



Practical Consequences of Elevated Pressure in Liquid Chromatography

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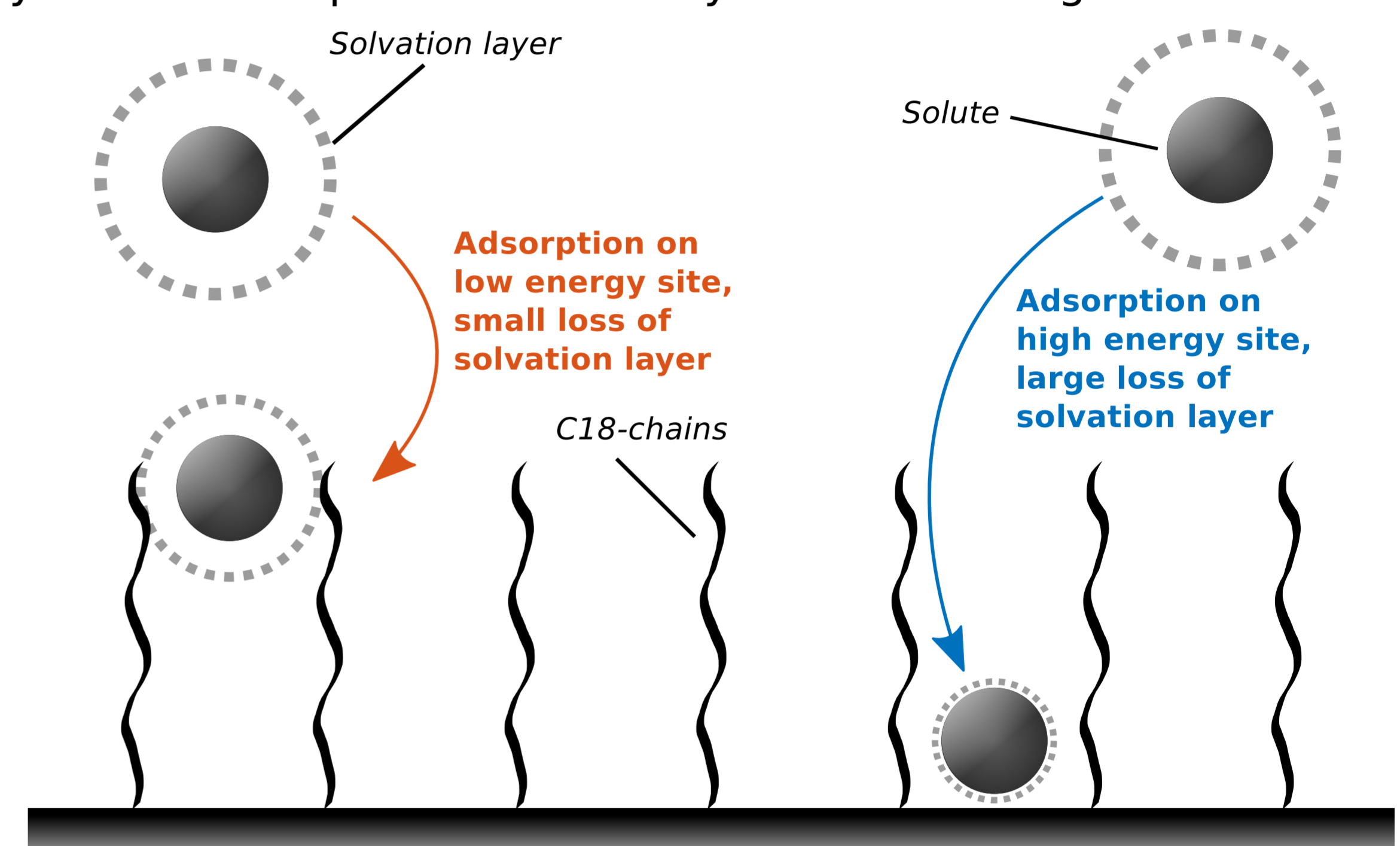
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Introduction

Many compounds show increased retention when the pressure is increased in Reversed-Phase Liquid Chromatography (RPLC) (Åsberg, *J. Chromatogr. A* 1401(2015)52). A new model that explains this in more detail is proposed and validated experimentally. Since compounds can have very different pressure dependence and hence the selectivity can change with pressure, it is important to understand the mechanism behind the pressure effect. When working with UHPLC the chromatographer should have some understanding of what effects the pressure can have on the performance and the model presented here aim at providing this understanding.

In RPLC, the solute is surrounded by a solvation layer which is partly lost when adsorbing on the stationary phase. The solute molar volume is therefore lower when the solute is adsorbed. When the pressure is increased, the system will counteract this change, according to Le Chatelier's principle, by decreasing its volume and thereby driving the solute phase equilibrium towards the stationary phase. This increases the retention with pressure.

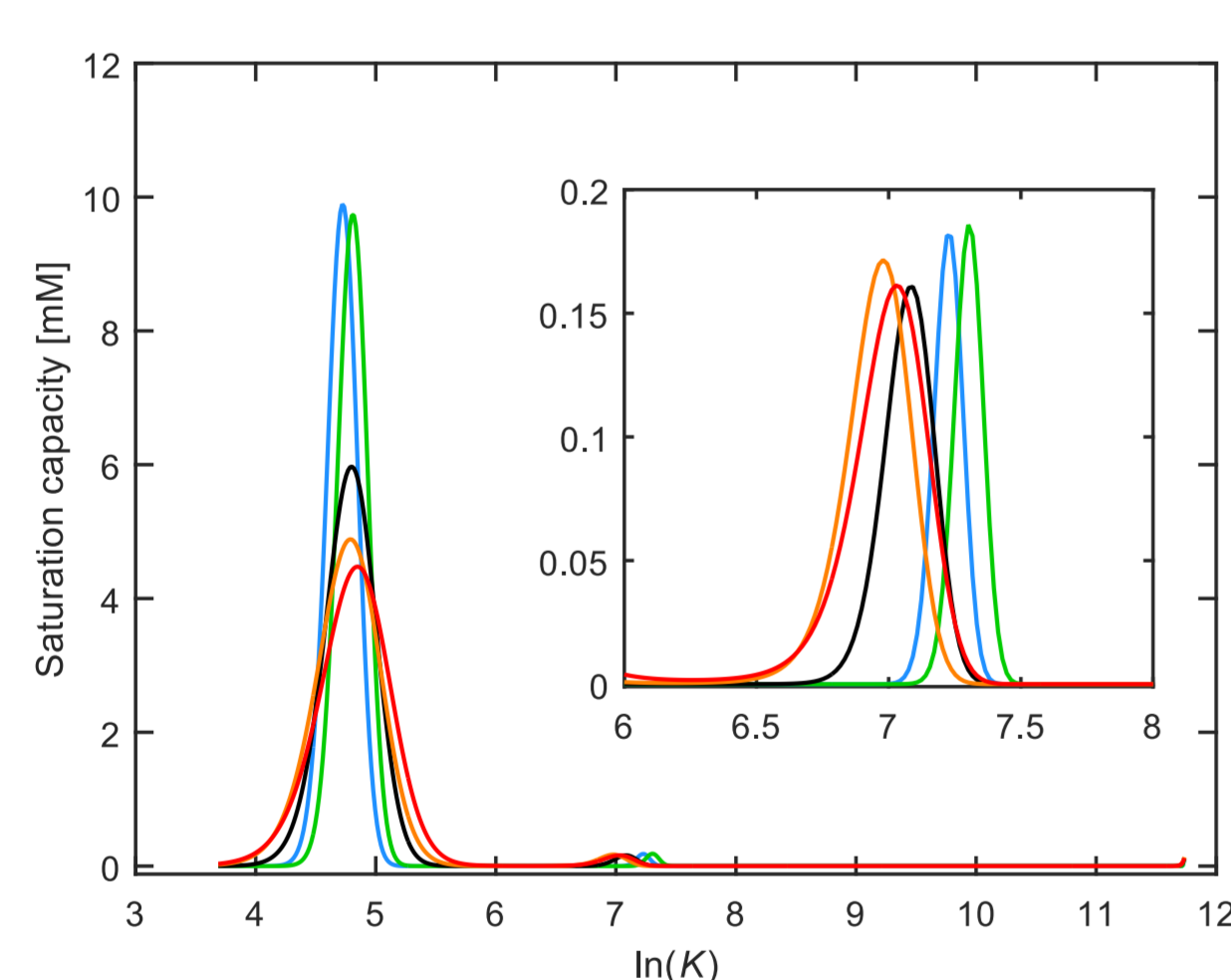
In RPLC, solutes usually adsorb on two type of sites which have different adsorption energy (equilibrium constant). Depending on the type of site, the solute loses different amounts of its solvation layer. A site deeper in the C18-layer causes a larger loss.



The model was tested experimentally using three solutes; one neutral, one organic cation and one organic anion

Using a restrictor after the column, the pressure could be adjusted while keeping the constant flow rate.

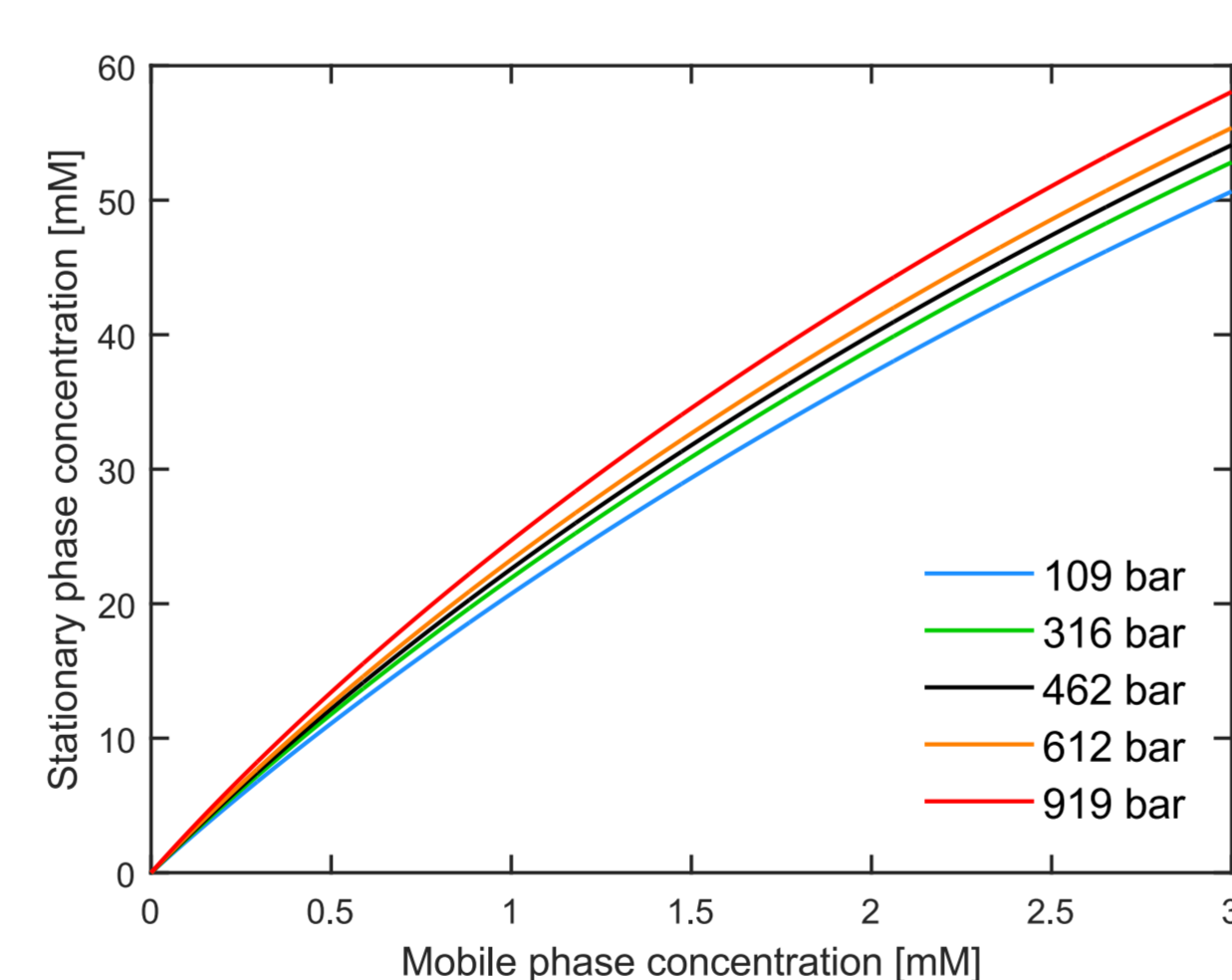
1. We measured the Adsorption Energy Distribution (AED) for each solute at five different pressures and concluded that: (i) the AED showed two adsorption sites and (ii) the number of adsorption sites did not change with pressure. Hence a two site adsorption isotherm would be correct at all pressures. Below the AED of the neutral compound is shown.



2. By measuring the adsorption isotherm data for each solute and fit different models to the data, we found that the bi-Langmuir model,

$$q(C) = q_{s,1} \frac{K_1 C}{1 + K_1 C} + q_{s,2} \frac{K_2 C}{1 + K_2 C}$$

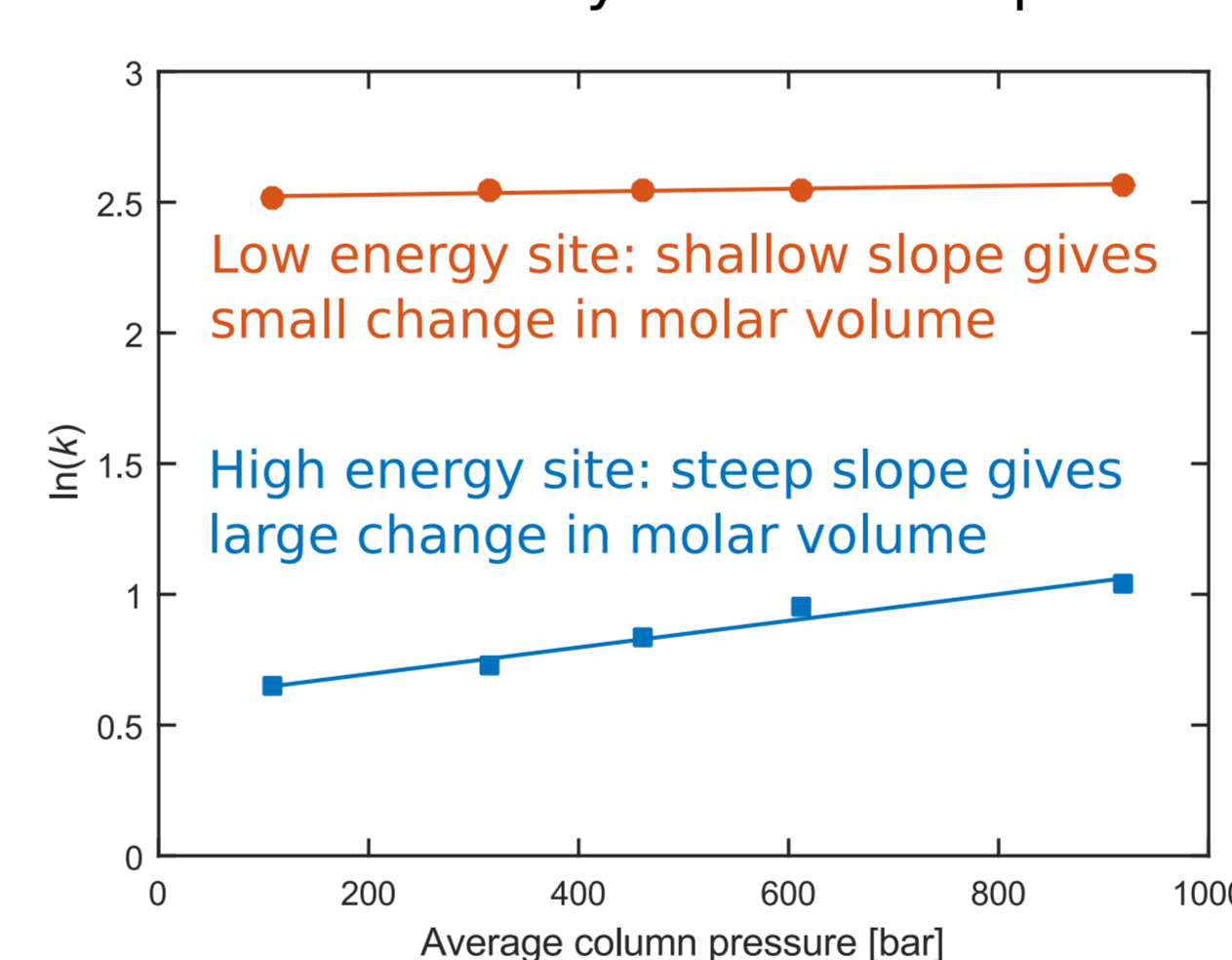
best described the data. The parameters K and q_s changed with pressure.



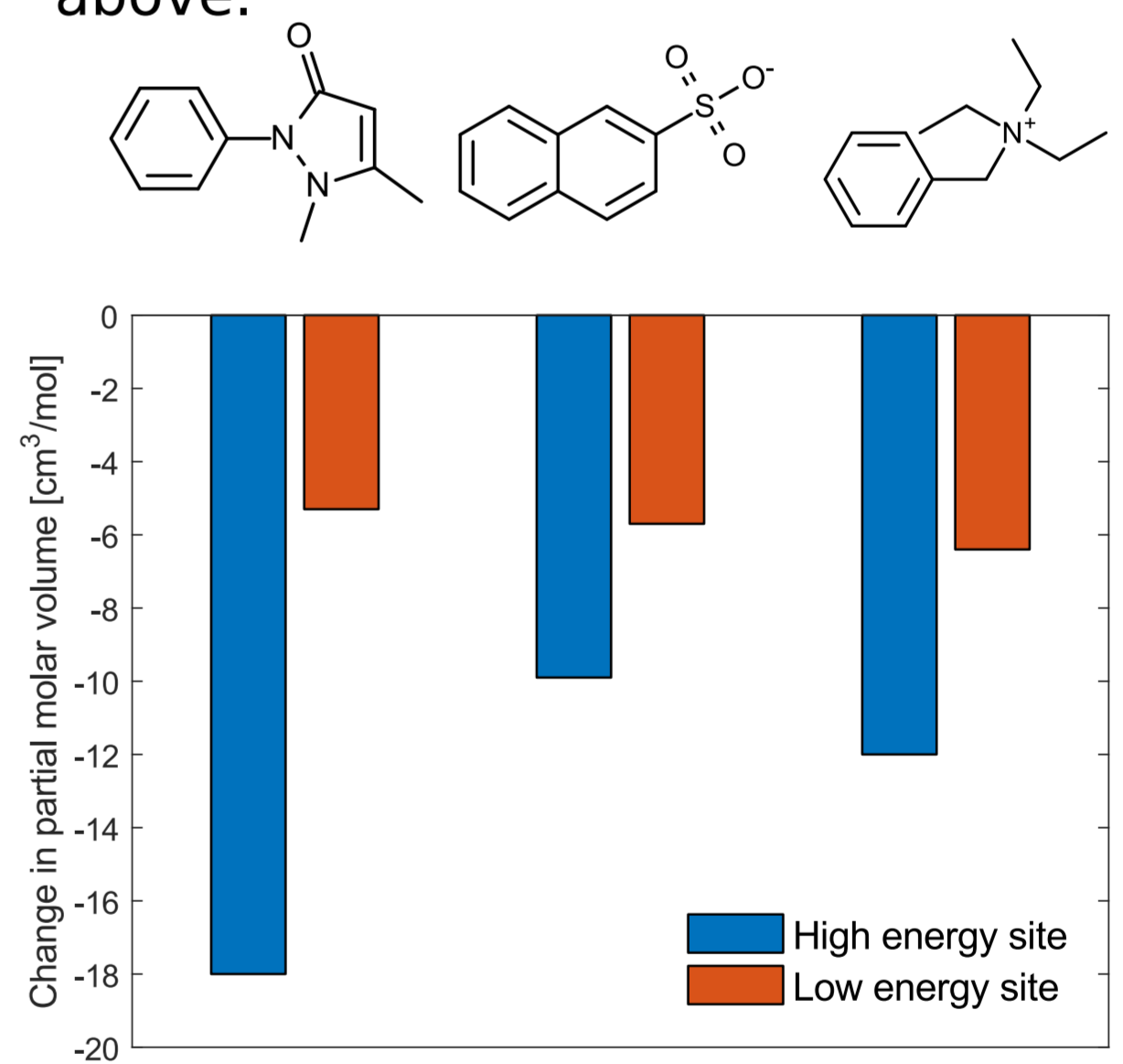
3. From the adsorption isotherm, we could calculate the retention factor for each of the two adsorption sites. The retention factor (k) is related to the pressure (P) through:

$$\ln(k) = -\frac{\Delta V}{RT} P + \ln(k_0) + \ln\left(\frac{F}{F_0}\right)$$

where ΔV is the change in molar volume between stationary and mobile phase.



4. Comparing the experimental change in molar volume upon adsorption on the two sites we see that the high energy site, where the solute penetrates deeper into the C18-layer, gives a larger reduction in molar volume. This agrees with the theoretical model above.

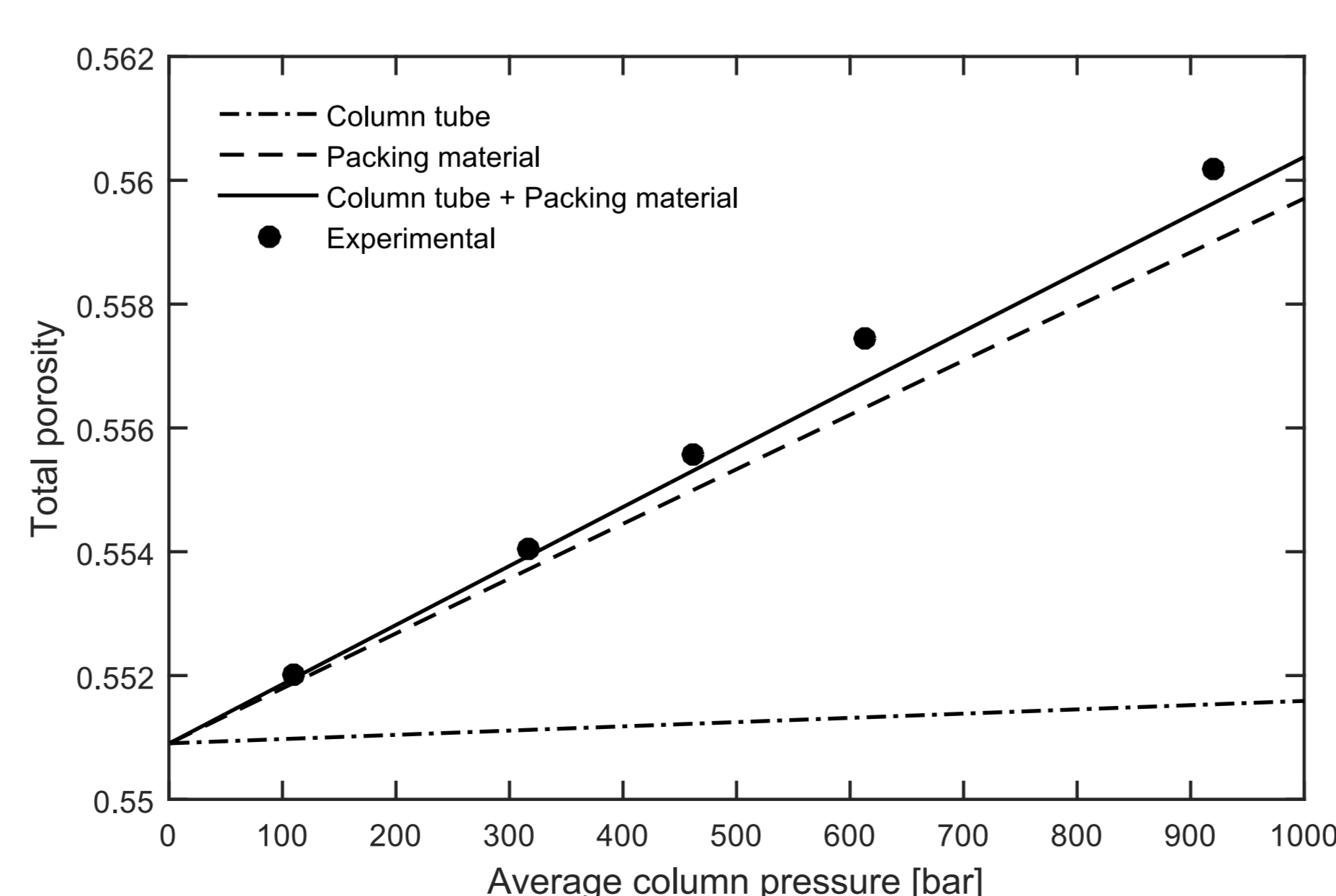


The pressure affects column void volume and stationary phase porosity

The total porosity increases 1.6% with a 1000 bar pressure increases

Measuring the column void volume with the unretained compound NaNO_3 , shows that it increases with pressure. This is due to a slight expansion of the column steel tube and a compression of the stationary phase at high pressures. Theoretical calculations of these two effects is in very good agreement with the experimental measurements.

When conducting accurate measurements of the retention factor at different pressures, this effect should be accounted for. However, in routine work the variations in void volume and total porosity due to pressure can be approximated as constant.



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