CCV.—*The Dissociation Constants of Quinine, Cinchonine, and Cinchonidine.*

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KOLTHOFF'S comprehensive account of the dissociation constants of alkaloids (*Biochem. Z.*, 1925, **162**, 289) contains a summary of previous work with references. Most of his own results were obtained by the aid of indicators, and the individual values of constants calculated from each measurement of p_H, etc., show considerable variations from the means. Kolthoff also used the quinhydrone electrode, but only in the case of quinine, for which the second constant is given as 0.9—2.3×10^{-10} (colorimetric) and 1.2—3.6×10^{-10} (electrometric). Titrations of the most important alkaloids with the quinhydrone electrode against the half-cell, $0.01N$-HCl,$0.09N$-KCl|HgCl,Hg, have been carried out by Rasmussen and Schou (*Z. Elektrochem.*, 1925, **31**, 189). As emphasised by Kolthoff (*loc. cit.*), these results are intended only to establish the p_H of end-points for use in titrations with indicators; nevertheless, we have been able to use them in checking the constants of quinine and cinchonine. The titrations of Wagener and McGill (*J. Amer. Pharm. Assoc.*, 1925, **14**, 288) do not afford material suitable for this purpose.

We considered that results much more concordant than those previously available could be obtained by the use of the quin-hydrone electrode. The accurate values of the second constants could then be used to recalculate the first constants, which have hitherto depended on colorimetric and conductimetric methods. For the three alkaloids selected, the first dissociation constants are extremely close to one another, and so also are the second, but the evidence referred to above is not sufficient to show whether they are identical.

EXPERIMENTAL.

Purity of Alkaloids.—These were of good quality, supplied by manufacturers of high standing. *Quinine.* The hydrate, after being heated at 130° to constant weight, had m. p. 174.8°. It was free from ammonium salts and sulphates. 1 G. dissolved completely in a mixture of 6 c.c. of alcohol and 3 c.c. of ether, proving the absence of salts and also of cinchonine and cinchonidine. It gave no colour with concentrated nitric acid, and only the faintest colour with concentrated sulphuric acid. It was proved to be free from hydroquinine by oxidation, followed by extraction with ether.

Cinchonine. This was precipitated from the hydrochloride with alkali, washed, and crystallised from alcohol; m. p. 264°. No quinine was found by the thalleioquin test.

Cinchonidine. The tetrasulphate was purified by recrystallisation from alcohol and sulphuric acid, the base precipitated with alkali, and recrystallised from alcohol; m. p. 207·2°. It gave no thalleioquin reaction.

Measurements of Potential.—These were made on a Gambrell metre bridge, with extensions having resistances equal to that of the bridge. When a single accumulator was connected across the bridge and extensions, the potential gradient was about 0·7 millivolt per mm. The null potential was registered by a Crompton I 3 reflecting galvanometer with bifilar suspension and a coil of 300 turns. The half cells were tested in various ways. The $N/10$-potassium chloride calomel electrode was checked against a hydrogen electrode in standard acetate. The three platinum electrodes were compared with one another, both in standard acetate and in one of the solutions containing the alkaloids. Diffusion potentials were eliminated by salt bridges of saturated potassium chloride.

A weighed quantity of the base was dissolved in a known excess (about 2 equivs.) of standard hydrochloric acid, saturated with quinhydrone, and titrated back with standard alkali. In some cases a current of nitrogen was passed through the solution : this had no noticeable effect on the titrations, but prevented a darkening of the quinhydrone in the more alkaline solutions. The potentials were measured at temperatures of about 15°, uniform in each series, and corrected to 18°.

The last series, in the $0·002M$-solutions, affords the closest comparison which is possible between the constants of the bases, since the ionisations of the salts $(BH^{\cdot})Cl'$ and $(BH_2^{\cdot\cdot})Cl'_2$ are nearly complete and still more nearly equal; moreover, the experimental conditions were very carefully controlled. $0·0002$ Mol. of each base was dissolved in acid which had been standardised, both by weight and by volume, with calcium carbonate and as silver chloride, and had been used to standardise the alkali by weight and by volume. In the titration, the acid was weighed and measured from a weight burette, and the alkali measured from a microburette. The volume of the initial solution was made up to 500 c.c. in each case. The plots of $\Delta p_H/\Delta x$ (where x is the fractional degree of neutralisation in the stage $BH^{\cdot} \longrightarrow BH_2^{\cdot\cdot}$) gave the positions of the theoretical end-points, within the limits of accuracy with which reagents could be added. Since the individual constants are concordant, their means will be more trustworthy than those derived from colorimetric measurements.

Methods of Calculation.—We are concerned with bases whose first and second dissociation constants, K_1 and K_2, differ sufficiently to be calculated separately, over the central part of the neutralisation range, from the simple equation

$$p_H = p_{K_w} - p_{K_b} + \log (1 - x)/x \quad . \quad . \quad . \quad (1)$$

in which K_b refers to either the first or the second constant, and x is the fraction either of base converted into kation BH˙, or of kation BH˙ converted into BH_2˙˙. As our measurements were corrected to 18°, we have used $p_{K_w} = 14.13$ $(K_w = 7.4 \times 10^{-15})$: Kolthoff's value of 14.20 refers to a mean temperature of 16°.

Generally, equation (1) is applicable for the range $x = 0.13$— 0.80, *i.e.*, from 13% to 80% of B,2HCl, but this range depends also on the total concentration.

In the more acid solutions it is necessary to correct for the " acid error " by means of the equations

$$K_a = \frac{[H˙](x' + [H˙])}{(c - x' - [H˙])} = \frac{K_w}{K_b}$$

or

$$K_b = \frac{K_w(c - x' - [H˙])}{[H˙](x' + [H˙])} \quad . \quad . \quad . \quad (2)$$

in which x' is the amount of alkali added to the salt BH_2Cl_2, and c is the total concentration of the salt. If $[H˙]$ is large compared with c, an appreciable part of the acid will be present as hydrogen ions and will not have formed BH_2˙˙ by the reaction BH˙ + H˙ $\longrightarrow$ BH_2˙˙. In these acid solutions it is possible, and in solutions near the first equivalence point it is necessary, to use an equation similar to that proposed by one of us (*Proc. Roy. Soc.*, 1915, **91**, *A*, 535) for dibasic acids, which may be transposed into a form suitable for diacid bases, *viz.*,

$$R = \frac{2 + \dfrac{[OH']}{K_2} + K_w\left\{\dfrac{[OH'] + K_2}{[OH']K_2 c}\right\}}{1 + \dfrac{[OH']}{K_2} + \dfrac{[OH']^2}{K_1 K_2}} \quad . \quad . \quad . \quad (3)$$

in which R is the total number of equivalents of acid added to 1 mol. of base. As an example of the use of this to calculate K_2 in an acid solution of low total concentration ($c = 0.002$), a value may be selected from the data referring to cinchonidine (p. 1593): $x = 0.533$; $R = 1.533$; $p_H = 4.06$: $K_w = 7.4 \times 10^{-15}$; $K_1 = 1.3 \times 10^{-6}$; hence $K_2 = 0.82 \times 10^{-10}$. The other use of the equation is illustrated by the table on p. 1593.

Since the equivalent point B,HCl can be determined with a considerable degree of accuracy by the quinhydrone electrode

in dilute solutions of these bases, it is even possible * to calculate K_1 at this point by means of the equation $[OH'] = \sqrt{K_1 K_2 c/(K_1+c)}$ from which the terms c and $K_1 + c$ can be omitted if c is large compared with K_1. Thus, in the case of cinchonine the maximum of $\Delta p_H/\Delta x$ gives $p_H = 6\cdot25$ at B,HCl; therefore $p_{OH} = 7\cdot88$, and since, in the special case just mentioned,

$$p_{OH} = \tfrac{1}{2}(p_{K_1} + p_{K_2}) \quad . \quad . \quad . \quad . \quad (4)$$

we have $\qquad p_{K_1} = 5\cdot86$, and $K_1 = 1\cdot38 \times 10^{-6}$

Results in 0·1M-*Solutions.*—*Quinine.* The first constant of this base has been given as $2\cdot6 \times 10^{-6}$ (Barratt, *Z. Elektrochem.*, 1910, **16**, 130); $1\cdot7 \times 10^{-6}$ (Mauz, Physik. Chem. Untersuchung. über Alkaloide, Diss., Stuttgart, 1904); 3×10^{-6} (Kolthoff, *loc. cit.*, by conductivity); $1\cdot0$—$1\cdot44 \times 10^{-6}$, mean $1\cdot08 \times 10^{-6}$ (*idem, ibid.*, colorimetric). Our value calculated from K_2 as described above is $p_{K_1} = 5\cdot94$, $K_1 = 1\cdot15 \times 10^{-6}$.

The previous values of the second constant are given in the table on p. 1595. Our determinations were carried out in 0·1, 0·05, 0·01, and 0·002M-solutions, partly by the back titration of the base dissolved in an excess of acid, partly by the back titration of the diacid salt, and partly by forward titration of the monacid base with acid. Since the variations in total concentration have little effect on the form of the curves or the numerical values of the constant, we have included only the results in 0·1M-solutions :

x	0·918	0·782	0·600	0·537	0·500	0·400	0·300
p_H	3·08	3·67	4·06	4·19	4·25	4·475	4·51
p_{K_2}	10·01	9·92	9·89	9·88	9·88	9·83	9·98

x	0·278	0·250	0·206	0·134	0·060		
p_H	4·68	4·71	4·87	5·075	5·35 (precipitation occurs)		
p_{K_2}	9·84	9·84	9·85	9·97	9·83		

The results at the acid end are less trustworthy on account of the correction for [H·] (equation 2). Those at the end near the stage B,HCl may be calculated by equation (3).

The mean value of p_{K_2} is 9·88, while that corresponding to the median at $x = 0\cdot5$ is also 9·88. A forward titration of a 0·05M-solution of B,HCl gave $p_{K_2} = 9\cdot83$. Precipitation occurs at the end-point B,HCl in the more concentrated solutions.

The p_H at the end-point in 0·02 and 0·01M-solutions was found to be 6·2 and 6·3 respectively, agreeing with the results of Rasmussen and Schou (*loc. cit.*). If p_{K_2} in the more concentrated solutions is taken as 9·88, the end-point which we have called p_t (titration exponent) appears to be at 6·10. A slight extrapolation

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* This method is not usually practicable, *e.g.*, in the case of high constants which give sharp inflexions in the neutralisation curves.

shows, however, that the end-point (maximum of $\Delta p_H/\Delta x$) has come a little late in the back-titration with alkali. Correcting for this, we find that, in agreement with the results of forward titrations, in $0.1M$-solutions the most probable value of p_t is 6·2—6·3, and therefore $p_{K_1} = 9.83$, $K_2 = 1.3 \times 10^{-10}$.

Cinchonine. The first constant appears from colorimetric measurements to be nearly the same as, or perhaps slightly greater than, that of quinine. This cannot be verified potentiometrically in the more concentrated solutions on account of precipitation. The second constant, deduced either from titrations in $0.05M$-solution (Rasmussen and Schou, *loc. cit.*) or colorimetrically (Kolthoff, *loc. cit.*), is $p_{K_2} = 9.92$, and the constant would therefore be very slightly less than that of quinine. This seems to be borne out by our results given below, which are very concordant. Although the form of the corresponding curve is undoubtedly correct, its position on the x axis cannot be confirmed by the potentiometric end-point (which can only be approached up to $x = 0.10$); the position of the curve in this case, therefore, depends entirely upon the purity and concentration of the alkaloid.

x	0·965	0·872	0·677	0·552	0·377	0·326	0·251	0·201
p_H	2·625	3·31	3·85	4·05$_5$	4·36	4·47	4·64	4·88
p_{K_2}	—	10·01	9·96	9·99	9·99	9·97	9·97	9·90

The mean value of p_{K_2} is 9·96, $K_2 = 1.1 \times 10^{-10}$.

On application of the same small correction to the x co-ordinates as was found in the case of quinine, the values became $p_{K_2} = 9.83$, $K_2 = 1.3 \times 10^{-10}$.

Cinchonidine. The first constant ($p_{K_1} = 5.80$), determined colorimetrically in $0.005M$-solutions, appears to be the same as that of cinchonine in $0.0033M$-solutions. In this case, it is possible from our results to estimate the constant in $0.1M$-solutions (see below). The second constant, determined colorimetrically in $0.01M$- or less concentrated solutions, appears to be lower than those of quinine and cinchonine under the same conditions. The corresponding curve is plotted in the figure, the right-hand scale of ordinates being required.

x	0·782	0·600	0·537	0·500	0·400	0·300	0·278
p_H	3·51	4·00	4·14	4·20	4·41	4·61	4·65
p_{K_2}	10·06	9·95	9·95	9·93	9·90	9·89	9·89

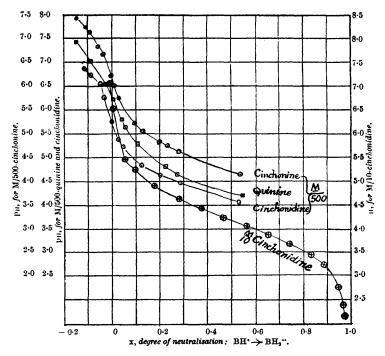| x	0·206	0·134	0·06	0·025	0·011	0·004	−0·004
p_H	4·83	5·06	5·45$_5$	5·92	6·29	6·52	6·77
p_{K_2}	9·89	9·89	9·87	9·80	9·79	—	—

The mean value of p_{K_2} is 9·92, $K_2 = 1.2 \times 10^{-10}$.

In spite of the statement that cinchonidine has a slightly lower solubility product, $[BH^·][OH'] = 1.38 \times 10^{-9}$, than that of quinine,

$4\cdot4 \times 10^{-9}$ (Kolthoff, *loc. cit.*), it is possible to add alkali to the cinchonidine salt up to a higher p_{H}, and beyond the point B,HCl, without precipitation. Thus, the value of p_t can be obtained potentiometrically from the maximum of $\Delta p_{\mathrm{H}}/\Delta x$, and is $6\cdot30$. This maximum actually occurs at $x = + 0\cdot01$, so the neutralisation curve is very slightly displaced on the x axis, but only to an extent which has no appreciable effect upon the constant obtained : $p_{K_2} = 9\cdot92$, $K_2 = 1\cdot2 \times 10^{-10}$.

From equation (4), $p_{K_1} = 5\cdot74$, whence K_1 is equal to $1\cdot8 \times 10^{-6}$ and is therefore slightly higher than the (colorimetric) K_1 values of

quinine and cinchonine in more dilute solutions. The truly comparable value of K_1 in the $0\cdot002M$-solutions is, however, decidedly lower than those of the other two alkaloids (see p. 1594).

Results in $0\cdot002M$-Solutions.—The constants in the more dilute solutions were determined in a thermostat at $18°$ by the method outlined above. The results are shown in the figure, one or other of the left-hand scales of ordinates being applicable.

Quinine. $0\cdot06488$ G., dissolved in $4\cdot968$ g. of $0\cdot0878N$-hydrochloric acid, which is $0\cdot410$ g. in excess of that required for B,2HCl, was diluted to 100 c.c. and titrated back with $0\cdot0795N$-sodium

hydroxide. Thus, after the addition of 1·616 c.c. of alkali, $x =$ 0·539, and so on.

x	0·539	0·273	0·222	0·108	0·059	
E	0·1231	0·0973	0·0887	0·0608	0·0395	
p_H	4·214	4·662	4·810	5·294	5·662	
p_{K_2} ...	9·89	9·92	9·90			
$\Delta p_H/\Delta x$					7·5	10·9

x	0·0455	0·007	−0·025	−0·086	−0·142
E	0·0312	0·0076	−0·0130	−0·0395	−0·0622
p_H	5·810	6·216	6·572	7·033	7·423
$\Delta p_H/\Delta x$ 10·9	10·6	11·1	7·5		

<div align="center">Mean $p_K = 9·91$; $K_2 = 1·23 \times 10^{-10}$.</div>

Cinchonine. 0·0588 G., dissolved in 5·151 g. of the acid (*i.e.*, 0·595 g. in excess of B,2HCl), was treated as above.

x	0·538	0·282	0·234	0·200	0·133	0·097	0·029	0·011
E	0·1252	0·0983	0·0912	0·0861	0·0730	0·0644	0·0335	0·0193
p_H	4·178	4·644	4·766	4·856	5·083	5·231	5·766	6·012
p_{K_2} ...	9·95	9·92	9·90	9·90	9·88			
$\Delta p_H/\Delta x$						4·11	7·87	13·66	20·5

x	−0·002	−0·018	−0·036	−0·054	−0·090	−0·106	−0·144
E	0·0071	−0·0115	−0·0203	−0·0288	−0·0476	−0·0527	−0·0632
p_H	6·224	6·545	6·699	6·845	7·171	7·259	7·442
$\Delta p_H/\Delta x$ 20·5	20·1	8·55					

<div align="center">Mean $p_K = 9·91$; $K_2 = 1·23 \times 10^{-10}$.</div>

Cinchonidine. 0·0588 G., dissolved in 4·960 g. of the acid (*i.e.*, 0·404 g. in excess of that required for B,2HCl), was treated as above.

x	0·533	0·285	0·202	0·134	0·051	0·026
E	0·1322	0·1072	0·0983	0·0872	0·0628	0·0554
p_H	4·057	4·489	4·644	4·837	5·259	5·390
p_K	10·09	10·065	10·01			
$\Delta p_H/\Delta x$					5·24	14·44

x	0·001	−0·030	−0·048	−0·083	−0·110
E	0·0324	0·0041	−0·0091	−0·0217	−0·0314
p_H	5·750	6·276	6·504	6·722	6·892
$\Delta p_H/\Delta x$ 14·44	17·0	12·7	6·23		

<div align="center">Mean $p_K = 10·05$; $K_2 = 8·9 \times 10^{-11}$.</div>

Calculation of the First Constants.—By interpolation of the smoothed curves at intervals of 0·1 unit of p_H, the maxima of $\Delta p_H/\Delta x$ are determined, and hence the values of the titration exponents, p_t; *e.g.*, in the case of cinchonine :

p_H	6·0—6·1	6·1—6·2	6·2—6·3	6·3—6·4
$\Delta p_H/\Delta x$	10	17	20	13

Therefore $p_t = 6·25$. Equation (4) is then applied to these values. The experimental points near B,HCl are dealt with by equation (3), the values of K_2 stated above being introduced.

Quinine.

R	1·00	0·98	0·90	0·86	
p_H ...	6·25	6·50	7·21	7·44	
p_{K_1} ...	5·85	5·91	5·96	5·90	Mean $= 5·90$; $K_1 = 1·2 \times 10^{-6}$.

Cinchonine.

R	1·00	0·97$_5$	0·924	0·86	
p_H ...	6·25	6·57	7·03	7·42	
p_{K_1} ...	5·85	5·97	6·01	5·94	Mean $= 5·94$; $K_1 = 1·15 \times 10^{-6}$.

Cinchonidine.

R	1·00	0·97	0·95	0·89	
p_H ...	5·80	6·28	6·50	6·89	
p_{K_1} ...	6·57	6·36	6·37	6·36	Mean $= 6·41$; $K_1 = 3·9 \times 10^{-7}$.

Correction for Activities, and Limiting Constants.—The effect of changes in the ionic strengths of the alkaloidal solutions upon their constants is relatively slight, and this is also true of the addition of neutral salts. Thus, a $0·05M$-solution of quinine mono- and di-hydrochloride was saturated with potassium chloride. The *E.M.F.* changed by 4 millivolts, or the p_H by 0·066 unit. The observed constants are not, therefore, very different in any case from the limiting constants. The latter have been calculated from the formula $\mu = \frac{1}{2}\Sigma c_i^r$, in which c_i is the concentration and z the valency of an ion (Lewis and Randall, " Thermodynamics "). At $c = 0·1M$: $\mu(B,HCl) = 0·1$, $\mu(B,2HCl) = 0·105$, therefore $\mu(B,HCl + B,2HCl) = ca. \ 0·102$. At $c = 0·002M$: $\mu(B,HCl + B,2HCl) = 0·002$. The activity coefficients α are now calculated from the equation $- \log \alpha = 0·5z^2/(1 + 3·3 \times 10^7 a\sqrt{\mu})$, in which a is the ionic diameter. The average value of a for the ordinary ions has been estimated as 4×10^{-8}, and their average mobility, L, is about 50. If we assume that the relation between L and a is given by Stokes's law, then $L \propto 1/a$; and if, further, a mobility of 25 is assumed for a univalent alkaloid kation, $a = 8 \times 10^{-8}$. Therefore the denominator in the above expression becomes $1 + 2·64\sqrt{\mu}$. The correction factor will take the form, $\log \alpha_{BH\cdot} - \log \alpha_{BH_2\cdot\cdot}$, which is obtained by introducing the coefficients α into equation (1) and transforming it :

$$p_K = p_{K_w} - p_H + \log \frac{BH^\cdot}{BH_z^{\cdot\cdot}} + \log \alpha_H + \log \alpha_{BH\cdot} - \log \alpha_{BH_2\cdot\cdot}$$

At $\mu = 0·002$: $\log \alpha_{BH\cdot} - \log \alpha_{BH_2\cdot\cdot} = 0·060$.
　　$\mu = 0·102$: ,, 　,, 　,, $= 0·260$.

The limiting constants, corrected for ionic strength, are therefore as follows :

Alkaloid.	Conc., M.	p_{K_2}.	$K_2 \times 10^{10}$.
Quinine	0·100	9·83 + 0·26 = 10·09	0·81
	0·002	9·91 + 0·06 = 9·97	1·07
Cinchonine	0·100	9·96 + 0·26 = 10·22*	0·60
	0·002	9·91 + 0·06 = 9·97	1·07
Cinchonidine	0·100	9·92 + 0·26 = 10·18	0·66
	0·002	10·05 + 0·06 = 10·11	0·78

* Or maybe the same as that of quinine.

General Summary of the Relations at 0·1 *and* 0·002M- *and Some Intermediate Concentrations.*—Typical results are tabulated below, together with the methods by which the constants are obtained and the observers. The essential features may be indicated.

(1) The two corresponding constants of quinine and cinchonine remain practically identical at all concentrations.

(2) The second constant of cinchonidine is slightly lower than these in the dilute solutions, but practically identical with them in the 0·1M-solutions.

(3) The first constant of cinchonidine is considerably lower, a result which did not appear in the more concentrated solutions. Thus, it is seen in the figure that the whole neutralisation curve of cinchonidine lies below those of the other alkaloids.

Observer & method.	Quinine. c & R.	pK & K.	Cinchonine. c & R.	pK & K.	Cinchonidine. c & R.	pK & K.
K. col.	0·0033	$p_K=5·97$	0·0033	$p_K=5·85$	<0·005	$p_K=5·80$
	0·44—0·90	$K_1=1·08 \times 10^{-6}$	0·50—0·90	$K_1=1·4 \times 10^{-6}$	0·50—0·90	$K_1=1·6 \times 10^{-6}$
P. & W. elect.	0·002	$p_K=5·90$	0·002	$p_K=5·94$	0·002	$p_K=6·41$
	0·86—1·00	$K_1=1·2 \times 10^{-6}$	0·86—1·00	$K_1=1·2 \times 10^{-6}$	0·89—1·00	$K_1=3·9 \times 10^{-7}$
K. col.	<0·01	$p_K=9·82$	<0·00975	$p_K=9·92$	<0·01	$p_K=10·03$
	1·10—1·90	$K_2=1·5 \times 10^{-10}$	1·10—1·90	$K_2=1·2 \times 10^{-10}$	1·50—1·90	$K_2=0·93 \times 10^{-10}$
K. elect.	<0·01	$p_K=9·66$				
	1·04—1·09	$K_2=2·2 \times 10^{-10}$				
R. & S. elect.	0·05	$p_K=9·92$	0·05	$p_K=9·92$		
	1·25	$K_2=1·2 \times 10^{-10}$	1·25	$K_2=1·2 \times 10^{-10}$		
P. & W. elect.	0·100	$p_K=9·83$	0·100	$p_K=9·96$	0·100	$p_K=9·92$
	1·134—1·92	$K_2=1·3 \times 10^{-10}$	1·20—1·97	$K_2=1·1 \times 10^{-10}$	1·13—1·78	$K_2=1·2 \times 10^{-10}$
P. & W. elect.	0·02	$p_K=9·88$				
	1·47—1·66	$K_2=1·3 \times 10^{-10}$				
P. & W. elect.	0·002	$p_K=9·91$	0·002	$p_K=9·91$	0·002	$p_K=10·05$
	1·06—1·55	$K_2=1·2 \times 10^{-10}$	1·13—1·54	$K_2=1·2 \times 10^{-10}$	1·13—1·53	$K_2=0 \times 10^{-10}$

Abbreviations: K. = Kolthoff (*loc. cit.*); P. & W. = present authors; R. & S. = Rasmussen and Schou (*loc. cit.*); col. = colorimetric; elect. = electrometric.

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