Theoretical Plate Concept in Chromatography: Part II

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Part A of this paper is essentially a continuation of a previous paper on the theoretical plate concept in chromatography (*4*). It deals with some special cases of eluting conditions and zone shapes. A more general equation, which combines both elution and deposition, is derived, and methods for approximating a continuous zone by means of a discontinuous one are discussed.

Part B discusses the effect of the finite size of samples and the dead free volume at the bottom of the column on the shape of elution curves. The effect of representative sample volumes is calculated, and the deviation between the experimental and the true elution curves is illustrated.

A simple expression for the number of theoretical plates utilizing the ratio between the maximum concentrations at the top and bottom of the column is derived and corrected for the effect of the finite size of samples.

PART A

In a previous paper (*4*) the theoretical plate concept in chromatography was treated on the basis of continuous flow of eluant through the plates of the column. Deposition and elution equations were derived for important cases from the practical point of view. One more case of particular theoretical interest is that in which during the elution process the eluant entering the column contains the solute at a certain constant concentration, and so the process is really a combination of elution and deposition. The derived equation can be considered as a general elution-deposition equation from which both the general elution and the deposition equations can be deduced as two special cases. This general case also has practical applications, especially when enrichment rather than complete separation is required and the eluant or one or more of the components to be separated are valuable, as in the case of the separation of uranium isotopes. In such cases it is sometimes more economical to recycle a portion of the effluent which might contain one or more of the solutes at a certain small and constant concentration. A hypothetical flow sheet is shown in Appendix 1 through 6.*

Three cases of an initial zone shape have been treated previously: case 1, where the zone occupies only one plate (*1, 2, 3, 4*); case 2, where the zone is deposited evenly on a finite number of plates (*2, 4*); and case 3, where an actual zone is deposited at the top of the column with the assumption that the total number of plates during deposition is the same as it is during elution (*4*). The total number of plates in the column depends on many variables, especially the nature of the eluant, temperature, and flow rate. The first two factors can be made the same during the two processes; yet it is not always convenient to make the two rates of flow during deposition and elution equal. Since the flow rate during deposition is usually less, or can conveniently be made less, than the rate of flow during elution, a column will contain a larger number of theoretical plates during the former than during the latter process. If the ratio between the number of plates during deposition and that during elution is λ, then the initial distribution in reference to the column during elution will be $R_n^0 = P_{\lambda n}^{\lambda u_0}$, and if one substitutes this value in the general elution equation, it will be very difficult if not impossible to obtain an analytical solution. Therefore the initial concentration on each plate will have to be calculated and substituted in the general elution equation

$$y_n = \sum_{r=1}^{n} y_r^0 \phi_{r-1}^u$$

Since when u_0 is a large number the calculations will be tedious, the initial distribution is approximated by an even distribution ($\lambda = \infty$) if λ is large enough ($\lambda > 10$), or by the Poisson distribution $R_n^0 = P_n^{u_0}$, (i.e. $\lambda = 1$) if λ does not differ much from 1, ($1.1 > \lambda > 0.9$). For other values of λ one can replace $P_{\lambda n}^{\lambda u_0}$ by a discontinuous distribution made up of a series of uniform zones averaged over the actual zone such that the total amount of solute in both the actual and discontinuous zones is the same. An analytical expression is possible for such a zone, and the larger the number of steps in the discontinuous zone the better the approximation but the more elaborate the calculations. For $\lambda > 1$ a less elaborate but fairly satisfactory approximation is to replace the initial distribution $P_{\lambda_n}^{\lambda u_0}$ by a zone made up of two parts: a uniform zone consisting of n_1 plates with solute concentration ratio equal to unity, followed immediately by a zone having the concentration distribution $P_n^{u_1}$ such that $n_1 + u_1 = u_0$. This distribution also yields an analytical expression, and the ratio u_1/n_1 is determined according to a formula derived on the basis of theoretical reasoning.

Derivation of the General Elution-Deposition Equation

The only difference between this case case and the general elution case treated in the previous paper (*4*) is that here the eluant contains the solute at a certain constant concentration y_0 before entering the column.

A material balance around plate 1 gives

$$k(y_0 - y_1)\,dx = dy_1$$

Integrating and substituting the boundary condition $y_1 = y_1^0$ when $x = 0$ one gets

$$\frac{y_0 - y_1}{y_0 - y_1^0} = e^{-kx} = e^{-u}$$

$$\therefore \quad y_1 = y_0(1 - kx) + y_1^0 e^{-kx}$$

By means of a differential material balance around plate 2 one can show that

$$y_2 = y_0[1 - e^{-u}(1 + u)] + y_1^0 e^{-u} u + y_2^0 e^{-u}$$

Continuing to plate n one finds that

$$y_n = y_0 P_n^u + \sum_{r=1}^{n} y_r^0 e^{-u} \frac{u^{n-r}}{(n-r)!} \quad (1)$$

which is the general elution-deposition equation.

It is evident that by substituting $y_0 = 0$ in this formula one obtains the general elution equation and that by substituting $y_r^0 = 0$ the equation reduces to the deposition equation.

Elution of an Initially Discontinuous Zone

Any discontinuous zone can be treated during its elution by applying the general elution equation to it, but only a few cases will lead to simple analytical expressions. Two such cases which are also of practical interest are treated in this paper.

1st Case: a Series of Uniform Zones

$$y^0 = y_{\mathrm{I}}^0 \quad \cdots \quad 1 \le n \le n_1$$

$$y^0 = y_{\mathrm{II}}^0 \quad \cdots \quad n_1 < n \le n_2$$

$$\vdots$$

$$y^0 = y_i^0 \quad \cdots \quad n_{i-1} < n \le n_i$$

By substitution in the general elution equation

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$$y_n = \sum_{r=1}^{n} y_r{}^0 e^{-u} \frac{u^{n-r}}{(n-r)!}$$

$$\therefore y_n = \sum_{r=1}^{n_1} y_{\mathrm{I}}{}^0 e^{-u} \frac{u^{n-r}}{(n-r)!} + \sum_{r=n_1+1}^{n_2} y_{\mathrm{II}}{}^0 e^{-u} \frac{u^{n-r}}{(n-r)!} + \cdots + \sum_{r=n_{j-1}+1}^{n_j} y_j{}^0 e^{-u} \frac{u^{n-r}}{(n-r)!}$$

$$= y_{\mathrm{I}}{}^0(P_{n-n_1}{}^u - P_n{}^u) + y_{\mathrm{II}}{}^n(P_{n-n_2}{}^u - P_{n-n_1}{}^u) + \cdots + y_j{}^0(P_{n-n_j}{}^u - P_{n-n_{j-1}}{}^u) \qquad (2)$$

so that if $y_{\mathrm{I}}{}^0 = y_{\mathrm{II}}{}^0 = \ldots = y_j{}^0 = y^0$
Equation (2) reduces to

$$\frac{y_n}{y^0} = P_{n-n_j}{}^u - P_n{}^u$$

2ND CASE: A UNIFORM DISTRIBUTION FOLLOWED BY A POISSON DISTRIBUTION

$$y^0 = y_{\mathrm{I}}{}^0 \qquad \cdots 1 \leq n \leq n_1$$

$$y^0 = y_{\mathrm{II}}{}^0 P_{n-n_1}{}^{u_0} \qquad n_1 < n$$

It can be proved by applying the general elution equation, expanding it, and rearranging it that

$$y_n = y_{\mathrm{I}}{}^0(P_{n-n_1}{}^u - P_n{}^u) + y_{\mathrm{II}}{}^0(P_{n-n_1}{}^{u+u_0} - P_{n-n_1}{}^u) \qquad (3)$$

if $y_{\mathrm{I}}{}^0 = y_{\mathrm{II}}{}^0 = y^0$

$$R_n = \frac{y_n}{y^0} = P_{n-n_1}{}^{u+u_0} - P_n{}^u \qquad (4)$$

This case can arise in practice if a zone is introduced at the top of a column by first equilibrating a small part of the adsorbent with the solvent containing the solute and then adding the mixture as a slurry at the top of the column. In this case $y_{\mathrm{I}}{}^0 = y_{\mathrm{II}}{}^0$, and therefore the distribution during elution is calculated from Equation (4); n_1 represents the amount of solute adsorbed on the adsorbent in the slurry, while u_0 represents the amount of solute in the solvent in the slurry.

Approximation of a Continuous by a Discontinuous Zone

An initial zone having a Poisson distribution in reference to the column during deposition (total number of plates = N_d) can be approximated by a discontinuous zone consisting of two parts, the top part having an even distribution and the lower part having a Poisson distribution in reference to the column during elution (total number of plates = N_e). This simplifies the analytical treatment of the elution process, and analytical expressions can be obtained.

Only the case where $N_d > N_e$ will be considered here. This is also the case which is more common in practice, since the rate of flow during elution is usually greater than that during deposition.

If the distribution of the zone in reference to the column during elution is

$$R_n{}^0 = P_{\lambda n}{}^{\lambda u_0} \qquad (5)$$

and the approximate discontinuous distribution is

$$R_n{}^0 = 1 \qquad \cdots 1 \leq n \leq n_1$$

$$= P_{n-n_1}{}^{u_1} \qquad n_1 < n$$

Then, since the total amount of solute in the two zones is the same

$$n_1 + u_1 = u_0 \qquad (6)$$

To evaluate both n_1 and u_1 another relation between the two values is required.

After many trials it was found that it is convenient as well as reasonable to choose the ratio n_1/u_1 such that the fraction of solute beyond the point where $R_n{}^0 = \frac{1}{2}$ is the same for both the continuous and the discontinuous distributions. Appendix 2 shows that this condition leads to the simple relation

$$\frac{n_1}{u_1} = \sqrt{\lambda} - 1 \qquad (7)$$

and therefore two equations exist in two unknowns, and both n_1 and u_1 can be determined.

PART B

This part of the paper deals with the difference between experimental and true elution curves. An experimental elution curve does not represent accurately the concentration of solute in the effluent as it emerges from the bottom of the column as a function of u, usually because the sample taken for analysis has a finite volume, and there is always a space at the bottom of the column of a finite volume b in which the effluent is mixed.

Evidently, to get a true elution curve each of the two volumes should be infinitesimally small; the larger they get the more the experimental elution curve will deviate from the true one. To be compared with the theoretical curve the experimental curve should therefore be corrected for the error due to these two factors. In this way the true experimental curve will be compared with the true theoretical curve. An alternate approach is to compare the experimental curve with the true theoretical curve after correcting the latter for the mentioned factors. This procedure is usually simpler, since the equation for the theoretical curve is known. In this paper the method of correcting the theoretical curve has been applied to one of the simple, theoretical elution curves, and relatively simple analytical expressions were deduced.

The most widely used method for the determination of the number of theoretical plates N in a chromatographic column is a graphic method (5) whereby tangents to the elution curve at the points of inflection are drawn and the number of plates for an extremely thin initial zone is calculated from distances between certain points on the elution curve.

A method for the determination of N was derived by this author (4). It is applicable to both thin and thick zones and makes use of the ratio between the maximum concentrations at the bottom and top of the column. It requires the solution of two simultaneous equations or the use of prepared charts. When the initial zone is extremely thin, the calculations can be simplified, and a very simple relation in terms of the maximum concentration ratio deduced. Another simple relation is also deduced for a zone which is moderately thin.

The magnitude of the maximum effluent concentration and also the slope at the point of inflection are affected by the finite size of samples, and deviations from the true values increase with increase in the size of the sample. Values obtained for N by using the previously mentioned formulas can be improved further by applying a simple correction factor which is derived in this paper.

Effect of Finite Size of Sample and Dead Free Volume on the Shape of the Elution Curve

Both the finite size of samples and the dead free volume at the bottom of the column affect the shape of the elution curve, and the larger the sample and the dead free volumes the larger the deviation from the shape of the true curve. The theoretical elution curve represented by the simple relation $R_N = \phi_{N-1}{}^u$ will be considered; and the effect of each of these factors will be treated separately first and then combined.

Effect of Finite Size of Sample on the Shape of the Elution Curve

The size of each sample may be assumed to be s in the same units as u, and the experimental curve may be assumed to be obtained by plotting the concentration of solute in each sample vs. u such that $u = u_- + s/2$, where u_- represents the amount of eluant that left the column before taking the sample. If R_s denotes R_n corrected for the finite size of sample, then

$$R_s = \frac{1}{s}\int_{u-s/2}^{u+s/2} R_N \, du = \frac{1}{s}\int_{u-s/2}^{u+s/2} \phi_{N-1}{}^u \, du = \frac{1}{s}[P_N{}^{u+s/2} - P_N{}^{u-s/2}] \qquad (8)$$

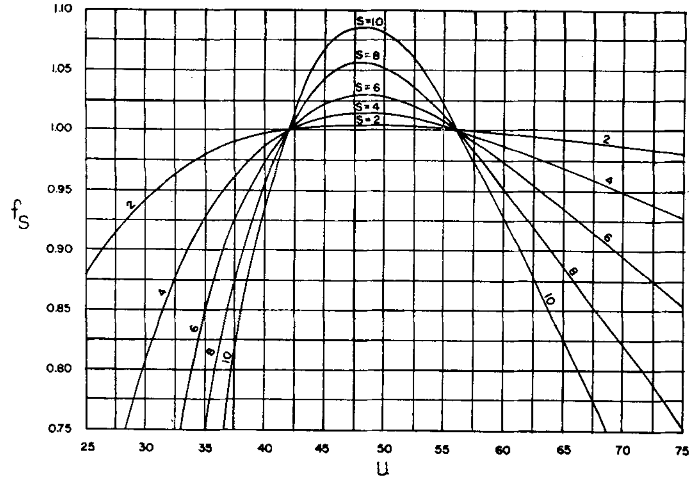

Fig. 1. Plot of f_s vs. u for $N = 50$.

By differentiating and equating to zero one can show that the maximum of the corrected curve lies at u_c, where

$$u_c = \frac{s}{2}\left[\frac{e^{s/n-1} + 1}{e^{s/n-1} - 1}\right] \qquad (9)$$

Since the maximum of the theoretical curve occurs at $u_m = n - 1$, the relation between u_c and u_m becomes

$$u_c = \frac{s}{2}\left[\frac{e^{s/u_m} + 1}{e^{s/u_m} - 1}\right] \qquad (10)$$

Effect of Dead Free Volume on the Shape of the Elution Curve

The dead free volume is the volume at the bottom of the column in which the effluent is mixed before the samples are taken.

It will be assumed that the weight of effluent in this volume is equal to b, that the effluent is completely mixed in it, and that it is filled with pure eluant at the start of the elution process.

A material balance on the solute in this free volume gives

$$\bar{y}_N \, dw - y_f \, dw = b \, dy_f$$

when one divides both sides by $\bar{y}_0$ and substitutes

$$R_N = \frac{\bar{y}_N}{\bar{y}_0} = \phi_{N-1}{}^u$$

$$\therefore \phi_{N-1}{}^u \, dw - \frac{y_f}{\bar{y}_0} \, dw = \frac{b}{\bar{y}_0} \, dy_f$$

When

$$\frac{y_f}{\bar{y}_0} = R_f$$

$$(\phi_{N-1}{}^u - R_f) \, dw = b \, dR_f$$

and since

$$u = w \frac{kN}{S}$$

$$\therefore (\phi_{N-1}{}^u - R_f) \, du = a \, dR_f$$

where $a = bkN/S =$ the dead free volume expressed in the same units as u

$$\frac{dR_f}{du} + \frac{1}{a} R_f = \frac{1}{a} \phi_{N-1}{}^u$$

Solving this first-order linear differential equation one gets

$$R_f = e^{-u/a}\left[\int \frac{1}{a} \phi_{N-1}{}^u e^{u/a} \, du + c\right]$$

Integrating and substituting the boundary condition $R_f = 0$ when $u = 0$ one finally gets

$$R_f = \frac{e^{-u/a}}{a\left(1 - \frac{1}{a}\right)^N} P_N{}^{(u-u/a)} \qquad (11)$$

Combined Effect of Finite Size of Sample and Dead Free Volume on the Shape of the Elution Curve

$$R_{sf} = \int_{u-s/2}^{u+s/2} \frac{e^{-u/a}}{a\left(1 - \frac{1}{a}\right)^N} P_N{}^{u(1-1/a)} \, du$$

Appendix 4 shows that by means of integration by parts,

$$R_{sf} = \left[P_N{}^u - \frac{e^{-u/a}}{\left(1 - \frac{1}{a}\right)^N} P_N{}^{u(1-1/a)} \right]_{u-s/a}^{u+s/a} \qquad (12)$$

Of these effects the effect of the finite size of samples is the more important, since in most cases it is unavoidable while the dead free volume can easily be made very small; consequently its effect becomes negligible. In the following discussions the effect of the sample size alone will be considered.

Figure 1 is a plot of f_s vs. u for $N = 50$ and for different values of s. For larger values of N the main features of the curves remain almost the same except that they become more and more symmetrical, and as N becomes very large the curves approach those obtained by sampling the normal distribution.

The sampling function $y(s)$ corresponding to any function $y = f(x)$ is represented by the equation

$$y(s) = \frac{1}{s} \int_{x-s/2}^{x+s/2} f(x) \, dx \qquad (13)$$

and in the case of the normal distribution

$$y(s) = \frac{1}{s\sqrt{2\pi}} \int_{x-s/2}^{x+s/2} e^{-x^2/2} \, dx$$

and

$$f(s) = y/y(s) = se^{-x^2/2} \Big/ \int_{x-s/2}^{x+s/2} e^{-x^2/2} \, dx$$

As shown in Appendix (6C) the right hand side is approximately equal to $1 - s^2/24 \, (x^2 - 1)$ for reasonably small values of s, say $s < 1$, and

$$f(s) = 1 - \frac{s^2}{24} (x^2 - 1) \qquad (14)$$

For different values of s Equation (14) represents a family of parabolas intersecting at the points $(\pm 1, 0)$ which are inflection points on the normal distribution. They also cross the ordinate at their maximum values which are equal to $1 + s^2/24$.

For the Poisson distribution, $f(s)$ can be satisfactorily calculated according to Equation (14) with the transformation $x = (u - N)/\sqrt{u}$ and with the following limitations

N is large, say $N > 50$

x is small, say $x < 1.5$

s is small, say $s < 1$.

Number of Theoretical Plates in a Chromatographic Column

According to the recommended method for the determination of the number of theoretical plates (*5*)

$$N = 16\left(\frac{\mu}{\theta}\right)^2 \qquad (15)$$

where θ represents the length of the intercept on the abscissa between the tangents to the elution curve at the points of inflection, and μ represents the distance between the origin and the midpoint of the intercept.

In spite of the large uncertainties in drawing tangents Equation (14) has the advantage of being dependent on the effluent concentrations only; yet when the concentration of solute at the top of the column is known, a more representative value for the number of theoretical plates is obtained from the formula

$$N = 2\pi\left(\frac{\alpha}{\gamma}\right)^2 \qquad (16)$$

Equations (15) and (16) both assume an extremely thin initial zone.

Equation (16) has the following advantages over Equation (15): The total volume of effluent need not be recorded; only a small part of the elution curve around the maximum point need be drawn; and it is easier to correct Equation (16) for the effect of the finite size of samples. When the initial zone is moderately thin, better values for N are obtained by using

$$N = 2\pi\left(\frac{\alpha}{\gamma}\right)^2\left[1 + \frac{\alpha^2}{2}\right] \qquad (17)$$

The difference between Equations (16) and (17) is in the term $\alpha^2/2$, neglected in the first equation, which depends upon initial zone thickness and the number of theoretical plates; it increases when γ and N increase. Equations (16) and (17) are derived in Appendix 5.

Formulas (15), (16), and (17) are subject to errors owing to the finite size of samples, and in Appendix 6C it is shown that

$$\epsilon_r(\alpha) \cong h^2/24$$

where

$$h = s/\sqrt{N} = w_s k\sqrt{N}/S$$

To correct for the sampling effect the method of successive approximations is used. One of the aforementioned formulas is chosen, and a preliminary value $N_{(0)}$ is calculated which is used to calculate $\epsilon_r(\alpha)$ giving a better value for α from which a better value $N_{(1)}$ for N is obtained and so on. Since $h^2/24$ is usually a small fraction, one approximation will be enough.

Illustrative Example

To calculate N for the following two cases: 10 mg. of solute were introduced by 2 g. of solvent at the top of a chromatographic column made up of 12 g. of adsorbent. The same solvent (pure) was used as the eluant, and the maximum concentration in the effluent was found to be 2.23 mg. solute/g. solvent. The weight of the sample = 1.50 g., and k = 0.122; 11 mg. of solute were introduced at the top of a 15 g. adsorbent column by 1.2 g. solvent and eluted by a different eluant. Maximum concentration in the effluent was 2.1 mg. solute/g. effluent. The weight of the sample = 2.20 g.; k for solvent = 0.037, and k for eluant = 0.093.

Solution

a) $\gamma = 0.122 \times 2/12 = 0.0203$

Maximum concentration in eluant at top of column = 10/2 = 5 mg. solute/g. eluant

$$\alpha = 2.23/5 = 0.446$$

$$N_{(0)} = 2\pi\left(\frac{0.446}{0.0203}\right)^2 \cdot\left[1 + \frac{0.446^2}{2}\right] = 3{,}340$$

$$h = \frac{s}{\sqrt{N}} = w_s k\sqrt{N}/S$$

$$= 1.5 \times \frac{58}{12} \times 0.122 = 0.88$$

$$\epsilon_r(\alpha) = (0.88)^2/24 = 0.032$$

$$N_{(1)} = 2\pi\left(\frac{0.446 \times 1.032}{0.0203}\right)^2 \cdot\left[1 + \frac{(0.446 \times 1.032)^2}{2}\right] = 3{,}590$$

It is evident that a second approximation is not necessary, and $N \cong N_{(1)} = 3{,}590$ theoretical plates

b) $$\gamma = \frac{0.037 \times 1.2}{15} = 0.00296$$

$$\bar{y}_0 = \frac{11}{1.2} \times \frac{0.093}{0.037} = 23.0$$

mg. solute/g. eluant. $\alpha = 2.1/23.0 = 0.0913$ α is small, and $\alpha^2/2$ can be neglected.

$$\therefore N_{(0)} = 2\pi\left(\frac{\alpha}{\gamma}\right)^2$$

$$= 6.28.\left(\frac{0.0913}{0.00296}\right)^2 = 5{,}950$$

$$h = w_s k\sqrt{N}/S$$

$$= 2.2 \times \frac{77.2}{15} \times 0.093 = 1.053$$

$$\epsilon_r(\alpha) = \frac{h^2}{24} = 0.046$$

$$N_{(1)} = 5{,}950 \times 1.046^2 = 6{,}510$$

$$\therefore N \cong N_{(1)} = 6{,}510$$

theoretical plates.

NOTATION

a = bkN/S = dead free volume in the same units as u
b = weight of effluent in dead free volume
f_s = ratio between ordinate on the true elution curve and corresponding ordinate on the experimental curve
j, r = positive integer numbers
k = adsorption or exchange coefficient
N = total number of theoretical plates in column
N_d = total number of theoretical plates during deposition
N_e = total number of theoretical plates during elution
$N_{(r)}$ = value of N after r successive approximations
n = plate number from top of column—top plate number $n = 1$ and bottom plate number $n = N$
n_1 = number of plates occupied by uniform zone at start of the elution process
P_n^u = $\sum_{r=n}^{\infty} \phi_r^u$
R_n = y_n/y_0
R_n^0 = y_n^0/y_1^0 = concentration ratio on plate n before elution
R_N^{um} = maximum value of R_N
R_f = R_N corrected for the dead free volume
R_s = R_N corrected for the sample size
R_{sf} = R_N corrected for both sample size and dead free volume
s = weight of sample in same units as $u = w_s kN/S$
S = total weight of adsorbent in column
u = kx during elution
u_0 = kx during deposition
u_1 = $u_0 - n_1$
u_c = u_m corrected for size of sample
u_- = value of u before taking sample
u_m = value of u when peak of zone has reached plate N
w = weight of eluant or solvent that has passed through any plate in column
w_s = weight of sample
x = wN/S
y_n = concentration of solute on plate n, g. solute/g. adsorbent, for the elution process
$\bar{y}_n$ = concentration of solute in eluant in equilibrium with plate n during elution
y_0 = $\bar{y}_0/k$ = concentration of solute on adsorbent if in equilibrium with solvent containing solute at a concentration $\bar{y}_0$
$\bar{y}_0$ = concentration of solute in solvent before entering plate 1
y_f = concentration of solute in dead free volume
y_n^0 = concentration of solute on plate n before elution

Greek Letters

α = ratio between the maximum concentrations of solute at bottom and top of column
γ = fraction of column loaded with solute at start of elution process
ϕ_n^u = $e^{-u}u^n/n!$ = Poisson exponential function
$\epsilon_r(\alpha)$ = relative error in α
λ = N_d/N_e

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