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The kinetics of heparin adsorption with Dowex 1x1 ion exchange resin

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Abstract

The recovery of heparin from the porcine intestinal mucosa by using the ion exchange resin was studied. The procedure involved the following steps: alkaline hydrolysis, ion exchange resin adsorption, and elution. The activity of heparin was measured by an antifactor Xa assay. The ammonium chloride/ammonium hydroxide was used as a buffer, and the effect of salt concentration on the adsorption efficiency was evaluated. The results showed that the highest heparin yield (86.17%) was achieved at the lowest concentration of ammonium chloride. The pseudo-first-order model was shown as more adequate to describe heparin adsorption onto the resin.

Keywords: adsorption; heparin; kinetics; ion exchange resin.

Practical Application: Adsorption system design for heparin recovery from the porcine intestinal mucosa.

1 Introduction

Processing of agricultural raw materials generates by-products that still contain a considerable quantity of valuable bioactive functional compounds and can be useful for both technological and pharmaceutical purposes (Shen et al., 2022; Subiria-Cueto et al., 2022). Heparin is a natural, linear, and complex sulfated polysaccharide composed of alternating units of D-glucosamines and uronic acids (Lee et al., 2004). It has been used as a clinical anticoagulant for over 80 years (Baytas & Linhardt, 2020). The animal tissues and organs are the only sources of natural mucopolysaccharides without alternative synthetic routes to produce mucopolysaccharides. The animal organs used for heparin production varied over time from the primary tissue as dog liver to beef lung and finally to the porcine intestine (Linhardt, 2003). The porcine intestinal mucosa as a byproduct of the meat industry has stood out as a rich source of heparin.

Generally, the heparin isolation methods include the collection of pig intestines from slaughterhouses and separation of the mucosal tissues, alkaline or enzymatic hydrolysis to extract the heparin and polysaccharide derivatives, the recovery of raw heparin, purification of heparin and recovery of purified heparin (Evans & Mozen, 1962; Williams, 1967; Okuyama et al., 1975; Vidic, 1981; Linhardt et al., 1992; van Gorp et al., 1997). The most laborious step in heparin production is the extraction of heparin from tissues and the removal of ballast substances, mainly proteins and mucopolysaccharides. Thus, quite harsh conditions, such as autolysis or alkaline extraction, heating, and acidification, are required for heparin extraction from the protein complexes. There is also a process that requires moderate conditions, such as enzymatic hydrolysis. However, this process is longer and may undergo bacterial contamination. van Houdenhoven et al. (2001) developed an enzymatic hydrolysis procedure at a temperature of 50-75 °C, which included the heparin recovery from the protein hydrolysate by using an ion exchange resin.

Generally, patent procedures differ mainly in the choice of extraction agent, the method of breaking the heparin protein complex, and the final purification method. The ion exchange resins were used for the isolation and purification of different products derived from plant or animal tissues, as it supports the reliable capture and removal of biologically active substances with a high molecular weight (Flengsrud et al., 2010; Huang et al., 2022; Pan et al., 2022). The different type of resins such as Dowex® (Warda et al., 2003; Flengsrud et al., 2010), Amberlite (Vreeburg & Baauw, 2010), Lewatit (Linhardt et al., 1992), and Sephadex A-25 anion-exchange resin (Luppi et al., 2005) was designed and tested for heparin recovery. In addition, the synthetic polycations such as cellulose nanocrystals (Liu et al., 2021) and cationic micelles (Vieira et al., 2017) have also shown a good efficiency in recovery of heparin. The efficiency of heparin recovery depends on temperature, pH, and salt concentration. The deviations on any of these parameters could increase the adsorption of contaminants that hinder the binding of heparin (van der Meer et al., 2017).

The studies on heparin recovery by using resin-based adsorption provided information on isolation and characterization of heparin but scarce in the kinetic data for the adsorption of heparin onto resin. The newly published study of Abdolmaleki et al. (2022) has considered the kinetic modeling of heparin adsorption onto zeolite imidazolate framework-8. However, to the best authors' knowledge, the kinetics of heparin adsorption onto Dowex® 1x1 resin has not been reported.

In this study, the recovery of heparin from the porcine intestinal mucosa using Dowex® 1x1 resin and column chromatography was investigated. For this purpose, the heparin extraction was performed by alkaline hydrolysis in the presence of ammonium

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chloride as a buffer component. The effect of ammonium chloride concentration on the adsorption efficiency was evaluated to determine the optimal concentration. The heparin was recovered by adsorption onto resin and elution with sodium chloride solution. Furthermore, the kinetics of heparin adsorption onto the resin was analyzed to propose the adequate kinetic model. Therefore, this study provides information on kinetics of heparin adsorption using resin (Dowex® 1x1) that lacks in literature.

2 Materials and methods

2.1 Material

Porcine intestinal mucosal tissue for heparin isolation and bovine blood for preparation of citrate plasma were collected from the meat processing industry "Neoplanta"- Novi Sad. USP Sodium heparin reference standard (140 USP heparin units/mg), poly(styrene-co-divinylbenzene) 200-400 mesh particle size, Dowex® 1x1 ion exchange resin was obtained from Serva (Heidelberg, Germany).

2.2 Heparin extraction

The alkaline hydrolysis of porcine intestinal mucosal tissue (500 g) was performed by adding water (250 mL) and ammonium chloride (in amount that corresponded to following concentrations: 0.54 mol/L, 0.74 mol/L, 1.0 mol/L, 1.56 mol/L g), while pH-value of slurry was adjusted at 9 by ammonium hydroxide. The slurry was heated at 78 °C until boiling and then boiled for 10 min. The obtained hot slurry was filtered through the multilayer fabric. The coagulate was submitted to subsequent extraction with an ammonium chloride buffer solution three times to extract the remained heparin. The collected filtrate was mixed with 12 g of resin (Dowex® 1x1) for 4 h. The samples were taken every 30 min. Afterward, the resin was separated by filtration and rinsed, first, with water three times, then with the solution of sodium chloride (0.5 mol/L, pH = 8).

2.3 Antifactor Xa assay

The biological determination of heparin activity was performed according to the procedure of United States Pharmacopeial Convention (1994). The substrate S-2222 was reconstituted in distilled water (20 mL) to final concentration of 1mmol/L. Antithrombin and Factor Xa solutions were prepared as specified by the manufacturer.

All experiments were performed in triplicate, and the average values with standard deviations (SD) at a confidence level (CL) of 95% calculated by Microsoft Excel, were presented.

2.4 Kinetic study

To analyze the kinetics of heparin adsorption onto the resin and to determine which model provide the best correlation of the experimental data, two models were employed, the pseudo-first-order and pseudo-second-order model.

The Lagergren's pseudo-first-order model assumes that adsorption occurs through diffusion through the interface. It is based on the assumption that the rate of change of solute uptake with time is directly proportional to the difference in the amount at saturation and the amount of absorbed solute (Sahoo & Prelot, 2020). The Lagergren's pseudo-first-order equation can be written as follows (Equation 1):

$$\frac{dq_t}{dt} = k_1 \cdot \left(q_e - q_t\right) \tag{1}$$

where q_e (IU/mL) is the amount adsorbed at equilibrium expressed through specific activity of adsorbed heparin; q_t (IU/mL) is the amount adsorbed at time t; k_1 is the rate constant in the pseudo-first-order model (min^{-1}).

After the integration of Equation 1 for boundary conditions $q_t = 0$ at t = 0 and $q_t = q_t$ at t = t to obtain (Equation 2):

$$q_t = q_e \cdot \left(1 - e^{-k_1 t}\right) \tag{2}$$

The linearized form of Equation 2 was used for determination of kinetic parameter k_1 (Equation 3):

$$ln\left(q_e - q_t\right) = -k_1 t + ln\left(q_e\right) \tag{3}$$

The pseudo-second-order kinetic model assumes that the rate-limiting step is chemical sorption or chemisorption. The adsorption rate is dependent on the adsorption capacity, not on the concentration of adsorbate (Sahoo & Prelot, 2020). The pseudo-second-order kinetics can be presented by following differential equation (Equation 4):

$$\frac{dq_t}{dt} = k_2 \cdot \left(q_e - q_t\right)^2 \tag{4}$$

where k_2 is the equilibrium rate constant in the pseudo-second-order model (mL/(IU·min).

The integration of Equation 5 by taking into account the boundary conditions resulted in:

$$q_t = \frac{q_e^2 \cdot k_2 \cdot t}{1 + q_e \cdot k_2 \cdot t} \tag{5}$$

Then Equation 5 is linearized to (Equation 6):

$$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e} \tag{6}$$

or (Equation 7)

$$\frac{1}{q_t} = \frac{1}{k_2 \cdot q_e^2} \cdot \frac{1}{t} + \frac{1}{q_e} \tag{7}$$

3 Results and discussion

3.1 Heparin isolation

The raw extracts usually contain a low content of heparin thus the application of ion exchange resins for its isolation and purification is recommended. In this way, a purification of heparin

that yields a higher concentration could be achieved. Dowex® 1x1 was employed for isolation and purification of heparin from raw alkaline extract. To avoid the contamination of ion exchange resin, the raw alkaline extract was filtrated. First, it was heated to boiling to ease the filtration of the coagulate. Apparently, the certain amount of heparin remained in the coagulate. Therefore, the coagulate was submitted to subsequent extraction with ammonium chloride buffer solution for three times. Total activity of combined filtrates was 713.5 IU and correspond to activity that remains in solid phase during filtration. Introducing the filtration step into the heparin isolation procedure had an advantage over the one without. Namely, the resulting solution had lower viscosity that alleviated heparin adsorption onto the resin and lowered the mixing intensity.

The other types of resins were successfully employed in heparin separation such as D254 resin (Shu et al., 2018) and Q-Sepharose (method of Volpi, Sarwar et al., 2015). The comparative study of adsorption performance of quaternized-CS/PS microbeads, CS/PS microbeads and Amberlite FPA98 Cl resin showed that the quaternized-CS/PS microbeads had a better adsorption performance than others (Eskandarloo et al., 2018). However, the multi-porous quaternized-CS/PS microbeads showed even the excellent adsorption capacity over the above mentioned because of the higher surface-to-volume ratio that provides more available reactive sites (Eskandarloo et al., 2018).

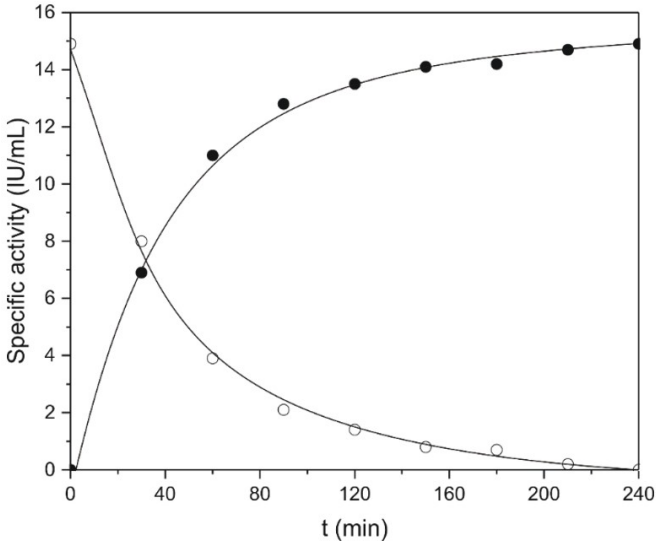

Figure 1. The change of heparin specific activity (●- bonded heparin, ○ - unbonded heparin) with time (average SD = ± 0.15 CL = 95%).

Considering that ammonium chloride as a buffer component could affect the heparin adsorption onto the resin, the effect of its concentration on the heparin adsorption efficiency was investigated. The results are presented in Table 1.

The high yields of purified heparin were achieved in the range of ammonium chloride concentration of 0.54-1 mol/L. It can be observed that the highest concentration of ammonium chloride concentration did not favor the heparin adsorption onto the resin. The highest adsorption efficiency was achieved at the lowest concentration of ammonium chloride. This way, a higher yield of recovered heparin (86.17%) from porcine intestinal mucosa was achieved than in the process using zeolite imidazolate framework-8 (70%) as an adsorbent (Abdolmaleki et al., 2022).

3.2 Kinetic study

In order to investigate the kinetics of heparin adsorption onto the resin, the alkaline extract of heparin specific activity 14.9 IU/mL (i.e. total activity 6285 IU) was used for heparin recovery. The change of heparin specific activity with time is presented in Figure 1.

It could be noticed that the adsorption of heparin onto the resin was resembled by exponential curve. The adsorption was fast at the beginning, and 46.3% of heparin was adsorbed for 30 min. Then, the process of adsorption slowed gradually and 95.4% of heparin was adsorbed for 3 h. Recent investigation on extraction of heparin by arginine-functionalized flowered mesoporous silica nanoparticles reported that the adsorption efficiency increased with adsorption time up to 30 min (Zhang et al., 2021).

The fast adsorption at the beginning of process indicated the application of pseudo-first-order model for adsorption kinetic analysis. The value of k_1 was determined from the slope of linear dependence of $ln(q_e - q_t)$ and t (Figure 2a).

This value was calculated to be 0.02 min^{-1}. The determined value of $q_{e,calc}$ (14.2 IU/mL) was close to the experimental one (14.7 IU/mL). Considering the relatively low relative deviation between these two values (3.40%) and the relatively low mean relative percent deviation (MRPD) between the predicted and experimental data (6.98%, based on 16 data), the pseudo-first-order model can be recommended for a description of the heparin adsorption from the raw alkaline extract onto the resin.

The pseudo-second-order model has often been employed for modeling adsorption kinetics. Considering that most of the data points in the case of heparin adsorption onto resin were close to equilibrium, the pseudo-second-order model can be applied. The estimated value of k_2 from the slope of linear

Table 1. The effect of NH_4Cl concentration on the sorption of heparin onto resin.

NH_4Cl concentration (mol/L)	Raw extract		Purified heparin		
	Activity (IU/mL)	Total activity (IU)	Activity (IU/mg)	Total activity (IU)	Yield* (%)
0.54	15.8 ± 0.20	3128.4	29.3 ± 0.30	2695.6	86.17
0.74	16.5 ± 0.10	3382.5	37.9 ± 0.20	2880.4	85.16
1.00	14.0 ± 0.20	2800	30.5 ± 0.10	2290.5	81.80
1.56	16.1 ± 0.10	3252.5	32.6 ± 0.20	2347.2	72.17

*The yield of purified heparin based on raw extract.

approximation of $\frac{t}{q_t}$ vs t was 0.002 mL/(IU·min) (Figure 2b). The higher value of $q_{e,calc}$ (16.7 IU/mL) was determined in the case of pseudo-second-order model. Nevertheless, the lower value of MRPD (3.62%, based on 16 data) between the predicted and experimental data was calculated. This result indicated a better prediction of heparin adsorption kinetics by the pseudo-second-order model.

However, further analysis is required to ensure the selection of an adequate model. Although both models had a high value of the coefficient of determination (R^2) (Figure 2), they cannot compare because of the involved transformation of function, i.e., $ln(q_e - q_t)$ and $\frac{t}{q_t}$ (Scott & Wild, 1991; Simonin, 2016).

Therefore, R^2 values of the same function q_t for the pseudo-first-order and pseudo-second-order need to compare to obtain a relevant results (Simonin, 2016). This implies a non-linear fitting of Equations 2 and 5 which results are presented in Tables 2-3.

The first procedure included the estimation of both model parameters, $q_{e,calc}$ and k_1 or k_2, while the second procedure involve estimation of one parameter, k_1 or k_2, using the experimental value of $q_{e,exp}$.

According to obtained results (Tables 2-3), the two-parameters fitting provide the high and very close values of R^2 for both pseudo-first-order and pseudo-second-order model.

Despite the high value of R^2, the value of $q_{e,calc}$ determined for pseudo-second-order model did not correspond to $q_{e,exp}$ and higher value of MRPD was obtained. In the case of one-parameter fitting, the lower value of R^2 for pseudo-second-order model and a significant higher value of MRPD were obtained. Considering that the two-parameters fitting yielded a value of $q_{e,calc}$ that corresponded to $q_{e,exp}$, the higher value of R^2 and the lowest value of MRPD recommended the pseudo-first-order over pseudo-second-order model. Compared with the results obtained by linear fitting for the pseudo-first-order model, the values corresponded to values estimated by nonlinear fitting, referring that the pseudo-first-order model provided a better fit of heparin adsorption onto the resin.

It can be concluded that adsorption of heparin onto the resin (Dowex® 1x1) occurred via diffusion through the interface. In the case of adsorption kinetics of heparin onto the zeolite imidazolate framework-8 (Abdolmaleki et al., 2022) or ectoine onto the Dowex HCR-S (Wu et al., 2021), it was described by

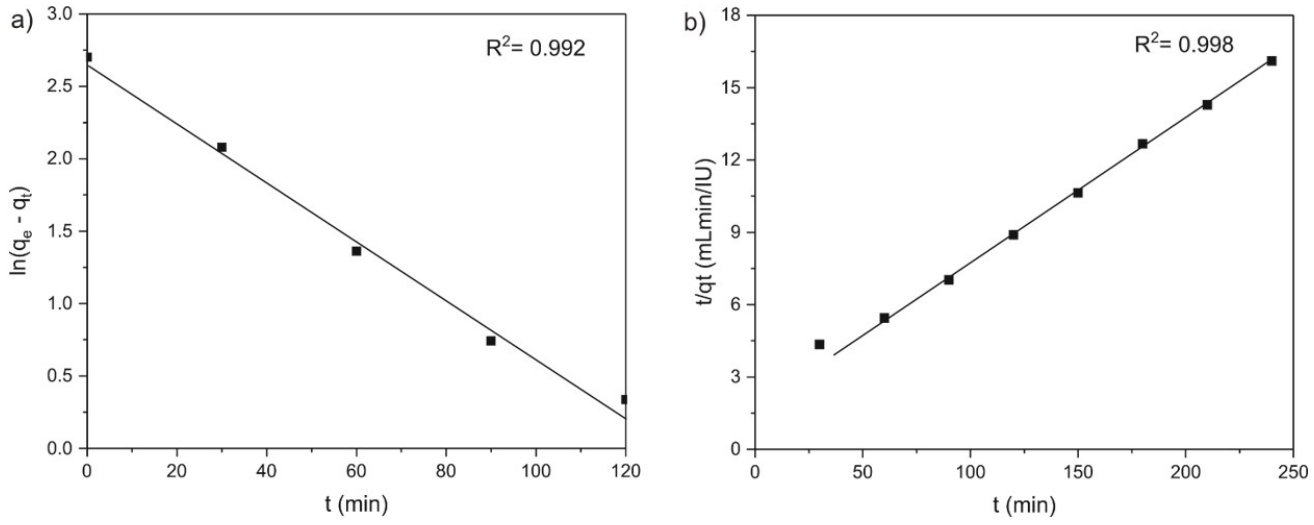

Figure 2. The linear dependence of (a) $ln(q_e - q_t)$ and t, and (b) $\frac{t}{q_t}$ and t.

Table 2. The two-parameters nonlinear fitting of Equations 2 and 5.

Kinetic model	$q_{e,calc}$ (IU/mL)	k_1 (min^{-1})	k_2 mL/(IU·min)	R^2	MRPD* (%)
pseudo-first-order model/Equation 2	14.75	0.022	-	0.998	1.51
pseudo-second-order model/Equation 5	17.64	-	0.0014	0.994	3.13

*Mean relative percent deviation between experimental and calculated values of q_t.

Table 3. The one-parameter nonlinear fitting of Equations 2 and 5.

Kinetic model	$q_{e,exp}$ (IU/mL)	k_1 (min^{-1})	k_2 mL/(IU·min)	R^2	MRPD* (%)
pseudo-first-order model/Equation 2	14.70	0.022	-	0.998	1.54
pseudo-second-order model/Equation 5		-	0.0036	0.946	9.24

*Mean relative percent deviation between experimental and calculated values of q_t.

the pseudo-second-order model better than the pseudo-first-order model.

4 Conclusion

The efficient process of heparin recovery from porcine intestinal mucosa using the ion exchange resin was established. The process included alkaline hydrolysis, ion exchange resin adsorption, and elution. The concentration of ammonium chloride as a component of buffer (ammonium chloride/ammonium hydroxide) affected the heparin adsorption onto the resin in way that the highest adsorption efficiency was achieved at the lowest concentration of ammonium chloride. The kinetic study of heparin adsorption onto resin revealed that the pseudo-first-order model fitted the experimental data better than pseudo-second order model.

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