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MOLECULAR ADSORBENT RECIRCULATING SYSTEM (MARS): A CHEMICAL ENGINEERING ANALYSIS OF IN VIVO EXPERIMENTAL DATA

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Dialysis and adsorption units are commonly used in liver support devices for the removal of albumin-bound toxins, such as bilirubin, and water-soluble low-molecular-weight toxins, such as creatinine. In this paper, the consolidated approach of chemical engineers' to process design is applied to the analysis of the performance of a MARS treatment. The theoretical analysis of the detoxification process is used to discuss clinical data obtained during MARS treatment sessions, that refer to bilirubin and creatinine concentration in plasma and different parts of the device circuit.

1. INTRODUCTION

The treatment of patients suffering from acute and acute-on-chronic liver failure is presently carried out with extracorporeal devices aimed at bringing them out of the acute phase safely or to bridge them to organ transplantation.
The main function provided by these liver support devices (LSDs) is blood detoxification from a wide range of noxious substances, including water-soluble and albumin-bound toxins. In order to remove selectively and effectively also toxins of this latter class, LSDs implement different separation processes that, under a general point of view, consist in some combination of dialysis and adsorption (Jalan et al., 2004; Stegmayr, 2005; Rozga, 2006).
One of the most commonly used LSDs in clinical practice is the Molecular Adsorbents Recirculating System (MARS, Gambro, Lund, Sweden). Basically, the detoxification process implemented in MARS consists in blood dialysis across a special albumin impregnated membrane against a concentrated albumin solution (albumin dialysate). The particular structure of the membrane and the presence of a binder in the dialysate allow also for albumin-bound toxin transfer across the membrane, while the cut-off of the membrane is chosen so as to avoid transfer of albumin and higher molecular weight substances. The albumin dialysate is continuously regenerated by conventional dialysis and adsorption on activated carbon and anionic resin, and recirculated (Fig.1).
Analysis and design of the unit operations implemented in MARS as well as other LSDs is a typical competence of chemical engineers; therefore, it is reasonable to believe that the application of the common methodology of chemical engineering could produce significant improvements in this field.
In this paper, the consolidated approach of chemical engineers' to process design is applied to the analysis of the performance of a MARS treatment. To that end, mathematical models of dialysis and adsorption processes, with parameters obtained by in-vitro data, were used to simulate single units and the whole detoxification process of MARS. The results obtained allowed to make qualitative and semi-quantitative considerations on the process efficiency, and detect important factors affecting the performance of the device.
The theoretical analysis provided the base for the discussion of some clinical experimental data obtained during MARS treatment sessions. The data presented refer to two toxins, namely, bilirubin and creatinine, that belong to two different classes of compounds: the former is a standard marker of the clinical state of liver-failure patients

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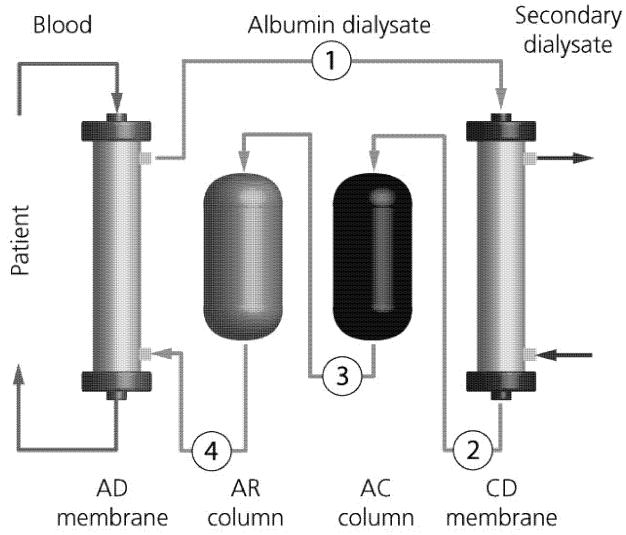

Figure 1: Schematic representation of the MARS device. The numbers indicate the points at which albumin dialysate samples were collected for the acquisition of the data reported in Table 1 *and* 2.

and can be considered as representative of the class of strongly albumin-bound toxins, the latter is a water-soluble low-molecular-weight molecule, which is present in plasma as free solute.

2. THEORETICAL ANALYSIS OF THE DETOXIFICATION PROCESS

Models of dialysis and adsorption units included in MARS were developed previously and used to simulate single parts (Piemonte et al., in press) and the whole detoxification process (Annesini et al. 2008b, 2009). As for bilirubin, the thermodynamic and kinetic parameters needed by these models were obtained by in-vitro data that include batch adsorption equilibrium tests (Annesini et al. 2005, 2008a), fixed-bed adsorption tests (Piemonte et al., in press) and dialysis (Annesini et al. 2009). Here, only the basic features of the models and the main results of the simulations will be reported.

2.1 Dialysis process

The model of the hollow fiber dialyser combines the toxin transfer rate across the membrane with the toxin mass balance in the feed and dialysate solution.
In the case of toxins not bound to albumin, the model is well known (Galletti et al., 1995). In the case of albumin dialysis of strongly albumin-bound toxins such as bilirubin, the model is corrected to account for bilirubin partition equilibrium between two albumin-containing aqueous phases: in this case, equal bilirubin-to-albumin molar ratios are present in the two phases at equilibrium.
As for bilirubin transfer, diffusion through the membrane is considered as the rate controlling step. In the framework of a diffusion-solution model, the bilirubin transmembrane flux is given by

$$J_{tox} = \frac{D_{tox}^{m}}{\delta}\left(c_{tox}^{m,\alpha} - c_{tox}^{m,\beta}\right) \qquad (1)$$

where superscript m refers to the membrane phase; $c_{tox}^{m,\alpha}$ and $c_{tox}^{m,\beta}$ are the bilirubin concentrations in the membrane at the interface with phase α and β, respectively; D_{tox}^{m} is bilirubin diffusivity inside the membrane and δ is the membrane thickness. Assuming equilibrium conditions at the interfaces, the bilirubin flux may be

rewritten in terms of total bilirubin and albumin concentrations in the liquid phases in contact with the membrane as

$$J_{tox} = K_0 \left(\frac{c_{tox}^{\alpha}}{c_{alb}^{\alpha} - c_{tox}^{\alpha}} - \frac{c_{tox}^{\beta}}{c_{alb}^{\beta} - c_{tox}^{\beta}} \right) \tag{2}$$

where K_0 is a modified membrane mass transfer coefficient. It is worth noting that the driving force for bilirubin transfer is the difference in the ratios between bilirubin and free albumin in the two phases and, obviously, the flux becomes zero when equilibrium is achieved.
The model of the albumin dialysis module is then obtained by combining the mass transfer rate equation (2) with the bilirubin mass balance in the solution that must be detoxified (α phase, patient's blood in the case of the AD module) and in the cleansing solution (β phase, albumin dialysate in the case of the AD module); for a hollow fiber module in counter-current flow, bilirubin mass balance in the two compartments are given by

$$\frac{dc_{tox}^{\alpha}}{dz} = \frac{K_0 A}{L Q^{\alpha}} \left(\frac{c_{tox}^{\alpha}}{c_{alb}^{\alpha} - c_{tox}^{\alpha}} - \frac{c_{tox}^{\beta}}{c_{alb}^{\beta} - c_{tox}^{\beta}} \right) \tag{3}$$

$$\frac{dc_{tox}^{\beta}}{dz} = \frac{K_0 A}{L Q^{\beta}} \left(\frac{c_{tox}^{\alpha}}{c_{alb}^{\alpha} - c_{tox}^{\alpha}} - \frac{c_{tox}^{\beta}}{c_{alb}^{\beta} - c_{tox}^{\beta}} \right) \tag{4}$$

where A is the membrane area, L the module length and Q the volumetric flow rate. Equations (3) and (4) can be integrated with the following boundary conditions

$$z = 0 \quad c_{tox}^{\beta} = c_{tox}^{\beta,in} \quad ; \quad z = L \quad c_{tox}^{\alpha} = c_{tox}^{\alpha,in}$$

in order to calculate the membrane module clearance respect to the α phase, defined as

$$CL = Q^{\alpha} \frac{c_{tox}^{\alpha,in} - c_{tox}^{\alpha,out}}{c_{tox}^{\alpha,in}} = Q^{\beta} \frac{c_{tox}^{\beta,out} - c_{tox}^{\beta,in}}{c_{tox}^{\alpha,in}}$$

It is worth noting that CL is an increasing function of $1/Z = Q^{\beta} c_{alb}^{\beta} / Q^{\alpha} c_{alb}^{\alpha}$ and approaches an asymptotic value, CL_{∞}, for $1/Z \to \infty$. Assuming a negligible inlet toxin concentration in the β phase ($c_{tox}^{\beta,in} \approx 0$), the limiting clearance value can be obtained as the solution of the following non-linear equation

$$\frac{CL_{\infty}}{Q^{\alpha}} \frac{c_{tox}^{\alpha,in}}{c_{alb}^{\alpha}} + \ln\left(1 - \frac{CL_{\infty}}{Q^{\alpha}}\right) = -k \tag{5}$$

where $k = K_0 A / Q^{\alpha} c_{alb}^{\alpha}$.
If the α phase has a very low toxin-to-albumin molar ratio, equations (3) and (4) can be analytically integrated to give:

$$\frac{CL}{Q^{\alpha}} = \frac{1 - \exp[k(1 - Z)]}{Z - \exp[k(1 - Z)]} \tag{6}$$

Again, equation (6) clearly shows that the membrane module clearance increases with increasing $1/Z$, i.e. increasing the dialysate flow rate or its albumin concentration.
It is worth noting that Eq. (6) applies also to dialysis of toxins not bound to albumin, with $Z = Q^{\alpha} / Q^{\beta}$ and $k = PA/Q^{\alpha}$ (where P is the toxin's permeability trough the membrane) while, in this case

$$\ln\left(1 - \frac{CL_{\infty}}{Q^{\alpha}}\right) = -k \tag{7}$$

It can be easily shown that, if $1/Z$ is above unity, the clearance is close to its asymptotic value for any value of k and a further increase of this parameter results in a negligible increase of the module clearance. Furthermore, the clearance is an increasing function of k.
This means that, if dialysis is operated at conditions corresponding to $1/Z > 1$, the performance of the process cannot be significantly improved just by changing the process operating conditions, such as the dialysate flowrate and (in the case of albumin-bound toxins) albumin concentration. Therefore, if the clearance is to be increased when $1/Z > 1$, it is necessary to use different membrane modules, with larger exchange areas or more permeable membranes, corresponding to a higher value of k.
These considerations can be used to discuss the performance of the two dialysis modules implemented in MARS in its present configuration. To that end, it is first of all necessary to define the operating conditions. A typical flow rate of about 170 ml/min can be assumed in all of the compartments of MARS (the design flowrate range reported in MARS' data sheet is 150-250 ml/min); as for albumin concentrations, the plasmatic value is about 40 g/l, while the albumin concentration in the albumin dialysate can be 110 g/l or higher (see section 3). With these operating conditions, $1/Z$ is close to 1 for water-soluble toxins such as creatinine in the CD membrane, and about 3 or higher for strongly albumin-bound toxins like bilirubin in the AD membrane. This suggests that both dialysers are working close to their respective limiting value of clearance CL_{∞} and no significant improvement can be obtained by changing the operating conditions.
In order to assess the efficacy of the AD module in removing albumin-bound toxins from blood, the bilirubin clearance in this unit can be estimated by assuming $K_0A \approx 2.6$ μmol/min (Annesini et al., 2009). With this assumption, solution of Eq. (5) gives $CL_{\infty} / Q^{\alpha} \approx 0.04$. This very low value suggests that the AD membrane currently implemented in MARS is under-dimensioned for bilirubin detoxification.
As for the CD dialyser, this unit is used in MARS to remove low-molecular weight, water soluble toxins from the albumin dialysate (that becomes, in this unit, the solution that must be detoxified). One such compound is creatinine, for which it can be assumed $(P\ A) \approx 300$ ml/min. In this case, solution of Eq. (7) gives $CL_{\infty} / Q^{\alpha} \approx 0.82$, suggesting that, with the present configuration, removal of creatinine from the albumin dialysate is significant, but not complete.

1.1 Adsorption process

The model of the adsorption columns is obtained by coupling the differential unsteady toxin mass balance in the liquid phase with mass transfer kinetics from the liquid to the adsorbed phase:

$$\varepsilon \frac{\partial c_{tox}}{\partial t} + (1-\varepsilon)\rho \frac{\partial n_{tox}}{\partial t} = D \frac{\partial^2 c_{tox}}{\partial z^2} - v \frac{\partial c_{tox}}{\partial z} \tag{8}$$

where c_{tox} is the toxin concentration in the liquid phase, n_{tox} the toxin adsorbed amount per unit sorbent mass, ε is the bed porosity, ρ is the intrinsic density of the solid adsorbent, v the liquid superficial velocity and D the toxin axial dispersion coefficient.
Assuming linear driving force (LDF) mass transfer kinetics, the toxin mass balance in the adsorbed phase may be written as

$$\frac{\partial n_{tox}}{\partial t} = \frac{3}{R} K_c (n^*_{tox} - n_{tox}) \tag{9}$$

where n^*_{tox} is the specific toxin adsorbed amount in equilibrium with the toxin concentration in the liquid phase, R the adsorbent particle radius and K_c is the LDF mass transfer coefficient. Equations (8) and (9) can be integrated with the initial and boundary condition

$$t = 0 \quad 0 \le z \le H \quad c_{tox} = 0 \quad n_{tox} = 0 \tag{10}$$

$$t > 0 \quad z = 0 \quad v c^{in}_{tox} = -D \frac{\partial c_{tox}}{\partial z} + v c_{tox} \tag{11}$$

$$t > 0 \quad z = H \quad \frac{\partial c_{tox}}{\partial z} = 0 \tag{12}$$

where H is the column bed height and c^{in}_{tox} is the bilirubin concentration in the inlet solution.

The adsorptive media used in MARS are activated carbon, and anionic resin. Thermodynamics (Annesini et al., 2005, 2008a) and fixed-bed kinetics (Piemonte et al., in press) of bilirubin adsorption on these media from albumin-containing aqueous solutions have been thoroughly investigated in-vitro and the parameters of the model have been estimated. It is worth mentioning some important results of these papers. Firstly, activated carbon proved to have a significant affinity for a wide range of toxins, even if its adsorption capacity for negatively charged compounds such as bilirubin is not very high; this latter class of toxins, on the other hand, is highly adsorbed by anionic resin.

Furthermore, in fixed-bed bilirubin adsorption tests on anionic resin, mass transfer kinetics resulted to be a very slow; as a consequence, in operating conditions similar to those used in MARS, bilirubin was not completely removed in the laboratory column.

1.2 Simulation of the MARS device

The models presented in the previous sections can be combined to simulate a complete LSD, including different units as reported by Annesini et al. (2009). The simulations carried out in the aforementioned work, based on operating conditions used in real MARS treatment sessions, give an overall bilirubin clearance as low as 3-4%, in agreement with the simple calculations performed in section 2.1.

A two-fold effect of albumin in the dialysate on the overall detoxification process is observed: on one hand, an higher albumin concentration enhances bilirubin transfer to the dialysate in the membrane module, but, on the other hand, impairs the regeneration of dialysate by adsorption. Furthermore, the overall bilirubin clearance decreases during the treatment, due to the incomplete regeneration and build-up of bilirubin concentration in the dialysate.

2. ANALYSIS OF CLINICAL DATA

In order to validate the considerations presented in the previous section, data were acquired during clinical MARS sessions.

Blood and albumin dialysate samples (withdrawn at the points indicated in Fig.1) were collected and analyzed to measure the concentration of albumin and some important toxins. Sample collection was performed before and at different times after the beginning of the treatment. Table 1 and 2 present an example of the data obtained for total bilirubin and creatinine, respectively, referring to the treatment of two different patients. In both cases, during the treatment, the flow rates of the blood (Q_B) and dialysate (Q_D) circuits were set to 170 ml/min and the concentration of albumin in the dialysate was about 110 g/l.

As for bilirubin, the data show that, although a decrease of the plasmatic concentration was actually observed, the efficiency of the treatment for the removal of this toxin was very low during the session considered. This can be most clearly shown by calculating bilirubin clearances as follows

$$CL = Q_D \frac{C_{(1)} - C_{(4)}}{C_{(patient)}} \tag{13}$$

where $C_{(i)}$ is the toxin concentration measured at point (i) of the albumin dialysate circuit (see Fig. 1 and Tab. 1). The values obtained are only a few percent of the blood flow-rate, in agreement with the prediction of the model. Furthermore, the comparison of the concentration measured at points 2, 3 and 4, shows that, in the albumin dialysate circuit, bilirubin is mainly cleared in the anionic resin column (maximum fractional concentration reduction observed, η=0.35) and, to a much lesser extent, in the activated carbon column (η=0.08), while the conventional dialyser is virtually ineffective for the removal of this toxin.
It is significant to point out that the effluent of the resin column contains a non-negligible bilirubin concentration even at early operating times, when the sorbent is far from saturation. This finding, clearly shows that albumin is never completely regenerated.
As for creatinine, the data reported in Tab.2 show that, as expected, this water-soluble toxin is efficiently cleared by the conventional dialyser. Indeed, by calculating the CD module clearance defined as

$$CL_{CD} = Q_D \frac{C_{(1)} - C_{(2)}}{C_{(1)}} \tag{14}$$

it is obtained that CL_{CD}/Q_D spans from 0.5 to 0.82 during the MARS treatment.
A modest amount of creatinine is also removed in the activated carbon column (10% after 8h), confirming the wide range of non-charged substances that can be cleared by this sorbent medium.

3. CONCLUSIONS

An engineering analysis of the MARS liver support device was presented. The analysis is based on simple physical models of dialysis and adsorption units implemented in MARS and aimed at identifying possible issues of the present configuration of this device.
The removal of bilirubin and creatinine from blood by MARS treatment was considered. The two toxins were chosen as representative of two different classes of substances that must be removed in case of liver failure: highly albumin-associated, water-insoluble (bilirubin) and low-molecular-weight, water-soluble (creatinine) compounds.
The results show that, with the operating conditions presently used in MARS sessions, bilirubin transfer across the albumin dialysis membrane is a kinetically limited processes and, consequently, a small difference between inlet and outlet bilirubin plasma concentration is expected; indeed, very low clearances of this toxin were estimated with the mathematical models proposed. A similar result was found for the adsorption process that is intended to remove bilirubin from the recirculating albumin dialysate.
Both of these findings are in good agreement with experimental data acquired during two clinical sessions with MARS in which, within the first 6 hours of treatment, bilirubin clearance in the albumin dialysis module was below 5% of blood flowrate, and the fractional reduction in bilirubin concentration in the adsorption column was below 35%.
Another important fact suggested by the model is that this poor performance respect to bilirubin removal cannot be improved just by changing the operating conditions, but a different albumin dialysis module should be considered to this aim.
As for creatinine, both the mathematical model and experimental data show that a satisfying, even if not complete, removal of this toxin from the albumin dialysate is obtained in the secondary dialyser of MARS.
Bilirubin and creatinine are not the only noxious substances that must be removed from blood in the treatment of acute liver failure; therefore, a complete assessment of MARS' performance cannot be performed only by

Table 1: Total bilirubin concentration in patient's blood and albumin dialysate at different points of the MARS circuit (see Fig.1) measured during a clinical MARS session

	Total Bilirubin [mg/dl]			
	Before	After 1h	After 3h	After 6h
Patient	16.57	15.56(*)	13.74	11.48
Point 1	-	1.3	1.6	1.1
Point 2	-	1.3	1.6	1.0
Point 3	-	1.2	1.5	0.9
Point 4	-	0.8	1.2	0.9
CL[ml/min]	-	5.5	4.9	3

(*)Extrapolated on the basis of the time course of plasmatic concentration

Table 2: Creatinine concentration in patient's blood and albumin dialysate at different points of the MARS circuit (see Fig.1) measured during a clinical MARS session

	Creatinine [mg/dl]			
	Before	After 10min	After 2h	After 8h
Patient	4.0	-	-	-
Point 1	-	1.7	1.5	1.8
Point 2	-	0.3	0.4	0.9
Point 3	-	0.1	0.2	0.8
Point 4	-	0.1	0.2	0.9

considering these two toxins, neither can the optimal operating conditions be chosen on this basis. For this reason, the analysis proposed in this paper should be extended to other substances, such as specific cytokines.

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