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Improvement in the generation of adsorption isotherm data in the elution by characteristic points method—The ECP-slope approach

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ABSTRACT

The elution by characteristic points (ECP) method is a very rapid and precise method for determination of the phase system equilibrium of phase systems in broad solute concentration ranges. Thus, the method is especially suitable for rapid characterization of high efficient separation systems. One important source of error, the effects by the post-loop dispersion, was eliminated in a recent investigation. In this study, the systematic error caused by the selection of the integration starting point at concentration equal to 0 is eliminated. This is done by developing and validating a new procedure for isotherm data generation; the ECP-slope method. The method generates raw slope data of the adsorption isotherm instead of raw adsorption data by integrations as the classical ECP does. Both numerical and experimental data were used for the comparison of the classical ECP approach with the slope-ECP method.

1. Introduction

In chromatography partition of a substance between the mobile and stationary phase depends on the local concentration of this compound. The functions describing this behavior are called adsorption isotherms. Different adsorption isotherm models describe different adsorption mechanisms, and are essential for the understanding of the separation process. There are many methods for determination of adsorption isotherms, such as frontal analysis (FA) the perturbation method (PM) [1], frontal analysis by characteristic point (FACP) and elution by characteristic point (ECP). All methods have its pros and cons in terms of time and solute consumption and accuracy [2,3] A very promising method for competitive cases is the inverse method; however it is not actual here [4].

In the ECP (or FACP) method the data points are generated from the diffuse part or an overloaded band profile for a type I or III adsorption isotherm [5,6]. The method generates huge amount of data point from just a single injection. Thus, the ECP method is one of the most rapid methods for determination of the adsorption isotherms. But, the ECP method has some serious disadvantages.

Firstly, the ECP theory is derived assuming rectangular injection-profiles. Surprisingly the ECP method has been used for years with traditional injection techniques producing large deviations from rectangular profiles. This has been a large problem especially since the use of very large injection volumes is necessary in order to determine low adsorption energy sites. However, this important disadvantage of the ECP method can easily be avoided using a new injection technique proposed and successfully validated in a recent study [7].

Secondly, the method, as based on the ideal model, assumes infinite column efficiency. Since the efficiency of an actual column is finite this results in inherent errors in the derived adsorption isotherms. For a homogenous surface at least a number of plates (N) of 2000 is required to have an error less than 3% [5] whereas a heterogeneous surface represented by the bi-Langmuir model requires a minimum N of 5000 to have an error less than 5% [6]. The size of this error decreases with increasing N. The data points on an elution profile close to the top and near the baseline are more affected of the source of band broadening should ideally be excluded when determining the adsorption isotherm. The data points contain high concentrations could be neglected. However, this is not the case for the low concentration data, since the ECP method integrates the profile from 0 to C. This causes a systematic error due to the use of the inaccurate low-concentration data for the determination of the proper adsorption isotherm parameters for low capacity sites as well as for high capacity sites [5,8]. Thus, in order to reach a complete census of all interactions in the

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characterization study this very important source of error must be eliminated.

The aim of the study is to develop a new ECP approach and compare it to the classical ECP-method for determination of adsorption isotherm data. We call this new approach the ECP-slope method. In the classical ECP method raw adsorption isotherm data (points on the adsorption isotherm) is generated by integrating the elution profile from concentration 0 to C. In the ECP-slope method we do not integrate the rear profile but use the raw slope data. By using slope data we do not need to determine an adsorption data point where the concentration is zero but could instead use an arbitrary selected low concentration point. This is an important advantage (see above). To compare adsorption isotherms determined using the slope method to the classical ECP method, three different numerical constructed systems and three different experimental systems were investigated.

2. Experimental

2.1. Apparatus

Agilent 1100 chromatographic system (Agilent Technology, Palo Alto, CA, USA) consisting of a binary pump module, auto sampler, manual injector (Rheodyne, Cotati, CA, USA) and a diode array UV-detector controlled with ChemStation v. 6.04 LC software. The temperature were controlled with a column jacket connected to a heating circulating water bath, LAUDA type B (LAUDA Dr. R Wobser, GMBH & Co: KG, Lauda-Koeningshofen, Germany) this setup was used for the "CUT-injections" [7]. Kromasil-Eternity C18 (Eka Chemicals AB, Bohus, Sweden) with dimensions of 150 mm × 4.6 mm, 5 μm nominal particle diameter were used.

2.2. Chemicals

Thiourea, >99% (Riedel-de Haën), 3-phenyl-1-propanol, 98% (PP), and *rac*-Metoprolol (+)-tartrate, >99% (ME). Eluents were prepared using: sodium hydroxide (FIXAMAL 1.0 M), orthophosphoric acid 85% (Fluka), ammonium bicarbonate > 99.5%, methanol (Fisher-scientific, HPLC-grade). All chemicals stated above were bought from Sigma–Aldrich (Sigma–Aldrich Sweden AB, Stockholm, Sweden) except methanol that was bought from Fisher-scientific, Gothenburg, Sweden. The water used was from a Milli-Q ZMQS 5000Y purification system from Millipore (Molsheim, France). Basic Metoprolol and Metoprolol hydrochloride were prepared according to previously published procedures [9].

2.3. Eluents

The eluent compositions (% v/v, methanol/buffer) were 43% for 3-phenyl-1-propanol at pH 3, 30% for Metoprolol at pH 3, and 50% at pH 11. All buffers were filtrated through a 0.22 μm type-GV DURAPORE membrane filter from Millipore (Cork, Ireland).

2.4. Procedures

The chromatographic separations were conducted at a flow rate of 0.70 mL/min at a temperature of 25.0 °C controlled by the circulating water bath. All solute components were dissolved in the eluent. The experimental overloaded injection profile is 3.5 mL cut injections [7]. Detector responses were converted to concentrations by using a calibration curve fitted to a piecewise polynomial. The data for the calibration curves were acquired by stepwise pumping known concentrations through the detector after replacing the column with a capillary.

3. Theory

Adsorption isotherm models describe the solute concentration in the stationary phase as function of the solute concentration in the mobile phase at a constant temperature [1,10]. One of the simplest nonlinear model is the Langmuir isotherm.

$$q = \frac{aC}{1 + bC}, \tag{1}$$

where a is the distribution coefficient, b is the association equilibrium constant, C is the solute concentration in the mobile phase and q is the solute concentration in the stationary phase. Here $a = q_s b$, where q_s is the monolayer saturation capacity.

The bi-Langmuir model assumes two independent ideal adsorption sites.

$$q = \frac{a_1 C}{1 + b_1 C} + \frac{a_2 C}{1 + b_2 C}, \tag{2}$$

where index 1 is for the first adsorption site and index 2 is for the second site.

The diffuse rear of an overloaded peak using the ideal model, assuming convex upwards adsorption isotherm (e.g. Langmuir and bi-Langmuir model) could be described by:

$$V_R(C) = V_{inj} + V_0 \left(1 + \frac{V_a}{V_0} \frac{dq}{dC}\right), \tag{3}$$

where $V_R(C)$ is the elution volume corresponding to the mobile phase concentration C. V_{inj} is the injected volume, V_0 is the hold-up volume and V_a is the stationary phase volume. dq/dC is the slope of the adsorption isotherm. The elution by characteristic points (ECP) method determines the adsorption isotherm by integrating the rear of the profile:

$$q(C) = \frac{1}{V_a} \int_0^C (V_R(C) - V_{inj} - V_0)\, dC \tag{4}$$

There are several sources of error with ECP (see Section 1). One source of error that we are going to investigate is that the adsorption isotherm is needed to be integrated from concentration zero. Experimentally it is hard to find the exact point and at extreme low concentrations the elution profile will be dispersed and in that way introduce errors in the calculation.

Instead of integrating Eq. (4) we use the raw slope of the adsorption isotherm. This is achieved by rewriting Eq. (3) to:

$$\frac{dq(C)}{dC} = \frac{V_R(C) - V_{inj} - V_0}{V_a} \tag{5}$$

Prior to model fitting the raw adsorption data are analyzed by using Scatchard plots and adsorption energy distribution (AED) calculations [11,12]. Depending on the shape of the Scatchard plot some models could be excluded. To plot a Scatchard plot from the slope data we have to integrate the data. The AED will provide if the adsorption process is unimodal (1-site, e.g. Langmuir) or multimodal (several-sites, e.g. bi-Langmuir) and could be calculated using both raw data and raw slope data [13]. The fitting of data to different models were done by using the Levenberg–Marqardt algorithm, as implemented in MATLAB 7.5.0342 (R2007b) (MathWorks Inc., Natick, MA, USA).

4. Results and discussion

First, an investigation based on synthetic data was done to investigate if the ECP-slope method, using slope data and classical ECP will predict correct adsorption isotherm parameters. Second, some selected experimental data, similar to the models used in the synthetic part, are used to verify the ECP-slope method.

Table 1
Adsorption isotherm calculated using classic ECP (ECP in table) and the new slope data (slopeECP in table) for three different adsorption isotherms (Model 1, Model 2 and Model 3).

	a_1	K_1	a_2	K_2
Model 1-ECP	10.01	9.97	Na	Na
Model 1-slopeECP	10.03	10.01	Na	Na
True	10.00	10.00	Na	Na
Model 2-ECP	2.00	9.61	5.80	910
Model 2-slopeECP	2.05	10.04	5.98	1009
True	2.00	10.00	6.00	1000
Model 3-ECP	5.93	9.750	12.08	98.54
Model 3-slopeECP	6.02	9.895	12.02	100.00
True	6.00	10.00	12.00	100.00

4.1. Synthetic data

To investigate if the ECP-slope method will be able to predict adsorption isotherms, profiles were generated using the Rouchon algorithm for solving the equilibrium dispersion model. The simulations are done using 10,000 plates and injecting 5000 μL of 400 mM samples using the rectangular injection profiles as boundary condition. Profiles were made from three different adsorption isotherm models describing typical solute adsorption mechanisms (see Table 1). Model 1 is a Langmuir model, the model is generally used to describe the separation of uncharged compounds in ODS systems, e.g. methyl mandelate and 3-phenyl-1-propanol (PP) [9]. The model is a high capacity system with a single low energy interaction site. Model 2, is a bi-Langmuir model where the two interaction sites have large differences in both adsorption energy and monolayer capacity. The model has been successfully used for describing charged compounds adsorption to ODS separation systems, e.g. Metoprolol (ME) at low pH and 2-pheylbutyric acid [9]. Model 3 represents a bi-Langmuir model where the differences in adsorption energy and capacity between the sites are small. The model is generally determined for phenol on RPLC columns and Metoprolol (ME) at high pH, i.e. ME in uncharged form [9].

In Fig. 1 the results from Model 1, using classical ECP is plotted in the left column and the ECP slope method is plotted in the right column. The middle row is the Scatchard plots and the bottom row the corresponding AED. The grey lines are the true model and the black lines are the estimated models. In Fig. 1a_I the raw adsorption isotherm data is plotted and in Fig. 1a_{II} the raw slope of the adsorption isotherm data is plotted, visually both methods predict the adsorption data well. The Scatchard plots (Fig. 1b) have a better agreement with the raw adsorption data (Fig. 1b_I) compared with the slope data (Fig. 1b_{II}). This is due to the fact that for slope data the lowest concentration point is selected at $C = 50$ μM and for raw adsorption data $C = 0$ M. This will lead to large errors in the integrated adsorption isotherm for the slope data. The error will be particular manifested in the low concentration part of the Scatchard plot. However, we must stress that we do only integrate the data to get the Scatchard plot and use raw slope data both for the following AED calculations and fitting procedure. The AED plot c_I and c_{II} is both unimodal, as should be expected for a one-site model. Here we also can see a sharper peek in c_{II} using slope data compared to c_I using classical ECP, indicating faster convergence in AED calculation for slope data. The Langmuir parameters were recreated with excellent congruence for both methods (see Table 1).

In Fig. 2 results from calculations using Model 2 is presented. The slope of the adsorption isotherm is visually better predicted than the raw adsorption isotherm. The predicted adsorption isotherm parameters deviates less from the true value with the raw slope data compared to the raw adsorption isotherm data. However, both methods predicted adsorption parameters agrees well with the true adsorption isotherm (see Table 1). The Scatchard plot is convex downwards and the AED is bimodal as suspected for a two-site model, the equilibrium constant and monolayer capacity for each site is estimated well.

Finally, calculations from Model 3 are presented in Fig. 3. The raw adsorption data (Fig. 3a_I) and the raw slope of the adsorption isotherm data (Fig. 3a_{II}) have excellent agreement with the true data (grey line). Fitting the data to the bi-Langmuir model resulted in a more accurate parameter prediction from the raw slope data compared with the raw adsorption isotherm data (see Table 1). As in the previous cases, the Scatchard plot does not have as good agreement with the experimental due to the same reason. The AED is bimodal as suspected for this two-site model and the adsorption isotherm parameters are well conserved in the solution. Generally one could note that the AED converts faster using the slope data compared with the raw adsorption data; this is in line with our previous findings [13].

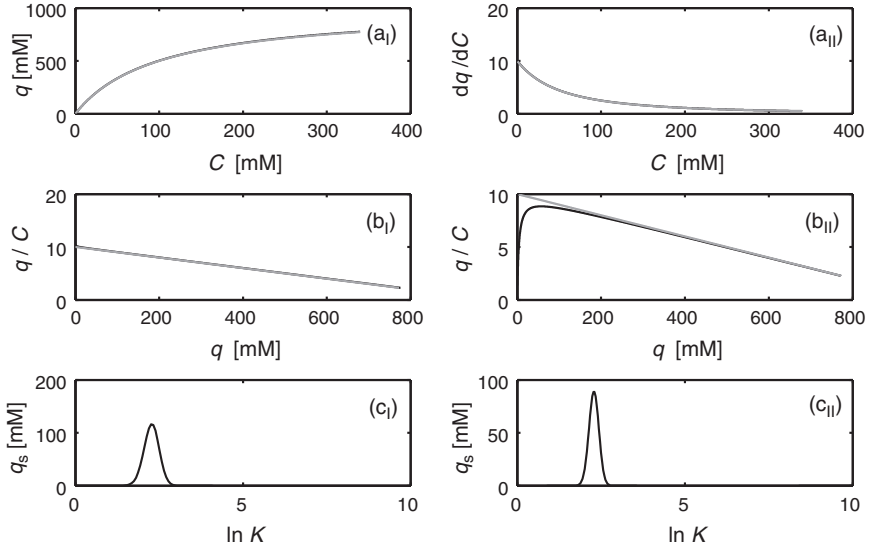

Fig. 1. Adsorption isotherm determined using classical ECP and the ECP-slope for Model 1 (see Table 1). In the left column (I) data from the classical methods are presented and in the right column (II) the ECP-slope method. Grey lines are true data and black lines are estimates of the adsorption data. In (a_I) the adsorption isotherm is plotted, (a_{II}) the raw slope data, (b) the Scatchard plots and (c) the AED were calculated using 100,000 iterations and the energy space were spanned using 350 grid points.

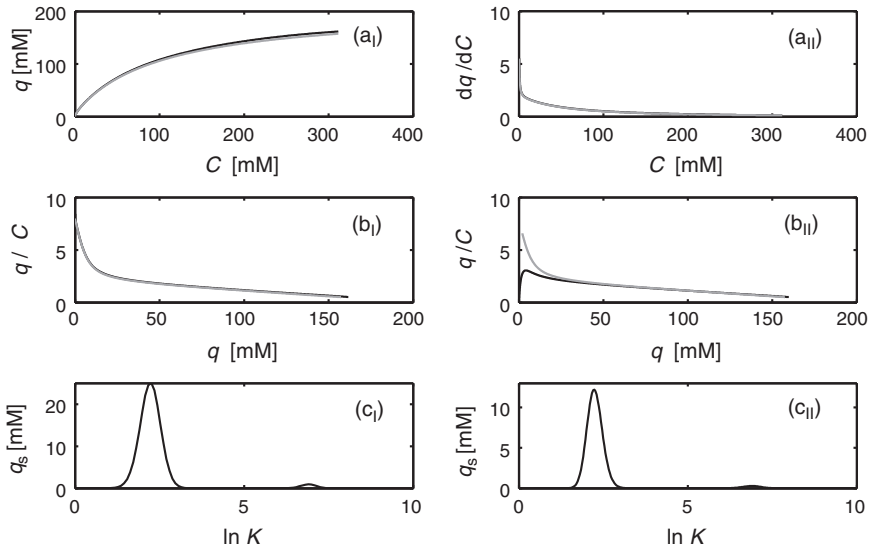

Fig. 2. Adsorption isotherm determined using classical ECP and the ECP-slope for Model 2 (see Table 1). In the left column (I) data from the classical methods are presented and in the right column (II) the ECP-slope method. Grey lines are true data and black lines are estimates of the adsorption data. In (a_I) the adsorption isotherm is plotted, (a_{II}) the raw slope data, (b) the Scatchard plots and (c) the AED were calculated using 100,000 iterations and the energy space were spanned using 350 grid points.

In Fig. 4, the ratios between the ECP method and the true adsorption isotherm are plotted versus concentration for both Model 1 (a) and Model 2 (b). This gives an illustration to the errors induced with classical ECP (grey lines) and with the ECP-slope approach (black lines), respectively. For the classical ECP method, distortions are noticed at low concentrations for both models, while the data derived using the ECP-slope methods is hardly affected at all. On the other hand, at higher concentrations, the ECP-slope method gives a slightly larger error when using Model 2. Generally the errors for both methods are small and of the same magnitude.

As can be seen from Table 1 the determined adsorption isotherm parameters are at least as well estimated using the ECP-slope method compared to the classical version. The advantages of the slope method are that we do not have to use the really low concentration data in search of the point where the concentration is zero. This advantage is especially important for experimental data at very low concentrations which contains noise and severe peak tailing due to slow kinetics.

4.2. Experimental data

So far we have shown that the new method is capable to produce accurate adsorption isotherms parameters from numerical data now we are going to investigate three corresponding experimental systems.

One problem that we have addressed is the difficulty in finding the point where $C = 0$, using experimental data. In Fig. 5a, a 3500 μL

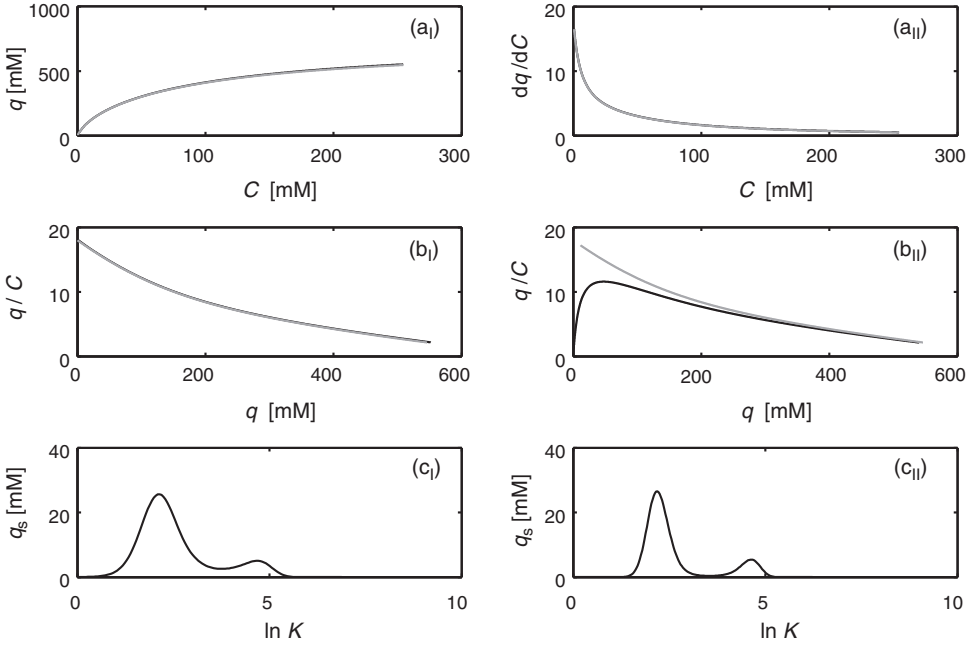

Fig. 3. Adsorption isotherm determined using classical ECP and the ECP-slope for Model 3 (see Table 1). In the left column (I) data from the classical methods are presented and in the right column (II) the ECP-slope method. Grey lines are true data and black lines are estimates of the adsorption data. In (a_I) the adsorption isotherm is plotted, (a_{II}) the raw slope data, (b) the Scatchard plots and (c) the AED were calculated using 100,000 iterations and the energy space were spanned using 350 grid points.

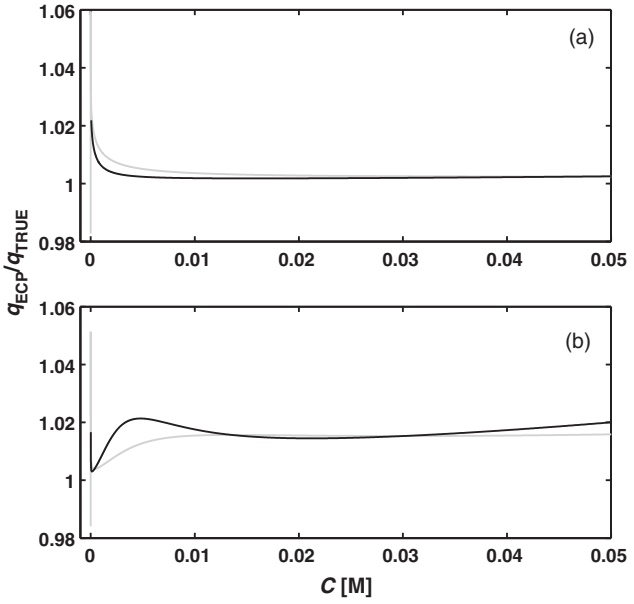

Fig. 4. Accuracy of the different variants of the ECP method as function of C. q_{ECP} is simulated data and q_{TRUE} is an experimental true value. Gray line is the use of raw adsorption isotherm data and black line is the raw slope data. Figure (a) shows the accuracy using Model 1 and (b) shows the accuracy for Model 2 (*cf.* Table 1).

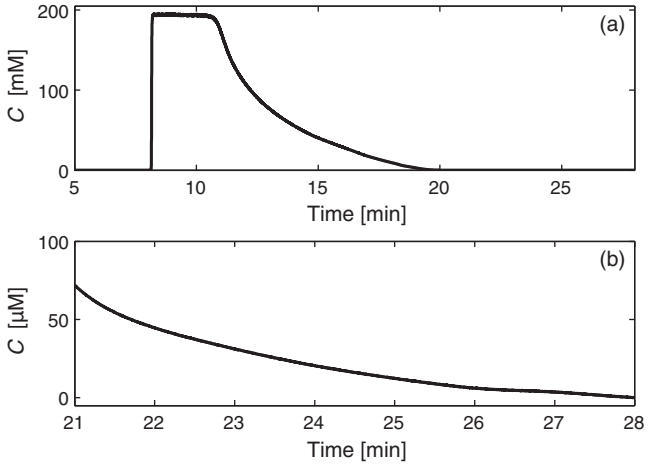

Fig. 5. (a) The elution profile for a 3500 μL injection of 3-phenyl-1-propanol on the Eternity column. (b) Zooms-in the low concentration part. For more experimental conditions, see Section 2.4.

Table 2
Adsorption isotherm parameters calculated using classic ECP (ECP in table) and the new slope data (slopeECP in table) for three different experimental setups (PP at pH 3, ME at pH 3 and ME at pH 11).

	a_1	K_1	a_2	K_2
PP-ECP	10.23	6.06	Na	Na
PP-slopeECP	10.34	6.12	Na	Na
pH 3-ME-ECP	1.59	4.64	6.59	844.90
pH 3-ME-slopeECP	1.71	6.88	6.64	939.27
pH 11-ME-ECP	5.61	9.58	10.26	81.52
pH 11-ME-slopeECP	7.38	13.01	9.10	114.34

cut injection [7] is presented for 200 mM 3-phenyl-1-propanol on Eternity C18 column using 43% (v/v) methanol/20 mM pH 3 phosphate buffer as eluent. As can be seen from the figure the elution profile is compact. From the top figure one would guess that the $C = 0$ would be found at around 20 min. However, if the low concentration area is zoomed in, we could see a pronounced tailing profile that last much longer (see Fig. 5b). One must also observe that this data set contains unusual small amounts of noise as compared to what is common.

To investigate adsorption isotherms determined using the two variants of the ECP method we used three experimental cases: PP at pH 3, followed by ME at pH 3 and at pH 11.

For the neutral compound PP at pH 3, the cut injection is presented in Fig. 6. The AED is unimodal (Fig. 6c) and the Scatchard plot is linear (Fig. 6b), indicating the Langmuir model fits the data. The Langmuir model is fitted to the data and is presented by lines in Fig. 6a (both I and II), the experimental data are circles. In Table 2,

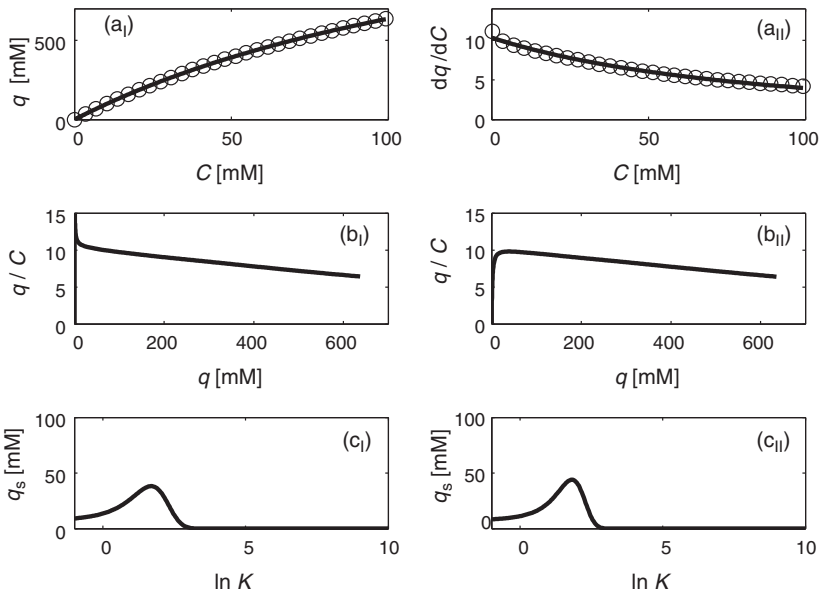

Fig. 6. Adsorption isotherm determined using classical ECP and the ECP-slope for cut injection with PP at pH 3 (see Table 2). In the left column (I) data from the classical methods are presented and in the right column (II) the ECP-slope method. Circles are experimental data and black lines are estimates of the adsorption data. In (a_I) the adsorption isotherm is plotted and in (a_{II}) the raw slope data. In (a) circles are fitted data from the Langmuir model and (b) and (c) shows the corresponding Scatchard and AED plots, respectively. The AEDs were calculated using 100,000 iterations and the energy space were spanned using 350 grid points.

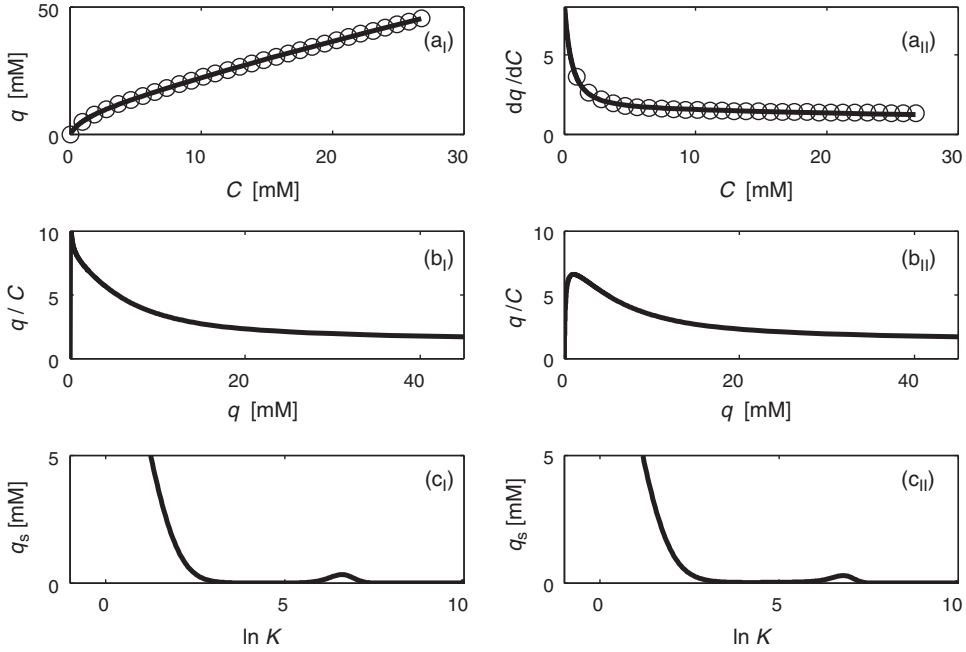

Fig. 7. Adsorption isotherm determined using classical ECP and the ECP-slope for cut injection with ME at pH 3 (see Table 2). In the left column (I) data from the classical methods are presented and in the right column (II) the ECP-slope method. Circles are experimental data and black lines are estimates of the adsorption data. In (a_I) the adsorption isotherm is plotted and in (a_{II}) the raw slope data. In (a) circles are fitted data from the bi-Langmuir model and (b) and (c) shows the corresponding Scatchard and AED plots, respectively. The AEDs were calculated using 100,000 iterations and the energy space were spanned using 350 grid points.

the calculated adsorption parameters are presented. The parameters for both methods are of similar magnitude.

For the basic compound ME at pH 3, the cut injection is presented in Fig. 7. The AED is bimodal (Fig. 7c) indicating a two-site model such as bi-Langmuir and the Scatchard plot has a clear concave curvature (Fig. 7c) with two asymptotes, one at low concentration and the other at high concentration. This is classical for a two-site adsorption model with large difference between the adsorption sites. In Table 2, the fitted bi-Langmuir adsorption parameters are presented.

For the basic compound ME at pH 11, the cut injection is presented in Fig. 8. The AED is bimodal (Fig. 8c), although with smaller difference between the adsorption energies and capacities of the adsorption sites compared to ME at pH 3. The Scatchard plot is concave although without the two asymptotes and now a more continuous fashion could indicate a Tóth or bi-Langmuir model.

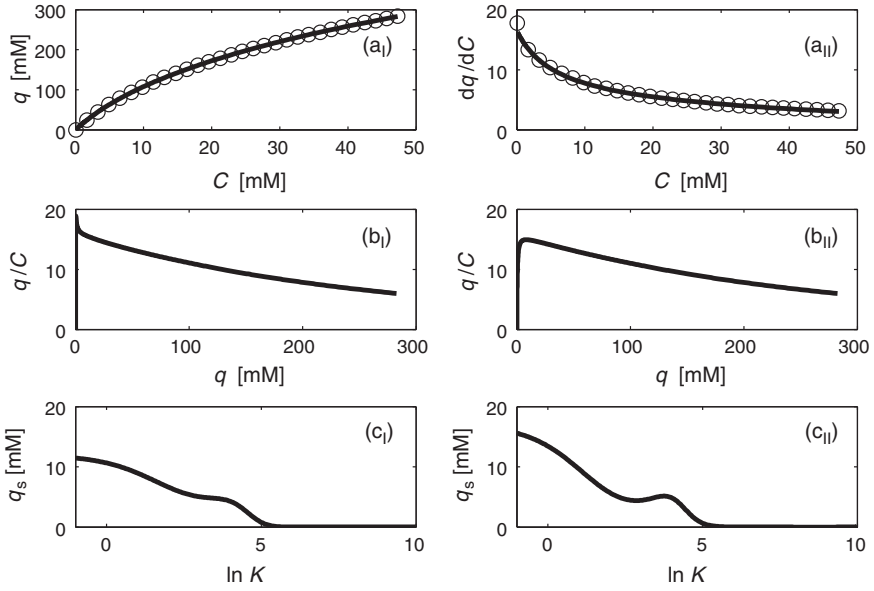

Fig. 8. Adsorption isotherm determined using classical ECP and the ECP-slope for cut injection with ME at pH 11 (see Table 2). In the left column (I) data from the classical methods are presented and in the right column (II) the ECP-slope method. Circles are experimental data and black lines are estimates of the adsorption data. In (a_I) the adsorption isotherm is plotted and in (a_{II}) the raw slope data. In (a) circles are fitted data from the bi-Langmuir model and (b) and (c) shows the corresponding Scatchard and AED plots, respectively. The AEDs were calculated using 100,000 iterations and the energy space were spanned using 350 grid points.

However, the AED is bimodal so the Tóth model could be ruled out. The data were fitted to a bi-Langmuir model and the predicted parameters are presented in Table 2.

As a general observation one could see that the predicted parameters are very similar which indicates that the slope method could be used to generate accurate adsorption data.

5. Conclusions

In this investigation the important source of error was eliminated for caused by the integration of the profile from 0 to *C*. When the classical integration method of the rear is used it is not possible to reject the inaccurate, noisy and dispersed low concentration data points close to the baseline. Moreover, it is very hard to find the, necessary, exact zero point. Instead of the classical integration of the rear of a band shape, we here introduced the use of raw slope data, an approach that was validated to eliminate this systematic error. Also, the need to assign an experimental point for the concentration zero is eliminated. The ECP-slope method was shown to yield excellent prediction of synthetic data, and also similar results as the classical ECP for experimental data. In addition, the AED calculations using adsorption isotherms generated by the ECP-slope method converted faster as compared to the classical approach, this is in line with our previous observation for perturbation peak adsorption data.

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