JOINT INSTITUTE FOR NUCLEAR RESEARCH

FLEROV LABORATORY OF NUCLEAR REACTIONS

FINAL REPORT ON THE

SUMMER STUDENT PROGRAM

Determination of the free volume of a chromatographic column using modeling in Matlab

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1. Introduction

Liquid chromatography is a useful analytical method for separating two or more compounds in a mixture based on the differences in the property of each individual substance. The chromatographic process for a typical liquid phase system is a simple process of adsorption and desorption of a solute. The separation of individual components is dependent on the interaction of the sample with the mobile and stationary phases and it occurs mainly due to differences in their affinity to the stationary phase. The main target of partition experiments is the determination of the distribution coefficient, Kd, by the ratio: concentration of the substance in the stationary phase (Cs) to concentration of the substance in the mobile phase (Cm).

$$Kd = Cs/Cm$$

For optimizing the HPLC technique the free volume needs to be determined as it is simply folded into the numerical solution of the diffusion equation.

In Flerov Laboratory of Nuclear Reactions, Joint Institute for Nuclear Research a research of transactinide elements chemistry was done by the process of liquid and gas chromatography. The purpose of this paper is to determine the free volume of the chromatographic column and make some predictions with modeling in Matlab in order to achieve a fast and optimal resolution as well as to make a comparison with the laboratory experiment.

2. Theoretical overview

2.1. Chromatographic key parameters

The primary purpose of liquid chromatography is the efficient separation performance of the compounds in the solution measured in “plates”. The plate concept as a separation metric is part of the theoretical plate model proposed by Archer Martin and Richard Synge, and provides the opportunity to remark many achievements in the field of chemistry and biology since the year of 1941 (Martin and Synge, 1941). The theoretical plate model is based on the idea that the chromatographed substance passes through the sorbent layer not in a continuous flow, but in batches, distributed between the mobile and stationary phases in separate sections of the layer, the so-called plates. Thus according to the theoretical plate model the chromatography column could be divided into hypothetical layers in which the stationary and the mobile phase balance the mass exchange between them. The analyte together with the eluent falls on the first plate. A new portion of the eluent fed to the first plate leads to a new distribution of the substance between the mobile and stationary phases, with some of the material from this plate being transferred to the next one. On the current plate, equilibrium is also instantaneously established, and part of the substance is transferred to the next plate. In such cases the separation efficiency depends on the presence of a series of such equilibrium stages and increases with the increase in their number. Therefore the presence of more theoretical plates leads to an increased efficiency of the separation process. The number of the theoretical plates is a measure of the efficiency of the column, associated with the dependence:

$$H = L/N,$$

where, H is the height equivalent to a theoretical plate, L is the length of the column and N is the number of theoretical plates. The height of the equivalent theoretical plate is the thickness of the sorbent layer necessary to establish the equilibrium distribution of substances between the mobile and stationary phases. Hence, the number of theoretical plates (N) and the height of the equivalent theoretical plate (H) are the values characterizing the efficiency of the chromatographic column.

The number of the theoretical plates (N) of a real chromatography column can be measured after elution with the equation:

$$N = 5.54\ (tR/W1/2)^2$$

or

$$N = 16\ (tR/Wb)^2$$

where, t_R is the retention time, Wb is the peak width at baseline, W1/2 is the peak width at half height. The width of the peak decreases with the efficiency of the column.

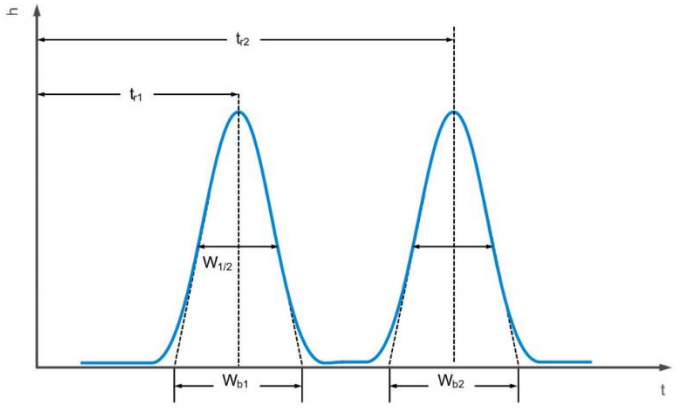

Figure 1. Visualization of peak width and retention time on a chromatogram.

Another crucial parameter to be determent is the retention factor, k, which indicates the time residence/retention volume of the solute in the stationary phase considered in relation of the time residence/retention volume of the solute in the mobile phase, calculated throughout:

$$k = (t_R - t0)/t0$$

in this case t_R is the retention time for sample peak and t0 is the retention time for unretained peak.

The separation factor (selectivity), α, is a measurement of the distance between the maxima of two peaks:

$$\alpha = k2/k1$$

where k1 is the retention factor of the first peak and k2 is the retention factor of the second peak. High separation factor values indicate more efficient separation.

The resolution, R_s, is a measure of a differentiation of two elution peaks which is significant to ensure the proper separation of the sample components. It is calculated as the difference in retention times of the two peaks, divided by the combined widths of the elution peaks:

$$R_s = 2(t_{R2} - t_{R1})/W_{b1} + W_{b2}$$

Efficiency, retention factor and separation factor affect the resolution as it shown by the fundamental resolution equation:

$$R_s = \sqrt{N}/4.[\alpha-1/\alpha].[k/k+1]$$

Each of these parameters have a meaningful contribution which is explained in details in the paper of Stuart et al, 2003.

2.2. The impact of longitudinal diffusion on chromatographic efficiency

The existence of few more factors, besides the retention time and volume, could affect the efficiency of the column, such as flow (linear velocity) of the mobile phase and particle size. The smaller the particles, the higher the number of theoretical plates. The flow velocity is also directly related to the number of theoretical plates, hence an optimum flow velocity of a certain column provides more theoretical plates. Without an optimum flow the particles would gradually diffuse to the ends of the column until an implementation of equal concentration throughout the whole column. Even with an optimum flow some particles transfer through the column with a higher velocity than other particles, and some of them diffuses slightly backward. The rate of diffusion of the sample's particles is dependent on the nature of the compound and the type of the mobile and stationary phase. The longitudinal diffusion is proportional to the diffusion coefficient D of the mobile

phase. Longitudinal diffusion contribution is inversely proportional to the velocity of the mobile phase.

$$\partial c/\partial t + ux\ \partial c/\partial x + uy\ \partial c/\partial y + uz\ \partial c/\partial z = -D\ [\partial 2c/\partial x2 + \partial 2c/\partial y2 + \partial 2c/\partial z2]$$

At the distribution coefficient Kd=0, in the absence of diffusion, the chromatogram has a characteristic shape that repeats the initial conditions and on the basis of which the experiment is made. Subsequently the free volume can be determined.

3. Experimental part

3.1. Preparation of reagents and chromatographic process

All reagents had a clear qualification and did not undergo additional purification. An α-HIBA solution is prepared from its definite solid mass mixed with distilled water with additional 25% NH_4OH in order to be achieved pH=4.5. For the purpose of the experiment we prepared a cation-exchange chromatographic column with a length of 10cm and a diameter of 0,2cm. Aminex A5 resin was transferred in NH_4^+-form. After that the column was conditioned with α-HIBA. A solution of 0.5M NH_4NO_3 (1.18ml) was used to achieve a pH=4.5.

Ten kBq ^{249}Cf was dissolved in 60 µl solution of 0,3M α-HIBA with consequent feeding into the top of the chromatographic column and pressure of 2atm was applied on the column. In such conditions the flow velocity was 35 µl/min. An indication of switching off the pressure is the vanishing of the water solution on the top of the column. A following step is to add 1 ml α-HIBA (0.3 mol/l) with pH=4.65 and to collect the eluted activity in eppendorfs [one drop (32.2µl) per eppendorf]. Out of all samples only 2 showed any activity. The activity concentration was measured with gamma-spetroscopy with high-purity Ge detector “Canberra” for 300 seconds as it is shown on the following table, as well as the whole evaluation data:

Sample number	Imp (388 keV)	Efficency	Standard divation	Measuring time, s
7	7350	0.0752	93	300
8	6970	0.0752	97	300

3.2. Matlab modeling

The parameters that were set for the free volume to be determent are shown in the following script:

```
%Height of the theoretical plate
h=0.4;
%Loading volume of the sample
V0=0.050;
%Ratio of the free volume of the chromatographic column to the volume of solid
%phase
beta=1.6;
%Cross-sectional area of column
s=0.0314;
%Volume of the theoretical column
Vl=(h*s)/(1+1/beta)
%Number of application steps
Nfeed=ceil(V0/Vl);
%Column length
L=10;
%Number of volumes in the theoretical plates
numVl=220;
%Number of theoretical plates
n=ceil(L/h);
%Matrix of initial conditions and iterations (number of theoretical plate
%volume)
%Number of theoretical plates
H=zeros(n+1,numVl+1);
%Distribution coefficient
Kd=1;
for k = 1:Nfeed
      H(1,k) = 15,000/Nfeed;
end
%Assigning the initial state from the previous array
%for r = 1: n
%     H1 (r, 1) = H (r, 600);
%end
%Solution of the differential equation (diffusion equation) without
%diffusion.
for j=1:numVl
      for i=1:n
            H(i+1,j+1)=H(i+1,j)-(H(i+1,j)-H(i,j))/(1+Kd)
            if H(i+1,j+ 1)<1e-3
                  H(i+1,j+1)=0;
            end
      end
end
%Display the last line
chrom=H(n+1,:)
%Output time out of the last theoretical plate
time=[1:numVl+1]
```

```
%Chromatogram output
plot(time,chrom)
%Output Control
S=sum(chrom)
%Output value at maximum point
M=max(chrom)
%Display time maximum
M=find (chrom1==max(chrom1))
%Display the standard deviation without diffusion, which depends on the volume
%deposition and number of theoretical plates.
B=std(chrom1)
%Time to maximum in minutes
t = M1*Vl/0.035
```

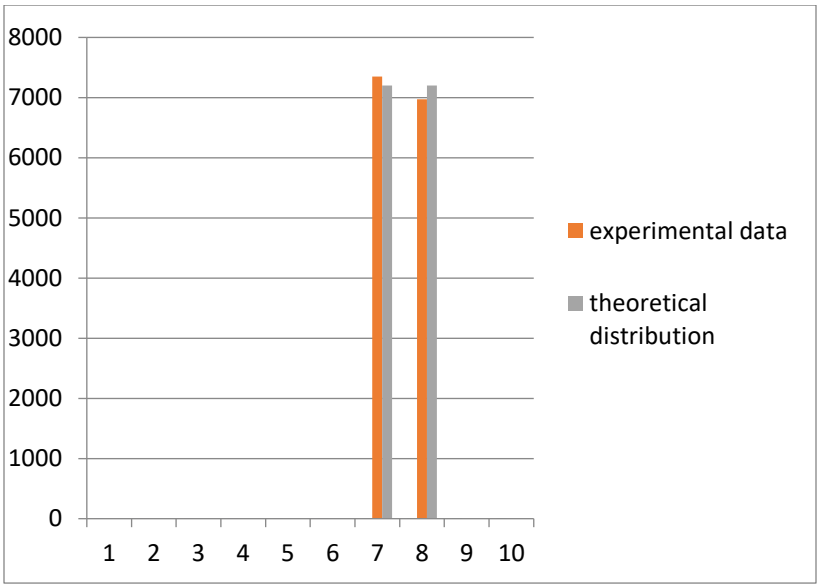

Figure 2: Visualization of the experimental data compared with the theoretical distribution

The final modeling results showed decent agreement with the experimental data, the simulation describes well the distribution. The free volume was determined to be 193.2µl (six drops without activity, each drop with a volume of 32.2µl). The detector measurement showed values of 7350 (sample 7) and 6970 (sample 8) which are close to the predicted modeling value of 7200, thus the chromatographic process was indeed optimized.

4. Conclusion

Difficulties with the separation of transactinide elements via liquid chromatography may occur in the downstream processing. The optimization suggested in this paper has been proven to be efficient by testing it in the laboratory to see if the modeling has made a correct prediction. The prediction was close to the achieved experimental results, although the numerical results showed the viability of our model as a perspective tool to aid the HPLC technique, further experiments are needed to prove its wide validity.

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