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Simultaneous Removal of Albumin-Bound Toxins in Liver Support Devices: Bilirubin and Tryptophan Adsorption on Activated Carbon

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Adsorption processes are commonly implemented in liver support devices for the removal of albumin-bound toxins such as bilirubin andtryptophan.A quantitative knowledge of the reduction of the adsorption capacity due to the simultaneous presence of albumin and several toxins in the liquid phase is essential for a rational design of an adsorption unit used in the liver support devices.In this work, simultaneous adsorption of bilirubin and tryptophan from albumin-containing solutions was investigated. In particular, experimental runs have been carried out in order to evaluate the presence of a competitive adsorption on the activated carbon of the two examined toxins. The IAS model has been used to account for multicomponent adsorption equilibrium.

1. Introduction

Toxins involved in liver failure are often tightly bound to albumin and simple hemodialysis cannot remove them selectively and effectively. Bilirubin and tryptophan are among the albumin bound toxins involved in liver failure. Bilirubin is a hydrophobic product of the catabolism of heme and in case of liver-failure, bilirubin plasmatic levels increase and the accumulation of this molecule in the organism can cause secondary pathologies (Ostrow et al., 1994). Bilirubin exhibits an extremely high affinity for albumin and is often considered as a marker for assessing the effectiveness of liver-failure treatments. Tryptophan is an essential amino acid; nevertheless, when it is not cleared by the failing liver, it accumulates in plasma, causing a branched-chain/aromatic amino acid imbalance which in turn has been suggested to play a causal role in hepatic encephalopathy (Dejong et al., 2007). As bilirubin, tryptophan form complexes with albumin, but, due to its lower binding constant (10^4-10^5 M^{-1}), it is also present in plasma as free-solute in non negligible concentrations. Therefore, bilirubin and tryptophan can be chosen as representative of a wide class of albumin bound toxin to be removed during liver failure.
Specific devices such as MARS(Gambro AB, Lund, Sweden) and Prometheus (FreseniusMedical Care AG, Bad Homburg, Germany) (Rozga, 2006; Stange et al., 1993, Annesini et al. 2009) for albumin-bound toxins removal have been developed; adsorption plays an important role in most of these devices and, in some cases, activated carbon (AC) is chosen as adsorptive medium especially to remove polar compounds such as tryptophan.
Even if several papers aimed to the analysis of liver support devices have been published in the literature, there is still a lack of fundamental works devoted to the investigation of equilibrium and kinetics of adsorptivedetoxification processes. Bilirubin adsorption onto activated carbon has been studied in different albumin containing media (Dunlop et al., 1978, Nikolaev et al., 1991; Fesenko et al., 1999, Sarnatskaya et al., 2002; Ash et al., 2006), even if the data reported are not sufficient for a rational design of a liver support device. As for tryptofan, Ribeiro et al. (1995) investigated tryptophan fixed-bed adsorption on

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different media, including activated carbon; however, in that work, only albumin-free solutions were used, so that the effect ofalbumin binding was not considered.
In previous works, adsorption of bilirubin and tryptophan as single solute onto polymeric resin (Annesini et al., 2005), anionic resin (Piemonte et al., 2010; Annesini et al., 2012) and activated carbon (Annesini et al., 2008; Annesini et al., 2010) were considered; in this work, simultaneous adsorption of bilirubin and tryptophan from albumin-containing solutions was investigated. In particular, several batch adsorption tests have been carried out in order to evaluate the presence of a competitive adsorption on the activated carbon of the two examined toxins. The effect of albumin concentration on the adsorption process was also taken into account. The Ideal Adsorbed Solution (IAS) model has been used to analyse the multi-solute adsorption isotherms.
A quantitative knowledge of the reduction in toxin adsorption caused by the simultaneous presence in the liquid phase of two or more toxins, as well as the presence of albumin, is essential for a rational design of an adsorption unit used in the liver support devices.

2. Materials and Methods

2.1 Reagents

Bovine serum albumin (Cohn fraction V, MW = 66 000), bilirubin (mixed isomers, MW = 585) and L-tryptophan (MW = 204) were purchased from Sigma–Aldrich (Milano, Italy) and used as received.
Activated carbon for gas chromatography 05112 (Fluka, Milano, Italy) with a specific surface area of 900m^2/g, apparent density of 0.41 g/cm^3 and particle size in the range of 0.3–0.5mm was used as adsorbent. All the chemicals used were reagent grade.
All operations were conducted in a dark room in order to avoid photodegradation of toxins.
All the solutions were prepared in phosphate buffer 0.15M at pH 7.4. Albumin–bilirubin-tryptophan solutions were prepared by dissolving solid bilirubin in aqueous NaOH 20mM and then adding albumin-tryptophan solution in phosphate buffer. The final pH was set to 7.4.

2.2 Adsorption Experiments

All adsorption experiments were carried out in magnetically stirred flasks, at 25 ± 0.1 ◦C, contacting 40 ml of a solution of known composition with different amounts of activated carbon. All solutions had the same initial albumin and tryptophan concentration(75.7 and 450 μmol/l, respectively), while 3 different initial bilirubin concentrations were used (46.1, 80.1 and 96.4 μmol/l). A summary of the solute concentrations used in the experimental tests is presented in Table 1.
After 16 h, the suspension was settled and centrifuged at 4500 rpm;then, the supernatant was filtered on a nylon 0.45 μm filter and analysed (preliminary tests showed that albumin-bound toxins adsorption on the filter is negligible).
The analysis of albumin-bilirubin-tryptophan solutions was carried out spectrophotometrically by a LAMBDA 25 UV–vis spectrophotometer (PerkinElmer,Waltham, MA, USA). Since the bilirubin absorption spectrum depends on the albumin concentration in the solution, the total bilirubin concentration was evaluated by calibration at the isosbestic point (λ= 416 nm), where the bilirubin extinction coefficient does not depend on albumin/bilirubin molar ratio (Annesini et al., 2005). Since the absorption peaks of tryptophanand albumin overlap (279 nm), spectrophotometric measurements have been performed against an equal concentration aqueous albumin solution. The albumin concentration was assumed as constant during each experimental test because a previous study showed that albumin adsorption on the activated carbon considered in this work is negligible (Annesini et al., 2008). The calibration line was obtained by taking account of bilirubin adsorption at the same wavelength.In each run, the toxin adsorbed amount was evaluated by a mass balance in the liquid phase.

3. Results and Discussion

Figure 1 reports as points the tryptophan adsorbed amount per unit sorbent mass, n_{try}, Vs. total (i.e. free and bound) tryptophan concentration in the liquid phase, C_{try}; the different point types correspond to different initial bilirubin concentration in the liquid phase. The figure also shows as a solid line the tryptophan adsorption isotherm for a two-solute tryptophan-albumin (i.e. without bilirubin) aqueous solution with the same albumin concentration (Annesini et al., 2008). It is clear that the tryptophan adsorbed amount is reduced when bilirubin is present in the liquid solution; obviously, the higher the bilirubin concentration, the higher the effect on tryptophan adsorption.
As previously reported (Annesini et al., 2008), bilirubin is poorly adsorbed on activated carbon and no significant effects due to tryptophan presence in solution could be observed in the experimental tests

performed in this work. Therefore, bilirubin adsorption data form three-solute solutions are not reported here.

In order to understand the adsorption equilibrium for three-solute albumin-tryptophan-bilirubin aqueous solutions, both albumin-toxin binding and competitive adsorption onto activated carbon must be accounted for. Expressions for bilirubin and tryptophan adsorption isotherms from two-solute albumin-toxin solutions on the same activated carbon used in this work were proposed previously (Annesini et al., 2008). Such expressions were derived on the basis of a model that accounts for albumin-toxin binding in the solution. For both toxins, it was possible to obtain an apparent isotherm relating the toxin adsorbed amount to the total toxin concentration; however, the parameters of the apparent isotherms depend on albumin concentration. For bilirubin, the apparent isotherm is linear

$$n_{bil}^{0} = \bar{m}_{bil} C_{bil} \tag{1}$$

$$\bar{m}_{bil} = \frac{m}{C_{alb}} \tag{2}$$

whereC_{bil} is the total bilirubin concentration in the liquid, n_{bil} is the bilirubin specific adsorbed amount at equilibrium and the superscript 0 refers to two-solute bilirubin-albumin aqueous solutions.

For tryptophan,an apparent Langmuir isotherm was obtained

$$n_{try}^{0} = \bar{n}_{try} \frac{C_{try}}{\bar{k}_{try} + C_{try}} \tag{3}$$

$$\bar{n}_{try} = n_{m,try}^{*} \left(1 - a \frac{C_{alb}}{b + C_{alb}}\right) \tag{4}$$

$$\bar{k}_{try} = \frac{k_{try}^{*} K_{AT}}{1 - k_{try}^{*} K_{AT}} C_{alb} \tag{5}$$

The values of the constants contained in Equations (2), (4) and (5) are reported in Table 2. With the albumin concentration used in this work (C_{alb}=75.7 μmol/L), $\bar{m}_{bil}$=0.56 L/g,$\bar{n}_{try}$=810.7 μmol/g and$\bar{k}_{try}$=11.6 μmol/g.

Since the binding site of bilirubin (Domain II, Subdomain A) and tryptophan (Domain III, Subdomain A) on albumin are different (Brodersen, 1979; McMenamy and Oncley, 1958), it has been supposed that no competitive binding between the two toxins occurs. On the other hand, competitive adsorption of toxins onto activated carbon is considered (see the scheme reported in Figure 2), assuming that the hypotheses of the Ideal Adsorbed Solution (IAS) theory (Radke and Prausnitz, 1978) hold.

According to the IAS theory, for the three-solute solution the total specific adsorbed amount of toxins per unit sorbent mass is

$$n_{bil} + n_{try} = \left(\frac{C_{bil} \bar{m}_{bil}}{\Pi^2} + \frac{C_{try}}{\bar{n}_{try} \bar{k}_{try} \left[\exp\left(\frac{\Pi}{\bar{n}_{try}}\right) - 1\right] \left[1 - \bar{k}_{try} \exp\left(-\frac{\Pi}{\bar{n}_{try}}\right)\right]} \right)^{-1} \tag{6}$$

And the molar fraction of bilirubin and tryptophan in the adsorbed phase ($z_{bil}$and z_{try}, respectively) are given by

$$z_{bil} = \frac{C_{bil} \bar{m}_{bil}}{\Pi^2} \tag{7}$$

$$z_{try} = \frac{C_{try}}{\bar{k}_{try} \left[\exp\left(\frac{\Pi}{\bar{n}_{try}}\right) - 1\right]} \tag{8}$$

The modified spreading pressure, Π,appearing in Equations (6), (7) and (8) can be calculated from the congruence equation

$$z_{bil} + z_{try} = 1 \tag{9}$$

Thus, the three-solute isotherms of bilirubin and tryptophan are

$$n_{bil} = \frac{C_{bil}\overline{m}_{bil}}{\Pi}\left(\frac{C_{bil}\overline{m}_{bil}}{\Pi^2} + \frac{C_{try}}{\bar{n}_{try}\bar{k}_{try}\left[\exp\left(\frac{\Pi}{\bar{n}_{try}}\right)-1\right]\left[1-\bar{k}_{try}\exp\left(-\frac{\Pi}{\bar{n}_{try}}\right)\right]}\right)^{-1} \quad (10)$$

$$n_{try} = \frac{C_{try}}{\bar{k}_{try}\left[\exp\left(\frac{\Pi}{\bar{n}_{try}}\right)-1\right]}\left(\frac{C_{bil}\overline{m}_{bil}}{\Pi^2} + \frac{C_{try}}{\bar{n}_{try}\bar{k}_{try}\left[\exp\left(\frac{\Pi}{\bar{n}_{try}}\right)-1\right]\left[1-\bar{k}_{try}\exp\left(-\frac{\Pi}{\bar{n}_{try}}\right)\right]}\right)^{-1} \quad (11)$$

The calculated tryptophan isotherm (i.e. Equation 11) obtained for an initial bilirubin concentration of 96.4μmol/l is plotted in Figure 1 as a dashed line. The agreement between calculated curve and experimental data is quite satisfactory, especially considering that the IAS model relies only on data obtained with two-solute solutions.

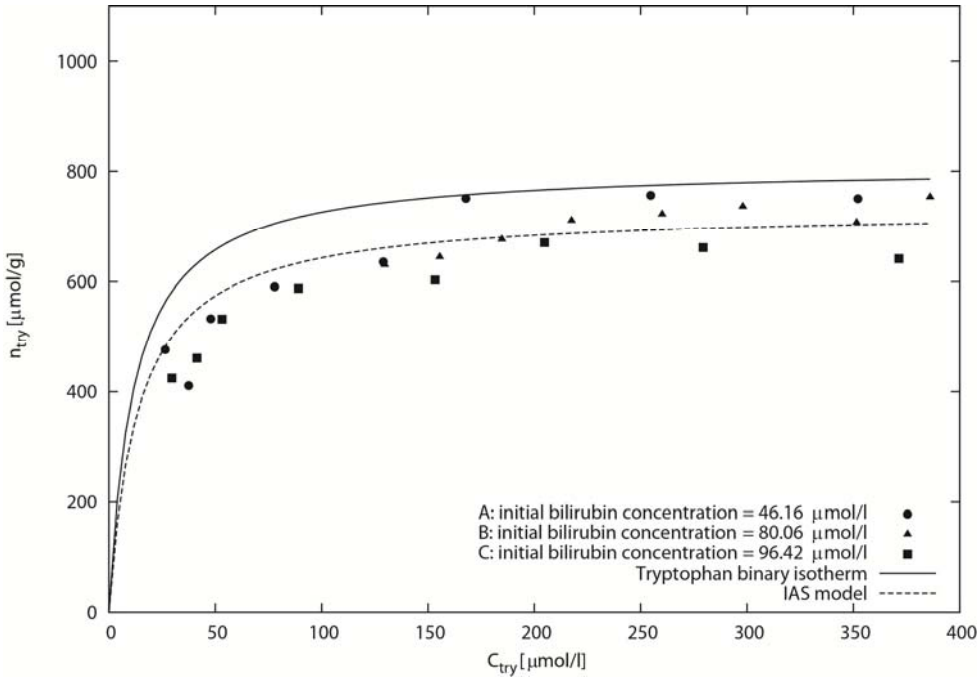

Figure 1: Tryptophan adsorption isotherms from a three-solute albumin-bilirubin-tryptophan aqueous solution. Points: experimental data. Dashed line: isotherm calculated with the IAS model (initial bilirubin concentration 96.42 μmol/l). Solid line: isotherm for a two-solute albumin-tryptophan aqueous solution (Annesini et al., 2008).

Table 1: Concentrations used in batch adsorption tests

Data Set	Albumin conc. [μmol/L]	Initial tryptophan conc. [μmol/L]	Initial bilirubin conc. [μmol/L]
A	75.7	450	46.1
B	75.7	450	80.1
C	75.7	450	96.4

Table 2: Constants of tryptophan adsorption isotherm in two-solute tryptophan-albumin aqueous solutions (Annesini et al., 2008)

Constant	Units	Value
m	μmol/g	43.4
$n^*_{m,try}$	μmol/g	1330
k^*_{try}	μmol/L	11.6
K_{AT}	L/mol	10^4
a	μmol/g	0.644
b	μmol/L	49.3

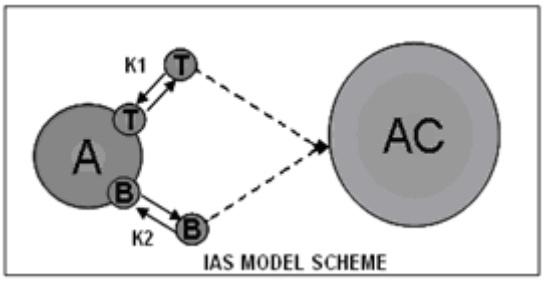

Figure 2: Schematic representation of the approach used to model toxin adsorption from three-solute albumin-bilirubin-tryptophan aqueous solutions. A: Albumin, B: Bilirubin; T: Tryptophan; AC: Activated Carbon; K1: Albumin-Tryptophan equilibrium binding constant; K2: Albumin-Bilirubin equilibrium binding constant.

4. Conclusions

Several experimental runs have been carried out on simultaneous adsorption of bilirubin and tryptophan from albumin-containing solutions. The experimental results obtained here show a competitive adsorption on activated carbon between tryptophan and bilirubin. This behaviour becomes more evident at higher bilirubin concentrations in the liquid phase.

The data collected have been compared with a calculated isotherm obtained by assuming that the two toxins exhibit competitive adsorption on the carbon and non-competitive binding with albumin in the liquid phase. Competitive adsorption has been accounted for by using the IAS theory and bilirubin and tryptophan adsorption isotherms obtained in previous works for two-solute albumin-toxin solutions. The agreement between the data and the calculated curve is satisfactory, especially considering that the model relies only on data obtained with two-solute solutions.

Activated carbon is one of the most commonly used adsorptive media in liver support devices for the removal of albumin-bound toxins such as tryptophan. Bilirubin is poorly adsorbed on activated carbon and other sorptive media are used for its efficent removal; however, in detoxification processes, activated carbon is contacted with albumin-rich liquid systems like plasma or special dialysate solutions containing both tryptophan and bilirubin. Therefore, it is important to assess the effect of bilirubin on the adsorption of other toxins andthe data and the analysis presented in this paper can provide important information for a deeper understanding blood detoxification devices and help in detecting and addressing design issues.

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