A Model Free Method for Estimation of Complicated Adsorption Isotherms in Liquid Chromatography

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Abstract

Here we show that even extremely small variations in the adsorption isotherm can have a tremendous effect on the shape of the overloaded elution profiles and that the earlier in the adsorption isotherms the variation take place, the larger its impact on the shape of the elution profile. These variations are so small so that they can be “hidden” by the discretization and in the general experimental noise when using traditional experimental methods, such as frontal analysis, to measure adsorption isotherms. But as the effects of these variations are more clearly visible in the elution profiles the Inverse Method (IM) of adsorption isotherm estimation is an option. However, IM usually requires that one selects an adsorption isotherm model prior to the estimation process. Here we show that even complicated models might not be able to estimate the adsorption isotherms with multiple inflection points that small variations might give rise to. We therefore developed a modified IM that, instead of fixed adsorption isotherm models, uses monotone piecewise interpolation. We first validated the method with synthetic data and showed that it can be used to estimate an adsorption isotherm which accurately predicts an extremely “strange” elution profile. For this case it was impossible to estimate the adsorption isotherm using IM with a fixed adsorption model. Finally, we will give an example of a real chromatographic system where adsorption isotherm with inflection points is estimated by the modified IM.

1 Introduction

Today chromatography is increasingly important for analysis and purifications of pharmaceuticals, and other valuable chemicals such as antioxidants and intermediates, both in industry and in academia. However, improved technical and numerical methods for obtaining detailed knowledge about the thermodynamics and mass transfer kinetics of the processes are necessary. Estimation of adsorption isotherms are crucial for computer-assisted optimizations of preparative systems [1–3] and also give deeper understanding of the separation processes and its molecular interactions [4,5]. Chiral preparative chromatography stationary phases are much more expensive than achiral ones and also have more limited capacities. Therefore in this case it is especially important to accurately determine the, often very complex, adsorption isotherms in order to perform computer assisted optimization to determine the optimal operational conditions [1,6]. “Optimal conditions” often mean maximal production rate and/or minimum solvent consumption but it depends in fact on the particular goal of the separation; e.g. it desirable to have as robust, safe and environmentally friendly processes as possible [7].

Adsorption isotherms are often classified according to their shapes [8]. The most common ones is Type-I (Langmuir or similar) that are convex functions with a horizontal asymptote equal to the surface capacity. Type-III adsorption isotherms, sometimes called anti-Langmuir, on the other hand are concave with a vertical asymptote. Type-II, Type-IV, Type-V and Type-VI adsorption isotherms are more complex and contain at least one inflection point. Although there are many cases where the adsorption is best described by complex adsorption isotherms containing inflection points [9–12], for most liquid separation systems the adsorption is best described with Type-I adsorption isotherms that can have one (e.g. Langmuir) or several different adsorption sites (e.g. bi-Langmuir). The Langmuir model has a unimodal energy distribution and the bi-Langmuir has a heterogenic bimodal energy distribution. The bi-Langmuir model has successfully been used to describe the adsorption of enantiomers to protein and cellulose derivatized stationary phases [5], the adsorption of charged

solutes [13] and the adsorption of uncharged solutes having both polar and nonpolar properties, e.g. phenol and caffeine [14]. The Tóth adsorption isotherm is an example of a one-site adsorption model that has a unimodal heterogeneous adsorption energy distribution and therefore accounts well for some energetically heterogeneous surfaces, e.g. polar hydrogen bindings between a polar surface and hydrogens at different positions in a peptide [15].

There are several chromatographic methods that can be used to measure adsorption isotherms, all with their advantages and drawbacks. Some methods are based on experiments where a constant stream of the solute molecules is introduced in the column, so called plateau methods. Other methods are based on processing of a few overloaded elution profiles. The Frontal Analysis (FA) plateau method is usually carried out in a series of programmed concentration steps, each step resulting in a so called breakthrough front giving one point on the adsorption isotherm curve. In the Perturbation Peak (PP) plateau method, a plateau is established and a small sample, with composition deviating from the plateau, is injected. The disturbance of the established equilibrium generates perturbation peaks with retention times related to the adsorption at that particular plateau level. The FA method is traditionally considered to be the most accurate one for determination of adsorption isotherms and it can be used for any type of adsorption [16]. However, it was recently showed that the PP method is as accurate as the FA method [17]. In both methods it is important to cover a large range of concentration plateau levels which is time-consuming, tedious and consumes large amounts of, often, expensive solutes.

The simplest method to obtain adsorption isotherms directly from overloaded elution profiles is Elution by Characteristic Points (ECP) where the diffuse tail of a large overloaded profile is integrated [1]. The ECP method is derived from the ideal model that assumes infinite column efficiency, but since the efficiency of a real column is finite this results in an error in the derived isotherms; the lower the column efficiency the larger the error [18]. Furthermore, the ECP theory assumes rectangular injection profiles which leads to large errors for the large injection volumes that are

necessary to obtain sufficient overloading. Due to considerable post-loop dispersion for large injections the injection profiles will have extremely tailed rears. However, it was recently demonstrated, and validated, how easily this this source of error can be eliminated by using the so called ECP-CUT method where a sharp slice is made on the rear of the injection sample zone before introduction into the column [19]. Interesting, and logically, is that the post-loop dispersion is not a problem when using ECP for Type-III adsorption isotherms [2].

The most recently developed method for adsorption isotherm determination is the so called Inverse Method (IM) [20–22]. Here adsorption isotherm parameters are determined from a few overloaded elution profiles in a fitting procedure that uses the whole profile, not only the rear as in ECP. The solute consumption and time requirements are very modest compared to plateau methods. However, as opposed to plateau methods, such as FA, adsorption data are not obtained directly from IM. Instead parameters in an adsorption isotherm model are estimated by solving an inverse partial differential equation problem by iteratively simulating elution profiles until the difference is small between the simulated and the experimental elution profiles.

To summarize, plateau methods, such as FA and PP, are usually more accurate for adsorption isotherm determination compared to methods based on overloaded elution profiles, such as ECP and IM [1,17]. On the other hand the latter methods are much faster since the whole adsorption isotherm can be obtained from a few overloaded experiments. The ECP method has serious inherent problems and is limited to a few types of adsorption isotherms (mainly Type-I and III) and can only be used for single component adsorption isotherms. IM on the other hand can be used for multi-component problems and for separations with low column efficiency; IM is therefore the primary choice today for preparative and process chromatography. The great advantage with IM is the saving of laboratory time and solvents. One serious disadvantage with IM is that it cannot provide adsorption isotherm data directly; it can only estimate adsorption isotherm parameters in an

adsorption isotherm model. This requires that one selects an appropriate adsorption isotherm model prior to estimation.

It should be noted that small variations in the adsorption isotherm can be difficult to detect using plateau method as these small variations can be "hidden" between the measured data points and in the general experimental noise. However, extremely small variations, barely visible in the raw adsorption isotherm plot, can have a tremendous impact on the eluted overloaded profile. An interesting example of this is given in [3]. Here the elution profile, see Fig. 9 in [3], has a strange shape where the retention initially increases with increasing sample concentration, but further increases in the sample concentration decreases the retention. Such elution profiles can only be the result of having an inflection point in the corresponding adsorption isotherm. However, this inflection point is barely visible in the adsorption isotherm, see Fig. 4 in [3]. This illustrates how extremely sensitive the elution profiles are to a change in the adsorption isotherm. There are also several examples of complicated adsorption isotherms, with inflection points, that gives very "strange" elution profiles [9].

Because of the above, IM, that utilizes the whole elution profiles, could be a much better alternative than the plateau methods to handle very complicated adsorption behavior with, barely visible, (multiple) inflection points in the adsorption isotherm. However, IM is currently restricted by the need to choose an appropriate adsorption isotherm model prior to estimation. One should of course always strive to understand the adsorption behavior of the system and use this understanding to select a proper adsorption isotherm, or to derive an new adsorption isotherm; e.g. see [23] for an example of how a complex adsorption isotherm is derived. However, this might be very hard, or even impossible, for more complicated adsorption behavior. The purpose of this article is to solve the problem in these cases by approximating the adsorption isotherm with monotone piecewise interpolation, in a modified IM, instead of using a closed adsorption isotherm model.

The idea of using interpolation instead of a closed adsorption isotherm model has previously been investigated by Haghpanah et al in [24]. They used the Transport Dispersive model with a Linear Driving Force mass transfer model and estimated piecewise linear adsorption isotherms by the Inverse Method (IM) with a Sequential Quadratic Programming algorithm. They successfully applied the method to Type I and III adsorption (no inflection points) and to simple Type II adsorption (one inflection point). Here we will instead use Stineman interpolation [25] that offers significant advantages over the linear interpolation used in [24]. Significantly fewer segments are needed to estimate a nonlinear function with Stineman interpolation than with linear interpolation. In Fig. 1(a) it is shown that only 8 segments are needed to estimate a non-linear adsorption isotherm with Stineman interpolation whereas 24 is needed using linear interpolation to achieve the same accuracy. This means that the numbers of unknown parameters in the inverse problem will be considerably less when using Stineman interpolation compared to linear interpolation. More important is that Stineman interpolation has continuous derivatives whereas linear interpolation has discontinuous ones, see Fig. 1(b). This means that adsorption isotherms estimated using linear interpolation cannot be used by the reliable algorithms for chromatographic calculations that requires continuous adsorption isotherm derivate, such as Orthogonal Collocation On Finite Elements [26] or the Finite Volumes [27,28] algorithm for the Equilibrium-Dispersive model [1] that is used here.

When we are dealing with complicated adsorption behavior with multip le inflection points it is of utmost importance the algorithm converge to achieve an acceptable solution and to not get stuck in local minima. For this purpose, we need to use a global optimization algorithm in IM, instead of a local one as used in [24]. Here we will use a derivative free parallelized pattern search optimization algorithm [29]. Notice that because we are dealing with inflection points that are barely visible in the adsorption isotherm we use IM to estimate dq/dC (i.e., the derivative of the stationary phase concentration with respect to the mobile phase concentration) instead of $q(C)$ as in [24].

Initially we will study how small very small perturbations in a Type I adsorption isotherm, that generates inflection points, will affect the corresponding overloaded elution profiles. We will then study a specific a synthetic system where the inflection points in the adsorption isotherm are "hidden" and show how the modified IM can successfully estimate an adsorption isotherm in this case. Finally we will test our approach on a real chromatographic separation, with possible inflection points in the adsorption isotherm, and show that the modified IM can successfully be used also in this case. It should be emphasized that the goal here is to use the estimated adsorption isotherms to improve process chromatography and to optimize the purification processes, not necessarily to obtain deeper mechanistic knowledge.

2 Theory

In order to simulate the chromatographic process we need a column model and an adsorption model. As the column model we will use the Equilibrium Dispersive model [1]. Instead of the usual closed expressions, e.g. the Langmuir adsorption model, as adsorption model we will use monotone piecewise interpolation to estimate dq/dC, i.e., the derivative of the stationary phase concentration with respect to the mobile phase concentration. Here we divide the mobile phase concentration range into a finite number of segments and in each segment we will have a monotone function interpolating between the segments boundary points such that continuity, up to a certain order, is preserved across the boundaries. Note that, for example, ordinary cubic splines do not have monotone functions interpolating between segment boundaries (knots) and we will use Stineman interpolation [25], that have continuous derivative, instead.

The Inverse Method (IM) of adsorption isotherm estimation is based on adjusting the adsorption isotherm until the difference between experimental elution profiles and elution profiles simulated using the adsorption isotherm is small [22], i.e., we solve the inverse problem. In the usual IM the

adsorption isotherm is adjusted by changing the adsorption isotherm parameters in some adsorption model, here we will instead adjust the values of dq/dC at the interpolation segment boundary points.

3 Procedures & Experimental

3.1 Synthetic System

The synthetic system investigated in section 4.1 - 4.3 was a 250 x 5 mm column with porosity 0.6 and 7 000 theoretical plates. The flow rate was 1.0 mL/min and we simulated a single 500 µL injection with square injection profile and concentration 0.1 g/L.

3.2 Materials and Experimental System

Here the material and chemicals used for the experimental system investigated in section 4.4 is given. The mobile phase was made from HPLC grade methanol (Fisher Scientific, Loughborough, UK) and water from a Milli-Q Plus 185 water purification system (Merck Millipore, Billerica, MA, USA). The buffer in the water part of the mobile was prepared using analytical grade sodium phosphate dibasic dehydrate and sodium phosphate monobasic dihydrate (Sigma-Aldrich, St. Louis, MO, USA). The solute omeprazole sodium monohydrate (> 99%) was from AstraZeneca R&D Mölndal, Sweden, while sodium nitrate (≥ 99.0%) from Sigma-Aldrich was used to determine the column hold-up time. Aqueous buffers and sample solutions were filtered through a 0.2 µm nylon filter membrane (Whatman, Maidstone, UK) before use. The HPLC instrument was an Agilent 1200 chromatograph system (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump, an auto sampler, a diode-array UV-detector and a thermostat-column oven. The stationary phase was an XBridge BEH C_{18}, 100 x 4.6 mm column packed with 3.5 µm particles (Waters, Milford, MA, USA), with porosity was 0.6157 and number of theoretical plates were 13 895, was operated at 30°C. The mobile phase methanol/phosphate buffer with 35% v/v methanol and pH 8.0 was pumped at a flow rate of 0.7 mL/min. Four injections were done, 5 µL of 0.02 g/L Omeprazole and 300, 400, 500 µL of 3.53 g/L

Omeprazole, and the corresponding injection and elution profiles were measured. The 5 µL analytical peak was detected at 220 nm while the other overloaded peaks were detected at 342 nm

3.3 Calculations

A finite volume solver with a Koren flux limiter was used to numerically estimate the solution to the Equilibrium-Dispersive model [27,28]. In the Inverse Method (IM) l_1-norm, i.e., absolute distance was used to measure the difference between experimental and simulated elution profiles and the elution profile areas were normalized so that all experimental elution profiles had the same weight. To solve the inverse problem we used a global, derivative free parallelized pattern search optimization algorithm [29].

All calculations were done using MATLAB R2012a on computer cluster consisting of five Intel® Core™ i7-3770S 3.10 GHz CPUs with in total 20 calculation cores.

4 Results

In section 4.1 we will begin by studying how a small perturbation to a Type I adsorption isotherm will affect the corresponding overloaded elution profiles. In section 4.2 we will study a specific synthetic chromatographic system and in section 4.3 we will estimate this systems complicated adsorption isotherm by using a modified Inverse Method (IM). Finally, in section 4.4 we will validate our approach for estimation of an adsorption isotherm with inflection points for a real system.

4.1 Impact of Inflection Points

Here we will investigate how the position of very small variations in an adsorption isotherm, that generates inflection points, will influence the shape of the corresponding elution profile. The adsorption isotherm studied in this section will be the Tóth adsorption isotherm,

$$q = q_s \frac{C}{\left(1/K + C^{\nu}\right)^{1/\nu}}, \qquad \frac{dq}{dC} = q_s \frac{1}{\left(1 + KC^{\nu}\right)\left(1/K + C^{\nu}\right)^{1/\nu}}, \tag{1}$$

where q_s = 2.563, K = 1.369 and ν = 0.882. Now let´s modify the adsorption isotherm in Eq. (1) by adding a very small, normal distributed, perturbation according to,

$$\tilde{q} = q_s \frac{C}{\left(1/K + C^{\nu}\right)^{1/\nu}} + \frac{a}{\sigma\sqrt{2\pi}}\left(e^{-\frac{(C-C_0)^2}{2\sigma^2}} - e^{-\frac{C_0^2}{2\sigma^2}} \right), \qquad \frac{d\tilde{q}}{dC} = q_s \frac{1}{\left(1 + KC^{\nu}\right)\left(1/K + C^{\nu}\right)^{1/\nu}} - \frac{a(C - C_0)}{\sigma^3\sqrt{2\pi}} e^{-\frac{(C-C_0)^2}{2\sigma^2}}, \tag{2}$$

where C_0 is the location of the perturbation in the adsorption isotherm, $a = 10^{-5}$ is the amplitude of the perturbation and $\sigma = 2 \cdot 10^{-3}$ is the width of the perturbation. In Fig. 2 (a) the Tóth adsorption isotherm in Eq. (1) is shown together with the modified Tóth adsorption isotherm in Eq. (2) at location C_0 = 0.012 g/L. In the insert the corresponding derivative is shown. Notice that the very small perturbation is almost invisible in the adsorption isotherm, but the difference in the corresponding derivative is clearly visible and we see that the perturbation introduces several inflection points. The corresponding elution profiles for a 500 µL injection of 0.1 g/L sample are shown in Fig. 2 (b). As can be seen the very small perturbation in the Type I adsorption isotherm has a very large effect on the shape of the corresponding elution profile.

Now we want to see how the location in the adsorption isotherm of the perturbation generating the inflection point, i.e., C_0 in Eq. (2) above, will affect the difference in elution profiles. We will study the same injection as in Fig. 2, i.e., a 500 µL injection of 0.1 g/L sample, and use the l_1-norm of the residual vector to measure the difference between the elution profiles. The result is shown in Fig. 3 and as can be seen a perturbation in the adsorption isotherm will have larger effect the earlier it appears. The effect of the perturbation on the shape of the elution profile initially decreases very

rapidly, thereafter the decrease in effect is more modest until the maximum eluted concentration where the effect again decreases very rapidly up to the injected concentration. After the injected concentration, the perturbation has almost no effect on the elution profile (notice that the highest concentration inside the column might be higher than the injected concentration).

4.2 Example Chromatographic System

Assume that we have measured 20 data points on the adsorption isotherm for the system (see section 3.1 for system details), e.g. by using frontal analysis. These data points are shown as symbols in Fig. 4 (a). From the data points and the Scatchard plot in the inset it is "obvious" that we have heterogeneous Type I (Langmuir Type) adsorption and therefore a heterogeneous Type I adsorption isotherm model should be used. Here we fitted the Tóth adsorption model in Eq. (1), with three parameters, q_s, K, ν, to the data points and got that $q_s \approx 2.563$, $K \approx 1.369$ and $\nu \approx 0.882$ (the same values used in section 4.1). As can be seen in Fig. 4 (a) the fit is excellent.

In Fig. 4 (b) the measured elution profile is shown together with an elution profile simulated using the estimated Tóth adsorption isotherm above. As can be seen the shape of the experimental profile looks strange and the tail of the elution profiles doesn't match at all. This might lead to the conclusion that there is something wrong with the experiments, e.g. that the injected sample contains impurities.

Now let us take a closer look at the true adsorption isotherm that is shown together with the estimated Tóth isotherm in Fig. 5. In Fig. 5 (a) there is a very slight difference in the initial part of the adsorption isotherms but this difference falls between the measured data points and is therefore not detected in the experiments. In Fig. 5 (b) the derivative dq/dC of the adsorption isotherms are shown and as can be seen the small deviation not only gives rise to large difference in the derivate of the adsorption isotherm but also to two inflection points, i.e., extreme points in the derivative. This is what actually causes the large difference in the overloaded elution profiles seen in Fig. 4 (b).

4.3 Modified Inverse Method

As the effect of the slight deviation in the true adsorption is clearly visible in the overloaded elution profile in Fig. 4 (b) the Inverse Method (IM) should be able to estimate the true adsorption isotherm. However in Fig. 5 (b) we see that we have two inflection points and if we are going to use IM we need to select an adsorption isotherm model with two inflection points. One option is the bi-Moreau model,

$$q = q_{s,1}\frac{K_1 C + h_1 K_1^2 C^2}{1 + 2K_1 C + h_1 K_1^2 C^2} + q_{s,2}\frac{K_2 C + h_2 K_2^2 C^2}{1 + 2K_2 C + h_2 K_2^2 C^2}, \tag{3}$$

with six parameters $q_{s,*}$, h_*, K_*. As the inflection points are more clearly visible in the derivative of the adsorption isotherm, see Fig. 5 (b), it is better to try to estimate the derivative of the adsorption isotherm by IM. For the bi-Moreau adsorption isotherm we have that,

$$\frac{dq}{dC} = K_1 q_{s,1}\frac{1 + 2h_1 K_1 C + h_1 K_1^2 C^2}{\left(1 + 2K_1 C + h_1 K_1^2 C^2\right)^2} + K_2 q_{s,2}\frac{1 + 2h_2 K_2 C + h_2 K_2^2 C^2}{\left(1 + 2K_2 C + h_2 K_2^2 C^2\right)^2}. \tag{4}$$

If we estimate $q' = dq/dC$ the corresponding adsorption isotherm q is of course,

$$q(C_0) = \int_{C=0}^{C_0} q'(C)dC. \tag{5}$$

In order to check if the bi-Moreau adsorption isotherm is a viable choice for the IM in this case, we begin by checking if we can fit dq/dC in Eq. (4) to the derivative of the actual adsorption isotherm in Fig. 5 (b), at least up to the highest eluted concentration. The fitting was done by using several runs with a global genetic algorithm [30] combined with a Levenberg-Marquardt local least squares solver [31]; the best fit achieved is shown in Fig. 6. As can be seen from the figure the bi-Moreau adsorption isotherm can most likely not be used to model the adsorption in this case. This means that it is very hard, and can even be impossible, to find an adsorption isotherm model that can be used here.

Because of the above we will instead try to estimate a monotone piecewise interpolation approximation to the adsorption isotherm by IM rather than using an adsorption isotherm model of any kind, i.e., we do not assume any adsorption isotherm model, see Theory section. The only starting user input needed is to select the number of interpolation points and where they should be placed on the mobile phase concentration axis of the adsorption isotherm. Thereafter, the corresponding stationary phase concentration at these points, and the interpolation between them, is estimated by the optimization procedure. There is no exact rule giving the number of interpolation points needed. If the number of interpolations are too few one will not be able to accurately estimate a piece-wise approximation to the actual adsorption isotherm and hence will not be able to get a good fit to the elution profiles. On the other hand, if the number of interpolation points are too more that required, the optimization procedure will take very long time. From our experience in this study, as a rule of thumb ~10 interpolation points is usually sufficient for Langmuir or anti-Langmuir shaped elution profiles whereas for more unusual and strange elution profiles around 20 – 30 points should be used. Note that one can easily add more interpolation points to an obtained solution and rerun to refine the solution if the fit to the experimental elution profiles is not satisfactory. In this case we will use 20 interpolation points (19 segments). As we saw in section 4.1 the initial part if the adsorption isotherm is more important and we will therefore use an uneven distribution of the interpolation points, i.e., uneven length of the segments. Up to 20% of the maximum eluted concentration we will place 8 equally spaced interpolation points, from 20% up to 100% of the maximum eluted concentration we will place 8 equally spaced interpolation points and from 100% of the maximum eluted concentration up to two times the sample concentration we will place 4 equally spaced interpolation points.

It should be noted that estimation of monotone piecewise interpolation approximation to an adsorption isotherm is a considerably harder and more costly inverse problem than estimation of parameters in an adsorption isotherm function. To solve this problem we will use a parallelized global pattern search algorithm on a computer cluster with 20 calculation cores and as starting guess

we will use discretization of the Tóth adsorption isotherm in Fig. 5. The estimated discretized adsorption isotherm is shown in Fig. 7 (a-b). In the figures there are clear differences between estimates and the true adsorption isotherm, note especially that we have very large difference above the maximum eluted concentration. However, this part of the adsorption isotherm has almost no effect on the position and shape of the elution profile, see section 4.1. In Fig. 7 (c) the experimental elution profile is shown together with an elution profile simulated using the discretized adsorption isotherm, as can be seen the fit shows now excellent agreement with the experimental data.

4.4 Real Systems

We tested our approach on a chromatographic HPLC system consisting of an XBridge BEH C10 column with methanol/phosphate buffer (35%/65% v/v, pH 8.0) as mobile phase, section 3.2 for more experimental details. Different injection of Omeprazole was done; in Fig. 8 (a) the elution profiles for overloaded 300 and 400 µL (main figure) and an analytical 5 µL injection (inset) are shown. Notice the extremely shallow and quite long plateau at the end of the large volume profiles The experimental overloaded profiles has indeed strange shapes and for this reason here serves as a challenging real system case. This plateau might have several reasons, e.g. degradation of the sample or impurities, or pH-instability, and warrants further investigation. Regardless of the reason such profiles are not impossible; even stranger profiles have been reported in the literature and some of them can also be explained [32,33].

Here the strange profiles will serve as a challenging case example and we will show that the plateau *might* also be the result of inflection points in the adsorption isotherm. Here we used the elution profiles from four injections in the modified IM to estimate a discretized adsorption isotherm with 30 interpolation points (29 segments). As in section 4.3 we will use an uneven distribution of the interpolation points: Up to 20% of the maximum eluted concentration we will place 12 equally spaced interpolation points, from 20% up to 100% of the maximum eluted concentration we will place 12 equally spaced interpolation points and from 100% of the maximum eluted concentration

up to two times the sample concentration we will place 6 equally spaced interpolation points. Please note that we does not use an adsorption isotherm model of any kind, i.e., we do not assume any adsorption isotherm model, see Theory section. The results presented in Fig. 8 (a) shows an excellent agreement was achieved between the experimental and the calculated profiles and we were able to estimate an adsorption isotherm that accounted well for the small plateau at the end. The estimated discretize adsorption isotherm is shown in Fig. 8 (b), please notice the inflection points in the inset.

5 Conclusions

We have shown that even very small variation in the adsorption isotherm might give rise to inflection points in the adsorption isotherm that have a tremendous impact on the shape of the elution profiles, see Fig. 3. The earlier in the adsorption isotherm these variations occur the larger the impact, but the impact decreases rapidly with increasing adsorption isotherm mobile phase concentration. We have also shown that inflection points might be missed when using traditional experimental plateau methods for adsorption isotherm determination, such as Frontal Analysis (FA). Here it should be noted that these traditional methods also have less accuracy at the lower concentration regions of the isotherm [17]. Furthermore we have shown that it might not be possible to find a closed adsorption isotherm model that account for these inflection points. We have therefore developed and validated a modified Inverse Method (IM), which uses monotone piecewise interpolation instead of a fixed adsorption isotherm model, to solve the problem of estimating adsorption isotherms where small variations give rise to inflection points. We demonstrated, both for a synthetic and a real case, that the method was able to successfully estimate adsorption isotherm with multiple inflection points.

Plateau methods, such as FA, measures points on the adsorption isotherm curve and the modified IM can be viewed as a way to indirectly estimate points on the adsorption isotherm curve (or the derivative of it) from overloaded elution profiles. Here we have studied single component cases, but

the principle of using IM with interpolation could, in principle, be extended to also two component cases. However, in this case we need to estimate an interpolated competitive adsorption isotherm surface which is a considerably harder problem.

It should be noted that it is not possible to draw conclusions about the adsorption mechanism from monotone piecewise interpolation approximations in the same way as it is for the parameters in a closed adsorption isotherm model. However, if the goal is to optimize the purification process piece-wise approximation is sufficient. Moreover, the piece-wise approximation can also be used as a starting point to derive a closed expression for the adsorption isotherm, e.g., the putative closed expression can be fitted to the piece-wise approximation and one can then both judge if the closed expression is a good option and, if it is, get the adsorption isotherm parameters in the closed expression.

It should also be noted that estimation of monotone piecewise interpolation approximations is a considerably harder and more costly inverse problem than determination of parameters in a closed adsorption isotherm model using IM. Therefore the modified IM should only be used as an option for estimation of complicated adsorption isotherms in cases where nothing else works.

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Figure Captions (ONLINE)

Figure 1: Linear (red curve and circle symbols) vs. Stineman (blue curve and square symbols) interpolation: in (a) interpolation of an adsorption isotherm (solid curve) and in (b) the corresponding derivatives.

Figure 2: In (a) a Tóth adsorption isotherm (red curve), see Eq. (1), and the same adsorption isotherm modified with a small perturbation (blue curve) at the location indicated by the vertical line, see Eq. (2); in then inset the derivative of the curves is shown. In (b) elution profiles simulated using the adsorption isotherms in (a).

Figure 3: How the difference between elution profiles, simulated using the Tóth and the modified Tóth adsorption isotherm, varies with the location of the perturbation in modified Tóth adsorption isotherm, see Eq. (1) and Eq. (2). The vertical dotted line indicates the injected concentration and the vertical dashed line indicates maximum eluted concentration in the Tóth adsorption isotherm simulation.

Figure 4: In (a) measured adsorption isotherm data points (symbols) and a fitted Tóth adsorption isotherm (red curve); in the inset the corresponding Scatchard (q/C) plot is shown. In (b) Experimental (black curve) and simulated (red curve) overloaded elution profiles.

Figure 5: In (a) measured adsorption isotherm data points (symbols) together with true (blue curve) and fitted Tóth (red curve) adsorption isotherms. A zoomed in view of the initial part in the box is shown in the inset. In (b) derivate of true (blue curve) and fitted Tóth (red curve) adsorption isotherm, the symbols indicate inflection points and the max eluted concentration for the elution profile in Fig. 4 (b) is shown as a vertical dashed line.

Figure 6: True (blue curve) and fitted bi-Moreau (red curve), see Eq. (3) and Eq. (4), adsorption isotherm.

Figure 7: Estimated (red curve and symbols) and true (blue curve) adsorption isotherm. In (a) the derivative of the adsorption isotherms up to the highest eluted concentration and in (b) the corresponding adsorption isotherm up to 0.014 g/ L, cf. inset in Fig. 5 (a). The insets in (a) and (b) shows the derivative of the adsorption isotherms and the adsorption isotherm, respectively, up to 2 times the injected concentration. In (c) experimental (black curve) and simulated (red curve) overloaded elution profiles.

Figure 8: In (a) experimental (black curves) and simulated (red curves) elution profiles for 5 (inset), 300 and 400 µL injections of Omeprazole. In (b) the estimated discrete adsorption isotherm (red curve), the line is a diagonal that makes it easier to see deviation from linearity. A zoomed in view of the initial part in the box is shown in the inset. See Section 3.2 for more experimental details.

Figure Captions (PRINT)

Figure 1: Linear (dotted curve and circle symbols) vs. Stineman (dashed curve and square symbols) interpolation: in (a) interpolation of an adsorption isotherm (solid curve) and in (b) the corresponding derivatives.

Figure 2: In (a) a Tóth adsorption isotherm (dotted curve), see Eq. (1), and the same adsorption isotherm modified with a small perturbation (dashed curve) at the location indicated by the vertical line, see Eq. (2); in then inset the derivative of the curves is shown. In (b) elution profiles simulated using the adsorption isotherms in (a).

Figure 3: How the difference between elution profiles, simulated using the Tóth and the modified Tóth adsorption isotherm, varies with the location of the perturbation in modified Tóth adsorption isotherm, see Eq. (1) and Eq. (2). The vertical dotted line indicates the injected concentration and the vertical dashed line indicates maximum eluted concentration in the Tóth adsorption isotherm simulation.

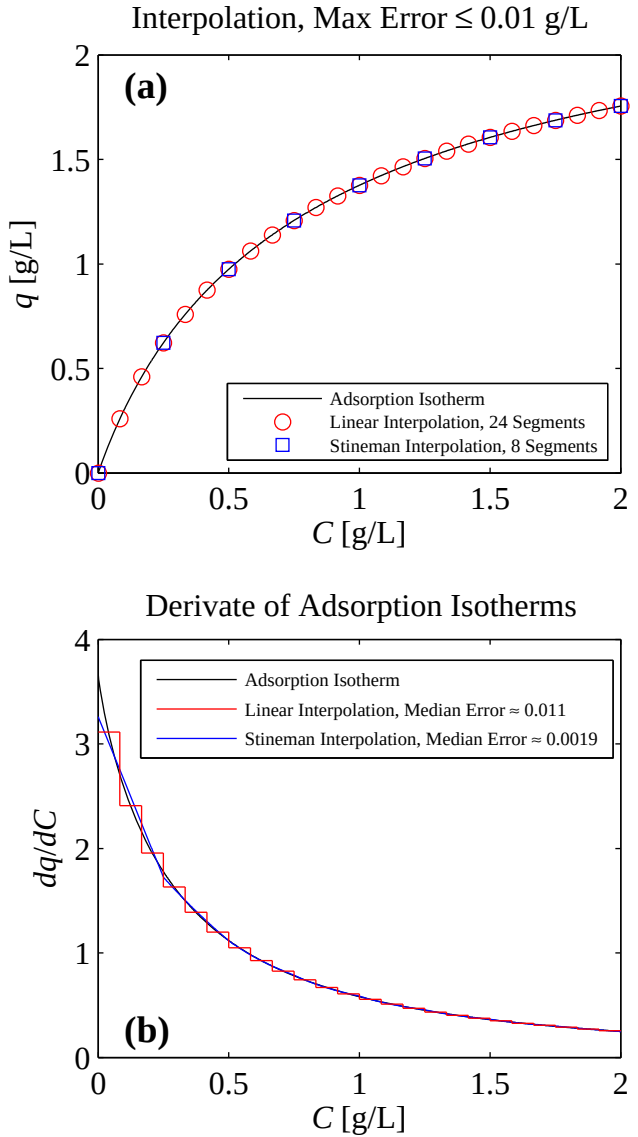
Figure 4: In (a) measured adsorption isotherm data points (symbols) and a fitted Tóth adsorption isotherm (dotted curve); in the inset the corresponding Scatchard (q/C) plot is shown. In (b) Experimental (solid curve) and simulated (dotted curve) overloaded elution profiles.

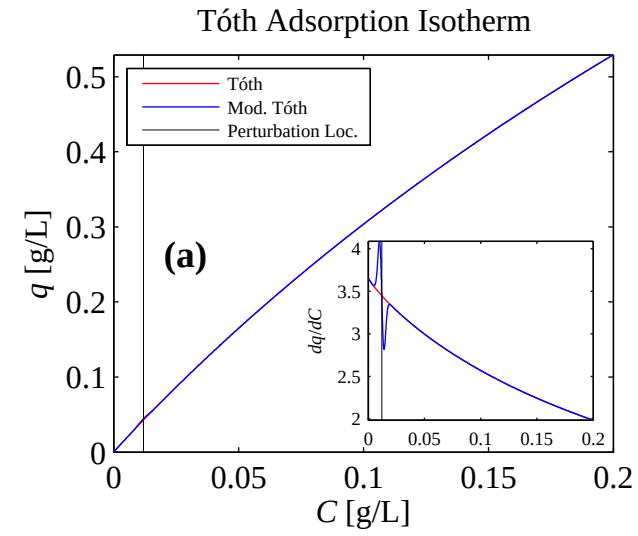
Figure 5: In (a) measured adsorption isotherm data points (symbols) together with true (dashed curve) and fitted Tóth (dotted curve) adsorption isotherms. A zoomed in view of the initial part in the box is shown in the inset. In (b) derivate of true (dashed curve) and fitted Tóth (dotted curve) adsorption isotherm, the symbols indicate inflection points and the max eluted concentration for the elution profile in Fig. 4 (b) is shown as a vertical line.

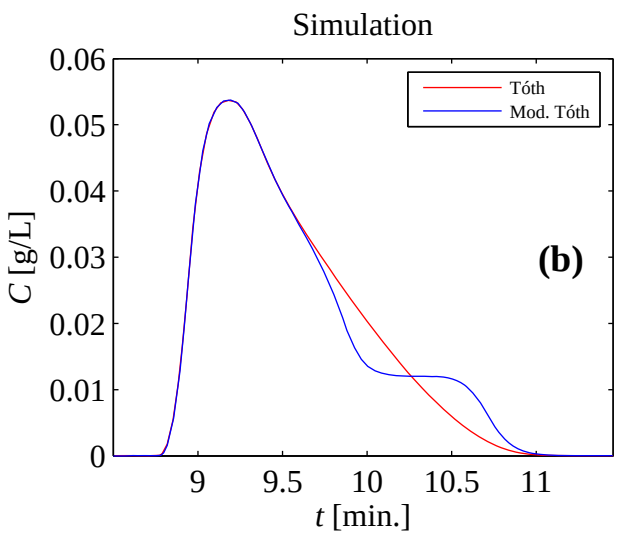
Figure 6: True (dashed curve) and fitted bi-Moreau (dotted curve), see Eq. (3) and Eq. (4), adsorption isotherm.

Figure 7: Estimated (dotted curve and symbols) and true (dashed curve) adsorption isotherm. In (a) the derivative of the adsorption isotherms up to the highest eluted concentration and in (b) the corresponding adsorption isotherm up to 0.014 g/ L, cf. inset in Fig. 5 (a). The insets in (a) and (b) shows the derivative of the adsorption isotherms and the adsorption isotherm, respectively, up to 2 times the injected concentration. In (c) experimental (solid curve) and simulated (dotted curve) overloaded elution profiles.

Figure 8: In (a) experimental (solid curves) and simulated (dotted curves) elution profiles for 5 (inset), 300 and 400 µL injections of Omeprazole. In (b) the estimated discrete adsorption isotherm (dotted curve), the line is a diagonal that makes it easier to see deviation from linearity. A zoomed in view of the initial part in the box is shown in the inset. See Section 3.2 for more experimental details.


Interpolation, Max Error ≤ 0.01 g/L
(a)
q [g/L]
C [g/L]
Adsorption Isotherm
Linear Interpolation, 24 Segments
Stineman Interpolation, 8 Segments
Derivate of Adsorption Isotherms
(b)
dq/dC
C [g/L]
Adsorption Isotherm
Linear Interpolation, Median Error ≈ 0.011
Stineman Interpolation, Median Error ≈ 0.0019


Tóth Adsorption Isotherm
Tóth
Mod. Tóth
Perturbation Loc.
(a)
q [g/L]
C [g/L]
dq/dC
0
0.05
0.1
0.15
0.2
0.3
0.4
0.5
2
2.5
3
3.5
4


Simulation
Tóth
Mod. Tóth
(b)
C [g/L]
t [min.]
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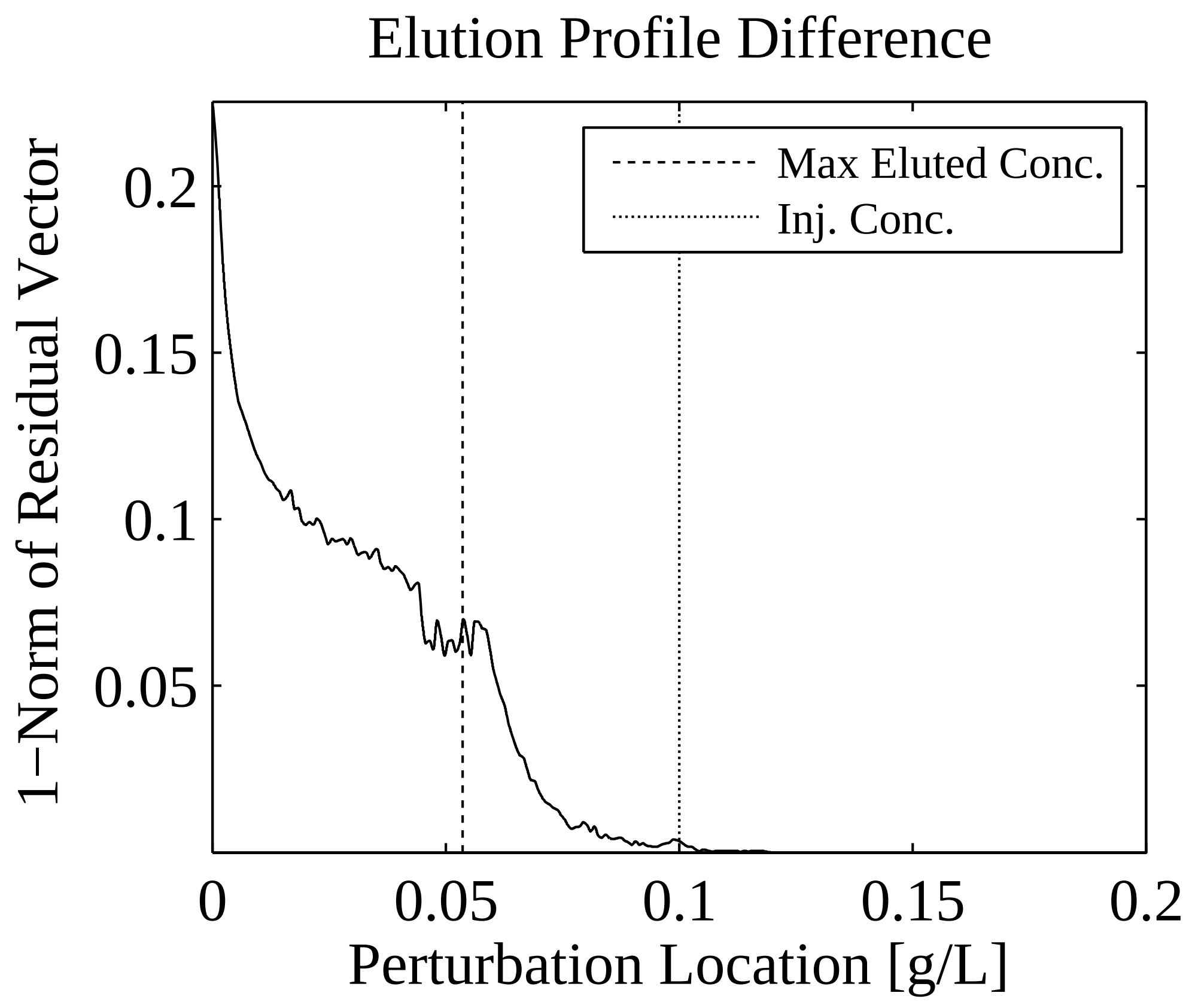
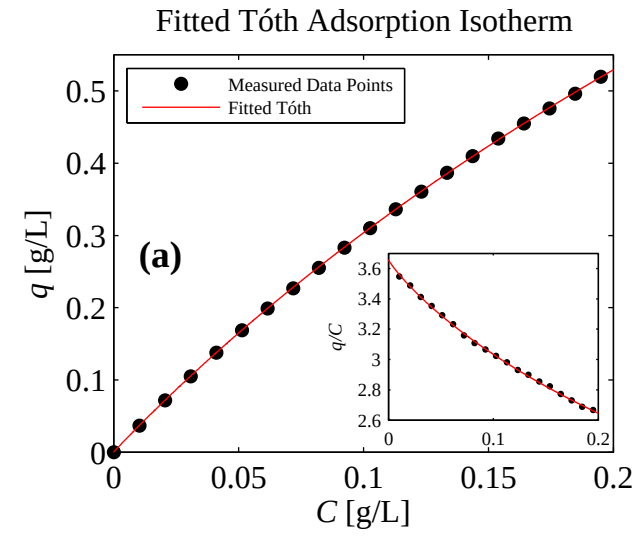
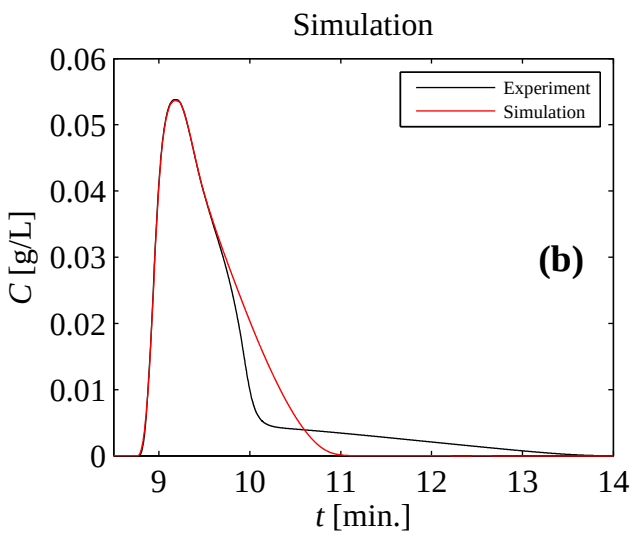
0.01
0.02
0.03
0.04
0.05
0.06
9
9.5
10
10.5
11


Elution Profile Difference
Max Eluted Conc.
Inj. Conc.
1−Norm of Residual Vector
0.2
0.15
0.1
0.05
0
0.05
0.1
0.15
0.2
Perturbation Location [g/L]


Fitted Tóth Adsorption Isotherm
Measured Data Points
Fitted Tóth
(a)
q [g/L]
C [g/L]
q/C
0
0.05
0.1
0.15
0.2
0.1
0.2
0.3
0.4
0.5
2.6
2.8
3
3.2
3.4
3.6


Simulation
Experiment
Simulation
(b)
C [g/L]
t [min.]
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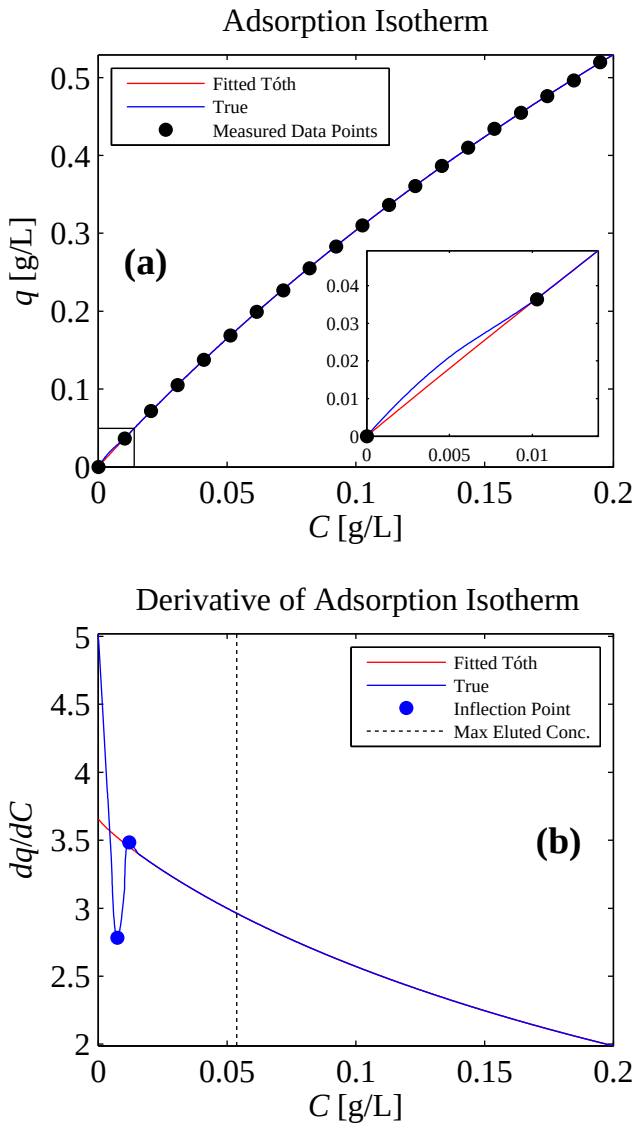
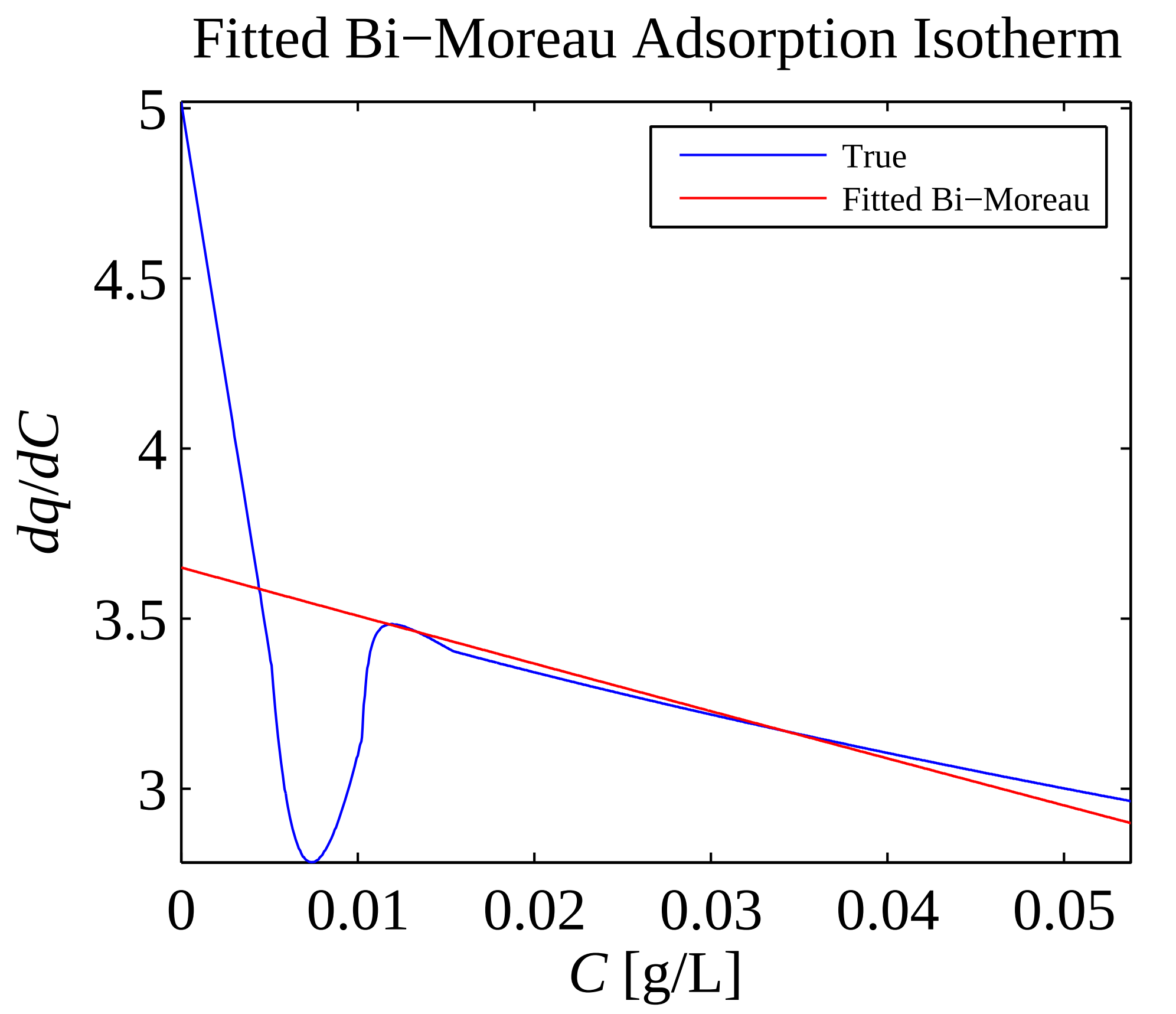
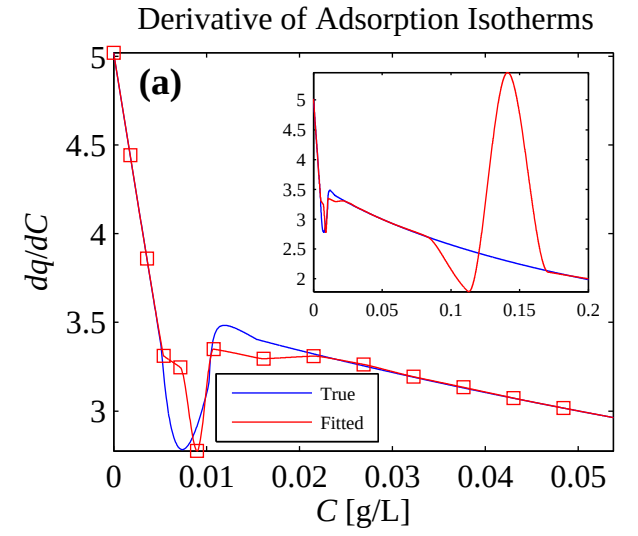
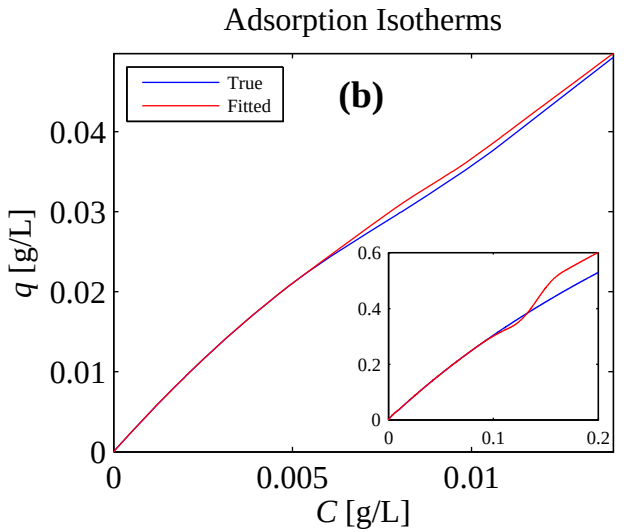
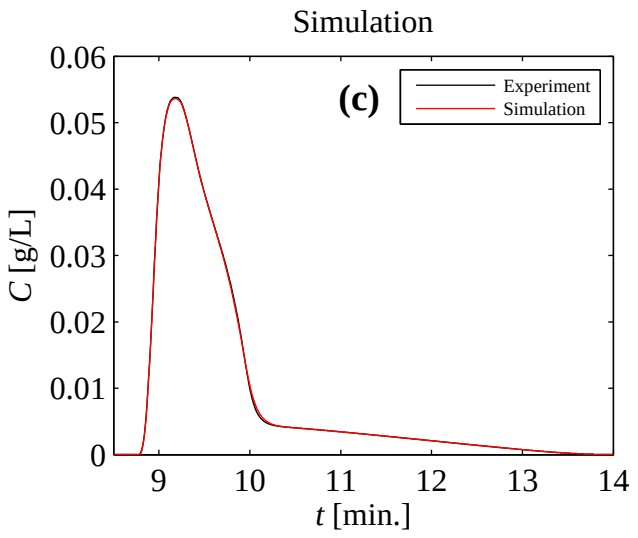
0.01
0.02
0.03
0.04
0.05
0.06
9
10
11
12
13
14

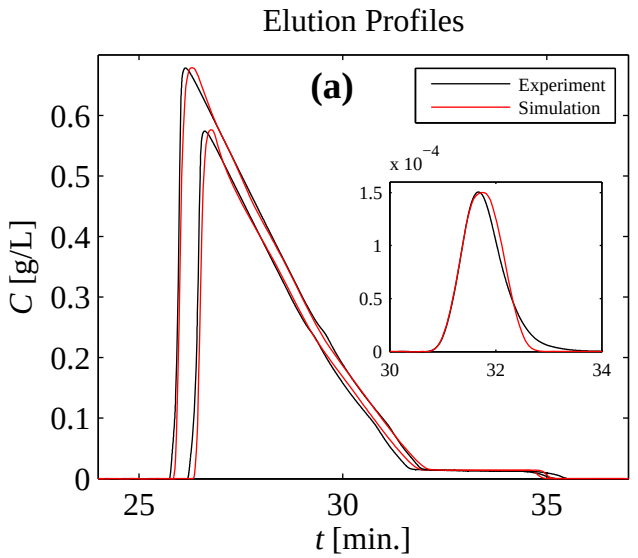

Adsorption Isotherm
Fitted Tóth
True
Measured Data Points
(a)
q [g/L]
C [g/L]
0
0.1
0.2
0.3
0.4
0.5
0.05
0.15
0.01
0.02
0.03
0.04
0.005
Derivative of Adsorption Isotherm
Fitted Tóth
True
Inflection Point
Max Eluted Conc.
(b)
dq/dC
2
2.5
3
3.5
4
4.5
5

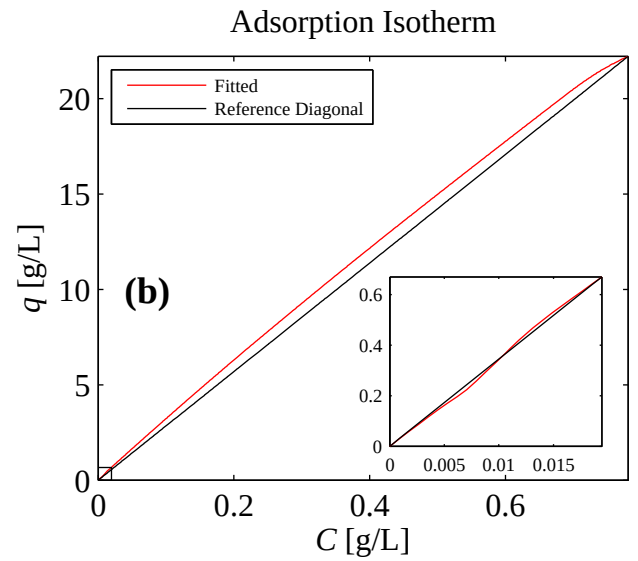

Fitted Bi−Moreau Adsorption Isotherm
True
Fitted Bi−Moreau
dq/dC
C [g/L]
5
4.5
4
3.5
3
0
0.01
0.02
0.03
0.04
0.05


Derivative of Adsorption Isotherms
(a)
dq/dC
C [g/L]
True
Fitted


Adsorption Isotherms
(b)
q [g/L]
C [g/L]
True
Fitted


Simulation
(c)
C [g/L]
t [min.]
Experiment
Simulation


Elution Profiles
(a)
Experiment
Simulation
x 10^−4
C [g/L]
t [min.]


Adsorption Isotherm
Fitted
Reference Diagonal
(b)
q [g/L]
C [g/L]