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An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

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Abstract

An innovative approach to separate amphiphilic biomolecules in a fixed bed adsorption plant is discussed and experimental results are presented. High hydrostatic pressure of up to 360 MPa is utilized to control the sorption equilibrium. Major advantages of this approach are that no auxiliary substances are required for the separation that have to be removed afterwards. Furthermore, of the two physical parameters pressure and temperature that can be used to control reaction or sorption equilibria, pressure is the less harmful one towards the activity of biomolecules.

To realize this approach, interdisciplinary researches were necessary. The surfaces of different silica gels were chemically modified in order to synthesize adsorbents with the desired properties. Two high pressure plants were designed and built. One is an circulation plant to investigate equilibrium states. It was utilized to record adsorption isotherms under a pressure of up to 300 MPa. The second one is a semi-continuous plant for a fixed bed reactor. It was hydrodynamically characterized and afterwards used to look into breakthrough curves and separation cycles. The adsorption capacity of the tailor made adsorbents and its pressure dependency was examined via these plants in equilibrium (isotherm) and dynamic (breakthrough) experiments. Therefore, the nonionic surfactant Triton X-100 was applied as a model substance for e.g. glycolipids, which are within the scope of the separation method.

During these experiments a surface modification was identified that showed a high adsorption capacity under high pressure conditions. At the same time at ambient pressure, which represents the desorption condition, it had nearly none adsorption capacity. Furthermore, the adsorption isotherms become more favorable with increasing pressure, indicating an increasing affinity towards the applied surfactant.

Experimental data were analyzed utilizing the Langmuir, Frumkin, and Temkin isotherm models. It was found that the best fit could be achieved with the Temkin-type isotherm. The dynamic experiments matched the findings from the isotherms. With increasing pressure the breakthrough of the surfactant through the fixed bed arrangement occurred later.

Full separation cycles including adsorption (breakthrough), washing, desorption, and regeneration proofed that the surfactant can be regained and hence the method is feasible. In first steps up to 75% of the initial concentration could be desorbed. The process was then optimized and it was found that there is an optimum pressure for the system examined. If the goal is to maximize the regained concentration, the best adsorption pressure is around 300 MPa. For an optimal efficiency, defined as amount of pressure desorbed substance in relation to unbound and removed compounds in the washing step, the adsorption pressure should be closer to 200 MPa. An influence of the utilized adsorbent's silica structure was discovered as well and a best pore size is suggested.

Key Words:

High pressure, adsorption, affinity separation cycle, isotherms, pressure controlled equilibrium

Introduction

In various applications pressure becomes more and more interesting as a parameter to control chemical equilibria and biotechnological processes. However, it is rarely considered as a tool to control adsorption equilibria for affinity separations. Since purifying proteins of interest [1] and bio molecules such as glycoconjugates is still a substantial bottleneck, in the following, an approach is given to utilize pressure in a semi-continuous selective separation process aiming at these substances.

To take advantage of high pressure in a semi-continuous separation process, joint results of researches in different branches are necessary. At first it had to be assured that bio molecules of interest like enzymes and glycoconjugates withstand the aimed working conditions unharmed. In literature can be found that in contrast to temperature induced changes to these molecules, most effects caused by pressure are reversible [2-4]. This is especially the case for ambient temperature and pressures up to 300 MPa. Researches concerning the treatment of foodstuff lead to similar conclusions. Significant deactivation of enzymes is found only above 350 MPa [5], BSE contaminated food was exposed to 1200 MPa at 410 K to reach sufficient decontamination [6]. Suitable food preservation parameters are reported to be 350 MPa pressure at a temperature of at least 343 K [7]. It is unlikely that high pressure-low temperature treatment for enzyme inactivation can substitute high temperature treatment [8]. In general, high pressure affects bio molecules in very different ways, partly even enhances enzyme activity [9], but no subunit dissociation of oligomeric proteins is observed [10] and no covalent bond breaking expected [11]. Pressure effects on Y-ADH were elaborately studied by this working group and the enzyme was found to withstand pressures of up to 300 MPa with only a minor loss of activity

[12, 13]. To realize the separation process, an automated high-pressure plant for a continuous flow through a fixed bed at pressures of up to 360 MPa was designed and built and its hydrodynamic behaviour was investigated [14]. Silica-based matrices were chemically modified to show a selective interaction with the aimed component to adsorb [15, 16] and were characterised by means of kinetic investigations and isotherms. To record isotherms at high pressure, a circulation plant for a maximum operational pressure of 360 MPa was built and isotherms at a pressure of up to 300 MPa were recorded.

Experimental

Material

The silica gel XWP-P005 was delivered by Grace Davison, Worms, Germany. XWP-P005 provides a specific surface of 80 m²/g, an average pore diameter of 50 nm, a pore volume of 1 to 1.2 mL/g, a density of 2120 kg/m³, and a void fraction of 0.82. SP 60-20 P, SP 200-20 P, and SP 1000-20 P are from Daiso Co. Ltd., Japan. They have a specific surfaces of 461 m²/g, 170 m²/g, and 24 m²/g and an average pore diameters of 7 nm, 23 nm, and 109 nm, respectively. For all Daisogels the pore volume is approximately 0,9 cm³/g and the particle size 20 µm. 3-mercaptopropyl-trimethoxysilane, diethyleneglycoldiglycidylether, and 2,2,2-trifluoroethanesulfonyl-chloride are obtained from Fluka (Fluka Chemie GmbH, Buchs, Switzerland); hydrochloric acid, sodium chloride, acetone, toluene, sulphuric acid, potassium permanganate (Titrisol) and Triton X-100 were purchased from Merck (Merck, Darmstadt, Germany). All not otherwise specified chemicals are of analytical-reagent grade.

Preparation of the adsorbents

The silica gels are covered with over molecular sieve dried toluene and 3-mercaptopropyl-trimethoxysilane is added. This mixture is heated up to 80°C. After 90 minutes of reaction time the support is filtered and extracted with acetone [16]. The result is a preliminary stage of the employed adsorbents that is utilized to proof the efficiency of the further modifications.

Water and diethyleneglycoldiglycidylether are then added and the mixture reacts 24 h at a temperature of 45°C under constant rotation in a rotary evaporator. The supports

are washed with sodium chloride solution (1 mol/L) and water. Afterwards they are covered with hydrochloric acid (0.05 mol/L) and rotated for 24 h at 65°C in a rotary evaporator. The resulting substances are filtrated, washed with acetone and dried at 60°C. These adsorbents are referred to as HSU (Helmut-Schmidt-University) 125-1.2.1, based on XWP-P005 and HSU 134-1.1.1, HSU 135-1.1.1, and HSU 136-1.1.1, based on SP 60-20 P, SP 200-20 P, and SP 1000-20 P, respectively.

The adsorbate Triton X-100

Triton X-100 is a non-ionic surfactant that is used as adsorbate as a model substance for amphiphilic substances such as glycolipides which provide large biological and pharmaceutical effects. Its formula is C_8H_{17}-C_6H_4-$(OCH_2$-$CH_2)_n$-OH where n equals 8…10 and it has an average molecular weight of 600 g/mol. Its structural formula for n = 8 is given in Fig. 1. In aqueous solution, Triton X-100 builds micelles. Its critical micelle concentration CMC and the aggregation number of the micelles are pressure dependent [17-19]. The CMC ranges from 0.15 g/L at ambient pressure, reaches a maximum at 100 MPa (0.17 g/L) and decreases to 0.11 g/L at 360 MPa. The corresponding aggregation numbers are 250 at ambient pressure, 100 at 100 MPa, and 150 at 360 MPa.

To quantify Triton X-100 concentrations in aqueous solution, an easy and reliable photometric analysis method has been developed. 300 µL solution are added to 900 µL half-concentrated sulphuric acid and 300 µL potassium permanganate. The sulphuric acid consists of 2 volume parts sulphuric acid, 99%, and 3 volume parts water. The concentration of potassium permanganate is 0.02 mol/L. This solution reacts under constant shaking in 1.5 mL reaction caps at a temperature of 30°C. The

resulting redox reaction gradually achromatises the potassium permanganate. After a reaction time of exactly 90 minutes, 300 µL of the solution are pipetted into a microtiter plate (HJ-Bioanalytik, Moenchengladbach, Germany). The absorption of light of a wavelength of λ = 490 nm is measured with a 1420 VICTOR^2 Multilabel Counter (Perkin-Elmer Life Sciences – Wallac Oy, Turku, Finland) and compared to standard solutions.

High pressure plants

The high pressure plant used to carry out the pressure-controlled fixed bed adsorption and desorption experiments with Triton X-100 is described in detail in a previous publication in this journal [14]. The plant is fully automated and designed for a continuous flow through a fixed bed reactor at pressures of up to 360 MPa. The feed, for the experiments at hand an aqueous solution of Triton X-100 of various concentrations, is filled into a pipe loop, where it is pressurized by an MhR 150/7 (ProMinent Orlita, Gießen, Germany) motor driven diaphragm pump to the desired pressure. The feed is conducted through the fixed bed reactor and is than depressurized by the pneumatic valve. A fraction sampler from Pharmacia (Amersham Biosciences Europe GmbH, Uppsala, Sweden) collects time-based up to 175 samples in caps of 1.5 mL size.

The circulation plant to investigate equilibrium states such as adsorption isotherms is shown in Fig. 2. It is connected to the fixed bed adsorption plant via valve V1. The plant is pressurized by the diaphragm pump of the semi-continuous plant and the feed is delivered out of its pipe loop through valve V1. The volume of the circulation plant is 32.8 mL. In order to record isotherms, 164 mg of the adsorbent are weight

into the reactor TS. By this, a constant ratio of the adsorbent to the feed is assured and the adsorbent's loading can be easily determined from the difference between equilibrium and initial concentration of the feed solution. Valves V2, V3, and V4 are installed to ensure the plant can be completely filled with the feed solution without any remaining bubbles within the system. After the plant is entirely filled and the desired pressure is set, V1 is closed and the feed is circulated by the opposed piston pump BS (Sitec Sieber Engineering, Zurich, Switzerland). Samples are taken through the lock that consists of the double valve ZV and the outlet valve FV. The piping between those two valves is empty except from air at ambient pressure and has a volume of 1.5 mL. To take a sample, FV is closed. By opening the left hand side of ZV, solution is pressed into the pipe. Because of this the pressure in the circulation plant drops about 100 MPa. ZV is closed immediately to ensure that the sample within the pipe reflects the situation in the plant before the pressure drop and is not affected by any changes that occur afterwards. The sample is released by carefully opening valve FV and the right hand side of valve ZV.

Methods

Isotherms were recorded using static methods [20]. At ambient pressure, the batch method was utilized. For this, kinetic investigations were carried out first and it was determined that equilibrium is reached after 30 minutes at the latest. [21]. From the kinetic investigations equilibrium isotherms were derived. Investigated was the adsorption behaviour of the adsorbents HSU 125-1.2.1, HSU 134-, 135-, and 136-1.1.1 as well as that of preliminary stages of the modified carriers. It could be proofed that the surface modification of the silica gels is effective. High pressure isotherms

were recorded by the circulation method, utilizing the high pressure circulation plant. Samples were drawn after 60 minutes of circulation time.

For the breakthrough curves and the adsorption-desorption experiments, the concentration of the solution leaving the semi-continuous plant was plotted not over the time but over the cumulative eluted volume. By this, better insight into the breakthrough behaviour and an easier comparison of different adsorbents is achieved [22].

Results

Isotherms

Adsorption isotherms at ambient pressure were recorded for the adsorbents HSU 125-1.2.1, 134-, 135-, and 136-1.1.1 [23]. Due to its large specific surface and especially the small pore diameter, which is of the same order of magnitude as the size of the adsorbate Triton X-100, HSU 134-1.1.1 shows a different adsorption behaviour than the other adsorbents. It is assumed that different adsorption and transport mechanisms (e.g. Knudsen diffusion) come into play. Because of this results for HSU 134-1.1.1 are not discussed in detail. Focus is laid on the carriers HSU 135- and 136-1.1.1. Isotherms recorded with these adsorbents at pressures between ambient pressure and 300 MPa are presented in Figs. 3 and 4.

Both adsorbents show a significant pressure dependency of their adsorption capacity. At ambient pressure, the adsorption capacity equals zero within the analysis' certainty. This assures optimum desorption and regeneration possibilities under these conditions. The adsorption capacity increases with pressure, the most distinctive pressure dependency is found between 100 MPa and 200 MPa. Furthermore, at higher pressure the isotherms become more favourable, showing a steeper slope at lower concentrations and hence a higher affinity towards the employed surfactant Triton X-100. It is noteworthy that none of the high pressure isotherms crosses the coordinate system's origin.

Figures 5 and 6 show the effect of a reduction of pressure in the circulation plant on the adsorption equilibrium with HSU 135-1.1.1 as adsorbents. It can be seen that the adsorption capacity of the adsorbents decreases with decreasing pressure. In accordance with the findings from the isotherms, the capacity decrease is bigger if

the pressure is reduced from 200 MPa to 120 MPa than it is for a reduction from 300 MPa to 210 MPa.

Breakthrough curves

To look into the pressure effects on the adsorption capacity under dynamic conditions, breakthrough curves were recorded with HSU 125-1.2.1 at various pressures, presented in figure 7. For these investigations the high pressure plant specified prior [14] has been utilized. The separating column was filled with 0.70 g of the adsorbents HSU 125-1.2.1, making up the fixed bed. It was renewed after every experiment. The mass flow for all experiments was 1.2 mL/min. In Fig. 7 the solute concentration of the eluted fluid is plotted against the total volume of the fluid that has passed the plant.

Experiments carried out at a pressure of 0.8 MPa match the findings from the kinetic investigations and the isotherms. The adsorbent shows nearly no adsorption. The breakthrough occurs after 15 mL solution have passed the plant, almost immediately after the plant's dead volume of 9 mL. A strong pressure dependency of the adsorption capacity is found, indicated by the right-shift of the breakthrough. At a pressure of 125 MPa the Triton X-100 concentration begins to rise after 30 mL feed volume have passed the plant and at a pressure of 250 MPa the breakthrough starts at the 38 mL point. Its slope, however, is not as steep as that for the curves recorded at lower pressures. This indicates a pressure influence on adsorption kinetics and mass transfer that was observed by Jansen and Niemeyer [14] in prior works.

Pressure controlled adsorption and desorption

Full separation cycles were investigated at pressures up to 360 MPa utilizing the adsorbents HSU 125-1.2.1, 135-, and 136-1.1.1. After the breakthrough had occurred, the feed was switched from the original Triton X-100 solution to water. The desorption was than initiated by stopping the high pressure pump after the plant was flushed with a sufficient amount of water to wash away any not-adsorbed molecules under high pressure. Stopping the pump causes a pressure decrease at a rate of approximately 15 MPa/min. All times at which the respective actions occurred and all relevant process parameters are specified in table 1 by means of the volume that had passed through the plant at the time.

Experimental results are shown in Figs. 8 and 9. Fig. 8 represents the pressure dependency of the adsorption/desorption equilibrium. While for an adsorption at 100 MPa the maximum concentration that was reached during the desorption process is 1/2 of the initial concentration, it is about 70% for the experiment at 300 MPa. With an operating pressure of 50 MPa (data not shown) only about 1/3 of the initial surfactant concentration can be regained. If the adsorption pressure exceeds 300 MPa, better results than 70% of the feed concentration can not be achieved. In fact, for an adsorption pressure of 360 MPa the regained concentration during the desorption is slightly lower than for an adsorption pressure of 300 MPa. The impact of the adsorbent's silica structure can be seen in Fig. 9. For all experiments shown in this figure the adsorption pressure was 300 MPa. The best results can be achieved with Daisogel SP 200-20 P as carrier matrix, providing a specific surface of 170 m^2/g and a pore size of 23 nm. Utilizing this silica gel in its modified form as HSU 135-1.1.1, 80% of the initial concentration can be regained.

Discussion

The isotherm model after Langmuir is one of the few that fits, under certain assumptions, for liquid-solid adsorption equilibria [24] and it can be used to describe the adsorption behaviour of non-ionic surfactants [25]. On the first glance, the recorded isotherms presented in Figs. 3 and 4, appear to be of a favourable Langmuir type [20]. However, it is not possible to explain isotherms that do not cross the coordinate system's origin with Langmuir's theory. Hence, two modifications of the Langmuir model are reviewed, the isotherms after Frumkin and Temkin [26]. The Langmuir equation can be written in the following form:

$$bc = \frac{\Theta}{1-\Theta}, \tag{1}$$

where Θ is the relative surface coverage, c the equilibrium concentration, and b a numerical coefficient reflecting a bonding energy [27]. With an interaction constant a_F Frumkin added another degree of freedom to the equation:

$$bc = \frac{\Theta}{1-\Theta}\exp(-2a_F\Theta), \tag{2}$$

and finally Temkin suggested a modification that allows for isotherms that do not cross the coordinate system's origin:

$$bc = \frac{\exp(a_T\Theta - 1)}{1-\exp[-a_T(1-\Theta)]}. \tag{3}$$

Applications of Temkin's theory can be found in literature [28-30].

To determine the respective model parameters, in a first step the experimental results with HSU 135-1.1.1 as adsorbent were shifted in a way that a fitted isotherm crosses the origin. To this modified data isotherms according to Langmuir could be properly fitted and the maximum load could be determined [23]. This value is assumed to be reliable. It is used to determine the constants a_F and a_T from

Frumkin's and Temkin's models, respectively. Isotherms fitted using Frumkin's equation are shown in Fig. 10. It is obvious that the fit is not satisfactory for low concentrations. Fig. 11 represents the fit based on Temkin's theory. It can be seen that with this model the results can be well explained. All parameters are summarized in table 2.

The recorded high pressure isotherms indicate that below a certain surfactant concentration in the solution no adsorption occurs. This explains the phenomenon that while recording breakthrough curves and separation cycles nowhere a surfactant concentration of zero was found. It can be assumed that below this minimal concentration the surfactant simply 'flows' through the fixed bed and no adsorption occurs. This effect again could be due to the formation of micelles that occurs above a certain concentration, the so called CMC (Critical Micelle Concentration). It would indicate that single surfactant molecules adsorb barely or not at all, while micelles do.

Looking into the influence of the silica structure of the basic materials, it appears that the shape of the desorption peak, and hence the desorption kinetic, is stronger affected than the overall adsorption capacity. The adsorption capacity for carriers with significant different specific surfaces, e.g. 24 m^2/g for SP 1000-20 P and 170 m^2/g for SP 200-20 P, is very similar, which as a start is a very surprising result. A possible and likely interpretation is that the silica gel's pore size is more important than the surface area. Pore sizes are connected to the adsorbent's specific surface, because a larger specific surface for particles of a similar size can only be realized through more – and smaller – pores. Considering micelle rather than single molecule adsorption as mentioned above, a high adsorption capacity can be realized with a comparatively small specific surface. By this, the unexpected small differences of the

adsorption capacities can be explained. Pore sizes, however, impact the adsorption kinetic in a way that for very large pores come along with lengthy diffusion while too small pores can get clogged. Hence it is expected that there is an optimum pore size for each system, going along with a specific surface of the respective silica gel.

By investigating the pressure dependence of the adsorption/desorption behaviour in the circulation plant it was proofed that the pressure induced change of the adsorption capacity is reversible. Furthermore, since these experiments are equilibrium investigations and pressure was the single parameter that was changed during the experiments, it can be stated the shift of the adsorption/desorption equilibrium can be controlled by pressure. Considering only the separation cycles, one can not be entirely sure that secondary effects, such as a small but unavoidable change in the mass flow while stopping the high pressure pump, have a significant impact on the desorption. However, regarding the equilibrium experiments shown in Figs. 5 and 6 it is obvious that the decreasing pressure is the decisive factor that initiates and controls the desorption.

Summary

A new sorptive separation process, using high hydrostatic pressure to control the adsorption/desorption equilibrium was established. The feasibility of the process was proofed. The impact of two main parameters, the adsorption pressure und the structure of the silica gel, on the affinity separation cycle, especially the regained concentration of the valuable material, was elaborately investigated. The results are summed up in Figs. 12-14.

Fig. 12 shows the influence of the adsorption pressure on the regained concentration for three examined adsorbents. It can be seen that the regained concentration rises with increasing adsorption pressure up to a pressure of 300 MPa. Above 300 MPa the regained concentration remains almost constant. Since 360 MPa marks the maximum operation pressure of the plants at hand, it can not be predicted whether the concentration will rise again at significant higher pressures or will eventually drop. From the high pressure isotherms it can be stated that the pressure dependence of the adsorption capacity does not rise linearly with pressure. Starting at a pressure of 200 MPa, the increase of the adsorption capacity becomes significantly smaller. Therefore it appears likely that the regained concentration in the separation cycles remains constant or rather drops at adsorption pressures above 360 MPa. In addition, the influence of the silica structure of the adsorbents can be determined. The adsorbent HSU 135-1.1.1, that is based on the Daisogel SP 200-20 P shows the best performance. This becomes more obvious in Fig. 13. Here the influence of the adsorbent's specific surface is shown. Again, SP 200-20 P with a specific surface of 170 m²/g reaches the best results. The adsorbent HSU 134-1.1.1, based on SP 60-20 P with a specific surface of 461 m²/g, shows entirely different results and is therefore not discussed in detail. In any case it does not match the performance of

HSU 135-1.1.1. The plotted results in Fig. 13 can be well fitted with a quadratic function. Because of this it can be assumed that there is an optimum silica structure for this process with a specific surface of approximately 250 m²/g. Such a carrier would provide a calculated pore size of 18 nm.

At last, a process efficiency can be defined as the ratio of the amount of desorbed surfactant to the amount of the surfactant that was eluted during the washing step, presented in Fig. 14. These results are in accordance with all prior findings. It can be stated that 200 MPa to 300 MPa adsorption pressure marks the best operating area for the system investigated. Looking at the adsorbents, best results are again achieved utilizing HSU 135-1.1.1.

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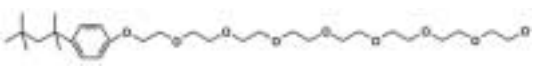

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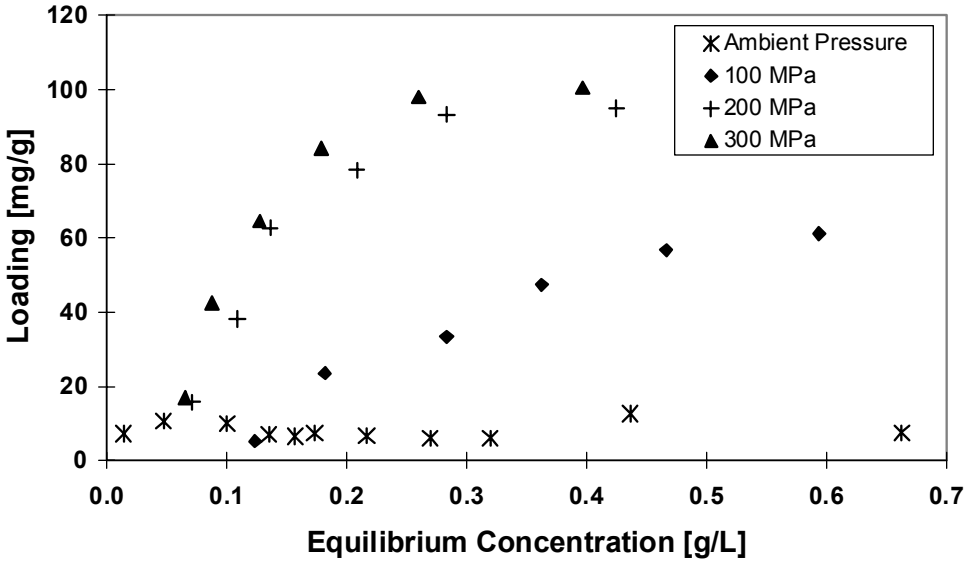

Fig. 3: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

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Figure(s)

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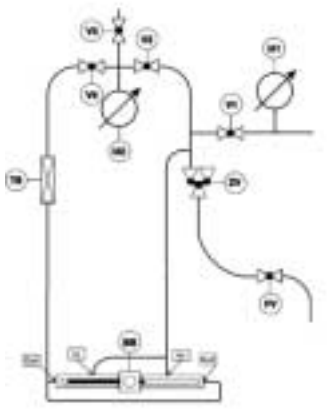

Figure(s)

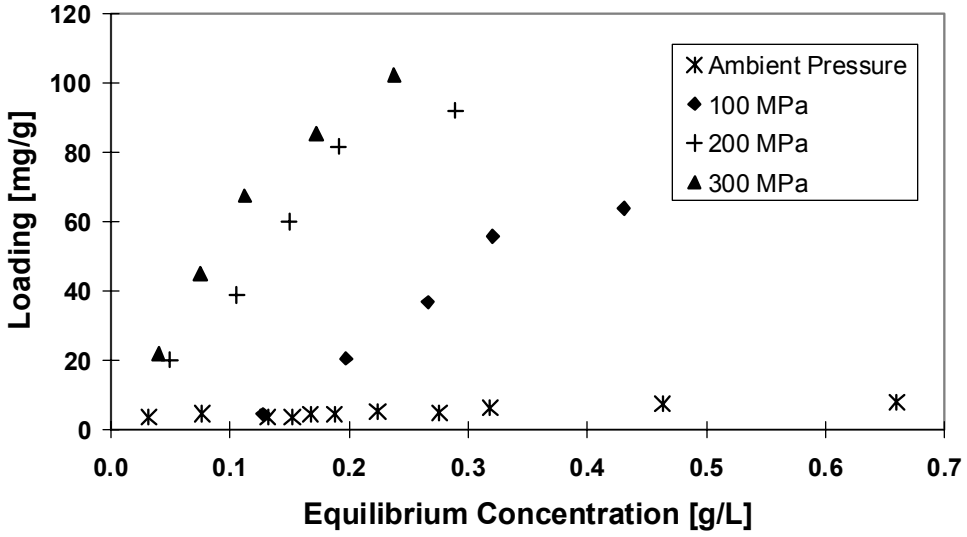

Fig. 4: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

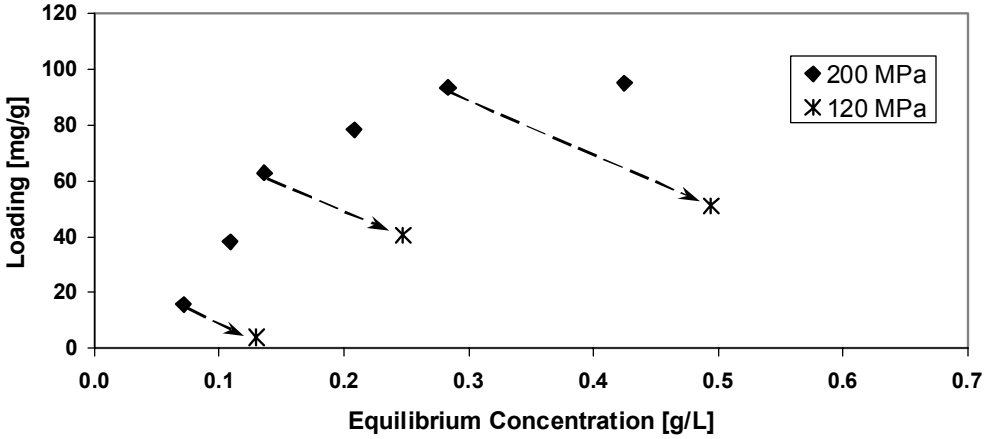

Fig. 5 An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

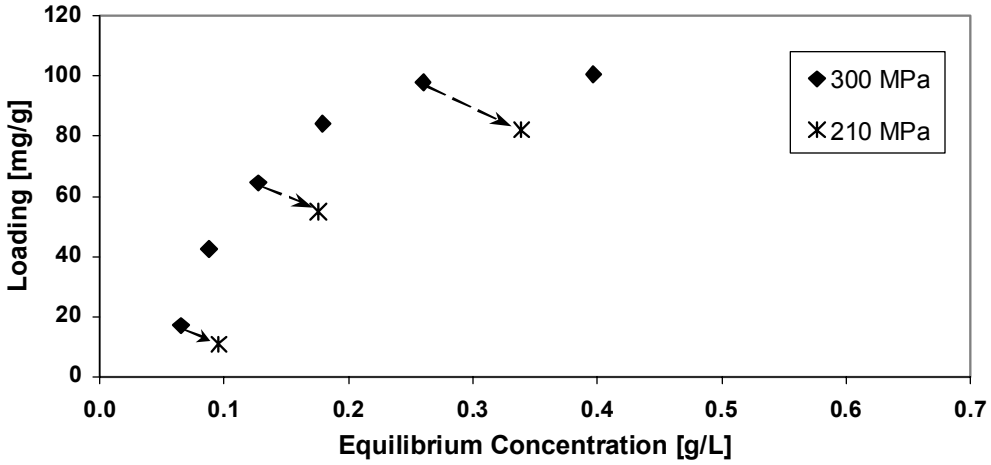

Fig. 6: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

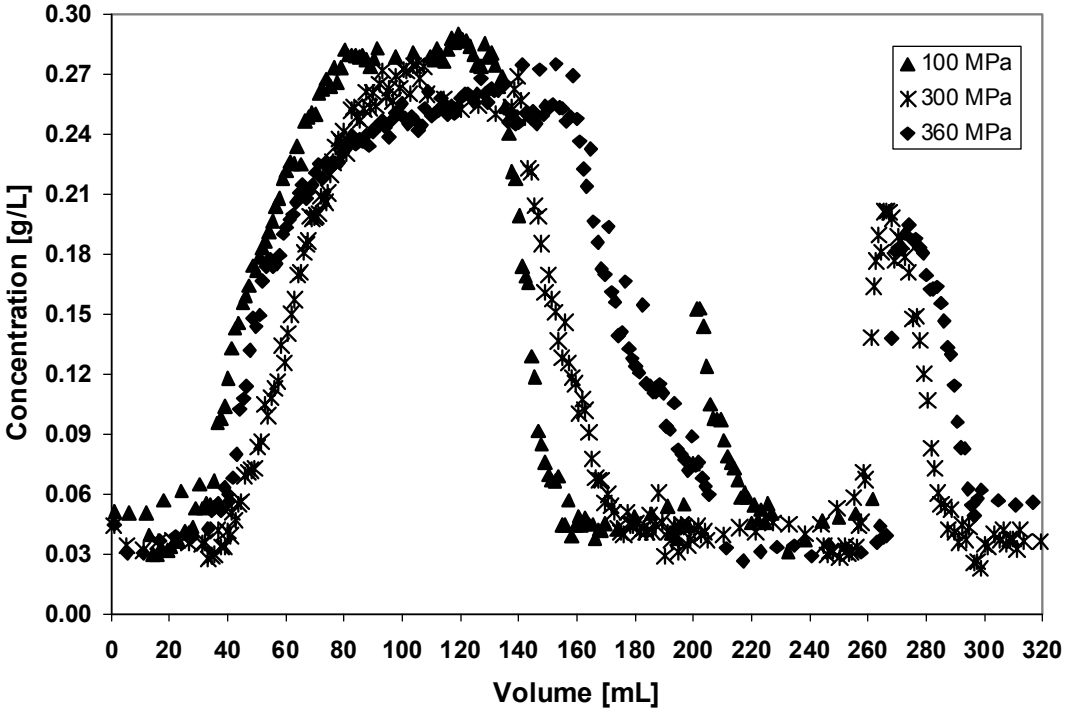

Fig. 8: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

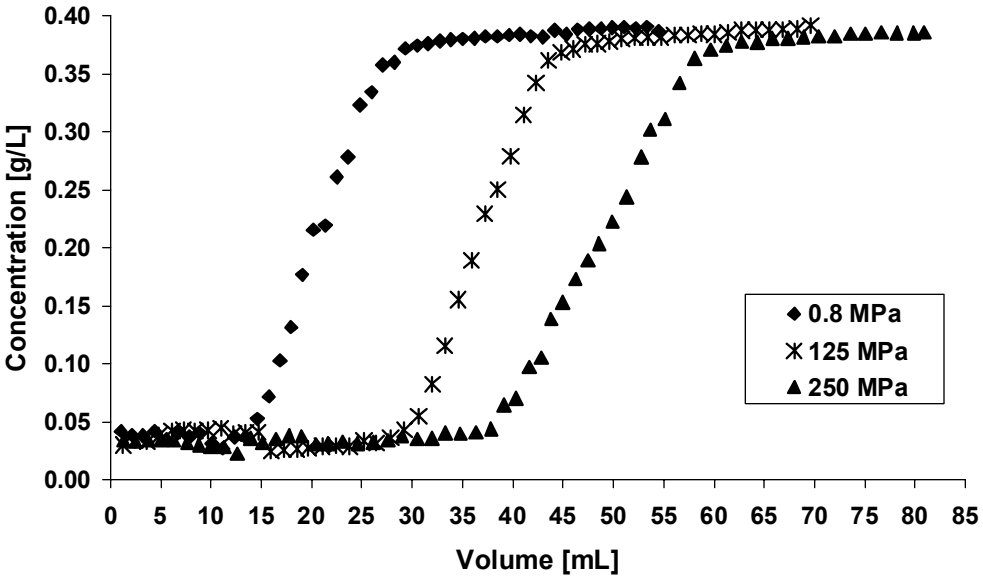

Fig. 7: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

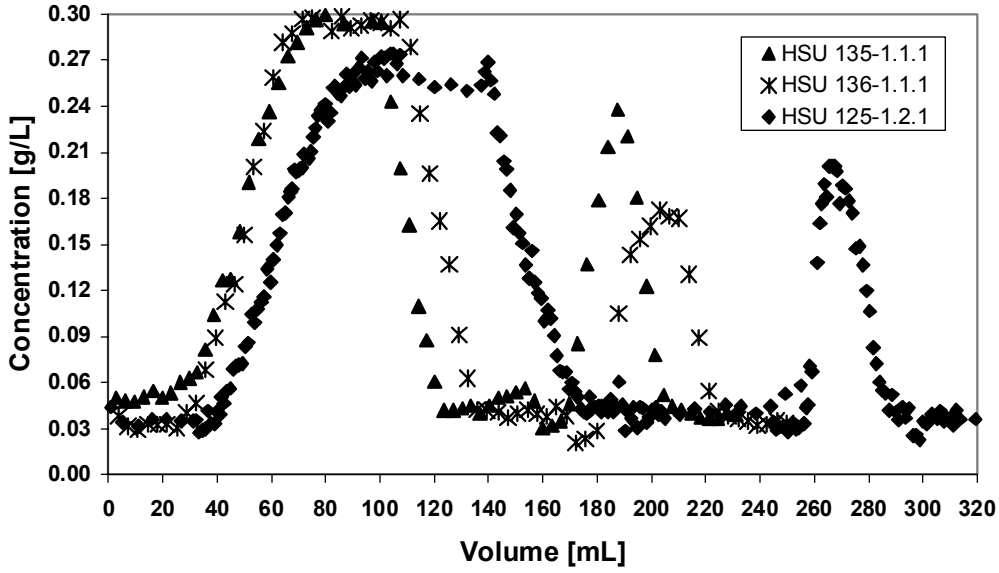

Fig. 9: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

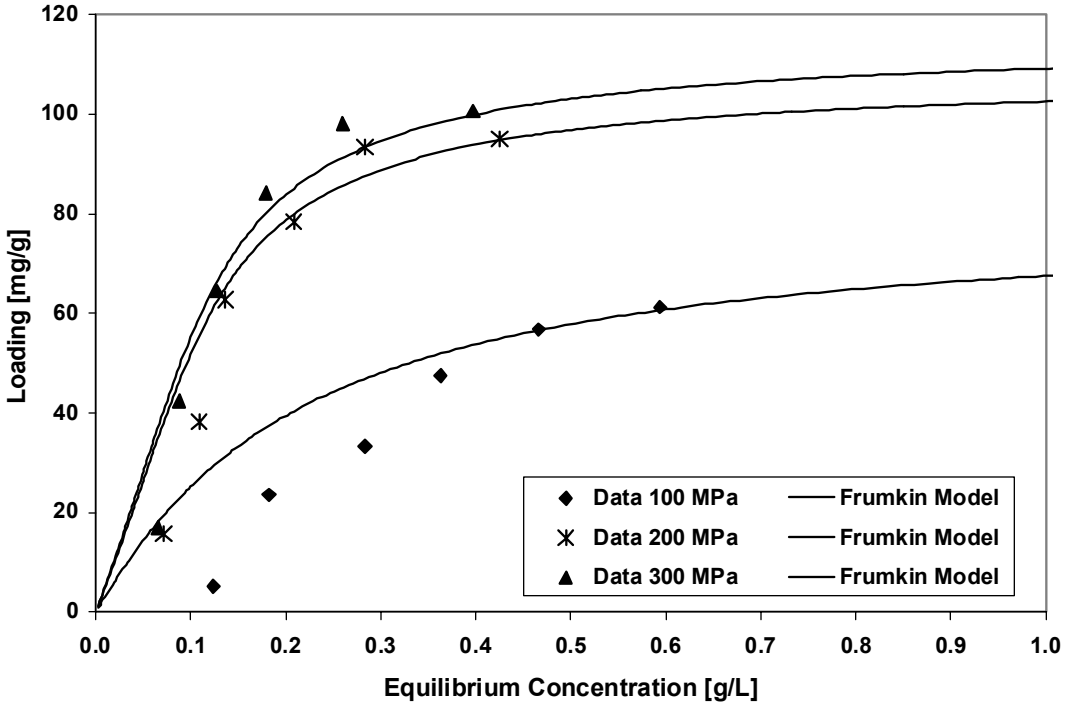

Fig. 10: Jan Jansen – An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

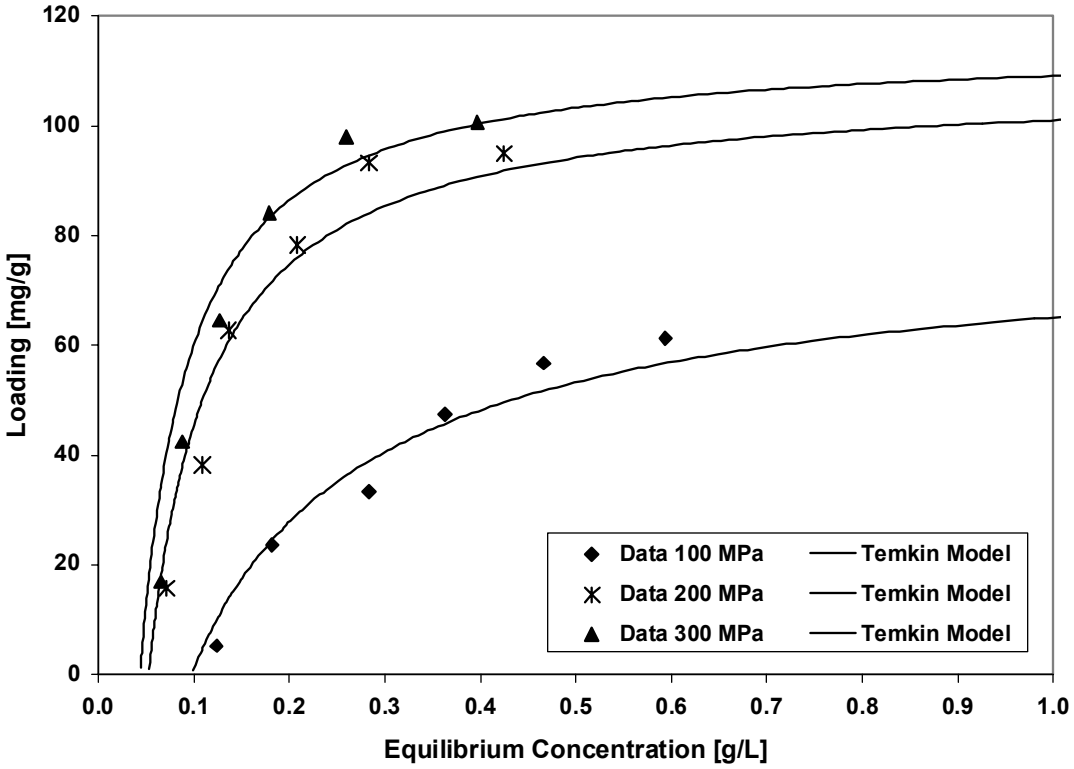

Fig. 11: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

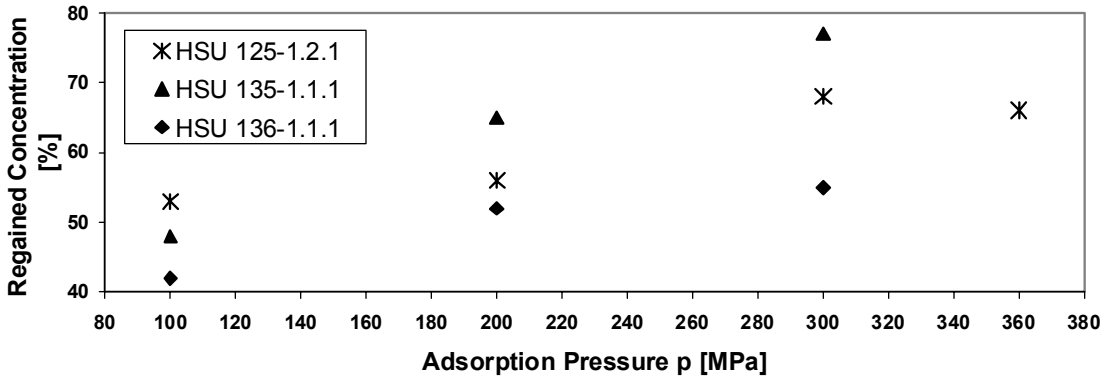

Fig. 12: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

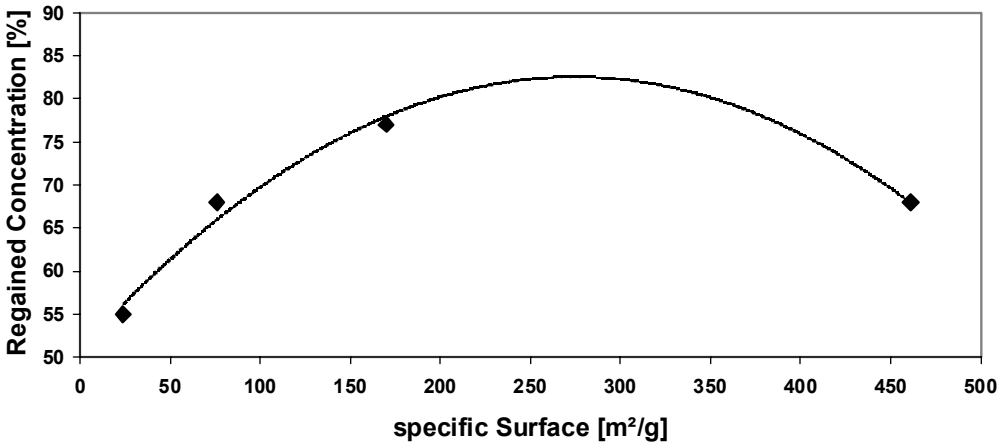

Fig. 13: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

Niemeyer - Jansen

Figure(s)

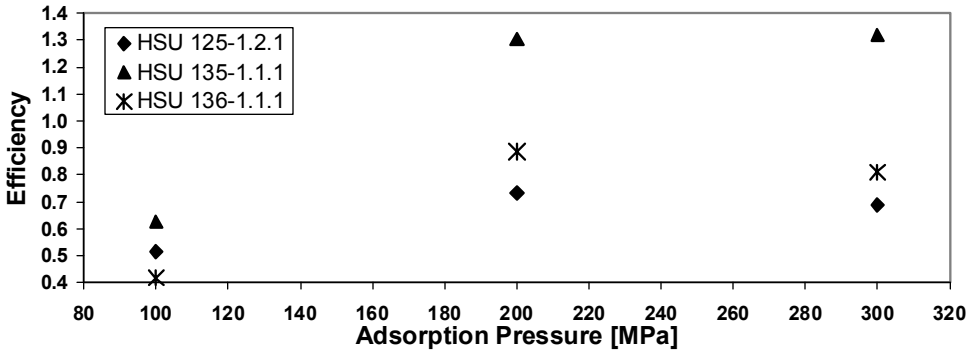

Fig. 14: An Innovative Approach for Sorptive Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

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Table(s)

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Table 1: Process parameters for the separation cycles

Adsorbent	Pressure [MPa]	Adsorbent mass [g]	Concen-tration [g/L]	Start of washing [mL]	Pressure reduction [mL]
HSU 125-1.2.1	0.8	0.709	0.290	N/A	N/A
	50	0.709	0.290	80	150-155
	100	0.705	0.290	120	190-200
	200	0.714	0.304	115	185-200
	300	0.712	0.294	126	245-275
	360	0.705	0.290	146	252-270
HSU 135-1.1.1	0.8	0.878	0.309	N/A	N/A
	100	0.878	0.309	90	157-168
	200	0.878	0.309	90	158-176
	300	0.878	0.309	88	155-179
	300	0.877	0.109	94	140-163
HSU 136-1.1.1	0.8	0.828	0.312	N/A	N/A
	100	0.828	0.312	92	162-170
	200	0.828	0.312	94	166-183
	300	0.828	0.312	95	167-190
	300	0.829	0.109	96	140-167

Table(s)

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Table 2: Fitted parameters for the high pressure isotherms

		Langmuir	Frumkin		Temkin	
Pressure [MPa]	max. Loading [mg/g]	b [L/g]	a_F	b [L/g]	a_T	b [L/g]
100	80	6.0	0.15	4.2	1.4	5.0
200	108	21	0.75	4.5	0.43	20
300	115	23	0.75	4.5	0.33	30

Cover Letter

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Einreichung eines Manuskriptes zur Publikation in der Zeitschrift *Journal of Supercritical Fluids*

Sehr geehrter Herr Kollege Brunner,

anbei reiche ich Ihnen anliegend das Manuskript in dreifacher Ausfertigung mit dem Titel:

Jan Jansen, Bernd Niemeyer

An Innovative Approach for Sorption Separation of Amphiphilic Biomolecules Applying High Hydrostatic Pressure

ein. Es handelt über die Beschreibung der experimentellen Ergebnisse zur Verfahrensentwicklung der sorptiven Stofftrennung aus komplexen verfahrenstechnisch bzw. biotechnologisch relevanten Stoffsystemen durch hohe Drücke. Da dies beides inhaltliche Schwerpunkte der Zeitschrift ***Journal of Supercritical Fluids*** sind, dachte ich an eine Veröffentlichung in dieser Zeitschrift. Die Entwicklungsarbeiten sind neu und noch an keiner anderen Stelle publiziert. Ich bitte Sie daher, das Manuskript im Zuge einer Begutachtung auf die Veröffentlichungsfähigkeit im zu prüfen/prüfen zu lassen und wäre Ihnen dankbar, wenn Sie bei positiver Rückmeldung die Publikations-Prozedur einleiten würden.

HERZLICHEN DANK!

Mit freundlichen Grüßen

Bernd Niemeyer

Anlage

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