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In situ determination of adsorption kinetics of proteins in a finite bath

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Abstract

A method for fast in situ measurement of adsorption kinetics based on a finite bath was developed. We modified the conventional finite bath by replacing the external loop by a dip probe which enables in situ measurement of the concentration change in the contactor. Deposition of adsorbent particles on the reflection surface of the dip probe compromised measurements. Different membranes, a polyamide, a polypropylene and a nylon membrane were tested to protect the internal reflection surface of the dip probe from fouling with adsorbent particles. The nylon membrane provided efficient protection and high mass transfer evaluated by response time experiments. Unspecific adsorption of the model protein on the membrane could also be excluded. To corroborate the measurements of the dip probe the results were compared to a conventional finite bath and to a shallow-bed. The uptake curves for human polyclonal IgG at different concentrationes (0.1–3 g/l) on rProtein A Sepharose FF and MabSelect were used as model system. The effective diffusion coefficients were determined using a pore diffusion model. These values were in good agreement for all methods.

Keywords: Adsorption kinetics; Adsorption isotherm; Dip probe; Finite bath; Shallow-bed; Immunoglobulin; Staphylococcal protein A

1. Introduction

Adsorption kinetics and equilibria are key parameters characterising a chromatography system [1]. A large panel of experimental methods have been reported to determine these parameters [2–5]. A key question is how fast this parameters can be estimated. Another criteria of applicability of such a method is the material consumption. In a lot of development steps the amount of material available for such studies is limited [6]. This is often the case during the development of a biotechnological product. Therefore, methods have to be developed which do not consume a lot of solute.

The simplest method for determination of adsorption kinetics and equilibiria is mixing adsorbent and adsorbate in a small vessel. After certain time intervals samples are drawn and analysed for solute concentration. From the concentration change with time the kinetics and equilibria can be estimated. The most common method is the frontal analysis where the amount of protein bound at a specified concentration is determined from the breakthrough curve [7]. In the perturbation method the column is initially equilibrated with solutes at a given concentration. Then a negative or positive perturbation of this background is introduced by injection of a small rectangular pulse of pure solvent or solutes [4,7]. Though these method show good results for the determination of isotherms, they are not suited for the determination of adsorption kinetics. The most common methods for determination of adsorption kinetics are the batch experiments where the compound and the adsorbent are presented in a stirred vessel and the concentration is monitored by continuously recirculating a small stream through a spectrometer. This experimental set-up is also known as batch contactor or finite bath. The disadvantage of this method is the response time due to the external fluid circuit [2,8]. Another experimental set-up is the shallow-bed which has been described by Boyd et al. [9] and successfully used to determine adsorption kinetics of ion-exchangers [10,11]. The shallow-bed is also known as zero length column. A small amount of adsorbent is placed in a column and the compounds are percolated through. The uptake of solute by the adsorbent is negligible small thus the concentration in the mobile phase is assumed to

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be constant. Therefore, the shallow-bed is characterised as an infinite bath. Other methods such as confocal laser scanning microscopy [3] monitor the in situ uptake of mixtures into chromatography particles. The extraction of reliable kinetic constants is still a problem and the method is often used in a qualitative way. There is a lack of in situ methods to probe the uptake of solutes by adsorbents.

In this work, we tried to develop a fast and simple in situ method for determination of adsorption equilibrium and kinetics. We improved the method of finite bath by replacing the external loop by a dip probe which enables in situ measurement of the concentration in the vessel. Therefore, we expected reduced response time. A further advantage is that several solutions can be measured in parallel reducing the time for measurement. In order to check if the modified system produces reliable estimates for adsorption kinetics the experimental data were compared to shallow-bed and conventional finite bath. For this purpose we used rProtein A Sepharose FF and MabSelect as adsorbent and human polyclonal IgG as solute.

2. Theory

As shown in prior work [12] biospecific adsorption in a finite bath is governed by film mass transfer, pore diffusion and the dynamics of the adsorption reaction. However, for the protein-A media it was shown by Horstmann and Chase [13] that the surface reaction is infinitely fast. Therefore, the model used for prediction of the adsorption curves considered only film mass transfer and pore diffusion whereas the concentration of the mobile and the stationary phase are assumed to be in instantaneous equilibrium.

The conservation equation and the initial condition for the bulk fluid are given as:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -\frac{3k_\mathrm{f}}{R_\mathrm{p}}\frac{V_\mathrm{m}}{V}(C - c|_{r=R_\mathrm{p}}) \tag{1}$$

$$t = 0, \quad C = C_0 \tag{2}$$

with C as the concentration in the bulk fluid, k_f the external film mass transfer coeffcient, R_p the particle radius, V_m the volume of particles, V the volume of the bulk fluid and $c|_{r=R_\mathrm{p}}$ as the concentration of the mobile phase at the particles surface. C_0 is the initial concentration of the bulk fluid.

The transport within a spherical particle is described using:

$$\varepsilon_\mathrm{p}\frac{\partial c}{\partial t} + (1-\varepsilon_\mathrm{p})\frac{\partial q}{\partial t} = \frac{D_\mathrm{e}}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c}{\partial r}\right) \tag{3}$$

$$t = 0, \quad c = 0, \quad q = 0 \tag{4}$$

$$r = 0, \quad \frac{\partial c}{\partial r} = 0 \tag{5}$$

$$r = R_\mathrm{p}, \quad D_\mathrm{e}\frac{\partial c}{\partial r} = k_\mathrm{f}(C - c) \tag{6}$$

with c as the concentration in the mobile phase of the particle and q as the concentration in the stationary phase of the particle. The particle porosity is given as ε_p and the effective pore diffusion coefficient as D_e.

Due to the fast adsorption of IgG to protein A media [13] the equilibrium between the mobile and stationary phase concentration can be described using a favourable isotherm, e.g. a Langmuir isotherm:

$$q = \frac{q_\mathrm{m}K_\mathrm{a}c}{1 + K_\mathrm{a}c} \tag{7}$$

The Langmuir isotherm is described using the maximum binding capacity q_m and the Langmuir isotherm equilibrium parameter K_a.

The model described by Eqs. (1)–(7) presents two unknown variables of the system, the external film mass transfer coefficient and the effective pore diffusion coefficient. Weaver and Carta [8] showed that the estimation of the external film mass transfer coefficient for a suspension in a finite bath using a correlation of Armenante and Kirwan [14] is in good agreement with the value estimated from experiments with low protein concentration. This correlation (Eq. (8)) uses the power input of the stirrer P, the diameter of the chromatography particles d_p, the kinematic viscosity ν and the Schmidt number Sc.

$$Sh = 2 + 0.52\left(\frac{P^{1/3}d_\mathrm{p}^{4/3}}{\nu}\right)^{0.52} Sc^{1/3} \tag{8}$$

With an power input, P of approximately 450 $\mathrm{cm}^2/\mathrm{s}^3$ we derived an external film mass transfer coefficient of 0.0009 cm/s for MabSelect and rProtein A Sepharose FF. Do and Rice [15] showed that for negligible surface diffusion the Biot number $Bi = k_\mathrm{f}R_\mathrm{p}/D_\mathrm{e}$ is typically larger unity and external mass transfer is therefore negligible. It has been discussed at several scientific conferences that surface diffusion is extremely difficult to explain for macromolecules such as proteins. Therefore, we assumed that the external mass transfer was of little interest for our models. Additional data from shallow-bed experiments [16] was evaluated for comparison. The same model (Eqs. (1)–(7)) can be applied as for finite bath experiments though for shallow-bed the volume of the media V_m is infinitely small compared to the volume of the protein solution V, simplifying the conservation equation for the bulk fluid (Eq. (1)) to:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = 0 \tag{9}$$

For the calculations using the data from the shallow-bed the following correlation was used for calculation of the Sherwood number:

$$Sh = 1.15\left(\frac{Re}{\varepsilon_\mathrm{p}}\right)^{1/2} Sc^{0.33} \tag{10}$$

using the Reynolds number Re, the particle porosity ε_p and the Schmidt number Sc.

For nonlinear isotherms the above model needs a numerical solution. This was obtained using orthogonal collocation on finite elements [17,18]. All simulations were carried out using MatLab 6.5 Release 13 (MathWorks).

3. Materials and method

3.1. Adsorbents and proteins

The resins, rProtein A Sepharose FF and MabSelect, were purchased from Amersham Biosciences (Uppsala, Sweden). Both have a matrix of highly cross-linked agarose with an estimated intraparticle void fraction ε_p of 0.96 for the rProtein A Sepharose FF and 0.9 for the MabSelect. The particle size ranges from 45 to 165 μm with an mean particle diameter of 90 μm [19] for the rProtein A Sepharose FF and from 40 to 130 μm with an mean particle diameter of 85 μm for the MabSelect. The polyclonal IgG solution (Octagam, 5%) was a gift from Octapharma (Vienna, Austria). The diluting and working buffer was phosphate buffered saline (PBS) composed of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4.

3.2. Finite bath experiments

Finite bath experiments were carried out for determination of adsorption equilibrium and kinetics. For all experiments 30 ml of an IgG solution of known concentration (0.1–3 g/l) was transferred in a test tube and mixed by rotation. After addition of approximately 0.06 ml of pre-equilibrated adsorbent samples were drawn at different time intervals, centrifuged and the protein concentration in the supernatant quantified employing UV 280 measurements. The last measurements, drawn after about 20 h, were used for determination of the parameters of the Langmuir equilibrium isotherm.

3.3. Protection of the dip probe and response experiments

For stable measurements the dip probe had to be protected excluding the adsorbent particles from the measurement gap. This was achieved using filters made of polyamide (Amersham, Uppsala, Sweden) with a pore diameter of 10 μm, polypropylene (Amersham, Uppsala, Sweden) with an pore diameter of 23 μm or nylon (Millipore, Bedford, USA) with a pore diameter of 41 or 60 μm, respectively. The filters were welded to tubes that covered the dip probe and were fixed using an elastic band. In order to evaluate the increase in response time of the dip probe due to the filters an IgG solution was provided in a vessel and stirred at different velocities (300–600 rpm) using a hanging stirrer (FishClip, Buerkle, Lörrach, Germany). At specified time steps the solution was diluted and the time to reach equilibrium determined. The measurements were compared to measurements with an unprotected dip probe. The lowest velocity where the response time was comparable to the response time of the unprotected system was used for the measurements.

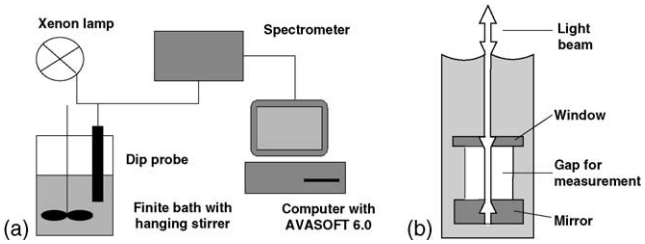

Fig. 1. The experimental set-up (a) for finite bath measurements with a dip probe (b).

3.4. Dip probe measurements in a finite bath

For the experiments 30 ml of an IgG solution of known concentration (0.1–3 g/l) was provided in a vessel stirred at 300–600 rpm by an hanging magnetic stirrer (FishClip, Buerkle, Lörrach, Germany) to reduce the shear forces applied on the proteins. The dip probe (FDP7UV200-2-ME, Avantes, Eerbeek, The Netherlands) was then placed in the solution and connected to a light source (AvaLight-DHS Deuterium Halogen Light Source, Avantes, Eerbeek, The Netherlands) and a spectrometer (AvaSpec-2048, Avantes, Eerbeek, The Netherlands). The data were transferred from the spectrometer to the computer where it was processed and saved using AVASOFT 6.0 (Avantes, Eerbeek, The Netherlands). The data could then be extracted as ASCI-files and used for further evaluation. Additional to the measurements with the dip probe samples were drawn from the suspension, centrifuged to separate the adsorbent and the concentration in the supernatant evaluated using UV 280 measurements. These measurements were used to check the mass transfer through the protection filter of the dip probe.

4. Results and discussion

The core part of the new finite bath for determination of adsorption kinetics is a dip probe which has been inserted into a stirred vessel. The schematic drawing of the experimental set-up is shown in Fig. 1. A light beam coming from the xenon lamp passes through the window of the dip probe as illustrated in Fig. 1b. The fluids to be measured flow constantly through the gap. The light beam is reflected by a mirror, passes the gap a second time and the intensity of the light beam is measured in a spectrometer. A computer is used for data storage and processing. As seen in Fig. 1b the optical path is twice the gap length which can be freely positioned. A decrease of gap length corresponds to a dilution of the fluid measured. Goal of our work was comparison of the estimated parameters (either kinetic or equilibrium) with other methods which have been frequently used for the same purpose to corroborate the obtained results.

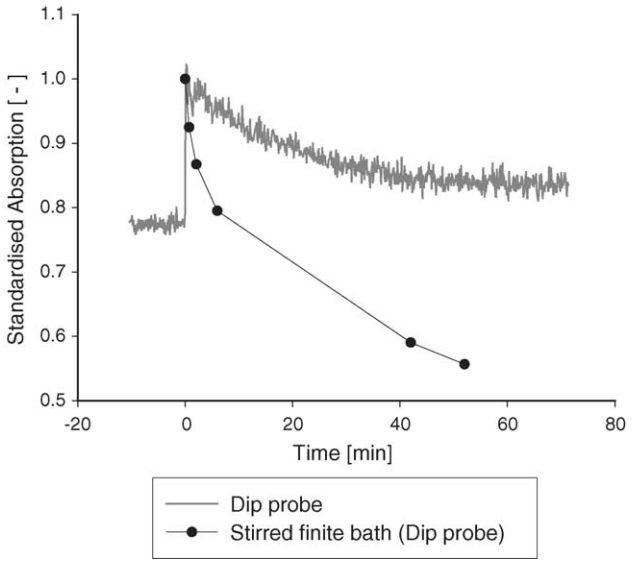

Fig. 2. Comparison of measurement with dip probe (solid grey line) to finite bath experiments (circles linked with solid line). The standardised absorption was determined with reference to the absorption of the suspension immediately after addition of the adsorbent.

For initial studies the dip probe was placed in the vessel and after reaching a constant baseline a known amount of pre-equilibrated adsorbent was added. A leap of absorption was observed in the dip probe measurements after addition of adsorbent. This is explained by the light scattering and absorption of the particles introduced into the system (Fig. 2). Furthermore, in the dip probe experiment equilibrium was reached much earlier at a higher absorption than for the finite bath experiments. Consequently different pore diffusion coefficients calculated according to Eqs. (1)–(7) were obtained. We observed that particles were deposited on the reflection surface of the dip probe further increasing the absorption. To avoid this effect the dip probe was protected by a filter, which excluded a major fraction of the adsorbent particles, while maintaining fast mass transfer. Three materials with hydrophilic character were tested; a polypropylene, a polyamide and a nylon filter, respectively. First response tests as described in the experimental section were carried out at different stirrer velocities. After comparison of the response time to the response time of the system with an unprotected dip probe the stirrer velocity necessary for the protected dip probe was determined. To check unspecific binding the filters were incubated with different concentrations of human polyclonal IgG (0.1–3 g/l). Though unspecific binding of IgG has been described for nylon membranes [20] we could not observe a decrease in IgG concentration. We therefore, assumed that unspecific binding of IgG to the membranes did not occur or was negligible small.

Polypropylene and polyamide filters are used in the construction of chromatography columns to retain the resins packed in the column. Due to the large pore size of the filters we assumed good mass transfer properties for these materials. The polyamide filter with a pore diameter of 10 μm showed a

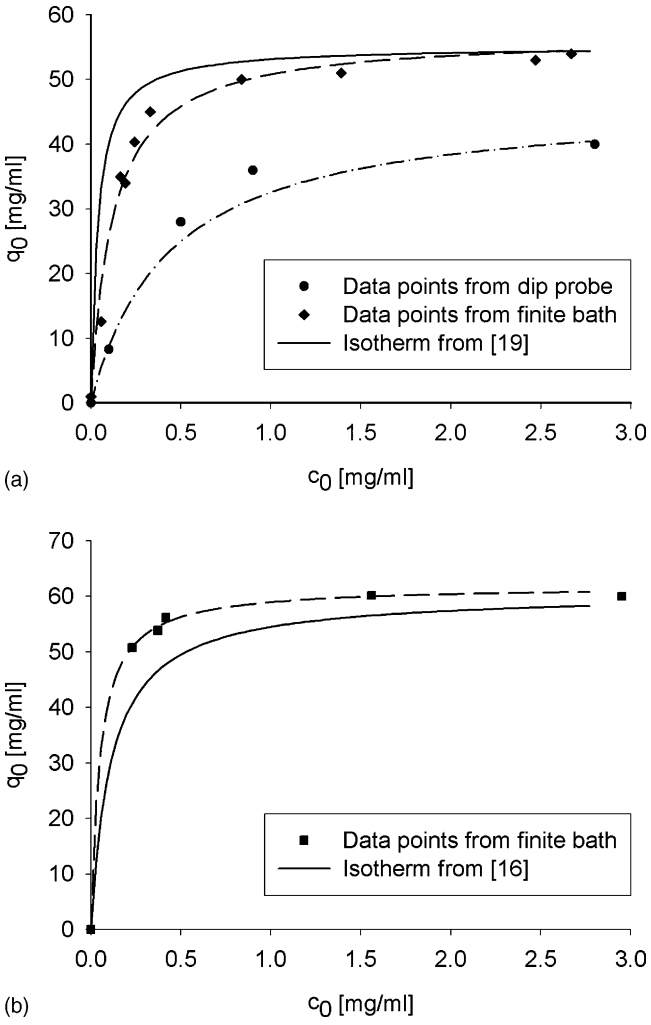

Fig. 3. Adsorption isotherms for rProtein A Sepharose FF (a) and MabSelect (b) for polyclonal IgG. (a) Data points derived from finite batch experiments (diamonds) and dip probe measurements (circles) are fitted (dashed lines) and compared to data from literature (solid line) [19]. (b) Data points from finite batch experiments (squares) are fitted (dashed line) and compared data from literature (solid line) [19].

faster mass transfer than the polypropylene filter with a pore diameter of 23 μm (data not shown). We assumed that this was due to higher hydrophobicity of polypropylene. However, for the polyamide filter a stirrer velocity of 600 rpm was necessary to have a mass transfer comparable to a system with an unprotected dip probe. High shear forces induced aggregation of IgG and this resulted in false pore diffusion coefficients. We estimated the equilibrium concentrations in mobile and stationary phase for different concentrations of IgG on rProtein A Sepharose FF from a set of uptake curves and fitted those to the Langmuir isotherm (Eq. (7)). We also obtained the isotherms for IgG on rProtein A Sepharose FF and MabSelect from finite bath experiments (Fig. 3). However, the isotherm for rProtein A Sepharose FF derived from the dip probe measurements deviates significant from the isotherms derived from finite bath experiments or literature [19]. Small deviations of the fitted isotherm from the finite

Table 1
Parameters for Langmuir isotherm fitted from the data points given from finite bath and dip probe measurements

	rProtein A Sepharose FF		MabSelect	
	q_m (mg/ml)	K_a (ml/mg)	q_m (mg/ml)	K_a (ml/mg)
Finite bath	56.9 ± 1.8	8.3 ± 1.8	61.9 ± 0.5	20.0 ± 1.7
Literature [16,19]	55.1 ± 1.5	27.0 ± 5.1	60.6 ± 1.3	8.9 ± 0.8
Dip probe	46.6 ± 2.9	2.3 ± 0.6		

bath and the isotherms from literature [19] were probably due to incorrect measurement of the amount of adsorbent added. The data of the isotherms are given in Table 1. We assumed that the problem for the dip probe measurement was due to mixing by agitation. This hypothesis was proven by comparing the curves to finite bath experiments where the suspension was mixed by end-over-end action. In Fig. 4, we compared the curves from the stirred finite bath using the continuous measurements from the dip probe and single measurements from centrifuged samples measured in a photometer with the measurements from a rotating finite bath. The adsorption of a 3.5 mg/ml IgG solution onto rProtein A Sepharose FF was measured. For the first 30 min both curves from the stirred finite bath, the dip probe and the single measurement curve were in fairly good agreement. Only after 60 min the absorbance of the dip probe system increased, probably due to deposition of adsorbent or more likely aggregates of IgG onto the reflection surface of the dip probe. However, adsorption of IgG onto the glass of the reflection surface can neither be excluded. For the end-over-end system the absorbance curve showed a stronger and longer decrease than for the curves from the stirred finite bath. We then calculated the capacity for the rProtein A Sepharose FF from the estimated final protein concentration to about 44 mg/ml for the dip probe and to about 54 mg/ml for the end-over-end system. Comparing these results to the maximum binding capacity of rProtein A Sepharose FF (55 mg/ml) we concluded mixing by agitation posed a problem for the measurement with IgG. However, from Fig. 4 we also concluded that measurements of IgG adsorption curves with the dip probe protected by a 10 μm polyamide filter should be stable up to 30 min. We therefore, assumed that fitting the effective diffusion coefficient to the adsorption curves measured for up to 30 min should lead to the same results as the fits for a rotating bath. In further experiments, nylon membranes with pore diameters of 41 or 60 μm, respectively were checked for their response time. As seen in Fig. 5 the 60 μm membranes showed better mass transfer rates than the 41 μm filters allowing a stirrer velocity of 300 rpm. However, due to the particle size distribution of the adsorbents only 41 μm membranes were used. Nevertheless, they showed better mass transfer efficiency than the polyamide or polypropylene filters although they also required a high stirrer velocity of approximately 450 rpm. Due to this velocity it was assumed that the stability of IgG was also only given for approximately 30 min. This was verified by superimposing curves from the dip probe system and the stirred finite bath experiments with the rotating finite bath experiments (Fig. 6). Twenty to thirty minutes after addition of adsorbent a deviation can be already observed.

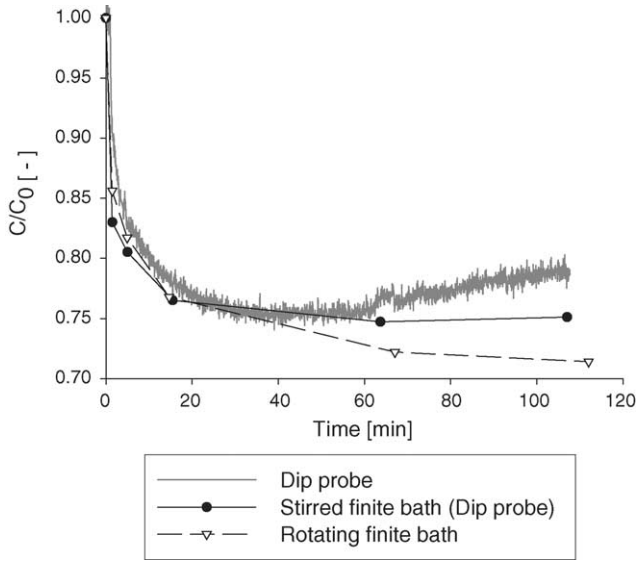

Fig. 4. The dip probe curve protected with the 10 μm polyamide filter (grey line), the single measurements from the single samples drawn from the dip probe vessel (circles with solid line) and the finite bath experiments using end-over-end action (inverse triangles with dashed line) are compared.

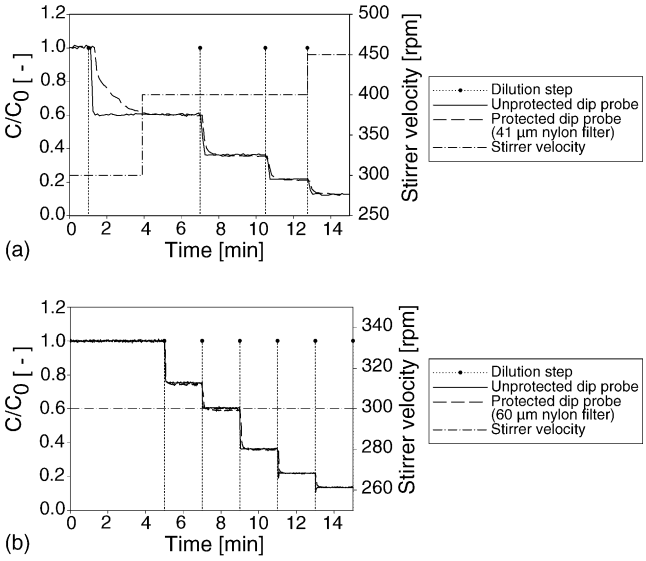

Fig. 5. The response time of the 41 μm nylon filter (a, dashed line) and the 60 μm nylon filter (b, dashed line) is compared to the response time of the unprotected system (a and b, solid line). The dilution steps are indicated by the dotted vertical lines.

Adsorption curves presented Figs. 7 and 9 were derived from a dip probe protected by a 41 μm nylon filter, from samples drawn from the agitated dip probe vessel and from a finite bath system with end-over-end mixing. The samples drawn from the dip probe vessel were used for checking the mass transfer through the filters into the dip probe and the measurement using the end-over-end mixing was used for

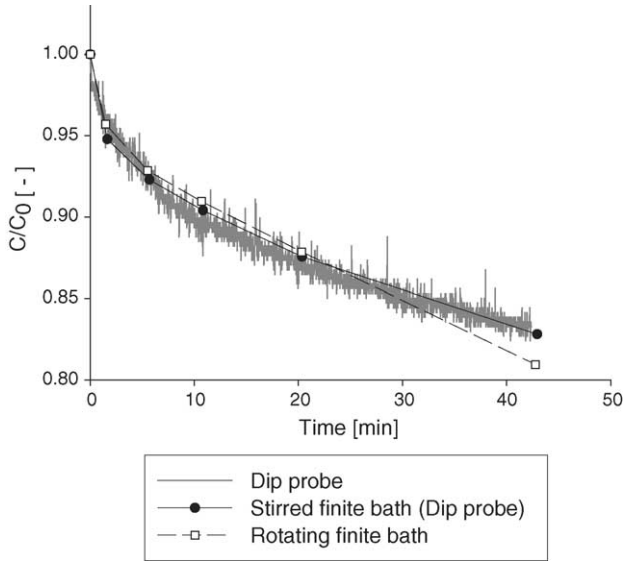

Fig. 6. The curve from the dip probe protected with the 41 μm nylon membrane (grey solid line), the samples drawn from the dip probe vessel (squares and dashed line) and the finite bath experiments (circles with solid line) are compared.

comparison to the system with a magnetic stirrer. A magnetic stirrer generates higher shear forces. As for the 10 μm polyamide filter also for the 41 μm nylon filter IgG stability was assumed to be only given up to 30 min. Nevertheless, it was possible to determine the effective diffusion coefficient. Better results might be achieved if the mixing technique could be improved. Fig. 7 shows adsorption curves for

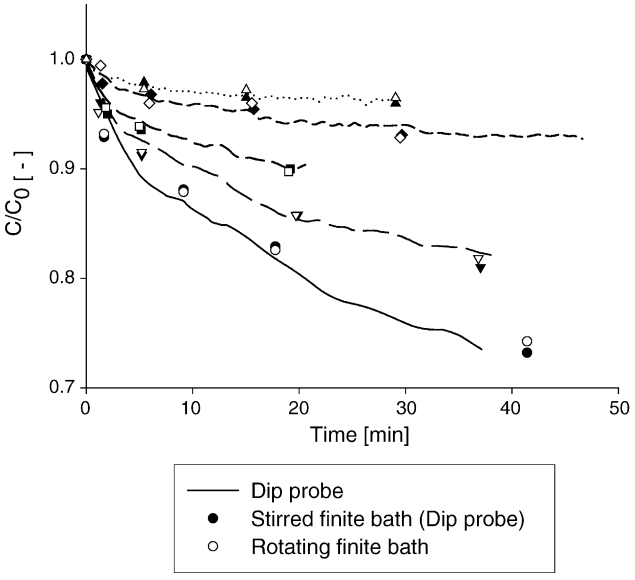

Fig. 7. Smoothed dip probe curves (lines), measurement points from single samples from dip probe vessel (filled symbols) and measurement points from the finite bath experiments (white symbols) are depicted for different concentrations of IgG onto rProtein A Sepharose FF. A 0.1 g/l IgG (solid line or circle), 0.25 g/l IgG (long dashed line or inverse triangle), 0.5 g/l IgG (medium dashed line or squares), 1 g/l IgG (short dashed line or diamonds) and 3 g/l (dotted line or triangles).

Table 2
Effective pore diffusion coefficient (D_e) for IgG on rProtein A Sepharose FF as calculated from the experimental data for different IgG concentrations

Concentration (mg/ml)	Dip probe D_e (cm^2/s)	Finite bath[a] D_e (cm^2/s)	Finite bath D_e (cm^2/s)
3.00	3.30E−08	3.00E−08	3.00E−08
1.50	3.80E−08	3.90E−08	3.80E−08
1.00	4.10E−08	4.00E−08	4.00E−08
0.50	4.00E−08	3.90E−08	3.90E−08
0.25	6.50E−08	6.50E−08	6.50E−08
0.10	1.40E−07	1.00E−07	1.00E−07

Data from shallow-bed is not presented here.
[a] Samples drawn from dip probe vessel.

rProtein A Sepharose FF for different protein concentrations (0.1–3 g/l) comparing the different methods. The methods showed good agreement though for 0.5 g/l (medium dashed line or squares) stability of the IgG solution was only given up to 20 min whereas for the 1 g/l solution (small dashed line or diamonds) a longer stability was achieved. Using the estimated external mass transfer coefficient the curves were fitted using Eqs. (1)–(7) and the effective diffusion coefficient was estimated. The values derived from the dip probe curves are given in Table 2 and concentration dependency is depicted in Fig. 8. Comparison to conventional methods, such as finite bath experiments or shallow-bed data has also been conducted [16]. A good agreement for the experimental values could be observed.

The adsorption curves for different IgG concentration for MabSelect were depicted in Fig. 9 for different methods. Also in this case stability of the dip probe measurement was given

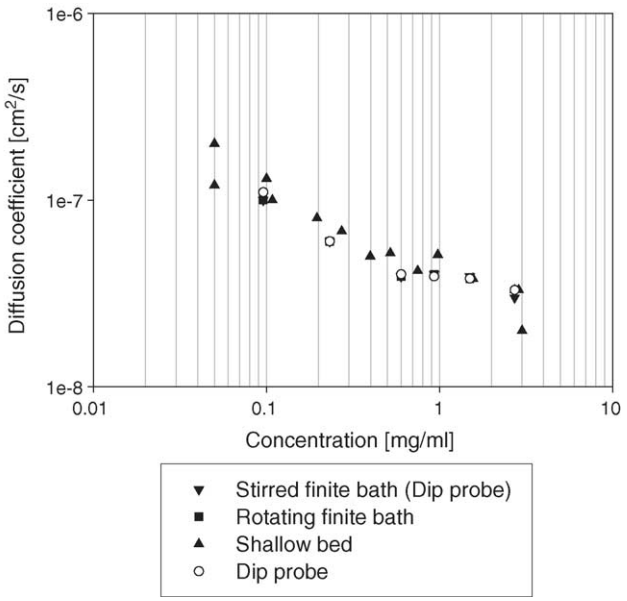

Fig. 8. Comparison of effective diffusion coefficients for IgG (0.1–3 g/l) onto rProtein A Sepharose FF derived from measurements using the dip probe (white circles) and samples drawn from the dip probe vessel (filled inverse triangle), shallow-bed measurements (filled triangles) and measurements with a finite bath with end-over-end agitation (filled squares).

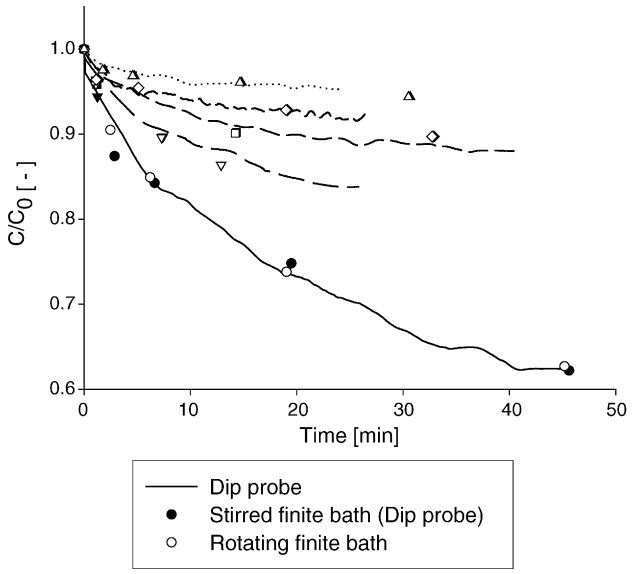

Fig. 9. Smoothed dip probe curves (lines), measurement points from single samples from dip probe vessel (filled symbols) and measurement points from the finite bath experiments (white symbols) are depicted for different concentrations of IgG onto MabSelect. 0.1 g/l IgG (solid line or circle), 0.25 g/l IgG (long dashed line or inverse triangle), 0.5 g/l IgG (medium dashed line or squares), 1 g/l IgG (short dashed line or diamonds) and 3 g/l (dotted line or triangles).

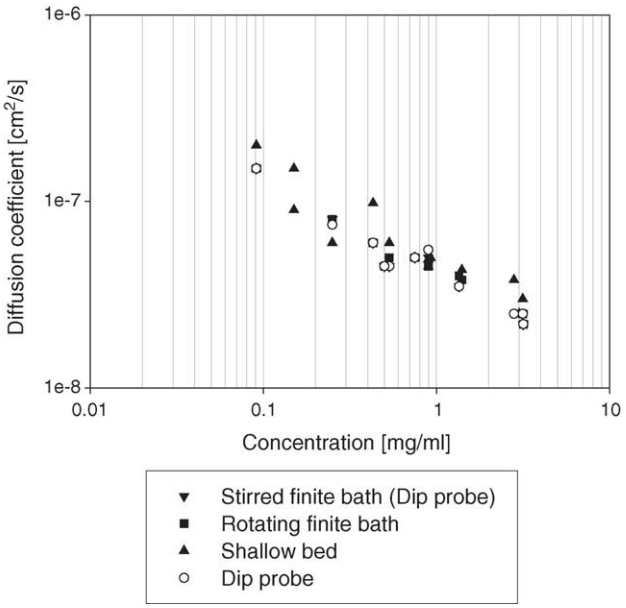

Fig. 10. Comparison of effective diffusion coefficients for IgG (0.1–3 g/l) onto MabSelect derived from measurements using the dip probe (white circles) and samples drawn from the dip probe vessel (filled inverse triangle), shallow-bed measurements (filled triangles) and measurements with a finite bath with end-over-end agitation (filled squares).

for maximal 30 min. For some concentrations, 0.1 g/l IgG (solid line) and 0.5 g/l IgG (medium dashed), the dip probe curves were stable over 30 min. The estimated effective diffusion coefficients were given in Table 3 and were compared to conventional methods (Fig. 10). As shown Figs. 8 and 10 the estimated effective diffusion coefficients were for all methods dependent on the concentration of the protein solution. This is due to the model chosen for parameter estimation since the main factor contributing to changes in diffusion is the pore diffusion coefficient. However, it has been discussed [8,21] that for decreasing concentration the contribution of the external mass transfer increases. Weaver and Carta [8] introduced a dimensionless group for estimation of the importance of external or intraparticle mass transfer resistance. According to the mechanism of mass transfer control two different analytical solutions can be applied. Another method [21] introduces a factor for the thickness of the adsorption layer enabling a continuous solution of the pore diffusion model. A different approach was introducing an apparent effective diffusion coefficient where the results are explained in terms of parallel surface and pore diffusion. However, it has been suggested that surface diffusion is of little interest for macromolecules like proteins. We fitted our data nevertheless using the pore diffusion model as introduced above accepting the concentration dependency of the pore diffusion coefficient. We did furthermore not account for the viscosity difference caused by the protein concentration.

Table 3
Effective pore diffusion coefficient (D_e) for IgG on MabSelect as calculated from the experimental data for different IgG concentrations

Concentration (mg/ml)	Dip probe D_e (cm^2/s)	Finite bath[a] D_e (cm^2/s)	Finite bath D_e (cm^2/s)
3.10	2.50E−08	2.50E−08	2.50E−08
1.40	3.50E−08	3.50E−08	4.00E−08
1.00	5.50E−08	5.00E−08	5.00E−08
0.40	6.00E−08	6.00E−08	6.00E−08
0.25	7.30E−08	7.30E−08	7.30E−08
0.10	2.00E−07	2.00E−07	2.00E−07

Data from shallow-bed is not presented here.
[a] Samples drawn from dip probe vessel.

It could be shown that a dip probe for continuous in situ determination of adsorption kinetics in a finite bath must be used with caution. For proteins with a high tendency of aggregation such as IgG several adjustments had to be made. These adjustments were necessary in all finite bath experiments of the same experimental set-up using a magnetic stirrer. A further problem arises from the low mass transfer of IgG through the protective filter of the dip probe. The transport of IgG through the mesh is most likely due to convection driven by shear and turbulence in the agitated vessel. Therefore, it was necessary to apply high stirrer velocities increasing the tendency of IgG to aggregation. Still it was possible to derive the effective diffusion coefficients whereas the equilibrium data had to be estimated using batch experiments.

5. Conclusion

One of the biggest advantages of this method was, besides measuring the adsorption kinetics in situ, that in our

set-up up to six dip probes could be applied for measuring different protein concentrations or buffer conditions in parallel. The use of fibre optic multiplexers, splitting the light emitted from the UV-lamp or the light returned to the spectrometer, can increase the number of dip probes significantly. This makes it an extremely time-saving and rapid method for the determination of adsorption kinetics.

In our experimental set-up we were limited to a minimal volume of 30 ml due to the size of the stirrer and the size of the dip probe. Besides the possibility of using commercially available smaller dip probes and a smaller stirrer the principle of this method, continuous measuring of the protein concentration using fibre optics, can be adapted to even smaller scale. A further target is the adaptation of this method for multi-component adsorption measurements. This should be achieved by labelling the proteins with fluorescent dyes and measuring the fluorescence at different wavelength in parallel. This enables rapid in situ determination of multi-component adsorption isotherms.

6. Nomenclature

Bi	Biot number ($k_f R_p/D_e$)
c	concentration in fluid phase of particle [mg/ml]
C	concentration in solution [mg/ml]
C_0	initial concentration [mg/ml]
d_p	particle diameter [cm]
D	molecular diffusion coeffcient [cm^2/s]
D_e	effective pore diffusion coefficient [cm^2/s]
k_f	external film mass transfer coefficient [cm/s]
K_a	Langmuir isotherm equilibrium parameter [ml/mg]
P	power input per unit mass of fluid [cm^2/s^3]
q	concentration in solid phase of particle [mg/ml]
q_m	Langmuir isotherm equilibrium parameter [mg/ml]
r	particle radial coordination [cm]
Re	Reynolds number ($d_p \varepsilon v/\nu$)
R_p	particle radius [cm]
Sc	Schmidt number (ν/D)
Sh	Sherwood number ($k_f d_p/D$)
t	time [s]
v	interstitial velocity [cm/s]
V	colume of solution [ml]
V_m	volume of particles [ml]

Greek letters

ε	void fraction of packing
ε_p	intraparticle void fraction
ν	kinematic viscosity [cm^2/s]

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