# Grisorixin, an Ionophorous Antibiotic of the Nigericin Group. Part IV.[1] Complexation of Monovalent Cations

By **Pierre Gachon,** Institut National de la Santé et de la Recherche Médicale, Laboratoire de Chimie Organique Biologique, Université de Clermont, B.P. 45, 63170 Aubière, France

Gérard Chaput and **Georges Jeminet,*** Laboratoire de Chimie Organique Biologique, E.R.A. n° 392 du C.N.R.S., Université de Clermont, B.P. 45, 63170 Aubière, France

Jean Juillard and **Jean-Pierre Morel,** Laboratoire d'Etude des Interactions Soluté-Solvant, Université de Clermont, B.P. 45, 63170 Aubière, France

Data on interactions between monovalent alkali metal, thallium, and silver cations and a new antibiotic grisorixin in methanol are reported. These were obtained by conductimetric and potentiometric measurements. Two types of interactions were studied, on one hand association of the salts formed between cations and grisorixin carboxylate ion, on the other complexation of the cations by the neutral antibiotic molecule. The magnitude of the equilibrium constants for the first process was *ca.* 200 times greater than for the second in the case of potassium. For association of salts the following order of selectivity was found: $Ag^+ > Tl^+ > K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$, the association constants varying from 10^2 to 10^7. The same order of selectivity was found using a liquid phase galvanic cell.

GRISORIXIN (Gr) (1) is an ionophorous antibiotic isolated[2] from a culture of *Streptomyces griseus*. It is a poly-cyclic polyether related to nigercin (2) and has a partially dissociated carboxy-group at physiological pH. Anti-biotics of this type perturb the potential of internal

dissociation of grisorixin, $RCO_2H \rightleftharpoons RCO_2^- + H^+$; the dissociation of its salts, $RCO_2^-, M^+ \rightleftharpoons RCO_2^- + M^+$; and the complexation of alkali metal cations by the neutral molecule of grisorixin. The cations under consideration are Na^+, K^+, Rb^+, Cs^+, Tl^+, and Ag^+.

(1) R = H
(2) R = OH
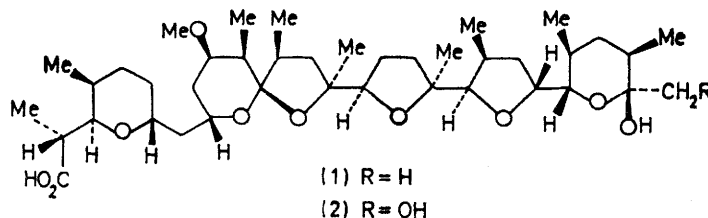
mitochondrial membranes, inducing through these membranes the transference of alkali metal cations. They determine the departure of K^+ ions from mito-chondria and a concurrent accumulation of H^+ ions within these organelles.[3] This property can be ex-plained through the ability of these antibiotics to form lipo-soluble complexes with the monovalents cations. The study of the complexation of alkali metal cations by the grisorixin provides information pertaining to the behaviour of this compound.

The lipophilic nature of grisorixin and its salts pro-hibits a study in water. Measurements on this sort of antibiotic are generally carried out in a polar medium, such as methyl alcohol which is closely related to water. Relative effects are expected to be comparable in both solvents, therefore methanol can be used to infer the properties in aqueous media such as are found under physiological conditions.

A conductimetric method was used. Unlike potentio-metry and enthalpimetry, conductimetry, although more precise, has been rarely used for similar measure-ments on other antibiotics.

The following equilibria are considered: the acid

We have also studied the e.m.f. of a galvanic cell com-posed of liquid phases. Such a cell allows independent information to be obtained on the selectivity of grisorixin for various cations.

METHODS AND RESULTS

Acid Dissociation of Grisorixin in Methyl Alcohol.—The pK_a of grisorixin was obtained from potentiometric titration of the acid by tetrabutylammonium methoxide. This base was preferred to alkali metal methoxide since the tetra-butylammonium salts are, unlike alkali metal salts, strongly dissociated in this medium. The standardization, measure-ments and calculations were done as previously described.[4a] A pK_a of 9.83 ± 0.05 (molar scale of concentration) was obtained, slightly higher than that obtained for other common carboxylic acids in the same solvent (acetic acid pK_a 9.41; benzoic acid pK_a 9.16).[4a]

Dissociation of Grisorixin Salts in Methyl Alcohol.—The conductimetric study shows that the silver and thallium salts are weak electrolytes as are the alkali metal salts except lithium which is relatively strong. On the contrary, the tetrabutylammonium salt is a strong electrolyte. The phoreogram[4b] $\Lambda = f(c^{1/2})$ is almost a straight line. Results obtained using concentrations from 3×10^{-4} to 2×10^{-3}M were treated within the scope of the Fuoss–Onsager theory.

[1] Part I, P. Gachon, A. Kergomard, C. Esteve, and T. Staron, *J. Antibiotics*, in the press; Part II, P. Gachon and A. Kergo-mard, *ibid.*, in the press; Part III, M. Duteurtre, A. Kergomard, G. Kergomard, and M. F. Lhomme, unpublished data.

[2] P. Gachon, A. Kergomard, H. Veschambre, C. Esteve, and T. Staron, *Chem. Comm.*, 1970, 1421.

[3] H. A. Lardy, S. N. Graven, and S. Estrada-O, *Fed. Proc.*, 1967, **26**, 1355.

[4] (a) J. Juillard, *Bull. Soc. chim. France*, 1966, 1727; (b) R. M. Fuoss and F. Accasina, 'Electrolytic Conductance,' Interscience, New York, 1959, pp. 266, 268.

A conductibility equation corresponding to fully dissociated electrolyte [5] was employed and limited to the c term (1) where Λ and $\Lambda°$ are molar conductivity and limiting

$$\Lambda = \Lambda° - Sc^{1/2} + Ec \log c + Jc \qquad (1)$$

molar conductivity, respectively, S and E are parameters dependent only on the properties of the solvent (viscosity η_0, dielectric constant ϵ), and J a term which is also dependent on an interionic distance parameter a. $\Lambda°$ and J were obtained using a method previously described from experimental results concerning (c, Λ) combinations.[6]
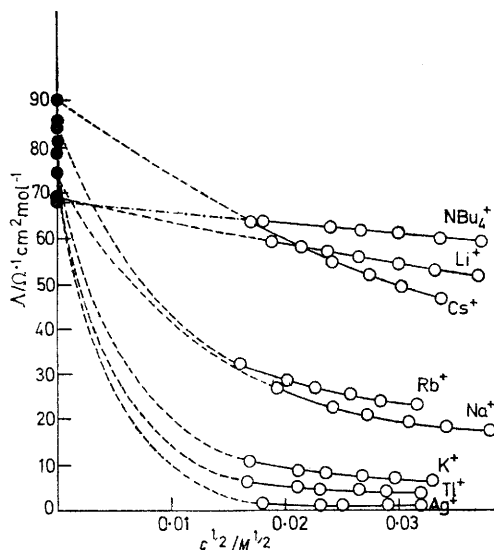

FIGURE 1 Conductivity of monovalent cation salts of grisorixin at 25 °C: molar conductivity *versus* square root of molarity

Mean values from three runs are $\Lambda° = 67.8 \pm 0.2$ and $J = 900 \pm 100$. This value of J is acceptable, corresponding to a value of 3 Å for the ionic distance parameter. If the critical Bjerrum distance [7] in methanol is taken for $a = (8.5 \text{ Å})$ and the Fuoss-Onsager equation for partially dissociated electrolyte is used, the dissociation constant K_d thus obtained is *ca.* 5×10^{-2}.

The molar conductivity of the grisorixin anion is calculated from the Kay value for the tetrabutylammonium cation: [8] $\lambda°(Bu_4N^+) = 38.9$ and thus $\lambda°$ $(Gr^-) = 28.9$. Molar conductivity of the alkali metal ions is obtained from the $\Lambda°$ values of their chlorides [9,10] using $\lambda°$ $(Cl^-) = 52.36$ (ref. 8).

The value for the silver ion is obtained from data for silver nitrate,[11] $\Lambda°$ $(AgNO_3) = 110.88$, and lithium nitrate,[12] $\Lambda°$ $(LiNO_3) = 100.7$. On account of the absence of information about Tl^+ in methanol in the literature, thallium nitrate was studied in the same manner as the tetrabutylammonium salt. A value of $\Lambda°$ of 115.9 was obtained.

The molar limiting conductivities of the grisorixin salts are calculated by the Kolraush relationship and dis-

sociation constants are determined from dissociation ratios corresponding to various values for c and Λ [equation (2)]

$$K_d = \alpha^2 cy/^\pm 1 - \alpha \qquad (2)$$

and the activity coefficient $y^\pm$ is obtained from the Debye-Hückel extended law (3), using Bjerrum's q as the ionic

$$\log_{10} y^\pm = -A I^{1/2}/1 + Bq I^{1/2} \qquad (3)$$

distance parameter. The dissociation ratios α are calculated using successive approximations, as in the MacInnes-Shedlovsky method; [13] the nth value of α is obtained from equation (4) with $\alpha_1 = \Lambda/\Lambda°$. Approximations can be frequently stopped at $n = 2$.

$$\alpha_n = \Lambda/[\Lambda° - S(c\alpha_{n-1})^{1/2}] \qquad (4)$$

A more sophisticated treatment was not found to be of any help, taking into account the accuracy of the measurements, except in the case of the weakly associated lithium salt. The Fuoss expression (5) [14] for α is then used where

$$\alpha = \Lambda/[\Lambda° f(z)] \qquad (5)$$

$z = S(c\Lambda)^{1/2}/(\Lambda°)^{3/2}$. $f(z)$ is a mathematical function of z tabulated by Fuoss. Results thus obtained are in the last column of Table 1.

TABLE 1

Conductimetric study of the dissociation of the monovalent cation salts of grisorixin at 25 °C

M+	Ionic radius (Å) (ref. 15)	$\lambda°$ (M+) [a]	pK_d [b]
Li+	0.86	39.7	2.39 ± 0.03 [c]
Na+	1.12	44.9	4.01 ± 0.03
K+	1.44	52.5	4.98 ± 0.09
Rb+	1.58	55.9	4.11 ± 0.07
Cs+	1.84	61.0	3.22 ± 0.03
Ag+	1.27	49.9	6.86 ± 0.08
Tl+	1.54	54.9	5.84 ± 0.06

[a] Ω^{-1} cm² mol⁻¹. [b] Mean values from three runs corresponding to concentrations from 5×10^{-4} to 5×10^{-3}M. [c] Mean deviation.

A direct treatment,[14] less precise but allowing both $\Lambda°$ and K_d to be obtained, was also applied to the conductimetric results for lithium grisorixinate. It yielded values comparable to the previous ones, $\Lambda° = 67$ and p$K_d = 2.4$. Then the $\lambda°$ value of grisorixinate ion, which appears to be high, considering the size of this ion, is confirmed.

Dissociation of Complexes formed by Grisorixin and its Methyl Ester with Monovalent Cations in Methyl Alcohol.— Formation of complexes with potassium was studied using a potentiometric method. The e.m.f. of the galvanic cell composed of a cationic glass electrode and the reference electrode, here described, is given by a formula of the kind (6) in the absence of any cation other than K^+.

$$E = E^\ominus + RT \log c(K^+)y^\pm/f \qquad (6)$$

E Measurements for various concentrations of KBr and calculation of activity coefficients through the extended Debye-Hückel law allow $E^\ominus$ to be determined. The constancy of $E^\ominus$ values obtained (standard deviation

[5] R. M. Fuoss, *J. Amer. Chem. Soc.*, 1959, **81**, 2659.
[6] J. Juillard, J. P. Morel, and L. Avedikian, *J. Chim. phys.*, 1972, **69**, 787.
[7] J. C. Justice, *J. Chim. phys.*, 1968, **65**, 353.
[8] R. L. Kay, C. Zawoyski, and D. F. Evans, *J. Phys. Chem.*, 1965, **69**, 4208.
[9] R. L. Kay, *J. Amer. Chem. Soc.*, 1960, **82**, 2099.

[10] R. L. Kay, *J. Phys. Chem.*, 1965, **69**, 2787.
[11] R. E. Busby and V. S. Griffiths, *J. Chem. Soc.*, 1963, 902.
[12] J. Lange, *Z. phys. Chem.* (*Leipzig*), 1941, **A188**, 284.
[13] D. MacInnes and T. Shedlovsky, *J. Amer. Chem. Soc.*, 1932, **54**, 1429.
[14] R. M. Fuoss, *J. Amer. Chem. Soc.*, 1935, **57**, 488.
[15] M. F. C. Ladd, *Theor. Chim. Acta*, 1969, **12**, 333.

<1 mV) is a good justification for the method used; it shows that the reference electrode diffusion potential is practically constant. Addition of ligand in increasing amounts allows the determination of the dissociation constant from the calculation of the K^+ free ion concentration [equation (7)] where c_s and c_a are the analytical

$$K_d = \frac{c(K^+) \times c(GrH)}{c(GrH, K^+)} = \frac{c(K^+) \times [c_a - c_s + c(K^+)]}{c_s - c(K^+)} \quad (7)$$

molar concentrations respectively of salt and ligand.

In the case of grisorixin, the absence of Gr^- ion was assumed, which is justified from knowledge of the acid pK_a (9·8) and assay of pH during the salt addition. It varies from 7·6 to 7·4 for a concentration range of 1·1 $\times$ 10^{-3}—3 $\times$ 10^{-3}M in KBr. Dissociation constants for the K^+ complexes of grisorixin and its ester are respectively 2·66 $\pm$ 0·02 (mean value from five determinations) and 2·27 $\pm$ 0·04 (mean value from 12 determinations). It was not possible to study sodium complexation using this method, the formation constant being too weak. A study of the thallium complex with grisorixin ester was made by a conductimetric method proposed by Evans.[16] Association is very weak, $pK = 0·5$.

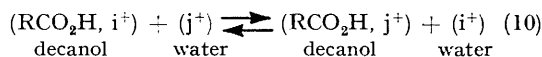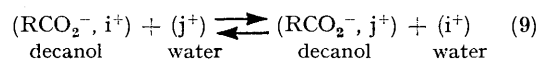*Selectivity of Grisorixin for Various Alkali Metal Cations from Liquid Membrane Electrode Measurements.*—The e.m.f.
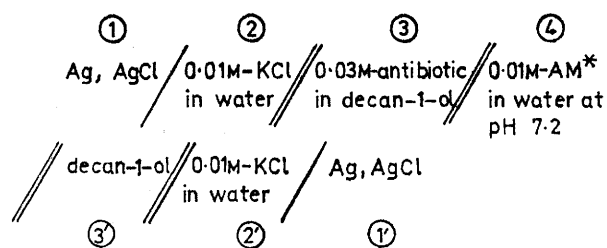
(A)

* A = anion, M = metal

of a galvanic cell such as (A) was measured. This method has been developed by Simon *et al.*[17] The measured e.m.f.s are indicative of grisorixin selectivity for various cations. Considering the symmetry of such a cell it can be assumed that the e.m.f. depends only on the nature of the cations in the two aqueous phases ② and ④. Therefore it can be expressed as (8) where a_i is the ionic activity and

$$E = \frac{RT}{F} \log \frac{④a_i + ④K_{ij}④a_j + ④K_{ik}④a_k + \cdots}{②a_i + ②K_{ij}②a_j + ②K_{ik}②a_k + \cdots} \quad (8)$$

$$(RCO_2^-, i^+) + (j^+) \rightleftharpoons (RCO_2^-, j^+) + (i^+) \quad (9)$$
decanol $\quad$ water $\qquad$ decanol $\qquad$ water

$$(RCO_2H, i^+) + (j^+) \rightleftharpoons (RCO_2H, j^+) + (i^+) \quad (10)$$
decanol $\quad$ water $\qquad$ decanol $\qquad$ water

K_{ij} can be taken, as a first approximation, as being identical to the constants of heterogenous equilibria such as (9) and (10).

An extended discussion of the meaning of the K_{ij} constants for the various kinds of ligands has been provided.[18] If AM is KCl in phase ④, equation (8) reduces to

[16] D. F. Evans, S. L. Wellington, J. A. Nadis, and E. L. Cussler, *J. Solution Chem.*, 1972, **1**, 499.

[17] W. K. Lutz, H. K. Wipf, and W. Simon, *Helv. Chim. Acta*, 1970, **53**, 1741.

(11) which can be experimentally verified. If AM is

$$E = \frac{RT}{F} (\log{}^④a_{K^+} - \log{}^②a_{K^+}) \approx 0 \quad (11)$$

different from KCl one obtains (12). The e.m.f. of the

$$E = \frac{RT}{F} \log \frac{K_{K^+,M^+}{}^④a_{M^+}}{{}^②a_{M^+}} \approx \frac{RT}{F} \log K_{K^+,M^+} \quad (12)$$

galvanic cell is thus related to the exchange constants of the previous equilibria. The results obtained correspond to the following sequence of selectivity: $Ag^+ > Tl^+ \sim K^+ > NH_4^+ > Rb^+ > Na^+ > Cs^+ \gg Li^+$.

DISCUSSION

There is good agreement between the two selectivity scales obtained, one from the determination of the dissociation constants of the salts in methanol, the other from e.m.f. measurements of liquid phase galvanic cells. This is clearly shown in Figure 2.

TABLE 2

Dissociation of some electrically neutral complexes (nigericin group) and positively charged complexes (valinomycin group) for sodium and potassium in methanol at 25 °C (pK_d)

		K^+	Na^+	References *
Nigericin	Monensin	4·6	6·0	a
group	Nigericin	5·6	3·9	a
	Grisorixin	4·9	4·0	
Valinomycin	Valinomycin	3·9	1·0	b
group	Enniatin B	2·9	2·4	b

* For an extensive tabulation of stability constants for carrier antibiotic complexes see ref.18.

^{a} W. K. Lutz, P. U. Früh, and W. Simon, *Helv. Chim. Acta*, 1971, **54**, 2767. ^{b} H. K. Wipf, L. A. R. Pioda, Z. Stefanac, and W. Simon, *Helv. Chim. Acta*, 1968, **51**, 377.

From the determination of the dissociation constant it can be concluded that there is no strong selectivity for
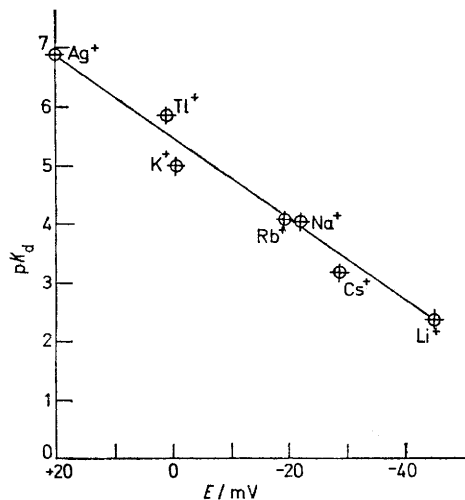

FIGURE 2 Comparison between the variation of the dissociation constant of the grisorixin salts and the e.m.f. of liquid phase galvanic cell for the same cations

a single cation as observed in the case of some macrocyclic neutral antibiotics (Table 2). For example

[18] W. Simon, W. E. Morf, and P. C. Meier, in ' Structure and Bonding,' Springer Verlag, Berlin, 1973, vol. 16, p. 113.

K^+/Na^+ selectivity is <15. Nevertheless if one considers the series of alkali metal cations, a weaker association is obtained for both the smallest and the largest: Li^+ (r 0.86 Å) and Cs^+ (r 1.84 Å); pK_d is 4—5 for the cations of intermediate ionic radius.

The occurrence of a selectivity maximum for K^+ among the alkali metal cations, shown in Figure 3, can be related to the existence of an equilibrium cavity where the steric interactions are minimised. A maximum of stability would be obtained, taking into account the size of the cavity, for a particular value of the ionic radius. For small cations such as Li^+, the low stability of the complexes can be attributed to the large distance between the ligand sites and the cation. For large cations such as Cs^+, it can be related to a noticeable
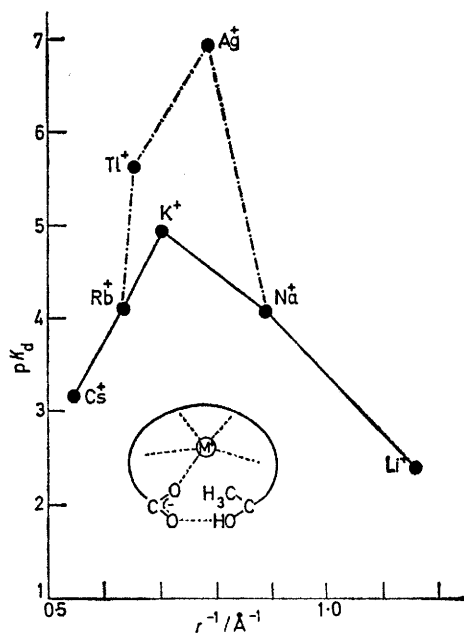

FIGURE 3 Variation of dissociation constants of grisorixin salts with the size of the cation (r = ionic radius)

increase in this of the cavity. The crystallographic studies of Ag^+ and Tl^+ salts confirm the existence of such a cavity.[19]

However, a more refined analysis can be proposed. Solvation Gibbs energies (passage from standard gaseous state to standard state in methanol) of alkali metal cations are known or can be calculated from results in the literature. Solvation of the complex metal salts is essentially a function of its external shape. So it seems a worthwhile assumption that solvation Gibbs energies of all alkali metal cations complexes are identical. This allows us to obtain intrinsic Gibbs energies of complexation for alkali metal cations; such intrinsic energies can be obtained in the absence of solvation for example in the gaseous state. The intrinsic Gibbs energy of complexation vary in a regular way with the cation size, lithium being the more associated.

[19] M. Alleaume and D. Hickel, Chem. Comm., 1970, 1422; 1972, 175; unpublished data.

The effects found here in methanol and probably arising in most other solvents (a weak variation in association depending on the nature of the cation and a maximum of selectivity for a given size of the complex ion) are thus due to a compensation. The experimental complexation Gibbs energy is the algebraic sum for an intrinsic complexation energy decreasing with the size of the cation and a solvation energy increasing with the size of the cation.

Table 2 shows the similarity for K^+/Na^+ selectivity between nigericin and grisorixin, although not with monensin. The difference between grisorixin and monensin is thus found at the level of the intrinsic Gibbs energies of complexation. These energies decrease more rapidly for the former antibiotic than for the latter, when the cation size increases; this can be related to the fact that the cavity of monensin is of smaller dimensions. Such an assumption, on the size of cavities, was proposed by Simon et al.[18] They thus justified the difference found between nigericin and monensin, which are due, according to these authors, to the deformation energy necessary to adapt the size of their preformed cavity to the cationic radius.

The study of the dissociation of the complexes formed by grisorixin and its methyl ester shows that the complexing power of the chain alone is weak. The neutralisation of the cationic charge by the carboxylate anion increases the stability of the complex in that the association increases by a factor of 200. This amply supports the importance of the carboxy-function in the mechanism of complexation for this kind of ionophore [20] and may explain the antibiotic inactivity observed for the methyl ester.

EXPERIMENTAL

Preparation of Grisorixin Salts.—The potassium salt was obtained by neutralisation in 50% ethanol and was crystallized from water–methanol. The silver salt was precipitated from an aqueous alcoholic solution of the potassium salt by addition of silver nitrate and then crystallized from water–acetone. The thallium salt was prepared by addition of thallium chloride to an aqueous alcoholic solution of the silver salt. It is obtained by evaporation of the solvent after filtration of the silver chloride precipitate and crystallized from water–acetone.

The purity of grisorixin and all its solid salts was checked carefully by elemental analysis and mass spectrometry (see ref. 1).

Stock solutions of the other salts were prepared by neutralisation of a given quantity of grisorixin using a concentrated solution of either methoxide (Na^+, Bu_4N^+, Cs^+) or of hydroxide (Cs^+, Rb^+) in methanol.

Tetrabutylammonium methoxide solutions in methanol were obtained by Cundiff and Markunas' method.[21] Pure anhydrous methanol was prepared as previously described.[4a]

Potentiometric Measurements.—A Radiometer pH meter 4 was employed. A calomel electrode was used as reference

[20] W. K. Lutz, F. K. Winkler, and J. D. Dunitz, Helv. Chim. Acta, 1971, 54, 1103.
[21] R. H. Cundiff and P. C. Markunas, Analyt. Chem., 1962, 34, 584.

with a junction composed of 0·1M-tetramethylammonium iodide in methanol. All experiments were carried out at 25·0 °C. Radiometer 202 B, sensitive to H$^+$, and the Beckman **39137** glass electrodes, sensitive to alkali metal cations, were used. Liquid membranes electrodes were prepared as described by Simon *et al.*[17] Conductimetric measurements were taken at 25·00 °C using a Beckman RCA 18 conductivimeter and a Tacussel dipping conductivity cell as previously described.[6]