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Reproducibility between Modern Supercritical Fluid Chromatographic Systems

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Abstract

This short communication is an investigation of adsorption isotherm estimation reproducibility between different commercially available Supercritical Fluid Chromatography (SFC) instruments: Thar Super Pure Discovery Series SFC, Waters UPC^2 and Agilent 1260 Infinity SFC. All experiments were conducted using identical instrumental settings and identical column and the results clearly shows that analytical retention times and "overloaded" elution profiles depends on both the instrument model and the particular instrument of that model that is used. This means that dynamically determined adsorption isotherm will also depend on the instrument, however it must be stressed that this do not mean that the adsorption is different. Instead it means that identical instrumental settings will not reflect the conditions inside the column, most likely due to a slight pressure-modifier difference between the systems despite of the identical operational settings.

This study highlights the importance of understanding the SFC-systems in order to to successfully transfer method between systems, have reliable adsorption isotherm determination, to do analytical quality work and to scale up from analytical to preparative mode. Additionally, despite the high complexity of SFC, the study shows that the simplest column mass balance model, the ideal model assuming infinite column efficiency, can be used to simulate elution profiles.

23 Keywords: Reproducibility; Supercritical fluid chromatography, overloaded elution profiles; modern SFC
24 systems

1 Introduction

Recently, both instrument manufacturers and the pharmaceutical industry have increased interest in Supercritical Fluid Chromatography (SFC). The reasons for this growing interest are the lower environmental impact and the considerably shorter separation times. Therefore a proper characterization and understanding of the thermodynamics and kinetics of the adsorption processes is important, both for analytical and preparative applications. Detailed thermodynamic information can be gained by conducting adsorption isotherm measurements over a broad concentration range. If properly processed, these adsorption data gives not only correct information about the degree of heterogeneity, but also the energy of interactions and monolayer capacities of each individual type of adsorption site in the phase system [1,2]. We recommend the following book and review of classical adsorption isotherms determination methods [3,4] and the recently proposed work flow, and numerical tools, for collecting, processing and interpreting adsorption data [4–7].

In SFC, only a handful papers deals with the determination of adsorption isotherms in preparative chromatography [8–12] and in even fewer cases the estimated adsorption isotherms have been verified by comparing simulated and experimental overloaded elution profiles [8–10]. We suspect three reasons for this (i) the lack of fundamental understanding of SFC, (ii) the lack of commercial SFC systems that has been properly evaluated for fundamental studies and (iii) the relatively low interest for SFC during the past 20 years prior to the recently growing interest. The lack of fundamental understanding of SFC is related to the state of the mobile phase, i.e., its density and how it governs adsorption. Recently Tarafder published an article which explains the origin of the variations of volumetric flow using a Thar system [13] and have quantified the density and volumetric flow variations by measuring the mass flow. Also, models taking into account both heat- and mass balance has recently been developed [14,15]. All of the above clearly motivates further development of methods to determine and interpret adsorption isotherms as well as studies on how to properly simulate elution profiles.

48 The investigation in this study is limited to methanol as modifier at operational conditions, temperature and
49 back pressure most typically observed when utilizing SFC to separate polar compounds [10]. Here we will use
50 the Elution by Characteristic Points (ECP) method [16–20] to determine the adsorption of antipyrine on neat
51 silica utilizing a fluid mixture of CO_2 and methanol. ECP requires that the separation system have high column
52 efficiency [17,18], which is often encountered in SFC, and in recent years the ECP method for liquid
53 chromatography (LC) has been considerably improved [16,19,20]. Because of this the ECP method is an
54 interesting candidate for SFC; still only a few reports exist on the use of the ECP method in SFC [21]. We will
55 perform identical experiments on a semi preparative Thar system and on analytical systems from Agilent and
56 Waters. The reason for choosing the two analytical systems from Agilent and Waters is that they represent two
57 of the latest commercial available systems while the semi-preparative instrument was added to widen the
58 study to include instruments used also for preparative purposes.

59 The first aim of this study is to investigate the reproducibility of adsorption isotherm determination between
60 different commercial available SFC systems and the second aim is to investigate if the ideal model can be used
61 to simulate elution profiles in SFC.

62 2 Experimental Section

63 Three different SFC systems were used in the study: a Thar Super Pure Discovery Series SFC (Waters
64 Corporation, Milford, MA, USA), an Agilent 1260 Infinity SFC system (Agilent Technologies, Palo Alto, CA, USA)
65 and a Waters UPC^2 system (Waters Corporation, Milford, MA, USA). Two Waters UPC^2 systems were used, one
66 placed at Karlstad University and one placed at Waters Corporation Saint-Quentin-en-Yveline (France). The
67 Thar system has only a circulating air oven with no dedicated heating of the incoming eluent, while both the
68 Agilent and Waters system have dedicated heaters prior to the column. The systems are hereafter referred to
69 as the “Thar”, “Agilent”, “Waters” and “Waters-Paris” system. Liquid CO_2 was supplied to the Thar/Waters
70 systems (AGA Gas AB, Sweden) and the Agilent system was fed gaseous CO_2. HPLC grade MeOH was used as

organic modifier and PT-100 resistance temperature detectors (Pentronic AB, Gunnebo, Sweden) connected to a PT-104 data logger (Pico Technology, Cambridgeshire, United Kingdom) were used to measure the column temperature.

A Kromasil neat silica 150×4.6 mm column, packed with 5 µm nominal particle size, 100 Å pore size (AkzoNobel Eka, Bohus, Sweden) was used throughout the entire study. Antipyrine analytical standard was purchased from Sigma-Aldrich (Sigma-Aldrich, Steinheim, Germany).

The chromatographic conditions: a back-pressure of 150 bar, a column temperature of 35°C and an eluent composition of 85/15 (v/v) % CO_2 - MeOH. All experiments were carried out at volumetric flow of 2 mL/min. A 350 g/L stock solution of antipyrine dissolved in MeOH was used throughout the study. The obtained chromatograms were then converted from UV-response to concentration by assuming a non-linear relation between concentration and response [9].

The dead volume of the system capillaries and connections from the injector to the detector was estimated by using injections of highly diluted samples of antipyrine when the column inlet and outlet capillaries were connected using a union. The system dead volumes were estimated to be 190, 80 and 80 µL for the Thar, Waters and Agilent system respectively.

Using a number of replicates of 5 µL injections of 0.35 g/L antipyrine the number of theoretical plates was estimated to be approximately 9 500 on the Thar system and approximately 10 000 on the other systems.

3 Results and Discussion

First the reproducibility between different systems was studied by injecting 20 µL (full-loop) of 350 g/L antipyrine on the four different SFC systems using identical instrumental settings and the same column. The resulting overloaded elution profiles, corrected for system void volumes, is clearly system dependent as can be seen in Fig. 1. This figure also shows that the elution volume is larger on the Thar system and the Waters and

Agilent system have lower, and similar, retention volumes. However the Waters-Paris system (same model as the Waters system, see experimental section) had a retention volume that was more than 0.5 mL larger than the Waters system when using the same column and the same set conditions. This indicates that either the set conditions are not manifested in the systems or that the column conditions were different. Below we will discuss and analyze the possible reasons for these differences prior to analyzing the estimated adsorption isotherm and finally we will use the determined adsorption isotherms to predict elution profiles.

3.1 Investigation of System Differences

First we investigated if the volumetric flow rate was the reason for the observed difference between the elution profiles shown in Fig 1. The column dead volume was determined and was estimated to be approximately 1.9 mL on each system. This means that the volumetric flow rate was more or less identical on all systems; otherwise the void volume would have been different. If the estimated void-volume is incorrect this will lead to incorrect adsorption isotherm parameters and in the worst case even to the use of the wrong adsorption isotherm model [22,23]. This issue is of major importance in a mechanistic investigation of the adsorption. However, in this short study the main goal is to study differences in experimental elution profiles between system and not to perform a mechanistic investigation.

Other important parameters governing retention are the density of the mobile phase, the fraction of organic modifier and the column temperature. The density is a non-linear function of pressure and temperature [24] and in order to calculate the density of the carbon dioxide and methanol stream inside the column, the pressure at the column inlet and outlet, as well as the actual column temperature must be known. None of the systems measures pressure directly prior and after the column, only at a point upstream the column and this pressure reading cannot simply be correlated between different systems because the pressure drop upstream to the back-pressure regulator will be divided between capillaries and column [25]. Pressure drop in the capillaries will depend on their length and diameter, which cannot be assumed to be identical in all systems.

To investigate how the retention is affected by changes in system back pressure and modifier content we made analytical injections, 5 µL of 0.350 g/L antipyrine, at back-pressures between 140 - 160 bar and modifier fractions between 14 - 16 %, on the Waters system. As seen in Fig. 2 the retention volume is strongly affected by changes in the modifier concentration and to a smaller extent to the set pressure. We noticed that the Thar system has a much lower pressure drop over the system compared to the Waters and Agilent systems. This sensitivity and back-pressure difference between systems are probably the explanation for the observed difference in retention volume between the systems.

3.2 Adsorption Isotherm Determination

To determine adsorption isotherms using ECP it is advantageous if the volumetric flow rate is constant over the column. At a set volumetric flow rate in an LC- instrument, both the mass flow and volumetric flow can be assumed to be constant regardless of rather large pressure gradients across the column due to the incompressibility of the solvents typically used. The mixture of 15 % MeOH and CO_2 is a compressible fluid, albeit much less compressible than pure CO_2. Because of this, the volumetric flow at any point in an SFC system will be determined by the mass flow and density at that point. In this study, we will assume that we have constant volumetric flow for both the simulations and adsorption isotherm determination. This assumption is valid only if the inlet and outlet pressure are identical or such that, despite a non-zero pressure gradient, the density gradient along the column is very small. We have also assumed that potential density gradients throughout the column do not significantly affect the adsorption isotherm.

To validate the assumptions of constant volumetric flow, isothermal and iso-pycnic conditions, measurement of the relative pressure drop and temperature profile of the column were performed on the Waters system. At the column inlet and outlet PT-100 temperature probes were attached using a high strength and high thermally conductive silver-epoxy adhesive. The recorded temperature was equal to 35°C with an uncertainty of 0.5°C. By comparing the upstream pressure reading with the column installed and replaced by zero volume union, a

pressure gradient of 10 bars along the column was record at the studied conditions. This corresponds to inlet and outlet densities of 871 and 867 kg/m^3, a relative decrease of less than 0.5%, according to the REFPROP software developed by National Institute of Standards and Technology. Also, by studying Fig. 2 in more detail, the retention volume at 15 % (v/v) MeOH decreased from 6.6 to 6.5 mL, a relative decrease of 1.5 % for a pressure drop increase from 150 to 160 bar. The indication of close to iso-pycnic and isothermal conditions with constant volumetric flow through the column enables us to draw the conclusion that the approach of LC, regarding determination of adsorption isotherms and simulation of elution profiles, is valid here, or at least a good approximation.

To determine adsorption data, 20 µL injections of 350 g/L and 140 g/L, were done; see black lines in Fig. 3a and Fig. 3b (note that both figures show the same experiments). The measurements repeatability was assured by overlaying duplicated injections and by monitoring the back-pressure and temperature readings from the system. It should be noted that the repeatability on the Thar system was worse than on the Agilent and Waters systems. Only replicates with stable and low back-pressure ripple were used, which meant that much more time had to be spent when using the Thar system. The adsorption isotherms were calculated from the 350 g/L injection using the ECP approach, see Fig. 4. Prior to the model fit the adsorption data were analyzed using Scatchard plots which were concave. This indicates that the adsorption describing the system is heterogeneous. Tóth and bi-Langmuir models were fitted to the adsorption data and the bi-Langmuir model was found to describe the data best. The ECP method requires that the separation system have high efficiency and the determined efficiency for all systems were around 10 000 plates. Because all separations hade high efficiency, and only data from the injection with the highest loading were used for the adsorption determination, the accuracy of this ECP can be expected to be good [18].

3.3 Simulation of Elution Profiles

Simulated elution profiles, using the determined adsorption isotherms and the Equilibrium-Dispersive (ED) model [4], are plotted in Fig. 3a (gray lines). Since preparative SFC typically utilizes smaller particles, and the diffusion is much faster than in liquid-based systems, it is reasonable to assume that the ideal model, to a much larger extent than in LC, can accurately describe the column mass transfer phenomena, especially if the column load is large [26]. To test this assumption, the same elution profiles were calculated using the ideal model and compared with overlaid with experimental profiles, see Fig. 3b (gray lines). The agreement between the experimental profiles and simulated profiles using both the ED and the ideal model were very good. The results are not surprising because it is well established that the solution of the ideal model approaches that of the ED-model when the Shirazi-number [26] increases. The Shirazi-number is a unit-less measurement of combined column load and column efficiency and is approximately 70 for the 350 g/L injection which clearly indicates the usefulness of the ideal model.

4 Conclusions

This study shows that the same instrumental set conditions does not necessarily give the same actual working conditions over the column on different systems. In this study, recorded elution profiles on three different systems, at the same set conditions, are clearly different. This means that it can be hard to transfer an established separation method from one system to another, even if one is using the same column and identical instrument settings. This has implications both for analytical methods and preparative applications, for example different separation methods are usually screened on an analytical instrument and then the selected method is transferred to a larger preparative system.

These issues can most likely be solved by measuring mass flow, pressure and temperature along the column, together with an understanding of the density variations of the mobile phase [13,25]. We showed that in our case the most probable explanation of the difference is that different system delivers different amount of

modifier (see Fig. 2) and that the column pressure is different. In other words: one SFC-system's 15% modifier content is not the same as another system's 15% modifier content.

Despite the greater degree of complexity in SFC compared to LC, SFC separations generally have much higher efficiency compared to the same separation conducted on an LC system. The increased efficiency, up to 10 000 theoretical plates in this case, enables us to use a much less complex mass balance model, the ideal model, see Fig. 3, to calculate the elution profiles. This could be favorable for numerical optimization of SFC separations where the mass balance model has to be solved thousands of times.

It should be noted that the investigation has focused on the case where one uses using carbon dioxide and methanol mixtures as the mobile phase. The situation when using pure carbon dioxide, or other combinations of polar co-solvents, remains to be investigated.

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Figure captions

Fig. 1: Experimental chromatograms recorded on the Thar (solid line), the Waters (dotted line), the Waters-Paris (dash-dotted line) and the Agilent system SFC-system (dashed line). The set volumetric flow was 2 mL/min and the injection volume was 20 µL of a 350 g/L sample.

Fig. 2: Retention volume of analytical peaks as a function MeOH content and pressure determined using 14, 14.5, 15, 15.5 and 16 (v/v) % MeOH content at 140, 145, 150, 155 and 160 bar on the Waters system. All analytical injections were 5 µL of a 0.35 g/L sample.

Fig. 3: Black lines are experimental chromatograms from the Thar (solid line), the Waters (dotted line) and the Agilent system (dashed line). The corresponding gray lines are simulations in (a) using the equilibrium dispersive model and in (b) using the ideal model. The set volumetric flow was 2 mL/min and the injection volume was 20 µL of a 350 g/L sample.

Fig. 4: Raw slope of the adsorption isotherm for all three systems determined using the ECP method: Thar (solid line), Waters (dotted line) and Agilent (dashed line).

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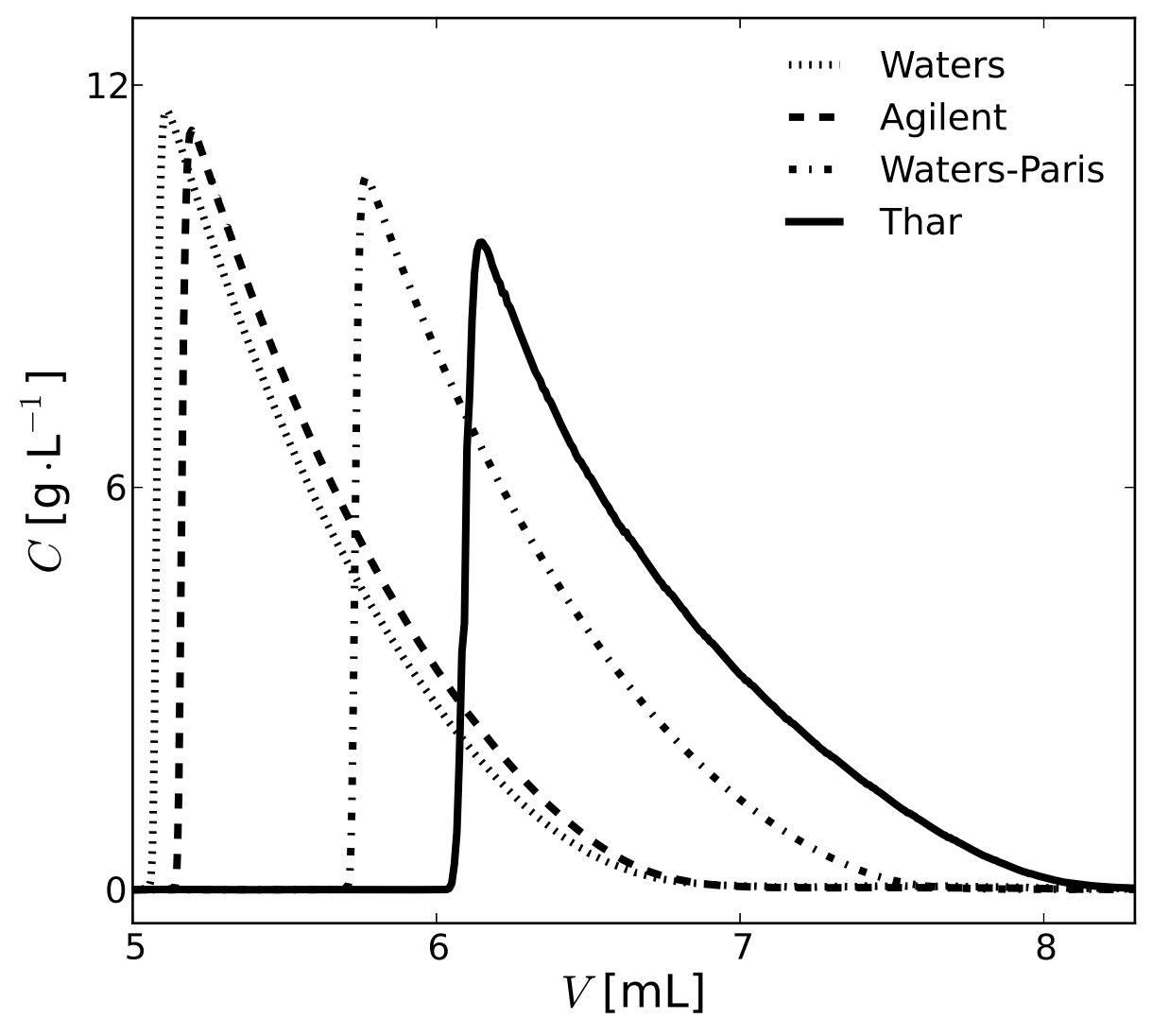
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Agilent
Waters-Paris
Thar
12
6
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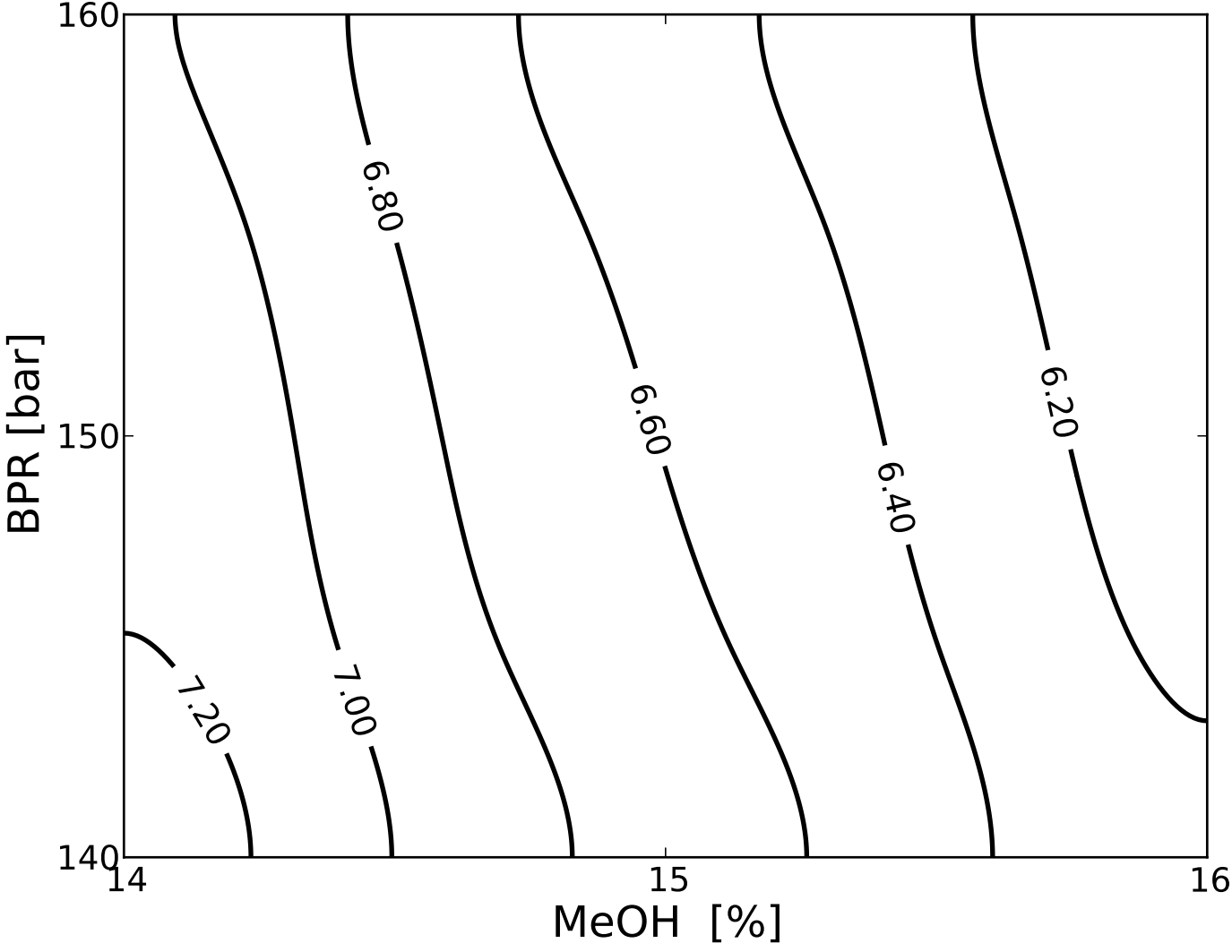
C [g·L^{-1}]
V [mL]


160
150
140
BPR [bar]
14
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MeOH [%]
7.20
7.00
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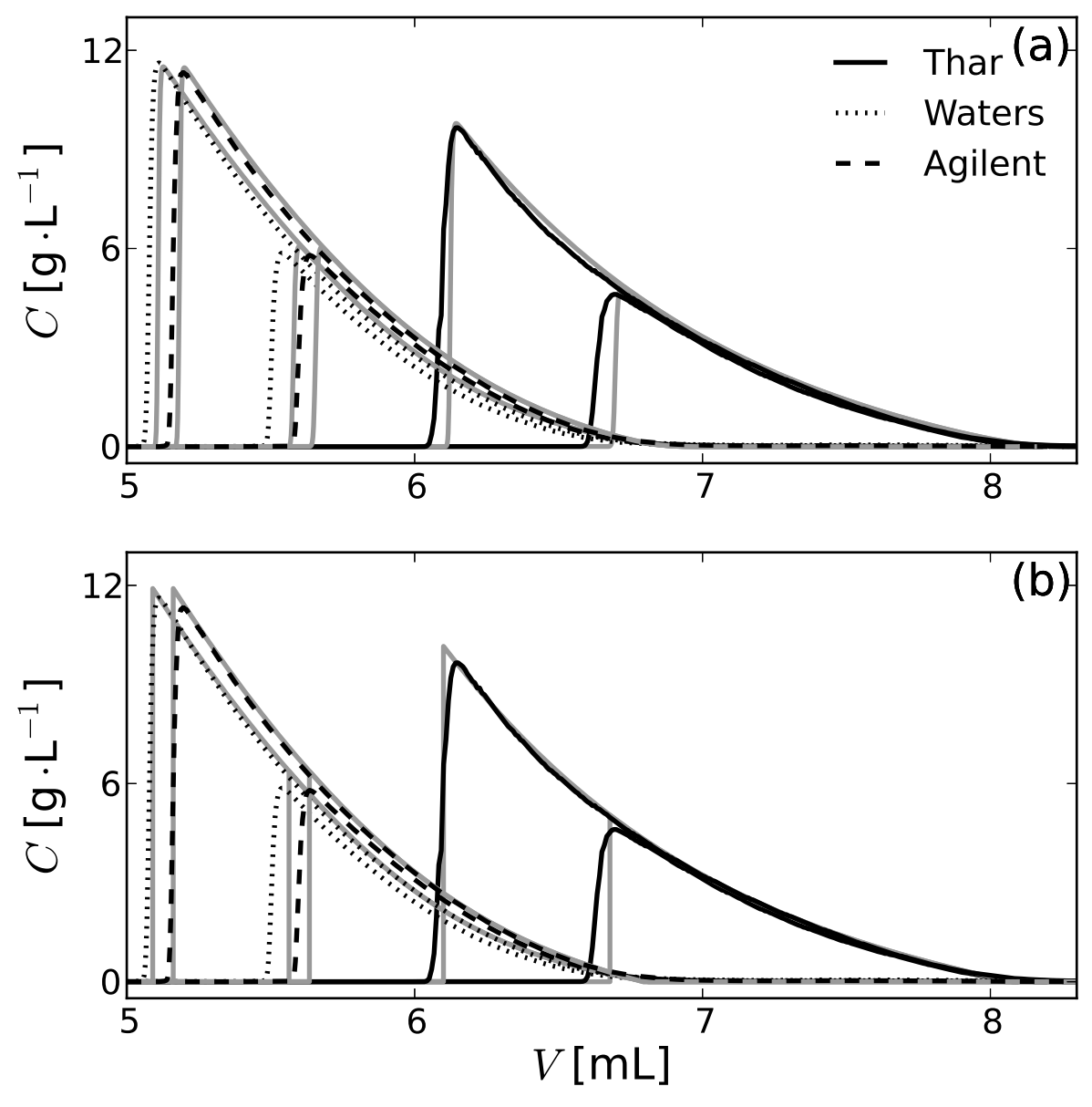
(a)
Thar
Waters
Agilent
12
6
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C [g·L^{-1}]
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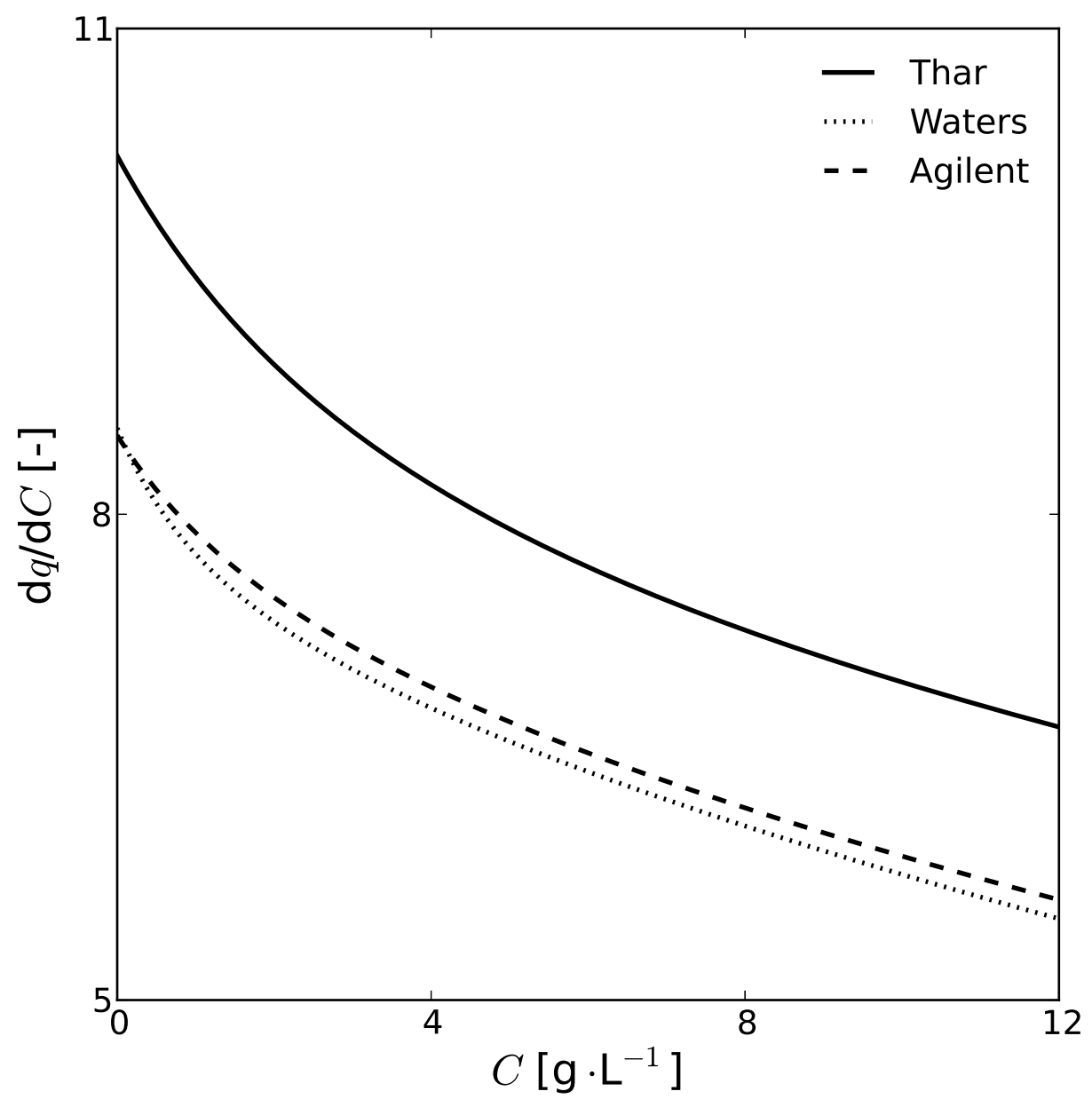
11
8
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Thar
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$\mathrm{d}q/\mathrm{d}C$ [-]
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