

Protein mass transfer studies on a spray column using the PEG-Reppal PES 100 aqueous two-phase system

A. Venâncio, J.A. Teixeira

251

Abstract The characterization of Bovine Serum Albumin mass transfer mechanisms in a spray column using an aqueous two-phase system composed of poly(ethylene glycol) and a modified starch-Reppal PES 100-is done. The poly(ethylene glycol) rich phase is used as the dispersed phase and protein transfer takes place from the dispersed phase to the continuous phase. The effect of dispersed phase superficial velocity, system composition, continuous phase height and distribution system design on either overall protein mass transfer coefficient or column hold-up is described. It is shown that continuous phase superficial velocity and phase composition are the main controlling factors for protein transfer. It is also observed that, with the tested system, only at very low dispersed phase superficial velocities it is possible to operate the spray column as an extraction column. In this system the upper operating limit of the dispersed phase velocity is ten times smaller than in other aqueous two-phase systems.

List of symbols

ATPS		Aqueous Two-Phase System
BSA		Bovine Serum Albumin
C_i	kg m^{-3}	inlet dispersed phase protein concentration
C_0	kg m^{-3}	outlet dispersed phase protein concentration
C_d	kg m^{-3}	dispersed phase protein concentration
C_c	kg m^{-3}	continuous phase protein concentration
D	m	column internal diameter
H		hold-up
h, h_d	m	dispersion height
h_0	m	initial dispersion height (initial continuous phase height)
k_{da}	s^{-1}	overall mass transfer coefficient
m		protein partition coefficient
n		number of holes of distribution system

PEG

Q $\text{m}^3 \text{s}^{-1}$

S m^2

V m^3

Poly(ethylene glycol)

dispersed phase volumetric flow rate

column internal area

dispersion volume

1

Introduction

The recovery and purification of biological products is one of the main areas of concern in biotechnological processes.

Among the techniques used for protein recovery and purification, liquid-liquid extraction using aqueous two-phase systems (ATPS) is one of the most attractive, due to the gentle environment they provide to proteins and other biological molecules (1, 2).

The selection of a liquid-liquid extraction system, requires that some conventional rules used in chemical engineering processes are applied: density differences between two phases greater than 5% are desirable, viscosities of the phases should be less than 10 mPa s and interfacial tension values should be in the range 1–50 mPa m in order to create a droplet dispersion (3).

ATPS have physical properties that make them, when considering mass transfer phenomena, very different from all the conventional processes. In ATPS, the heavy phase usually has a viscosity larger than 10 mPa s (4, 5). Only in the case of PEG/salt systems, the heavy phase (salt rich phase) presents a smaller viscosity (6). For PEG/sulphate and PEG/phosphate systems, interfacial tensions lower than 0.55 mPa m have been reported (4). PEG/Dextran systems exhibit interfacial tensions in the range 0.02–0.32 mPa m (7). Density differences between phases is the only parameter that fits in the above referred criteria

In spite of these drawbacks, liquid-liquid extraction systems using ATPS have been successfully applied, even at an industrial scale (8). Among the several liquid-liquid contacting devices, spray columns due to its simplicity-easy of construction and operation-are particularly attractive. The main objective in applying spray columns is to eliminate the use of expensive centrifuges, since in the columns, phase separation occurs by gravity. Furthermore spray columns have the advantage that they can provide up to five theoretical stages and in many applications more than one theoretical stage is required for achieving the desired product quality (4). The disadvantage of spray columns is the large backmixing in the continuous phase.

Only a small number of ATPS have been tested in spray columns. Reported results refer to PEG/Dextran (4),

Received 2 January 1995

A. Venâncio, J.A. Teixeira
Centro de Engenharia Biológica da Universidade do Minho, Campus de Gualtar, 4709 Braga codex, Portugal

Correspondence to: J.A. Teixeira

A. Venâncio was supported by a JNICT (Junta Nacional de Investigação Científica e Tecnológica) grant.

PEG/maltodextrin (5) and PEG/salts (sulphate or phosphate) (6) systems. As it has been pointed out (9), there is a need to develop new and less expensive systems for large scale processes. Among these, starch derivatives have been considered. In order to design a spray column for protein separation, data on mass transfer coefficients of the protein, dispersed phase hold-up, degree of mixing in both phases and droplet hydrodynamics is required. These parameters depend on phase velocities, physical properties of the phases, sparger design and spray column dimensions.

In this work, the characterization of BSA mass transfer in a laboratory spray column using the PEG/Reppal PES system is reported. BSA mass transfer takes place from the PEG rich phase to the Reppal PES rich phase. The PEG rich phase will be used as the dispersed phase and will be distributed at the bottom of the column using a sieve plate.

2

Materials and methods

2.1

Chemicals

The chemicals used in this experiment were standard grade PEG (number average molecular weight 8000) and bovine serum albumin (BSA), obtained from Sigma Chemical Co (USA), Reppal PES 100, obtained from Reppe AB (Sweden) and monopotassium phosphate and dipotassium phosphate, obtained from Merck (Germany). Protein concentration was assayed by measuring absorbance at 280 nm.

2.2

Two-phase systems

Systems composition was as shown in Table 1. ATPS were prepared by weighting appropriate amounts of all chemicals. Sodium azide was added (0.05% w/w). Systems were stirred for 6 hours and then transferred to a decanter, where they were allowed to separate for 48 hours. Dissolution and settling was performed on a temperature controlled chamber at 27 °C.

For partition coefficient determination 10 g systems were prepared by mixing stock solutions of chemicals. Systems were allowed to separate and appropriate samples of both phases were withdrawn for protein quantification. Partition coefficient, m , was defined as the ratio between protein concentration in the top and bottom phases, respectively.

2.3

Equipment

The schematic representation of the spray column is shown in Fig. 1. The diameter of the column and the number of holes were varied. The design details for the distributors are presented in Table 2.

Table 1. Phase compositions and BSA partition coefficient

System	Composition (% (w/w))	Partition coefficient, m
1	18% Reppal-3.0% PEG8000	1.06
2	15% Reppal-5.0% PEG8000	0.84
3	25% Reppal-4.2% PEG8000	0.27

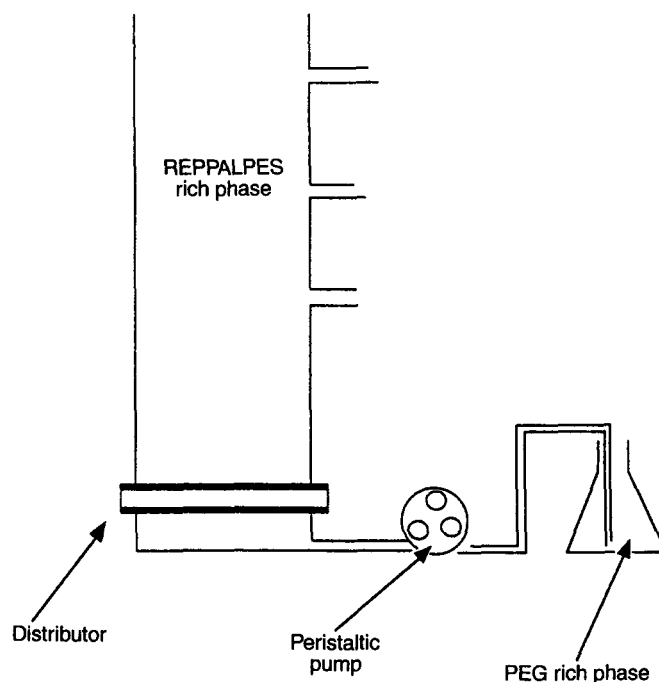


Fig. 1. Schematic representation of the experimental set-up

Table 2. Design characteristics of distribution systems

Column diameter, $D(m)$	Number of holes, n
0.036	4
	7
	11
0.052	23

2.4

Experimental procedure

The spray column was operated in a semi-batch mode. In all experiments the Reppal PES rich phase formed the continuous phase and the PEG rich phase was continuously sparged at the bottom of the column.

Dispersed phase droplets coalesced at the top of the continuous phase and were collected through the overflow outlets.

For mass transfer studies, protein was transferred from the dispersed to the continuous phase. Protein concentration in the dispersed phase at the inlet of the column was $3 \times 10^{-3} \text{ kg l}^{-1}$.

2.5

Hold-up and mass transfer coefficient determination

The dispersed phase hold-up (H) was obtained by measuring the clear liquid height (h_0) and the height of the dispersed phase (h_d):

$$H = (h_d - h_0)/h_d \quad (1)$$

In systems in which the interfacial area is not known, as is the case, a mass balance for the enzyme over a differential height

can be used to calculate the overall transfer coefficient:

$$Q dC_d = K_d a (C_d - mC_c) S dh, \quad (2)$$

where Q , C_d , C_c , $K_d a$, m , S , h represent the dispersed phase volumetric flowrate, dispersed phase protein concentration, continuous phase protein concentration, overall mass transfer coefficient, protein partition coefficient, column internal area and column height, respectively.

Since protein concentration in the continuous phase is much smaller than in the dispersed phase at any time in the experiment, the value of mC_c may be considered constant with respect to height.

The integration of eq. 2 with the boundary conditions

$$h = 0; \quad C_d = C_i$$

$$h = h_d; \quad C_d = C_o$$

gives:

$$K_d a = Q/V \ln((C_i - m \cdot C_c)/(C_o - m \cdot C_c)), \quad (3)$$

where V , C_i and C_o represent the dispersion volume, inlet dispersed phase protein concentration and outlet dispersed phase protein concentration, respectively.

An average C_c value, between the initial and final continuous phase protein concentration, was used.

3

Results and discussion

3.1

Effect of dispersed phase superficial velocity

The effect of superficial velocity was studied in the range 0 m s^{-1} to $2 \cdot 10^{-5} \text{ m s}^{-1}$. Above this value, a complete mixing of both phases was observed.

As can be seen in Fig. 2 an increase in superficial velocity V_d leads to an increase in either mass transfer or hold-up.

This result is similar to results described for other ATPS (4, 5) and may be explained by a decrease in drop size for the largest superficial velocities and a corresponding increase in the number of formed drops: the decrease in drop size and drop superficial velocity results in an increase in the volumetric mass transfer coefficient and hold-up.

3.2

Effect of column height

No significant effect of column height on mass transfer coefficient was observed for columns higher than 0.6 m and superficial velocities in the range $0-2 \cdot 10^{-5} \text{ m s}^{-1}$ (Fig. 2). However, in what concerns mass transfer coefficients, there is a significant difference between the 0.4 m height and the other two (0.6 m and 0.8 m). Mass transfer coefficients are much lower, suggesting that at this height conditions for mass transfer are diminished. It was not possible to obtain hold-up values for the 0.4 m height, since a continuous increase in hold-up, during the entire experiment was observed. An emulsion was formed and drops were retained inside the column. This is similar to results obtained, at superficial velocities greater than $2 \cdot 10^{-5} \text{ m s}^{-1}$, for the 0.6 m continuous phase height. This kind of observations were already reported by Save et al. (10).

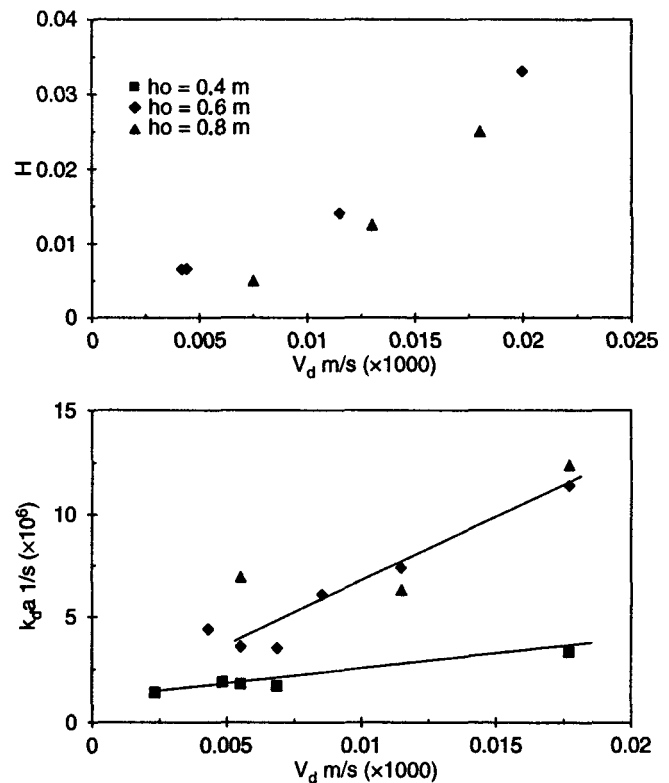


Fig. 2. Experimental hold-up, H , and overall mass transfer coefficients, $k_d a$, for a column with 0.036 m of internal diameter as a function of the superficial velocity, V_d , and initial dispersion height, h_o

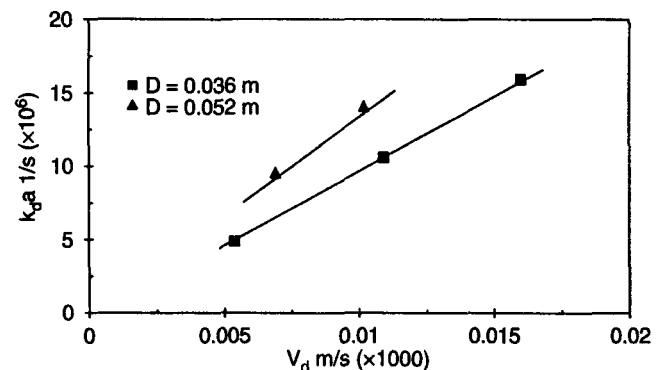


Fig. 3. Experimental overall mass transfer coefficient, $k_d a$, as a function of the superficial velocity, V_d , and column internal diameter, D

3.3

Effect of column diameter

To investigate the effect of the column diameter on mass transfer coefficients, the number of holes of each distribution system was chosen in order to maintain a constant ratio between the distribution system hole area and the column internal area. A slight reduction on mass transfer coefficients are observed for the smaller diameter (Fig. 3). This reduction may be explained by a wall effect phenomena.

3.4

Effect of number of holes of sparger

Mass transfer coefficients slightly increase with an increase in the number of holes. This is similar to results reported for

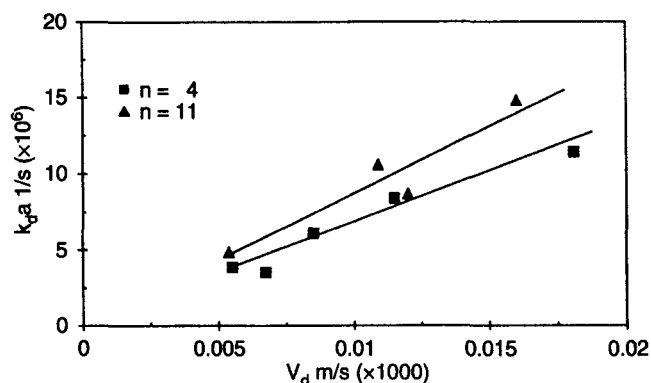


Fig. 4. Experimental overall mass transfer coefficient, $k_d a$, as a function of the superficial velocity, V_d , and the number of holes of the distribution system, n

other ATPS (4, 6). In this situation, an increase in the number of holes causes a decrease in the height above the sparger where drop formation occurs. This means that, for spargers with a larger number of holes, the region where drops are well established and mass transfer is enhanced, is larger (Fig. 4).

3.5

Effect of system composition

For all the tie-lines, as expected, mass transfer coefficients and hold-up increase with an increase in dispersed phase superficial velocity (Fig. 5).

The mass transfer coefficients for the longer tie-lines are smaller. In Table 3, it can be seen that, with an increase in system composition, density difference between phases and viscosity of each phase increase. Also as described by Forciniti et al. (7) interfacial tension increases with increasing polymer concentration. The overall effect of the increase in phases viscosity results in a reduction in protein diffusivity and the film thickness for mass transfer increases. As a consequence volumetric mass transfer coefficients are smaller for the largest tie-lines.

For hold-up, it is clear that the tie-line closer to the critical point presents the highest values. This is in agreement with results reported by Jafarabad et al. (6). Between the other tie-lines no differences in hold-up values are observed, although a significant increase in continuous phase viscosity occurs. According to the same authors and Sawant et al. (5) an increase in dispersed phase hold-up, should be expected.

Anyway, it may be concluded that in systems with a composition closer to the critical point, either the mass transfer coefficients or the hold-up are larger.

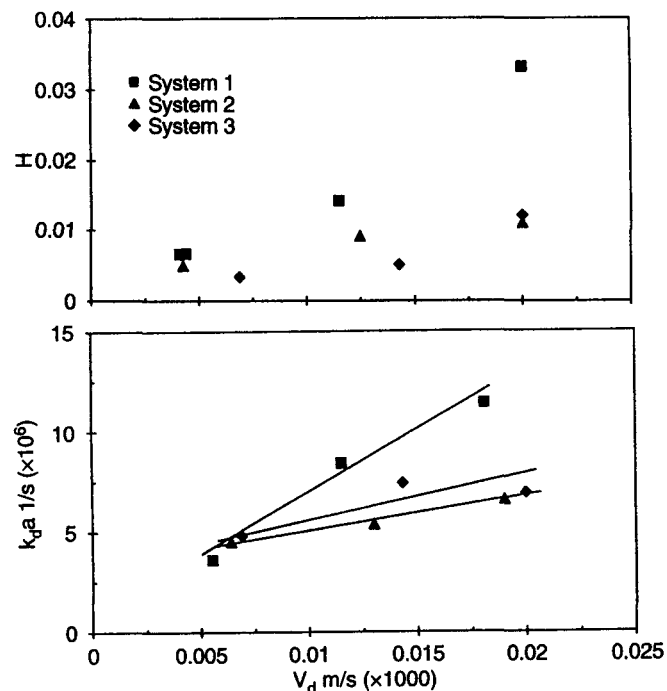


Fig. 5. Experimental hold-up, H , and overall mass transfer coefficients, $k_d a$, for a column with 0.036 m of internal diameter as a function of the superficial velocity, V_d , and system composition

3.6

Some considerations on the mechanisms of drop formation in PEG/Reppal PES systems

A simple analysis on the mechanisms of drop formation in a single orifice, indicates that the diameter of the initially formed drop is the result of a force balance between buoyancy forces and restraining (interfacial tension) forces. At flowrates below a certain value individual drops are formed (11, 12). Above this flowrate-critical flow rate-no more individual drops are formed and a jet leaves the orifices. Drop formation occurs by break-up of the formed liquid jet. Interfacial forces are a controlling factor on this mechanism. As a consequence of the low interfacial tension values in ATPS, it might be expected that in spray columns operating with these systems, drop formation would be in the jetting regime. Results described for other systems confirm this reasoning.

In the PEG/Reppal PES system the flow profiles of the dispersed phase are presented in Fig. 6. It may be seen that for extremely low superficial velocities, plug flow is observed. As velocity increases drop formation occurs. However, for a very small increase in dispersed phase velocity, drops coalesce, i.e.,

Table 3. Physical properties of tested systems

System	PEG rich phase		Reppal rich phase	
	density (kg m^{-3})	viscosity (mPa s)	density (kg m^{-3})	viscosity (mPa s)
1	1043	8.3	1084	26.8
2	1037	8.5	1096	48.5
3	1041	12.5	1134	176.5

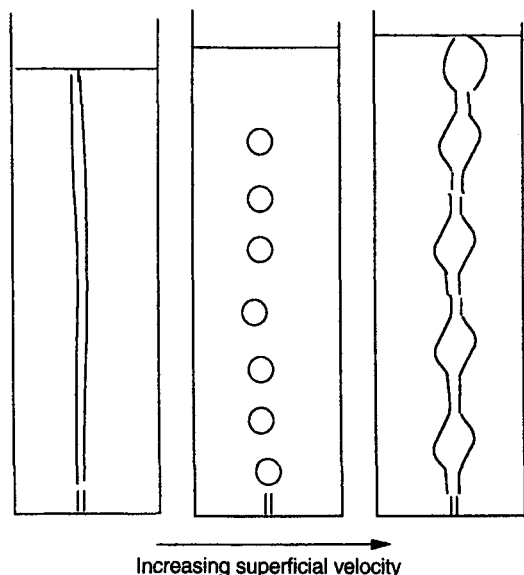


Fig. 6. Relationship between dispersed phase superficial velocity and flow profile

new formed drops are entrapped by the wake of the previously formed ones. This situation reduces the conditions for mass transfer.

At slightly higher flow rates, no stable drops are formed. A complete mixing of both phases occurs, with the formation of an emulsion of many microscopic drops of the dispersed phase in the continuous phase. These drops take more than three hours to separate at the top of the column. This is confirmed visually and by observing the increase in the height of the uniform dispersion inside the column.

The range of operating light phase superficial velocities in the PEG/Reppal PES system is very different and much smaller than in other reported ATPS (4, 5, 6). For this system the upper limit of operation, for the dispersed phase superficial velocity before complete mixing occurs, is $0.02 \cdot 10^{-3} \text{ m s}^{-1}$. For other systems-PEG-maltodextrin (5) or PEG-salts (6)-it is possible to operate till $0.6\text{--}0.7 \cdot 10^{-3} \text{ m s}^{-1}$. The worst situation occurs for PEG-Dextran systems with a reported upper limit of $0.3 \cdot 10^{-3} \text{ m s}^{-1}$.

However, in the range of operating conditions, it seems that no significant differences occur in mass transfer coefficients between this system and above mentioned ones.

This set of observations leads us to conclude that in some ATPS, due to its physical properties, the balance between the forces that control drop size-dynamic pressure, surface tension forces and viscous forces-may strongly limit its application in continuous extraction processes.

4

Conclusions

The characterization of the performance of a spray column using the PEG/Reppal PES system was done. It is clearly

demonstrated that dispersed phase superficial velocity and phase concentration are key factors on protein transfer. As expected, an increase in superficial velocity leads to an increase in volumetric protein transfer mass coefficient and phase composition closer to the critical point allows for the higher mass transfer rates.

Unfortunately, only at very low superficial velocities is it possible to operate with this system. These results suggest that to obtain purification factors of the same order of magnitude of those reported for other systems it is necessary to use columns with enormous dimensions, which makes PEG/Reppal PES systems unattractive. Anyway, due to the low price of crude starch derivatives, the use of spray columns using ATPS composed of PEG/crude starch derivatives must be considered. Such a consideration is reinforced by the observation that ATPS with similar physical properties present very different performances for mass transfer. It may be concluded that the effect of phase forming compounds on mass transfer must be considered for each individual system, before considering its implementation at large scale.

References

1. Albertsson, P.-A.; Johansson, G.; Tjerneld, F.: Aqueous Two-Phase Separations. In: J.A. Asenjo (ed.): Separation processes in Biotechnology, pp. 287-327, New York: Marcel Dekker (1990)
2. Walter, H.; Johansson, G.: Partitioning in aqueous two-phase systems: An overview. *Anal. Biochem.* 155 (1986) 215-242
3. Cusack, R.W.; Fremaux, P.; Glatz, D.: Though basically simple, the technique has a surprisingly wide range of useful variations. *Chem. Eng. Feb* (1991) 66-76
4. Sawant, S.B.; Sidkar, S.K.; Joshi, J.B.: Hydrodynamics and mass transfer in two-phase aqueous extraction using spray columns. *Biotechnol. Bioeng.* 36 (1990) 109-115
5. Raghav Rao, K.S.M.S.; Szlag D.C.; Sidkar, S.K.; Joshi, J.B.; Sawant, S.B.: Protein extraction in a spray column using poly(ethylene glycol) maltodextrin two-phase system. *Chem. Eng. J.* 46 (1991) B75-B81
6. Jafarabad, K.R.; Sawant, S.B.; Joshi, J.B.; Sidkar, S.K.: Enzyme and protein mass transfer coefficient in aqueous two-phase system-I. Spray extraction columns. *Chem. Eng. Sci.* 47 (1992) 57-68
7. Forciniti, D.; Hall, C.R.; Kula, M.-R.: Interfacial tension of polyethylene glycol-dextran-water systems: influence of temperature and polymer molecular weight. *J. Biotechnol.* 16 (1990) 279-296
8. Hustedt, H.; Kroner, K.H.; Kula, M.-R.: Applications of phase partitioning in Biotechnology. In H. Walter, D.E. Brooks and Fisher, D.: Partitioning in Aqueous Two-Phase Systems, pp 529-587, London. Academic Press (1985)
9. Venâncio, A.; Teixeira, J. A.; Mota, M.: Evaluation of Crude Hydroxypropyl Starch As a Bioseparation Aqueous-Two-Phase-Forming Polymer: *Biotechnol. Prog* 9 (1993) 635-639
10. Save, S.V.; Pangarkar, V.G.; Kumar, S.V.: Intensification of mass transfer in aqueous two-phase systems. *Biotechnol. Bioeng.* 41 (1993) 72-78
11. Bailey, J.E.; Oliis, D.F.: Biochemical Engineering Fundamentals, McGraw Hill, Inc. (1986)
12. Clift, R.; Grace, J.R.; Weber, M.E.: Bubbles, Drops and Particles. New York: Academic Press (1978)