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Unifying sorption isotherms in reversed-phase liquid chromatography

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# Abstract

The use of adsorption isotherm models has contributed to understanding retention in chromatography. However, the common isotherm models (such as Langmuir, BET, and GAB) assume site-specific, layer-by-layer adsorption on a uniform surface, which is at odds with the reality of chromatographic interfaces (heterogeneous surfaces with sorbate mobility and adsorption-partitioning duality). Our goal is to clarify the interactions underlying chromatographic retention while overcoming the above contradiction caused by the overly idealized or unrealistic assumptions of the traditional isotherm models. This can be achieved by the statistical thermodynamic fluctuation theory and the ABC isotherm derived from it, which is capable not only of capturing the mono-, di-, and tri-sorbate interactions as its parameters but also of encompassing the Langmuir, BET, GAB, and even anti-Langmuir isotherm models as its special cases. We demonstrate how the parameters from the traditional models can be reinterpreted via sorbate interactions. The sorbate-sorbate interaction plays a key role in elucidating the origin of different IUPAC isotherm types: Type I (convex upward) and Type III (convex downward) represent the opposite end of sorbate-sorbate repulsion and attraction that are independent of sorbate concentration, whereas Type II lies in-between, in which sorbate-sorbate interaction, which is repulsive at low concentration, turns attractive once sufficient quantity of sorbate is present at the interface (convex upward to convex downward).

# 1 Introduction

Retention in reversed-phase liquid chromatography (RPLC) is governed by the distribution of the solute between the mobile phase and the solid sorbent surface. However, the retention mechanism involving these two phases has often been simplified as partitioning between the interface and bulk or as site-specific, layer-by-layer adsorption [1-3], although it is reportedly better described by a combinatory approach, somewhere between these two extremes [4-8]. Understanding the sorbate-surface and solvent-surface interactions underpinning retention is crucial for the appropriate modelling and subsequent optimization of a separation, which involves an appreciation of the complex physical properties of the sorbent surface that mediate the interactions. This necessitates a universal theoretical foundation to reveal the molecular interactions which dictate retention.

The measurement of adsorption isotherms offers a comprehensive method for capturing these key interactions between sorbate, solvent, and sorbent, which control sorption at the solid-liquid interface. The practical and theoretical relevance of isotherm measurement and modelling to liquid chromatography is well established, particularly in the design of large-scale preparative chromatographic processes [9-11], column characterization [12, 13], overloaded band profile modelling [14-16], and elucidation of retention mechanisms [17, 18]. Moreover, dynamic methods of isotherm determination are well suited to liquid chromatography [19], offering a more rapid and accurate approach in contrast to static methods [20] while requiring lesser quantities of solute. Numerous techniques exist for the acquisition of single-component isotherm data [21-27], with the possible extension to competitive sorption of multiple solutes, either by direct measurement or approximation from single-component data [28, 29]. Among these approaches, frontal analysis is most widely employed owing chiefly to its accuracy [16, 20] and independence of column efficiency [17, 30]. However, the validity of mechanistic insights drawn from sorption isotherms has been limited due to the overly idealized assumptions embedded in common isotherm models (e.g., site-specific, layer-by-layer adsorption), which are inconsistent with the practical reality in a column, as will be detailed below. The inconsistency in modelling principles complicates analysis, and model specificity impedes comparison on account of the lack of a universal language and parametrization.

An appreciation of the poor suitability of common isotherm models (namely Langmuir, BET, and GAB) may be achieved by examining their common assumptions:

1. the adsorbent surface is homogeneous and is composed of statistically independent site-specific binding sites of equal binding strength;
2. subsequent layer-by-layer adsorption proceeds without any restrictions on thickness (for the case of BET and GAB models);
3. no lateral interactions exist between sorbates in the same layer of the adsorbed phase.

To address these assumptions, we must first appreciate the surface chemistry and physical properties of common sorbents. Silica-supported stationary phases are ubiquitous in liquid chromatography [31]; silica itself is inherently heterogeneous with the existence of multiple distinct adsorption sites on its surface [32-34], each characterized by their differing energies [35-38]; this energetic heterogeneity is well known to contribute to peak asymmetry and a reduction in peak resolution [39]. The need for reversed-phase separations necessitated the functionalization of porous silica supports; their encountered surface chemistries are dominated by alkyl octadecyl and octyl phases [40], the bonding densities of which are pertinent to the total number and nature of adsorption sites [41, 42]. Full derivatization of the silica surface is unachievable, partially owing to the steric hindrance effects of the reacted group [43-46]; bare silica regions with unreacted silanol groups, with possibly high adsorption energies [47, 48], can remain accessible to both the mobile phase components and sorbate [49]. In most modern columns, end-capping aims to minimize these secondary interactions involving the exposed residual silanol groups. An additional aspect of surface heterogeneity may be introduced by considering solvent-induced structural changes [50, 51]; the chemical properties and physical structure of the stationary phase are intrinsically linked to the nature of the mobile phase employed in analysis [52]. For the aliphatic chains of alkyl-silicas, this concerns their ordering and conformation on the silica surface, which has an influence on both the mechanism of retention and adsorption capacity [6, 50]. It is apparent that assumption (i) is contradictory to a given column’s surface heterogeneity, and assumption (ii) is impossible for their porosity and steric hindrance, alongside the physical constraints imposed by the column geometry and void volume. In view of a complex interplay between the structures of the stationary and mobile phases, the assumption of layerwise adsorption without any lateral interactions (assumptions (ii) and (iii)) is unrealistic. Thus, the assumptions underlying the simple adsorption isotherm models, founded on site-specific, layer-by-layer adsorption, do not agree with the structural complexity at chromatographic interfaces.

Despite their oversimplified assumptions (i)-(iii), the simple adsorption models have been successful in fitting experimental isotherms from chromatography that involve complex interfaces. This paradox, which was also observed for solid-gas and simpler solid-liquid isotherms, can be resolved by the adoption of statistical thermodynamic fluctuation theory, which can be applied to sorption involving (i) interfaces of any geometry and porosity, (ii) structural changes to the interface, and (iii) high surface heterogeneity [53, 54]. This novel approach enables the derivation of isotherm equations from the two exact (approximation- and model-free) fundamental equations of the statistical thermodynamic fluctuation theory: the generalized Gibbs isotherm and the fluctuation relationship [53-55]. One of the resultant isotherms, termed the ABC isotherm, is a truncated sorbate activity expansion whose parameters represent surface-sorbate, di-sorbate, and tri-sorbate interactions at the interface. This isotherm is free of the overly idealized assumptions of site-specific, layer-by-layer adsorption inherent to Langmuir, BET, and GAB models, which are restricted specific cases of the ABC isotherm, while also capturing interface/bulk partitioning as an additional special case. Consequently, the ABC isotherm has been demonstrated to replace the Langmuir, BET, and GAB models and provide them with a new, unified interpretation via the mono-, di, and tri-sorbate terms [53, 56].

Thus, our goal is twofold: firstly, to capture the interfacial complexity between the mobile and stationary phases without introducing overly simplified model assumptions and secondly, to establish a unified approach to model adsorption isotherms from RPLC, encompassing, as specific cases, the conventional view of site-specific adsorption occurring in a layer-by-layer manner, interface/bulk partitioning, and their combination. Our first objective is to establish the isotherm theory that can handle sorption on inhomogeneous and complex interfaces of sorbates in mixed solvents (Section 2). This will lead to our second objective, i.e., a universal mechanistic elucidation of sorption isotherms in chromatography, replacing many isotherm models with the universality of the ABC isotherm, enabling a systematic comparison between isotherms previously modelled via isotherm models with different mechanistic assumptions, on a universal mechanistic basis.

# 2 Theory and Methods

## 2.1 A statistical thermodynamic fluctuation theory for heterogeneous interfaces

Throughout this paper, we consider a solid-liquid interface of finite-range and arbitrary geometry consisting of a surface formed of a sorbent, denoted as , which is exposed to the solution phase consisting of solvent (species 1) and sorbate (species 2).

The sorption from solution is quantified by the surface excess defined by IUPAC as “the difference between the amount of component actually present in the system, and that which would be present (in a reference system) if the bulk concentrations in the adjoining phases were maintained up to a chosen geometrical dividing surface” [57]. While the reduced surface excess is experimentally accessible [58], the relative surface excess of 2 with respect to 1, , which is related to via [55, 57], has a direct link to the thermodynamics of sorption, defined as

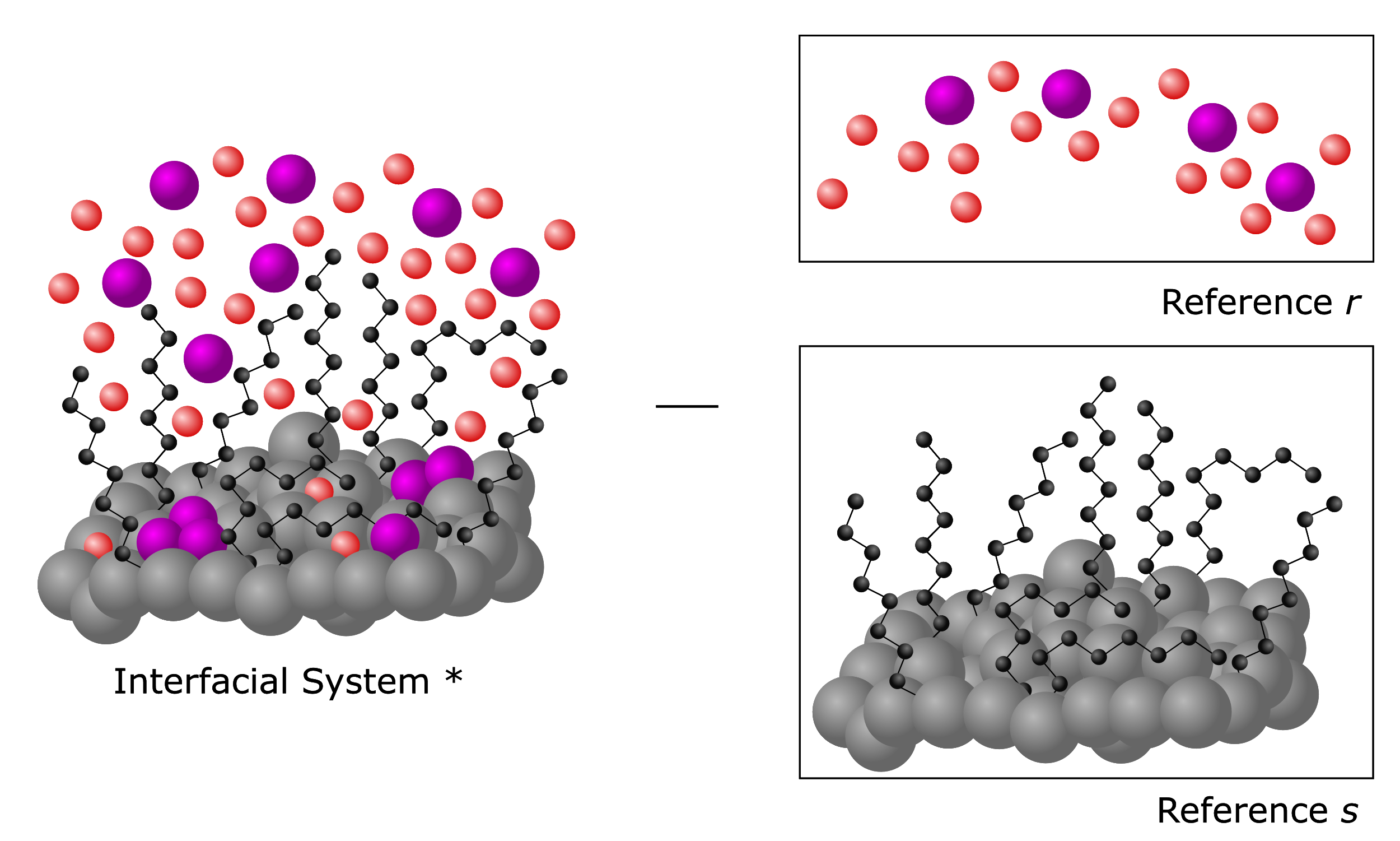
(1)

where and () are the numbers of molecules in the interfacial and reference (solution) subsystems, respectively, as depicted in **Figure 1**. Now, we incorporate surface heterogeneity into eq 1. Let an interface be composed of different surface patch types denoted by the Roman numeral superscripts (e.g., or ). Generalizing our previous theory on gas sorption on heterogeneous surfaces to sorption from solution [59], can easily be shown (Supporting Information A) to be expressed

(2)

namely, as the sum of sorption isotherms on individual patch types [59]. This isotherm additivity (eq 2), derived under the statistical independence of sorption on individual patches (Supporting Information A), is the theoretical foundation for the summation of simple isotherm equations.

This is analogous to classical approaches employing the summation of local isotherm equations, including multi-Langmuir, Langmuir-BET, and adsorption energy distribution functions (AEDFs), where there is also an assumed statistical independence of the discrete patches. Further assumptions are introduced concerning the adsorption mechanism, i.e. site-specific, layer-by-layer binding on a uniform patch. Within this assumption, multi-Langmuir and Langmuir-BET divide a surface into multiple patch types with different binding, and AEDFs into a continuous distribution of patch types characterized by their adsorption energy (related to the “local” binding constant, often Langmuir or BET). Problems arise when a Langmuir isotherm is assumed for the entire surface or a surface patch, as the breakdown of the Langmuir mechanism has been reported (see [53] and references therein). Consequently, we adopt the AB and ABC isotherms, free of simplifying assumptions, while encompassing the Langmuir and BET models as specific cases. If multiple Langmuir terms are demonstrated to be necessary for accurately modelling an isotherm (without any need for an AEDF calculation), we propose translation to a sum of ABC isotherms. This way, the postulate of patch decomposition is preserved, while the restrictions of conventional isotherm models can be overcome.



**Figure 1** Schematic depiction of the difference between the interfacial system (\*) and the two reference systems and . The molecules of sorbent (), solvent (1), and sorbate (2) are represented by grey, red, and purple spheres respectively.

## 2.2 The ABC isotherm for each patch

Here, we consider an individual patch with the aim of establishing an isotherm equation applicable to a chromatographic interface. The ABC isotherm, derived from the statistical thermodynamic fluctuation theory [55, 60], captures how for a given patch changes with sorbate concentration in the reference bulk solution, via

(3)

where is the mole-fraction of the sorbate in the bulk. Conceptually, its parameters, , , and , capture the preferential sorbate-surface interaction, the surface-bulk self-association difference, and triplet interactions, respectively. This interpretation is founded in the distribution functions between molecular species in the solution phase (, as the distribution function between the molecular species and (, , ) at separation ), as well as the surface-solvent and surface-sorbate distribution functions ( and , at separation from the surface). This relates to the isotherm parameters through the Kirkwood-Buff integrals (KBIs) [61, 62] of the distribution functions, defined between two molecular species in the solution phase via

(4a)

which represents the net excess (positive) or deficit (negative) distribution of species molecules around a species molecule. The KBIs were originally introduced for the solution system yet can be generalized to an interfacial system [53, 60]; the surface-molecule KBI is defined as

(4b)

which is the measure of net accumulation (positive) or exclusion (negative) of the molecules of species at the interface as compared to the bulk. Following this, the preferential sorbate-surface interaction, , is the difference between surface-sorbate and surface-solvent KBI interactions, and , as

(5)

where is the pure bulk solvent concentration. The surface-bulk self-association difference, ,

(6)

is linked to KBIs through the and are the Kirkwood-Buff parameters that quantify self-interaction at the interface and in the bulk, defined in terms of the solvent-solvent (), solvent-sorbate (), and sorbate-sorbate () Kirkwood-Buff integrals, via

(7a)

(7b)

with as the partition coefficient representing the exchange process of “one solvent at the interface and one sorbate in the bulk” with “one sorbate at the interface and one solvent in the bulk”

(7c)

where and are defined as the sorbate/solvent mole ratios in the interface and bulk. The expression for the parameter , whose rigorous expression is presented in Supporting Information B, can be interpreted as the sorbate triplet interaction at the interface.

For the application to heterogeneous surfaces comprised of multiple distinct patches, we employ isotherm additivity (eq 2, Section 2.1) and the ABC isotherm as an isotherm for a patch (see eq 3) such that

(8)

Thus, we have established a universal isotherm theory for heterogeneous surfaces (see our first objective in Section 1). It is important to note that a heterogeneous sorption mechanism necessitating isotherm additivity is not restricted exclusively to topographically heterogeneous surfaces, just as a topographically heterogeneous surface does not always equate to heterogeneous sorption.

## 2.3 Adapting the ABC isotherm to mixed solvents

The extension of this theory beyond the three-component formalization for single-solvents to that for multicomponent solvent systems is essential for application to liquid chromatography, where mixed solvent systems are near-universal. To this end, we establish here the conditions upon which a binary solvent mixture (comprised of solvent components and ) can be treated as a single mean solvent. The theoretical foundation for this has been set out in Supporting Information C, and D. Supporting Information C presents a rigorous yet complex mathematical expression for the surface excess that is related to the sorbate activity derivative of the interfacial free energy while Supporting Information D details the mathematical expression for the change of the sorbate-to-solvent mole ratio when a sorbent is introduced to the system. This expression generalizes the experimental procedure for determining surface excess in binary sorbate-solvent mixtures. Unlike the case of binary mixtures, it is not straightforward to equate the results from these two appendices unless the solvent composition difference between the bulk and the interface is negligible compared to the adsorbate composition difference, namely,

(9)

The adherence to this condition is evident when examining the simulated density distributions of sorbate and analyte molecules obtained from molecular dynamics simulations performed for various analytes in acetonitrile-water mixtures within C18-silica mesopores. There is a greater disparity between interfacial analyte concentration versus the bulk than the solvent composition difference; the surface excess of the analyte from the mixed solvent is far greater than that of the strong solvent component [8, 63-66].

## 2.4. Simplifying the ABC isotherm

Interpreting the parameters of the ABC isotherm can be simplified significantly when the specific reduced surface excess can be approximated as the actual amount adsorbed ( in the conventional notation); such an approximation has been implicit in the majority of publications related to analyte adsorption, a practice which IUPAC advises against [57]. However, recently the experimentally-tractable condition for the validity of has been established for dilute sorbates in single-component solutions [67]. For this purpose, the surface excess must be divided into individual isotherms for solvent, , and sorbent, ,

(10a)

(10b)

where , , and are the respective partial molar volumes of the solvent, sorbate, and sorbent and is the volume per unit mass of the sorbent phase. The relative surface excess may be expressed solely in terms of (eq 11), enabling a comparison of the volumetric data with and allowing the demonstration of the contribution of to ,

(11)

The quantitative assessment of is reliant on the use of scarce volumetric data, the absence of which precludes the direct examination of the contribution of to the surface excess. This practical limitation may be circumvented by carrying out an order of magnitude comparison, involving the estimation of the volume of the interfacial layer () using an appropriate value for the sorbent surface area, , and interfacial layer thickness, . This is the approach employed in Supporting Information E, where the dominance of in is established for sorption of multiple sorbates in both acetonitrile-water and methanol-water mixtures, demonstrating that the interpretation of the ABC may be simplified, focusing only on the exchange coefficient and Kirkwood-Buff at the interface.

# 3 Results and Discussion

## 3.1 Unification of traditional sorption isotherm models through ABC

As discussed in Section 2.2, conventional isotherm models may be reinterpreted via the relation of eq 3 with their respective expressions. This allows a universal interpretation of microscopic interactions between the sorbate and its surroundings in terms of the mono-, di-, and tri-sorbate interactions (**Figure 2**) captured by the parameters A, B, and C.

### 3.1.1 The Langmuir model

Here, we show that the Langmuir model is a special case of the ABC isotherm. The Langmuir model [68, 69] is widely employed in the analysis of experimental isotherm data, including that of solid-liquid systems, despite its formulation for a solid-gas interface (**Figure 2**). The fundamental presumptions made in its conception are (i) “the forces acting between adjacent adsorbed atoms or molecules are negligible” so that each is “independent of the presence of others on the surface”, (ii) “atoms condense only on that part of the surface which is bare”, and (iii) uniformity of the binding sites [70, 71]. These assumptions clearly restrict its application to real systems [72], with Langmuir himself attesting that due to the “simplifying assumption made in its derivation, it should, of course, not be looked upon as a general equation of the ‘adsorption isotherm’” [70]. The amount of adsorption, , is related to the monolayer capacity , Langmuir constant , and adsorbate concentration () via

(12)

which may be extended to heterogeneous surfaces as the sum of multiple Langmuir isotherms, each corresponding to a statistically independent individual patch type. Converting the Langmuir model to a form comparable to the ABC isotherm requires the conversion of to the mole fraction (which is carried out at the limit, as the ABC isotherm is founded on an expansion of at the limit [55]) via , leading to

(13a)

where is the molarity of bulk solvent (or solvent mixture). Comparing eq 13a with the ABC isotherm (eq 3) establishes the correspondence between the two via [60]:

, , and (13b)

### 3.1.2 The Anti-Langmuir Model

The anti-Langmuir model is used to account for cooperative sorption, as is encountered in concave upward isotherms (Type III of the IUPAC classification for the physisorption of gases) [73]. It represents indefinite successive adsorption without saturation, via

(14)

where the parameters and are merely empirical and lack any physical meaning. Through examination of the form of eq 14, we can see that when is realized, there is an infinite amount of adsorbed sorbate in the stationary phase, a clearly unattainable scenario [73, 74]. These features of the anti-Langmuir isotherm pose challenges to the traditional approaches to isotherms: Type III isotherms cannot be elucidated using a common language of layer-by-layer adsorption that has been applied to Types I and II. To enable a practical interpretation of anti-Langmuir parameters, we must establish the correspondence to the ABC parameters at the dilute sorbate limit,

(15a)

which, via comparison of eqs 3 and 15a, yields

, , and (15b)

Thus, eq 15b shows that the anti-Langmuir isotherm can be reinterpreted in the framework of the ABC isotherm.

### 3.1.3 The BET Isotherm

The BET isotherm model for solid-gas equilibria accounts for multilayer formation at the sorbent surface (**Figure 2**). The adsorption of a molecule occurs on a uniform surface, and the adsorbed molecule may then act as an adsorption site for a subsequent layer in the formation of a multilayer with unlimited thickness [56]. Interactions between molecules within the same layer are ignored, and the energy of adsorption of the initial layer is both unique and greater than that of further layers, the adsorption energies of which are considered equal [75]. When applied to a solid-liquid interface, this yields unphysical parameters [76], which has prompted the extension of the BET theory to a solid-liquid interface, formulated under the same principles as BET, leading to [77, 78]

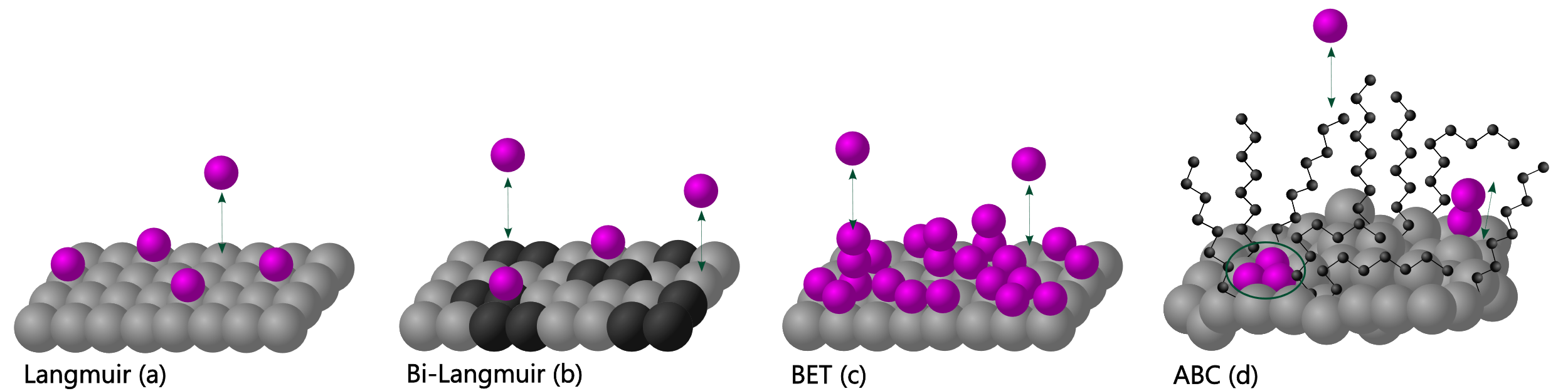
(16)

where is the monolayer saturation capacity of the sorbent, describes the equilibrium over the bare surface, and is the equilibrium constant for the adsorption-desorption process over adsorbed molecules (corresponding to multilayer formation). Note that the parameters and retain the same physical meaning as the standard BET isotherm equation [12]; despite a closer resemblance between eq 16 and the GAB model, we will follow the convention by referring to it as the extended liquid-solid BET (LS-BET) model. In order to facilitate comparison with the ABC isotherm, we rewrite eq 16 as

(17a)

the comparison of eq 17a with the ABC isotherm (eq 3) yields

(17b)



**Figure 2** Simplified schematic representation of presented isotherm theories: (a) Langmuir, (b) Bi-Langmuir, (c) BET, and (d) ABC, showcasing the assumed planarity and site-specificity of adsorption inherent to the conventional models. Anti-Langmuir has been omitted due to its lack of physical interpretation.

### 3.1.4 ABC parameters as a universal descriptor for chromatographic sorption isotherms

The ability of the ABC isotherm to encapsulate conventional isotherm models allows universal comparison of isotherm parameters, as summarised in **Table 1** and demonstrated in Sections 3.1.1-3.1.3. Through the use of KBIs as the foundational theory describing interactions, complications arising from the site-specificity of these conventional models may be avoided. As addressed in Section 2.4, when the surface excess may be equated to the actual amount adsorbed (eq 18a is satisfied), the parameters of the ABC isotherm may be simplified (the full theoretical justification of which may be found in Supporting Information F).

(18a)

To demonstrate the simplification of the and parameters, we start with a definition of ,

(18b)

we may use eq 18a and the definition of the sorbent-surface KBI, , to define as

(19)

such that the parameter is predominantly the measure of sorbent-sorbate interaction. When (Supporting Information F), can be simplified as

(20)

signifying its origin in the interface/bulk partitioning. can be simplified as

(21)

which signifies the amount of sorption per sorbate deficit number ().

Thus, we have established that the parameters and have a clear statistical thermodynamic interpretation that is applicable to interfaces with any geometry or porosity.

**Table 1.** ABC isotherm parameters expressed in terms of the conventional Langmuir, anti-Langmuir, and LS-BET model parameters.

|  |  |  |  |
| --- | --- | --- | --- |
| Model |  |  |  |
| Langmuir |  |  | 0 |
| Anti-Langmuir |  |  | 0 |
| LS-BET |  |  |  |

The successful modelling by a single ABC isotherm shows that mono-, di-, and tri-sorbate interactions can sufficiently capture overall sorption without requiring surface patch decomposition. The demonstrated capacity of the bi-ABC or tri-ABC isotherms (as the generalization of the bi- and tri-Langmuir models) to reproduce experimental isotherms shows that only a few surface patch types are needed to capture the effect of surface heterogeneity on sorption isotherms. Previously, adsorption energy distribution functions (AEDF) have been evaluated to characterize surface heterogeneity [39, 77]. Here, we point out that AEDF depends on implicit assumptions (choice of a local isotherm model or an imposed range of binding constants, in addition to a definition of adsorption energy via one of its parameters), which can suffer from oversimplification of the sorption mechanism similar to what we have already outlined for classical models themselves. Thus, we propose our approach not only as a more general approach valid for both site-specific and mobile sorbates but also as a simpler approach to capture surface heterogeneity.

## 3.2. Binding and partitioning within statistical thermodynamic fluctuation theory

In interpreting retention in RPLC, two primary mechanisms are routinely offered: adsorption (displacement) and partitioning (or a combination thereof). Numerous publications spanning the last several decades have aimed to address the relevance of these processes to sorption in reversed-phase systems [1, 3, 5, 79-83], forming the basis of a fierce debate surrounding the correct mechanism of retention. Various attempts to model retention have been hampered by simplifying assumptions concerning the role and structure of both the mobile and stationary phases [3, 4]. The modern consensus, supported by nonlinear chromatographic data [17] and molecular simulations [5-8, 63, 65, 84], appears to hold that both partitioning and adsorptive processes can dually occur (with a clear dependence on the nature of the analyte, eluent, and sorbent [4, 6, 7, 82, 83, 85]) leading to a mixed partitioning-adsorption model. We note that partitioning here should not be directly equated with bulk liquid-liquid partitioning [4, 6, 81, 84]. Hence, any theory purported to appropriately detail retention at the RPLC interface must incorporate a suitable description of both partitioning and adsorption. Here, we show that the amount of sorption, characterized by the statistical thermodynamic fluctuation theory, contains adsorption and partitioning as special cases. This can be shown by demonstrating how when the surface excess, , can be approximated as (see Supporting Information: Sections E and F), the use of the interface/bulk partition coefficient of the sorbate, defined as

(22)

is sufficient to simplify as

(23)

Now we can show that eq 23 encompasses binding and partitioning: is expressed in terms of the partitioning coefficient, ; hence it encompasses partitioning and binding is the special case when or ; in this case, and is interpreted as the amount bound.

In contrast to the above clarification, traditional isotherm models (such as Langmuir, BET, or GAB) consider adsorption only as site-specific binding on the adsorption sites distributed uniformly on an interface. Consequently, it was impossible to encompass binding and partitioning. Note that the evidence for the “dynamic” nature of the adsorbates at the interface is beyond the reach of the binding models, as has been well-recognized [86]. Indeed, the aforementioned strong evidence for the dual character of RPLC, incorporating both partitioning and adsorption, restricts the appropriateness of conventional isotherm models. This is yet another factor in support of our proposal to adopt the ABC isotherm, founded on the fluctuation theory, for RPLC because of its capacity to handle both “localized” and “mobile” [53, 86, 87] modes of sorption.

## 3.3 Sorbate-sorbate interactions as the key to isotherm classifications from Types I-III

In Section 3.1, we have demonstrated how the Langmuir, BET, and anti-Langmuir isotherms are (i) the special cases of the ABC isotherm and (ii) the overly idealized assumptions underlying the Langmuir, BET and anti-Langmuir parameters can be overcome, simply by using them as the empirical fitting equations from which the mono-, di-, tri-sorbate interactions can be evaluated via the ABC parameters. Based on this achievement, here we show that sorbate-sorbate interaction at the interface is the key factor in distinguishing the IUPAC Types I, II, and III of the vapor sorption isotherms.

First, we focus on the difference between Type I and Type III. We have shown that

* the Langmuir isotherm, a common model for Type I, corresponds to , signifying sorbate-sorbate repulsion unchanged over sorbate concentration;
* the anti-Langmuir isotherm, a common model for Type III, corresponds to , signifying sorbate-sorbate attraction unchanged over sorbate concentration.

Consequently, Types I and III are distinguished by sorbate-sorbate repulsion or attraction. We emphasize that a common language spanning these types (repulsive or attractive sorbate-sorbate interactions) has been beyond the reach of the traditional models based on site-specific adsorption (e.g., Langmuir).

Second, we clarify the sorbate-sorbate interaction underlying Type II. We have shown that

* the BET (and GAB) models correspond to with and , signifying sorbate-sorbate repulsion at low concentration, which turns attractive at high concentration.

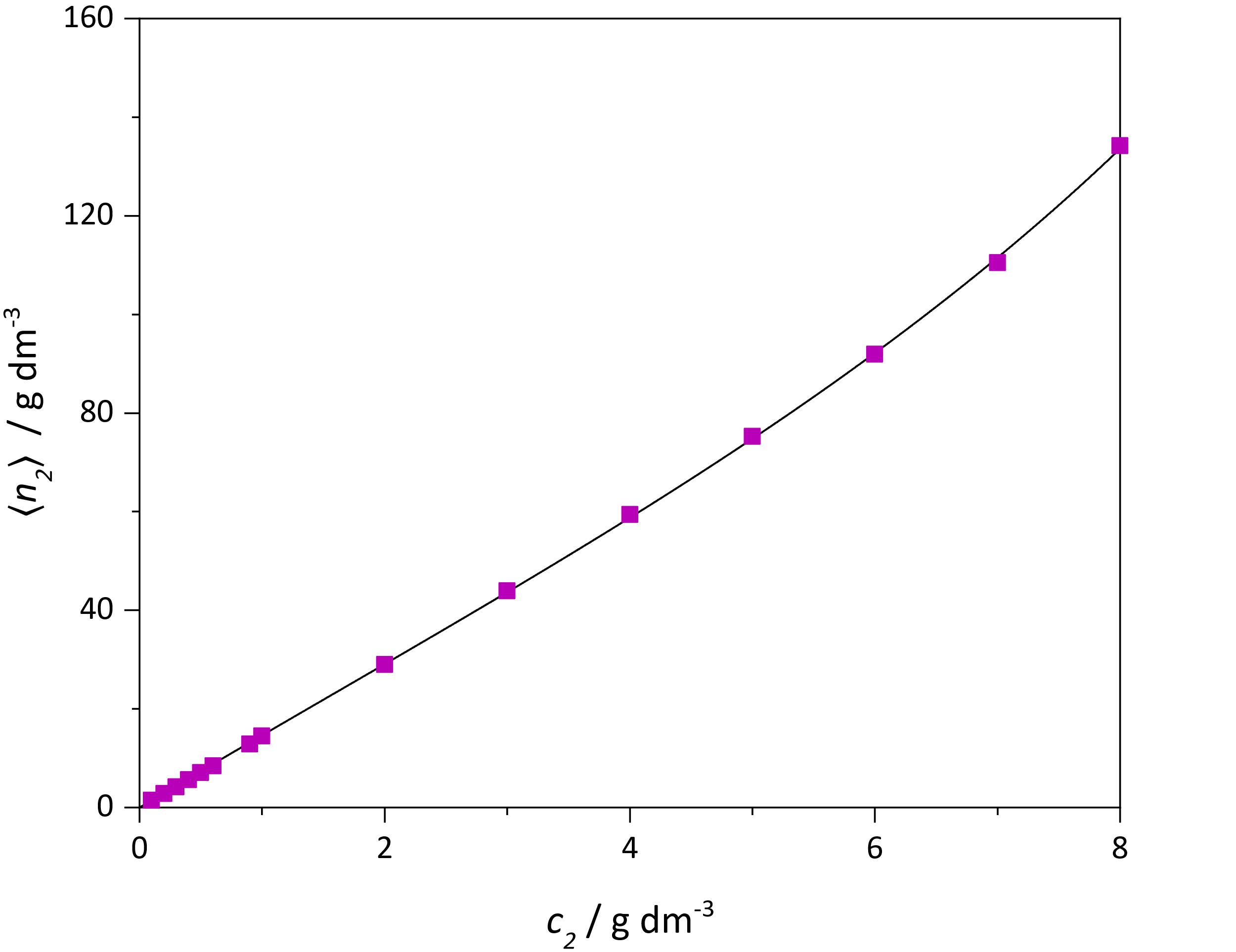
Consequently, the difference between Type II and III has been made clear: attractive sorbate-sorbate interactions span the entire concentration range for Type III, whereas initial sorption (where sorbate-sorbate interaction is repulsive, just like Type I), acting as the new interface, brings in more sorbate molecules via attractive sorbate-sorbate interactions for Type II.

We have demonstrated that a change from sorbate-sorbate repulsion to attraction at the interface can cover isotherm Types I-III. Noteworthily, the BET model is capable of mathematically reproducing Type I-III isotherms [88], although its application in describing Type III behavior is limited [89]; such a fitting involves a weak binding between the surface and the first layer and strong binding between the outer layers (e.g., ) implying a formation of further layers rather than a sufficient growth in coverage of the first layer, which is at odds with the layer-by-layer adsorption mechanism that is fundamental to the BET model. In contrast to such a difficulty, the ABC isotherm considers Type III behavior simply as favourable sorbate-sorbate interaction at the interface, without any restriction posed by the assumption of layer-by-layer adsorption.

To illustrate the application and merit of the ABC isotherm in the direct fitting of chromatographic adsorption isotherm data, we have taken the data tabulated by Gubernak et al. [90, 91] for amylbenzene adsorption on a LiChrospher 100 RP-18e column from methanol-water (80:20, v/v) and performed fitting through sequential quadratic programming using IBM SPSS Statistics (version 29). Standard errors and confidence intervals (CI) were obtained via bootstrapping, whereby repeated random sampling of the original dataset generates multiple resampled datasets [90], each fitted to the ABC isotherm. The standard error is reported as the standard deviation of the bootstrapped estimates, while the 95% confidence interval is defined by the 2.5th and 97.5th percentiles of the bootstrap distribution. The resulting best-fitting parameters are presented in **Table 2** alongside standard errors, confidence intervals, and the residual sum of squares (RSS). At first inspection, the functional shape of the isotherm (**Figure 3**) does not show a stereotypical isotherm type. The ABC parameters show that initial sorbate-sorbate repulsion () soon turns to sorbate-sorbate attractive via a strong trisorbate contribution (reflected by the value of ).

**Table 2.** Best-fit ABC isotherm parameters for amylbenzene sorption (80:20, v/v, methanol-water) on a LiChrospher 100 (RP-18e) column [90, 91]. Note that the standard error associated with each isotherm parameter is due to the limited size of the fitted dataset ( 15).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Isotherm | Model Parameters | | Standard Error / % | 95% CI | RSS |
| ABC | 0.00219  -0.194  380 | dm3 mol-1  dm3 mol-1  dm3 mol-1 | 0.966  14.3  5.27 | [0.00215, 0.00223]  [-0.249, -0.138]  [340, 420] | 2.88 |

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**Figure 3**. Experimental isotherm of amylbenzene on a LiChrospher 100 RP18e column with a methanol-water (80:20, v/v) mobile phase (data from [91, 92]). Fitting to the ABC isotherm is shown by the solid black line.

Thus, we have demonstrated how sorbate-sorbate interactions, as well as their change over sorbate concentration, are a unifying principle in distinguishing different isotherm types. We now present **Table 3** as an illustrative example of the encompassing nature of the ABC theory; its parameters allow consistency in interpretation and classification across isotherm Types I-III. Here, attractive sorbate-sorbate interactions are significant in the retention of alkylbenzenes; for the larger butyl- and amylbenzene, there is no saturation barrier (Type III), whereas for toluene and ethylbenzene, an initial amount of sorbate must first accumulate before the sorbate-sorbate interactions become favorable (Type II). The magnitude of for this homologous series increases with increasing molecular size (and, in turn, decreasing polarity), reflecting the enhanced preferential sorbate-surface interaction.

**Table 3.** Example literature isotherm parameters for sorbate sorption from methanol-water mixtures on C18 columns from fitting to Langmuir, anti-Langmuir, and LS-BET models [12, 73, 77] and their converted , , and parameters.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Model | Sorbate | MP (v/v) | Model Parameters | |  |  |  |
| / dm3 mol-1 | / dm3 mol-1 | / dm3 mol-1 |
| Langmuir | 3-Phenyl-1-propanol | 50:50 | = 1.33  = 5.41 | mol dm-3  dm3 mol-1 | 3.48 x 10-3 | -7.52 x 10-1 | 0 |
|  | 4-*tert*-Butylphenol | 60:40 | = 0.939  = 9.30 | mol dm-3  dm3 mol-1 | 3.10 x 10-3 | -1.07 x 100 | 0 |
|  |  |  |  |  |  |  |  |
| Anti-Langmuir | Butylbenzene | 80:20 | = 9.5  = 2.95 | dm3 mol-1 | 3.42 x 10-3 | 3.11 x 10-1 | 0 |
|  | Amylbenzene | 80:20 | = 15.1  = 4.15 | dm3 mol-1 | 2.15 x 10-3 | 2.75 x 10-1 | 0 |
|  |  |  |  |  |  |  |  |
| LS-BET | Toluene | 80:20 | = 4.02  = 0.739  = 0.316 | mol dm-3  dm3 mol-1  dm3 mol-1 | 1.10 x 10-2 | -3.60 x 10-2 | 2.77 x 100 |
|  | Ethylbenzene | 80:20 | = 1.57  = 2.72  = 1.16 | mol dm-3  dm3 mol-1  dm3 mol-1 | 7.59 x 10-3 | -9.56 x 10-2 | 2.60 x 101 |

# 4 Conclusion

Adsorption isotherm models have found application in the analysis of chromatographic retention despite a well-acknowledged contradiction between the basic assumptions of the isotherm models (site-specific, layer-by-layer adsorption on a uniform surface) and the reality of chromatographic interfaces (duality of adsorption-partitioning on heterogeneous surfaces with sorbate mobility). Our goal was to investigate interactions responsible for retention at the RPLC interface, which requires clarification of the underlying interactions without the overly idealized or unrealistic assumptions of the traditional isotherm models. This was achieved by the statistical thermodynamic fluctuation theory and the ABC isotherm derived from it, which not only captures the mono-, di-, and tri-sorbate interactions as its parameters but also contains the Langmuir, BET, GAB, and anti-Langmuir isotherm models as its special cases. The key was the ability of the ABC isotherm to capture both the attractive and repulsive interactions between sorbates, which enabled the mechanistic elucidation of Type III. Moreover, the dual character of adsorption and partitioning was also shown as the special limiting cases of our theory.

Previously, the Langmuir, BET, GAB, and anti-Langmuir models have been used successfully to fit experimental isotherms derived from chromatographic retention, even though their overly idealized assumptions do not represent the complex reality of the chromatographic interface. This contradiction was overcome by adopting these traditional models as the empirical fitting equations, from which the mono-, di-, and tri-sorbate interaction parameters (ABC) can be evaluated.

We have clarified that sorbate-sorbate interaction at the interface is the key which gives rise to differences in isotherm types. Type I and Type III represent the opposite end of sorbate-sorbate repulsion and attraction that are independent of sorbate concentration. Type II lies in-between, in which sorbate-sorbate interaction, which is repulsive at low concentrations, turns attractive once a sufficient quantity of sorbate is present at the interface.

Thus, the present work paved the way towards a universal interpretation of chromatographic isotherms without the need to oversimplify complex chromatographic interfaces while providing an easily implementable method for rendering fittings to such models to yield the statistical thermodynamic interpretation.

# Acknowledgements

The authors would like to thank Julie Lynch and Nicholas Garland for constructive discussions. We are grateful to Merck KGaA for the funding contributions supporting this study.

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