

THEORIA

Ligand-Leakage in Affinity Chromatography: a Note on the Mathematical Approach

In a recent paper, GRIBNAU and TESSER¹ derive mathematical expressions for the concentration of free ligands and the half-time of leakage. The authors use graphical solutions and the Newton-Raphson method to solve their equation (3)

$$e^{-k\tau} \sum_{p=0}^{n-1} \frac{(k\tau)^p}{p!} = \frac{1}{2} \quad (1)$$

This equation can easily be solved using a wellknown relation in mathematical statistics. Equation (1) is the cumulative probability function of the Poisson-distribution with parameter $\lambda = k\tau$ and is related to the χ^2 -distribution with ν degrees of freedom by

$$Q(\chi^2|\nu) = \sum_{p=0}^{n-1} e^{-kt} \frac{(kt)^p}{p!} \quad (2)$$

Table I. Computation of $k\tau_n$, using equation (2) and tabulated values of the χ^2 -distribution

n	ν	$\chi^2_{Q;\nu}$	$k\tau_n$
1	2	1.38629	0.69315
2	4	3.35670	1.67835
3	6	5.34812	2.67406
4	8	7.34412	3.67208
5	10	9.34182	4.67091
6	12	11.3403	5.67015

Table II. Values of kt for varying values of n and C_N/a , using tabulated values of the χ^2 -distribution³.

C_N/a		10^{-2}		10^{-4}	
n	ν	χ^2	kt	χ^2	kt
1	2	0.02010	0.01005	0.00020	0.00010
2	4	0.29711	0.14856	0.02842	0.01421
5	10	2.55821	1.27911	0.88892	0.44446
10	20	8.26040	4.13020	4.39516	2.19758

where $\chi^2|_\nu$ is the Q -quantile of the χ^2 -distribution with $\nu = 2n$ degrees of freedom and $kt = (\chi^2)/2$. Hence the value of $k\tau_n$ can be found from any table of the χ^2 -distribution^{2,3} by taking half of the tabulated χ^2 -value for $\nu = 2n$ degrees of freedom and $Q = 0.5$ (Table I). A very accurate approximation for τ_n can be derived from the WILSON-HILFERTY-approximation of the χ^2 -distribution² for large ν ($\nu > 30$).

$$\chi^2_{Q;\nu} \simeq \nu \left\{ 1 - \frac{2}{9\nu} + u_Q \sqrt{\frac{2}{9\nu}} \right\}^3 \quad (3)$$

where u_Q is the Q -quantile of the standard normal distribution. Setting $Q = 0.5$, $u_Q = 0$, the approximation yields

$$\tau_n \simeq \frac{n}{k} \left(1 - \frac{1}{9n} \right)^3 \simeq \frac{n}{k} \left(1 - \frac{1}{3n} \right) \quad (4)$$

This approximation is even accurate enough for n as small as 5 (it should be noted that for $n = 1$, $\tau = (\ln 2)/k$ as given in¹ is exact!). Using relation (2), solutions to kt for varying values of C_N/a can be found using appropriate tables of the χ^2 -distribution³ and setting $P = 1 - Q = C_N/a$ (Table II).

Zusammenfassung. Für die von GRIBNAU und TESSER angegebene Auswaschfunktion wird eine Lösungsmöglichkeit mit Hilfe der χ^2 -Verteilung angegeben.

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¹ T. C. J. GRIBNAU and G. I. TESSER, *Experientia* 30, 1228 (1974).

² M. ABRAMOWITZ and I. A. STEGUN, *Handbook of Mathematical Functions* (Dover Publications, New York 1965).

³ E. S. PEARSON and H. O. HARTLEY, *Biometrika Tables for Statisticians*, vol. 1 3rd edn. (Cambridge University Press 1970), vol. 2 (1972).

PRO EXPERIMENTIS

Simplifications to Substrate Preparation for the Cultivation of Dissociated Nerve Cells

Reconstituted collagen¹ has been used with success as substrate for the cultivation of nerve tissue explants² and of dissociated nerve cells^{3,4}. However, the presence of toxic ammonia vapours used for the reconstitution process necessitates a time-consuming rinsing procedure, making the method inconveniently long, especially in

laboratories where a great number of experiments have to be performed. Plastic substrate has been used without collagen, but in this case the settlement and the onset of differentiation of the nerve cells were retarded⁵. A method of photo-reconstitution for the collagen, that makes the preparation of the substrate more rapid, was