Artificial Liver Optimization: Analysis of Albumin Bound Toxin Adsorption

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1. Introduction

Acute on Chronic liver disease is associated with high plasmatic levels of toxins that are responsible for life-threatening multi-organ disfunction. In such a condition, liver support devices can help to bring the patients out of the acute phase or bridge them to an organ trasplantation (Stange et al., 2002).

Toxins accumulating in the blood in liver failure are both water soluble and hydrophobic, protein-bound compounds. Clinical treatment of patients with simple haemodialysis or haemofiltration didn't prove to have any significant effect on the clinical outcome, since these processes are unable to remove protein-bound toxins effectively and selectively; therefore, specific processes, mainly based on toxin adsorption, have been developed.

In the Molecular Adsorbent Recirculating System (MARS), the patient's blood is dialysed across a special albumin impregnated membrane against a concentrated albumin solution which takes up albumin-bound toxins; the albumin dialysis solution is continuously regenerated by conventional low flux dialysis and adsorption onto activated carbon and anion exchange resin. After regeneration, the solution is recirculated to the primary dialysis unit (fig. 1). The adsorption columns are the true depurative elements for protein-bound toxins and the regeneration capacity of the adsorption units strongly affects the effectiveness of the whole process.

Several clinical data on the clearance of MARS or similar liver support devices are reported in the literature, but an engineering analysis of these systems, based on a deep knowledge of the thermodynamic and kinetic behaviour of each unit, has not yet been carried out. Thus it is reasonable to believe that significant improvements in the performance of these devices can still be achieved.

This paper reports some results of a research project aimed both to aquire experimental data on albumin-bound toxin adsorption on solid materials and develop a theoretical model to simulate each detoxifying unit and the system as a whole . The model can be a valuable tool for optimising the device and/or its operating conditions and, potentially, it

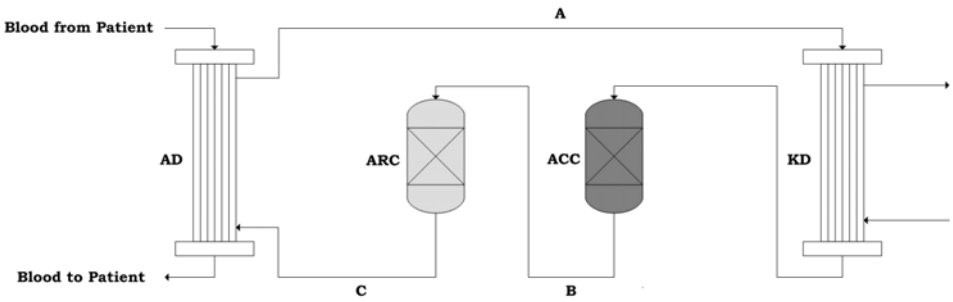

Fig. 1: *MARS scheme*

could also be used in the future to tailor the treatment conditions to the clinical state of the patient.

In particular, this paper focuses on experimental data and theoretical models of adsorption in fixed bed columns; the effects of adsorption kinetics on the behaviour of MARS treatment is also considered.

As model albumin-bound toxins, bilirubin and tryptophan have been selected. The former is a standard marker of the clinical state of liver-failure patients and of the detoxifying efficiency of liver support devices; it is mainly removed by adsorption in the anionic resin column. The latter is an aromatic amino acid relevant for the onset of hepatic coma; it is less tightly bound to albumin and it is removed both in low flux dialyser and in the activated carbon adsorption column.

2. Background on the thermodynamic basis of albumin bound toxin removal

Albumin binds bilirubin and tryptophan in solution with an extremely high (10^6-10^7 M^{-1}) and a lower (10^4-10^5 M^{-1}) binding constant respectively.

In previous works (Annesini et al., 2005 and 2007), a simple chemical model was proposed as a first approach to describe albumin-bound toxin adsorption, assuming that 1) only 1:1 toxin-albumin complexes are formed, with an equilibrium constant *K*; 2) only free toxin is adsorbed on the solid support. According to these assumptions, the amount of adsorbed toxin per unit adsorbent mass, $n_{tot,}$ is related to the total (albumin-bound and free) toxin concentration, c_{tox}, by:

$$n_{tox} = n_{\max} \frac{\alpha c_{tox}}{k_{tox} + \alpha c_{tox}} \tag{1}$$

For tightly bound toxins like bilirubin, equation (1) can be rewritten as:

$$n_{tox} = \bar{n} \frac{c_{tox}}{\bar{k} + c_{tox}} \qquad \bar{n} = \frac{n_{\max}}{1 - k_{tox} K} \qquad \bar{k} = \frac{k_{tox} K}{1 - k_{tox} K} c_{alb} \tag{2}$$

where c_{alb} is the total albumin concentration in the liquid phase.

The experimental results show that this chemical approach may be satisfactory to describe bilirubin adsorption onto anionic resin and the reduction of the resin uptake observed with increasing albumin concentration in the liquid solution. As for tryptophan adsorption onto activated carbon a further reduction of the maximum

Table 1: Adsorption Isotherm Parameters (n, μmol/g, c_{alb} and k, μmol/l)

Bilirubin onto IRA 400 eq. (2)	$\bar{n}$ =41.7	$\bar{k}$ =0.258 c_{alb}
Tryptophan onto activated carbon eq.(1)	$n_{\max} = 1330\left(1 - 0.644\frac{c_{alb}}{49.3 + c_{alb}}\right)$	k =11.6

adsorption capacity with increasing albumin concentration is observed. In order to describe this experimental evidence, that may be due to albumin competitive adsorption and/or steric hindrance effects, dependence of the $n_{\max}$ on the albumin concentration is also considered.

The adsorption isotherm parameters, obtained by the fitting of experimental data, are reported in table 1.

A chemical model accounting for albumin-toxin association is also useful to describe bound toxin transfer in albumin dialysis (Patzer, 2006). A similar approach has been used to model the mass transfer in MARS dialyser (Turchetti, 2005), that contains a carrier which shuttles the toxin from the blood to the dialysate side of the membrane; the following expression for the toxin flux across the membrane is obtained

$$N = k_m\left(\frac{c_{tox,B}}{c_{alb,B} + \beta c_{tox,B}} - \frac{c_{tox,D}}{c_{alb,D} + \beta c_{tox,D}}\right) \tag{3}$$

where subscripts *B* and *D* stand for blood and dialysate side, respectively. As for bilirubin removal, β=13.43 and k_m =0.02 μmol/m^{2}s were obtained by fitting of the experimental data reported by Stange and Mitzner (1996) .

3. Experimental

In order to investigate adsorption kinetics, breakthrough curves of albumin-toxin solutions and free toxin solutions were obtained in a fixed bed column (10 mm ID, 60 mm bed length) at 25±0.5°C. The column was loaded by a fixed amount of adsorbent (anionic resin IRA 400 or activated carbon 05112 Fluka) and rinsed with phosphate buffer (pH=7.4 and ionic strength 0.15 M) before the experiments.

Toxin concentrations in the outlet stream have been measured differently, depending on the solution type (Annesini et al., 2005, 2007). For bilirubin-albumin and tryptophan solutions, the column outlet stream was sent to an UV-VIS spectrophotometer (Perkin Elmer Lambda 25) equipped with a quartz flow cell and absorbances at 416 and 279 nm were continuously recorded to evaluate toxin and albumin concentrations. For tryptophan-albumin solutions, samples of the outlet stream were collected and analysed by HPLC with a Spherisorb ODS2 (Agilent) column; both albumin and tryptophan concentration were evaluated from the peak area detected at 279 nm.

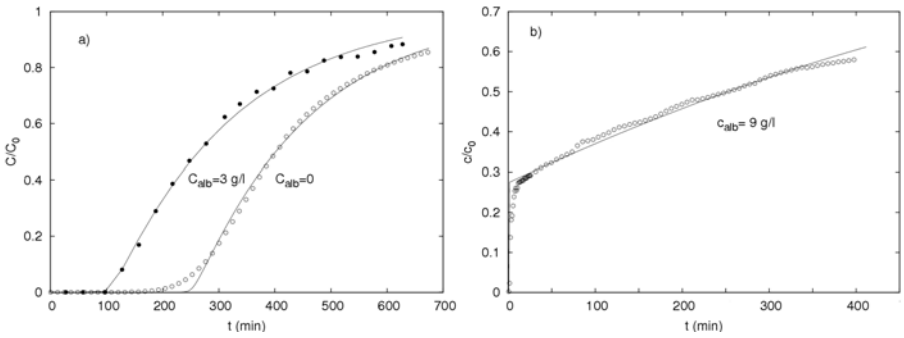

Fig. 2 *Typical tryptophan and bilirubin breakthrough curves on activated carbon and anionic resin, respectively. Line: fitted mode, circles: experimental data..*

(a) Tryptophan: Q=6 ml/min ; M=2 g ; c_{try}^{in} *=800 µmol/l ;* k_c*=1.4* 10^{-6} *cm/s*

(b) Bilirubin: Q=1 ml/min ; M=3 g ; c_{bil}^{in} *=100 µmol/l ;* k_c*=2.1* 10^{-7} *cm/s*

Fig. 2 reports typical breakthrough curves for tryptophan on activated carbon and bilirubin on anion exchange resin. As for tryptophan, breakthrough curves obtained with and without albumin in the solution are compared. The figure clearly shows that albumin reduces the ability of the activated carbon bed to remove tryptophan from solution; in any case, the adsorption rate is high enough to have an almost complete purification of the solution until the column bed is nearly saturated.

Bilirubin adsorption from buffer solution has not been studied, due to the very low solubility of bilirubin at neutral pH; breakthrough curve from albumin containing solution shows that bilirubin adsorption is quite slow, so that the toxin is never completely removed and a significant concentration in the outlet stream is obtained even in the early operating time. Also in this case, the higher the albumin concentration, the lower the resin adsorption capacity (data not reported).

4. Fixed-bed adsorption model

Even if albumin bound toxin adsorption is a complex phenomenon, in a first attempt to provide a physical model of the behaviour of a fixed-bed column the classical linear driving force (LDF) model was used. According to this model, solute mass balance in the fluid phase and in the adsorbed phase are given by:

$$\varepsilon \frac{\partial c_{tox}}{\partial t} + (1-\varepsilon)\frac{\partial q_{tox}}{\partial t} = D\frac{\partial^2 c_{tox}}{\partial z^2} - v\frac{\partial c_{tox}}{\partial z} \tag{4}$$

$$\frac{\partial q_{tox}}{\partial t} = \frac{3}{R}K_c(q_{tox}^{*} - q_{tox}) \tag{5}$$

In the above equations, q_T is the amount of free adsorbed toxin per volume of adsorbent, q_T^{*} the adsorbed amount of toxin in equilibrium with the toxin concentration in the liquid phase and K_c is the mass transfer coefficient; furthermore, ε is the bed porosity, v the interstitial fluid velocity, *D* the toxin dispersion coefficient

and R the radius of adsorbent particles. Equations (6) and (7) are integrated with the classical initial and boundary conditions

$$t = 0 \qquad 0 \le z \le 1 \qquad c_{tox} = 0 \quad ; \quad q_{tox} = 0 \tag{6}$$

$$t > 0 \qquad z = H \quad \frac{\partial c_{tox}}{\partial z} = 0 \qquad z = 0 \quad vc_{tox}^{in} = -D\frac{\partial c_{tox}}{\partial z} + vc_{tox} \tag{7}$$

The mass transport coefficient K_c should account for both liquid and solid phase resistance; reasonably K_c depends on the fluidodynamics and on the albumin concentration of the liquid phase; nevertheless, in this work only the main effect of albumin on the toxin adsorption isotherm was considered.

As for tryptophan, K_c value was obtained from the fitting of the albumin-free solution data and then used to predict the breakthrough curve of the albumin-tryptophan solution, accounting for the reduced carbon adsorption capacity at the equilibrium: fig. 2a shows the very good agreement between the experimental data and the predicted curve and confirm the validity of the model. In a similar way, fig. 2b shows a comparison between the theoretical model and the experimental data for bilirubin adsorption from albumin-containing solution. Also in this case, the effect of albumin concentration on breakthrough curves (data reported by Piemonte, 2007), is correctly predicted only accounting for its effect on the adsorption equilibria, at least in the first part of the breakthrough curve; then a further reduction of bilirubin adsorption rate is observed.

4. MARS model

The theoretical models of adsorption columns and albumin dialysis unit have been included in a complete model of the MARS detoxification process (fig. 1). In particular, the model considers: 1) a single compartment model for the patient, without toxin production during the treatment; 2) a countercurrent albumin dialysis units (AD), with a toxin flux across the membrane given by equation (3); 3) a secondary dialysis module (KD) for low molecular weight, free toxin removal; 4) an activated carbon adsorption column (ACC); 5) an anionic resin adsorption column (ARC). In a first attempt to gain understanding of the effects of the operating parameters on the efficiency of the process, in this paper the analysis is limited to bilirubin removal, so that both the secondary dialyser and carbon adsorber are not effective for toxin removal.

Fig. 3 reports some model simulation results. In particular, the model describes the progressive saturation of the anionic resin adsorption column and the bilirubin removal from the patient blood: in the ongoing treatment, bilirubin is firstly transferred from patient blood to the albumin circuit, where a steep concentration increase is observed; the bilirubin is then slowly removed by adsorption in the fixed bed columns.

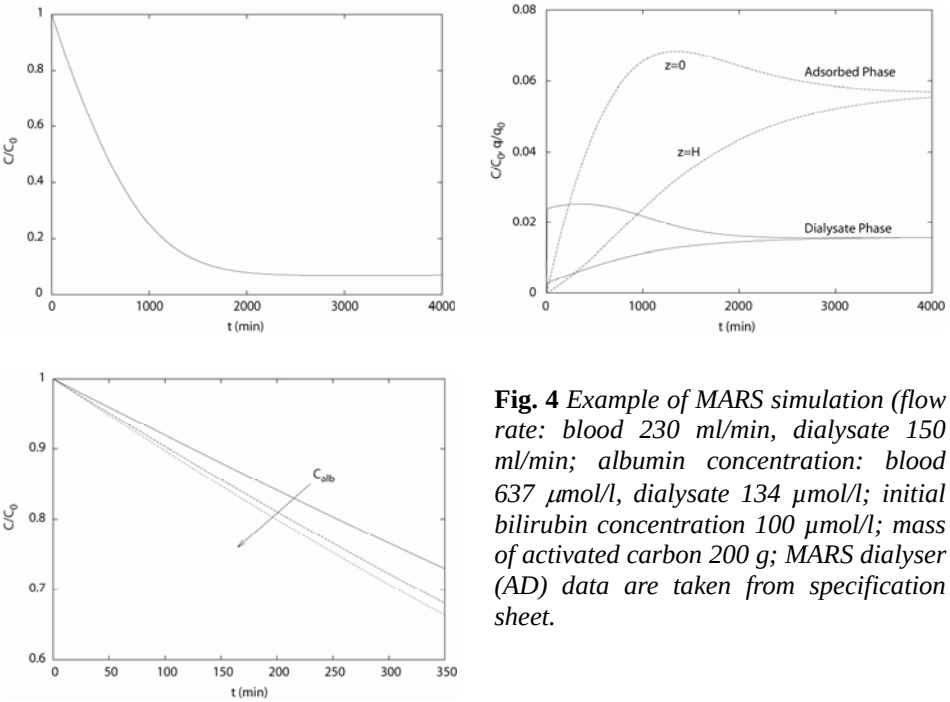

Fig. 4 *Example of MARS simulation (flow rate: blood 230 ml/min, dialysate 150 ml/min; albumin concentration: blood 637 µmol/l, dialysate 134 µmol/l; initial bilirubin concentration 100 µmol/l; mass of activated carbon 200 g; MARS dialyser (AD) data are taken from specification sheet.*

In the same figure, we also report the effect of albumin concentration in the recirculating albumin solution on the bilirubin profile of patient: in the tested concentration range and focusing on clinical treatment period (6 hours), the higher the albumin concentration, the faster bilirubin removal.

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