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Influence of the column hold-up time measurement accuracy on the prediction of chromatographic band profiles

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Abstract

The influence of the column hold-up time measurement accuracy on the determination of equilibrium isotherms by classical frontal analysis and the prediction of overloaded elution band profiles were investigated. The ideal model of chromatography in combination with a Langmuir isotherm was used. Breakthrough curves and overloaded elution profiles were computer generated with a known hold-up time value (true hold-up time). Then these data were evaluated the same way as it is done with experimental chromatographic data where the true hold-up time is unknown, i.e. to determine the equilibrium isotherm by the frontal analysis procedure, to fit the isotherm data to the Langmuir model and then to predict chromatographic band profiles using, e.g. the ideal model of chromatography. A comparison of overloaded elution profiles obtained with different deviations of the hold-up time from its true value shows that the effect of its measurement error is significant in preparative liquid chromatography because the isotherm is usually strongly nonlinear in this case.

Keywords: Frontal analysis; Preparative chromatography; Hold-up time; Void volume; Prediction of band profiles; Ideal model

1. Introduction

Many scale-up optimizations in preparative chromatography are conducted by using trial and error techniques [1]. The success of such procedures depends to a large extent on the experience and skill of the scientist involved as well as on the complexity of the separation problem. These techniques are usually only adequate for small-scale preparative applications where only a few grams of sample need to be isolated, or for separations that are carried out only once to produce some initial sample, e.g. bulk drug substance material for pre-clinical development in the pharmaceutical industry. For such cases the yield, recovery and solvent consumption often do not play a significant role, but only the purity and production rate are important; in other words, time is the most important factor. Trial and error approaches will fail to give acceptable results in large-scale preparative separations or process-scale purification processes where many kilograms or tons of material need to be purified [1,2]. For these cases, the optimization of the scale-up of the separation has to be done using a more sophisticated procedure, i.e. by first accurately measuring the equilibrium isotherms and mass transfer kinetics of the components involved. This is done on the lab-scale, i.e. using a small analytical size column. These experimentally determined data can then be used for the chromatographic scale-up, to numerically calculate band profiles and predict optimal separation conditions on a larger column configuration using a suitable chromatographic model, e.g. the ideal model, the equilibrium-dispersive model, the transport model or the general rate model [1,2]. Such a procedure is the only feasible one, which can consistently provide acceptable results for the chromatographic scale-up. It also permits the accomplishment of the scale-up with a minimum amount of wasted chemicals. Using this more sophisticated scale-up approach will objectively provide the best separation conditions. The optimal conditions depend on the objective of the preparative separation, which

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can be, e.g. the production rate based on maximal recovery or based on the yield of the compound of interest.

The column hold-up time can be defined as the total volume of liquid phase in the column [3]. This definition is well suited for a theoretical investigation using the ideal model of chromatography. The hold-up time is one of the key parameters in the chromatographic scale-up investigation. The reason for this is because the determination of the equilibrium isotherm, using, e.g. classical frontal analysis or other chromatographic methods [1], is directly dependent on its accurate value. Furthermore, the hold-up time is connected to the column linear velocity of the mobile phase in the column. The exact calculation of chromatographic band profiles will necessary be dependent on an accurately determined hold-up time because both the equilibrium isotherm and the linear velocity in the mass balance equation are needed for the calculation.

The experimentally determined column hold-up time value is often not very accurate and many different methods have been employed in the past for its measurement [3–17]. The use of an inert, un-retained marker is a very common approach, but adsorption of the hold-up time marker on the stationary phase can never be ruled out completely, especially because of the wide array of different stationary phases available for chromatography [8,9]. This problem is more significant for preparative chromatographic stationary phases because these phases have generally a higher surface area and a more complex pore structure than analytical stationary phases. Therefore, in many cases a hold-up time which is too large is observed in experimental practice, however, also underestimation of the hold-up time can occur in some cases when the marker is partially excluded. Other methods for the determination of the hold-up time include the use of isotopically labeled markers [17], the minor disturbance method [15] and the derivation of the hold-up time from linearization plots of the logarithm of homologous series [7]. Direct weighing methods, i.e. filling the column with different solvents of known density, have also been used [13]. These latter methods are often not very easy to employ for preparative HPLC applications because of the size, weight and setup of typical preparative columns. The exact definition, and also the true physical meaning, of the hold-up time is another problem to consider [3,16–17]. The question, whether the adsorbed modifier (adsorbed solvent layer on the stationary phase) is a part of the mobile or the stationary phase is also of considerable interest. The difference in a hold-up time defined including or excluding the extracted mobile phase layer can be quite significant considering that preparative packing materials have a very high surface area and complex pore structure [17]. For the chromatographic scale-up procedure using, e.g. the ideal model of chromatography the exact physical meaning of the hold-up time is insignificant, but only its accuracy is important. The hold-up time is needed for the determination of the equilibrium isotherm and in the mass balance equation for the prediction of chromatographic band profiles. Besides the just discussed problems in the accurate measurement of the column hold-up time there are other errors to consider, e.g. the exact measurement of the extra column volume (the volume before and after the column), the flow rate stability and band dispersion. All these considerations make it clear that the exact determination of the column hold-up time is not a trivial problem.

The goal of this paper is to study the influence of the column hold-up time measurement accuracy on the prediction of overloaded elution band profiles. Since it is not possible to know the extent of a systematic error when performing actual experiments because a reference value of the hold-up time is not known, it is convenient to use computer experiments in which various values of the hold-up time can be introduced to generate chromatographic band profiles and equilibrium isotherms. The computer generated data, i.e. breakthrough curves and overloaded elution profiles, can be treated the same way as it is done with actual experiments. Various deviations of the hold-up time from its true value are considered in this study along with different levels of non-linearity of the equilibrium isotherm.

2. Theory

2.1. Chromatographic model

The ideal model of chromatography was used in this study [1]. The ideal model assumes that the mass transfer kinetics are infinitely fast and that, in the chromatographic column, no axial dispersion is present, i.e. the axial dispersion coefficient $D_a = 0$. The mass balance for the ideal model is given by

$$\frac{\partial C}{\partial t} + \phi\frac{\partial q}{\partial t} + u_z\frac{\partial C}{\partial z} = D_a\frac{\partial^2 C}{\partial z^2} = 0 \quad (1)$$

where C and q are the sample concentrations in the mobile and stationary phases, respectively, t is the time and z the position in the column, ϕ represents the phase ratio (volume of stationary phase divided by volume of mobile phase) and u_z the linear mobile phase velocity. The phase ratio is related to the total column porosity ε by $\phi = (1 - \varepsilon)/\varepsilon$.

A Langmuir isotherm was used to describe the relationship between the sample concentration in the stationary and mobile phases, q and C. The Langmuir equation is written as

$$q = \frac{aC}{1 + bC} \quad (2)$$

where a and b are numerical coefficients. The ratio a/b represents the sample saturation capacity q_s of the column.

2.2. Solution of the mass balance equation

The mass balance can be solved analytically for the ideal model ($D_a = 0$). The calculation of the band profile is divided into the calculation of the frontal shock and the calculation of the diffuse boundary [1,18]. The frontal shock of the peak is stable. It propagates at the same velocity while being eroded.

The diffuse boundary or rear shock is unstable because the concentration decreases on the band rear. The band broadens at the band rear because each concentration is associated with a different velocity. The following equation gives the rear diffuse profile:

$$t_R = t_p + t_0\left(1 + \phi\frac{\partial q}{\partial C}\right) = t_p + t_0\left(1 + \frac{\phi a}{(1+bC)^2}\right) \quad (3)$$

where t_p is the duration of the injection pulse and t_0 the hold-up time. The retention time of the shock is given by:

$$t_R = t_\mathrm{p} + t_0\left(1 + \phi a\left(\frac{1 - L_\mathrm{f}(1+bC_0)}{(1+bC_0)}\right)^2\right) \quad (4)$$

where L_f is the loading factor or amount of sample injected in the sample pulse, and C_0 is the concentration. L_f is defined as:

$$L_f = \frac{t_p C_0 b}{t_0 \phi a} \quad (5)$$

3. Generation and evaluation of chromatographic data

The chromatographic experiments, i.e. break through curves and overloaded elution band profiles, were computer generated. The following parameters were held constant throughout this study: column length L = 10 cm, inner diameter of column d = 0.46 cm and volumetric flow rate F = 0.499 mL/min. Three cases were studied with Langmuir isotherm parameters a = 10 and b = 1.0, 0.5 and 0.1 L/g, respectively. These parameters represent, e.g. a fixed column configuration using a constant flow rate and three samples that have different adsorption properties.

3.1. Generation of breakthrough curves and calculation of retention times

The retention time of a breakthrough curve is given by the solution of the ideal model of chromatography as:

$$t_{R,n+1} = t_0\left(1 + \phi\frac{\Delta q}{\Delta C}\right) = t_0\left(1 + \phi\frac{q_{n+1} - q_n}{C_{n+1} - C_n}\right) \quad (6)$$

Retention times of breakthrough curves were calculated for concentration steps from C_n = 0 to C_{n+1} = 0.5, 1, 2, 3, 4 and 5 g/L. The hold-up time used for the calculations was t_0 = 100 s. This value will be referred to as true hold-up time in this study. While performing actual experiments the true hold-up time value cannot be known exactly, however in this study we know the true value because we chose it to be 100 s. Table 1 shows the calculated retention times for the concentration steps for the three sets of the isotherm coefficients a and b. These sets of retention times will be treated the same way as experimental data are treated, and used for the re-calculation of isotherm data using the classical frontal analysis procedure, later for the determination of the Langmuir isotherm coefficients via nonlinear fitting, and finally for the prediction of overloaded elution band profiles. These calculations will be performed with various deviations of the hold-up time from its true value of 100 s.

Table 1
Computer generated retention times of breakthrough curves for various sample concentration steps and isotherm parameters

C_n (g/L)	C_{n+1} (g/L)	t_R (s)		
		I, a = 10.00, b = 1.000 L/g	II, a = 10.00, b = 0.500 L/g	III, a =10.00, b = 0.100 L/g
0	0.5	766.7	900.0	1052.4
0	1.0	600.0	766.7	1009.1
0	2.0	433.3	600.0	933.3
0	3.0	350.0	500.0	869.2
0	4.0	300.0	433.3	814.3
0	5.0	266.7	385.7	766.7

Three sets of Langmuir isotherm parameters were used, a = 10, b = 1, 0.5 and 0.1 L/g. The retention times are based on the column dimension of 10 cm × 0.46 cm, a volumetric flow rate of 0.499 mL/min and a hold-up time t_0 = 100 s.

3.2. Error in the hold-up time

The theoretically possible hold-up time values depend on the column dimensions and the volumetric flow rate. In the case studied here, a column length of 10 cm, an inner diameter of 0.46 cm and a volumetric flow rate of 0.499 mL/min, the hold-up time can theoretically vary from 0 to 200 s. These values represent the two limits, a column which is empty, i.e. without stationary phase, and a column completely filled with the stationary phase, i.e. there is no space left for the liquid phase. Calculated phase ratios and total porosities for hold-up times between 0 and 200 s are shown in Table 2. The column hold-up volume is calculated by $V_0 = Ft_0$ (F: volumetric flow rate) and the phase ratio by $\phi = (V_{total} - V_0)/V_0$ (V_{total}: total volume of the empty column). In reality the hold-up time will vary within much narrower limits [19]. Nevertheless, it is possible to study the problem for variations of the hold-up

Table 2
Dependence of the calculated void volume V_0, phase ratio ϕ and total porosity ε on the assumed hold-up time value

t_0 (s)	V_0 (mL)	ϕ	ε
0	0.00	∞	0.00
20	0.17	9.00	0.10
40	0.33	4.00	0.20
60	0.50	2.33	0.30
80	0.66	1.50	0.40
100	**0.83**	**1.00**	**0.50**
120	1.00	0.67	0.60
140	1.16	0.43	0.70
160	1.33	0.25	0.80
180	1.50	0.11	0.90
200	1.66	0.00	1.00

The calculations are based on the column dimension of 10 cm × 0.46 cm and a volumetric flow rate of 0.499 mL/min; t_0 = 100 s is selected as the true hold-up time.

time over the whole theoretically possible range. This paper considers variations of the hold-up time of 60, 80, 120 and 140 s.

3.3. Calculation of isotherm data points using classical frontal analysis

In classical frontal analysis a series of n concentration steps from C_n to C_{n+1} is performed to obtain isotherm data points. These concentration steps result in a series of n breakthrough curves with the retention times $t_{R,n+1}$ (see generated retention time data in Table 1). The sample concentrations in the stationary phase are calculated by the integrated mass balance equation:

$$q_{n+1} = q_n + \frac{C_{n+1} - C_n}{\phi} \frac{t_{R,n+1} - t_0}{t_0} \tag{7}$$

where q_n and q_{n+1} are the initial and final sample concentrations in the stationary phase in equilibrium with C_n and C_{n+1}, respectively. The retention time $t_{R,n+1}$ is represented by the area over the breakthrough curve

$$t_R = \int_0^\infty \frac{C_{n+1} - C}{C_{n+1} - C_n} \, \mathrm{d}t \tag{8}$$

For the purpose of this study the experimental retention times of the breakthrough curves were calculated using the ideal model as previously described, hence the generated retention data from Table 1 were used, and therefore there is no error associated with the determination of retention time from breakthrough curves. In experimental practice, the retention time at half height, i.e. at the concentration $(C_{n+1} + C_n)/2$, is often used. It has been shown previously that the use of this retention time gives satisfactory results in most cases where the mass transfer is not extremely slow and independent of the concentration [20–22].

3.4. Determination of isotherm coefficients

The isotherm data points, i.e. sample concentrations in the stationary and mobile phases, respectively, which were obtained by the frontal analysis procedure discussed in the previous section, were fitted to the Langmuir equation using a nonlinear fitting procedure. A commonly used Marquardt–Levenberg algorithm was used for the best parameter estimation of a and b [23–25].

3.5. Calculation of overloaded elution profiles

Overloaded elution profiles were calculated for sample concentrations C_0 = 0.5, 1, 3 and 5 g/L by using the ideal model of chromatography. The duration of the injection was held constant at t_p = 100 s which represents an injection volume of 0.8 mL at a flow rate of 0.499 mL/min. Calculations were performed for hold-up times t_0 = 60, 80, 100, 120 and 140 s using the initial (true) isotherm coefficients a = 10 and b = 1.0, 0.5 and 0.1 L/g, respectively, and the isotherm coefficients obtained by the previously described nonlinear fitting procedure.

4. Results and discussion

In the following section first the errors observed in the determination of the isotherms data points that are caused by using an incorrect determined hold-up time are discussed. Afterwards the nonlinear fitting of these data to the Langmuir isotherm and the prediction of overloaded elution band profiles using various hold-up times and isotherm parameters are presented.

4.1. Influence of the error in the hold-up time on the equilibrium isotherms and Langmuir isotherm coefficients

An example of calculated equilibrium isotherm data points and fittings to the Langmuir equation is shown in Fig. 1. The isotherm data points that were used to generate the as experimental data treated retention times were obtained with isotherm parameters a = 10.0 and b = 1.0 L/g and a hold-up time of 100 s. The calculations show isotherms for t_0 = 60, 80, 100, 120 and 140 s. It can be seen in Fig. 1 that an overestimation in the hold-up time leads to a proportional overestimation in the amount adsorbed. For an under-estimation of the hold-up time the converse is true. For a very large overestimation of the hold-up time the error becomes quite apparent and the resulting isotherm does not show the typical Langmuir saturation behavior. This is seen, e.g. in Fig. 1

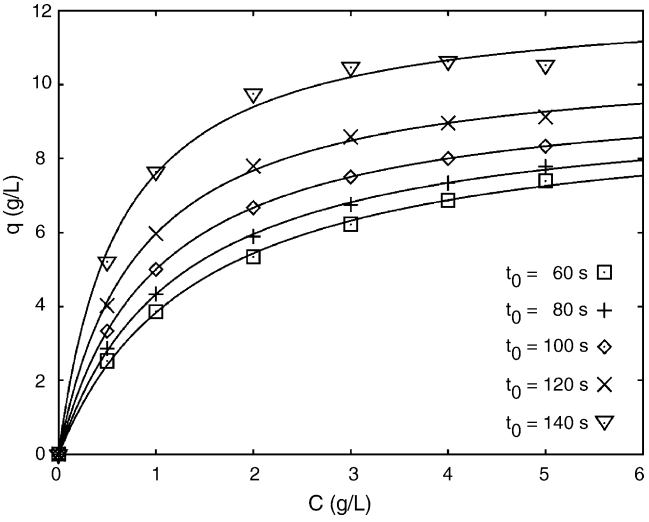

Fig. 1. Dependence of the calculated isotherms on the value of the column hold-up time. Calculated isotherm data points and best fittings to the Langmuir equation. The original Langmuir isotherm parameters are a = 10, b = 1 L/g for the true hold-up time t_0 = 100 s. Calculations for t_0 = 60, 80, 120 and 140 s.

for t_0 = 140 s. After an initial increase the amount adsorbed decreases again at higher mobile phase concentrations. This behavior can also be easily verified by examining Eq. (7), e.g. when $t_{R,n+1}$ becomes smaller than t_0. It seems obvious that in this case the t_0 value must be wrong, however experimental data are often not available for concentrations that are high enough and therefore the t_0 value does usually not exceed $t_{R,n+1}$ for the experimental data available. This observation is one reason why it is always advisable to acquire adsorption data up to the highest possible concentration. It is also important to acquire as many data points as possible because the adsorption data could in some experimental cases have a large experimental error and therefore appear to decrease with higher mobile phase concentrations. This is, of course, not the case for this study because the experimental data were computer generated. The problem described above that for some cases the typical Langmuir behavior is not seen does not arise for an underestimation of the hold-up time.

An error in the hold-up time results in errors in the isotherm coefficients. The best Langmuir coefficients, *a* and *b*, for three cases studied are shown in Table 3. The original isotherm parameters were *a* = 10.0 and *b* = 1.0, 0.5 and 0.1 L/g. The quality of the fitting of the adsorption data to the Langmuir model deteriorates with increasing error in the hold-up time. This is seen in Fig. 1 where the fitting of the isotherm data for t_0 = 60 and 140 s is worse than the other fittings t_0 = 80 and 120 s. Table 3 also shows the sum of the squared residuals and confirms numerically the visual result found in Fig. 1. The quality of the fitting decreases with increasing error in t_0 (see Fig. 1) as well as with increasing non-linearity of the isotherm (Figure not shown), i.e. with increasing *b*. An overestimation of t_0 results in a larger error based on s^2 than an underestimation. For an overestimation of t_0 the Langmuir parameters *a*, *b* and the saturation capacity q_s are overestimated too. For an underestimation of t_0 these parameters are underestimated. It is interesting to note that although the fitting to the Langmuir equation becomes worse for more nonlinear systems, i.e. larger s^2 values, the relative error made in the determination of the saturation capacity q_s is smaller. For example for a 20% deviation of the hold-up time from its true value, i.e. for t_0 = 80 and 120 s, the deviation of q_s from its original true value is <1% for case I with *a* = 10.0 and *b* = 1.0 L/g. The deviation is >10% for the less nonlinear case III with *a* = 10.0 and *b* = 0.1 L/g. This finding reinforces the previously made statement that isotherm data should be acquired for sample concentrations as high as possible.

4.2. Comparison between predicted and original overloaded band profiles

The influence of the magnitude of the error in the hold-up time was studied by a comparison of the generated band profiles at t_0 = 100 s and the predicted band profiles that were obtained using different values of t_0. Fig. 2a and b show band profile calculations for t_0 = 60 and 140 s and t_0 = 80 and 120 s, respectively. An over-estimation of the hold-up time leads to a predicted or calculated band profile with an earlier eluting and less eroded front shock as compared to the original band profile (C_0 = 5 and 3 g/L). The diffuse boundary is more curved and more tailed, i.e. the concentration 0 is reached later. For an underestimation of the hold-up time the converse is true. For lower injection concentrations, i.e. C_0 = 1 and 0.5 g/L, the front shock of the band profile with an overestimated hold-up time elutes later. The difference between the original and re-calculated band profiles increases with an increasing deviation of the hold-up time from its true value t_0 = 100. These results are important for practical purposes, because when considering a multi-component problem there will be an error in the band profiles and hence an error in cut points chosen [27]. The calculations of the yield, purity and the production rate will therefore be influenced by an error in the hold-up time determination.

The influence of the increased non-linearity of the isotherm on elution band profiles was studied by changing the second Langmuir coefficient *b* from 1 to 0.05 and 0.01 L/g and

Table 3
Original isotherm coefficients used for the generation of computer experimeFnts, recalculated isotherm coefficients *a*, *b*, saturation capacities q_s and sums of squared residuals s^2 for different assumed values of the holdup time t_0

t_0 (s)	Recalculated Langmuir isotherm parameters and squared residuals											
	I, *a* = 10.00, *b* = 1.000 L/g[a]				II, *a* = 10.00, *b* = 0.500 L/g[a]				III, *a* = 10.00, *b* = 0.100 L/g[a]			
	a	*b* (L/g)	q_s (g/L)	s^2 (g^2/L^2)	*a*	*b* (L/g)	q_s (g/L)	s^2(g^2/L^2)	*a*	*b* (L/g)	q_s (g/L)	s^2 (g^2/L^2)
20	4.81	0.520	9.25	0.094	5.52	0.349	15.81	0.035	5.96	0.088	67.73	0.001
40	5.52	0.597	9.25	0.076	6.19	0.378	16.38	0.027	6.59	0.090	73.22	0.000
60	6.50	0.696	9.34	0.050	7.09	0.412	17.21	0.017	7.41	0.093	79.68	0.000
80	7.89	0.825	9.56	0.020	8.28	0.453	18.27	0.007	8.48	0.097	87.42	0.000
100	**10.00**	**1.000**	**10.00**	**0.000**	**10.00**	**0.500**	**20.00**	**0.000**	**10.00**	**0.100**	**100.00**	**0.000**
120	13.39	1.245	10.76	0.056	12.56	0.557	22.55	0.018	12.21	0.104	117.40	0.000
140	19.80	1.608	12.31	0.161	17.03	0.626	27.20	0.141	16.01	0.107	149.63	0.002
160	34.44	2.193	15.70	2.715	26.16	0.710	36.84	0.798	23.65	0.112	211.16	0.010
180	86.73	3.265	26.56	22.787	54.34	0.816	66.59	6.473	46.88	0.116	404.14	0.075

[a] Original Langmuir isotherm parameters. The true hold-up time is t_0 = 100 s.

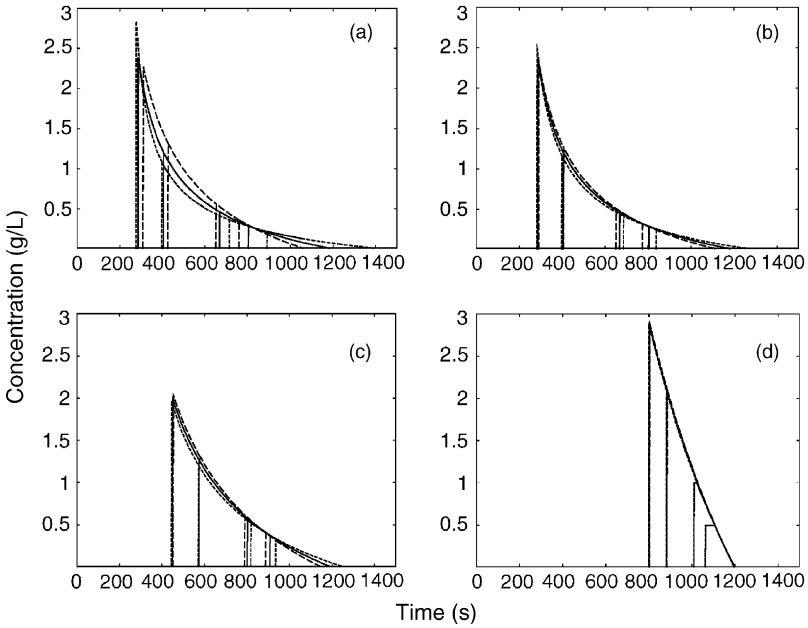

Fig. 2. Comparison of calculated overloaded band profiles. Injection concentration C_0 = 5, 3, 1 and 0.5 g/L. Injection duration t_p = 100 s. True hold-up time t_0 = 100 s (solid lines). (a) Assumed hold-up times t_0 = 60 s (dashed lines) and t_0 = 140 s (dotted lines). Original isotherm parameters a = 10.00, b = 1.0. (b) Assumed hold-up times t_0 = 80 s (dashed lines) and t_0 = 120 s (dotted lines). Original isotherm parameters a = 10.00, b = 1.0. (c) Assumed hold-up times t_0 = 60 s (dashed lines) and t_0 = 140 s (dotted lines). Original isotherm parameters a = 10.00, b = 0.5. (d) Assumed hold-up times t_0 = 60 s (dashed lines) and t_0 = 140 s (dotted lines). Original isotherm parameters a = 10.00, b = 0.1.

keeping the dimensionless coefficient a constant at 10. This changes the saturation capacity by a factor of 100, i.e. the saturation capacity increases from 1 to 100 g/L. The product bC which characterizes the non-linearity of the system changes from 5 to 0.01. At the highest concentration, the surface coverage is 83% for the first case and 5% for the second case, respectively. The values presented in this study are realistic examples and have been found, e.g. for phenol or 2-phenyl 1-propanol on a C-18 stationary phase [20,26]. Fig. 2a, c and d shows predicted and original band profiles for the three isotherm sets investigated, and values of the hold-up time t_0 = 60, 100 and 140 s. As seen from a comparison of Fig. 2a, c and d the differences in the profiles, i.e. the band profiles using t_0 = 100 s, and calculated band profiles decrease with decreasing b, hence with decreasing non-linearity of the equilibrium isotherm. Therefore, the prediction of band profiles is less accurate for chromatographic systems that exhibit more non-linerarity of the isotherm, i.e. systems that have a low saturation capacity and are overloaded easily, or systems that have a reasonable high saturation capacity but are overloaded heavily to increase the production rate. The latter is the case for most large-scale preparative separations and it shows the importance of the hold-up time accuracy.

5. Conclusion

An error in an experimental hold-up time determination causes an error in the determined isotherm and in the calculated overloaded band profile. The significance of this error increases with the non-linearity of the equilibrium isotherm and with an increasing deviation of the column hold-up time from its true value. The shape of the calculated band profile is affected by this error. The hold-up time measurement error is of great significance in preparative liquid chromatography because the isotherm is usually strongly nonlinear in this case. The observation that the best fitting of the isotherm data to the Langmuir equation is obtained for the true hold-up time leads to the conclusion that an actual measurement of the hold-up time could be validated by the measurement of the equilibrium isotherm using classical frontal analysis and subsequently fitting the isotherm data to the Langmuir equation. The hold-up time is then chosen as the value that gives the best fitting, i.e. a minimum in the sum of squared residuals. The applicability of such a procedure is however based on the assumption that the adsorption behavior of a sample follows a Langmuir isotherm model. The findings in this paper show that the right choice of isotherm model is important, i.e. the equilibrium isotherm model that fits best the experimental data, whether it is Langmuir or not will give the most accurate calculation of overloaded elution profiles.

6. Nomenclature

a	first numerical coefficient of the Langmuir isotherm
b	second numerical coefficient of the Langmuir isotherm (L/g)
C	sample concentration in the mobile phase (g/L)

C_0 initial sample concentration (g/L)
C_n sample concentration in the mobile phase of the nth concentration step (g/L)
C_{n+1} sample concentration in the mobile phase of the [n + 1]th concentration step (g/L)
d inner column diameter (cm)
F volumetric flow rate (mL/min)
L column length (cm)
L_f loading factor
q sample concentration in the stationary phase (g/L)
q_n sample concentration in the stationary phase of the nth concentration step (g/L)
q_{n+1} sample concentration in the stationary phase of the [n + 1]th concentration step (g/L)
q_s sample saturation capacity (g/L)
t time (s)
t_0 hold-up time (s)
t_p injection time (s)
t_R retention time (s)
$t_{R,n+1}$ retention time of the [n + 1]th concentration step (s)
u_z linear velocity of the mobile phase (cm/s)
V_0 column hold-up volume (mL)
V_{total} total (geometrical) volume of the empty column (mL)
z location in the column (cm)

Greek letters
ε total column porosity
ϕ volume ratio of stationary to mobile phase

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