase obtained at a concentration of 15% w/w and filtered (press filter Seitz Enziger Noll, filter sheet 4946 Seitz-Filterwerke, Germany) to remove undissolved material. The permeate was used as the salt-rich phase for the experiments in the Graesser contactor. All experiments were carried out at room temperature.

2.3. Determination of α-La and β-Lg concentration

A chromatographic method was developed for the simultaneous determination of both proteins. A calibration curve was constructed with an aqueous solution of α-La–β-Lg (1:2.5 average whey concentration) in a Pharmacia fast protein liquid chromatography (FPLC) system. The FPLC system was equipped with a Mono Q HR 5/5 anion-exchange column [21] and operated at 1.5–2 MPa pressure and 1.0 ml/min flow-rate. The sample size at ambient temperature was 1 ml and the absorbance of the eluate was measured at 280 nm.

Before injection the samples were desalted by gel filtration (PD-10 Sephadex column-Pharmacia) and filtered (0.2 μm).

The buffer systems given in Table 1 were investigated. System No. 4 allowed the best separation with the following stepwise gradient shown in Table 2.

Table 2
Stepwise gradient used in this study

	Time (min)	Buffer B (%)
Gradient	0–20	10
Step	20–25	10
Step	25–40	30
Gradient	40–50	100

The Mono Q column should be cleaned after each run with 2 *M* NaCl, 1 *M* NaOH, 1 *M* HCl and 2 *M* NaCl and equilibrated with 100% buffer B. This procedure is necessary to obtain reproducible results.

2.4. Experiments in the Graesser contactor

The 100 cm × 10 cm I.D. Graesser contactor employed was manufactured by QVF Glastechnik. (Wiesbaden, Germany). The extractor consists of a glass cylinder with an internal rotor containing 36 compartments. Each of them is formatted for 6 buckets. A 0.25-kW motor is used to drive the rotor, the speed is set via a variable gear box. The connection between rotor and motor is propitiated by gears and linked chains. The direction of rotor movement had been reversed to obtain more stable operational conditions [22]. The interface level is kept at the centre of the contactor by a hydrostatic control.

In all the experiments the salt-rich phase (permeate of filtration) was the heavy (bottom) and the PEG-rich phase was the light (top) phase. The upper and lower phases were pumped (peristaltic pump, Verder, Düsseldorf, Germany) into the extractor above and below the rotor axis at opposite ends of the contactor.

2.5. Operational conditions

Rotor speeds of 3, 5, 7 and 9 rpm and top–bottom phase flow-rate ratios of 2:1, 1.2:1, 0.8:1 and 0.5:1 were investigated during the residence time and hold-up experiments. For the mass transfer experiments these values were 2 and 5 rpm and 2:1 and 1.5:1. Correspondent phase flow-rates were 36, 66 and 88 ml/min for the PEG-rich phase and 44 and 70 ml/min for the salt-rich phase.

2.6. Residence time distribution experiments

The PEG phase residence time distribution was determined by the pulse injection method. A pulse of cibacron blue was used as the dye (15

mg/ml, 1 ml, injection time 2 s) and was injected into the system at the PEG phase inlet. The tracer concentration was determined at the PEG phase outlet by measuring the absorption at 595 nm (spectrophotometer LKB Biochrom 4049).

The applicability of the dispersion model for this real situation was examined. The basic differential equation which represents this model in its dimensionless form [23]

$$\frac{dc}{d\theta} = \left(\frac{E_A}{UL}\right) \cdot \frac{d_2 c}{dz^2} - \frac{dc}{dz} \quad (1)$$

where c is the dimensionless dye concentration, θ is the dimensionless time, z is the dimensionless space coordinate, (E_A/UL) is the dimensionless inverse of the Bodenstein number (Bo). E_A is the coefficient of axial mixing, U is the linear phase flow velocity and L is the extractor length.

The response function obtained when the system is considered as an open vessel is

$$E_\theta = \frac{1}{2 \cdot \sqrt{\pi\theta\left(\frac{E_A}{UL}\right)}} \cdot \exp\left[-\frac{(1-\theta)^2}{4\theta\left(\frac{E_A}{UL}\right)}\right] \quad (2)$$

with mean

$$\theta_c = 1 + 2 \cdot \left(\frac{E_A}{UL}\right) \quad (3)$$

and variance

$$\sigma_\theta^2 = 2 \cdot \frac{E_A}{UL} + 8 \cdot \left(\frac{E_A}{UL}\right)^2 \quad (4)$$

2.7. Hold-up experiments

After steady state was reached for defined operation conditions the fractional hold-up of the PEG-rich phase was measured. This was performed by the simultaneous stop of the inlet and outlet streams and the rotor, followed by rapid removal of the extractor contents and volume determination of the two phases. This method leads to an average hold-up measurement that is calculated by

$$H_d = \frac{V_t}{V} \quad (5)$$

where H_d is the fractional hold-up of the top-phase, V_t and V are the volume of the top phase and total volume.

The top-phase hold-up at the top and bottom of the contactor was calculated after samples of 10 ml of the top and bottom phase had been withdrawn from the sample port at the middle of the extractor.

2.8. Mass transfer experiments

The direction of mass transfer was from bottom- to top-phase. The samples were collected at the outlets of the heavy and light phases. Up to 6 mean residence times of unchanged experimental conditions in continuous mode of operation were maintained to reach steady-state data. The concentration of the proteins was determined by FPLC as described above.

3. Results and discussion

3.1. Hydrodynamic characterization

Our experiments describing contactor hydrodynamics were based on measurements of the residence time distribution (RTD) of the Graesser contactor under defined conditions after application of a dye pulse. The time course of the outlet dye concentration was plotted *vs.* time to evaluate the first momentum of the RTD, that stands for the mean residence time. As the experimental setup was considered as an open system, the normalized first momentum had to be used for the determination of the mean residence time [23]. The result was then used to plot the value of the $C(\theta)$ function *vs.* the dimensionless time θ, to achieve independence of the RTD from flow-rate. The diffusion model of the RTD was then applied by non-linear regression and the result was compared to the experimental data. As described above, the diffusion model yields the Bodenstein number, a dimensionless number expressing the relation of convective and diffusional flows, containing the coefficient of axial mixing E_A. This coefficient was determined for each RTD and then used as a measure of axial mixing under different conditions. Fig. 2 shows an RTD after a dye pulse, the experimental values (points) show a reasonable correlation to the model calculations (line)

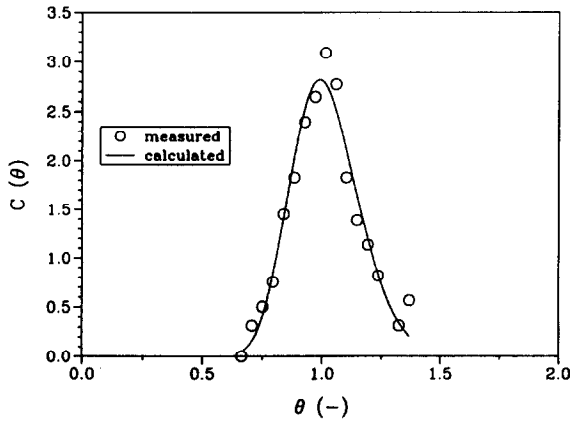

Fig. 2. Residence time distribution after dye-pulse. Experimental conditions: 14% PEG 1550–18% potassium phosphate pH 7; dye pulse: 1 ml of 2 mg/ml aqueous solution of Cibachron Blue.

thus validating the use of RTD measurements to represent axial mixing in our case.

Another important hydrodynamic parameter is the fractional hold-up of the phases. For the dispersed phase this parameter describes the amount of solvent actually available to remove the desired product from the feed at a certain point of the equipment. In this specific case, hold-up signifies the percentage of PEG-rich phase capable of extracting α-La from the salt-rich phase containing the protein mixture. Thus measurements of hold-up are valuable for the estimation of the mass transfer capabilities of an extraction unit under defined conditions.

A very important result of RTD measurements was the fact, that the flow of the salt-rich phase did not influence the residence time in the PEG-rich phase. This was evaluated by applying a dye pulse into the PEG-rich phase at different flows of the salt-rich phase and a fixed flow of the PEG-rich phase. The dye solely partitioned into the PEG-rich phase. All the experiments performed under these conditions yielded the same mean residence time, underlining the main feature of the Graesser contactor — the existence of two independently flowing phases, that are dispersed into each other by gentle mixing.

Another experiment to visualize this fact was performed by examining the PEG-rich phase hold-up at two different vertical positions in the contactor: The contactor was loaded with the two-phase system (14 % PEG 1550–18% phosphate, w/w) and operated at different rotor speeds. The port in the middle of the contactor was used to take samples from the upper and the lower half of the horizontal glass cylinder. Measurements of the PEG-rich phase hold-up of the respective samples demonstrated that the upper half of the contactor was predominantly (96–98%) filled with PEG-rich phase, the section below the interface line was filled with salt-rich phase (PEG-rich phase hold-up 2–3%). Fig. 3 shows the independence of the PEG-rich phase hold-up above and below the interface line from rotor speed.

To characterize axial mixing in the PEG-rich phase, the dependency of E_A from rotor speed was examined. Fig. 4 shows the independence of axial mixing from rotor speed, at least at the low speeds applied in our experiments. The low rotor speeds were chosen, to minimize shear forces, that may prove harmful for labile proteins.

Axial mixing may also be influenced by the linear velocity of the two phases as well as by the ratio of the two respective linear flow velocities. To examine this dependency the coefficient of axial mixing E_A was measured as a function of PEG- and salt-rich phase linear velocity. To determine the influence of phase velocity on axial mixing, the experiments were performed at

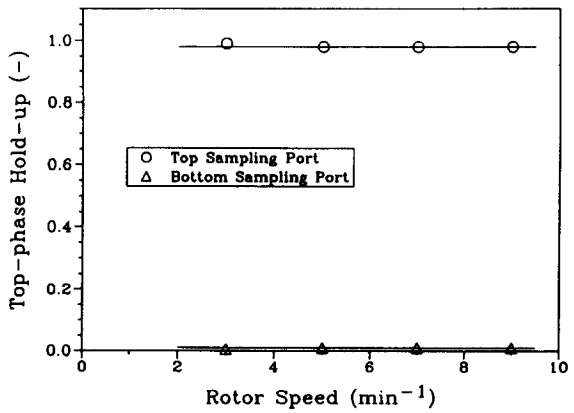

Fig. 3. Fractional hold-up at top and bottom of the extraction unit. Experimental conditions: 14% PEG 1550–18% potassium phosphate pH 7.

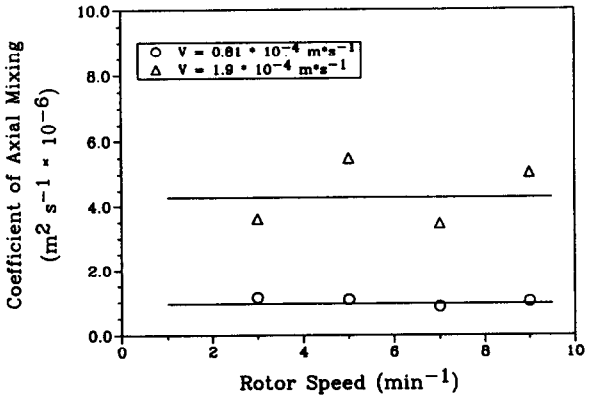

Fig. 4. Axial mixing *versus* rotor speed; parameter: linear phase velocity. Experimental conditions: 14% PEG 1550–18% potassium phosphate pH 7.

different top-phase linear velocities. As can be seen from Fig. 4, higher linear velocities of the top-phase lead to an increased degree of axial mixing expressed by higher E_A values, which seems to be independent from rotor speed at constant phase velocity. In Fig. 5 it is demonstrated that the ratio of the linear phase velocities does not influence the degree of axial mixing in the Graesser contactor. Again the independence of E_A from rotor speed is evident. Since in our case the salt-rich phase can be regarded as the feed and the PEG-rich phase as the solvent stream, this means that increasing the process capacity by a higher solvent-to-feed flow-ratio does not affect the quality of counter-current extraction.

The performance of a continuously operated extraction unit is strongly dependent on the amount of solvent, that is present in the extractor to remove the solute from the feed. At high amounts of solvent compared to feed the solute concentration gradients are favourable for mass transfer from feed to solvent. In our case top-phase hold-up stands for the amount of PEG-rich phase (solvent) available to extract proteins (solute) from the whey-containing phosphate-rich phase (feed). Thus the influence of rotor speed and ratio of phase velocities on top-phase hold-up was examined. Fig. 6 shows the independence of the top-phase hold-up from rotor speed as well as from the ratio of phase velocities. Thus the capacity and performance of the extractor is not reduced by low rotor speeds, that provide favourable mixing conditions for labile products. Additionally, a high ratio of solvent-to-feed flow can be applied without affecting extractor performance.

Summarizing the results of the hydrodynamic measurements the special characteristics of the Graesser Raining Bucket contactor can be applied for operation with aqueous two-phase systems. There are two independently flowing phases with an interface line that can be controlled in the equatorial position of the contac-

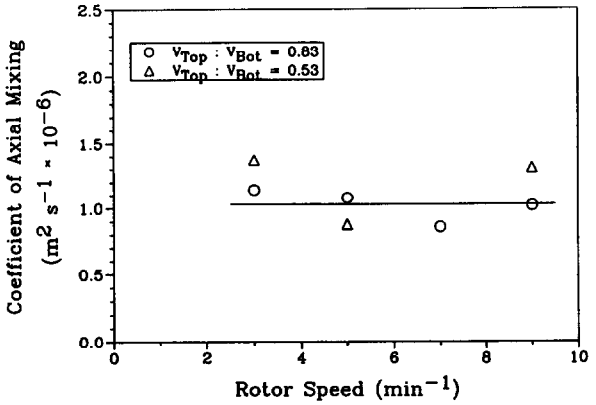

Fig. 5. Axial mixing *versus* ratio of linear phase velocities. Experimental conditions: 14% PEG 1550–18% potassium phosphate pH 7.

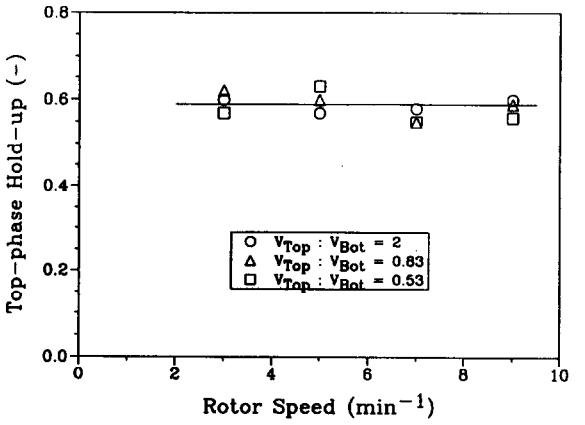

Fig. 6. Fractional hold-up *versus* ratio of linear phase velocities. Experimental conditions: 14% PEG 1550–18% potassium phosphate pH 7.

tor, both phases being dispersed into each other. An independence of the axial mixing from rotor speed and from the ratio of linear phase velocities can be stated. Additionally, both rotor speed and ratio of phase velocities do not affect top-phase hold-up, thus leading to constant extraction efficiency within the range of experiments performed here. Linear phase velocity strongly affects axial mixing, so there is a limiting phase velocity, above which the quality of counter-current extraction is severely diminished.

Comparing our results to the experiments described with kerosene–water systems [10,11] reveals several differences when operating the Graesser contactor with ATPS compared to the organic solvent–water systems. In the studies with the latter system a decrease in axial mixing in the organic phase was detected with increasing rotor speed, while the opposite behaviour was observed for the aqueous phase. The influence of linear phase velocity on back mixing was similar to our results. Contrary to the results presented here, the authors found increasing aqueous phase hold-up at increasing linear flow-rates, but also described the hold-up to be independent from rotor speed. These discrepancies can be attributed to the different properties of the phase system used in this work: ATPS are systems with especially low interfacial tension and increased viscosity, thus some deviations in their hydrodynamic behaviour compared to classical water–organic solvent systems are to be expected. Nevertheless, the special hydrodynamic features of the Graesser contactor also apply for operation with ATPS, thus allowing the use of phase systems with low density difference and interfacial tension.

3.2. Continuous separation of whey proteins

The promising results of the initial experiments to test the hydrodynamic behaviour of the Graesser contactor with ATPS prompted us to test the performance of continuous experiments with real solutions. As mentioned in the Introduction, our model system was the separation of α-La and β-Lg from whey with a PEG 1550–potassium phosphate system. Based on the work of Chen [20] we performed some preliminary experiments in order to find a useful phase system for the continuous experiment. From the partition coefficient and phase ratios obtained by several batch experiments (data not shown) we decided to use 14% PEG 1550–18% potassium phosphate at pH 7. Since the whey was delivered as spray dried powder, we used a 15% solution of whey powder in the salt-rich phase as feed. The experimental conditions for the continuous experiment were chosen according to the results of the hydrodynamic study. The continuous extraction was conducted at two different rotor speeds to confirm the independence of axial mixing and hold-up from rotor speed, as was already observed during the hydrodynamic measurements. In the second set of experiments two different ratios of phase velocities were used to show an increase of extraction efficiency by increasing the ratio of solvent-to-feed flow. Thus a ratio of PEG-rich phase linear velocity (solvent flow) to salt-rich phase linear velocity (feed-flow) of 2 and 1.5 were used.

The continuous experiments were performed for at least 6 residence times before a parameter was changed. To determine the quality of extraction, the phases were analyzed for content of α-La and β-Lg by FPLC as described above. The results of the variation of rotor speed is shown in Table 3 for the first set of experiments ($\tau = 1$ h, $V_{TOP} = 88$ ml/min, $V_{BOT} = 44$ ml/min). β-Lg completely partitioned into the salt-rich phase, while α-La had similar concentration in PEG-rich and salt-rich phase. Fig. 7 shows chromatograms of the feed solution and of the two phases at steady state in a continuous experiment. As the ratio of top-phase to bottom-phase linear velocity was 2:1, the extraction yield of α-La from the continuous process was 63%. Total protein recovery was 99% for β-Lg and 97% for α-La. As indicated from the hydrodynamic measurements the extraction yield was independent from rotor speed. Reducing the top-phase flow from 88 ml/min to 66 ml/min at constant flow of bottom-phase reduced the ratio of linear velocities from 2 to 1.5. As shown in Table 3 the PEG-rich phase concentration of α-La remained unchanged while α-La concen-

Table 3
Results of the continuous extraction of whey proteins

	α-Top-phase	α-Bottom phase	β-Top phase	β-Bottom phase
Rotor speed[a]				
2 rpm	0.42 mg/ml	0.45 mg/ml	n.d.	7.4 mg/ml
5 rpm	0.43 mg/ml	0.3 mg/ml	n.d.	7.2 mg/ml
Ratio of linear phase velocities[b]				
2:1	0.43 mg/ml	0.45 mg/ml	n.d.	7.4 mg/ml
1.5:1	0.41 mg/ml	0.61 mg/ml	n.d.	7.9 mg/ml

[a] Effect of rotor speed (starting conditions 1.4 Mg/ml α-La, 7.4 Mg/ml β-Lg).
[b] Effect of ratio of linear phase velocity (starting conditions 1.4 mg/ml α-La, 7.4 mg/ml β-LG).

tration increased in the salt-rich phase. Calculating the yield of α-La shows a reduction to 44%. However, the partitioning of β-Lg into the salt-rich bottom-phase still was complete. The total amount of protein processed in our experiments was 660 g of protein per day, the amount of α-La purified from whey was 70 g per day.

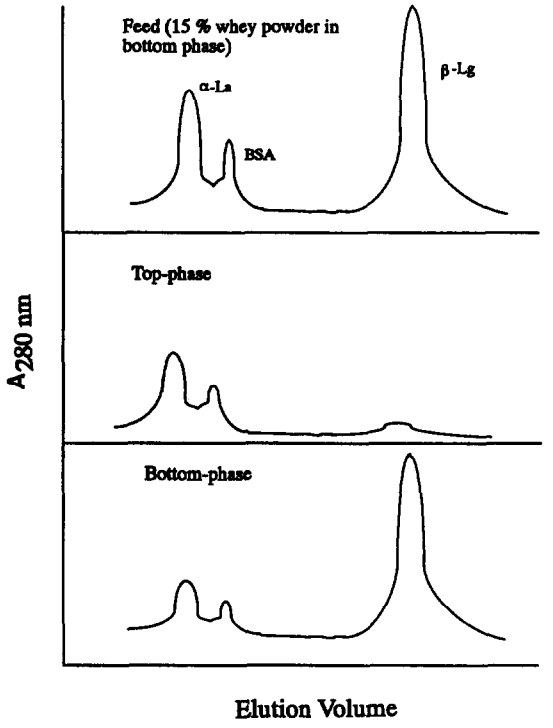

Fig. 7. Schematic presentation of the FPLC chromatograms of feed, top- and bottom-phase from the continuous experiment at steady state.

Comparing these results to the suggestions of Wang *et al.* [11] with respect to optimum extraction conditions in a Graesser contactor shows good agreement: low rotor speeds at high ratio of solvent-to-feed flow also yielded the best results in our experiments. The low rotor speeds prevent protein inactivation by shear forces and moreover simplify the handling of the extraction unit: phase separation in the end zones of the contactor is better at reduced rotor speed. A high ratio of solvent-to-feed flow increases the interface area per unit time that is available for mass transfer and thus favours efficient extraction.

4. Conclusions

The Graesser raining bucket contactor proved to be an extraction unit well suited for work with aqueous two-phase systems. The general hydrodynamic features as described in the literature —independent phases, that are separated at the equatorial line of the extraction vessel and are dispersed into each other— were also found in basic experiments. Examination of the influence of rotor speed, phase velocity and phase ratio resulted in a high extraction efficiency at conditions favourable for protein processing. The flow-rates that could be applied in continuous operation indicated a large capacity of the bench-scale contactor used in our experiments.

Transferring these results to the separation of α-La and β-Lg from whey confirmed the expectations from the initial measurements with PEG–salt systems: the Graesser contactor is capable of continuously separating α-La and β-Lg with satisfactory yields at a rotor speed of 2 rpm. The residence time of 1 h leads to short process times, allowing processing of 660 g of total whey protein per day under the conditions applied here (15% solution of whey powder in salt-rich phase). The PEG-rich phase was free of β-Lg, thus the primary goal of the process, the reduction of the content of the major allergen in infant nutrient formulations was achieved. The space time yield with respect to α-La production is 8.8 g/l per day. Increasing the ratio of linear phase velocities in favour of the PEG-rich phase velocity should lead to even higher space–time yields. Another possibility of increasing the process productivity might be the increase of protein load of the phase system above 15% of whey powder.

Interesting points, that remain to be examined are the distribution of other whey proteins, as for example serum albumin, lactoferrin and immunoglobulins. These proteins are important for retaining the immunologic properties of the artificial infant milk formulations from bovine whey [14]. Research with respect to these questions has been initiated. Another point of interest is the degree of back mixing under the hydrodynamic conditions applied. Sheik *et al.* [10] performed experiments with a kerosene–water system to evaluate back mixing in the Graesser contactor. The question of back mixing with ATPS in the contactor described here is presently under investigation.

Vernau and Kula [24] established a PEG–sodium citrate two-phase system to replace phosphate as the water-structuring salt for ecological and economic reasons. Preliminary experiments have been performed that try to transfer this technique to the separation of whey proteins. Based on the results of Vernau and Kula [24] the replacement of phosphate by citrate seems possible although thorough investigations must follow.

5. Acknowledgements

as financial support of the DAAD (Deutscher Akademischer Austauschdienst, Germany) and the CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Brazil). We also thank Meggle Milchindustie GmbH for the gift of whey powder.

6. References

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