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THE CALCIUM ELECTRODE

STUDY OF CALCIUM BINDING TO HEXACYANOFERRATES

BY

SIRVART A. ISRAELIAN

submitted in partial fulfillment of the requirements

for the degree Master of Science

in the Department of Chemistry

American University of Beirut

Beirut, Lebanon

June, 1968

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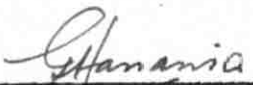
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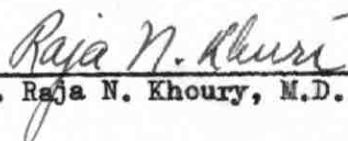
APPROVED:



Prof. George I.H. Hanania (Chemistry) Advisor



Prof. Samir K. Thabet (Chemistry) Member of Committee



Prof. Raja N. Khoury, M.D. (Physiology) Member of Committee

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ABSTRACT

This work is a study of the properties and limitations of the Orion liquid-liquid membrane electrode, its application to the determination of the ion-association constant at 25⁰ of a known system, calcium sulfate, and the thermodynamic study of ion-association equilibria involving calcium ions and hexacyanoferrate (II) and hexacyanoferrate (III) ions in aqueous solution.

The electrode was first shown to be reversible to calcium ion activity over the range of concentration 5.0×10^{-4} to $7.0 \times 10^{-3} M$ within which work was carried out. Effect of variation of pH, Mg^{2+} , Ba^{2+} , Na^{+} and K^{+} ion concentrations was considered at three different concentration levels of Ca^{2+} . All measurements were made against a selected commercial calomel electrode in saturated KCl solution, and an attempt was made to estimate the liquid junction potential of the electrode by calibrating it against Ag - AgCl electrodes.

The application of the potentiometric method to the study of the two equilibria involving Ca^{2+} and hexacyanoferrates involved determinations of the ion-association constants over a range of ionic strengths, at 15, 20, 25, 30 and 35⁰C, and the subsequent computation of thermodynamic parameters ΔH° and ΔS° .

The enthalpy change of both equilibria was also measured independently and directly using a microcalorimeter (Beckman Model 190B). The results show that the binding of Ca^{2+} to the hexacyanoferrate ions is more favored than univalent cations, and less than trivalent cations,

that the equilibria are weakly endothermic, and that ion-pair distances calculated on the basis of Bjerrum's theory are in reasonable agreement with hydrated ion radii. These results support the validity of the electrostatic view of ion-pair formation in aqueous solutions.

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INTRODUCTION

This work involves a potentiometric study of the recently introduced Orion liquid-liquid membrane calcium electrode, and its application to the study of ion-association phenomena in dilute aqueous solutions containing calcium and hexacyanoferrate ions.

The importance of the calcium electrode stems from the fact that it gives a direct and accurate measure of the thermodynamic activity of free (unbound) calcium ions in solution. Metal / metal-ion electrodes have of course been in use for many years, but these are of necessity limited to the less active metals, such as zinc, silver and mercury, and in some cases to amalgams of the more active metals under special and restricted conditions.

The novel approach in the new series of liquid-ion exchange (or solid state) membrane electrodes is their applicability to alkali and alkali-earth metals, and indeed to any other cation or anion for which a suitable and selective ion-exchanger can be found. Ion-selective electrodes therefore, open up a new field for potentiometric investigation of electrolytes in chemical, industrial and physiological systems.

Our primary concern here is with the thermodynamic application of such an electrode. Thus, if measurements with the calcium electrode show that the concentration of free calcium ions in a given solution is less than the total stoichiometric amount, it may be assumed that the difference represents the extent to which calcium ions are bound to some

counterions in solution, and this enables one to calculate an ion-association constant which is the thermodynamic constant for the ion-pair.

The method is straightforward except for the estimation of activity coefficients, a computation which requires an extension of the simple Debye-Huckel equation. Nevertheless the validity of such assumptions is justified by the fact that quantitative measurements are usually made in dilute solutions where physical interactions are minimal.

IONIC INTERACTIONS

The realization that aqueous solutions of salts consist of charged particles (ions) started with the work of Faraday on electrolysis (1830 to 1850). This led to the establishment of the concept that electrolytes break down into smaller components in solution.

Arrhenius (1887) was the first to suggest specifically an ionization theory which stated that undissociated molecules are in equilibrium with their ions in solution and that the equilibrium is governed by the law of mass action.

Discrepancies were however observed between the "apparent degree of ionization" as determined by static methods involving the colligative properties of electrolytes, and by dynamic methods such as conductivity. Noyes and Johnston (1909) pointed these discrepancies out in the case of the salt $K_4Fe(CN)_6$.

Debye and Huckel (1923) proposed the theory of complete ionization of strong electrolytes, and attributed the observed deviations from ideality (which is complete dissociation into ions) to physical inter-

actions within an ionic atmosphere. Thus the concept of thermodynamic activity of ions as distinct from their stoichiometric concentration was introduced, and the two quantities were related through a physical parameter, the activity coefficient.

An alternative approach to the problem of ionic interactions is due to Bjerrum (1926) who, in contrast to the Debye-Huckel theory, postulated a chemical effect, namely ion-pair formation. Bjerrum's argument was based on the idea that although there is constant interchange of ions in solution, there is also a finite probability for two oppositely charged ions to be closer than a certain critical distance "a" from each other, justifying the formation of a new chemical species which would persist through a number of collisions.

During subsequent years several independent lines of evidence have established the reality of ion-pair formation and indeed it has become evident that both the physical and the chemical factors are real and have to be taken into consideration in studying solutions of electrolytes.

A brief review of methods of investigating ion-association follows.

METHODS OF INVESTIGATING ION-ASSOCIATION

A major consideration in the study of the chemical phenomenon of ion-association is the need for establishing the composition and structure of ion-pairs in solution. This cannot be achieved unequivocally by one single method. In fact, a wide variety of experimental techniques have been used in the determination of equilibrium (ion-association) constants, and it is the cumulative agreement between results from independent methods that lends support to the theory of

chemical binding of ions in solution.

Among the techniques so far employed are: conductivity, spectrophotometry, Raman spectroscopy, nuclear magnetic resonance, ion exchange, solvent extraction, kinetics of reactions, polarography, potentiometry and various other methods like solubility, freezing point depression, viscosity and, quite recently, the measurement of partial molal volumes of solutions (Spiro, Revesz, Lee, 1968).

Conductance method: Historically, conductance measurements constitute the first method by which the association constant of 2:2 electrolytes were determined (Davies, 1962). The method is based on the idea that in a symmetrical electrolyte any ion-pairs formed will be effectively uncharged and will therefore not contribute to the conductivity of the solution. The conductance of free ions, in dilute solutions, is assumed to obey the Onsager limiting equation, and activity coefficients are calculated from a suitable Debye-Huckel equation. Under favorable conditions, measurements can be made with great precision. However, major difficulties arise in the evaluation of data obtained for unsymmetrical salts, data obtained at higher electrolyte concentrations, and data for salt mixtures.

Spectrophotometric method: This method takes advantage of the changes in absorption spectra of ions which accompany complexation. Such changes occur in various parts of the spectral range, and vary in intensity, depending on the type and extent of ionic interactions.

The advent of modern instrumentation covering 180 to 2500 nm has made possible very precise measurements on most aqueous solutions. The method assumes application of the Beer-Lambert law for the ions in a given solution, and apparent deviations from the law are attributed to

association.

The method works best where a big difference in absorption between the ligand and the complex ions can be found. This is often not the case with systems involving weak ion-associated pairs.

Raman Spectra. The method utilizes the scattering of light which follows the interaction of radiation with matter. The frequencies of scattered light depend on the rotational and vibrational levels of the molecule. Raman line intensities depend on the change of polarizability of scattering molecules with the change of internuclear distance associated with the vibrations which produce the Raman spectral lines. In this method, strong polarized lines are obtained only in cases where short-range interactions between the ions are involved. Thus in many systems, such as the metal sulfates, very weak lines are usually obtained, presumably because in the ion-pairs the ions are separated by water molecules. Another disadvantage of the method is its restriction to solutions of concentration $\geq 0.5M$.

Nuclear magnetic resonance. Nuclear magnetic moments are attributed to nuclear spins of all atoms, excluding those with even numbers of both neutrons and protons. One of the methods of studying and measuring nuclear magnetic moments is nmr. Nmr frequencies are determined by the electronic environment surrounding the nucleus due to the magnetic screening effect of the electron cloud. Any disturbance of the electronic distribution of the molecule or atom such as is caused by a foreign molecule or ion (whether it is paramagnetic or diamagnetic) gives rise to "chemical shifts" of the nmr frequencies. This could well be the case in ion-pair formation. The method has been successfully applied to

thallous Tl(I) solutions containing OH^- , F^- , CH_3COO^- , HCOO^- , NO_3^- , CO_4^- (Freeman, Gasser, Richards and Wheeler, 1959), $\text{Fe}(\text{CN})_6^{3-}$ and citrate ions (Gasser and Richards, 1959). In a recent review, Hinton and Amis (1967) summarize the problems that are exclusively answerable by nmr methods and compare nmr with results of other methods.

Ion-exchange method. This technique involves the establishment of an equilibrium between metal ion and a cation-exchanger, followed by adding salt of an anion which causes a redistribution of the metal ion in the resin and the solution. The distribution of concentrations is altered to an extent which is proportional to the formation constant of the ion-pair. Radioisotope and other techniques have been used for determining the distribution of the cation and hence the ion-association constant. The method also has the advantage of indicating the charge of the complex. Betts and Dahlinger (1959) have used a tracer radio method with Dowex 50 cation exchanger to measure the heats and entropies of association of the Lanthanide ions with EDTA.

Solvent extraction. The method is based on the partition of a solute between an aqueous solution and an immiscible organic solvent as a means of investigating equilibria in solution. It is useful only if the ion-pair is neutral because it is assumed that only the neutral ion-pair is present in the organic phase. The association constant is obtained by studying the effect of the ligand concentration on the partition equilibrium between the two phases. A modification by Connick and McVey (1949) makes use of an organic solvent containing a complexing agent, L, which forms complexes with the metal ion but is insoluble in the aqueous phase. Assuming that the metal exists wholly

in the form of ML in the organic phase, the association constant of complex MX is found by studying the distribution of M, between the two phases at various concentrations of added anion X.

Reaction kinetics. In the general field of reaction kinetics, results are often interpreted only if one assumes the existence of pre-equilibria involving ion association of the reacting ligand with counterions in solution. Davies (1961) has applied the idea in the study of ionic strength effects on reaction rates, and Campion (1963) has used this method in his study of electron-exchange redox reaction between hexacyanoferrates II and III.

Polarography. Polarography constitutes a very useful method for studying complex ions because it gives direct evidence on the number of ligand molecules. Experimentally, it utilizes a micro-dropping mercury electrode. In presence of a ligand the half-wave potential of the metal ion is shifted to more negative values by an amount which is related to the stability (i.e., formation constant) and the formula (i.e., composition) of the complex. The usual ionic strength of solutions in polarography is over 0.1M, a fact which limits its thermodynamic significance.

Potentiometry. In theory, potentiometric methods present the most direct approach to the problem of investigating ion-association. The application depends upon having an electrode which is thermodynamically reversible to a given ion in solution; measurement of the emf then gives directly the activity of the free ion which is stoichiometrically related to the activities of the other ions involved.

The subject of the development of ion-selective electrodes very useful in this method, is treated in some detail in the next chapter.

Other methods. A number of other methods have also been used, for instance relaxation spectrometry, viscosity, freezing point, solubility and changes in volume which accompany ion association. Nancollas (1966) and Prue (1966) have both reviewed the subject of ionic equilibria and interactions.

Application of some of the above methods to the investigation of ion-association equilibria in hexacyanoferrates has been fairly extensive. Sillen (1964) has tabulated available data for H^+ , K^+ , Mg^{++} , Ca^{++} , Ba^{++} , La^{+++} , UO_2^{++} , Mn^{++} , Fe^{+++} , Co^{++} , Ni^{++} , Cu^{++} , Ag^+ , Zn^{++} , Cd^{++} , Hg_2^{++} , Hg^{++} , Ga^{+++} , In^{+++} , Tl^+ and Pb^{++} complexing with $Fe(CN)_6^{-4}$ and for some of these ions with $Fe(CN)_6^{-3}$, most determinations having been made from conductivity, spectrophotometric, solubility or potentiometric studies. However, in only two cases (Tl^+ and La^{+++}) are there enthalpy and entropy data. Eaton et al. (1967) have published thermodynamic data for K^+ association with hexacyanoferrates II and III ions.

So far thermodynamic parameters of divalent cations have not been reported. The advent of the Ca electrode has made it possible to do so now.

ELECTRODES

I. GENERAL TYPES

Potentiometry provides a direct, continuous and a highly sensitive method of measuring activities of ions in solutions consisting of one or more components. What is involved in all cases is the measurement of a difference in potential between two electrodes.

Classically, electrodes can be classified into two general groups: Direct. This type consists either of a metal in equilibrium with a solution containing its ion, e.g. $\text{Ag(s)} / \text{Ag}^+(\text{aq})$, or an inert collector of electrons, such as Pt, in contact with a gas and an ion, such as the H_2 and O_2 electrodes. In both cases the electrode is reversible to the ion in question. Such electrodes however, are useful only for metals of intermediate activity, and for acids and bases. Alkali and alkaline earth metals are therefore excluded and can only be used in amalgamated forms. In practice, these electrodes are subject to various limitations; notably the ease of poisoning through adsorption of ions, the effects of redox side reactions, and the fact that they can be used only in concentrations above 0.1M.

Indirect. In an indirect electrode, a metal may be in contact with an insoluble salt of the metal, thermodynamic reversibility being with respect to a counterion of the metallic cation. Thus the well known and accurate $\text{Ag(s)} / \text{AgCl(s)}$ electrode is reversible to Cl^- ions. This idea may be extended further. Thus a metal may be in contact with two insoluble salts, one involving the metal and the second involving another

cation to which the electrode is reversible. As an example, one can set up a calcium-ion electrode from a lead metal electrode impregnated with lead and calcium oxalates and dipping in a calcium salt solution. The use of such an electrode requires knowledge of solubilities and activity coefficients in saturated solutions, and is therefore limited and not so accurate.

II. THE GLASS ELECTRODE

The most widely used electrode in recent years has been the glass electrode which can be considered to belong to the direct type of electrodes as it is in fact an H^+ electrode. Since the present series of ion-selective electrodes came through the development of the glass electrode, it is relevant to consider first the main features of the regular glass electrode.

Origin. The idea of a glass electrode may be traced back to Lord Kelvin (Thomson, 1875) who suggested that glass was an electrolytic conductor. Next came the discovery (Cremer, 1906) that an electric potential could be observed across a thin glass membrane interposed between two aqueous solutions, and that the membrane was sensitive to changes in acidity. This was followed by quantitative assessment of the potential and by numerous improvements in the manufacture and design of glass electrodes.

Features. The glass electrode consists of (1) an inner reference electrode, usually $Ag(s) / AgCl(s)$, in 0.1M HCl or a buffered chloride solution; (2) a pH sensitive glass membrane, usually less than 0.1 mm thick and with a resistance less than 5×10^8 ohms; and (3) good electrical insulation between the test solution and the inner reference system.

Mechanism of response. Several theories have been put forward which have the following two points in common. First, they attribute the potential difference across glass membranes to a change in free energy caused by the transfer of H^+ across the phase boundary. Second, they propose thermodynamic reversibility to H^+ in the range of pH 1 to 9, at least for the special Corning 015 glass.

The three most widely accepted theories are the adsorption-potential, the membrane potential, and the phase-boundary-potential theories.

The adsorption-potential theory postulates that glass surfaces acquire charges by the adsorption of ions from solutions with which they are brought into contact, and thus act as adsorption electrodes the potentials of which arise from the differences in free energy between the free and the adsorbed ions. This theory cannot explain the electrode behavior in strongly acidic and basic media, and attributes less than 59 mv per pH unit change.

The membrane-potential theory considers a glass membrane to be permeable to H^+ and other cations and attributes its potential to the difference between the diffusion rates of ions through glass. The theory predicts correctly the behavior of glass membranes in the strongly acidic and basic regions. However, recent radio tracer experiments (Schwabe and Dahms, 1961, and others) have shown that H^+ ions become bonded in the Si-O network of the hydrated gel layer of glass and so contribute little to the slight current running through the glass which is thought to be carried mostly by Na^+ ions.

The phase-boundary (or Donnan) potential theory postulates that

only the gel layer at the glass surface participates directly in cationic exchange equilibrium because the potential is found to be established faster than would be expected from a true phase equilibrium. Thus the inner regions of the glass have little effect on the potential formed.

The recent trend is to consider the glass-electrode potential to be due to an ion-exchange process in the gel layer of the glass membrane. Eisenman (1967) has reviewed critically the experimental results in this field, and has shown that the theory not only predicts the acid and alkaline errors of glass electrodes but also gives a satisfactory explanation of the behavior of other ion-exchange electrodes.

Acid and alkaline errors of the glass electrode. The ordinary type of glass electrode is well known to deviate from thermodynamic behavior in solutions of $\text{pH} < 1$. This "acid error" is time dependent, and has been attributed by various workers to acid sorption by the gel layer, to changes in the activity of H_2O , and to the presence of H^+ acceptor sites of abnormally low energy in the gel surface.

"Alkaline error" is a negative pH error due to positive emf deviation from thermodynamic behavior in solutions of very high pH. The phenomenon was first observed by Hughes (1922) who noticed electrode response to cations other than H^+ in highly alkaline solutions. Furthermore, if the composition of the glass was changed so as to contain more Al_2O_3 , and B_2O_3 , the electrode became more sensitive to Na^+ relative to H^+ ions.

The above observations led the way to the development of special $\text{Al}_2\text{O}_3 - \text{B}_2\text{O}_3$ glasses which were thermodynamically reversible to Na^+ , with K^+ and Li^+ as interfering ions, and to many later improvements.

Eisenman and coworkers (1957) have made a systematic study of simple glasses containing Na_2O , Al_2O_3 and SiO_2 , and have shown that glass sensitivity to cations was a reproducible property of glass compositions. The way was thus set for the development of a variety of other electrodes, all within the past few years only.

III. ION SELECTIVE ELECTRODES

The systematic survey of electrodes by Eisenman (1957), the tracer experiments of Schwabe & Dahms (1961), and several other investigations in the field, provided the necessary insight for the development and manufacture of practical electrodes having wide applications in different areas of chemistry.

The electrodes available to date may be classified (Rechnitz 1967) into four classes: glass electrodes, solid state electrodes, precipitate electrodes, and liquid-liquid membrane electrodes. They all seem to involve an ion-exchange process in the potential-determining mechanism. Their potentials are, within a range, described by the Nernst equation; they are proper thermodynamic electrodes.

Glass membrane electrodes. The main criterion that determines the selectivity of this class of electrodes is the composition of its glass. The structure and the mode of operation are very similar to the H^+ glass electrode.

There are three subtypes in this group which have the following selectivity characteristics (Rechnitz, 1967):

pH type - selectivity order: $\text{H}^+ \gg \text{Na}^+ > \text{K}^+, \text{Rb}^+, \text{Cs}^+ \dots \gg \text{Ca}^{2+}$

Cation-sensitive type - selectivity order: $\text{H}^+ > \text{K}^+ > \text{Na}^+ > \text{NH}_4^+, \text{Li}^+ \dots \gg \text{Ca}^{2+}$

"Sodium"-sensitive type - selectivity order: $\text{Ag}^+ \gg \text{H}^+ \gg \text{Na}^+ \gg \text{K}^+$,
 $\text{Li}^+ \dots \gg \text{Ca}^{2+}$.

Precipitate electrodes. These consist of sparingly soluble inorganic salts whose anions are the same as the one to be measured, embedded in a neutral matrix, such as silicone-rubber and paraffin, to increase mechanical manageability. They are also called heterogeneous or precipitate-impregnated membranes. Examples include electrodes sensitive to Cl^- , Br^- , I^- , S^{2-} and SO_4^{2-} . The grain size and the ratio of precipitate to matrix are two factors which influence electrode behavior; the low sensitivity limit is dictated by the solubility of the salt. Such electrodes are insensitive to cations and the absence of cation effects in salts containing the same anion but different cations suggests that the membrane acts as an anion exchanger. Such electrodes have an advantage over the Ag / Ag halide electrodes in that they are not affected by redox interferences or surface poisoning.

Solid-state electrodes. This type of electrode consists of an inorganic synthetic single crystal doped with a rare earth salt to lower its electrical resistance. It is capable of functioning effectively in solutions of extremely low concentrations due to the low solubility of the inorganic electrode material. Some solid-state electrodes for the halides contain solid cast pellets of silver halides as the active membrane materials.

Examples of commercially available solid-state electrodes are:
 F^- , Cl^- , Br^- and S^{2-} .

Liquid-liquid electrodes. This type consists of an internal Ag-AgCl reference electrode dipping into the internal filling solution. A porous

glass or plastic membrane constitutes the liquid-liquid interface between an ion-exchanger and the external test solution. Necessary characteristics of the ion-exchanger include high selectivity for the ion concerned, low solubility in sample solution, high viscosity to prevent rapid flow across the interface, photochemical and thermal stability, moderate cost, availability in high purity and compatibility with the internal reference element and high exchange capacity.

Electrodes available so far, based on this principle, are the Corning and Orion calcium electrodes, the divalent cation electrode, equally responding to Ca^{2+} and Mg^{2+} , the cupric ion electrode, as well as anion electrodes for Cl^- , ClO_4^- and NO_3^- .

The subject of ion selective electrodes is a very recent- and no doubt due partly to industrial potential- a rapidly developing subject. Most of the information available to us is derived from commercial pamphlets and brochures. An overall summary is given in Table I.

Table I

Ion-Selective Electrodes, available 1968

Type	Electrode	Manufacturer	Conc. range	Interfering Ions, main
Glass Electrodes	H ⁺	Beckman, Orion		
	Na ⁺	Corning, Beckman	10 ⁻⁴ -10 ⁻¹ M	H ⁺ , Li ⁺ , K ⁺
	K ⁺	Corning, Beckman	10 ⁻⁴ M-	K ⁺
	Li ⁺		1.0 - 20 m	Na ⁺
	Rb ⁺			
	Cs ⁺			
	Ag ⁺			
	NH ₄ ⁺	Beckman		OH ⁻ and F ⁻
Liquid-liquid membrane electrodes	Ca ²⁺	Beckman (BHL15)	10 ⁻⁴ -1M	Na ⁺
	Divalent Ca ²⁺ , Mg ²⁺	Orion, Corning	10 ⁻⁴ -	Mg ²⁺ , Ba ²⁺ , Na ⁺ , H ⁺
	Cu ²⁺	Orion	10 ⁻⁴ -sat.	H ⁺ , OH ⁻ , Zn ²⁺ , Fe ²⁺ , Cu ²⁺ , Ni ²⁺
	Cl ⁻	Orion	10 ⁻⁵ -10 ⁻¹ M	Mg ²⁺ , Sr ²⁺ , Ca ²⁺ , Zn ²⁺ , Ni ²⁺ , H ⁺
	ClO ₄ ⁻	Orion	10 ⁻⁵ -10 ⁻¹ M	ClO ₄ ⁻ , SO ₄ ²⁻ , I ⁻ , F ⁻ , NO ₃ ⁻ , Br ⁻
	NO ₃ ⁻	Orion	10 ⁻⁵ -10 ⁻¹ M	H ⁺ , OH ⁻ , Cl ⁻ , F ⁻ , HCO ₃ ⁻
	Cl ⁻	Pungor	10 ⁻⁴ ?	ClO ₄ ⁻ , I ⁻ , Br ⁻ , S ²⁻
	Br ⁻	Pungor	10 ⁻⁵ ?	I ⁻ , Br ⁻
	I ⁻	Pungor	10 ⁻⁶ ?-0.1M	Cl ⁻ , I ⁻
	S ²⁻	Pungor		Br ⁻ , Cl ⁻
Ppt-impregnated membrane electrodes	PO ₄ ³⁻	Pungor		
	SO ₄ ²⁻	Pungor	10 ⁻⁶ -10 ⁻¹ M	PO ₄ ³⁻ , Cl ⁻

Table I Cont'd.

Type	Electrode	Manufacturer	Conc. range	Interfering Ions, main
Solid-state membrane electrodes	F ⁻	Orion	10 ⁻⁶ -10 ⁰ M	OH ⁻
	Cl ⁻	Orion	5.0x10 ⁻⁵ -1M	OH ⁻ , Br ⁻ , S ²⁻ , CN ⁻ , NH ₃ *, S ₂ O ₃ ²⁻ *
	I ⁻	Orion	0.01ppm-sat.	S ²⁻ , S ₂ O ₃ ²⁻ , CN ⁻
	S ²⁻	Orion	10 ⁻¹⁷ -1M	no ions
	Br ⁻	Orion	10 ⁻⁷ -sat.	S ²⁻ ** , OH ⁻ , Cl ⁻ , I ⁻
	CN ⁻	Orion	10 ⁻⁵ -10 ⁻³ M	S ²⁻ , I ⁻

* form complexes

** must be absent

STUDY OF THE CALCIUM ELECTRODE

I. HISTORY

Thermodynamically, the obvious method of measuring calcium ion activity in solution is the use of a $\text{Ca(s)} / \text{Ca}^{2+}$ ion electrode. Such an electrode is impossible to construct for aqueous solutions because of the high reactivity of calcium metal (reduction potential -2.87 volts). In fact, the first literature reference to a calcium electrode is a calcium amalgam electrode $\text{Ca(s)} / \text{Hg(l)} / \text{Ca}^{2+}$ (aq.) used for determining activity coefficients and transference numbers of alkali earth chlorides (Lucasse, 1924).

Fosbinder (1929) used a similar electrode to measure activity coefficients of CaCl_2 and CaSO_4 in mono-cationic solutions. However, the use of these electrodes is very limited. Work with them is laborious, they cannot be used in solutions containing other cations such as Na and K, and measurements cannot be made in very dilute solutions.

Corten and Esterman (1928) employed an electrode of the "third kind", $\text{Ag(s)} / \text{Ag}_2\text{C}_2\text{O}_4\text{(s)} / \text{CaC}_2\text{O}_4\text{(s)} / \text{Ca}^{2+}$ (aq.), but their results were disputed and later improved upon by several other workers. Nevertheless such electrodes are difficult to prepare, slow in response and cannot be used in calcium salt solutions $< 10^{-3}\text{M}$.

Modern glass, membrane, and liquid ion-exchanger membrane electrodes have provided a new approach to the thermodynamic measurement of Ca^{2+} activity. Each of these electrodes, in turn, has its limitations and merits.

Glass electrodes using natural, phosphate and alkali aluminosilicate glasses have been reviewed by Truesdell and Christ (1967). Li-Ca-Al silicate glasses do not give reproducible results. Phosphate glass electrodes are fragile, with high electrical resistance, and sluggish response, and cannot be used in presence of Mg^{2+} ions. Alkali silicate glasses which are essentially alkali ion-sensitive glasses, show higher selectivity to K^+ and can only be used in non-cationic solutions.

Membrane electrodes of various types composed of calcium fluoride, sintered clay minerals, collodion, paraffin and ion exchange resins have been used. These generally show a nonspecific selectivity to Ca^{2+} ions. Shatkey (1967) has studied the suitability of paraffin membranes and solid polymer membranes for Ca^{2+} activity measurements.

An interesting non-potentiometric method of measuring Ca^{2+} activity is provided by the McLean-Hastings (1934) frog-heart method which makes use of the measurement of the amplitude of the contractions of a vital frog heart in the test solution as compared to the amplitude of contractions in a known solution. Clearly the method cannot be applied to all systems and is not convenient to use except in physiological work.

There are at present two commercial liquid ion-exchange calcium electrodes. They are manufactured by Orion and by Corning companies and they appear to have similar features. These electrodes are convenient to use, give rapid and reproducible results under optimum conditions and can be used in the presence of many ions within certain limits of concentration.

II. THE ORION CALCIUM ELECTRODE

The main features of the Orion calcium electrode (Section IVB, below) include an internal half cell consisting of a $\text{Ag(s)} / \text{AgCl(s)}$ reference electrode which provides a stable contact to the potentiometer and an internal filling solution of CaCl_2 which contacts the inside surface of the porous inert membrane disc. The electrode also includes a liquid ion-exchanger which is a calcium salt of an organophosphoric acid having very high selectivity to Ca^{2+} ions and very low solubility in water. The inert membrane, in contact with both the ion-exchanger and the internal CaCl_2 solution from the inside and with the test solution on the outside, prevents mixing of the liquid phases.

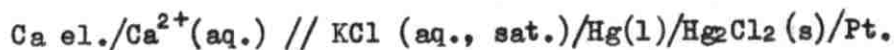
The electrode is provided in the form of a kit which includes the body, bottom cap and the membrane spacer assembly, all made from Fluoro-carbon plastic, together with the ion-exchanger and the internal filling solution vials and the syringes and a box of porous membranes.

The tip of the electrode is illustrated in Fig. 1 which also shows the main features described above.

III. THEORY

In this, as in all potentiometric methods, one measures the potential difference between the electrode in question and another (reference) electrode, using a potentiometer and taking into account the necessary precautions such as control of temperature, pH, ionic strength, and ionic composition.

The electrochemical cell comprising a calcium electrode and the reference electrode is



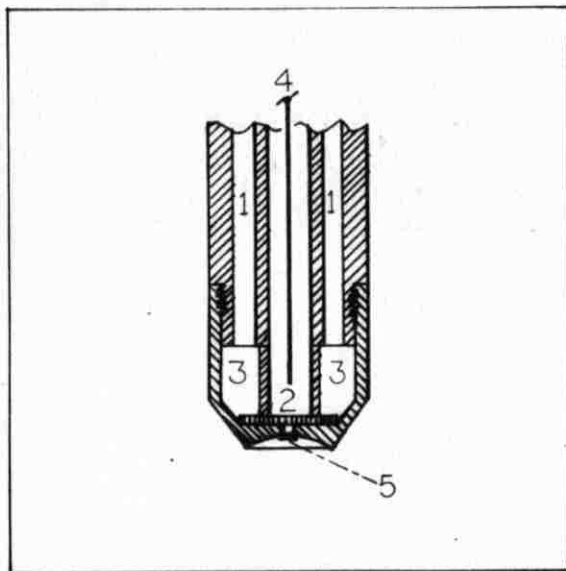


Figure 1 - The Calcium electrode tip.

1. Ion exchanger
2. Internal aqueous filling solution (CaCl_2)
3. Ion-exchange reservoir
4. Ag-AgCl reference electrode
5. Porous membrane

for which

$$E = E_{\text{cal}} - E_{\text{Ca}} = E_{\text{cal}} - (E_{\text{Ca}}^0 + \frac{RT}{2F} \ln a) \quad (1)$$

where E is the measured emf, E_{cal} the potential of a calomel electrode in saturated KCl (244.4 mv at 25⁰), E_{Ca}^0 the "standard" potential of the calcium electrode (about 90 mv, assumed to be constant during an experiment), $RT/2F$ the thermodynamic constants (29.58 mv at 25.0⁰), and "a" the molar ionic activity of free Ca^{2+} in the solution.

Because of the uncertainty about E_{Ca}^0 , measurements have to be made relative to a standard solution, such as CaCl_2 , where ion-association is known to be practically negligible in dilute solutions. Thus a given (standard) solution of CaCl_2 will have a concentration of calcium ions c_s exactly equal to the stoichiometric molarity of the salt, whereas some other solution of a calcium salt with unknown extent of ion association will have a concentration c_x . This quantity c_x is obtained in the following manner:

If E_s and E_x are, respectively, the emfs of the above cell with standard and unknown calcium salts, both emfs measured against the same reference calomel electrode, it follows from equation 1 that

$$\begin{aligned} E_x - E_s &= \frac{RT}{2F} \ln \frac{a_x}{a_s} \\ &= \frac{RT}{2F} \ln \frac{c_x}{c_s} \cdot \frac{y_x}{y_s} \end{aligned} \quad (2)$$

The symbols are as defined above, and y_x and y_s are the ionic molar activity coefficients of Ca^{2+} ions calculated on the basis of the Debye-Huckel relation:

$$-\log y = Az^2 I^{\frac{1}{2}} / (1 + BaI^{\frac{1}{2}}) \quad (3)$$

where the constants A and B . a are given in Table II, z is the ionic charge +2, and I is the total molar ionic strength of the solution.

Equation 2 can be used in two ways. On the one hand it can yield the Ca^{2+} ionic activity a_x in an unknown solution. For this, one needs the measured quantities E_x and E_s , together with a calculated value of y_s and the known stoichiometric quantity c_s .

Alternatively, equation 2 can be used in the following form:

$$\log c_x = \log c_s + \frac{E_x - E_s}{RT/2F} + \log (y_s / y_x) \quad (4)$$

to yield the molar concentration c_x of free Ca^{2+} ions in an unknown solution using the experimentally measured quantities E_x and E_s , the ratio of the two activity coefficients y_s/y_x calculated from equation 3, and the stoichiometrically known quantity c_s .

In our work, this form of the equation was used as it has the additional advantage of requiring only the ratio of the two activity coefficients. The application of this method to the study of ion-association is discussed in section VI below.

IV. EXPERIMENTAL DETAILS

A. Chemicals

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (M.W. 147.03) Merck pro-analysis. Not purified further, but its solutions were titrated against standard EDTA.

CaCO_3 (M.W. 100.09) Merck pro-analysis (99.7%) used for standardizing EDTA solutions.

EDTA = Diaminoethanetetra-acetic acid, disodium salt $(\text{CH}_2\text{COOH})_2\text{N} \cdot \text{CH}_2\text{CH}_2 \cdot \text{N}(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ (M.W. 372.25) B.D.H. Analar (99.99%).

Approximately 0.01M solutions were standardized against standard

Table II

Data on Debye-Huckel Constants

Temp °C	A *	(1) B.a	(2)	$\frac{2.303}{F^2/F}$ * (mv)
15	0.503		2.0	57.17
20	0.507		2.0	58.16
25	0.512	1.6	2.0	59.16
30	0.516		2.0	60.15
35	0.521		2.0	61.14

* Robinson and Stokes (1960), p. 468

Data for Debye-Huckel extended equation (Equation 3)

(1) Solutions of CaCl_2 and CaSO_4 for the CaSO_4

experiments.

(2) Solutions of CaCl_2 , $\text{K}_4\text{Fe}(\text{CN})_6$ and $\text{K}_3\text{Fe}(\text{CN})_6$

CaCl₂ prepared by taking 1.000 g CaCO₃, dissolving it in 5 ml of 6N HCl and making up to a liter. 25.0 ml of the standard CaCl₂ were used in the titrations using Eriochrome T (0.25 g/50 ml absolute alcohol) as indicator.

KCl (M.W. 74.56) Analar (99.8%) dried at 110° and stored in a desiccator. Solutions were used for studying the effect of 1:1 salts on calcium electrode behavior.

NaCl (M.W. 58.44) Analar, used without further purification in testing the effect of 1:1 salts on calcium electrode.

BaCl₂·2H₂O (M.W. 244.306) Analar (99.3%), used without further purification. Solutions were made from a stock 1M solution by appropriate dilutions. These were used in studying the effect of 2:1 salts on the calcium electrode.

CaSO₄ (anhydrous powder) (M.W. 136.146) Baker Analyzed (98.9%). A 0.01M stock solution was prepared and diluted appropriately.

MgCl₂·6H₂O (crystals) (M.W. 203.31) Analar (99.96%). A 1M stock solution was diluted to about 10⁻²M and standardized against EDTA solution. The diluted solutions were used in studying the effect of 2:1 salts on calcium electrode.

KOH (M.W. 56.11) U.S.P. pellets approximately 85%, used without further purification.

B. Apparatus

Faraday black metal box for protection of solutions and electrodes from light and electrical pick-up. It contained the cell which consisted of the electrodes, two glass jacketed vessels, saturated KCl agar bridge and a magnetic stirrer.

- Electrodes: (1) Calcium electrode (Orion Res. Inc., Mass., model 92-20). Membrane was replaced when the potential of a $1.0 \times 10^{-3} \text{M}$ solution of CaCl_2 deviated from zero millivolts. The electrode elutes about $5 \times 10^{-6} \text{M}$ Ca^{2+} due to the solubility of ion-exchanger in water. Only the tip of the electrode was allowed to dip into the solution during measurements; hence the Ag-AgCl internal electrode was essentially at room air temperature.
- (2) Saturated calomel commercial electrode was selected on basis that it gave reproducible readings and compared within 0.2mv with similar calomels. This electrode was always deeply immersed in thermostated, saturated KCl solution.

Salt bridge. A saturated KCl-agar salt bridge was prepared by injecting the gel, prepared by dissolving 4g of Agar in 100 ml of hot saturated KCl solution, into narrow polyethylene tubing of appropriate length. Salt bridges were kept under saturated KCl solution.

The electrodes were set up in two separate glass jacketed vessels adjusted over magnetic stirrers and thermostated by circulating water from a constant temperature bath.

Temperature control. A constant temperature water bath with heating and cooling was used (Wilkens-Anderson Co., Chicago). Thermostated water was pumped to the glass jacketed cells. Thermometers were calibrated against a set of standard thermometers (National Physical Laboratory, England). This was done at every temperature. The control of temperature, as measured in the actual solution was found to be constant within $\pm 0.05^\circ$ or better.

Potentiometer. A Beckman Research pH meter was used in making all measurements. The instrument has a readability of 0.02 mv and precision of 0.1 mv.

Recorder. A Beckman 10" linear recorder was used in conjunction with the potentiometer at 10 mv full range to record the output ($E_x - E_s$) in some of the experiments. This was done to check the pattern of variation of the reading with time.

C. Procedure

Different techniques were tried in an effort to minimize errors and to overcome the difficulties which were encountered in emf measurements. Difficulties were of two types: a drift in potential with time for the same solution, and shift of all the potential readings to higher positive values. The second problem, when encountered, was solved by replacing the membrane and regenerating the electrode until the potential reading for the $1.0 \times 10^{-3} M$ $CaCl_2$ solution was around zero mv.

To minimize effects due to time drifts in potential the following uniform procedure was followed:

1. Solutions were thermostated before use and the electrode was preconditioned with the solution to be used.
2. Solution S was always put first and the electrode was washed repeatedly with the solution, with constant stirring between washings. This was carried on for at least 10 minutes.
3. The cell emf was read until two consecutive readings of two samples of the same solution read the same within ± 0.1 mv.
4. The electrode was washed two or three times with solution X, containing an estimated free Ca^{2+} concentration which is close to that of

solution S, and then two constant consecutive readings were recorded. Usually two or three different X solutions were chosen to be measured with the same S but in all cases the sequence (S₁ - X₁), (S₁ - X₂) or (S₁ - X₁ - S₁), (S₁ - X₂ - S₁), was followed.

The choice of the concentration of X was important. In order to minimize the contribution of the factor $(E_x - E_s) / 29.6$ (equation 4), E_x should be as nearly equal to E_s as possible.

A Beckman recorder was used during preliminary runs to observe the changes and the drifts in the potential. No special advantage, however, was found in the recording of emf; all subsequent work was therefore carried out by visual reading of emf to the nearest 0.02 mv.

V. RESULTS

A. Thermodynamic reversibility of calcium electrode.

The thermodynamic significance of the calcium electrode is its direct reversibility to Ca²⁺ ion activity in aqueous solutions. Before using the electrode in any quantitative study this assumption had to be verified. The test was made as follows:

1. A series of solutions of CaCl₂ in water ranging from 1.00x10⁻²M to 1.00x10⁻⁵M were prepared.
2. A commercial calomel electrode was selected and tested against similar electrodes to ensure its stability and reversibility.
3. A number of Ag-AgCl electrodes were prepared electrolytically (Tayim, Thesis 1964) and a matching pair was selected.
4. The cell emf ($E_{ref} - E_{Ca}$) was measured for every solution, using the procedure described above. The measurements were made on the Ca electrode against calomel and Ag-AgCl electrodes. This type of experiment

was made on several occasions and gave concordant results.

The results of one set of measurements are summarized in Table III and illustrated in Fig. 2, where:

M = molar concentration.

I(M) = total molar ionic strength assuming complete ionization.

y = molar ionic activity coefficient of Ca^{2+} calculated from

Debye-Huckel extended equation (3) and constants (Table II).

a = molar ionic activity of Ca^{2+} = (CaCl₂ conc.)·y .

E_{Ca} = potential of Ca electrode obtained in two ways:

- 1) Cell comprising calcium electrode / calomel electrode,
gives $E_{\text{Ca}} + E_j$ (including liquid junction potential E_j).
- 2) Cell comprising calcium electrode / Ag - AgCl electrode,
gives E_{Ca} (without liquid junction potential).

Inspection of Table III and Figure 2 leads to the following conclusions about the properties of the Ca^{2+} electrode.

1. The limit for reproducible quantitative measurement of Ca^{2+} concentration is $5.0 \times 10^{-4} \text{M}$. At lower concentrations the relation is not linear probably due to the appreciable solubility of the ion-exchanger in the aqueous phase. (Claimed to be $5 \times 10^{-6} \text{M}$ Ca^{2+} by the manufacturers).

2. The theoretically predicted slope of 29.6 mv/pCa at 25°C of a plot of E vs -log a confirms the thermodynamic reversibility of the electrode to Ca^{2+} activity.

3. The slope of the E vs -log c line is about 25 mv/pCa which again shows that the electrode does not thermodynamically measure concentration. Nevertheless, the latter can be obtained from the data on a purely empirical basis.

Table III

Thermodynamic Test of Calcium Electrode at 25.0°

as described in text V.A

CaCl ₂ 10 ³ M	I 10 ³ M	y	a 10 ³ M	Calomel Reference			Ag-AgCl Reference		
				meas.E mv	E _{cal} mv	E _{Ca} mv	meas.E mv	E _{AgCl} mv	E _{Ca} mv
0.0738	0.218	0.935	0.0696	-21.0	244.4	223.4	-224.7	450.2	225.5
0.177	0.531	0.912	0.162	-18.8	244.4	225.6	-202.9	427.4	224.5
0.693	2.08	0.819	0.567	- 3.6	244.4	240.8	-151.8	392.6	240.8
1.72	5.16	0.747	1.28	6.1	244.4	250.3	-119.3	370.3	251.0
6.88	20.6	0.571	3.93	21.0	244.4	265.4	- 71.0	336.1	265.1

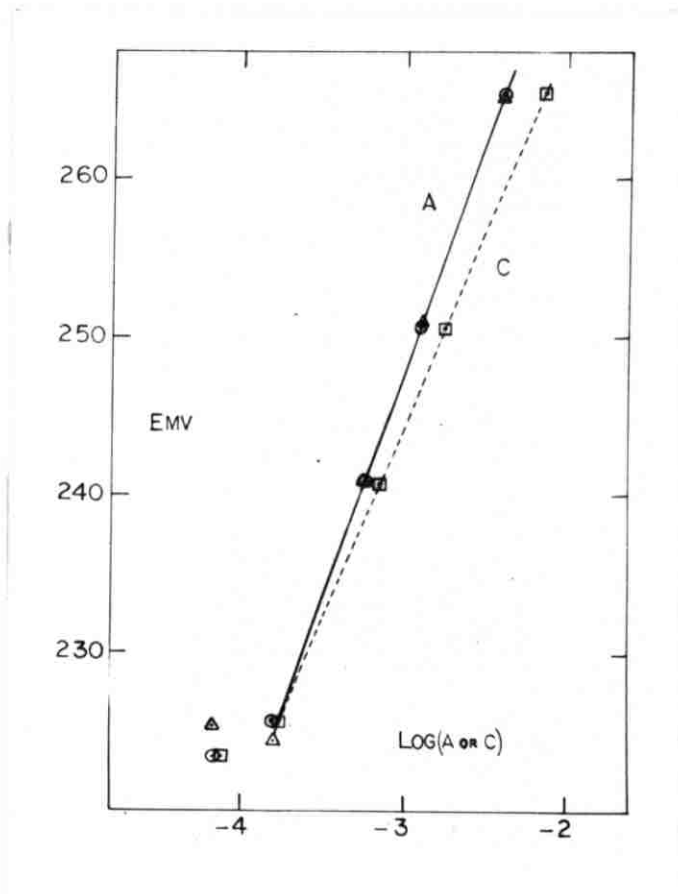


Figure 2 - The calibration of the Ca electrode

A. Plot of E_{Ca} versus $\log (Ca^{2+}$
activity) (equation 1).

Δ Ag-AgCl reference; \circ calomel reference.

C. Plot of E_{Ca} versus $\log (Ca^{2+}$
concentration); \square calomel reference.

4. The line A shows that agreement is close between results from measurements using a calomel reference and those using Ag-AgCl reference. Since the former case involves use of a salt bridge while the latter does not, it may be concluded that any residual liquid-junction potential between the salt bridge and the test solutions used must have been either reasonably constant or negligibly small. This is not unexpected since the solutions were around neutral pH.

B. Effect of pH and salts

Having established the thermodynamic reversibility of the electrode it was essential to check the claims of the manufacturers regarding the effect of foreign cations on electrode performance.

Effect of pH. The purpose of this study was to verify that the optimum range of pH in which the electrode could be used was pH 6.0 - 10. This was done by measuring the emf of a series of solutions containing a constant concentration of CaCl_2 at different pH values which were adjusted by the addition of appropriate amounts of 0.1M KOH.

It was indeed found that the electrode was sensitive to H^+ at $\text{pH} > 5$ and that reasonably constant emf readings could be obtained in the range of pH 6 to 9. The behavior in more alkaline solutions was not investigated due to drifts in emf readings starting around pH 9.

The conclusion to be drawn from this series of tests, which confirm the published statement of the manufacturers, is that the best region for use of the electrode is between pH 6 - 8 and that outside these limits, interference due to H^+ and OH^- becomes significant depending on the particular conditions.

Effect of salts. The electrode is claimed by the manufacturers to be

"approximately 50 times more sensitive to Ca^{2+} than Mg^{2+} and approximately 100 times more sensitive to Ca^{2+} than Ba^{2+} ". In a recent study of this electrode, Shatkay (1967) has refuted this claim. It was therefore appropriate to examine the effect of ions on electrode performance, particularly with regard to the question of whether the effects are due to changes in ionic strength or to ion-exchange phenomena, such as competition of foreign ions for sites in the ion-exchanger.

1:1 salts. Three sets of solutions were made each containing a different constant CaCl_2 concentration with varying concentrations of NaCl . In every case, solution (X) containing foreign ions was compared with a solution S containing the same level of CaCl_2 (with little KOH), and the emf difference, $E_X - E_S$, was obtained. Numerical analysis of the data is described in the next section and some results are given in Table IVa and illustrated in Figure 3.

Corresponding studies on KCl solutions were made on $1.00 \times 10^{-3} \text{M}$ CaCl_2 with up to $8.0 \times 10^{-3} \text{M}$ KCl which, covers the range of our experiments in $\text{K}_4\text{Fe}(\text{CN})_6$ and $\text{K}_3\text{Fe}(\text{CN})_6$ systems. No K^+ effects were detected under these conditions.

2:1 salts. Here the effects of Mg^{2+} and Ba^{2+} ions were investigated. At least two sets of solutions were made, each case with a different but constant concentration of CaCl_2 and varying concentrations of BaCl_2 and MgCl_2 ranging from $1.0 \times 10^{-4} \text{M}$ to $1.0 \times 10^{-1} \text{M}$. Again, the emf of solutions X was compared with the emf of S, a solution with the same level of CaCl_2 concentration. A sample of one set of results is given in Table IVb and shown in Figure 3.

Analysis and calculations. The above results were analyzed on the

Table IVa

Effect of 1:1 Salts on Electrode

T = 25.0°

1	2	3	4	5
C_{salt} $10^2 (M)$	I_x $10^2 (M)$	$-\log y_x$	$E_x - E_s$ calc. (mv)	$E_x - E_s$ obs (mv)
0.020	0.36	0.110	-0.20	
0.040	0.38	0.112	-0.30	
0.060	0.40	0.115	-0.40	
0.080	0.42	0.118	-0.50	
0.200	0.54	0.131	-0.80	
0.400	0.74	0.150	-1.40	
0.600	0.94	0.166	-2.00	
1.50	1.84	0.219	-3.50	
1.80	2.14	0.232	-3.70	
2.50	2.84	0.259	-4.60	
2.80	3.14	0.268	-5.00	
10.0	10.34	0.400	-9.00	-5.10
20.0	20.34	0.485	-11.4	-5.30
40.0	40.34	0.573	-14.1	-5.50
60.0	60.34	0.624	-15.6	-5.30
80.0	80.34	0.659	-16.4	-3.00

1. Conc. of 1:1 salt with $1.00 \times 10^{-3} M$ $CaCl_2$ and $4.0 \times 10^{-4} M$ KOH in solution X.

2. Total ionic strength of solution X.

3. y_x = molar ionic activity coefficient of Ca^{2+} (equation 3).

4. Theoretical $E_x - E_s$ calculated from equation 5.

5. Observed $E_x - E_s$

Table IVb

Effect of 2:1 Salts on Electrode

1	2	3	4	5	6
C_{salt} 10^2 (M)	I_x 10^3 (M)	$-\log y_x$	$E_x - E_s$ calc. (mv)	Mg^{2+} $E_x - E_s$ (mv)	Ba^{2+} $E_x - E_s$ (mv)
0.010	0.370	0.1110	-0.30	0.40	-1.70
0.015	0.380	0.1130	-0.30		
0.0175	0.390	0.1140	-0.40		
0.100	0.640	0.1460	-1.30	-2.15	-2.80
0.150	0.790	0.1545	-1.60		
0.200	0.940	0.1663	-1.90		
0.500	1.84	0.2185	-3.50		
0.700	2.44	0.2436	-4.20		
1.00	3.34	0.2742	-5.10	-11.4	-12.50
5.00	15.3	0.4494	-10.3		
7.00	21.3	0.4912	-11.5		
10.0	30.3	0.5361	-12.9	drift	-22.90
30.0	90.3	0.6710	-16.7		

1. Conc. of 2:1 salt with $1.00 \times 10^{-3} M$ $CaCl_2$ and $4.0 \times 10^{-4} M$

KOH in solution X.

2. Total ionic strength of solution X.

3. y_x = molar ionic activity coefficient of Ca^{2+} (equation 3).

4. Theoretical $E_x - E_s$ calculated from equation 5.

5. Observed $E_x - E_s$ for Mg^{2+} .

6. Observed $E_x - E_s$ for Ba^{2+} .

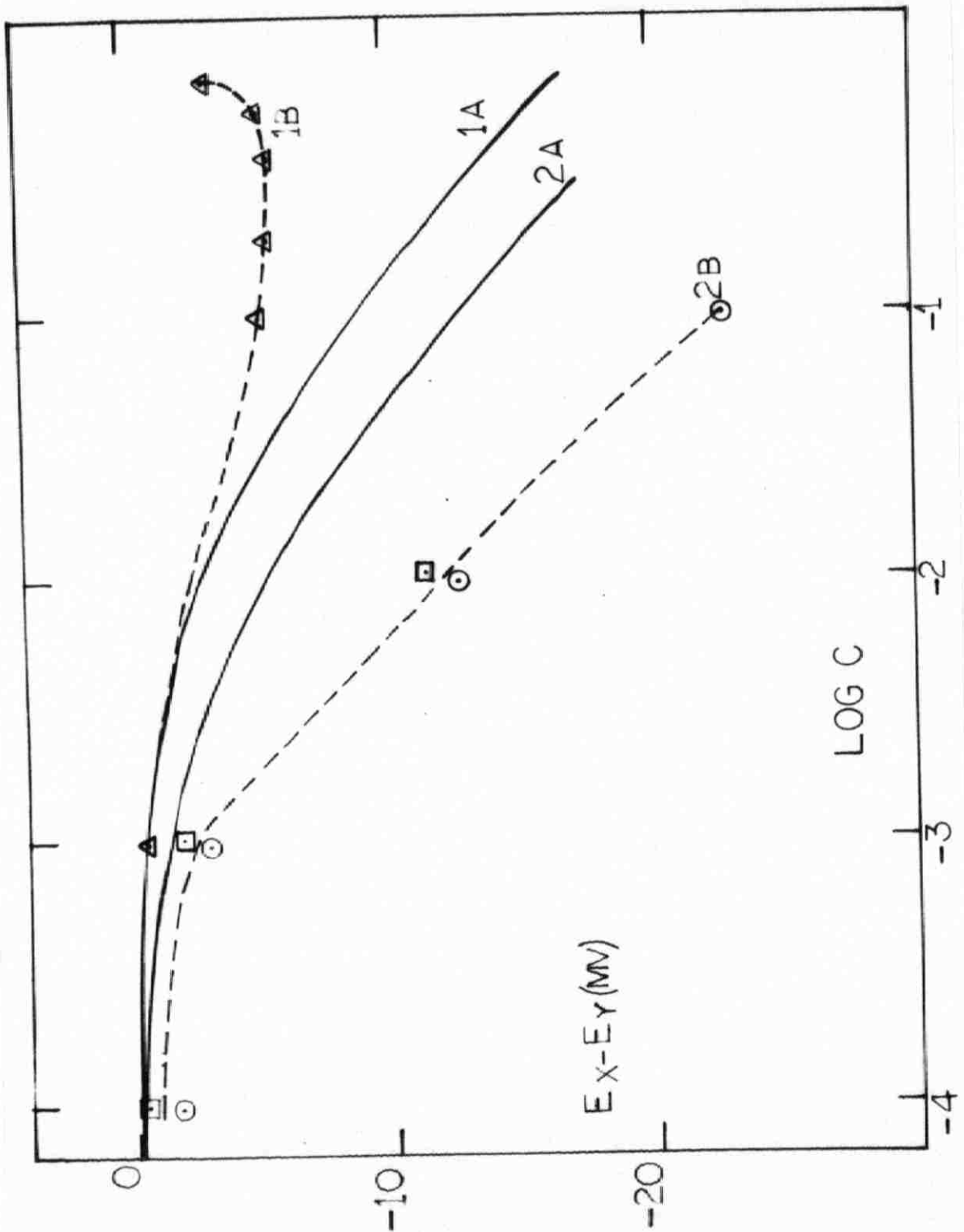


Figure 3 - Effect of 2:1 and 1:1 salts on $1.0 \times 10^{-3} \text{ M CaCl}_2$ (eq. 5)

1A Theoretical curve for
1:1 salt (—)
1B Line drawn through
experimental points
for Na^+ (Δ)

2A Theoretical curve for
2:1 salts (—)
2B Line drawn through experimental
points: Ba^{2+} (\circ), Mg^{2+} (\square)

assumption that changes in the observed emfs were due solely to changes in ionic strength. This was done as follows: At 25.0°, equation 2 may be rewritten in the form

$$E_x - E_s = 29.6 \log c_x y_x - 29.6 \log c_s y_s \quad (5)$$

where the symbols are as already defined. In this case, however,

$c_x = c_s$ (stoichiometric concentration of Ca is the same in solutions S and X), and

$y_x \neq y_s$ (due to changes in ionic strength in presence of added salts).

For $1.00 \times 10^{-3} M CaCl_2$, the second term in equation 5 is numerically equal to - 91.8 mv.

Theoretical curves for both 2:1 and 1:1 salts were drawn on the basis of this assumption by calculating $E_x - E_s$ at different ionic strengths. The results, as expected, first showed that the influence of the different ions is greatest at the lowest concentrations of Ca^{2+} ion or relatively high concentrations of foreign ions. In the case of $1.00 \times 10^{-3} M CaCl_2$, details of the results are given in Tables IVa and b, which also include the experimentally determined quantity $E_x - E_s$. Figure 3 shows the data in the form of theoretical and experimental curves.

Deviations from the theoretical curves are wide and clearly show that the phenomenon is not simply due to ionic strength effects. Furthermore, analysis of the results on basis of competition of ions for exchanger leads to the conclusion that deviations from theory should be positive whereas the observed $E_x - E_s$ values are negative. It is of course possible that the observed drifts in emf at higher foreign ion concentration might be due to an intermediate stage in the properties of the electrode falling

between sensitivity to Ca^{2+} and sensitivity to other cations.

The overall conclusions that can be drawn from the measurements done within the considered region of CaCl_2 and foreign salt concentration are the following:

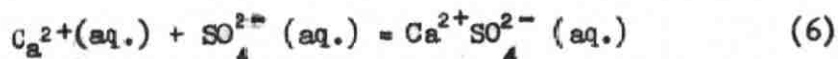
1. At low foreign ion concentrations the effects of foreign ions are adequately accounted for in terms of their contributions to ionic strength of the medium.

2. At high foreign ion concentrations, these ions appear to block ion-exchanger sites which reduce the effective Ca^{2+} concentration and cause a negative deviation.

3. At extremely high foreign ion concentrations one may expect the electrode to become sensitive, or partly selective, to the foreign ions. However, under such conditions no quantitative measurements are meaningful.

VI. THE CALCIUM-SULFATE SYSTEM

Having established the range of conditions of pH and various salt concentrations under which the Orion calcium electrode was thermodynamically reversible to Ca^{2+} ions, it remained necessary to find out if the same potentiometric method could be used for the study of ion-association. For this purpose, the aqueous calcium sulfate system was chosen. In this system, ion-pair formation has been well established, and the chemical equilibrium is



for which the thermodynamic ion-association (formation) constant is defined in terms of the activities of the ion-pair complex, the cation, and the anion, at equilibrium:

$$K^0 = a_{\text{ion-pair}} / a_{\text{Ca}^{2+}} \cdot a_{\text{SO}_4^{2-}} \quad (7)$$

K^0 has been determined from conductivity (Davies and Money, 1932) and

also from solubility studies (Bell and George, 1953). Its generally accepted value at 25° is 200.

The $\text{Ca}^{2+}\text{SO}_4^{2-}$ ion-pair is also of physiological interest and has been studied by Walser and Payne (1959) to establish the physiological role of ion association. The McLean and Hastings (1934) frog heart method was used for determining the free Ca^{2+} ion activity, and evidence was presented to negate any interferences due to precipitation of the insoluble salt or to toxicity of anions. The results were interpreted in terms of ion-association and a dissociation constant of 0.039 ± 0.009 at 0.11M ionic strength was reported.

A. Experimental

The experimental set-up and procedure used were as described above (section IVB & C). In these experiments, solutions X ranged in concentration from $5.00 \times 10^{-4}\text{M}$ to $1.50 \times 10^{-3}\text{M}$ in CaSO_4 with an appropriate amount of 0.10M KOH to adjust the solution to pH 7 to 8. Solutions S (CaCl_2) were prepared by dilution from a standardized 0.100M stock solution. The range of concentration of solutions S was between $4.00 \times 10^{-4}\text{M}$ to $1.50 \times 10^{-3}\text{M}$.

All experiments were carried out at 25.0° using saturated KCl-calomel electrode. The Ag-AgCl electrode was used in one set of measurements, and a Beckman 10" recorder was used in some cases for recording the output E_x and E_s .

B. Calculations

Details of the calculations are best illustrated with reference to Table V in which the columns are as follows:

1. Molar stoichiometric concentration of CaSO_4 in solution X.
2. Molar stoichiometric concentration of CaCl_2 in solution S.

Table V

Determination of Ion Association Constant for CaSO_4 at 25.0°

1	2	3	4	5	6	7	8
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_B$ (M) ^B	$10^3 I_x$ (M) ^x	$10^3 I_B$ (M) ^B	$10^3 c_x$ (M) ^x	K (M ⁻¹)	K ⁰ (M ⁻¹)
0.500	0.400	1.40	1.92	1.32	0.466	182	220
0.500	0.400	1.28	1.92	1.32	0.462	209	246
0.750	0.600	1.08	2.82	1.92	0.682	158	199
0.750	0.600	0.98	2.82	1.92	0.678	169	212
0.800	0.800	-1.50	3.00	2.52	0.729	144	182
0.800	0.800	-1.80	3.00	2.52	0.712	185	234
1.00	0.800	0.74	3.72	2.52	0.890	146	190
1.00	0.800	0.76	3.72	2.52	0.891	144	187
1.00	0.800	0.80	3.72	2.52	0.894	139	180
1.00	1.00	-1.86	3.82	3.22	0.901	150	195
1.00	1.00	-2.00	3.82	3.22	0.889	160	221
1.50	1.50	-2.24	5.64	4.74	1.30	132	181
1.50	1.50	-2.40	5.64	4.74	1.28	149	204
1.50	1.50	-2.44	5.64	4.74	1.28	149	204
1.50	1.50	-2.28	5.64	4.74	1.30	132	181
1.50	1.50	-2.30	5.64	4.74	1.30	132	181

3. The difference between cell emfs with the calcium electrode in solutions X and S, in both cases the potential being measured against a saturated calomel electrode reference.

4. Total molar ionic strength in solution X estimated on basis of 10% ion-association of $\text{Ca}^{2+}\text{SO}_4^{2-}$ for all concentrations, and including contributions from eluted CaCl_2 ($5 \times 10^{-6}\text{M}$) and from added KOH ($2.0 \times 10^{-4}\text{M}$).

5. Total molar ionic strength in solution S estimated on 100% dissociation of CaCl_2 , and including contribution from eluted CaCl_2 ($5 \times 10^{-6}\text{M}$) and from added KOH ($2.0 \times 10^{-4}\text{M}$).

6. Equilibrium concentration of free Ca^{2+} , c_x in equation 4, calculated as described in section III.

7. Equilibrium constant defined in terms of the equilibrium molar concentrations:

$$K = (\text{ion-pair}) / c_x \cdot (\text{SO}_4^{2-}) \quad (8)$$

where

$$(\text{ion-pair}) = X + (\text{CaCl}_2)_{\text{eluted}} - c_x$$

$$(\text{SO}_4^{2-}) = X - (\text{ion-pair})$$

8. Thermodynamic equilibrium constant K^0 (equation 7) related to K through the ionic activity coefficients:

$$K^0 = K (y_{\text{ion-pair}} / y_{2+} \cdot y_{2-}) \quad (9)$$

assume $y_{\text{ion-pair}} = y_{\pm}$

since $y_{2+} \cdot y_{2-} = y_{\pm}^2$, application of Debye-Huckel relation equation 3 leads to

$$\log K^0 = \log K + 4AI^{\frac{1}{2}} / (1 + 1.6 I^{\frac{1}{2}}) \quad (10)$$

Thus it is seen from Table V that application of the calcium

electrode method to the calcium sulfate system yields a value for the ion association constant ($K^0 = 198 \pm 17$ at 25.0°) which is in close agreement with the literature value 200. This may be taken as confirmation of the validity and reliability of the method.

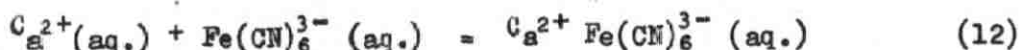
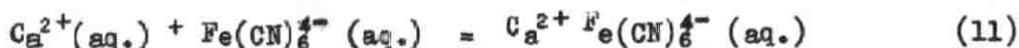
ION ASSOCIATION IN CALCIUM HEXACYANOFERRATES

The results obtained above suggested that the calcium electrode could be conveniently used for ion-association studies within a concentration range in which the assumptions about activity coefficients could be justified and formation of ion-triplets and other complexes could be neglected.

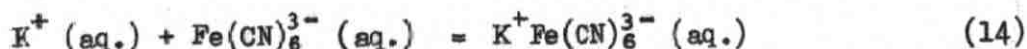
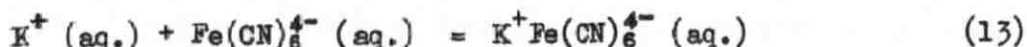
We have chosen to study in detail the binding of Ca^{2+} ions to hexacyanoferrates (II) and (III) ions. These two stable complex ions are known to have similar rigid octahedral structures, with net charges of -4 and -3 respectively. Comparison of thermodynamic parameters for their ion-association equilibria would show the influence of changing charge without concomitant structural effects. Furthermore, the well known salts $\text{K}_4\text{Fe}(\text{CN})_6$ and $\text{K}_3\text{Fe}(\text{CN})_6$ are very convenient to use, and their ion-association equilibria involving K^+ have already been investigated (Eaton, George and Hanania, 1967).

I. THERMODYNAMIC CONSIDERATIONS

The equilibria to be considered are the following:



The corresponding equilibria involving K^+ , which have to be taken into account in calculations are:



Considering equilibrium 11, the thermodynamic ion-association

constant is defined in terms of the activities of ion-pair, free Ca^{2+} and ligand at equilibrium.

$$K^0 = a_{\text{ion-pair}} / a_{\text{Ca}^{2+}} \cdot a_{\text{Fe}(\text{CN})_6^{4-}} \quad (15)$$

and the measured association constant is expressed in terms of the equilibrium molar concentration of the ions:

$$K = (\text{ion-pair}) / c_x \cdot (\text{Fe}(\text{CN})_6^{4-}) \quad (16)$$

where c_x stands for the free Ca^{2+} molar concentration. The relation between the two equilibrium constants involves the molar activity coefficients of the respective species:

$$K^0 = K \cdot \gamma_{2-} / \gamma_{2+} \cdot \gamma_{4-} \quad (17)$$

Assuming the applicability of a Debye-Huckel relation, it follows that

$$\begin{aligned} \log K^0 &= \log K + \log (\gamma_{2-} / \gamma_{2+} \cdot \gamma_{4-}) \\ &= \log K + 16AI^{\frac{1}{2}} / (1 + 2.0I^{\frac{1}{2}}) \end{aligned} \quad (18)$$

where A is the Debye-Huckel constant tabulated in Table II.

The corresponding equations for $\text{Ca}^{2+} \text{Fe}(\text{CN})_6^{3-}$ are similar. Thus,

$$K^0 = a_{\text{ion-pair}} / a_{\text{Ca}^{2+}} \cdot a_{\text{Fe}(\text{CN})_6^{3-}} \quad (19)$$

$$K = (\text{ion-pair}) / c_x \cdot \text{Fe}(\text{CN})_6^{3-} \quad (20)$$

and the relation between them is:

$$K^0 = k \cdot \gamma_{-} / \gamma_{2+} \cdot \gamma_{3-} \quad (21)$$

which leads to

$$\log K^0 = \log K + 12A \cdot I^{\frac{1}{2}} / (1 + 2.0 I^{\frac{1}{2}}) \quad (22)$$

In both cases, I is the total molar ionic strength of solution X after taking ion-association into account.

II. EXPERIMENTAL DETAILS

A. Chemicals

1. $K_4Fe(CN)_6 \cdot 3H_2O$ (M.W. 422.41). Analytical Reagent. The reagent was recrystallized from hot water and the crystals were dried in a black painted desiccator over a saturated aqueous solution of sucrose and sodium chloride (Kothoff and Stenger, 1947) for several months before use. The solutions were made by weight assuming the trihydrate form.

2. $K_3Fe(CN)_6$ (M.W. 329.26) 99.96% Merck pro-analysi was used from a fresh bottle without further purification.

3. $CaCl_2$ and KOH as described in section IVA above used.

B. Apparatus

The experimental set-up was the same as that used for the $Ca^{2+}SO_4^{2-}$ system. In some experiments, the calcium electrode was replaced by the Orion Divalent cation electrode (model 92-32). The two electrodes have similar characteristics and give concordant results.

C. Procedure

1. Preparation of solutions

Two sets of solutions were made as described in section IV C. Due to the unavailability of the corresponding calcium salts of ferro and ferricyanides, solutions X were composed of $CaCl_2$ and $K_4Fe(CN)_6$ in the ferrocyanide experiments, and of $CaCl_2$ and $K_3Fe(CN)_6$ in the ferricyanide experiments. Solution S contained $CaCl_2$ and were prepared by dilution of a standardized 0.10M stock solution. The stoichiometric calcium ion concentration in S was made to be very close to that in solution X.

Special precautions were taken in preparing solutions X containing hexacyanoferrate II to minimize oxidation by O_2 . Fresh solutions were

prepared a few minutes before the actual measurements using freshly boiled out doubly distilled water.

The solutions were in most cases equimolar with respect to CaCl_2 and the hexacyanoferrates (II) and (III).

The range of concentration for the hexacyanoferrate (II) solutions was 9.00×10^{-4} to 4.00×10^{-3} M with respect to both CaCl_2 and potassium ferrocyanide and the corresponding range of concentration of CaCl_2 in solutions S was 6.00×10^{-4} to 1.50×10^{-3} M.

The range of concentration for solutions X containing hexacyanoferrate (III) was 9.00×10^{-4} to 5.00×10^{-3} M with respect to both CaCl_2 and potassium ferricyanide and the corresponding range of concentration of CaCl_2 in solutions S was 8.00×10^{-4} to 3.50×10^{-3} M.

2. Emf measurements

The general technique described above in section IVC was used with slight modifications.

It was found that as solutions X contained ferro and ferricyanides it was necessary and convenient to wash the electrode with a concentrated 0.1M solution of CaCl_2 for at least 10 minutes after each emf measurement of X, before washing with solution S. This was done to minimize contamination of the electrode surface with the foreign ions.

The KCl-Agar bridge had to be cut between successive measurements, as it was found to be affected by ferricyanide solutions.

Measurements were carried out over the above range of concentrations and at several temperatures between 15 and 35°C.

D. Detailed sample calculation for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ at 25.0°

Initial stoichiometric concentrations

S: $7.00 \times 10^{-4} \text{M CaCl}_2$ ($4.0 \times 10^{-5} \text{M KOH}$)

X: Equimolar mixture containing $1.40 \times 10^{-3} \text{M CaCl}_2$ and
 $1.40 \times 10^{-3} \text{M K}_4\text{Fe}(\text{CN})_6$ ($4.0 \times 10^{-5} \text{M KOH}$)

Measurements (see equation 4)

$$E_x - E_s = 0.10 \text{ mv.}$$

$$\frac{E_x - E_s}{RT/2F} = \frac{0.10}{29.6} = 0.0034$$

Estimation of Ionic Strengths and Activity Coefficients

1. Solution S (assume 100% dissociation)

Contributing species	Conc. 10^4M	I 10^4M
CaCl_2	7.00	21.0
CaCl_2 (eluted)	0.05	0.15
KOH	0.40	0.40

$$\text{Total } I_s = 21.55 \times 10^{-4} \text{M}$$

using equation 3 it follows that

$$\begin{aligned} -\log \gamma_s &= 2.048 I_s^{\frac{1}{2}} / (1 + 2.0 I_s^{\frac{1}{2}}) \\ &= \frac{2.048 (21.55 \times 10^{-4} \text{M})^{\frac{1}{2}}}{1 + 2.0 (21.55 \times 10^{-4} \text{M})^{\frac{1}{2}}} = 0.0870 \end{aligned}$$

2. Solution X

- Assume 1. 35% $\text{Ca}^{2+} \text{Fe}(\text{CN})_6^{4-}$ formed (fit of the data).
2. 8.2% $\text{K}^+ \text{Fe}(\text{CN})_6^{4-}$ formed (Eaton, George & Hanania, 1967).
3. No contribution from ion-triplets etc.

Contributing species	Conc. $10^3 M$	I $10^3 M$
$CaFe(CN)_6^{2-}$	0.490 (0.35X)	0.980
$KFe(CN)_6^{3-}$	0.115 (0.082X)	0.518
$Fe(CN)_6^{4-}$	0.795 (X - (0.35X + 0.082X))	6.36
Ca^{2+}	0.915 (X - 0.35X)	1.83
K^+	5.48 (4X - $KFe(CN)_6^{3-}$)	2.742
Cl^-	2.81 (2X + 2(eluted))	1.405
KOH	0.04	0.04

$$\text{Total } I_x = 13.88 \times 10^{-3} M$$

$$-\log y_x = 0.1952 \text{ (using equation 3).}$$

Calculation of K

$$c_s = 7.05 \times 10^{-4} M \text{ (including eluted } Ca^{2+})$$

$$E_x - E_s / 29.6 = 0.0034$$

$$\log (y_s / y_x) = 0.1952 - 0.870 = 0.1082$$

Substituting into equation 4:

$$c_x = 9.12 \times 10^{-4} M$$

$$(\text{ion-pair}) = 1.405 \times 10^{-3} - 9.12 \times 10^{-4} = 4.93 \times 10^{-4} M$$

$$(Fe(CN)_6^{4-}) = X - (\text{ion-pair}) - (K^+Fe(CN)_6^{3-}) = 7.93 \times 10^{-4} M$$

Substituting into equation 6:

$$K = 4.92 \times 10^{-4} / 9.12 \times 10^{-4} \times 7.93 \times 10^{-4} M = 683 M^{-1}$$

Note: the procedure above was repeated if the calculated and the assumed (ion-pair) differed by more than 4%. In case of a discrepancy the calculations were repeated by assuming a different percentage of association.

Calculation of K^0

Using equation 18

$$\log K^0 = \log 683 + 16 \times 0.512 (13.88 \times 10^{-3})^{\frac{1}{2}} / (1.0 + 2.0 (.01388)^{\frac{1}{2}})$$
$$K^0 = 4115.$$

III. RESULTS

Following the above procedure and method of calculation, K and hence K^0 was determined for both systems, over a range of concentration and at several temperatures. For the calcium ferrocyanide system the data are given in Tables VIa - e, and for the calcium ferricyanide system the corresponding data are given in Tables VIIa - d. The results at 25° for both systems are summarized in Table VIII and are illustrated in Figure 4.

In Tables VIa - e and VIIa - d the columns are as follows:

1. Molar concentration of each of $K_4Fe(CN)_6$ or $K_3Fe(CN)_6$, and $CaCl_2$ with $2 \times 10^{-4} M$ KOH in solution of unknown extent of ion-association.
2. Molar concentration of $CaCl_2$ standard (with about $2 \times 10^{-4} M$ KOH).
3. Difference between measured cell emf (Ca electrode / sat. calomel electrode) of solutions X and S.
4. Percent ion-association of $K^+Fe(CN)_6^{4-}$ in solution X (data from Eaton, George and Hanania, 1967).
5. Molar ionic strength of solution X, after taking ion-association into account.
6. Molar ionic strength of Solution S, assuming complete ionization.
7. Equilibrium molar concentration of free Ca^{2+} ions in solution X calculated from equation 4 (method as described above in section IIa, using constants from Table II).

Table VIa

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ at 15.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv)	$K^+ \text{Fe}(\text{CN})_6^{4-}$ %	$10^3 I_x$ (M) ^x	$10^3 I_s$ (M) ^s	$10^3 c_x$ (M) ^x	K (M ⁻¹)	K^0 (M ⁻¹)
1.00	0.600	-0.50	6.75	10.74	2.015	0.715	631	3097
1.20	0.600	1.00	7.50	12.38	2.015	0.825	631	3388
1.40	0.800	-0.30	8.00	14.61	2.615	0.994	472	2844
1.60	0.800	0.90	8.00	16.68	2.615	1.123	433	2900
2.00	1.00	-0.20	9.00	19.90	3.215	1.30	489	3744
3.00	1.00	3.30	11.7	28.70	3.215	1.86	332	3476
4.00	1.50	2.80	11.2	35.82	4.715	2.69	217	2766
4.00	1.50	2.80	11.2	35.82	4.715	2.69	217	2766

mean $K^0 = (3.12 \pm 0.28) \times 10^3$

Table VIb

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ at 20.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv)	$K^+ \text{Fe}(\text{CN})_6^{4-}$ %	$10^3 I_x$ (M) ^x	$10^3 I_s$ (M)	$10^3 c_x$ (M) ^x	K (M ⁻¹)	K^0 (M ⁻¹)
1.00	0.800	-4.00	6.75	10.55	2.615	0.702	686	3366
1.20	0.800	-2.20	7.50	12.38	2.615	0.832	607	3306
1.20	0.600	0.90	7.50	12.38	2.015	0.819	651	3545
1.40	0.800	-1.30	8.00	14.05	2.615	0.914	673	4030
1.80	1.00	-0.60	8.50	18.46	3.125	1.24	416	3056
1.80	1.00	-1.25	8.50	17.96	3.125	1.18	525	3787
2.50	1.00	1.70	10.0	24.32	3.125	1.58	443	4075

$$K^0(\text{mean}) = (3.60 \pm 0.32) \times 10^3$$

Table VIc

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ at 25.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv)	$K^+ \text{Fe}(\text{CN})_6^{4-}$ %	$10^3 I_x$ (M) ^x	$10^3 I_s$ (M) ^s	$10^3 c_x$ (M) ^x	K (M ⁻¹)	K^0 (M ⁻¹)
0.900	0.800	-5.70	6.20	9.366	2.615	0.608	900	4123
1.00	0.600	-1.20	6.75	10.26	1.855	0.681	785	3839
1.20	0.600	0.40	7.50	12.00	2.015	0.785	776	4213
1.20	0.600	0.20	7.50	11.94	1.855	0.784	780	4224
1.40	0.700	0.10	8.20	13.88	2.155	0.912	683	4115
1.50	0.700	1.20	8.40	14.97	2.155	1.01	564	3595
1.60	0.700	2.00	8.50	16.09	2.155	1.09	506	3406
1.80	1.00	-1.00	8.50	18.09	3.045	1.21	463	3413
1.80	1.00	-1.00	8.50	18.09	3.045	1.21	463	3413
2.00	1.00	0.00	9.00	19.98	3.045	1.34	434	3462
2.00	1.00	-0.80	9.00	19.42	3.045	1.25	570	4437
3.00	1.20	1.30	11.7	28.54	3.815	1.86	406	4350

mean $K^0 = (3.88 \pm 0.36) \times 10^3$

Table VI

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ at 30.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv)	$K^+ \text{Fe}(\text{CN})_6^{4-}$ %	$10^3 I_x$ (M) ^x	$10^3 I_s$ (M) ^s	$10^3 c_x$ (M) ^x	K (M ⁻¹)	K^0 (M ⁻¹)
1.20	0.600	1.00	7.50	12.22	1.86	0.832	608	3403
1.40	0.800	-1.00	8.00	14.27	2.46	0.946	585	3658
1.60	0.800	0.00	8.00	15.90	2.46	1.05	581	3941
1.60	0.800	-0.10	8.00	15.90	2.46	1.04	594	4219
1.80	0.800	0.65	8.50	17.57	2.46	1.12	629	4613
1.80	0.800	1.60	8.70	18.08	2.46	1.22	460	3449
2.00	1.00	-0.80	9.00	19.58	3.22	1.25	573	3835
2.50	1.00	2.10	10.0	24.72	3.22	1.64	385	3741

mean $K^0 = (3.86 \pm 0.30) \times 10^3$

Table VIe

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ at 35.0°C .

1	2	3	4	5	6	7	8	9
10^3I (M)	10^3S (M)	$E_x - E_s$ (mv)	$K^+\text{Fe}(\text{CN})_6^{4-}$ %	10^3I (M) ^x	10^3I (M) ^s	10^3c (M) ^x	K (M ⁻¹)	K ⁰ (M ⁻¹)
1.20	0.600	0.60	7.50	12.03	1.86	0.805	699	3931
1.20	0.600	0.30	7.50	12.03	1.86	0.787	767	4313
1.20	0.600	0.50	7.50	12.03	1.86	0.800	718	4037
1.30	0.600	1.60	7.80	13.22	1.86	0.883	615	3702
1.30	0.600	1.20	7.80	12.96	1.86	0.854	706	4188
1.60	0.800	1.40	8.70	18.08	2.46	1.20	490	3752
1.80	1.00	-1.50	8.70	17.64	3.065	1.17	542	4064
2.00	1.00	-0.30	9.00	19.74	3.065	1.31	580	4714
2.50	1.50	-3.00	9.20	19.54	4.565	1.52	499	4060
2.50	1.50	-2.60	9.20	19.54	4.565	1.57	446	3628

$$\text{mean } K^0 = (4.04 \pm 0.23) \times 10^3$$

Table VIIa

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$ at 15.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv) ^s	$K^+ \text{Fe}(\text{CN})_6^{3-}$ %	$10^3 I_x$ (M) ^x	$10^3 I_s$ (M) ^s	$10^3 c_x$ (M) ^x	K (M ⁻¹)	K^0 (M ⁻¹)
1.00	0.900	-1.30	5.00	8.560	2.92	0.934	86.6	256
1.20	0.900	0.55	6.00	9.697	2.92	1.10	88.5	277
2.00	1.60	-0.90	8.00	16.43	5.02	1.80	71.0	293
2.50	2.00	-1.00	9.20	20.78	6.22	2.26	54.6	255
3.00	2.00	0.50	10.0	24.16	6.22	2.64	60.2	312
5.00	3.50	-0.60	14.0	39.06	10.72	4.33	43.2	312

$$\text{mean } K^0 = 284 \pm 21.5$$

Table VIIb

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$ at 20.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv)	$K^+ \text{Fe}(\text{CN})_6^{3-}$ %	$10^3 I_x$ (M) ^x	$10^3 I_s$ (M) ^s	$10^3 c_x$ (M)	K (M ⁻¹)	K^0 (M ⁻¹)
1.20	0.900	0.40	6.00	10.12	2.82	1.10	89.1	288
1.60	1.60	-3.00	7.00	13.51	5.02	1.47	67.5	253
2.00	1.60	-0.75	8.00	15.70	5.02	1.81	66.7	271
3.00	2.00	0.70	10.0	24.33	6.22	2.69	49.8	263
4.50	3.50	-1.60	13.0	35.47	10.72	3.92	44.6	304

$$\text{mean } K^0 = 276 \pm 16.2$$

Table VIIc

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$ at 25.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv)	$K^+ \text{Fe}(\text{CN})_6^{3-}$ %	$10^3 I$ (M) ^x	$10^3 I$ (M) ^s	$10^3 c$ (M) ^x	K (M ⁻¹)	K^0 (M ⁻¹)
0.900	0.800	-1.25	4.00	7.720	2.52	0.838	99.8	287
1.00	0.800	-0.200	5.00	8.583	2.62	0.921	105	317
* 1.60/ 1.42	1.40	-3.20	7.20	12.86	4.32	1.29	79.3	293
2.00	1.40	0.50	8.00	16.43	4.32	1.80	70.3	298
2.50	3.00	-5.90	9.20	20.22	9.22	2.19	73.4	351
3.00	2.00	0.60	10.0	24.16	6.22	2.66	54.3	290
4.00	3.50	-2.70	12.0	31.90	10.72	3.52	44.9	289
5.00	3.50	-0.76	14.0	39.06	10.72	4.30	45.5	339

* $1.60 \times 10^{-3} \text{M } \text{K}_3\text{Fe}(\text{CN})_6$
 $1.42 \times 10^{-3} \text{M } \text{CaCl}_2$

mean $K^0 = 308 \pm 21$

Table VIIId

Determination of Ion Association Constant for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$ at 30.0°C .

1	2	3	4	5	6	7	8	9
$10^3 X$ (M)	$10^3 S$ (M)	$E_x - E_s$ (mv)	$K^+ \text{Fe}(\text{CN})_6^{3-}$ %	$10^3 I$ (M) ^x	$10^3 I$ (M) ^s	$10^3 c$ (M) ^x	K (M ⁻¹)	K^0 (M ⁻¹)
1.00	0.900	-1.60	5.00	8.585	2.915	0.921	106.0	324
1.20	0.900	0.50	6.00	10.22	2.915	1.11	80.8	268
2.00	2.00	-3.50	8.00	16.43	6.215	1.80	68.8	295
4.50	3.50	-1.70	13.00	35.47	10.72	3.92	44.6	314
5.00	3.50	-0.90	14.00	38.62	10.72	4.26	49.6	371

mean $K^0 = 314 \pm 26$

Table VIII

Variation of Ion Association constant with Ionic Strength at 25.0°C.

$\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$			$\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$		
K (M^{-1})	log K	$I^{\frac{1}{2}}/1+2I^{\frac{1}{2}}$	K (M^{-1})	log K	$I^{\frac{1}{2}}/1+2I^{\frac{1}{2}}$
900	2.95	0.0811	45.5	1.66	0.1416
785	2.90	0.0842	44.9	1.65	0.132
775	2.89	0.0898	54.3	1.74	0.118
780	2.89	0.0897	73.4	1.87	0.111
685	2.83	0.0953	84.4	1.93	0.102
564	2.75	0.0984	70.3	1.85	0.102
505	2.70	0.1012	79.3	1.90	0.0924
465	2.67	0.1060	105	2.02	0.0782
465	2.67	0.1060	99.8	2.00	0.0748
434	2.64	0.1102			
570	2.75	0.1090			
405	2.61	0.1262			

Data from Tables VIc and VIIc

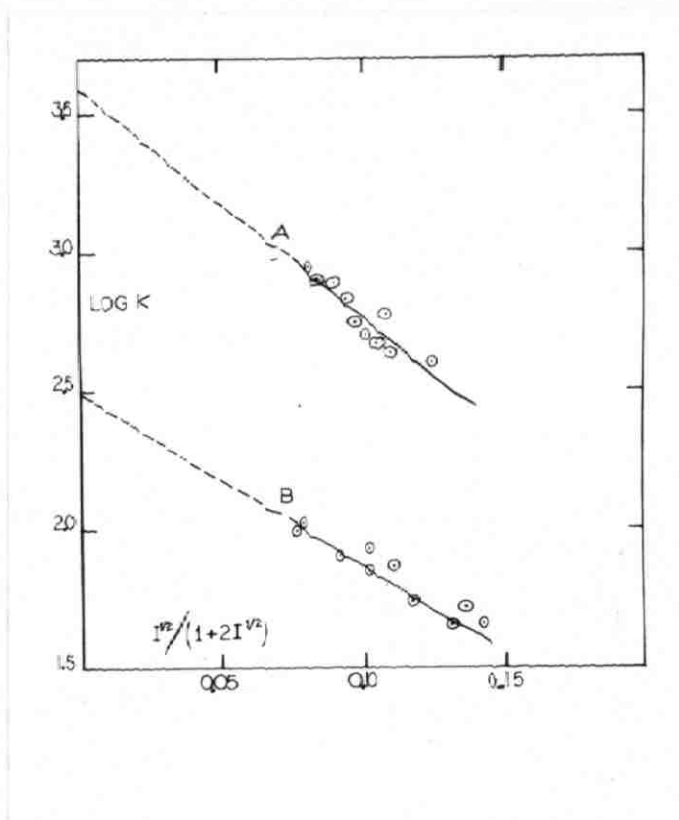
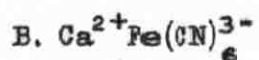
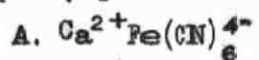


Figure 4 - Variation of K with ionic strength at 25.0°C.

Data from Table VIII. Lines indicate theoretical slopes (equations 18 and 22).



8. Ion-Association constants for equilibria 11 and 12 at ionic strength I_x , calculated from equations 16 and 20 (method as described in section IID).

9. Thermodynamic ion-association constant for equilibria 11 and 12, calculated from equations 15 and 19 (method as described in section IID).

The mean enthalpies and entropies of ion association for the two systems are obtained from the temperature variation of their formation constants using the thermodynamic relations:

$$\Delta H^{\circ} = -4.576 \, d \log K^{\circ} / d (1/T) \quad (23)$$

$$\Delta S^{\circ} = (\Delta H^{\circ} - \Delta G^{\circ}) / T \quad (24)$$

The data are given in Table IX and are plotted in Figure 5. The resulting ΔH° and ΔS° values are summarized in Table XI.

Table IX

Variation of Thermodynamic Ion Association Constant K° with Temperature

t $^{\circ}\text{C}$	$10^3/T(K^{\circ})$	$\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$		$\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$	
		$10^{-3} K^{\circ}$	$\log K^{\circ}$ (limits)	K°	$\log K^{\circ}$ (limits)
15.0	3.471	3.12 \pm .28	3.453 - 3.532	284 \pm 21.5	2.419 - 2.485
20.0	3.411	3.60 \pm .32	3.516 - 3.592	276 \pm 16.2	2.415 - 2.466
25.0	3.354	3.88 \pm .36	3.547 - 3.628	308 \pm 21.0	2.458 - 2.517
30.0	3.299	3.86 \pm .30	3.551 - 3.619	314 \pm 26.0	2.459 - 2.532
35.0	3.245	4.04 \pm .23	3.581 - 3.629		

Data from Tables VIa - e and VIIa - d

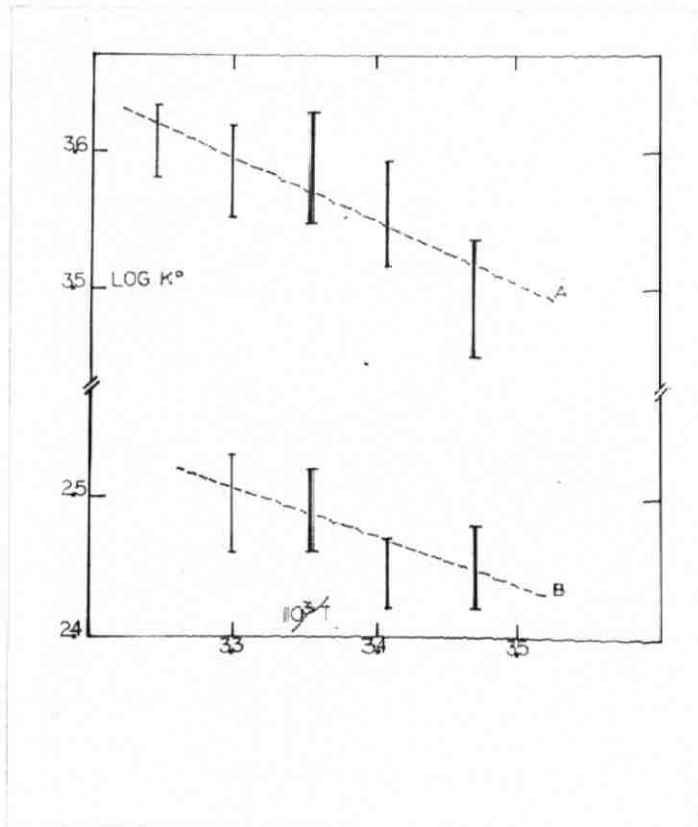
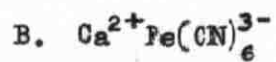
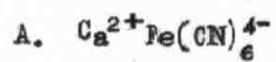


Figure 5 - Variation of K° with Temperature.

Data from Table IX. Lines indicate average slopes.



IV. CALORIMETRY

The enthalpy changes obtained above were also determined independently and directly using a microcalorimeter described below. The experiments were carried at room temperature which was $25 \pm 4^{\circ}$.

A. Apparatus

Instrument

A Beckman model 190B microcalorimeter was used in the calorimetric determinations of the enthalpy changes which had a minimum sensitivity of 5 millicalories and an accuracy of $\pm 3\%$ over 24 hours.

The instrument is designed to measure the heatburst resulting from the mixing of micromole quantities of reactants in a reaction vessel blanked against an appropriate reference vessel containing suitable quantities of the reference reagents. The mixing is achieved either automatically or manually by preparing a program up to seven phases which can rotate the reaction vessel to either, 0, 90, 180 or 270° positions, clockwise or counterclockwise.

The resulting heatburst is speeded through a thermopile into a heat sink and is proportional to the generated voltage in the thermopile.

A recorder plots both the generated voltage vs time and the integral of the thermopile voltage versus time. Once the instrument is calibrated, the integral plot yields directly the heat produced in millicalories.

Cells

Two types of cells were used in the determinations. One type consisted of glass vessels with annular space capacity of 15 ml and two drop wells with capacities up to 300 μ l each. The second type could

accommodate up to 8 ml in each of two compartments but had no drop wells.

For calibrating the instrument cells of the first type were used while for the other determinations it was found convenient to use the other cells.

B. Experimental procedure

1. Calibration. The instrument was calibrated by measuring the heat of neutralization of 0.0100M HCl with excess 0.010N NaOH. The following solutions were used:

1.00×10^{-4} M NaCl. The solution was prepared by taking 117.0 mg of Analar NaCl from a freshly opened bottle and making up to 2 liters with CO₂ free doubly distilled water. The solution was used both in the reference cell as a control reagent, and for making up the HCl and NaOH solutions to adjust the ionic strength.

1.00×10^{-2} M HCl, prepared from a standardized B.D.H. concentrate using the 1.00×10^{-4} M NaCl solution, as the diluent.

1.00×10^{-2} M NaOH, prepared from a stock which was standardized against potassium hydrogen phthalate, by dilution. The concentration of this solution was not crucial in the experiments because it was taken in excess.

The measurements were made using the drop well type cells described above. The quantities taken are shown in Tables Xa - b, which also give the heats obtained. A plot of the calibration curve traced by the pen recorder is shown in Figure 6.

The micro quantities were delivered using a Beckman micro pipette (200 μ l) and larger volumes through hypodermic syringes (20 cc.).

Heats were measured after 3 - 4 hours of equilibration when a steady

Table Ia

Calorimetric Measurement of Heat for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$

100 mv range

Expt. No.	Temp. °C.	Reaction cell		Reference cell		i.u. (ave.)	Heat (mcal)
		Left	Right	Left	Right		
1	26	HCl	NaOH	HCl	NaCl	-1110	53.28
	27	HCl	NaOH	HCl	NaCl	-1110	53.08
2	27	mixt.	H ₂ O	H ₂ O		-125	5.98
3	27	CaCl ₂	$\text{K}_4\text{Fe}(\text{CN})_6$	H ₂ O		320	15.3

HCl = 0.40 ml of 0.0100N

NaOH = 15.0 ml of 0.010N

NaCl = 15.0 ml of $1.0 \times 10^{-4}\text{M}$

$\text{K}_4\text{Fe}(\text{CN})_6$ = 7.0 ml of $4.00 \times 10^{-3}\text{M}$

CaCl₂ = 7.0 ml of $4.00 \times 10^{-3}\text{M}$

Mixture = 7.0 ml, $4.00 \times 10^{-3}\text{M}$ in $\text{K}_4\text{Fe}(\text{CN})_6$ and in CaCl₂

Table Xb

Calorimetric Measurement of Heat for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$

100 μv range

Expt. No.	Temp °C.	Reaction cell		Reference cell		i.u. (ave.)	Heat (mcal)
		Left	Right	Left	Right		
1	21	HCl	NaOH	HCl	NaCl	-555	-27.1
2	21	CaCl ₂	H ₂ O	H ₂ O	H ₂ O	-100	- 4.90
3	21	K ₃ Fe(CN) ₆	H ₂ O	H ₂ O	H ₂ O	- 85	- 4.15
4	21	mixture	H ₂ O	H ₂ O	H ₂ O	-158	- 7.71
5	25	CaCl ₂	K ₃ Fe(CN) ₆	H ₂ O	H ₂ O	190	9.14
6	21	CaCl ₂	K ₃ Fe(CN) ₆	Mixt.	H ₂ O	320	15.6

HCl = 0.20 ml of 0.0100N

NaOH = 15.0 ml of 0.010M

NaCl = 15.0 ml of 1.00×10^{-4} M

K₃Fe(CN)₆ = 7.0 ml of 1.00×10^{-2} M

CaCl₂ = 7.0 ml of 1.00×10^{-2} M

Mixture = 7.0 ml, 1.00×10^{-2} M in CaCl₂ and in K₃Fe(CN)₆

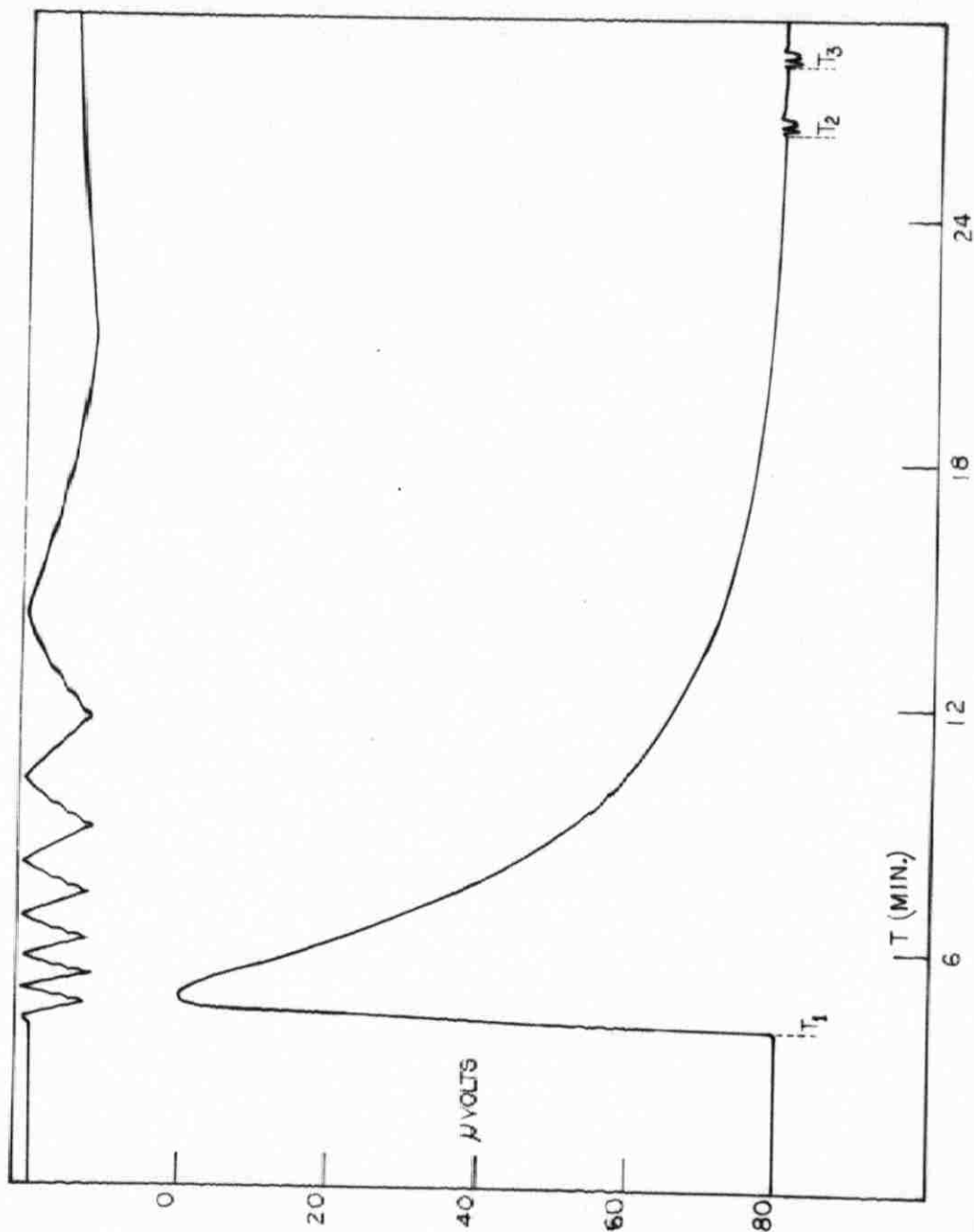


Figure 6 - Calorimetric measurement of heat of neutralization of HCl and NaOH (see text)

T₁ = time of initial mixing, showing heatburst

T₂, T₃ = time of second and third mixings

baseline was obtained by the differentiator pen.

2. Measurement of heats of ion-association for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ and $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$

Using cells with 8 ml double compartments, measurements were made of the heats produced upon mixing various combinations of the compounds involved. Details of conditions and quantities of reagents are given in Tables Xa - b, pages 63 and 64.

C. Calculations and results

The method of computing the heat involved makes use of the integrator trace of the type shown in Figure 6. Such a plot yields a number of integrator units, i.u., (number of chart graduations crossed by the integrator trace x 10), and these are directly proportional to the heat in millicalories (see below).

Calibration factor. In the calibration experiment reported in Table Xa, 4.00×10^{-6} moles of HCl are neutralized at 27° . However, as the subsequent experiments for measuring the heat of the calcium ferrocyanide system are carried at 26° a calibration factor at this temperature is needed:

Heat of neutralization = 13,270 cal/mole.

Therefore, 4.00×10^{-6} moles x 13,270 cal/mole = 53.08mcal evolved.
But the reaction gave -1110 i.u. (at 100 μv range of amplifier),

therefore, calibration factor = $-1110/53.08 = -20.91$ i.u./mcal.

In the calibration experiment reported in Table Xb, 2.00×10^{-6} moles of HCl were neutralized at 121°C . At this temperature, the heat of neutralization is 13,570 cal/mole,

therefore, 2.00×10^{-6} mole x 13,570 cal/mole = 27.14 mc cal evolved.

Calibration factor = $-555 \text{ i.u./}27.1 \text{ mcal} = -20.48 \text{ i.u./mcal.}$

For $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$, heat of reaction = $\frac{320 - (-125)}{20.91} = 21.3 \text{ mcal (Table Xa)}$

Moles reactant = $7.00 \text{ ml} \times 4.00 \times 10^{-3} \text{ M} = 28.0 \times 10^{-3} \text{ mmole.}$

Assume 35% ion-pair formation,

$$\text{product} = 0.35 \times 28.0 \times 10^{-3} = 9.80 \times 10^{-3} \text{ mmole}$$

$$\Delta H = 21.3 \text{ mcal}/9.80 \times 10^{-3} \text{ mmole}$$

$$= 2.17 \text{ Kcal/mole}$$

For $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$, heat of reaction = $\frac{320}{20.48} = 15.6$ (direct from Table Xb, 6)

or $9.14 - (-7.71) = 16.85$ (indirect from

Table Xb, 4 and 5)

$$= 15.6 \text{ or } 16.8 \text{ mcal.}$$

Assume 15% ion-pair formation,

$$\text{product} = 0.15 \times 7.00 \text{ ml} \times 1.00 \times 10^{-2} \text{ M} = 1.05 \times 10^{-2} \text{ mmole}$$

$$\Delta H = 15.6 \text{ or } 16.8 \text{ mcal.}/1.05 \times 10^{-2} \text{ mmole}$$

$$= 1.48 \text{ or } 1.60 \text{ Kcal/mole.}$$

Table XI

Summary of Thermodynamic Data at 25.0°C

	$\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$	$\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$
K°	3882 ± 361	308 ± 21
$\Delta H_{\text{pot}}^{\circ}$ (Kcal)	2.1 ± 0.5	1.5 ± 0.7
$\Delta H_{\text{Cal}}^{\circ}$ (Kcal)	2.17 ± 0.12	1.54 ± 0.06
ΔS e.u.	23.6 ± 1.2	16.5 ± 1.2

$\Delta H_{\text{pot}}^{\circ}$ - as obtained from potentiometric measurements.

$\Delta H_{\text{Cal}}^{\circ}$ - as obtained from calorimetric measurements.

DISCUSSION

In the work described above, a commercial liquid-liquid membrane calcium electrode was evaluated to establish its thermodynamic reversibility to free (unbound) Ca^{2+} ions in dilute aqueous solutions. On this basis, it was used in the determination of ion-association constants within specified ranges of conditions.

As with all quantitative work, the results are subject to certain limits of uncertainty. In our case, these depended on the following major factors: limiting properties of the electrode, necessary electrochemical assumptions, and the sensitivity of the measuring device (potentiometer).

The limiting properties of the electrode were the following:

- (1) Finite solubility of ion-exchanger in water which was assumed to contribute Ca^{2+} concentration of 5×10^{-6} (claimed by the manufacturers).
- (2) Drifts in potential with time which could be due to the uncertainty in E^0 . These caused non-reproducible results in some cases.
- (3) Drifts in potential in solutions containing foreign ions. This did not pose a serious problem because all measurements could be made within a range of conditions where outside effects did not interfere.

To minimize these uncertainties, the electrode was preconditioned and thoroughly washed with the solution, the potential of which was to be determined. Also, the time interval of recording the observed potentials was minimized and the solutions were chosen such that they had very close Ca^{2+} ion activities which minimized the contribution of the measured

potential to the uncertainties in the calculations. The electrode was used in the optimum range of concentration in which it had been shown to have Nernstian behavior and a range in which assumptions about activity coefficients and non-existence of ion-triplets could be justified.

The pH of the solutions was kept in the safe region 6 - 8.

The electrochemical assumptions were the following: (1) that the electrode had been equilibrated and was thermodynamically reversible to Ca^{2+} ion activity at the different temperatures; (2) that the reference calomel electrode was also equilibrated and reversible (to Cl^-); (3) that liquid junction potential of the system was either constant or negligible during the experiments; (4) that the Debye-Huckel extended equation could be used in the estimation of activity coefficients.

Temperature equilibration of the whole electrode was not practicable and so the assumption about equilibration could not be verified directly, but was indirectly checked through the internal agreement of results and from agreement between the calorimetric and potentiometrically determined heats.

The second assumption was justified on grounds that the calomel matched, within 0.2 mv, other similar electrodes, and that it was equilibrated at the different temperatures for at least four hours before use.

The assumption regarding liquid-junction potential was directly tested in the Ag-AgCl experiments (VA in previous section) which showed that changes in E_j , if any, were within the limits of error of potentiometric measurements, less than 0.1 mv. Assumptions about activity coefficients were justifiable on grounds of the concentration range covered

in our experiments. The uncertainties due to the precision of the potentiometer (± 0.05 mv) were within the range of other uncertainties about the electrode.

Altogether, these uncertainties are reflected in the degree of reproducibility of results. Tables VI to VII show a 10% mean deviation in the K^0 values. It should be noted that from every set of measurements a few results were ignored as their deviation from the mean was larger than twice the mean deviation of the set.

The enthalpies of ion-association were determined by two independent methods. From the temperature variation of the equilibrium constant at five temperatures, the uncertainty appears to be very large (25 to 40% respectively (Table IX)). However, the direct microcalorimetric determination yielded more precise results (5%) which were also in good agreement with the potentiometric results. It was on this basis that average enthalpies and hence entropies, were computed. Table XI.

These results on Ca^{2+} binding may be compared with other ion-association data on hexacyanoferrate systems. Unfortunately, there are very few cases for which complete thermodynamic studies have been reported; these are summarized at the top of Table XII. Other available data, chosen from the compilation of stability constants (Sillen 1964), are given in the lower part of Table XII. Also included in the Table are Bjerrum distances for ion-pairs calculated on the basis of the electrostatic theory of ion-association (Hanania, private communication (1968)) using the equilibrium constant data. Bjerrum distances may be taken as a measure of equilibrium distances between the centers of hydrated cations and anions in ion-pairs.

Table XII

Comparison of Data on Hexacyanoferrate Ion-pairs

T = 25°. Data on 1, 4, 5, 6 & 7 from "Stability Constants" * (The Chemical Society, London 1964);
 2 from Eaton, George and Hanania (1967); 3 from present work.

	Fe(CN) ₆ ³⁻				Fe(CN) ₆ ⁴⁻				Method	
	Cation	K ⁰	ΔH ⁰ Kcal/mole	ΔS ⁰ e.u.	Bjerrum distance Å	K ⁰	ΔH ⁰ Kcal/mole	ΔS ⁰ e.u.		Bjerrum distance Å
1	Tl ⁺	-	-	-	-	1.1x10 ³	1.1	17	-	spectroph.
2	K ⁺	30	0.5	8	6.6	2.2x10 ²	0.8	13	4.3	pot. & cal.
3	Ca ²⁺	3.1x10 ²	1.5	17	10.3	3.9x10 ³	2.1	24	6.5	pot. & cal.
4	La ³⁺	5.5x10 ³	2.0	24	7.3	1.2x10 ⁵	-	-	1.4	
5	Ca ²⁺	6.8x10 ²	-	-	-	-	-	-	-	condy.
6	Ca ²⁺	-	-	-	-	5.0x10 ³	-	-	-	soly.
7	Ca ²⁺	-	-	-	-	5.9x10 ³	-	-	-	condy.

* Sillen (1964).

It is noticed first of all that the present work has yielded ion-association constants which are smaller than the literature values reported. In the case of $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{4-}$ our result is about 20% less, but for $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$ it is less than half the value previously reported (Table XII). These discrepancies probably reflect the difficulties encountered in previous studies of association between ions of unequal charge by the conductivity method (Nancollas, 1966), and may also be partly due to underestimation of the extent of K^+ binding to hexacyanoferrate ions (Eaton, George and Hanania, 1967). An independent check on these results would be through the use of calcium hexacyanoferrate salts directly. An attempt was made, in this work, to prepare and crystallize $\text{Ca}_3(\text{Fe}(\text{CN})_6)_2 \cdot 12\text{H}_2\text{O}$, but no potentiometric measurements on this compound were made.

A comparison of the data in Table XII shows the main features of ion-association thermodynamics. Increasing charge on either ion favors stronger binding. Enthalpies of ion-pair formation are weakly endothermic, and Bjerrum ion-pair lengths are reasonably close to hydrated ionic radii. But there are two abnormal Bjerrum distances: (1) $\text{Ca}^{2+}\text{Fe}(\text{CN})_6^{3-}$ has a very large value which indicates extremely weak binding (as reflected in the small formation constant); (2) $\text{La}^{3+}\text{Fe}(\text{CN})_6^{4-}$ has an impossibly small value, clearly showing the failure of the Bjerrum electrostatic model for such a highly charged system. Apart from these exceptions the general conclusion may be drawn that the complexes are real ion-pairs held by electrostatic forces.

The present work has shown that the calcium electrode works thermodynamically under certain conditions of Ca^{2+} and of foreign ion

concentrations. As such it can be used for measuring calcium ion activity in natural, biological and chemical systems.

Calcium finds its importance in natural systems such as sea and natural water resources, and in different forms of rocks which are of interest to geochemists.

In biological systems free ionized calcium is said to control muscle contraction, blood clotting and nerve impulse transmission and as the physiological functions are related to calcium activity in contrast to concentration, the method could be a very convenient way of measuring Ca^{2+} activities. This would be in contrast to previously used methods such as EDTA, which gives the concentration of total free calcium, and McLean and Hastings' frog heart method which measures not only Ca activity but rather the ratio of Ca/Na as the activities of the two ions are very much related (Niedergeserke and Luttgau, 1957).

In chemistry, the electrode provides a method of studying the thermodynamic constants of different complexes, chelates or ion-pairs involving Ca. Indeed, Glauser, Ifkovits, Glauser & Sevy (1967) have evaluated the Corning calcium electrode, which works on the same principle as the Orion Ca electrode, and have used it in studying the EDTA complexometric titration of calcium at pH 7, for the determination of the thermodynamic parameters which could only be studied before, through the study of the ligand characteristics.

The application of the electrode to the problem of ion-association has confirmed the reality of ion-association equilibria and has yielded results which are consistent with an electrostatic theory of ion-pair formation.

The existence of ion-pairs necessitates modifications in calculations involving electrolytes in aqueous solutions. A recent treatment of the subject concerns the solubility and solubility product equilibria in the CaSO_4 system where large errors in previous calculations were pointed out (Meites, Pode and Thomas, 1966).

Ion-pairs are also important in physiological systems and a few illustrative examples may be noted here. Bianchi and Shanes (1959) have postulated that ion transport across all membranes may occur as ion-pairs. Walser and Browder (1959) have pointed out the important role of ion-pairs formation in the renal transport of certain electrolytes.

It is interesting to note that ion-pairs, such as calcium ferrocyanide, have been found by Walser (1960) to promote protein binding of calcium in contrast to chelates, such as calcium citrate.

So far, the calcium electrode has had very limited use in the study of ionic interactions and equilibria. But its development is a recent one, and the prospects of its application in analytical, biological and physicochemical research is certain to increase.

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