Voltammetry as a Virtual Potentiometric Sensor in Modelling of a Metal/ Ligand System and Refinement of Stability Constants

Part 2

Differential-Pulse- and Sampled-Current-Polarographic and Virtual Free-Metal-Ion Potentiometric Study of a Bismuth(III)/Picolinic Acid/Hydroxide System

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A mathematical conversion of data from nonequilibrium and dynamic voltammetric techniques (direct-current-sampled (DC) and differential-pulse (DP) polarography) into potentiometric, free-metal-ion sensor-type data is described and employed in the study of Bi^{III} complexes with the ligand picolinic acid (= pyridine-2-carboxylic acid) (labile and dynamic metal/ligand system). A novel procedure that allows evaluation of experimental data collected at very low pH values (acid-base titration) is proposed. Software ESTA dedicated to potentiometry was successfully employed in the refinement operations performed with virtual potentiometric (VP) data obtained from DC and DP polarography, the latter being performed at fixed pH (ligand titration) as well as at fixed $[L_T]/[M_T]$ ratio (acid-base titration). It was possible to refine stability constants either separately from VP-DC or VP-DP, or simultaneously from any combination of VP-DC and VP-DP obtained from ligand and/or acid-base titrations. The concept of VP-DC or VP-DP is employed for the first time in the study of an unknown Bi^{III}/picolinic acid/OH system, and numerous documented and possible advantages are discussed. Five complexes of bismuth, ML, ML_2, ML_3, ML_4, and $ML_3(OH)$ and their stability constants (as log β) 7.48 ± 0.01, 13.94 ± 0.01, 18.10 ± 0.04, 20.47 ± 0.25, and 26.65 ± 0.03, respectively, are reported at 0.5M $(Na,H)NO_3$ ionic strength and T 298 K. The proposed procedure can be easily utilized also by non-electrochemists who are interested in, *e.g.*, ligand-design strategies.

1. Introduction. – Evaluation of stability constants usually involves solution of mass-balance equations written for the total metal-ion, ligand, and proton concentrations $[M_T]$, $[L_T]$, and $[H_T]$[1]), respectively. Refinement of stability constants that involves the simultaneous solution of these mass-balance equations is possible only if either the free-metal-ion, free-ligand, or free-proton concentration [M], [L], or [H], respectively, which can vary over a wide concentration range, can be monitored with high accuracy throughout an experiment. It is well known that a glass electrode (GE) is still the best potentiometric sensor with the widest linearity range. Thus, it is not surprising that glass-electrode potentiometry (GEP) became the most powerful and most widely used analytical technique in the study of metal/ligand equilibria [1]. An additional and very important advantage of GEP is that the variation in the free-proton concentration always takes place when any kind of an organic ligand involved in the protonation

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[1]) For convenience, charges of ions and the brackets that should enclose the formula of a complex are omitted.

reactions is investigated with any kind of a metal ion involved in the complex-formation reaction with a protonated form of a ligand. The widely spread application of GEP also resulted in the development of powerful dedicated software, such as ESTA [2–4] or SUPERQUAD [5], allowing a study of any kind of complex $M_pL(1)_{q1}L(2)_{q2}H_r$[1]).

Polarography, as all other voltammetric techniques, is a dynamic and in principle nonequilibrium analytical technique; it does not allow direct measurement of any of the above-mentioned free-ion concentrations. Polarographic measurements, however, result in a signal, whose position and intensity depends on the solution composition. Two main approaches to the analysis of polarographic data from which stability constants can be evaluated might be identified, namely an interpretation of a shift or of the intensity of a polarographic signal. The analysis of the shift as a function of an excess of added ligand at fixed pH started over 60 years ago with the *Lingane* equation [6] allowing an evaluation of a stability constant for a single complex ML_q present in a solution when a fully labile M/L system and reversible electrochemical process were observed. Significant progress was made by *DeFord* and *Hume* [7][8], who modified the *Lingane* equation to the form that allows evaluation of stability constants of several complexes ML_q formed in consecutive fashion under exactly the same experimental conditions as described by *Lingane*, *i.e.*, titration of a metal ion solution by a ligand at fixed pH with the strict requirement of full reversibility of the electrochemical signal recorded for a dynamic (labile) metal/ligand system. *Schaap* and *McMasters* [9] extended the *DeFord* and *Hume* methodology to systems involving two competing ligands. All the above methodologies showed a very limited field of applications as they were based on a simple data interpretation without involving mass-balance equations. Possibly the most significant contribution in establishing stability constants by polarography comes from the Swiss school of *Schwarzenbach* [10][11], who derived a simple equation allowing analysis of systems involving two metal ions competing for a single ligand when a fully inert (nonlabile) complex is formed. Among many theories and methodologies described in a book by *Crow* [12], there is not a single contribution that involves mass-balance equations and is typical for a GEP analytical procedure, *e.g.*, acid-base titration.

The interaction of metal ions with polyelectrolytes was also studied by polarography [13–18]. However, theoretical considerations were of such complexity that only simplified solutions could be reached [13–20]. The analytical procedures employed as well as exclusion of mass-balance equations lead to significantly restricted applications, related mainly to the formation of ML_q complexes investigated by a ligand titration at a fixed pH value.

New methodology and theory of speciation by polarography was recently developed and tested on numerous metal/ligand/OH systems that show a diverse polarographic behavior. Fully labile [21–23], inert [24][25], or mixed [26] metal/ligand systems on the polarographic time scale were investigated under typical (in principle) GEP experimental conditions, *i.e.*, at fixed $[L_T]/[M_T]$ ratio and varied pH (acid-base titration). A single mathematical expression and a concept of complex-formation curves [21][27] (experimental and theoretical) were used in modelling and refinement operations that involve the simultaneous solution of the mass-balance equations for $[M_T]$ and $[L_T]$, allowing also successful use of polarography in the study of polynuclear species [28]. It has been demonstrated that the objective function used previously [21–

28] in the refinement of polarographic data is of general nature, and it was possible to refine stability constants from potentiometric data generated by ion-selective electrodes (ISE) [29]. Recent work [30] has shown that it is possible to evaluate potentiometric (ISE) and polarographic results either from acid-base or ligand titrations by the same mathematical expression [21–29] indicating significant and general limitations of the ligand-titration procedure when power of modelling and accuracy in refinement of stability constants are concerned.

All polarographic (or more generally voltammetric) theories of and applications to speciation studies reported to date [6–9][12–39] involve the evaluation of the shift in the position of the signal, and it has never been attempted to predict theoretically the position of a signal (*e.g.*, $E_{1/2}$ or E_p) when metal/ligand equilibria were investigated; the theoretically predicted reading of the recorded signal is a foundation of refinement operations employed in dedicated software for potentiometric studies of metal-ligand equilibria [2–5]. Evaluation of the shift requires as accurate as possible information on a position of a polarographic signal obtained for the free metal ion only, *i.e.*, of $E(\mathrm{M})$. There are many significant limitations related to this, which are discussed below, among them the fact that the value of $E(\mathrm{M})$ is not always available from an experiment [23]. Due to the nature of polarographic and potentiometric experiments, it was and is impossible to use directly the well-developed and tested powerful programs, such as ESTA or SUPERQUAD, in the refinement of polarographic data.

The aim of this work is to test further a newly reported [40] mathematical treatment of polarographic (voltammetric) data that results in a type of data that is suitable for refinement by dedicated potentiometric software. The new concept of conversion of a dynamic, nonequilibrium voltammetric technique into a 'virtual' potentiometric sensor is verified here with an unknown labile $\mathrm{Bi^{III}}$/picolinic acid/OH system (picolinic acid = pyridine-2-carboxylic acid) studied by two voltammetric techniques and two analytical procedures, *i.e.*, the ligand and proton titrations. A novel methodology is proposed that allows studying highly acidic metal ions at very low pH values. This metal/ligand system could not be studied by the equilibrium potentiometric technique GEP as it was necessary to collect data at as low pH as 0.3 (0.5M acid solution) and at very low total metal ion concentration (below 10^{-4} M). Many advantages and future applications of the proposed procedure are also demonstrated and discussed.

2. Theory and Method of Calculation. – *Polarography.* It has been shown recently that *Eqn. 1* can be successfully employed in the modelling and refinement operations performed on data collected for – on the polarographic time scale – fully labile [21–23], inert [24][25], or mixed [26] metal/ligand systems. In this equation, $E(\mathrm{M})$ and $I(\mathrm{M})$, and $E(\mathrm{comp})$ and $I(\mathrm{comp})$ stand for the potential (either half-wave ($E_{1/2}$) or peak potential (E_p)) and current (either limiting-diffusion (I_d) or peak current (I_p)) in the absence and in the presence of metal complexes, respectively. In this study, $X(i)$ stands for either pH(i) or $[\mathrm{L_T}](i)$ for the ith polarogram recorded during acid-base (at fixed $[\mathrm{L_T}]/[\mathrm{M_T}]$ ratio) or ligand titration (at fixed pH), respectively, performed with the metal/ligand system under investigation.

$$(E(\mathrm{M}) - E(\mathrm{comp}))_{X(i)} - \frac{RT}{nF}\ln\left(\frac{I(\mathrm{comp})}{I(\mathrm{M})}\right)_{X(i)} = \frac{RT}{nF}\ln\left(\frac{[\mathrm{M_T}]}{[\mathrm{M}]}\right)_{X(i)} \qquad (1)$$

The left-hand side of *Eqn. 1* is calculated directly from experimental data provided that $E(\mathrm{M})$ is available from the experiment. The plot of the left-hand side of *Eqn. 1 vs.* either pH or log [L] generates the experimental complex-formation curve (ECFC). The ECFC is used as the experimental objective function that has to be reproduced by a theoretical function, the computed complex-formation curve (CCFC), *i.e.*, the right-hand side of *Eqn. 1.* The refinement of stability constants is achieved by the computation of the free-metal-ion concentration [M] from mass-balance equations written for the assumed model. The computed [M] is used by the right-hand side of *Eqn. 1.* During the iterative operation, the values of refined stability constants are varied. The refinement operation is completed when the CCFC fits best the experimental objective function, *i.e.*, the ECFC.

The total metal ion and ligand concentrations $[\mathrm{M_T}]$ and $[\mathrm{L_T}]$ are known at any stage of the experiment. They can be expressed by the mass-balance equations, *i.e.*, *Eqns. 2* and *3*, where $\beta_{\mathrm{M_pL_qOH_r}}$ and $\beta_{\mathrm{k}}^{\mathrm{H}}$ stay for the overall stability and protonation constant, respectively, and [M], [L], [H], and [OH] are the free-metal-ion, free-ligand, proton, and hydroxide concentration, respectively. In case of the formation of complexes involving the protonated form of a ligand, *e.g.*, MLH, the term $[\mathrm{M}]^p[\mathrm{L}]^q[\mathrm{OH}]^r$ would be replaced by $[\mathrm{M}]^p[\mathrm{L}]^q[\mathrm{H}]^r$. For the final metal/ligand system proposed in this work, *Eqns. 2* and *3* would become *Eqns. 4* and *5*, where $K_{\mathrm{n}}^{\mathrm{H}}$ stands for the stepwise protonation constants of the ligand picolinic acid. The stability constants that were refined are printed in boldface. The stepwise protonation constants [41] of the ligand picolinic acid and the hydrolysis constants for Bi$^{\mathrm{III}}$ [41] were kept fixed during the refinement operations.

$$[\mathrm{M_T}] = [\mathrm{M}] + \sum_{p=1}\sum_{q=1}\sum_{r=0} p\beta_{\mathrm{M}_p\mathrm{L}_q\mathrm{OH}_r}[\mathrm{M}]^p[\mathrm{L}]^q[\mathrm{OH}]^r + \sum_{x=1}\sum_{y=1} x\beta_{\mathrm{M}_x\mathrm{OH}_y}[\mathrm{M}]^x[\mathrm{OH}]^y \quad (2)$$

$$[\mathrm{L_T}] = [\mathrm{L}] + \sum_{p=1}\sum_{q=1}\sum_{r=0} q\beta_{\mathrm{M}_p\mathrm{L}_q\mathrm{OH}_r}[\mathrm{M}]^p[\mathrm{L}]^q[\mathrm{OH}]^r + [\mathrm{L}]\sum_{k=1}\beta_k^{\mathrm{H}}[\mathrm{H}]^k \quad (3)$$

$$\begin{aligned}[\mathrm{M_T}] = {} & [\mathrm{M}] + \boldsymbol{\beta}_{\mathbf{ML}}[\mathrm{M}][\mathrm{L}] + \boldsymbol{\beta}_{\mathbf{ML_2}}[\mathrm{M}][\mathrm{L}]^2 + \boldsymbol{\beta}_{\mathbf{ML_3}}[\mathrm{M}][\mathrm{L}]^3 + \boldsymbol{\beta}_{\mathbf{ML_4}}[\mathrm{M}][\mathrm{L}]^4 \\ & + \boldsymbol{\beta}_{\mathbf{ML_3OH}}[\mathrm{M}][\mathrm{L}]^3[\mathrm{OH}] + \beta_{\mathrm{M(OH)}}[\mathrm{M}][\mathrm{OH}] + \beta_{\mathrm{M(OH)_3}}[\mathrm{M}][\mathrm{OH}]^3 \\ & + \beta_{\mathrm{M(OH)_4}}[\mathrm{M}][\mathrm{OH}]^4 + 6\beta_{\mathrm{M_6(OH)_{12}}}[\mathrm{M}]^6[\mathrm{OH}]^{12} + 9\beta_{\mathrm{M_9(OH)_{20}}}[\mathrm{M}]^9[\mathrm{OH}]^{20} \\ & + 9\beta_{\mathrm{M_9(OH)_{21}}}[\mathrm{M}]^9[\mathrm{OH}]^{21} + 9\beta_{\mathrm{M_9(OH)_{22}}}[\mathrm{M}]^9[\mathrm{OH}]^{22} \end{aligned} \quad (4)$$

$$\begin{aligned}[\mathrm{L_T}] = {} & [\mathrm{L}] + K_1^{\mathrm{H}}[\mathrm{H}][\mathrm{L}] + K_1^{\mathrm{H}}K_2^{\mathrm{H}}[\mathrm{H}]^2[\mathrm{L}] + \boldsymbol{\beta}_{\mathbf{ML}}[\mathrm{M}][\mathrm{L}] + 2\boldsymbol{\beta}_{\mathbf{ML_2}}[\mathrm{M}][\mathrm{L}]^2 \\ & + 3\boldsymbol{\beta}_{\mathbf{ML_3}}[\mathrm{M}][\mathrm{L}]^3 + 4\boldsymbol{\beta}_{\mathbf{ML_4}}[\mathrm{M}][\mathrm{L}]^4 + 3\boldsymbol{\beta}_{\mathbf{ML_3(OH)}}[\mathrm{M}][\mathrm{L}]^3[\mathrm{OH}] \end{aligned} \quad (5)$$

The refinement procedure employed in this work was performed according to the following steps. One starts with initial guesses for the values of stability constants $\boldsymbol{\beta}_{\mathbf{ML}}$,

$\boldsymbol{\beta}_{\mathbf{ML_2}}$, $\boldsymbol{\beta}_{\mathbf{ML_3}}$, *etc.* The initial guesses for stability constants can easily be calculated performing a single-point calculation at a particular pH value from *Eqn. 6*, where the free-ligand concentration [L] is obtained from a simplified form of *Eqn. 5*, solved with the assumption that all the ligand is involved only in the protonation reactions; [H] represents either the free-proton or -hydroxide concentration and is obtained from a direct pH measurement. *Eqns. 4* and *5* must hold at any stage of the experimental procedure (*e.g.*, after each addition of the standardized NaOH solution) and can be written in a simplified form as *Eqns. 7* and *8*, where exp and calc stand for experimental and calculated values. *Eqns. 7* and *8* are solved at each pH(i) value simultaneously (with particular values of refined stability constants) for the free-metal-ion and free-ligand concentrations. The free-metal-ion concentration obtained at each pH(i) value is then used in the right-hand side of *Eqn. 1*. The value generated by the right-hand side of *Eqn. 1* is compared with the left-hand side of *Eqn. 1* (fitting the computed complex-formation curve into the objective function, *i.e.*, the experimental complex-formation curve). This is followed by a change in the refined values of stability constants in such a way that the computed free-metal-ion concentration from *Eqns. 4* and *5* will reproduce best the objective function ECFC for all experimental points collected.

$$(E(\mathrm{M}) - E(\mathrm{comp}))_{X(i)} - \frac{RT}{nF}\ln\left(\frac{I(\mathrm{comp})}{I(\mathrm{M})}\right)_{X(i)} = \frac{RT}{nF}\ln\beta_{\mathrm{ML}_q\mathrm{H}_r} + q\frac{RT}{nF}\ln[\mathrm{L}]_{X(i)} + r\frac{RT}{nF}\ln[\mathrm{H}]_{X(i)} \tag{6}$$

$$\{[\mathrm{M_T}](\mathrm{exp})\}_{\mathrm{pH}(i)} = \{[\mathrm{M_T}](\mathrm{calc})\}_{\mathrm{pH}(i)} \tag{7}$$

$$\{[\mathrm{L_T}](\mathrm{exp})\}_{\mathrm{pH}(i)} = \{[\mathrm{L_T}](\mathrm{calc})\}_{\mathrm{pH}(i)} \tag{8}$$

Note that refinement operations employed in the evaluation of polarographic data do not include the mass-balance equation for the total-proton concentration [$\mathrm{H_T}$]. This is the fundamental difference between the voltammetric and potentiometric theories and mathematical procedures employed. The free-proton or -hydroxide concentrations ([H] and [OH]) were obtained in this work directly from the pH measurements by the calibrated glass electrode and K_w for water (here, the value for K_w at 25° and $I = 0.5\mathrm{M}$ was used [41]).

Glass-Electrode Potentiometry (GEP). The essence of the potentiometric experiment involving a glass electrode is the monitoring of the change in the free-proton concentration throughout the experiment. The initial total proton concentration [$\mathrm{H_T}$] (usually coming from the addition of a known amount of a standardized acid solution and often from the concentration of an acidic form of a ligand; *e.g.*, in this study, the ligand used is an acid with one COOH group) must be known as accurately as possible. The total proton concentration is known throughout the experiment since a standardized base solution is used, and K_w is included in the calculations. The free-proton concentration [H] varies throughout the experiment due to, *e.g.*, neutralization of a mineral acid or the formation of metal-containing complexes, *e.g.*, $\mathrm{M + HL = ML + H}$ or $\mathrm{M + 2\ HL = ML_2 + 2\ H}$. The change in [H] is monitored by the direct readings of the potential of a calibrated GE. The refinement operations used in potentiometry involve solving mass-balance equations, including the equation for the total proton concentration $[H_T]$, in such a way that the computed free-proton concentration, when used by the equation describing the response of the calibrated GE, reproduces the experimentally recorded potential of the GE as accurately as possible. Note that, in GEP experiments, well-defined mathematical expressions are used to directly compute the electrode response; this is another fundamental difference between GEP and voltammetry since, in the latter case, not the recorded potential but the shift of the potential is accounted for. This means that the ultimate experimental objective function in the GEP experiment is the variation in the recorded potential of the calibrated GE. All the above considerations related to optimization procedures employed in GEP have been well-known for years. They are described here in some detail to show clearly that the optimization operations employed in potentiometry are very much different from those used in polarography as described above.

It is also important to realize that, in case of potentiometry, the change in [H] must arise mainly from the formation of metal complexes to provide necessary information about the formation of complexes; otherwise a simple acid-base titration is performed. From this it becomes clear that potentiometry cannot be used in the study of metal complexes at very low pH values (below 2) with commonly employed $[M_T]$ in the region of 10^{-3} M. Polarography, on the other hand, can be performed below pH 2 [23][25][40][42] as it does not involve mass-balance equations for the total proton concentration.

Virtual Potentiometry. Polarography is a dynamic electrochemical technique, and any attempt to rigorously describe processes occurring at the electrode–solution interface must involve many complex processes, among them thermodynamics, kinetics (homogeneous and heterogeneous), transport, *etc.* Often, it is impossible to arrive at a solution that would allow theoretical reproduction of observed polarograms. This is the reason why, in polarography, the change in the observed signal, *i.e.*, $\Delta E_{1/2}$ or ΔE_p, rather than the theoretically predicted position of a curve is used when the study of metal complexes is of interest. This generally employed approach has, however, a significant weakness, and this is seen from *Eqn. 1*, where all recorded curves (*i.e.*, $\{E(\text{comp})\}_{\text{pH}(i)}$) are compared with a single value of $E(\text{M})$. It is well known that the nonlinear-curve-fitting operations will generate more-reliable values for fitted parameters with an increase in the number of experimental points recorded. The problem with refinement of many polarographic experimental data points collected with a solution adjusted to many pH(i) values is that all the recorded data are compared with a single experimental point, *i.e.*, the shift in the peak or half-wave potential is used and not the observed values (E_p or $E_{1/2}$) at each pH(i). As a result, the computed refined stability constants must change with the change in the value of $E(\text{M})$ even though the metal/ligand model, the overall fit of CCFC in ECFC, and standard deviations in stability constants will remain virtually the same. This means that one has to establish the value of $E(\text{M})$ with accuracy and certainty as high as possible. Unfortunately, it is not always possible to obtain a reliable value for $E(\text{M})$ [23]. A suitable example might be the study of highly acidic metal ions, such as Bi^{III}. Bismuth undergoes hydrolysis already at pH 0, which means that the recorded polarographic curve represents two bismuth species, Bi(aq) and Bi(OH). This implies that the value of $E(\text{M})$ is not directly available from the experiment. One of the advantages of studying metal complexes by polarography is the possibility of working with a large excess of ligand. This, however, might introduce significant uncertainty in the $E(\mathrm{M})$ value, which might be different in the absence and the presence of a large concentration of a ligand.

The term $I(\mathrm{comp})/I(\mathrm{M})$ in *Eqn. 1* represents the normalized change in the intensity of the recorded polarographic signal. The change in the recorded current (I_d or I_p) can be attributed to a change in the diffusion coefficients of different labile metal complexes formed [12]; this is an assumption made by *Lingane*, *DeFord*, and *Hume* and others who followed their assumptions. However, the voltammetric signal can also change due to the formation of a polarographically inactive metal complex [24–26][28]. The intensity of the recorded signal will also decrease with a decrease in the electrochemical reversibility; if this takes place, then direct-current-sampled polarography (DCP) is the technique recommended by us, as the limiting diffusion-controlled current does not depend on the degree of the electrochemical reversibility. From the above, it is clear that one is not able to distinguish all components that cause the change in the intensity of the recorded voltammetric signals.

For the purpose of this study, we assumed that, regardless of the cause of the variation in the intensity of the recorded signal, the term $(RT/nF)\ln(I(\mathrm{comp})/I(\mathrm{M}))$ can be interpreted as a fraction of a potential by which the recorded polarographic signal should have shifted more towards negative potentials, provided that there was no change in the value of the intensity of the recorded polarographic wave or peak. If the above supposition holds, the theoretically and thermodynamically expected position of the reversible polarographic signal along the potential scale ($E_{1/2}$ or E_p) can be written as in *Eqn. 9*, and it can be calculated at any $X(i)$ from available experimental data. There is a well-defined relationship between $E_{1/2}$ and the standard redox potential if the system under investigation can be regarded as thermodynamically fully reversible. For the purpose of this study, *i.e.*, the formation of metal complexes, the redox system considered would be $\mathrm{M}^{n+} + n\mathrm{e}^- = \mathrm{M}^0$ and the proposed virtual half-wave potential $E_{1/2}$ (virt) might be interpreted as a thermodynamic potential of a virtual potentiometric probe generated from dynamic, nonequilibrium polarographic data; the virtual probe should be sensitive to the change in the free-metal-ion concentration when metal complexes are formed. This virtual potentiometric probe is *metal-ion nonspecific* and should be able to work for any metal ion that is polarographically active and is reduced reversibly. If all the above holds, the virtual potentiometric sensor should have *unlimited linear-response* with a theoretical *Nernst* slope. The experimental parameters, such as pulse height and width, are kept fixed throughout the whole differential-pulse polarographic experiment. This means that the concept of $E_\mathrm{p}(\mathrm{virt})$ must also be valid with an unlimited linear response shifted in E° by a fixed number of mV when compared with a linear response of a virtual probe generated from $E_{1/2}(\mathrm{virt})$ values. The concept of a virtual potentiometric sensor implies that it should be possible to employ potentiometric software, such as ESTA, in the refinement of stability constants by using, in principle, data coming from a dynamic and nonequilibrium electrochemical technique. The use of ESTA in the refinement of stability constants would have numerous advantages, and some of them will be discussed below.

$$\left(E(\mathrm{comp}) + \frac{RT}{nF}\ln\left(\frac{I(\mathrm{comp})}{I(\mathrm{M})}\right)\right)_{X(i)} = E(\mathrm{virt})_{X(i)} \tag{9}$$

3. Results and Discussion. – *Voltammetric Data from the Ligand Titration.* As was stated above, the mathematical treatment of polarographic data applies to fully reversible electrochemical processes. Initially, three polarograms were recorded with a solution containing only Bi^{3+} ions. Analysis of DC curves will be discussed in some detail here. To estimate the values of I_d and $E_{1/2}$, *Eqns. 10–12* were used in fitting experimental curves, where I_F, I_b, and I_{obs} stand for the reduction, background, and observed total current, n stands for a number of electrons (here, 3), E_{appl} is the stepwise applied potential at which the total current was recorded, and γ is a coefficient describing how steep the polarographic wave is; for the fully reversible system, γ should be equal to 1. Analysis of three ligand-titration experiments (data collected at pH 0.90, 1.37, and 1.85) showed no change in the electrochemical reversibility throughout the total-ligand concentration range $1 \cdot 10^{-4}$ to $1 \cdot 10^{-2}$ M. The coefficient γ was *ca.* 0.97 ± 0.01, and, for any practical application, this could be considered as a reversible reduction process. Most importantly, however, there was no change in the coefficient γ throughout the experiments; hence, the computation of the shifts in the half-wave potentials $\Delta E_{1/2}$ made perfect physical sense when the formation of complexes is concerned. Also the differential-pulse-polarography (DPP) curves did not show any significant variation in the peak width which was *ca.* 50 mV throughout. That made possible to include DPP results in the modelling and refinement operations.

$$I_F = I_d / \left(10^{\left(\left(n\gamma (E_{appl} - E_{1/2}) \right) / 0.05916 \right)} + 1 \right) \quad (10)$$

$$I_b = a + bE_{appl} \quad (11)$$

$$I_{obs} = I_F + I_b \quad (12)$$

Modelling of a metal/ligand system was based on the analysis of slopes [43] where, commonly accepted, the variation in the observed peak potential or half-wave potential is plotted *vs.* the free-ligand concentration. In addition, we demonstrate here that the analysis of the corrected shift, *i.e.*, the right-hand side of *Eqn. 1*, when plotted *vs.* the free-ligand concentration provides the correct answers (see *Fig. 1*). In *Fig. 1*, the points and the solid lines represent the experimental objective and theoretically computed functions, *i.e.*, the ECFC and CCFC, respectively, obtained at the indicated pH values. It is seen that the computed functions reproduced the experimental ones quite well, even though they represent different metal/ligand models. Moreover, the slope obtained at pH 0.90 approaches a value of *ca.* 19 mV per log [L] unit, indicating the formation of ML as a major metal-containing species. At pH 1.85, the slope approaches the value of *ca.* 36 mV per log unit, confirming the formation of complex ML_2 besides ML. The refinement operations were performed with software dedicated to voltammetry [44]. All known $Bi_x(OH)_y$ species were also included in the M/L/OH models refined at the three pH values (see *Table 1*). Results obtained from DCP and DPP might be regarded as almost identical at a particular pH value. Note that the complex ML_2 was included in the models refined at all pH values to achieve best fits, even though the slope analysis did not indicate the formation of this complex at pH 0.90 at all. In addition, at the highest pH value, the incorporation of the complex ML_3 resulted in a slightly better overall fit (*OF*; see *Eqn. 13*) and more-consistent computed stability

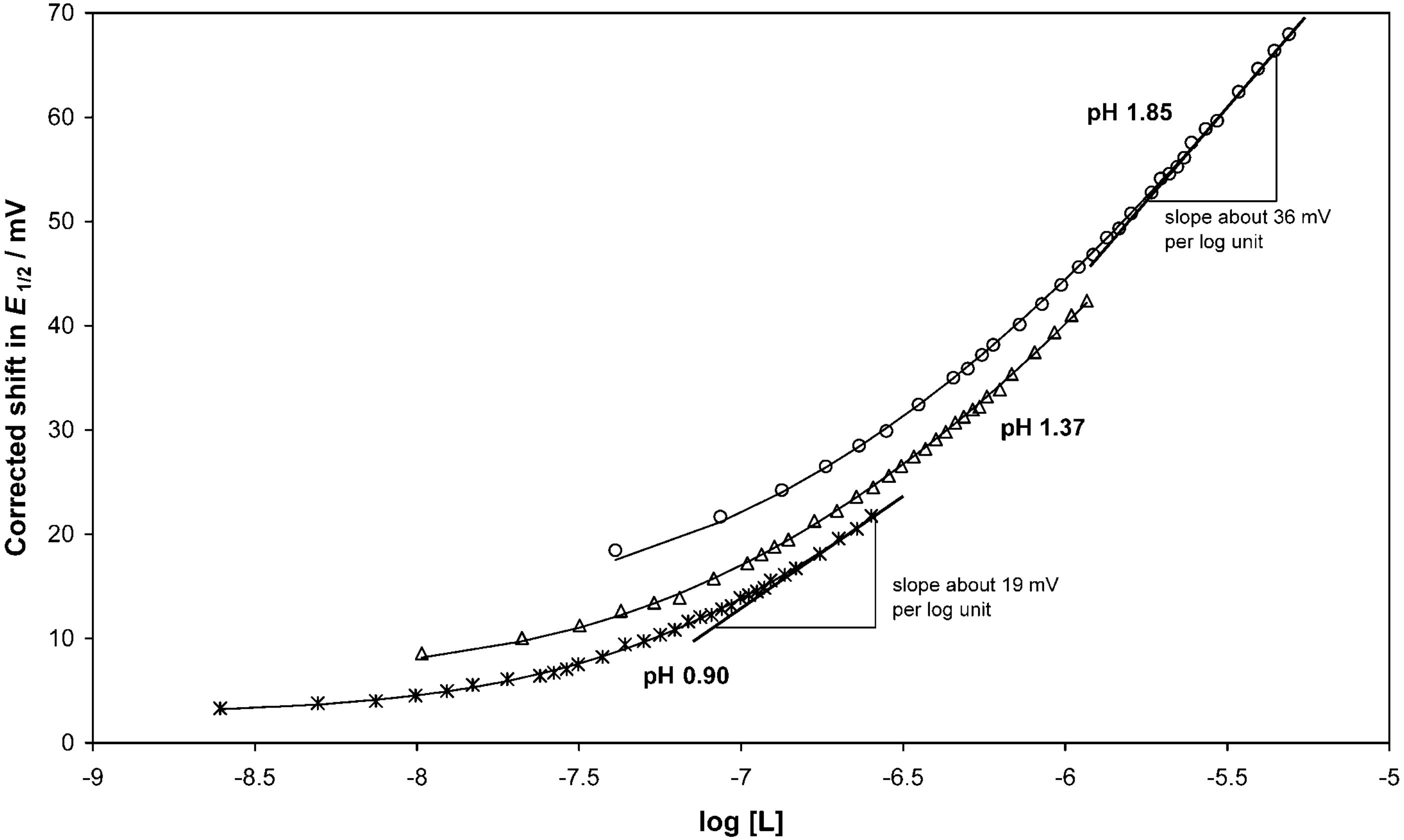

Fig. 1. *Complex-formation curves generated from the ligand titrations: experimental* (points, ○, △, *) *and computed* (—) *curves used in the refinement of real experimental DCP data obtained at the indicated pH values at 25° and* $\mu = 0.5$M. The free-ligand concentration was obtained from mass-balance equations solved with the refined stability constants of the BiL_j species included in the M/L/OH models (all known $Bi_x(OH)_y$ species shown in *Table 1* were included in the models). The total metal ion concentration was $3.95 \cdot 10^{-5}$, $4.65 \cdot 10^{-5}$, and $4.88 \cdot 10^{-5}$ M at pH 0.90, 1.37, and 1.85, resp. The $[L_T]/[M_T]$ ratio for all titrations varied between 2 and 240.

Table 1. *Values of the Formation Constants for Bi*^{III} *Complexes with Hydroxide* [41], *Protonation Constants of the Ligand* (L^-) *Picolinic Acid* [41], *and* pK_w *for Water* [41] *Used for the Computation of the Stability Constants and Species Distribution Diagrams at 25° and a Ionic Strength of 0.5*M *(Na,H)NO*$_3$

Equilibrium	Log β
$H^+ + OH^- \rightleftharpoons H_2O$	13.74
$Bi^{3+} + OH^- \rightleftharpoons Bi(OH)^{2+}$	12.42
$Bi^{3+} + 3\ OH^- \rightleftharpoons Bi(OH)_3$	31.88
$Bi^{3+} + 4\ OH^- \rightleftharpoons [Bi(OH)_4]^-$	32.98
$6\ Bi^{3+} + 12\ OH^- \rightleftharpoons [Bi_6(OH)_{12}]^{6+}$	162.78
$9\ Bi^{3+} + 20\ OH^- \rightleftharpoons [Bi_9(OH)_{20}]^{7+}$	266.92
$9\ Bi^{3+} + 21\ OH^- \rightleftharpoons [Bi_9(OH)_{21}]^{6+}$	276.76
$9\ Bi^{3+} + 22\ OH^- \rightleftharpoons [Bi_9(OH)_{22}]^{5+}$	287.30
$H^+ + L^- \rightleftharpoons HL$	5.17
$2\ H^+ + L^- \rightleftharpoons H_2L^+$	6.07

constants for ML_2. In *Eqn. 13*, n stands for the number of experimental points (polarographic curves) recorded with a particular metal/ligand-sample solution.

$$OF = \sqrt{\frac{\sum (\mathrm{ECFC} - \mathrm{CCFC})^2_{X(i)}}{n-1}} \quad [mV] \tag{13}$$

Virtual Potentiometry and Combined Refinement Operations on Data from the Ligand Titration. The dynamic voltammetric DCP and DPP data were converted into the virtual potentiometric type of data by use of *Eqn. 9*. The virtual potential, when plotted *vs.* the free-metal-ion concentration, resulted in a straight line as expected for the real potentiometric sensor generating its response towards the free-metal-ion concentration (in general, activity) according to the *Nernst* equation. Six linear responses are shown in *Fig. 2* representing two sets of virtual potentiometric (VP) data, VP-DC and VP-DP at all pH values (DC = direct current, DP = differential pulse). All the curves have an almost ideal *Nernst* slope for the three-electron redox couple $Bi^{3+} + 3e = Bi^0$ at 25°. All the curves representing a virtual DP potentiometric sensor are parallel to the VP-DC curves and shifted by *ca.* 22.5 mV, as expected for the applied differential pulse of 50 mV. Fitting all the experimental points with straight lines resulted in linear equations that, in this case, resemble the *Nernst* equation. For instance, let us consider the curve obtained from the DPP experiment at pH 1.85 that has the linear equation $y = 19.62x + 172.22$. This equation can be rewritten as $E(\mathrm{virt}) = 172.22 + 19.62 \cdot \log\ [\mathrm{M}]$ (in mV), and, in more-general form, as $E = E^\circ + (RT/nF) \cdot \ln[\mathrm{M}]$ (in V). As explained in the data-treatment section, dedicated potentiometric software requires two experimental parameters describing the response of the potentiometric sensor used, *i.e.*, the response slope and E°, as they are employed in the computation of the expected response of the sensor. Fitting the curves shown in *Fig. 2* provided these parameters. Since the electrochemical reduction process must be fully reversible and since the fitted curves have almost the ideal *Nernst* slope, it was assumed that it is reasonable to use the *Nernst* slope during the refinement operations performed with software ESTA. It was very pleasing to note that ESTA had no problem with the refinement of virtual potentiometric data (VP-DC and VP-DP) and that the

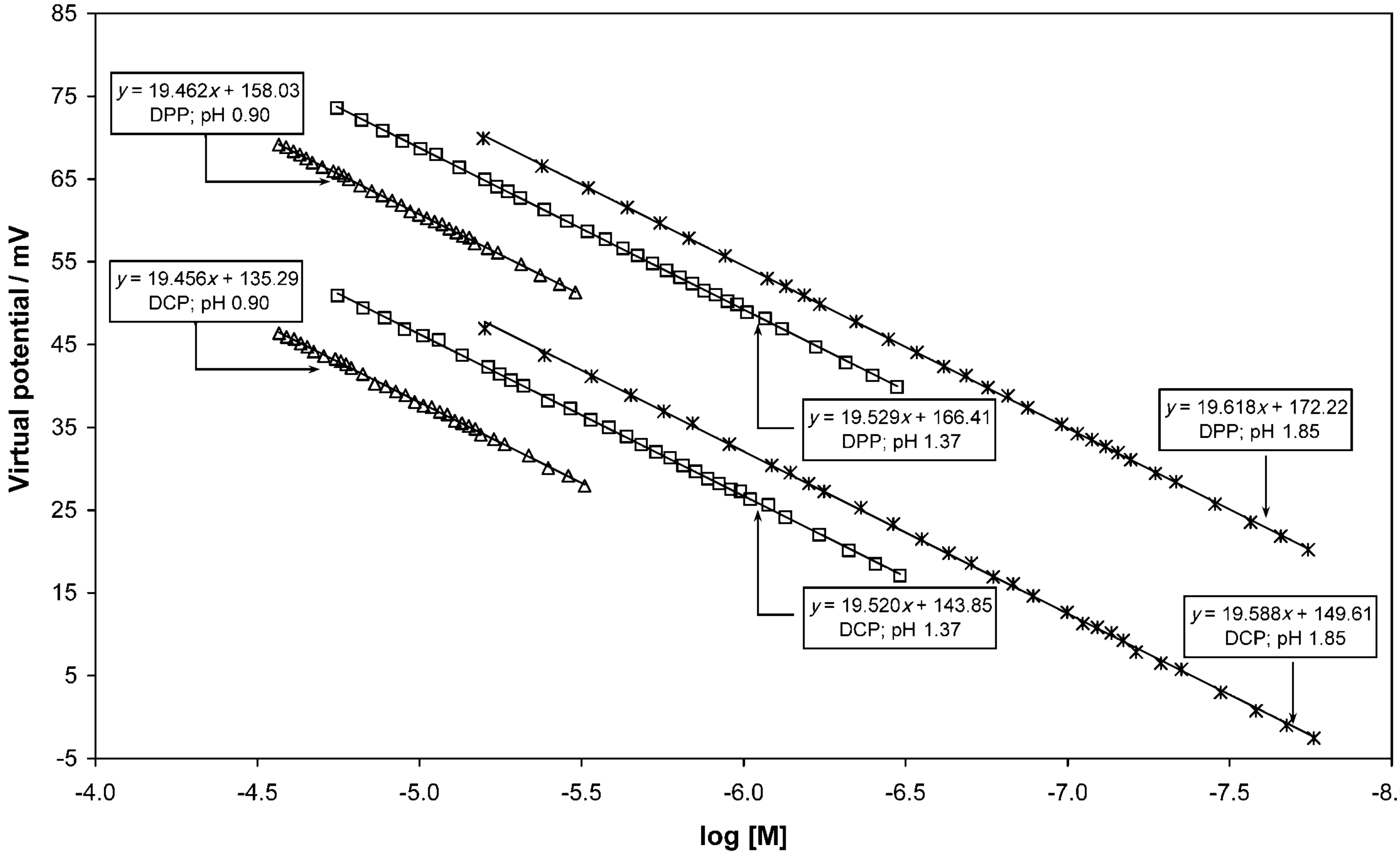

Fig. 2. *Response of virtual potentiometric sensors towards the free-*Bi^{III}*-ion concentration: virtual potentials* (points, *, □, △) *as calculated by* Eqn. 9, *solid-lines curves* (—) *from fitting the points at indicated pH values at which the ligand titration was performed*. Exper. conditions as for *Fig. 1.*

stability constants generated by ESTA were very much the same as those obtained from totally different refinement operations performed with real voltammetric data (see *Table 2*). It is important to stress that, in case of the VP data, the potential and not the shift in the potential was employed in the refinement operations; this is a fundamental difference between voltammetric and potentiometric theories and methodologies. It means that the peak (or half-wave) potential obtained with the solution of the metal ion (E_p(M) or $E_{1/2}$(M)) prior to the addition of a ligand was not taken into account at all.

The question arises now whether dedicated voltammetric software is necessary at all to obtain reliable stability constants and M/L/OH models. It was of great interest and importance to find out whether scientists who are using software such as ESTA and who are not trained electrochemists might make direct use of the proposed concept of the virtual potentiometric sensor. One must realize that the procedure described above,

Table 2. *Stability Constants for the Bi*^{III}*/Picolinic Acid/OH System Obtained from the Ligand Titrations Performed at the Indicated pH Values at* $[M_T] = 4.99 \cdot 10^{-5}$ M, *25°, and* I = *0.5*M *for* $[L_T]/[M_T]$ *Varying between 2 and 240.*

pH	Technique a)	Logβ			E°_{fin} (E°_{ini}) [mV] b)	R factor or O fit c)
		ML	ML$_2$	ML$_3$		
1.85	DCP	7.89 ± 0.03	13.99 ± 0.02	–	–	0.087
	DCP	7.91 ± 0.03	13.94 ± 0.02	18.50 ± 0.50	–	0.062
	DPP	7.88 ± 0.03	13.97 ± 0.02	–	–	0.027
	DPP	7.89 ± 0.04	13.94 ± 0.06	18.28 ± 0.79	–	0.017
	VP-DP	7.85 ± 0.01	13.90 ± 0.01	17.87 ± 0.19	172.29 (172 ± 30)	0.003
	VP-DC	7.80 ± 0.02	13.87 ± 0.01	17.88 ± 0.24	148.92 (149 ± 30)	0.007
	VP-DP and VP-DC	7.82 ± 0.01	13.88 ± 0.01	17.88 ± 0.32	d)	0.010
1.37	DCP	7.66 ± 0.03	13.78 ± 0.05	–	–	0.045
	DPP	7.64 ± 0.03	13.78 ± 0.05	–	–	0.022
	VP-DP	7.620 ± 0.005	13.662 ± 0.004	–	167.03 (166 ± 30)	0.002
	VP-DC	7.630 ± 0.009	13.666 ± 0.007	–	144.45 (144 ± 30)	0.005
	VP-DP and VP-DC	7.624 ± 0.002	13.664 ± 0.004	–	d)	0.003
0.90	DCP	7.50 ± 0.04	13.69 ± 0.18	–	–	0.027
	DPP	7.49 ± 0.04	13.61 ± 0.21	–	–	0.010
	VP-DP	7.480 ± 0.008	13.22 ± 0.05	–	159.24 (158 ± 30)	0.002
	VP-DC	7.487 ± 0.008	13.39 ± 0.04	–	136.49 (136 ± 30)	0.004
	VP-DP and VP-DC	7.483 ± 0.003	13.31 ± 0.03	–	d)	0.004
0.90, 1.37, and 1.85	VP-DP	7.45 ± 0.02	13.97 ± 0.02	–	d)	0.030
	VP-DC	7.47 ± 0.02	13.94 ± 0.01	–	d)	0.041
	VP-DP and VP-DC	7.46 ± 0.01	13.96 ± 0.01		d)	0.033

a) VP-DC and VP-DP stand for virtual potentiometric sets of data created from experimental DCP and DPP data, resp. b) E°_{fin} and E°_{ini} stand for the final (refined simultaneously with stability constants) and initial (the virtual E° value obtained from the analysis of the polarographic data (see *Fig. 2*) values of E° of a virtual potentiometric sensor, resp. c) The R factor is a statistical parameter generated by ESTA and applies to the VP data; O fit is a statistical parameter for voltammetric data and stands for the overall fit of CCFC in ECFC (± 1 mV). d) Refined E° values from appropriate individual titrations were used as fixed values during the refinement operations.

even though successful, involved refinement of real polarographic data as a necessary step to obtain the calibration plot of the virtual potentiometric sensor (*Fig. 2*). Let us assume now the following scenario. A set of polarograms was recorded on a sample solution adjusted to a particular pH (the ligand-titration procedure as described in the *Exper. Part*). Let us assume further that the polarogram of a solution of a metal ion (prior to the addition of the ligand) was not recorded at all. This implies that refinement of dynamic polarographic data could not be performed by use of dedicated software (such as that used in this work [44]) because shifts in the peak (or half-wave) potentials could not be computed. It implies that stability constants of complexes formed as well as E° of a virtual potentiometric sensor could not be established, hence, they are not known to the investigator. The only software available to a researcher is a commonly employed dedicated potentiometric program, such as ESTA, that requires direct potential measurements of a free-metal-ion-sensitive probe.

The proposed analytical procedure, describing treatment of data from DCP (in principle the same applies to any voltammetric technique), might be as follows. The recorded DC polarograms must be fitted to establish $E_{1/2}$, I_d, and coefficient γ for each recorded curve. Any program that allows nonlinear curve fitting might be used for that purpose. In case of a small change in the shape of the DC polarograms, *e.g.*, when a small departure from reversibility is observed, the corrected values in $E_{1/2}$ might be established by using a procedure described elsewhere [40]. Relationships, such as E(obs) *vs.* log [L], might be useful to identify at least some of the metal-containing species for which initial stability constants (used in refinement operations) might be calculated from *Eqn. 6*. Next, *Eqn. 9* can be used to calculate the set of $E_{1/2}(\text{virt})_{[\text{L}](i)}$ values. It is assumed that the virtual potentiometric sensor has a *Nernst* slope, hence, we are left with one operations parameter necessary for the potentiometric refinement, *i.e.*, E°. It is obvious that this parameter cannot be established directly from the polarographic experiment as the half-wave potential does not depend on the total metal ion concentration. The parameter E° must be provided to the program ESTA as an initial value to be refined simultaneously with the optimized stability constants. Initially, the E° values shown in *Fig. 2* were refined. It was pleasing to note that the refined E° values differed only by a fraction of a mV (see *Table 2*). *Table 2* shows that the program ESTA was able to refine simultaneously stability constants and E° for which the initial value could vary in the range of ±30 mV. This is a large range in potential, and with a little bit of a practice, it should not be difficult to predict the E° in that range. Moreover, it was noted that, if the initial guess were to be out of that range, then, even though ESTA failed to refine all the parameters (the number of 20 allowed optimization iterations was exceeded), the final value of E° reported by ESTA was always closer to the expected value indicating at the same time the direction of the necessary change in the initial E°_{ini} value. In general, it was quite a straightforward refinement procedure when virtual potentials generated from polarographic data were used by ESTA. It seems reasonable to assume that one could start the refinement operations with an initial estimate in E° set to the standard redox potential for the reduction of Bi^{3+} ions if no prior knowledge of the virtual E° from polarographic experiments was available.

The use of dedicated potentiometric software has the additional advantage that it allows refining stability constants arising from different titrations. A successful output from the refinement operations described above prompted us to explore the possibility of refinement operations performed with several titrations simultaneously. Numerous combinations and strategies in the refinement operations were tested, and selected results are shown in *Table 2*. Thus, one was able to combine data from polarography (VP-DC with VP-DP) at each individual pH, or to combine data from a single polarographic technique, *e.g.*, DPP, but obtained at different pH values, as well as to combine and refine simultaneously all the data obtained from both techniques and at all 3 different pH values. This has never been achieved previously (when a voltammetric study is concerned) and, in our opinion, opens up a new field in the study of metal complexes by different voltammetric techniques.

Even though all possible precautions were made to assure the highest quality of experimental data, a small difference in the computed stability constants is observed when results obtained at different pH values are compared. There seems to be a trend indicating the presence of a systematic error. One possibility might be a difference in the diffusion junction potential at different pH values. However, the computed stability constants are larger at higher pH values, where the diffusion junction potential is almost negligible. Analysis of the species-distribution diagrams shown in *Fig. 3* indicates that the formation of the complex ML is a competing reaction to the formation of the complex MOH. The extent of the formation of MOH increases with pH. If the stability constant for MOH included in the refinement operations (mass-balance equations) is not accurate, then it must cause some uncertainty in the computed log K_1 value. To test this supposition, the stability constants were refined again, but this time with log K_{MOH} decreased by 0.2 log units. It resulted in log K_1 values 7.65, 7.54, and 7.44 at pH 1.85, 1.37, and 0.90, respectively (a decrease by 0.15, 0.09, and 0.05 log units at appropriate pH values). One must realize that the value in log K_{MOH} is an estimate [24] at an ionic strength of 0.5M, as there is no experimental value available at this ionic strength.

The stability constants for ML and ML_2 have small standard deviations. In case of ML_3, the standard deviation is rather large. It is not due to experimental error but because the extent of the formation of this complex is very small (see data at pH 1.85 in *Fig. 3*). It was possible to refine the stability constant for ML_3, but, at the same time, the exclusion of ML_3 from the model did not result in a large change in the computed stability constants for ML, ML_2, as well as the value of the R factor at pH 1.85. From the above, one may conclude that the ligand titration provided reasonable estimates of the values for ML and ML_2 bismuth complexes with the ligand picolinic acid. At the same time, it is clear that ligand titration alone was not able to provide a full metal/ligand/OH model. To achieve that, an experiment involving acid-base titration was necessary.

Voltammetric Data from the Acid-Base Titration. Any voltammetric theory of speciation involved in the study of a labile metal/ligand system, including *Eqn. 1*, requires computation of shifts in recorded signals. This, in turn, requires a single experimental value, *i.e.*, the potential of a free metal ion. This, however, is not experimentally available when bismuth is investigated – one can see in *Fig. 3* that, even at pH 0.9 and even in the presence of a strongly complexing ligand such as picolinic acid, 26% of bismuth is in the form of MOH. In addition, the extent of bismuth hydrolysis increases with increasing pH. Clearly, a special procedure had to be worked

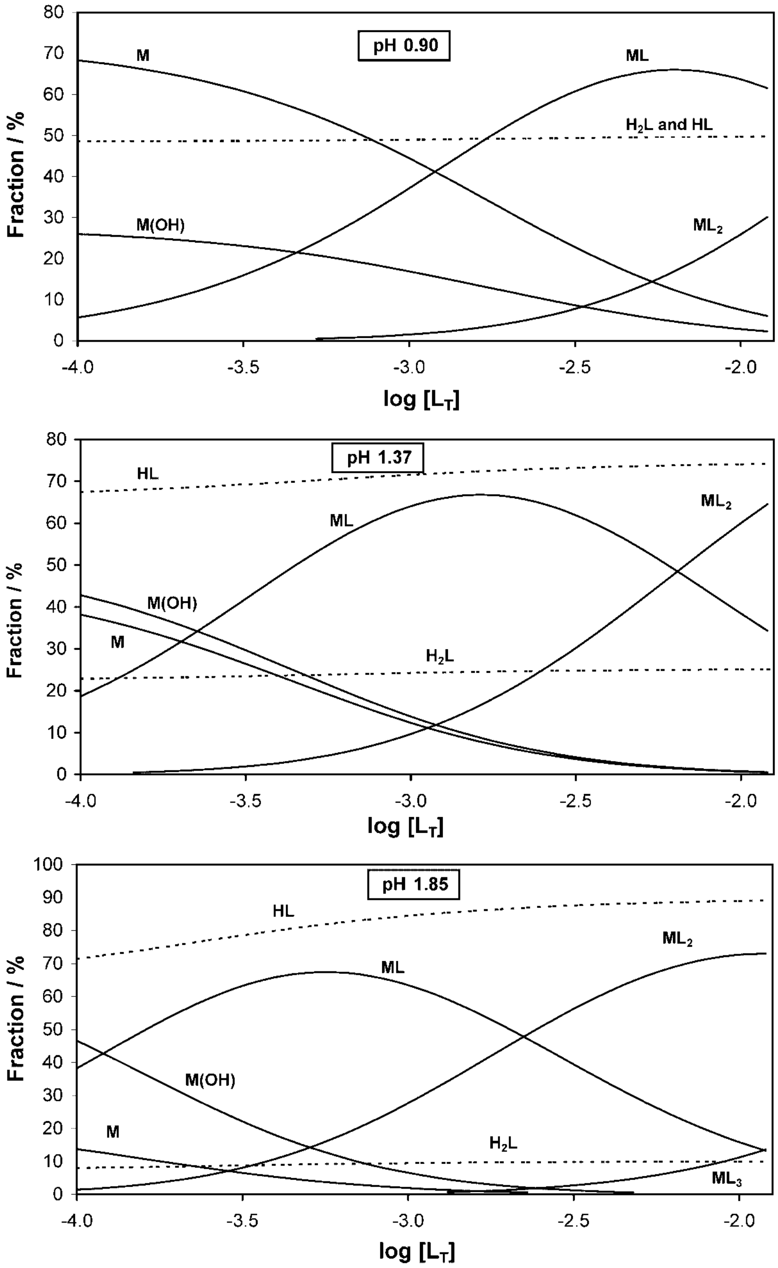

Fig. 3. *Species distribution diagrams computed for the M/L/OH models obtained from the ligand titrations at specified pH values.* Refined stability constants used are those from *Table 2*. Exper. conditions as for *Fig. 1*.

out to estimate mathematically the expected potential $E_{1/2}(\mathrm{M})$ at which the free bismuth would be recorded if no complexes of bismuth were formed.

The experimental points obtained with a solution containing only bismuth (no ligand present) as a function of pH are shown in *Fig. 4* (◆). It shows that the half-wave potential shifts towards more-positive values with an increase in pH. At pH of *ca.* 1.5, the recorded values are almost constant followed by the opposite shift, *i.e.*, towards more-negative values. Our interpretation of the potential variation *vs.* pH is as follows. There are two competing processes, namely the change in the diffusion junction potential E_j and the formation of the MOH complex. The value of E_j is largest at the smallest pH values, which is opposite to the extent of bismuth hydrolysis. It appears that both processes cause the recorded polarograms to shift towards more-negative values. It was possible to predict the expected shift due to the formation of MOH by use of our software [44]. Note that any software that solves appropriate mass-balance equations and provides the free-metal ion concentration can be used for this purpose as the free-metal-ion together with the total metal ion concentrations are required to predict the shift (see right-hand side of *Eqn. 1*). This furnished the corrected experimental points ($E_{1/2}(\mathrm{M})$; □) for the formation of MOH; they represent the potential of the 'free' bismuth at different pH values. It is seen that, *e.g.*, at pH *ca.* 1.9, the formation of BiOH caused a shift of *ca.* 14 mV towards more-negative values. The solid-line curve in *Fig. 4* was obtained by fitting the corrected experimental points (□) and represents the experimentally obtained change in the diffusion junction potential that caused the change in the recorded potential of the free bismuth. The solid-line curve approaches a constant value of $E_{1/2}(\mathrm{M}) \approx 85.3$ mV at pH 2.5. From this pH onwards, it is assumed that $E_{1/2}(\mathrm{M})$ must stay constant, and any experimental point obtained in the presence of the ligand can be compared with this single value to compute the shift in $E_{1/2}$ at an appropriate pH above 2.5. At pH values smaller than 2.5, a function is required to compute shifts in potentials. A third-order polynomial was fit in squares and was used to compute shifts of the potential due to the formation of bismuth complexes in the presence of the ligand picolinic acid, ΔE_n values shown in *Fig. 4* indicating how the shifts were computed throughout the whole pH range.

The computed shifts of the half-wave potential allowed the computing of the corrected shifts, *i.e.*, the objective function shown in *Fig. 5* (○). The objective function was reproduced very well by the theoretical one (see *Fig. 5*) that incorporated ML, $\mathrm{ML_2}$, $\mathrm{ML_3}$, $\mathrm{ML_4}$, and $\mathrm{ML_3(OH)}$ (see *Table 3*), besides all known $\mathrm{Bi}_x(\mathrm{OH})_y$ species. It was very pleasing to note that the log K_1 value obtained from the acid-base titration generated almost the identical stability constant when compared with the ligand titration. The incorporation of $\mathrm{ML_3(OH)}$ resulted in the best fit at the highest pH values (see *Fig. 5,b*). The dotted curve (····) represents the computed refined model without $\mathrm{ML_3(OH)}$. When the species $\mathrm{ML_3(OH)}$ is subtracted from the optimized model, then a drop of the computed complex-formation curve by *ca.* 10 mV results at the highest pH value.

Virtual Potentiometry and Combined Refinement of Data from the Acid-Base Titration. It was a straightforward matter to generate virtual potentials from the acid–base-titration experiment. The function (the polynomial) shown in *Fig. 4* was used in computing the values of E_j at each pH. Next, the experimentally obtained $E_{1/2}$ (or E_p) values were corrected by subtracting the E_j value. The variation in the virtual

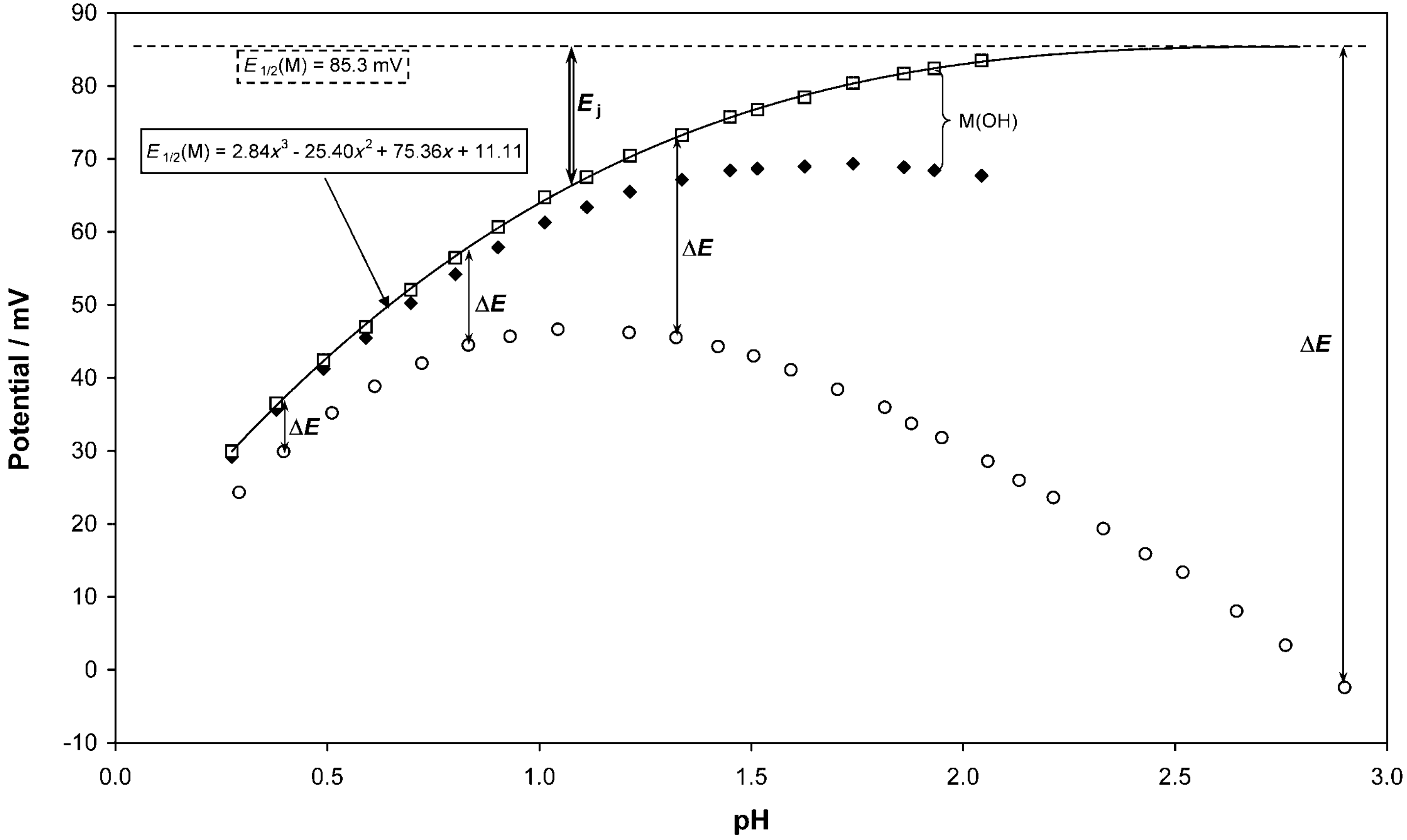

Fig. 4. *Analysis of experimental data at low pH values: shift in the* $E_{1/2}(M)$ *potentials obtained with a* Bi^{III} *solution in the absence of the ligand* (◆), *corrected* $E_{1/2}(M)$ *values* (□; the distance between ◆ and □ represents the contribution made by the formation of Bi(OH)), *solid-line curve* (—) *fit to the corrected* $E_{1/2}(M)$ *values* (□) *and describing the variation in diffusion junction potential* E_j vs. *pH* (used to predict the $E_{1/2}$(M) value of 85.3 mV one should obtained under conditions where E_j and the formation of M(OH) do not take place), *and experimental points* (○) *obtained from the acid titration of a sample containing* Bi^{III} *and the ligand*. Shifts in $E_{1/2}$ due to the formation of bismuth complexes are indicated by ΔE.

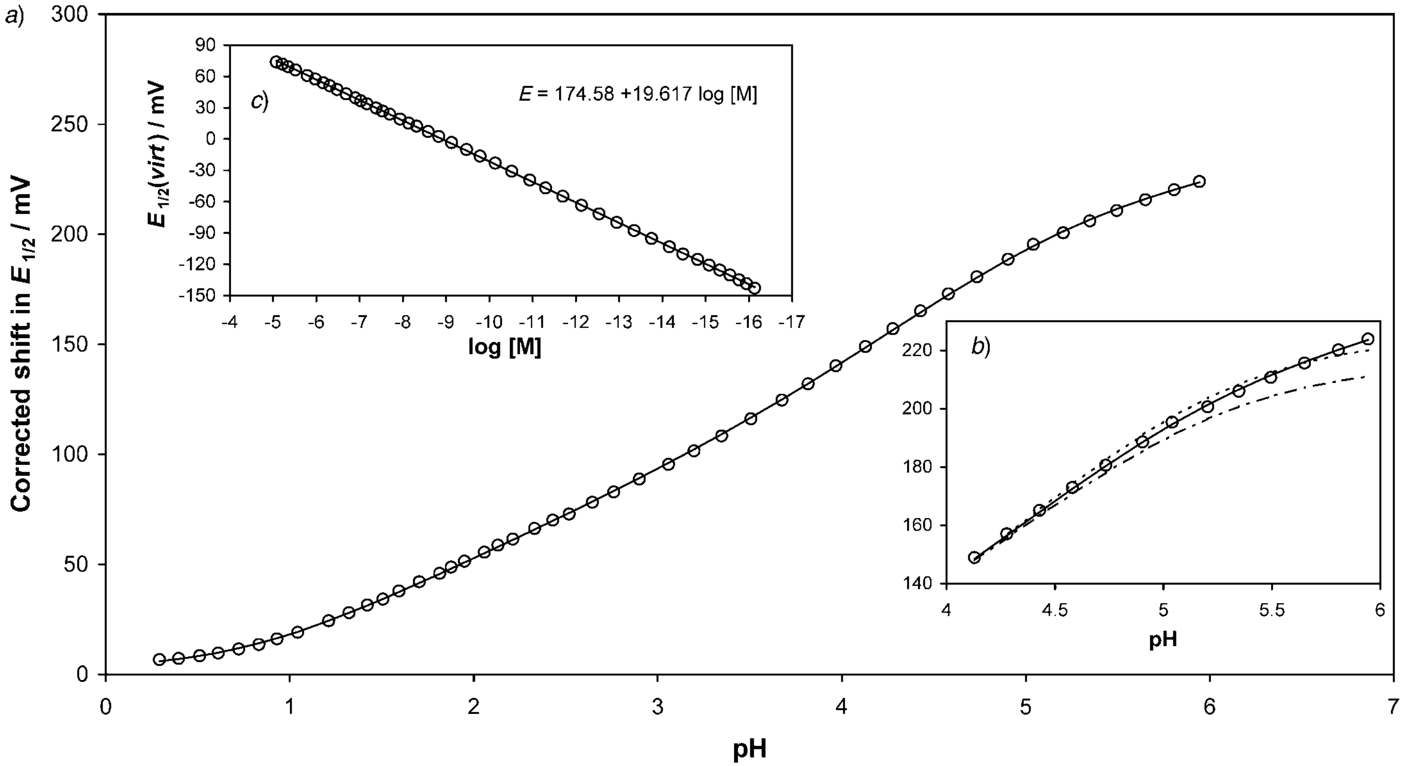

Fig. 5. *Optimization of the Bi^{III}/L/OH model and refinement of stability constants based on DCP data obtained from the acid-base titration:* a) *experimental points* (○) *and computed solid-line* (—) *complex-formation curves* (ECFC and CCFC, resp.) *as defined by* Eqn. 1 (CCFC for the final model consisting of ML, ML$_2$, ML$_3$, ML$_4$, and ML$_3$(OH) and their refined stability constants (see *Table 3*)); b) *upper part of the complex-formation curves computed for three models: final model* (—), *refined stability constants for ML, ML$_2$, ML$_3$, and ML$_4$ with ML$_3$(OH) excluded from the model* (····) *and CCFC for ML, ML$_2$, ML$_3$, and ML$_4$ with the refined stability constants of* Table 3 *but with exclusion of ML$_3$(OH) from the M/L/OH model* (– · – ·); c) *response of a virtual potentiometric sensor VP-DC generated from the DCP data* (virtual potential as computed by *Eqn. 9*; the free-metal-ion concentration was computed for each experimental data point for the refined M/L/OH model).

Table 3. *Stability Constants for the Bi^{III}/Picolinic Acid/OH System Obtained from the Acid-Base Titration* ($[M_T] = 4.99 \cdot 10^{-5}$ M, $[L_T]/[M_T] = 132$ at 25° and $\mu = 0.5$M) *and from all combined experiments* (ligand and acid-base titrations)

Technique[a]	Log β					E°_{fin} (E°_{ini}) [mV][b]	*R* Factor or O fit[c]
	ML	ML_2	ML_3	ML_4	$ML_3(OH)$		
DCP	7.47 ± 0.06	13.96 ± 0.02	18.06 ± 0.04	20.71 ± 0.20	26.70 ± 0.05	–	0.224
VP-DC	7.54 ± 0.03	13.91 ± 0.02	18.13 ± 0.04	20.36 ± 0.35	26.6 ± 0.03	174.58[d]	0.018
	7.46 (fixed)[e]	13.83 ± 0.03	18.05 ± 0.04	20.27 ± 0.34	26.58 ± 0.04	171.96 (173 ± 30)	0.018
	7.46 (fixed)[e]	13.96(fixed)[e]	18.08 ± 0.05	20.55 ± 0.22	26.65 ± 0.03	173.39 (174 ± 30)	0.018
VP-DC and VP-DP (all ligand and acid-base titrations)	7.48 ± 0.01	13.94 ± 0.01	18.10 ± 0.04	20.47 ± 0.25	26.65 ± 0.03	refined values[f]	0.027[i]
	7.48 ± 0.01	13.94 ± 0.01	18.07 ± 0.04	20.80 ± 0.13	26.53 ± 0.05	refined values[f]	0.0276[g]
	7.46 (fixed)[e]	13.94 ± 0.01	18.09 ± 0.04	20.50 ± 0.23	26.65 ± 0.03	173.39 (fixed)	0.0276
	7.46 (fixed)[e]	13.94 ± 0.03	18.07 ± 0.04	20.51 ± 0.22	26.64 ± 0.03	173.19 (173 ± 30)	0.0276[h]
	7.46 (fixed)[d]	13.96 (fixed)[e]	18.07 ± 0.04	20.57 ± 0.20	26.65 ± 0.03	173.37 (173 ± 30)	0.0277[h]

[a]) VP-DC and VP-DP stand for virtual potentiometric sets of data created from the experimental DCP and DPP data, resp. [b]) E°_{fin} and E°_{ini} stand for the final (refined simultaneously with stability constants) and initial (the virtual E° value obtained from the analysis of the polarographic data, see *Fig. 5*) values of E° of a virtual potentiometric sensor, resp. [c]) O Fit stands for the overall fit (± 1 mV) of CCFC in ECFC; R factor applies to the VP data refined by ESTA and stands for a statistical *Hamilton R* factor generated by the program ESTA. [d]) This value was obtained from fitting virtual $E_{1/2}$ values, see *Fig. 5,b*. [e]) These values were fixed at refined stability constants obtained from the ligand titration. [f]) Refined values from appropriate individual titrations were used as fixed values. [g]) The initial total ligand concentration was refined simultaneously. [h]) The E° value from acid–base titration was refined simultaneously. [i]) This is the final set of stability constants proposed in this work and used to compute the species distribution diagram shown in *Fig. 6*.

$E_{1/2}$ *vs.* the computed free-metal-ion concentration for the optimized metal/ligand system resulted in a straight line with a slope that is almost theoretical for the three-electron reduction. The computed line does not lose its linearity even at a free-metal-ion concentration smaller than 10^{-16} M. It is reasonable to assume that, if one would be able to extend the voltammetric experiment towards higher pH values, then the linearity range shown in *Fig. 5,c*, would be even wider. It appears that the virtual potentiometric sensor, generated by conversion of dynamic voltammetric data, does not have linearity limits.

The virtual potential values were refined by ESTA without a problem. Different strategies were tested, and some results obtained are shown in *Table 3*. It was also possible to combine all the results, the ligand and acid-base titrations together. It was interesting to note that the refined $\log K_1$ value, computed from the combined optimization operation performed by ESTA, did not differ much at all from the value obtained from the ligand titration.

Stability constants and the metal/ligand/OH model obtained from a joint refinement of all available data were used to compute the species-distribution diagram of *Fig. 6*. Thus, ML is already present even at pH 0. Also, the precipitation at pH *ca.* 6.5 is correctly predicted. According to this diagram, no polynuclear species should be formed in the pH range investigated (prior to precipitation), and this was confirmed experimentally as the shape (that means electrochemical reversibility) of recorded curves did not change significantly throughout the experiment. It is quite clear that glass-electrode potentiometry would be of no use in the study of bismuth complexes with picolinic acid. Besides the same problem related to the diffusion junction potential, it would be impossible to solve mass-balance equations for bismuth complexes in the pH range 0–2. Note that it is impossible to start the experiment

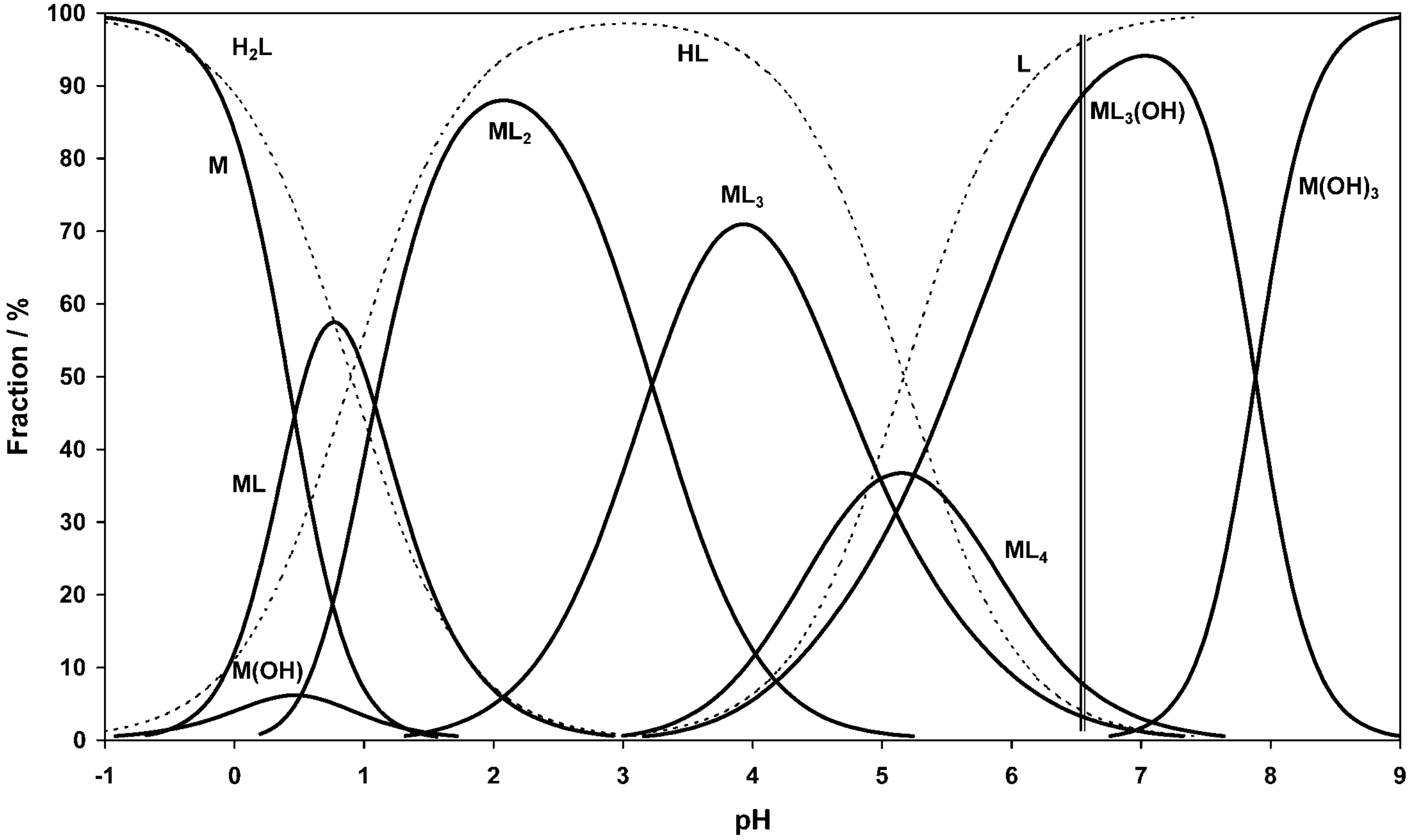

Fig. 6. *Species distribution diagram for a final Bi^{III}/picolinic acid/OH model as a function of pH*. $[M_T] = 5 \cdot 10^{-5}$ M. $[L_T]/[M_T] = 132$. Refined stability constants from *Table 3*. The double solid vertical line indicates the pH at which precipitation is predicted.

above pH 2 as precipitation occurs immediately. However, if a metal/ligand system would be suitable for the study by GEP, then it should not be a problem to refine stability constants by ESTA by using a set of data coming from GEP and the virtual potentiometric probe, both experiments performed at their most suitable experimental conditions, *i.e.*, GEP at large $[M_T]$ and small $[L_T]/[M_T]$ ratio, and voltammetry at small $[M_T]$ and large $[L_T]/[M_T]$ ratio. Also, the use of voltammetry and virtual potentiometric data allows one to work in a much larger $[H_T]$ range. This type of approach dramatically expands the experimental domain available in the field of a study of metal/ligand equilibria and makes the final model and stability constants much more reliable.

4. Conclusions. – This work has demonstrated that it is possible to employ dedicated potentiometric software, such as ESTA, in the refinement of data coming from two dynamic and nonequilibrium electrochemical techniques, DCP and DPP, to obtain stability constants and a final metal/ligand/OH model. This was achieved by a mathematical transformation of polarographic data into a type of data one would obtain from a real free-metal-ion potentiometric sensor. The block diagram of *Fig. 7* shows the general concept of procedures implemented in this work. The conversion of dynamic to virtual potentiometric data is, as far as we know, the first attempt of this kind reported in the literature for an unknown metal/ligand/OH system, and, in our opinion, it opens up a new chapter in the field of the study of metal complexes by different electrochemical techniques. The proposed new procedure was also verified as a useful and reliable tool for the data treatment at extremely low pH values; it should be now possible to study many bismuth systems with ligands that might have some potential biomedical applications.

Potentiometry has been widely used in the field for many decades, and this has resulted in the development of powerful dedicated programs, such as ESTA or SUPERQUAD. Polarography, even though employed in the field of metal/ligand equilibria for over half a century, has never been developed to a powerful and reliable analytical tool, and, as a result, it has nearly not been used at all, by researchers working in the development of new ligands [1]. The proposed procedures, involving dedicated potentiometric software, should now allow to: *i*) use polarography by non-electrochemists; *ii*) employ existing potentiometric software in rigorous refinement of voltammetric data; *iii*) use the same refinement and optimization operations on voltammetric and potentiometric data (separately and combined); *iv*) study a M/L system in different and much larger experimental domains; this should provide more reliable data for a system under investigation; *v*) avoid or minimize problems associated with solubility of a ligand or complexes formed when the GEP experimental domain is concerned; *vi*) perform experiments in a wider pH range; this should provide much more data on, *e.g.*, acidic metal ions such as Bi^{III} or In^{III}; *vii*) refine simultaneously results from different polarographic techniques; this should improve reliability and increase area of applications of polarography in the field; *viii*) eliminate the need of $E(M)$ values with which the shifts of the polarographic curves along the potential scale are calculated; this applies to systems studied by ligand titration at any pH and by acid-base titrations starting at pH 2 and above where E_j can be ignored.

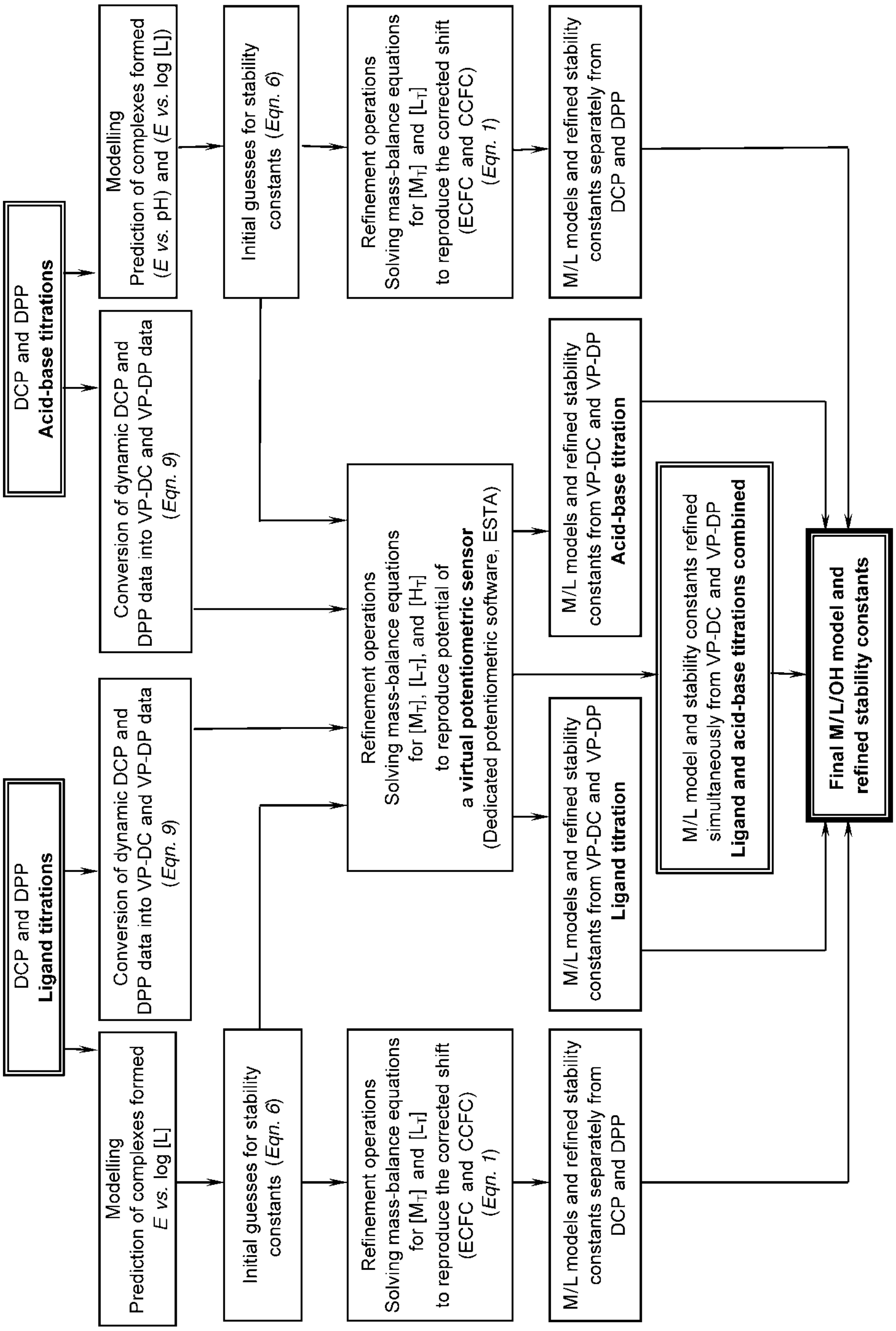

Fig. 7. *Block diagram of experimental, mathematical, and refinement procedures developed and tested in this work*

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Experimental Part

Instrumentation. All experiments were performed in a *Metrohm* (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer and thermostatted at $25.0 \pm 0.1°$ by water circulating from a constant-temperature bath. The potential of a combination glass electrode and $\mathrm{pH} = -\log_{10}$ [H] of solutions were measured to within ± 0.1 mV (± 0.001 pH unit) with a *pHI-72* pH meter (*Beckman*, Fullerton, CA, USA). A model *6.0234.100* combination glass electrode (*Metrohm*) was used. Differential-pulse and direct-current polarograms were obtained with a computer-controlled instrumental setup [45] and a model *663 VA* stand (*Metrohm*). A multi-mode electrode (*Metrohm*, model *6.1246.020*) was employed as the working electrode and used in the dropping-mercury-electrode mode with a drop time of 2 s. A Ag/AgCl electrode (3M KCl) and a Pt electrode (both from *Metrohm*) were used as reference and auxiliary electrode, respectively. A pulse height of 50 mV and step height of 4 mV were used. The pulse width and integration time were set to 200 and 90 ms, respectively. High-purity N_2 was used for de-aeration of the sample solutions.

Materials. The ligand picolinic acid (99%) was obtained from *Aldrich* (Milwaukee, USA) and used as received. All other reagents used were of anal. grade (*Merck*, Darmstadt, Germany). Pure H_2O was obtained by passing de-ionized water through a *Milli-Q*-water-purification system (*Millipore*, Bedford, MA, USA). The $5.00 \cdot 10^{-2}$ M stock soln. of bismuth nitrate was prepared in 1.00M standard HNO_3 solution. The 0.5M ligand stock solns. (used for the ligand-titration experiments) were prepared by solving the solid ligand in H_2O and adjusting the soln. to an appropriate pH by use of 0.5M standardized acid or base solns. Standard HNO_3 soln. was prepared by dilution of concentrated acid and titration with borax. The concentration of a standard NaOH soln. was established by titration with the standardized acid soln. All solns. were adjusted to a ionic strength of 0.5M by the addition of $(Na,H)NO_3$.

Analytical Procedure. The combination glass electrode (GE) was calibrated before and after a polarographic experiment. This operation involved titration of 25.0 ml of standardized acid soln. placed in a polarographic cell with the standardized base soln. Readings in the potential of GE were collected in the pH range 0.3–1.5 and 12.0–12.5. Exper. points were fitted with a straight line to establish the value of $E°$ and a response slope (to the third decimal place) of the GE; *e.g.*, the response of GE before and after the voltammetric experiment was $y = -58.235x + 406.664$ and $y = -58.190x + 406.219$, resp. The values of $E°$ and a response slope from the combined fitting operation performed with the two titrations were used later (provided that they did not differ much, *e.g.*, as shown above) to calculate $\mathrm{pH} = -\log_{10}$ [H] of a sample under investigation; the combined GE response would be $y = -58.21(\pm 0.03)x + 406.44(0.20)$ for the two separate GE response evaluations shown above.

Ligand Titration. In the polarographic cell, 25.0 ml of 0.5M $NaNO_3$, an appropriate volume of 0.5M HNO_3, and few grains of solid gelatine were placed, and DP as well as DC polarograms were recorded to test for impurities. The addition of 25 µl (*Hamiltonian*-type micro-syringes were used for the additions of very small volumes of solns.) of the acidified standard bismuth soln. resulted in $[M_T]$ of *ca.* $5 \cdot 10^{-5}$ M (constancy in pH was achieved by the addition of the standardized base soln.). Three consecutive polarograms (DCP and DPP) recorded with that soln., were used to evaluate $E_{1/2}(M)$ and $E_p(M)$ for a 'free' bismuth ion. The consecutive additions of the appropriate volume (µl) of the standard ligand soln. to the vessel containing a metal-ion soln. generated the $[L_T]/[M_T]$ ratio in the range 2–240. The constancy in pH was maintained to ± 0.003 pH unit by the addition of few µl of the standardized base soln. For each $[L_T]/[M_T]$ ratio, a DC and DP polarogram was recorded; a set of 32 polarograms was obtained for each pH value, *i.e.*, 0.90, 1.37, and 1.85. It was checked that equilibration of the metal/ligand solns. occurred within a few minutes.

Acid-Base Titration. In the polarographic cell, 25.0 ml of standardized (*ca.* 0.5M) HNO_3 and few corns of solid gelatine were placed. DP as well as DC polarograms were recorded to test for impurities, followed by the addition of 25 µl of the acidified standard bismuth soln. Three consecutive polarograms (DCP and DPP) were recorded with that soln. to assure the constancy in the recorded curves. The bismuth soln. was titrated with the standard 0.5M NaOH soln. up to pH *ca.* 2 (above that pH, precipitation takes place). The cell and electrodes were rinsed with nitric acid followed by the addition of a new portion of 25.0 ml of 0.5M HNO_3; a new polarogram was recorded to check for impurities again. After recording polarograms on a bismuth soln., as described above, a solid ligand was added to achieve the required $[L_T]/[M_T]$ ratio. New polarograms, DC and DP, were recorded at pH values increased stepwise by 0.1 units. The standardized base soln. was used to vary the pH.

REFERENCES

[1] A. E. Martell, R. D. Hancock, in 'Metal Complexes in Aqueous Solutions', Modern Inorganic Chemistry Series, Ed J. P. Fackler Jr., Plenum Press, New York, 1996.
[2] F. Marsicano, C. Monberg, B. S. Martincigh, K. Murray, P. M. May, D. R. Williams, *J. Coord. Chem*. **1998**, *16*, 321.
[3] P. M. May, K. Murray, D. R. Williams, *Talanta* **1988**, *35*, 23.
[4] P. M. May, K. Murray, *Talanta* **1988**, *35*, 927.
[5] P. Gans, A. Sabatini, A. Vacca, *J. Chem. Soc., Dalton Trans.* **1985**, 1195.
[6] J. J. Lingane, *Chem. Rev*. **1941**, *29*, 1.
[7] D. D. DeFord, D. N. Hume, *J. Am. Chem. Soc*. **1951**, *73*, 5321.
[8] D. N. Hume, D. D. DeFord, G. C. B. Cave, *J. Am. Chem. Soc.* **1951**, *73*, 5323.
[9] W. B. Schaap, D. L. McMasters, *J. Am. Chem. Soc.* **1961**, *83*, 4699.
[10] G. Schwarzenbach, H. Ackermann, *Helv. Chim. Acta* **1952**, *35*, 486.
[11] G. Schwarzenbach, R. Gut, G. Anderegg, *Helv. Chim. Acta* **1954**, *37*, 937.
[12] D. R. Crow, in 'Polarography of Metal Complexes', Academic Press, London – New York, 1969.
[13] H. G. De Jong, H. P. van Leeuwen, K. Holub, *J. Electroanal. Chem*. **1987**, *234*, 1.
[14] H. G. De Jong, H. P. van Leeuwen, *J. Electroanal. Chem*. **1987**, *234*, 17.
[15] H. G. De Jong, H. P. van Leeuwen, *J. Electroanal. Chem*. **1987**, *235*, 1.
[16] J. M. Díaz-Cruz, C. Ariño, M. Esteban, E. Casassas, *Electroanalysis* **1991**, *3*, 299.
[17] A. M. Nadal, C. Ariño, M. Esteban, E. Casassas, *Electroanalysis* **1991**, *3*, 309.
[18] J. M. Díaz-Cruz, C. Ariño, M. Esteban, E. Casassas, *J. Electroanal. Chem*. **1992**, *333*, 33.
[19] R. Guidelli, D. Cozzi, *J. Phys. Chem*. **1967**, 71, 3020.
[20] R. Guidelli, D. Cozzi, *J. Phys. Chem*. **1967**, 71, 3027.
[21] I. Cukrowski, M. Adsetts, *J. Electroanal. Chem*. **1997**, *429*, 129.
[22] I. Cukrowski, *Electroanalysis* **1997**, *9*, 1167.
[23] I. Cukrowski, S. A. Loader, *Electroanalysis* **1998**, *10*, 877.
[24] I. Cukrowski, R. D. Hancock, R. C. Luckay, *Anal. Chim. Acta* **1996**, *319*, 39.
[25] I. Cukrowski, *Electroanalysis* **1999**, *11*, 606.
[26] I. Cukrowski, *J. Electroanal. Chem*. **1999**, *460*, 197.
[27] I. Cukrowski, *Anal. Chim. Acta* **1996**, *336*, 23.
[28] I. Cukrowski, J. R. Zeevaart, N. V. Jarvis, *Anal. Chim. Acta* **1999**, *379*, 217.
[29] I. Cukrowski, G. Ngigi, *Electroanalysis* **2001**, *13*, 1242.
[30] I. Cukrowski, N. Maseko, *Electroanalysis*, in press.
[31] J. Buffle, *J. Electroanal. Chem*. **1981**, *125*, 273.
[32] J. Buffle, A. M. Mota, M. L. Gonçalves, *Port. Electrochim. Acta* **1985**, *3*, 293.
[33] D. S. Jain, O. Prakash, S. K. Bhasin, *J. Electrochem. Soc. India* **1976**, *25*, 139.
[34] A. M. Bond, *J. Electroanal. Chem*. **1969**, *20*, 223.
[35] A. M. Bond, *J. Electroanal. Chem.* **1969**, *23*, 227.
[36] G. A. Heath, G. Hefter, *J. Electroanal. Chem.* **1977**, *84*, 295.
[37] M. Fernández, C. Ariño, J. M. Díaz-Cruz, R. Tauler, M. Esteban, *J. Electroanal. Chem.* **2001**, *505*, 44.
[38] M. Fernández, C. Ariño, J. M. Díaz-Cruz, R. Tauler, M. Esteban, *Electroanalysis* **2001**, *13*, 1405.
[39] R. M. Town, H. P. van Leeuwen, *J. Electroanal. Chem.* **2002**, *535*, 11.
[40] I. Cukrowski, Z. M. Zhang, *Electroanalysis* **2004**, *16*, 612.
[41] 'NIST Standard Reference Database 46. NIST Critically Selected Stability Constants of Metal Complexes Database', Version 3.0, data collected and selected by R. M. Smith and A. E. Martell, U.S. Department of Commerce, National Institute of Standards and Technology, 1997.
[42] R. D. Hancock, I. Cukrowski, J. Baloyi, J. Mashishi, *J. Chem. Soc., Dalton Trans.* **1993**, 2895.
[43] I. Cukrowski, *Analyst* **1997**, *122*, 827.
[44] I. Cukrowski, dedicated to polarography program 3D-CFC, unpublished.
[45] I. Cukrowski, E. Cukrowska, R. D. Hancock, G. Anderegg, *Anal. Chim. Acta* **1995**, *312*, 307.

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