A New Method to Increase the Adsorption of Protein-Bound Toxins in Artificial Liver Support Systems

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Abstract: In this work, a new method, called the preconcentration method (PCM), is proposed to increase the adsorption of protein-bound toxins onto adsorbents in artificial liver support systems. In the PCM, a concentrator is installed before the inlet of the adsorbent cartridge. This method is validated in an experiment using activated carbon to remove albumin-bound bilirubin, and the mechanism of the increase in adsorption is theoretically explained with breakthrough curve and equilibrium adsorption analyses. Our results show that when this PCM is used, the mass transfer rate of bilirubin from solution to activated carbon is enhanced, the adsorbed bilirubin amount per unit mass of activated carbon is greatly increased, and more albumin-bound bilirubin molecules are quickly removed from the albumin solution. When the concentration ratio (the ratio of the inlet flow rate to the outflow rate of the concentrator) is 2.59, the adsorption efficiency of activated carbon at 120 min is increased by approximately 36%. Only approximately 60 min is required for the bilirubin concentration to decrease from 19.3 to 13.0 mg/dL; however, without the PCM, nearly 180 min is needed. In addition, by adjusting the concentration ratio, the adsorption of albumin-bound bilirubin onto activated carbon can be further increased. Key Words: Artificial liver support system—Adsorption—Protein-bound toxin—Mass transfer—Bilirubin—Albumin.

Liver failure, whether fulminant or acute-on-chronic, may become life-threatening. This condition is usually associated with high morbidity and mortality (1,2). Currently, the only accepted therapy for end-stage liver failure is liver transplantation. However, the shortage of available donor organs is prompting scientists to look for alternative methods. Therefore, in the past several decades, various artificial liver support systems have been developed (3,4). Because loss of the detoxifying function of the liver causes the accumulation of toxins and especially protein-bound toxins in the human body, the central task of artificial liver support systems is to remove these protein-bound toxins (5,6).

Adsorption technology is often used to remove protein-bound toxins (7,8). Many efforts have been made to improve the efficiency of adsorbents in terms of the speed of adsorption or mass transfer (to reduce the treatment time) and the quantity of adsorbed toxins (to reduce the treatment cost). The size of adsorbents can affect the speed of adsorption (9,10). For example, powdered activated carbon can provide faster adsorption for toxins than can granular activated carbon. However, very careful design of the detoxifying/adsorption cartridge is required to maintain an adsorbent suspension and to prevent any particles from making contact with the blood (10). An increase in the adsorbent mass can also enhance the speed of adsorption and the quantity of adsorbed toxins, but the use efficiency of adsorbents (i.e., the adsorbed toxin amount per unit adsorbent mass) may decrease. In other words, in this situation, adsorbents may be mostly wasted. The regeneration of adsorbents (during the treatment) is essentially equivalent to an increase in the adsorbent mass. This method allows the effective use of adsorbents but...
also presents other issues, such as operational complexity and uncontrollability while running. An increase in flow rates through the detoxifying cartridge is an effective way to increase the adsorption speed of toxins (11,12). However, this method does not ultimately increase the quantity of adsorbed toxins.

Regarding equilibrium adsorption, if we consider the patient and the artificial liver support system as a whole and assume that the operation time is sufficiently long, for a given initial condition, there is only one corresponding equilibrium status. A change in flow rates does not affect the final equilibrium status at all, but a change in the adsorbent mass does (an increase in the adsorbent mass can lower the equilibrium concentration of toxins in the plasma). For a given adsorbent, the adsorption capacity is determined, which does not mean that all binding sites are occupied during treatment. In fact, in most cases, the adsorbent in the detoxifying cartridge does not become saturated or is not fully used, even under the condition of adsorption equilibrium. For certain adsorbents, saturation of adsorption can only happen when the toxin concentration is very large; however, in practice, the toxin concentration cannot be extremely high. Therefore, there is still a substantial possibility for these adsorbents to absorb more toxins and thereby further lower the toxin concentration in patients’ bodies. The question is how to create a feasible way to fully use this space (void sites) in adsorbents.

In this study, we introduce a simple and practical engineering method, called the preconcentration method (PCM), to increase the use efficiency of adsorbents and the speed of adsorption (or the mass transfer rate from stationary phase to mobile phase). In this method, only a concentrator is needed before the detoxifying cartridge. As the protein size is much larger than the pore size of the separation membrane, the plasma, which is separated from the patients’ blood in advance, is concentrated in the concentrator. Because toxins in the plasma are mainly bound to proteins, the concentrations of these toxins are consequently increased before entering the cartridge. The filtrate, with very few toxins, is pumped out of the concentrator and mixed with the plasma from the outlet of the cartridge. Using the PCM, adsorbents can be used both adequately and efficiently. Currently, many adsorbents have been developed for artificial liver support systems (13). However, there is a need for cost savings because the treatment is still expensive. Therefore, the PCM presented here is of significance.

MATERIALS AND METHODS

Materials

Bovine serum albumin (Cohn fraction V, molecular weight = 66 000 Da), dimethyl sulfoxide (DMSO; ≥99.5%), sodium carbonate (anhydrous, ≥99.95%), and activated carbon (Norit RO 0.8) for liquid-phase adsorption were bought from Sigma-Aldrich (St. Louis, MO, USA). Bilirubin (molecular weight = 584.68 g/mol, 97%, Manufacturer’s Part No.: A17522-06) was obtained from Alfa Aesar (Ward Hill, MA, USA). Reagents for albumin (bromocresol green) and total bilirubin (diazonium salt) assays were obtained from Thermo Fisher Scientific (Middletown, VA, USA). Hydrochloric acid (1.0 N) was purchased from the University of Washington Chemistry Store. The hemodialysis machine was a Gambro Phoenix, and the hollow-fiber filter was a Gambro 6LR (Gambro, Lakewood, CO, USA). The two peristaltic pumps used (Masterflex Easy-Load II, model no. 77200-62) were obtained from Cole-Parmer, Vernon Hills, IL, USA.

Adsorbent packing

The fixed-bed structure was adopted for the detoxifying cartridge (14). The fluidized and suspended-bed structure could also have been used (10); however, the choice does not affect the validity of the PCM proposed here. Before all experiments, 76.5 g activated carbon (dry weight) was tightly packed into a stainless steel cartridge. The cartridge was 9 cm in length and 5 cm in diameter, and the packing porosity was approximately 0.271 (15). The activated carbon was approximately 4 mm in length and 0.8 mm in diameter, and the pore diameter was approximately 19 nm (16). Additionally, the void volume was approximately 0.5 mL/g (17). Deionized water was then continuously pumped through the cartridge for at least 48 h to completely remove air bubbles and carbon ash/debris. Afterward, the void volume in the cartridge was measured experimentally (it was approximately 50 mL).

Solution preparation

Albumin is one of the most important proteins involved in the detoxification process and has the highest concentration in the blood (3,18,19). Bilirubin is an albumin-bound toxin and is also a standard clinical marker in liver failure patients (20–22). Therefore, in this study, we used an albumin solution containing bilirubin as a substitute for plasma from patients and focused on the adsorption of the free bilirubin onto activated carbon in the detoxifying cartridge to validate the PCM. First, dialysate was...
produced by the hemodialysis machine. The dialysate was then used to prepare a solution containing 2% albumin and 20 mg/dL bilirubin in dim light. In the preparation, DMSO (4 mL) was added to the weigh boat to dissolve the bilirubin powder (200 mg), and 0.25 M Na₂CO₃ (6 mL) solution was used to help dissolution (the final concentration of DMSO was 0.4% v/v). Moreover, 1.0 N HCl solution was added drop by drop to adjust the pH value of the solution to approximately 7.3.

**Experimental design**

The aim of this study is to introduce a simple and practical design that can be embedded in an artificial liver support system to enhance the adsorption of protein-bound toxins. To validate the PCM, experiments were mainly designed to compare situations with and without the PCM. In our design (Fig. 1), a hollow-fiber filter, serving as a concentrator, is installed before the inlet of the activated carbon cartridge. As albumin molecules are much larger than the pore size of the hollow-fiber membrane, the albumin solution is concentrated on the lumen side of the filter before flowing through the cartridge. Meanwhile, because the bilirubin in the solution is mainly bound to albumin, the bilirubin concentration is consequently increased when entering the cartridge. The filtrate from the shell side of the filter is pumped to the outlet of the cartridge and mixed with the albumin solution again. Because nearly no albumin can pass through the fiber membrane, the bilirubin concentration in the filtrate is very low. The lumen and shell volumes of the concentrator were measured experimentally and were approximately 115 and 100 mL, respectively. Before experiments, the adsorption cartridge and the concentrator were primed with dialysate for 30 min (the flow rates during the priming process were the same as those in the real experiments). The solution volume in all experiments was 1.0 L.

In experiments without the PCM, 165 mL priming solution was removed from the cartridge outlet at the beginning, and the tubing outlet was then quickly placed in the container. The flow rates for pumps A and B were set to 120 and 0 mL/min, respectively. Samples were collected from locations 2 and 3 at the desired times. In experiments with the PCM, at the beginning, 165 and 100 mL priming solution was removed from the cartridge outlet and the filtrate outlet, respectively, and these two tubing outlets were then quickly placed in the container. The flow rate from pump A ($Q_A$) was always 120 mL/min. The flow rate from pump B ($Q_B$) was adjustable (pump B was used to change the concentration ratio). Samples were taken from locations 1–4 at the desired times. Each experiment was repeated three times, and all experiments were performed in dim light.

**Concentration test**

In this test, all samples were analyzed using clinical biochemistry methods and a spectrophotometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA, USA). In a test for albumin, the bromocresol green method was used (sample–reagent ratio, 2 μL : 200 μL; incubation time, 90 s; assay type, endpoint; absorbance, 630 nm; temperature, 37°C). In a test for total bilirubin, the diazonium salt method was used (sample–reagent ratio, 10 μL : 200 μL; incubation time, 10 min; assay type, endpoint; absorbance, 540 nm; temperature, 37°C). Each sample was tested three times, and an averaged value was used.

**Data analysis**

To facilitate comparison between situations with and without the PCM, an equivalent bilirubin concentration (EBC) was defined for the solution in the entire system. In the situation without the PCM, the total solution volume $V$ is mainly composed of three parts: the solution volume in the container $V_c$, the solution volume in the cartridge $V_r$, and the solution volume on the lumen side of the concentrator $V_{hl}$ (the solution volume in the tubing is assumed to be negligible). Because of the lack of preconcentration, the bilirubin concentration before the concentrator is equal to the concentration after the concentrator but before the cartridge (Fig. 1). The bilirubin concentrations before and after the cartridge are different. It is difficult to obtain an accurate average concentration of bilirubin for the solution in the cartridge. In this study, this value is approximated by the average value of the bilirubin concentration before and after the cartridge. The formula for calculating the EBC in the situation without the PCM is then defined as

$$C_{EBC} = \left(\frac{V_c + V_{hl}}{2} \cdot C_1 + 0.5V_r \cdot (C_1 + C_3)\right)/V$$
(C denotes the bilirubin concentration; subscripts 1, 2, 3, and 4 represent locations where samples are taken; and \( C_{EBC} \) is the EBC; bilirubin outside the activated carbon primarily consists of albumin-bound bilirubin, whereas bilirubin inside the activated carbon mainly consists of bilirubin that is bound to activated carbon or unbound, as few albumin molecules can enter the very tiny pores of activated carbon).

In the situation with the PCM, the total solution volume is mainly composed of four parts: the solution volume in the container, the solution volume in the cartridge, the solution volume on the lumen side of the concentrator, and the solution volume on the shell side of the concentrator \( V_{hs} \). In this situation, the concentrations at locations 1, 2, and 3 are different (Fig. 1). In this study, the average bilirubin concentration on the lumen side of the concentrator is approximated by the average value of the bilirubin concentration before and after the concentrator. The method for determining the average bilirubin concentration in the cartridge is the same as described above. The formula for calculating the EBC in the situation with the PCM is then defined as

\[
C_{EBC} = \left[ V_c \cdot C_1 + 0.5V_{hl} \cdot (C_1 + C_2) + 0.5V_r \cdot (C_2 + C_3) + V_{hs} \cdot C_4 \right]/V.
\]

RESULTS AND DISCUSSION

Bilirubin concentrations at different locations

In the experiment without the PCM, the concentration of bilirubin in the container (before the cartridge) decreased continuously, whereas the concentration at the cartridge outlet (after the cartridge) first increased and then decreased (Fig. 2a). After 15 min, the outlet concentration was only slightly lower than the inlet concentration. However, when the PCM was used, the concentration difference between the locations before and after the cartridge was much larger than the difference observed when the PCM was not used (Fig. 2b). The larger difference in concentrations means a larger driving force of mass transfer, making it possible for more bilirubin molecules to be adsorbed on the surface of the adsorbent. As a result, more bilirubin molecules are ultimately removed. In the PCM, the concentration at location 2 should be proportional to the concentration at location 1; however, a degree of deviation still exists because of the imperfect concentrator that was used in this work (the concentration ratio \( Q_1/Q_2 \) is equal to \( Q_1/(Q_1 - Q_4) \); the bilirubin concentration at location 4 is close to zero, which was validated experimentally; and the flow rate in all figures denotes the value at location 1).

Comparison of EBCs between situations with and without the PCM

Under the condition with the PCM, the albumin solution was enriched before passing through the activated carbon cartridge. Consequently, the bilirubin concentration entering the cartridge was higher than the concentration observed when the PCM was not used. The results shown in Fig. 3 indicate that the bilirubin removal efficiency with the PCM (concen-
tion ratio: 2.59) was significantly higher than when the PCM was not used (concentration ratio: 1.0). In the experiment without the PCM, nearly 180 min was required for the bilirubin concentration to decrease from 19.3 to 13.0 mg/dL; however, in the experiment with the PCM, only approximately 60 min was needed. Here, it is important to note that the PCM proposed here is effective not only for albumin-bound bilirubin but also other protein-bound toxins, such as tryptophan, nitric oxide, and cholic acid.

**Effect of concentration ratios with the PCM**

For a patient with liver failure, the flow rate of blood is limited, mainly depending on the hemodynamic status of the patient (23). In addition, due to the determinate hematocrit of the patient (11), the flow rate of the plasma that is separated from the blood by the filter is also limited; plasma flow rate = blood flow rate × (1 − hematocrit) (24). Here, we mimicked the case: the blood flow rate was 240 mL/min and the hematocrit was 50%; as a result, the maximum flow rate of the plasma was 120 mL/min.

In this work, we studied the effect of concentration ratios on the adsorption of bilirubin at 120 min (Fig. 4). In experiments, the flow rate of the plasma/albumin solution from pump A was always set to be 120 mL/min, the concentration ratio was changed by adjusting the filtrate flow rate from pump B, and the albumin concentration was set to 2%, which was based on the fact that it is usually lower in patients with liver failure (25). In the data analysis, the adsorbed bilirubin was defined by

\[ V(C - C_{EBC})/m \]

\( m \) is the activated carbon mass, \( V \) is the albumin solution volume, and \( C \) is the bilirubin concentration before experiments. Our results show that at 120 min, as the concentration ratio increased, the bilirubin amount adsorbed per unit mass of activated carbon increased. The use efficiency of activated carbon at a concentration ratio of 2.59 was increased by approximately 36% compared with the use efficiency observed when the PCM was not used. Therefore, in practice, by increasing the concentration ratio, one can further enhance the adsorption of albumin-bound bilirubin onto activated carbon.

In the experiment with the PCM, more albumin molecules could be trapped on the surface of the fiber membrane, attracting more bilirubin molecules and thus resulting in a transient pseudo-increase in the adsorption efficiency. As the experiment continued, the bilirubin molecules that were bound to these albumin molecules would still have been transported to the unbound albumin molecules in the solution due to the concentration difference and would then be removed by activated carbon. Regarding the clearance of bilirubin, the effect of the trapped albumin was slight; however, in a patient with liver failure, the effect would be negative because of the possible loss of albumin. Thus, a high-performance concentrator would be needed in practice. In addition, the PCM may cause an increase in the adsorption of albumin onto the adsorbent and thereby increase the adsorption efficiency and loss of albumin. However, in this work, the effect of the increase in the adsorption of albumin was not significant (15), which will be discussed in the next section.

**Mechanism of adsorption enhancement in the PCM**

**Breakthrough curve analysis**

In this study, the situation with the PCM showed equivalence to the case without the PCM. For
example, in the situation without the PCM, if the total plasma volume is $V$, the initial concentration of bilirubin is $C$, and the flow rate through the cartridge is $Q$, then with the PCM, the initial bilirubin concentration entering the cartridge will be $nC$ and the flow rate through the cartridge will be $Q/n$ ($n$ is the concentration ratio). Thus, the situation with the PCM is equivalent to the situation without the PCM, as follows: the plasma volume is $V/n$, the initial bilirubin concentration is $nC$, and the flow rate is $Q/n$. The lower flow rate through the cartridge caused an increase in the residence time for bilirubin adsorption; however, the average residence time for the entire bilirubin solution remained constant. Therefore, regarding the average residence time, the situations with and without the PCM were almost equivalent. However, the average residence time is not the exclusive factor affecting the adsorption of bilirubin onto activated carbon. Based on the linear driving force theory, the bilirubin concentration difference across the fictitious membrane near the surface of an activated carbon particle is another dominant factor (26). For the sake of the concentrator, the bilirubin concentration at the inlet of the cartridge was always higher than the concentration observed when the PCM was not used; accordingly, the bilirubin concentration difference (or the mass transfer driving force) in the cartridge was also higher. Therefore, more bilirubin molecules could be removed by the detoxifying cartridge if the PCM was used.

If the total plasma volume is very large, the mechanism of the increase in adsorption with the PCM can also be explained with the breakthrough curve. Figure 5 shows the comparison of theoretical breakthrough curves between situations with and without the PCM. Our results clearly indicate that the adsorption efficiency was increased when the PCM was used (the equations for simulation are shown in the Appendices).

**Equilibrium adsorption analysis**

A batch experiment was conducted to obtain the adsorption isotherm of bilirubin on activated carbon, which is similar to the Brunauer–Emmett–Teller (BET) isotherm because of multilayer adsorption (Fig. 6). To facilitate an understanding of why the adsorption efficiency increased with use of the PCM, we can suppose that experiments with and without the PCM keep running infinitely and that equilibrium is ultimately reached. One can then use the equilibrium isotherms $q_I = \frac{[V \cdot (C - C_i)]}{m} = f(C_i)$ and $q_{II} = \frac{[V \cdot (n \cdot C - C_{II})]}{m} = f(C_{II})$ to estimate the final status in the experiments without and with the PCM, respectively ($V$ is the total bilirubin solution volume; $m$ is the activated carbon mass; $f$ denotes an equilibrium isotherm function, which depends on the equilibrium property between the adsorbent and the adsorbate; $C$ is the initial bilirubin concentration; $C_i$ and $C_{II}$ are the equilibrium bilirubin concentrations without and with PCM implementation, respectively; and $q_I$ and $q_{II}$ are the adsorbed bilirubin amounts per unit carbon mass without and with PCM implementation, respectively, at equilibrium).

By solving these two equations, if $n > 1$, one could easily reach $C_i < C_{II} < n \cdot C_i$. The mass transfer driving force, that is, the concentration difference in the experiment with the PCM ($n \cdot C - C_{II}$), is then
much larger than the force achieved without use of the PCM \((C - C_f)\). This phenomenon is also consistent with the qualitative explanation in the previous section. In addition, one could obtain a direct explanation for the increase in adsorption efficiency by comparing the solute adsorption amount per unit carbon mass (Fig. 6). According to the above equations, \(q_I\) is less than \(q_D\) when \(n\) is greater than 1. In other words, more bilirubin molecules are removed when the PCM is used. In the adsorption isotherm in Fig. 6, the equilibrium point will move from A to B if the PCM is used.

In this study, for bilirubin, the adsorption isotherm was
\[
q = q_m \cdot k_s C / [(1 - k_1 C)(1 - k_t C + k_3 C)],
\]
where \(q_m = 5.316 \text{ mg/g}\), \(k_s = 7.469 \times 10^{-3}\), and \(k_1 = 4.856 \times 10^{-3}\). In contrast, for albumin, the adsorption isotherm was
\[
q = q_m \cdot b C / (1 + b C),
\]
where \(q_m = 0.0397 \text{ mg/g}\) and \(b = 1.92\). When the PCM is used and the concentration ratio is 2.59, at equilibrium, for bilirubin, \(q\) will increase from 1.19 to 1.88 mg/g, whereas for albumin, \(q\) will increase from 0.0385 to 0.0392 g/g. Therefore, the effect of the increase in the adsorption of albumin on the adsorption of bilirubin is not significant. In addition, our experiments show that when the bilirubin concentration is high, the pseudo-first- or second-order adsorption rate increases as the concentration of bilirubin increases (15). In other words, with the PCM, the high concentration difference contributes to the speed of the adsorption of bilirubin onto activated carbon.

The equilibrium isotherm in Fig. 6 can also explain why certain methods are not sufficient. A higher flow rate through the cartridge can shorten the clearance time of toxins to a certain extent; however, this change does not eventually increase the adsorbed toxin amount per unit mass of the adsorbent (the equilibrium point does not change). An increase in the adsorbent mass can remove more toxin molecules, but the use efficiency of the adsorbent decreases (the equilibrium point moves from A to D). In this situation, the adsorption amount per unit mass of the adsorbent is lower, that is, the adsorbent is not used fully and goes to waste.

Based on the above analysis, the advantage of the PCM is not only a larger quantity of toxins adsorbed but also an improvement in the adsorption rate. Theoretically, with the PCM, the higher the concentration ratio is, the higher the removal efficiency will be; however, one cannot excessively increase the concentration ratio because this ratio is limited by the hemodynamic status of the patient and the performance of the concentrator. Because the albumin concentration is relatively low in patients with liver failure, the PCM would make excellent use of space; however, one would still need to carefully consider the practical situation and then reasonably adjust the concentration ratio to satisfy the treatment requirement. The performance of the concentrator is another factor that could affect our method. In this study, a hollow-fiber dialyzer was used as a concentrator. One could choose other concentrators with high-performance ultrafiltration. The key point is that the membrane in the concentrator should be able to bear a certain transmembrane pressure; otherwise, the membrane could be broken, causing a decrease in the concentration ability. In addition, as the concentration ratio increases, the blockage of the membrane surface by albumin could become more and more serious, and the concentration ability could consequently decrease.

**CONCLUSIONS**

In this study, a new method (PCM) was designed to increase the use efficiency of adsorbents and the clearance of protein-bound toxins in artificial liver support systems. In this method, a concentrator is installed before the detoxifying cartridge. Using this method, one can reduce the treatment cost and time simultaneously. The method sacrifices a certain proportion of the flow rate through the detoxifying cartridge; however, the average retention time and the average mass transfer time in the cartridge do not change. Due to the preconcentration, the driving force of mass transfer for toxins from the solution to the adsorbent is greatly enhanced, and the toxin amount adsorbed per unit carbon mass is thereby increased. This PCM was validated experimentally in this work. To the best of our knowledge, this study is the first to present such a promising method for increasing the adsorption of protein-bound solutes in the field of blood purification.

In this study, we explained the mechanism of the increase in adsorption with the PCM with equivalent and equilibrium analyses and used a simple model to theoretically investigate the breakthrough curves in situations with and without the PCM, but a more comprehensive model is still needed to characterize adsorbents for various toxins, to study the mass transfer across the interface, and to investigate the uptake of toxins by adsorbents (14,27), which will be performed in our next work.

Although this PCM is simple and practicable, it is difficult to set up and apply. In liquid-phase separation, in most cases, the solutes to be separated are usually small and soluble. In these situations, concentration techniques are not feasible. When large and soluble solutes are separated, a common assumption
is that if the solution containing these solutes can be concentrated using a membrane, an adsorption method is not necessary at all because the filtration method can instead be used for separation. The possible application of preconcentration in the adsorption separation field can then be ignored easily.

In this study, the focus of separation/removal is protein-bound toxins. These toxins are small molecules but are mainly bound to large protein molecules, which are not expected to be removed. The binding between carrier proteins and toxins makes it difficult to effectively eliminate these toxins by conventional hemodialysis because the protein size is larger than the pore size of the dialysis membrane. However, these features are amenable to preconcentration, making the PCM especially significant. One could use the traditional adsorption method to remove protein-bound toxins; however, if the treatment cost and time are considered simultaneously in practice, the PCM is definitely promising.

In addition, it is important to note that the validation of the PCM was based on the adsorption of toxins onto activated carbon. One could use other adsorbents to reach the same conclusion. Although this PCM is proposed for artificial liver support systems, the method can also be extended to increase the clearance of protein-bound drugs for other applications in the field of blood purification.

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REFERENCES

APPENDIX A: BILIRUBIN TRANSPORT IN THE DETOXIFYING CARTRIDGE

If we assume that the flow in the detoxifying cartridge is even, all activated carbon particles are identical, and the distribution of these particles is uniform, then a one-dimensional mass transfer equation for albumin-bound bilirubin in the cartridge can be described as follows (26):

\[
\frac{\partial C_{c,b}}{\partial t} + F_c \frac{\partial \bar{q}_b}{\partial t} + u \frac{\partial C_{c,b}}{\partial z} = D_{c,b} \frac{\partial^2 C_{c,b}}{\partial z^2} \tag{A1}
\]

where \( C_{c,b} \) is the concentration of albumin-bound bilirubin in plasma or albumin solution in the detoxifying cartridge (in plasma, bilirubin, whether unconjugated or conjugated, is primarily bound to albumin) (4), \( u \) is the local flow velocity, \( F_c \) is the ratio of carbon volume to solution volume in the cartridge \( (F_c = (1 - \varepsilon_c)/\varepsilon_c, \text{ where } \varepsilon_c \text{ is the packing porosity of the activated carbon}) \), \( D_{c,b} \) is the diffusion coefficient of albumin-bound bilirubin (in this work, it is negligible because the convection transport is dominant, compared to the diffusion transport), and \( \bar{q}_b \) is the average concentration of the free bilirubin adsorbed by the activated carbon (the albumin molecule is large and it is assumed to rarely reach the internal space of very tiny pores).

In this work, due to a large ratio of length to radius, the length of a cylindrically shaped activated carbon is assumed to be infinite. Then, according to the mass conservation, the average concentration of the free bilirubin in the cartridge can be described as:

\[
\frac{\partial \bar{q}_b}{\partial t} = \frac{2}{r_p} k_{t,b} \left( C_{c,b} - C_{p,b} \big|_{r=p} \right) \tag{A2}
\]

where \( r_p \) is the activated carbon radius, \( C_{p,b} \big|_{r=p} \) is the concentration of bilirubin on the outer surface of the activated carbon, \( k_{t,b} \) is the mass transfer coefficient across the interface film near the outer surface of the activated carbon \( (k_{t,b} = D_{c,b} Sh/d_p, \text{ where } d_p \text{ is the activated carbon diameter}) \), \( Sh \) is the Sherwood number, which can be calculated by an empirical relationship \( Sh = 1.09Re^{0.33}Sc^{0.33}/\varepsilon_c \) according to the literature (28), where the Reynolds number \( Re = \rho u r_p d_p/\mu \), the Schmidt number \( Sc = \mu/(\rho D_{c,b}) \), \( D_{c,b} \) is equal to the diffusion coefficient of albumin \( D_a = k_B T/(6\pi \eta r_s) \), \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \mu \) is the viscosity of the albumin solution, and \( r_s \) is the albumin molecular radius. In this study, considering the effect of albumin, the equation for the mass transfer coefficient across the interface film was modified to \( k_{t,b} = D_a Sh[\beta + (1 - \beta) \cdot e^{-\beta d_p}] \) (26), where \( \alpha, \beta, \text{ and } \lambda \text{ are three empirical parameters that can be determined experimentally (15): } \alpha = 2.5 \times 10^{-3}, \beta = 4.0 \times 10^{-3}, \text{ and } \lambda = 0.88 \).

In addition, based on the flux conservation at the interface between the solution and the activated carbon, we have:

\[
D_{p,b} \frac{\partial C_{p,b}}{\partial r} \bigg|_{r=p} = k_{t,b} \left( C_{c,b} - C_{p,b} \big|_{r=p} \right) \tag{A3}
\]

where \( C_{p,b} \) is the concentration of the free bilirubin in the solution in the activated carbon, \( \varepsilon_p \) is the porosity of the activated carbon, \( C_{c,b} \) is the concentration of the free bilirubin adsorbed by the stationary phase, and \( D_{p,b} \) is the effective diffusion coefficient of the free bilirubin in pores, which is calculated with the equation \( D_{p,b} = D_0[\varepsilon_p/(2 - \varepsilon_p)]^2 \) (26), where \( D_0 = k_B T/(6\pi \eta r_b) \) is the free diffusion coefficient of the free bilirubin and \( r_b \) is the bilirubin molecular radius (in this work, the surface diffusion is assumed to be negligible, compared with the pore diffusion).

In calculation of the concentration of the free bilirubin adsorbed by the stationary phase, the linear driving force equation is used:

\[
\frac{\partial C_{c,b}}{\partial t} = k_b(C_{p,b} - C_{c,b}^*) \tag{B2}
\]

where \( k_b \) is the adsorption rate constant of the free bilirubin, which can be determined by fitting the experimental results (15) \( (k_b = 2.5 \times 10^{-3} \text{s}^{-1}) \), and \( C_{c,b}^* \) is the bilirubin concentration in pores, which is in equilibrium with \( C_{c,b} \). \( C_{c,b}^* \) is determined by the isotherm adsorption function.

In the simulation, the concentration of bilirubin was 19.3 mg/dL, the porosity of the activated carbon was 0.603 (15), the albumin molecular radius was 3.48 nm, the bilirubin molecular radius was 0.5 nm, the viscosity of the albumin solution was approximately 1.0 mPa·s (29), and the temperature was 25°C; other parameters can be found in the main text.

APPENDIX B: BILIRUBIN TRANSPORT IN THE ACTIVATED CARBON

The following equation can be used for bilirubin transport in the activated carbon:

\[
\varepsilon_p \frac{\partial C_{p,b}}{\partial t} + (1 - \varepsilon_p) \frac{\partial C_{c,b}}{\partial t} = D_{p,b} \left( \frac{\partial^2 C_{p,b}}{\partial r^2} + \frac{1}{r} \frac{\partial C_{p,b}}{\partial r} \right) \tag{B1}
\]

where \( C_{p,b} \) is the concentration of the free bilirubin in the solution in the activated carbon, \( \varepsilon_p \) is the porosity of the activated carbon, \( C_{c,b} \) is the concentration of the free bilirubin adsorbed by the stationary phase, and \( D_{p,b} \) is the effective diffusion coefficient of the free bilirubin in pores, which is calculated with the equation \( D_{p,b} = D_0[\varepsilon_p/(2 - \varepsilon_p)]^2 \) (26), where \( D_0 = k_B T/(6\pi \eta r_b) \) is the free diffusion coefficient of the free bilirubin and \( r_b \) is the bilirubin molecular radius (in this work, the surface diffusion is assumed to be negligible, compared with the pore diffusion).

In calculation of the concentration of the free bilirubin adsorbed by the stationary phase, the linear driving force equation is used:

\[
\frac{\partial C_{c,b}}{\partial t} = k_b(C_{p,b} - C_{c,b}^*) \tag{B2}
\]

where \( k_b \) is the adsorption rate constant of the free bilirubin, which can be determined by fitting the experimental results (15) \( (k_b = 2.5 \times 10^{-3} \text{s}^{-1}) \), and \( C_{c,b}^* \) is the bilirubin concentration in pores, which is in equilibrium with \( C_{c,b} \). \( C_{c,b}^* \) is determined by the isotherm adsorption function.