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Short communication

Weight adjustment in calculating surface ligand coverage or density for chromatographic bonded phases

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Abstract

A method based on successive approximation is proposed for the calculation of surface coverage or ligand density of chemically bonded stationary phases from elemental analysis data. The approach could be used to calculate surface coverage in many stationary phase preparations, including end-capping and stepwise couplings. Key in this procedure is the use of successive approximation to simplify the adjustment of weight changes in stationary phase preparation. The method does not involve complicated mathematic equations.

Keywords: Weight adjustment; Surface coverage; Ligand density; Stationary phase; End-capping

1. Introduction

Surface coverage of the chemically bonded stationary phases, α, defined as mmoles of anchored group per m^2 of native support, is an important parameter in stationary phase characterization. [1] This parameter is often calculated from elemental analysis data. [2] When preparation of the stationary phase involves only a single step modification of the native support such as bare silica gel, the calculation is straightforward. If the preparation involves more than one step such as in end-capping and stepwise amide coupling reaction on silica gel, [3] such calculation is more demanding. Although equations have been developed in the past to calculate surface coverage in these cases, they can be difficult to follow as they involve complicated mathematical equations. In addition, different equations are required for different stationary preparation procedures. Complication in these calculations is due to the need to adjust for the significant weight change of the stationary phase upon modification.

The author proposes an alternative method to account for the weight change. In the case of the modification with a single step reaction, simple weight adjustment was sufficient for the accurate calculation of surface coverage. When more steps are involved, successive approximation [4] provides the same surface coverage as obtained with the accurate equations. In my experience, the successive approximation method is easy to learn. It does not involve complicated equations and can be applied to different preparation procedures.

2. Results and discussion

The procedure is demonstrated with two examples. The first example involves end-capping. [5] A C18 stationary phase was prepared by first reacting silica gel (surface area 300 m^2/g) with $C_{18}H_{37}Si(CH_3)_2X$ and followed by end-capping with $(CH_3)_3SiX$. Carbon percentage of C18Silica (before end-capping) by elemental analysis is 8.4%, while that of C18E Silica (after end-capping) is 10.04%.

To calculate the C18 coverage in C18Silica, a simple weight adjustment is sufficient. Based on the carbon percentage 8.4%, in 100 g of C18Silica gel, there is 8.4 g/20 (there are 20 carbons)/12 (molar mass of C) = 0.035 mole of $C_{18}H_{37}Si(CH_3)_2$ (MW = 311) group. According to the immobilization equation, for every mole of $C_{18}H_{37}Si(CH_3)_2$ immobilized, 1 mole of proton is lost. Therefore, 100 g of C18Silica contains $100 - 0.035 \times 311 + 0.035 \times 1 = 89.15$ g of silica gel. Therefore, the surface coverage is $0.035/(89.15 \times 300) = 1.31 \times 10^{-6}$ or 1.31 μmol/m^2.

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Calculation of the surface coverage of the end-capping $(CH_3)_3Si$ group in C18ESilica (after end-capping) is more complicated. Here, it requires a successive approximation method and is shown in the following steps.

Step 1: First assume that weight gain of the stationary phase in the end-capping step can be ignored, it takes 100 g of C18Silica to make 100 g of C18E silica. Accepting this assumption, for 100 g of C18E silica, there is $(10.04 - 8.40)$ (C% difference)/3 [3C in $(CH_3)_3Si$]/12 = 0.0456 mole of $(CH_3)_3Si$ (MW = 73). For every $(CH_3)_3Si$ group, one proton will be lost. Therefore, making 100 g of C18E silica requires $100 - 0.0456 \times 73 + 0.0456 \times 1 = 96.72$ g of C8Silica, not 100 g of C18Silica.

Step 2: With this information, recalculate the mole of $(CH_3)_3Si$ group in 100 g of C18E silica. In 100 g of C18E silica, there is $(10.04 - 8.4 \times 96.72/100)/3/12 = 0.0532$ mole of $(CH_3)_3Si$, since it takes only 96.72 g of C18Silica to make 100 g of C18E silica according to step 1. According to this new mole of $(CH_3)_3Si$ group, to make 100 g of C18E silica requires $100 - 0.0532 \times 73 + 0.0532 \times 1 = 96.17$ g of C18Silica.

Step 3: Repeat Step 2 until the mole of $CH_3)_3Si$ converges. It takes three more iterations in this case. Mole of $(CH_3)_3Si$, 0.0545 mole, weight of C18Silica, 96.08 g; mole of $(CH_3)_3Si$, 0.0547 mole; weight of C18Silica, 96.06 g; mole of $(CH_3)_3Si$, 0.0547 mol; weight of C18Silica, 96.06 g.

Therefore, making 100 g of C18E silica requires 96.06 g of C18Silica, which needs to be made from $96.06 \times 89.15/100 = 85.64$ g of silica (it takes 89.15 g silica to make 100 g C18Silica). Surface coverage of $(CH_3)_3Si$ group equals $0.0547/(85.64 \times 300) = 2.13 \times 10^{-6}$ or 2.13 μmol/m^2, which is the same as the 2.13 μmol/m^2 reported in the literature [1].

The second example involves stepwise coupling in stationary phase preparation. In this example, a Dnb-Leu stationary phase (Dnb-Leu-AP-Silica) was prepared by coupling Dnb-Leu-OH to 3-aminopropylsilica gel (AP-Silica), which was obtained by reacting 3-aminopropyltriethoxysilane with silica gel. [6] The net reaction is Dnb-Leu-OH + AP-Silica = Dnb-Leu-AP-Silica + H_2O. Results of elemental analysis of aminopropyl silica (AP-Silica) are C: 1.96%; H: 0.64%; N: 0.67%, while results of elemental analysis of the Dnb-Leu stationary phase (Dnb-Leu-AP-Silica) are C: 5.30%; H: 0.81%; N: 1.54%.

To calculate the surface Dnb-Leu density of the Dnb-Leu stationary phase from the nitrogen analysis data, the following steps are taken.

Step 1: First assume that weight gain can be ignored, the surface Dnb-Leu coverage can be calculated easily according to: (1.54–0.67 g) (nitrogen percent gain)/3 (number of nitrogens in Dnb-Leu)/14(molar mass of N)/100 g of stationary phase = 0.000207 or 0.207 mmol/g.

Step 2: Using this surface ligand concentration to adjust for weight change of the stationary phase. To prepare 100 g of Dnb-Leu-AP-Silica, one needs $100\text{ g} - 100 \times 0.000207 \times 325$ (molar mass of Dnb-Leu-OH) + $100 \times 0.000207 \times 18$ (molar mass of H_2O) = 93.64 g of AP-Silica, as the coupling reaction results in a net loss of one H_2O molecule.

Step 3: Recalculate the surface ligand concentration considering this weight change. Since it takes 93.64 g of AP-silica to make 100 g of Dnb-Leu-AP-Silica, the surface Dnb-Leu concentration is $(1.54 - 0.67 \times 93.64/100)$ (adjusted N% increase)/3/14/100 = 0.000217 or 0.217 mmol/g.

Step 4: Repeat Steps 2 and 3 until convergence of the result. In this case, the surface Dnb-Leu concentration converges at 0.218 mmol/g with two more iterations.

3. Conclusions

Successive approximation is simple and effective in calculating the surface coverage in complicated stationary phase preparations. Although slow-convergence or non-convergence is theoretically possible, the author has not encountered such cases in his past experience regarding stationary phase preparations. If non-convergence does occur, one would have to resort to the accurate methods described in the literature.

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