Albumin-Bound Toxin Removal in Liver Support Devices: Case Study of Tryptophan Adsorption and Dialysis

M. Cristina Annesini, Vincenzo Piemonte, Luca Turchetti
Department of Chemical Engineering, University of Rome "La Sapienza"
via Eudossiana 18, 00184 Roma (Italy)

Dialysis and adsorption processes are commonly implemented in liver support devices for the removal of albumin-bound toxins such as tryptophan. In this work, dialysis and adsorption of tryptophan from albumin-containing solutions were investigated; in particular, the effect of albumin concentration on both processes was analysed. Theoretical models accounting for albumin-tryptophan binding were proposed and fitted to the experimental data in order to obtain equilibrium and kinetic parameters.
The results allowed to perform a simulation of an albumin-dialysis liver support device session and compare the performance of different dialysate recirculation/regeneration policies.

1. Introduction

Acute and acute-on-chronic liver failure are associated with high plasmatic levels of toxins that are responsible for secondary life-threatening multi-organ pathologies. In such clinical conditions, liver support devices aimed at removing toxins from blood offer a temporary solution, helping to keep patients alive in wait of a recovery of liver functionality or an organ transplantation (Stange et al., 2002).
Toxins not cleared by the failing liver include both small water-soluble molecules and hydrophobic molecules that are tightly bound to plasma proteins. While a conventional dialysis treatment can remove selectively the former class of toxins, more complex processes are required to remove molecules of the latter type. At present, the most widely used artificial liver devices are the Single Pass Albumin Dialysis (SPAD) (Sauer et al., 2004), MARS (Mitzner et al., 2001) and Prometheus (Rifai et al., 2003) systems. Schemes of these devices are reported in figure 1. Although several clinical studies on the performance of these apparatus are available, until now only few works have been devoted to the analysis of the fundamental phenomena involved in the detoxification processes, while this information could help in designing and optimizing these systems. In spite of the differences between clinically used liver support devices, all of them are based on membrane separation and adsorption processes. Modelling of these operations is a well consolidated knowledge of chemical engineering and the evaluation of the performance of these apparatus can be confidently carried out once equilibrium conditions and transport phenomena kinetics are known.
In order to obtain such information, in this work tryptophan removal from albumin-containing solutions is investigated. Tryptophan is an aromatic amino acid relevant for the onset of hepatic coma; it can form complexes with albumin, even if the binding

constant (10^4-10^5 M^{-1}, as reported by McMenamy et al., 1958, and Sun et al., 1993) is largely lower than that of other hepatic toxins such as bilirubin.
In the first part of this work, dialysis and adsorption process are considered separately: as for dialysis, the effect of albumin as tryptophan binder in the dialysate is investigated; as for adsorption, equilibrium conditions and fixed-bed adsorption kinetics on two different adsorbents (activated carbon and polymeric non-ionic resin) are reported. In the second part, theoretical models of the dialysis and adsorption units are used to simulate a complete liver support device session.

2. Experimental

Materials and Methods

Bovine serum albumin (Cohn fraction V, MW=66000) and L-Tryptophan (MW=204) were purchased from Sigma-Aldrich (St.Louis, MO, USA); activated carbon for gas chromatography 05112 (Fluka Chemie GmbH, Buchs, Switzerland) and polymeric resin Lewatit VP OC 1064 (Bayer AG, Leverkusen, Germany) were used as adsorbents. All the solutions were prepared in phosphate buffer 0.15 M at pH 7.4.
Analysis of tryptophan solutions was carried out by an UV-VIS spectrophotometer (Perkin Elmer Lambda 25) at 279 nm or, in presence of albumin, by HPLC with a Spherisorb ODS2 (Agilent Technologies, Santa Clara, CA, USA) column and an UV detector set at 279 nm (Annesini et al., 2005, 2007).

Dialysis

Dialysis experiments were carried out in a two compartment closed loop system, using a hollow fibre membrane module (Fresenius F40): 300 ml of an albumin-tryptophan solution (hitherto referred to as "feed") was recirculated at constant flow rate (150 ml/min) in the fibre-side of the dialysis module; 300 ml of dialysis solution (buffer solution or albumin-containing solution, hitherto referred to as "dialysate") was recirculated at the same flow rate in countercurrent in the shell-side of the membrane

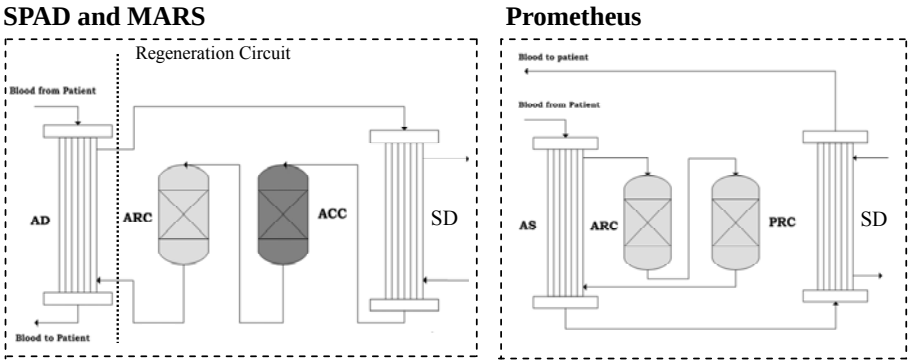

Figure 1: Artificial liver systems (*AD: albumin dialyser; KD: secondary dialyser; ARC: anionic resin column, ACC: activated carbon column; PRC: polymeric non-ionic resin column; AS: plasma fractionator). In SPAD, the albumin dialysis solution is discarded, while in MARS it is continuously regenerated and recycled to the membrane module; in Prometheus adsorption is performed directly after filtration of the albumin fraction on a polysulphone membrane.*

module. Samples were collected at the fibre and shell outlet and analysed at different times, to determine tryptophan and albumin concentrations. The transmembrane pressure was controlled in order to have a null net water flow across the membrane. In all the experiments, absence of albumin transfer across the membrane and tryptophan adsorption on the membrane was checked by a mass balance.

Adsorption

Adsorption equilibrium experiments were carried out contacting 40 ml of albumin-tryptophan solution with different amounts of adsorbent in stirred flasks for 16 hours; then the suspension was settled and the supernatant filtered and analysed. Preliminary experiments showed that no significant changes in the liquid concentration occur after this contact time.

Kinetic experiments were carried out in a fixed bed column (10 mm ID, 60 mm bed length) at 25±0.5°C, with a constant flow-rate tryptophan-albumin feed. Samples were collected at regular time intervals at the column outlet and analysed.

3. Dialysis process

The influence of albumin concentration both in feed (fibre-side) and dialysate (shell-side) on the membrane module clearance was investigated. Figure 2a reports the decrease in tryptophan concentration in the albumin-containing feed, obtained with a traditional dialysis process (albumin-free dialysate); it is evident that the higher the albumin concentration the lower the tryptophan clearance. This result is easily explained by tryptophan-albumin binding considerations: albumin in feed reduces the free tryptophan concentration gradient across the membrane, that is the true driving force of the dialysis process, since only free tryptophan can cross the membrane.

Addition of albumin to the dialysis solution (albumin dialysis process) results in a significant increase in tryptophan clearance, as shown in Figure 2b. Clearly, in this case, the higher the albumin concentration in the dialysis solution, the lower the free tryptophan concentration in the dialysate and the higher the dialysis driving force. This result agrees with similar findings reported in the literature, referring both to *in vivo* and *in vitro* experimental evidence (Stange et al., 1993), confirming that the presence of albumin in the dialysate enhances albumin-bound toxin transfer across the membrane. Therefore, albumin dialysis is indeed a more efficient process for albumin-bound toxin removal that traditional dialysis.

A simple model of the albumin dialysis process has been previously proposed by Patzer (2006), accounting for albumin-toxin equilibrium in the liquid phases and free-toxin diffusion across the membrane. According to this model, the solute mass balances in the feed-side and in the shell-side solutions are given by

$$\frac{\partial c_{t,f}}{\partial t}\left(1+\frac{k_{eq}c_{alb,f}}{\left(1+k_{eq}c_{t,f}\right)^2}\right)=-v_f\frac{\partial c_{t,f}}{\partial z}\left(1+\frac{k_{eq}c_{alb,f}}{\left(1+k_{eq}c_{t,f}\right)^2}\right)-a_fK_c\left(c_{t,f}-c_{t,s}\right) \quad (1)$$

$$\frac{\partial c_{t,s}}{\partial t}\left(1+\frac{k_{eq}c_{alb,s}}{\left(1+k_{eq}c_{t,s}\right)^2}\right)=-v_s\frac{\partial c_{t,s}}{\partial z}\left(1+\frac{k_{eq}c_{alb,s}}{\left(1+k_{eq}c_{t,s}\right)^2}\right)-a_sK_c\left(c_{t,f}-c_{t,s}\right) \quad (2)$$

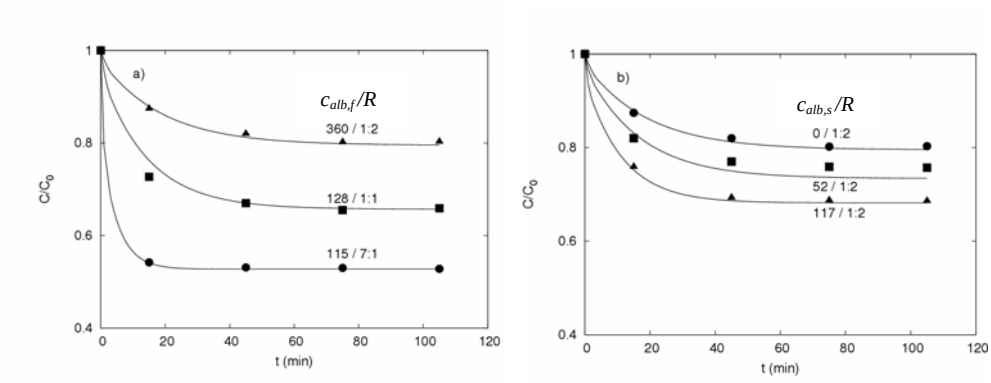

Figure 2 *Tryptophan concentration decrease during traditional (a) and albumin (b) dialysis processes. C/ C_0 : fraction of initial tryptophan concentration in feed.*
(a) Data sets refer to different albumin concentrations ($c_{alb,f}$) and different initial tryptophan-to-albumin molar ratios (R) in the feed.
(b) Data sets refer to different albumin concentrations in the dialysate ($c_{alb,s}$) and different tryptophan-to-albumin molar ratios in the feed. Albumin concentration in feed $c_{alb,f}$ =360 μM

Equations (1) and (2) can be integrated with the following initial and boundary conditions:

$$t = 0 \qquad 0 \le z \le L \qquad c_{t,f} = c_{t,f}^{0} \;\; ; \;\; c_{t,s} = 0 \tag{3}$$

$$t > 0 \qquad z = 0 \qquad c_{t,f} = c_{t,f}^{in} \; ; \;\; z = L \qquad c_{t,s} = c_{t,s}^{in} \tag{4}$$

In the above equations, c_t and c_{alb} are the free tryptophan and total albumin concentrations, respectively, k_{eq} the albumin-tryptophan binding constant, K_c is the overall mass transfer coefficient, a is the membrane specific surface and v the fluid velocity. Additional subscripts f and s refer to fiber-side and shell-side solutions, respectively. This model of the dialysis module was solved together with mass balances in the feed and dialysate reservoirs, which were considered perfectly mixed.
In the analysis of the experimental data, the albumin-tryptophan binding constant was set to 10^{-4} M^{-1}, according to the order of magnitude reported in the literature; this value was also confirmed by the experimental measurements of tryptophan concentrations (fibre and shell side) at long dialysis time (approaching equilibrium conditions).
The overall mass transfer coefficient K_c was obtained by fitting of the experimental data; both traditional and albumin dialysis data can be described with the same K_c value ($K_c = 7.5 \cdot 10^{-9}$ cm/s); figures 2a and 2b show a good agreement between the experimental data and the calculated curves and confirm the validity of the model.

3. Adsorption process

The design of an adsorption process to remove tryptophan requires information both on equilibrium and kinetics. As for tryptophan adsorption equilibrium in albumin-containing solutions, a simple model has been previously proposed (Annesini et al. 2005 and 2008). This model relies on the assumptions that 1) only 1:1 toxin-albumin complexes are formed, with an equilibrium constant K_{eq}; 2) only free toxin is adsorbed.

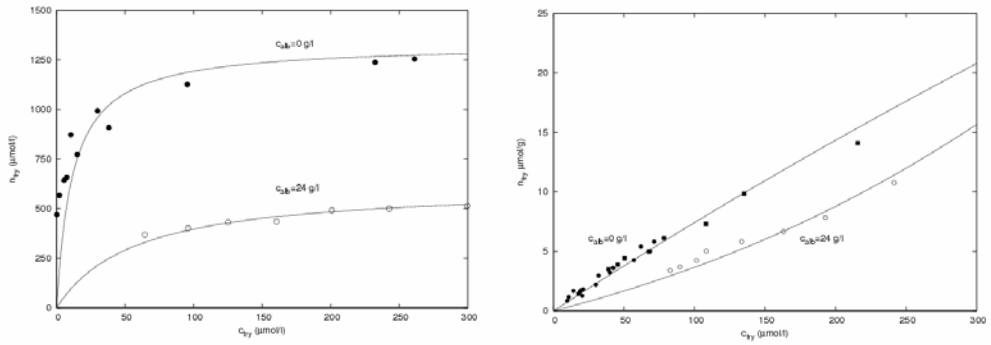

Figure 3 *Typical tryptophan adsorption isotherms on (a) activated carbon (data from Annesini et al., 2008) and (b) polymeric resin. c_{alb}: albumin concentration in the solution; lines: model fitting; circles: experimental data.*

Under these hypotheses, the amount of adsorbed tryptophan per unit adsorbent mass, n_t, is related to the total (albumin-bound and free) toxin concentration, c_{try}, by:

$$n_t = n_{\max} \frac{\alpha c_{try}}{k_{try} + \alpha c_{try}} \tag{5}$$

or , for $\alpha c_{try} << k_{try}$

$$n_t = \frac{n_{\max}}{k_{try}} \alpha c_{try} \tag{6}$$

where the free tryptophan fraction $\alpha = c_t / c_{try}$ depends on K_{eq}, c_{try} and c_{alb}. If albumin interaction with tryptophan adsorption is limited to tryptophan binding in solution, parameters $n_{\max}$ and k_{try} should coincide with the Langmuir parameters of the free tryptophan adsorption isotherm; however, effects such as competitive adsorption or steric hindrance could be significant and, in order to fit the experimental data, it could be necessary to consider a dependence of $n_{\max}$ on albumin concentration.

In figure 3 tryptophan adsorption equilibrium on polymeric resin and activated carbon are compared. As for adsorption onto the resin in albumin-free solutions, the adsorption isotherm is fairly linear ($n_{\max}$ / k_{try} =0.077 l/g); when albumin is added to the solution, it is sufficient to consider only the effect of albumin-toxin binding in the liquid phase, in order to predict the reduction of tryptophan uptake.

As for tryptophan adsorption onto activated carbon in albumin-free solutions, Langmuir parameters $n_{\max}$=1330 μmol/g and k_{try}=11.6 μmol/l are obtained; in this case, when albumin is added to the solution, both binding effects and a reduction of maximum tryptophan uptake must be considered in order to predict the reduction in tryptophan adsorbed amount (Annesini et al., 2008).

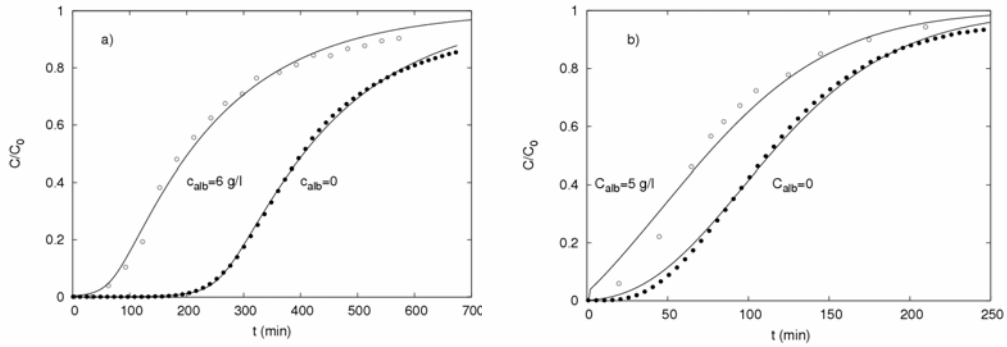

Figure 4 *Typical tryptophan breakthrough curves on activated carbon (a) and polymeric non-ionic resin (b). Line: model fitting; circles: experimental data.*
(a) Activated Carbon: Q=6 ml/min ; M=2 g ; C_{alb}=800 µmol/l ; K_c=1.4 10^{-6} cm/s
(b) Polymeric Resin: Q=1.3 ml/min ; M=2.4 g ; C_{alb}=400 µmol/l ; K_c=1.3 10^{-7} cm/s

The equilibrium experiments show that both the polymeric resin and the activated carbon tested can be considered as tryptophan adsorbents, even if the adsorption capacity of the activated carbon is much higher than that of the resin.
As for fixed-bed adsorption kinetics, figure 4 reports typical breakthrough curves for tryptophan on activated carbon (a) and polymeric resin (b). In both cases, tryptophan breakthrough curves from albumin-free and albumin-containing solutions are compared. Albumin presence causes a "back translation" of tryptophan breakthrough curves, as a consequence of the reduction of efficiency of the adsorption process.
Tryptophan adsorption from albumin-free solutions in fixed bed column was described assuming linear driving force (LDF) mass transfer kinetics. Under this hypothesis, tryptophan mass balances in the liquid and solid phases can be written as follows:

$$\varepsilon \frac{\partial c_{tox}}{\partial t} + (1-\varepsilon)\frac{\partial q_{tox}}{\partial t} = D\frac{\partial^2 c_{tox}}{\partial z^2} - v\frac{\partial c_{tox}}{\partial z} \tag{7}$$

$$\frac{\partial q_{tox}}{\partial t} = \frac{3}{R}K_c(q^*_{tox} - q_{tox}) \tag{8}$$

Equations (7) and (8) were integrated with the following initial and boundary conditions

$$t = 0 \qquad 0 \le z \le 1 \qquad c_{tox} = 0 \qquad q_{tox} = 0 \tag{9}$$

$$t > 0 \qquad z = H \qquad \frac{\partial c_{tox}}{\partial z} = 0; \quad t > 0 \quad z = 0 \quad vc^{in}_{tox} = -D\frac{\partial c_{tox}}{\partial z} + vc_{tox} \tag{10}$$

In the above equations, q_{tox} is the specific toxin adsorbed amount per unit volume of adsorbent, q^*_{tox} the specific toxin adsorbed amount in equilibrium with the toxin concentration in the liquid phase and K_c is the LDF mass transfer coefficient;

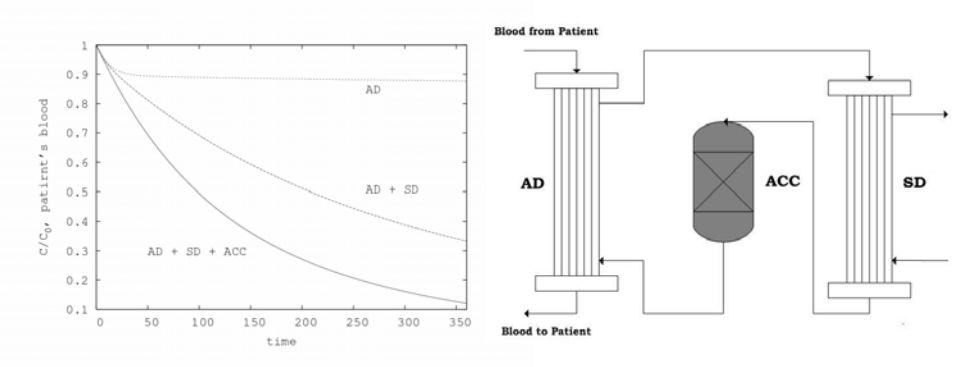

Figure 4 *Example of patient-artificial liver device simulation (flow rate: blood 230 ml/min, dialysate 150ml/min; albumin concentration: blood 600 µmol/l, dialysate from 300 to 3000 µmol/l; initial tryptophan concentration 471 µmol/l; mass of activated carbon 200 g; mass of polymeric resin 100 g; for the albumin dialyser and secondary dialysis module data are taken from specification sheet (Fresenius F40).*

furthermore, ε is the bed porosity, v the interstitial fluid velocity, D the toxin dispersion coefficient, calculated by the model proposed by Chung et al. (1968) and R the radius of adsorbent particles. This model was fitted to the experimental breakthrough curves obtained with albumin-free solutions, using K_c as adjustable parameter (see figure 4).

The same model proved to predict adequately the breakthrough curves obtained with albumin-containing solutions, accounting only for changes in equilibrium conditions caused by the presence of albumin in the liquid phase: figure 4 shows an example of prediction obtained assuming that the same K_c value applies to same flow-rate experiments, regardless of albumin concentration in the feed solution.

Finally, is worth noting that the overall mass transfer coefficient for activated carbon is one order of magnitude higher than that of the polymeric resin, confirming the better performance of activated carbon as tryptophan sorbent. Nevertheless, a polymeric resin can be chosen when an higher biocompatibility of the adsorbent is required, as in the Prometheus system, in which plasma comes into direct contact with adsorbents.

4. Simulation of a liver support device session

The theoretical models presented in the previous sections were used to model an albumin dialysis liver support device, including recirculation and regeneration of the dialysate solution (see scheme in figure 5). A single compartment model was used for the patient, neglecting endogenous tryptophan production.

Different recirculation/regeneration policies were compared in the simulations: recirculation without regeneration (AD), regeneration with a standard dialysis module (AD+SD) and regeneration with a standard dialysis module and an activate carbon adsorption column (AD+SD+ACC). Respect to tryptophan removal, the latter regeneration policy is equivalent to a MARS treatment; actually the, MARS device include also an anionic resin column in the regeneration circuit, but, as shown by (Look et al. 2002), this unit has a considerably minor adsorption capacity for tryptophan and its contribution to tryptophan removal from dialysate is negligible.

Some simulation outputs are reported in figure 5, where the performance of different dialysate regeneration policies are compared. It is evident that without dialysate regeneration, the detoxification process becomes rapidly ineffective, due to the build-up of tryptophan concentration in the dialysate. A traditional dialysis module in the regeneration circuit improves the effectiveness of the process, providing a continuous removal of tryptophan. An even better result is obtained if an activated carbon adsorption column is added to the regeneration circuit, at least until column saturation is reached. It is worth considering that an high albumin concentration in the dialysate improves the performance of the albumin dialysis module, but reduces the efficiency of both the standard dialysis module and adsorption column in the regeneration line. Further investigation of these processes could allow to evaluate a trade-off between these two aspects and help in designing albumin dialysis liver support devices.

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