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Prediction of calibration curves in ria of digoxin Influence of some variables

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Abstract

Background: Competitive protein binding radioimmunoassay (CPB-RIA) is a principal method for quantifying serum digoxin concentration. The accuracy of this method is critically dependent on factors that influence the substitution reaction between unlabelled (Q) antigen (digoxin) with ^{125}I-labelled antigen (M) bound to anti-digoxin antibody (P). We studied the influence of initial concentration of M, ionic strength, and viscosity on the substitution reaction between M and Q. In addition, we propose a kinetic model for this reaction.
Methods: We used a commercially available CPB-RIA for digoxin, a gamma counter, and a viscosimeter to study the effect of initial concentration of M, ionic strength, viscosity, and temperature on the substitution reaction between M and Q. Data were analyzed using Statistica software.
Results: The apparent rate constant for the reaction between M and Q in the formation of PM is dependent on the initial concentration of M, and the ionic strength, viscosity, and temperature of the reaction medium, and independent of the concentration of Q.
Conclusion: A kinetic model for the displacement of the ^{125}I-digoxin by the digoxin in its union to a specific antibody is proposed. Such model adjusts satisfactorily to the results and allows the prediction of the calibration curves of RIA (activity bound to the antibody vs. concentration of digoxin) showing the influence of the concentration of both species, the time of incubation, the viscosity and the ionic strength of the medium, on the sensitivity of the method of RIA on which the analytical determination of the digoxin is based.

Keywords: Displacement; Labelled digoxin; Antibody; Immunocomplex; Vicosity; Ionic strength

1. Introduction

Immunoassay has proved itself a particularly useful aid in determining drug overdoses in patients treated with cardiac glycosides. The technique is also useful (a) in clarifying situations where a patient's symptoms might be due either to intrinsic heart disease or to digitalis intoxication; (b) where there is doubt concerning the type of digitalis preparation the patient is taking—in this case, digitoxin immunoassay is also necessary; (c) for measuring the digoxin ingestion of patients with an inadequate history of previous dosage; (d) in documenting cases of underdigitalization as well as digitalis (digoxin) excess; (e) in monitoring the toxic response in patients with myocardial disease associated with hypokalemia, hypomagnesemia, hypercalcemia, hypoxia and alkalosis, which are particularly sensitive to digitalis; and (f) in preventing overdigitalization, particularly in patients whose renal function is deteriorating or for whom an increased digoxin dosage is contemplated. The high sensitivity of digoxin immunoassay is especially necessary in view of the small differences and occasional overlap that exist between therapeutic and toxic levels of circulating digoxin. Intoxication is defined in terms of arrhythmias and disturbances of cardiac conduction due to the drug's presence.

The procedure is a solid-phase radioimmunoassay [1], wherein ^{125}I-labelled digoxin competes for a fixed time with digoxin in the patient sample for antibody sites. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabelled digoxin. Counting the tube in a gamma counter then yields

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a number, which converts by way of a calibration curve to a measure of the digoxin present in the patient sample.

Kinetics and equilibrium in antigen–antibody reactions are determining factors in the sensitiveness and accuracy of immunoanalytical techniques [2–4]. A diffusion-controlled process must meet some typical requirements such as a considerable reaction rate decrease when medium viscosity is greater, and scarce temperature influence with a reduced energy demand with regards activation, thus causing activation enthalpy values to be the same order as the solvent's viscous flow energy (19000 J mol^{-1} for water) [5]. Diffusion control for this type of processes has been theoretically studied by Nigren, Stenberg et al. [6–10]. They proposed an application model for reactions produced in the solid–liquid interphase which provided an equation containing four diffusion influence parameters. Raman [11] also observed diffusion control for monoclonal antibody binding to cytochrome *c*. Xavier and Willson [12] studied the association and dissociation reactions of Anti-Hen Egg Lysozyme (HEL) with two of its specific antibodies (HyHEL-5 and HyHEL-10) under pseudo first order conditions for the association, and found diffusion control. The decrease in the reaction rate constants as a result of viscosity turned out to be more drastic than theoretically expected, this aspect being put down to potential osmotic effects. In addition, rate constants were found to approximately double when ionic strength goes down from 500 mM to 27 mM, which indicates that the process occurs between species with opposite charges that affect the orientational requirements of association.

Equilibrium data analysis is largely used in determining the capacity of a substance to bind to one or several receptor populations. Nonetheless, as pointed out by Weber [13], detecting two binding sites through such an assay requires the ligand to have very different affinity for the two binding sites.

In our previous research [14–23] different features relative to the kinetics of antigen–antibody reactions used by immunoanalytical techniques were analyzed. Theoretical models were prepared for an application to the immunocomplex formation processes produced in RIA (radioimmunoassay) and IRMA (immunoradiometric assay). We also studied the fitting of equilibrium results to several pre-set equations, and a mathematical deduction that justifies them theoretically was obtained.

We seek to develop a general model applicable to competitive immunoassays including the influence of several variables. Its validation comes from the fitting of the results to the equations obtained. The models of Stenberg, Rabany, and those of Zuber refer to the formation of the radioactive immunocomplex but not to the competition between labelled and unlabelled antigen, which is the basis of competitive immunoassays. Such models do not determine the influence of the variables studied here.

In line with the above research, this paper aims to:

- Produce a kinetic model applicable to the substitution of the labelled antigen bound to the antibody by the unlabelled one, this process being at the foundations of RIA.
- Distinguish between single-site and two-site binding models by analysing kinetic data.
- Determine potential diffusion control.

This must be done in different stages:

- Obtaining integrated rate equations for the overall processes.
- Studying the medium's viscosity influence on reaction kinetics.
- Complementary analysis of ionic strength influence in order to include or rule out the effect of electrical charges.
- To predict the calibration curves showing the influence of the mentioned variables.
- The results must be potentially applicable to the design of immunoanalytical techniques.

2. Materials and methods

2.1. Instruments

ILKB Gammamaster Automatic Gamma Counter. Brookfield DV–II digital viscosimeter. Viscosity measurements were performed at 60 rpm with a UL ADAPTER at room temperature.

2.2. Reagents

DM is the solution of ^{125}I-labelled digoxin in a protein-based buffer. PT denotes the plastic tubes with rabbit anti-digoxin immunoglobulin immobilized to the inside wall. DQ is the digoxin standard solution 8 nmol/L. These reagents were included in the Cot-A-Count digoxin kit provided by DPC; GL is glycerol (Merck, pro analysi) and DS is the solution of NaCl 2.05 M.

2.3. Experimental procedure

Several tube series were prepared as per Table 1.

They were left to react overnight. The next day, they were decanted and washed with 2 mL distilled water. Activity was measured on tube 1, 7, 13 and 19 using a gamma counter. The activity measured in tube 19 was taken as the initial activity for the experiments 4–13.

Solutions were as per Table 2.

Reaction kinetics were studied by placing 1 mL of the previously mentioned solutions in the plastic-coated tubes and letting them react at different times and at 48 h, this being considered infinite time. Each tube was washed to remove any unbound labelled antibody. Any radioactivity present in the remaining bound labelled antibody was then measured using a gamma counter.

Thirteen experiments were performed, arranged as follows:

Table 1
Composition of the 125I-Digoxin Solutions

PT	1–6	7–12	13–18	19–82
DM (mL)	0.25	0.50	0.75	1
H_2O (mL)	0.75	0.50	0.25	0

Table 2
Composition of the Digoxin Solutions

Solution	1	2	3	4	5	6	7	8	9	10
DQ (μL)	25	50	75	100	100	100	100	100	100	800
GL (mL)	0	0	0	1	2	3	0	0	0	0
DS (μL)	100	100	100	100	100	100	200	300	400	800
H_2O (mL)	7.875	7.850	7.825	6.8	5.8	4.8	7.7	7.6	7.5	62.4
(Dig) (pmol/L)	25	50	75	100	100	100	100	100	100	100

- *Experiments* 1–4: Study of the influence of ^{125}I-digoxin concentration (m) upon the global reaction using tubes 1–28 and solution 10.
- *Experiments* 4–7: Study of the influence of digoxin concentration (q) upon the global reaction using tubes 22–46 and solutions 10, 1, 2, and 3.
- *Experiments* 4, 8, 9 *and* 10: Study of the influence of viscosity (η) using tubes 22–28, 47–64 and solutions 10, 4, 5, and 6. The final viscosity of the solutions obtained in this manner was determined by comparison with a calibration curve drawn from standard glycerol–water mixes.
- *Experiments* 4, 11, 12 *and* 13: Study of the influence of ionic strength (I), using tubes 22–28, 65–82 and solutions 10, 7, 8, and 9.

2.4. Data analysis

The Statistica programme (Copyright©StatSoft, Inc., 1993) was used with specific non-linear regression equations. As the statistical criterion that allows a choice from different equations, SS and Corrected Akaikeĭs Information Criterion (AIC_c) was used, expressed as $AIC_c = N\ln(SS/N) + 2P + ((2p(p+1))/(N-p-1))$ where N is the number of points, SS the addition of residual squares, and p the number of parameters in the equation. The fitting with the lowest AIC_c must be chosen. In order to distinguish equations from monoexponential and biexponential models, AIC_c and ANOVA (F test) were used [24,25].

3. Results

See Table 3.

4. Discussion and conclusions

4.1. Analysis of results

4.1.1. Influence of m and q (Experiments 1–7)

The results of experiments 1–7 are fitted to the equation:

$$z = \frac{Am}{(1+Bq)} + \left(\frac{Cmq}{(1+Dq)}\right)\exp(-t(E+Fq+Gm)) + \left(\frac{Hmq}{(1+Jq)}\right)\exp(-t(K+Uq+Wm)) \quad (1)$$

Its parameters, coefficient of correlation (r) and sum of squares of residuals (SS) are: $A = 112.8$, $B = 0.00733$, $C = 1.128$, $D = 0.01041$, $E = 0.01180$, $F = -1.038 \times 10^{-4}$, $G = 1.378 \times 10^{-3}$, $H = 15.59$, $J = 0.274$, $K = 0.00537$, $U = -2.30 \times 10^{-4}$, $W = 6.82 \times 10^{-5}$, $r = 0.994$, and $SS = 10.0 \times 10^{6}$.

The Eq. (1) is identical to Eq. (A.13) (See Appendix A)

- *Conclusion* 1. The initial activity of the radioactive immunocomplex (z_0) is directly proportional to m. The apparent rate constant for the process (kf) is linearly dependent on the initial concentration of the radioactive immunocomplex and the digoxin concentration.
- *Conclusion* 2. Activity in equilibrium (z_e) depends on m as per Langmuir's equation. As a consequence, the RIA calibration curves obtained with these reagents must follow the model of the four parameters and provide a good logit-log linear fit.

4.1.2. Influence of m, q and η (experiments 1–10)

The results of experiments 1–10 are fitted to the equation:

$$z = \frac{Am}{q+B\eta} + \left(\frac{Cm}{1+Dq+E\eta}\right)\exp(-t(Fm+G-H\eta)) + \left(\frac{Jm}{(1+Kq+L\eta)}\right)\exp(-t(Nm+U-W\eta)) \quad (2)$$

Its parameters, coefficient of correlation (r) and sum of squares of residuals (SS) are: $A = 16761$, $B = 108.9$, $C = 146.0$, $D = -0.240$, $E = 3.76$, $F = 1.753 \times 10^{-3}$, $G = 1.256$, $H = -0.900$, $J = 23.9$, $K = -0.001473$, $L = -0.363$, $N = 3.30 \times 10^{-5}$, $U = -0.1357$, $W = 0.1054$, $r = 0.992$ and $SS = 17.2 \times 10^{6}$.

The Eq. (2) is identical to Eq. (A.14)

- *Conclusion* 3. The rate and equilibrium constants depends on the medium viscosity, as the Kramers Equation. For constant values of m, q and I, the activity in the equilibrium diminishes when it increases viscosity.

4.1.3. Influence of m, q,η and I (experiments 1–13)

The results of experiments 1–13 are fitted to the equation

$$z = \frac{Am\exp(BI^{0.5})}{q+C\eta} + \left(\frac{Dm}{1+Eq+F\eta}\right)\exp(-t\exp(GI^{0.5}) \times (Hm+Jq-K\eta)) + \left(\frac{Lm}{1+Nq+Rh}\right) \times \exp\left(-t\exp(SI^{0.5})\left(\frac{Um+Vq}{100-Wv}\right)\right) \quad (3)$$

Its parameters, coefficient of correlation (r) and sum of squares of residuals (SS) are: $A = 16675$, $B = 0.0322$, $C = 113.7$, $D = 26.4$, $E = -0.00377$, $F = 0.1545$, $G = -3.20$,

Table 3
z values for experiments 1–13

	t							m	q	η	I
	0	12	24	36	48	60	1440				
z_1	4592.1	3758.2	3424.4	3006.3	2926.8	2536.6	1650.6	25	100	1.385	0.0256
z_2	9306.5	6837.5	6228.4	5976.2	5307.4	5315.8	3087.5	50	100	1.385	0.0256
z_3	13685	10509	9198.6	8102.9	7394.9	7118.1	4564.8	75	100	1.385	0.0256
z_4	17094	11416	10313	10123	10413	9735.4	6058.6	100	100	1.385	0.0256
z_5	17094	14413	12885	12927	11944	12456	9168.3	100	25	1.385	0.0256
z_6	17094	13020	11786	11895	11788	11061	8888.3	100	50	1.385	0.0256
z_7	17094	13589	12791	11856	9870.1	9735.6	7740.6	100	75	1.385	0.0256
z_8	17094	12594	11054	10557	9682	8879.8	5898.6	100	100	1.478	0.0256
z_9	17094	12484	12599	10140	7971.2	7617.9	4882.7	100	100	1.677	0.0256
z_{10}	17094	12352	11189	9692.2	9101.1	8288.4	4240.1	100	100	1.98	0.0256
z_{11}	17094	13158	12923	11311	11094	9696.8	7296.5	100	100	1.385	0.0513
z_{12}	17094	13904	12257	10684	10712	9617	6994.6	100	100	1.385	0.0769
z_{13}	17094	12981	11679	10318	10010	9272.5	6337	100	100	1.385	0.1026

t = time (min); z = activity (cpm) of PM immunocomplex. The subscript indicates the experience number; m = M initial concentration (relative units); q = Q initial concentration (pmol L^{-1}); I = ionic strength (mol L^{-1}); η = viscosity (mPa s).

$H = 5.18 \times 10^{-3}$, $J = -1.721 \times 10^{-3}$, $K = -0.0782$, $L = 42.3$, $N = -0.00242$, $R = -0.1475$, $S = -0.868$, $U = 8.60 \times 10^{-5}$, $V = 6.85 \times 10^{-5}$, $W = -0.00294$, $r = 0.991$ and $SS = 24.3 \times 10^{6}$.

The Eq. (3) is identical to Eq. (A.15)

- *Conclusion* 4. The effect of the ionic strength is little important but appreciable. It suggests that the reacting chemical species have electrical charges of an opposite sign (G and $S < 0$). Therefore, the reaction becomes slower and the value of z_e is greater when I is upper.

The adjustment of the data to Eq. (3) can be seen in Fig. 1.

- *Conclusion* 5. The proposed theoretical model predicts the values of z with a mean deviation that can be estimated as: $(24.3 \times 10^6/91)^{1/2} = 517$ cpm. This represents a relative deviation of about 5%.

4.2. Prediction of calibration curves

z values are calculated by application of Eq. (3) for different q values as is plotted in the calibration curves of RIA. Figs. 2–5 represent such curves and they show the influence of the studied variables.

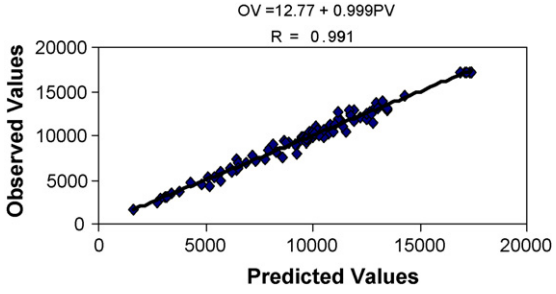

Fig. 1. z values observed in experiments 1–13 (Table 3) vs. values predicted for Eq. (3).

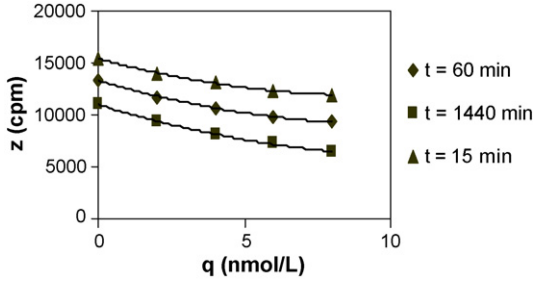

Fig. 2. Calibration curve calculated with the Eq. (3) for different times.

As it is observed in Fig. 2, the curves move towards smaller values of z at larger times. Nevertheless, the slopes change very little.

- *Conclusion* 6. The incubation time can be diminished up to 15 min without appreciable loss of sensitivity. This is important considering that the determination of digoxin can be urgent in some cases.

Fig. 3 indicates that the slopes of the curves are larger for larger concentrations of M. Therefore

- *Conclusion* 7. The sensitivity of the method increases when increasing the concentration of tracer.

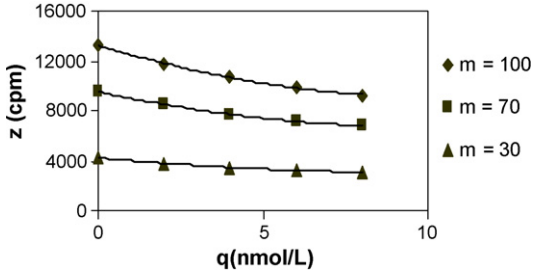

Fig. 3. Calibration curve calculated with the Eq. (3) for different tracer concentrations.

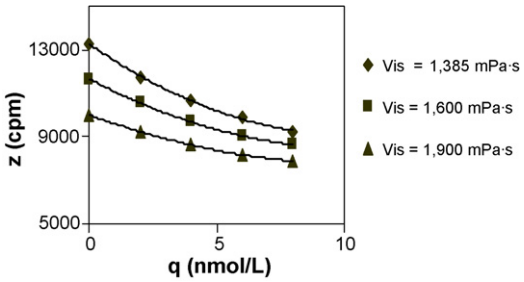

Fig. 4. Calibration curve calculated with the Eq. (3) for different viscosities.

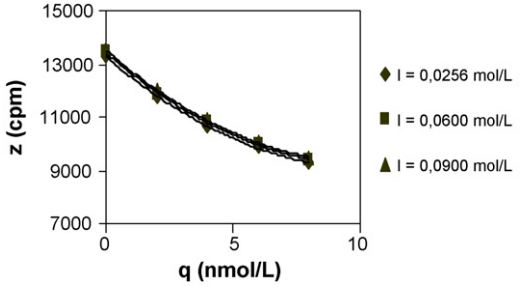

Fig. 5. Calibration curve calculated with the Eq. (3) for different ionic strengths.

Fig. 4 shows that the slope of the curves appreciably diminishes when increasing viscosity. It indicates that the studied process is influenced by the diffusion.

- *Conclusion* 8. The sensitivity of the method diminishes when increasing viscosity of medium.

Fig. 5 shows the influence of the ionic strength. It is observed that the curves appear overlapped, what explains why the influence predicted by the Eq. (3) has not practical relevance.

- *Conclusion* 9. The variation in the ionic strength does not affect sensitivity.

Appendix A

A.1. Theoretical model

This is the reaction studied:

$$\mathrm{PM} + \mathrm{Q} \leftrightarrow \mathrm{PQ} + \mathrm{M}$$

where P is the anti-digoxin antibody immobilized on the tube wall, M the ^{125}I-digoxin; PM the radioactive immunocomplex, Q the digoxin, and PQ is the non-radioactive immunocomplex.

The stoichiometry of the process indicates that PM and Q are consumed in equimolar amounts. The amounts of PQ and M formed must also be equimolar. Hence, the concentrations of PQ and M must be equal each other.Using these symbols:

$$(\mathrm{PM})_0 = w, \qquad (\mathrm{Q})_0 = q, \qquad (\mathrm{PM}) = w - x,$$
$$(\mathrm{Q}) = q - x, \qquad (\mathrm{PQ}) = (\mathrm{M}) = x$$

k_1 = direct rate constant, k_{-1} = reverse rate constant

then

$$\frac{\mathrm{d}x}{\mathrm{d}t} = k_1(w - x)(q - x) - k_{-1}x^2 \tag{A.1}$$

This must be true at equilibrium:

$$0 = k^1(w - x_\mathrm{e})(q - x_\mathrm{e}) - k_{-1}x_\mathrm{e}^2 \tag{A.2}$$

From (A.1) and (A.2) we have

$$\frac{\mathrm{d}x}{\mathrm{d}t} = (k_1 - k_{-1})(x_\mathrm{e} - x)\left[\left(\frac{wqk_1}{(k_1 - k_{-1})x_\mathrm{e}}\right) - x\right] \tag{A.3}$$

or

$$\frac{\mathrm{d}x}{\mathrm{d}t} = k'(x_\mathrm{e} - x)(u - x) \tag{A.4}$$

By integrating (A.4) we have

$$x = \frac{x_\mathrm{e}(1 - \exp(-(u - x_\mathrm{e})k't))}{(1 - (x_\mathrm{e}/u)\exp(-(u - x_\mathrm{e})k't))}$$

or

$$x = \frac{[x_\mathrm{e}(1 - \exp(-(wqk_1/(k_1 - k_{-1})x_\mathrm{e} - x_\mathrm{e})(k_1 - k_{-1})t))]}{[(1 - (x_\mathrm{e}^2(k_1 - k_{-1})/wqk_1)\exp(-(wqk_1/(k_1 - k_{-1})x_\mathrm{e} - x_\mathrm{e})(k_1 - k_{-1})t))]} \tag{A.5}$$

Taking into account that

$$1 - \left(\frac{x_\mathrm{e}^2(k_1 - k_{-1})}{wqk_1}\right)\exp\left(-\left(\frac{wqk_1}{(k_1 - k_{-1})x_\mathrm{e} - x_\mathrm{e}}\right)(k_1 - k_{-1})t\right) \approx 1$$

Eq. (A.5) becomes

$$x = x_\mathrm{e}\left(1 - \exp\left(-t\left(\frac{wqk_1}{(x_\mathrm{e} - x_\mathrm{e})(k_1 - k_{-1})}\right)\right)\right) \tag{A.6}$$

Our experiments measured PM activity, represented by z, directly proportional to (PM). Therefore

$$\frac{z_0}{w} = \frac{z}{w - x} = \frac{z_\mathrm{e}}{w - x_\mathrm{e}} = \frac{z_0 - z}{x}x = \frac{z_0 - z_\mathrm{e}}{x_\mathrm{e}} = \varepsilon \tag{A.7}$$

From (A.6) and (A.7) we obtain

$$z = z_\mathrm{e} + (z_0 - z_\mathrm{e})\exp\left(-t\left(\frac{z_0 q\varepsilon k'_1}{(z_0 - z_\mathrm{e})} + (z_0 - z_\mathrm{e})(k'_{-1} - k'_1)\right)\right) \tag{A.8}$$

$$\frac{z_0 q\varepsilon k'_1}{z_0 - z_\mathrm{e}} + (z_0 - z_\mathrm{e})(k'_{-1} - k'_1) = \text{apparent rate constant}$$

Considering that the used concentrations of tracer are significantly smaller than those of antibody, it can be assumed that the initial activities are directly proportional to m. So, it follows

$$z_0 = am \tag{A.9}$$

Assuming that the concentration of PM in the equilibrium follows the model of Langmuir we have

$$z_e = \frac{am}{(1+bq)}(\text{Langmuir}) \tag{A.10}$$

From (A.9) and (A.10) it follows

$$z_0 - z_e = am - \frac{am}{1+bq} = \frac{am + abmq - am}{1+bq} = \frac{abmq}{1+bq}$$

$$\frac{z_0}{z_0 - z_e} = \frac{am}{abmq/(1+bq)} = \frac{1+bq}{bq}$$

$$\frac{z_0 q\varepsilon k'_1}{z_0 - z_e} = \frac{(1+bq)qek'_1}{bq} = \frac{ek'_1(1+bq)}{b}$$

$$(z_0 - z_e)(k'_{-1} - k'_1) = abmq\frac{k'_{-1} - k'_1}{1+bq} \tag{A.11}$$

Introducing (A.11) into (A.8)

$$z = z_e + (z_0 - z_e)\exp\left(-t\left(\frac{z_0 q\varepsilon k'_1}{(z_0 - z_e)} + (z_0 - z_e)(k'_{-1} - k'_1)\right)\right)$$

$$= \frac{am}{(1+bq)} + \left(\frac{abmq}{1+bq}\right)\exp\left(-t\left(\frac{\varepsilon k'_1(1+bq)}{b} + \frac{abmq(k'_{-1} - k'_1)}{(1+bq)}\right)\right) \approx \frac{am}{1+bq} + \left(\frac{abmq}{1+bq}\right) \times \exp\left(-t\left(\frac{\varepsilon k'_1(1+bq)}{b} + abm(k'_{-1} - k'_1)\right)\right)$$

Grouping the constants, results

$$z = \frac{Am}{1+Bq} + \left(\frac{Cmq}{1+Dq}\right)\exp(-t(E + Fq + Gm)) \tag{A.12}$$

If in the reaction two simultaneous processes are ongoing, then Eq (A.12) is transformed in

$$z = \frac{Am}{1+Bq} + \left(\frac{Cmq}{1+Dq}\right)\exp\left(-t\left(\frac{E + Fq + Gm}{100}\right)\right) + \left(\frac{Hmq}{1+Jq}\right)\exp\left(-t\left(\frac{K + Uq + Wm}{1000}\right)\right) \tag{A.13}$$

For the rate constants, standard theory on diffusion-controlled reactions [5] provides the following expression

$$k = \frac{8RT}{3\eta}$$

which is valid for spherical, non-ionic, and similar-radius molecules. Kramers [26] pointed out that rate constants k^0 and k^v obtained in the absence and presence of a viscosizing agent such as glycerol relate to the corresponding viscosities through the equation

$$\frac{k^0}{k^v} = A + \frac{B\eta}{\eta^0}$$

which can be reduced to the previous one provided $A = 0$ and $B = 1$. Finding the value of k^v in the Kramersequation, substituting it in B, E, K Eq. (A.10), and simplifying, we then have

$$z = \frac{Am}{q + Bh} + \left(\frac{Cm}{(1 + Dq + Eh)}\right)\exp(-t(Fm + G - Hh)) + \left(\frac{Jm}{(1 + Kq + Lh)}\right)\exp(-t(Nm + U - Wh)) \tag{A.14}$$

The influence of the ionic strength on the rate constant is expressed as [5,8]$k = k_0\exp(2.344 z_A z_B I^{1/2})$ (Debye – Hückel)Introducing the expression of Debye-Hückel in A, F, N, and simplifying, it is

$$z = \frac{Am\exp(BI^{0.5})}{q + Ch} + \left(\frac{Dm}{1 + Eq + Fh}\right)\exp(-t\exp(GI^{0.5}) \times (Hm + Jq - Kh)) + \left(\frac{Lm}{(1 + Nq + Rh)}\right) \times \exp\left(-t\exp(SI^{0.5})\left(\frac{Um + Vq}{100 - Wv}\right)\right) \tag{A.15}$$

The parameters A, B, C, D, etc., represent therefore sets of constants. They have different meaning in the Eqs. (A.12)–(A.15).

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