Journal of Chromatography, 283 (1984) 89–98
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,285

DETERMINATION OF COMPLEXING ABILITIES OF LIGANDS FOR METAL IONS BY FLOW INJECTION ANALYSIS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. PRINCIPLE OF THE SUBSTITUTION REACTION METHOD

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(Received September 9th, 1983)

SUMMARY

A flow injection system, interchangeable as a high-performance liquid chromatographic detector, was designed for the spectrophotometric determination of colourless ligands (L) such as aminopolycarboxylic acids and polyphosphates and for the determination of their complexing abilities. L was allowed to react with a coloured methylthymol blue complex of magnesium or calcium (MR) to form a colourless metal complex (ML). By the spectrophotometric recording of negative peaks at 605 or 610 nm the rapid analysis of 60 samples per hour was possible. The complexing abilities of ligands and the stability constants of ML complexes can be calculated from their peak heights.

INTRODUCTION

The potential of flow injection analysis (FIA) has been demonstrated with a number of theoretical considerations and practical applications[1,2]. One of new trends in this field is the interchangeable use of FIA systems as post-column reaction detectors in high-performance liquid chromatography (HPLC)[3,4]. Another subject of interest is the development of FIA methodology by means of which physico-chemical parameters, such as stability constants and rate constants of chemical reactions, can be determined[5–7].

In a previous paper[5] we reported FIA profiles (negative peaks) obtained by injecting polyphosphate anions (L) into a reagent stream of a coloured magnesium complex (MgR). Such an FIA method based on the substitution reaction between L and MgR was found to be useful for the indirect determination of polyphosphate anions. During that work we noticed that the relative peak heights and the sensitiv-

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0021-9673/84/$03.00

ities of detection of polyphosphate anions increased in increasing order of the complexing abilities of polyphosphate anions for magnesium ions or the stability constants of magnesium polyphosphate complexes (MgL).

This work was undertaken to design an FIA system and a coupled FIA–HPLC system useful not only for the spectrophotometric detection of colourless ligands, but also for the determination of the complexing abilities of these ligands. Part II will give a detailed description of the characterization of multi-component samples by HPLC, and this first part deals with the FIA system, the precursory HPLC detector, in order to explain the principles of the substitution reaction method. A number of organic aminopolycarboxylic acids and inorganic oxoacids of phosphorus were used as ligands. Methylthymol blue (MTB) complexes of magnesium and calcium were used as colour reagents.

Calibration graphs for the spectrophotometric detection of these colourless ligands showed good linearity between FIA peak heights and concentrations of samples. Reasonable correlations were also observed between the relative peak heights of the ligands (H_L) and the conditional stability constants of the ML complexes (K_{ML}). The usefulness of log H_L *versus* log K_{ML} plots is discussed from the viewpoint of determining the complexing abilities of various ligands for magnesium and calcium ions.

EXPERIMENTAL

Reagents

Unless otherwise stated, all chemicals (Wako, Osaka, Japan) were used without purification. Linear phosphates of oxidation number 5 were orthophosphate (P_1), KH_2PO_4; diphosphate (P_2), $Na_4P_2O_7 \cdot 10H_2O$; triphosphate (P_3), $Na_5P_3O_{10} \cdot 6H_2O$; and tetraphosphate ($P_4$), $(NH_4)_6P_4O_{13} \cdot 6H_2O$. Cyclic phosphates of oxidation number 5 were trimetaphosphate (P_{3m}), $Na_3P_3O_9 \cdot 3H_2O$; tetrametaphosphate (P_{4m}), $Na_4P_4O_{12} \cdot 4H_2O$; and hexametaphosphate (P_{6m}), $Na_6P_6O_{18} \cdot 6H_2O$. Phosphorus compounds of lower oxidation states were phosphonate (P^{III}), $Na_2PHO_3 \cdot 5H_2O$; and hypophosphate (P^{IV}–P^{IV}), $Na_2H_2P_2O_6 \cdot 6H_2O$. P_3 from Wako was purified by the repeated recrystalization. P_4, P_{3m}, P_{4m}, P_{6m} and P^{IV}–P^{IV} were synthesized in our laboratory.

Methylthymol blue (MTB), sodium 3,3′-[bis-N,N-di(carboxymethyl)aminomethyl]thymol sulphonphthalate ($C_{37}H_{43}O_{13}N_2SNa$), was used as the chromogenic reagent. Abbreviated notations[8] for the organic ligands used as samples are as follows: TTHA for triethylenetetramine-N,N,N′,N″,N‴,N⁗-hexaacetic acid, $C_{19}H_{30}N_4O_{12}$; EDTA for ethylenediaminetetraacetic acid, $C_{10}H_{16}N_2O_8$; EDDHA for ethylenediamine-di(*o*-hydroxyphenylacetic acid), $C_{18}H_{20}N_2O_6$; CyDTA for *trans*-1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid, $C_{14}N_{24}N_2O_9$; DPTA-OH for 1,3-diaminopropan-2-ol-N,N,N′,N′-tetraacetic acid, $C_{11}H_{18}N_2O_9$; EDTA-OH for N-hydroxyethylethylenediamine-N,N′,N′-triacetic acid, $C_{10}H_{18}N_2O_7$; GEDTA for glycol ether diamine-N,,N,N′,N′-tetraacetic acid, $C_{14}H_{24}N_2O_{10}$; EDDA for ethylenediamine-N,N′-diacetic acid, $C_6H_{12}N_2O_4$; HIDA for hydroxyethyliminodiacetic acid, $C_6H_{11}NO_5$; IDA for iminodiacetic acid, $C_4H_7NO_4$; methyl-EDTA for 1,2-diaminopropane-N,N,N′,N′-tetraacetic acid, $C_{11}H_{18}N_2O_8$; DTPA for diethylenetriamine-N,N,N′,N″,N″-pentaacetic acid, $C_{14}H_{23}N_3O_{10}$; and NTA for nitrilotriacetic acid, $C_6H_9NO_9$.

Each reagent solution of MgMTB and CaMTB is composed of an equimolar mixture of metal chloride and MTB in ammonia–ammonium chloride buffer (pH 10). The concentration of each reagent in a reservoir was adjusted to $1 \cdot 10^{-4}$ *M* so that the concentration in the reaction coil was $3.3 \cdot 10^{-5}$ *M*. Unless stated otherwise, the ionic strength of the solution in the reaction coil was 0.1, *i.e.*, 0.1 *M* ammonia–ammonium chloride buffer (pH 10).

Apparatus

The apparatus for FIA consisted of a reciprocating pump with two channels (Seishin PSU-3.2W), a spectrophotometer (JASCO, UVIDEC-100W) with a flow-through cell (volume 8 μl), a loop valve sample injector (Seishin VMU-6, 100 μl) and a switching valve for the stopped flow (Kyowa KMU-4V2). The function of each component in the FIA manifold was described in previous papers[3,5].

RESULTS AND DISCUSSION

Principle of detection

Consider a substitution reaction in which a colourless ligand L reacts with a coloured metal complex MR in a solution to form a colourless metal complex ML:

$$MR + L \rightleftarrows ML + R \qquad (1)$$

If the spectrophotometric measurement is carried out at a wavelength where the molar absorptivity of MR, ε_{MR}, is different from that of R, ε_R, the absorbance of reaction system is expected to vary with the variation in concentration of L to be determined. The value of $\varepsilon_{MR} - \varepsilon_R$, positive or negative, is a measure of the sensitivity of detection. The plot of the variation in absorbance *versus* the sample concentration can be used as a calibration graph for the determination of L.

The MR reagent should have a simple composition, be labile and have a moderately high stability constant so that the free metal concentration becomes negligible compared with the MR concentration. MgMTB and CaMTB complexes with stability constants of $10^{5.2}$ and $10^{5.5}$ (pH 10), respectively[8,9], were used in this work. The absorption spectra for these reagents, each with an equimolar mixture of metal and MTB, at pH 10 are shown in Fig. 1. The values of $\varepsilon_{MR} - \varepsilon_R$ can be calculated to be *ca.* $1.2 \cdot 10^4$ for MgMTB reagent at 605 nm and *ca.* $8.0 \cdot 10^3$ for CaMTB reagent at 610 nm.

FIA manifold

In practical experiments the FIA manifold shown in Fig. 2 was employed to accomplish the substitution reaction in eqn. 1. A sample solution of colourless ligand L (100 μl) was injected via a loop valve injector (S) into a carrier stream of water (H_2O) and confluenced at a point M with a reagent stream of coloured metal complex (MR). The flow-rates of the water and MR reagent streams were controlled by a reciprocating pump with two channels (plungers P_1 and P_2) at 1.0 and 0.5 ml/min, respectively. In a reaction coil (C_4) immersed in a water-bath (30°C) the sample L reacted with the reagent MR to form the complex ML, which resulted in variation of the absorbance in the sample zone. A negative peak, corresponding to a positive

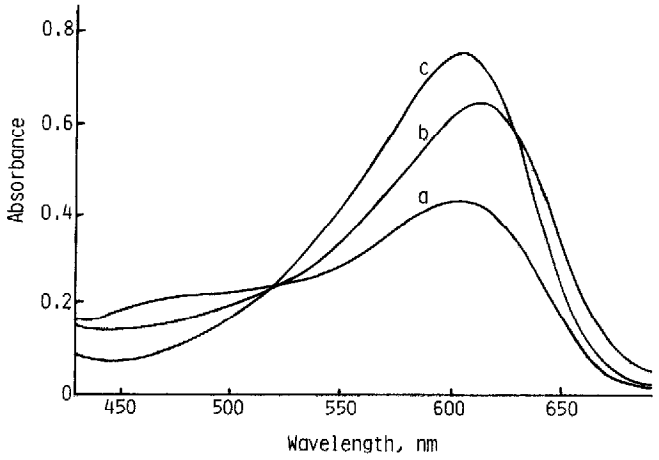

Fig. 1. Absorption spectra of MTB, MgMTB and CaMTB. (a) $3 \cdot 10^{-5}$ M MTB; (b) $3 \cdot 10^{-5}$ CaMTB; (c) $3 \cdot 10^{-5}$ M MgMTB.

($\varepsilon_{MR} - \varepsilon_R$) value, was recorded when the absorbance was monitored by employing a spectrophotometric detector (D) with a flow cell (8 μl) at 605 nm (MgMTB system) or 610 nm (CaMTB system). The residence time of the sample zone in the reaction coil was calculated to be *ca.* 80 sec.

A switching valve for stopped-flow experiments was located just before the detector to observe the variation in absorbance of the sample zone stopped in the flow cell. When the sample zone reached the flow cell, the valve was switched to direct the carrier stream to waste, W_2, and to stop the sample zone in the flow cell. The extent of the reaction was determined by recording the variation in absorbance for a desired period. The valve was then switched on-line to the detector again.

The manifold in Fig. 2 was designed to be useful as a high-pressure FIA system by which experiments can be carried out even at temperatures as high as 140°C[3]. The application of the back-pressure coil (C_5) becomes very important above 100°C, but is not essential at low temperatures such as in this work, although its incorporation is preferable in order to make the flow more stable.

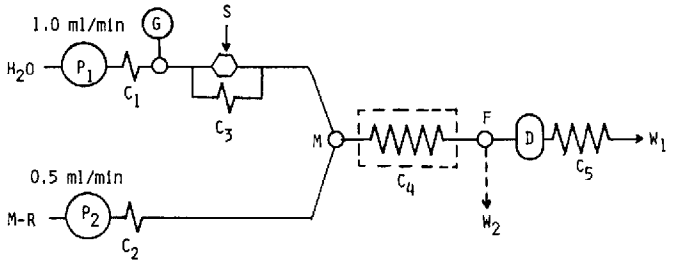

Fig. 2. FIA manifold. P_1 and P_2, reciprocating pump; G, Pressure gauge; S, loop valve sample injector; M, three-way joint; F, stopped-flow valve; D, detector; C_1 and C_2, damper tubing (30 cm, Technicon No. 065-116-0536-13); C_3, by-pass tubing (50 cm × 0.25 mm I.D., PTFE); C_4, reaction coil (500 cm × 0.5 mm I.D., PTFE); C_5, back-pressure coil (200 cm × 0.25 mm I.D., PTFE); W_1 and W_2, waste; MR, MgMTB or CaMTB solution.

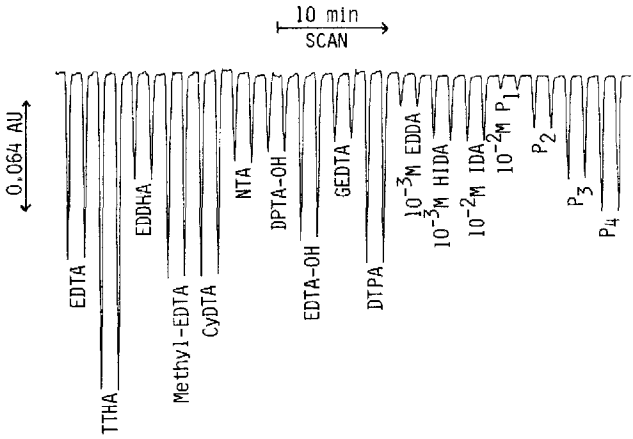

Fig. 3. FIA signals for seventeen organic and inorganic ligands in MgMTB system. Each sample concentration is $1 \cdot 10^{-4}$ *M*, except for EDDA, HIDA, IDA and P_1 as indicated.

FIA profiles in MgMTB system

FIA profiles obtained by the MgMTB system are shown in Fig. 3 for thirteen organic ligands and four inorganic ligands. The concentration of MgMTB reagent in the reservoir was $1 \cdot 10^{-4}$ *M* (pH 10). The baseline level corresponds to the absorbance at 605 nm due to $3.3 \cdot 10^{-5}$ *M* MgMTB reagent recorded at 0.32 absorbance unit full-scale (a.u.f.s.). Each sample (100 μl) was injected in duplicate to obtain two negative peaks. It is evident that each sample responds with different sensitivity or peak height. Ten organic aminopolycarboxylic acids on the left and three inorganic polyphosphates on the right can be sensitively detected at a low concentration of $1 \cdot 10^{-4}$ *M*, whereas very small or undetectable peaks are recorded for the three aminodicarboxylic acids EDDA, HIDA and IDA and monomeric orthophosphate (P_1). The sample concentrations must be increased from $1 \cdot 10^{-4}$ to $1 \cdot 10^{-3}$ *M* for EDDA and HIDA and to $1 \cdot 10^{-2}$ *M* for IDA and P_1 in order to obtain FIA signals (Fig. 3).

FIA profiles in CaMTB system

Similar experiments were also carried out using $3.3 \cdot 10^{-5}$ *M* CaMTB reagent in the reaction coil (pH 10). Measurements were made at 610 nm and 0.64 a.u.f.s. The general pattern of the FIA profiles for the CaMTB system (Fig. 4) is similar to that for the MgMTB system in Fig. 3. As in the MgMTB system, the sensitivities of four ligands, *viz.*, EDDA, HIDA, IDA and P_1, are lower than those of the other ligands. A marked difference is that the sensitivity of detection in Fig. 3 is better than that in Fig. 4, which may be partly attributable to the difference in $\varepsilon_{MR} - \varepsilon_R$ values between MgMTB and CaMTB reagents (Fig. 1).

Stopped-flow signals

The combined contribution of kinetic factors and equilibrium factors of chemical reactions is considered to effect the FIA profiles in Figs. 3 and 4. It can be simply expected from eqn. 1 that the greater the stability constant of ML, the higher the FIA signal becomes, provided that the reaction is rapid and that chemical equilibrium

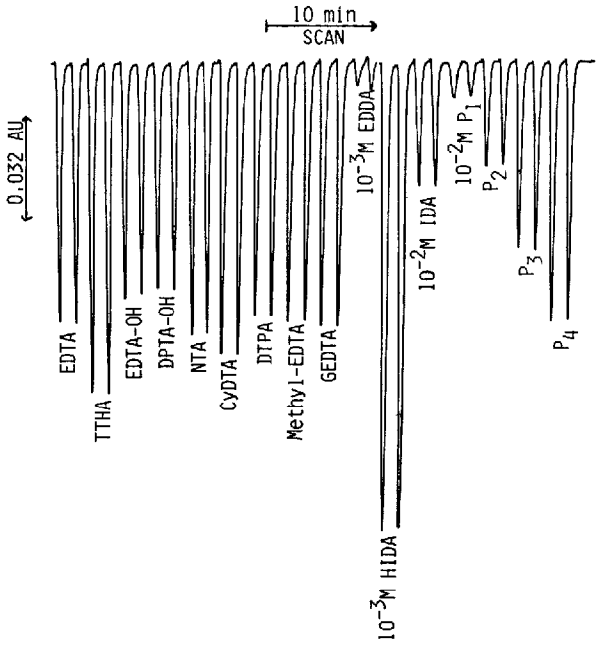

Fig. 4. FIA signals for sixteen organic and inorganic ligands in CaMTB system. Each sample concentration is $1 \cdot 10^{-4}$ *M*, except for EDDA, HIDA, IDA and P_1 as indicated.

has been attained before the sample zone passes through the detector. Some examples of slow reactions are known where the contribution of kinetic factors predominates, *e.g.*, the substitution reaction between orthophosphate and the cerium (III)–xylenol orange complex[5]. Therefore, it is necessary to examine by means of the stopped-flow technique whether the reactions are rapid or not, if one wants to observe the effects of the stability constants of ML complexes on the FIA profiles.

The stopped-flow patterns for several aminopolycarboxylic acids are shown in Fig. 5. For each component, the same sample was injected successively in duplicate

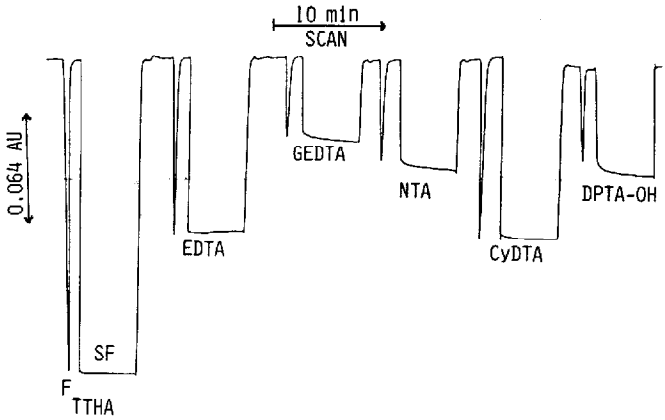

Fig. 5. Comparison of FIA signals (F) with stopped-flow signals (SF) in MgMTB system. Sample size: each $1 \cdot 10^{-4}$ *M*, 100 μl.

to obtain a normal FIA signal (F) and a broad stopped-flow signal (SF). The stopped-flow signal was recorded by stopping the sample zone, corresponding to the peak maximum, in the detector for *ca.* 5 min to observe the time dependence of absorbance or the further progress of the reaction. The absorbance was expected to vary with time if the substitution reaction had not been completed. The stopped-flow signals with plateaux in Fig. 5 lead to the conclusion that the equilibria of the substitution reactions have almost been reached. Similar stopped-flow signals were also observed for all ligands shown in Figs. 3 and 4, with a 10% increase in absorbance, at most, for IDA in the MgMTB system.

Relative peak height

As shown in Fig. 6 for EDTA as an example, the peak height, h_L, increases linearly with increase in ligand concentration, C_L. To compare the sensitivities of detection of various ligands, the relative peak height of a given ligand, H_L, was defined on the basis of the sensitivity of EDTA:

$$H_L = \frac{h_L}{C_L} \cdot \frac{C_{EDTA}}{h_{EDTA}} \tag{2}$$

where h_1 is the peak height at any concentration of L, C_L. $H_L = 1.0$ for EDTA, as $C_L = C_{EDTA}$ and $h_L = h_{EDTA}$. EDTA was selected as the standard ligand, because MgEDTA and CaEDTA complexes are well characterized as 1:1 complexes with stability constants of $10^{8.69}$ and $10^{10.96}$, respectively[8,9].

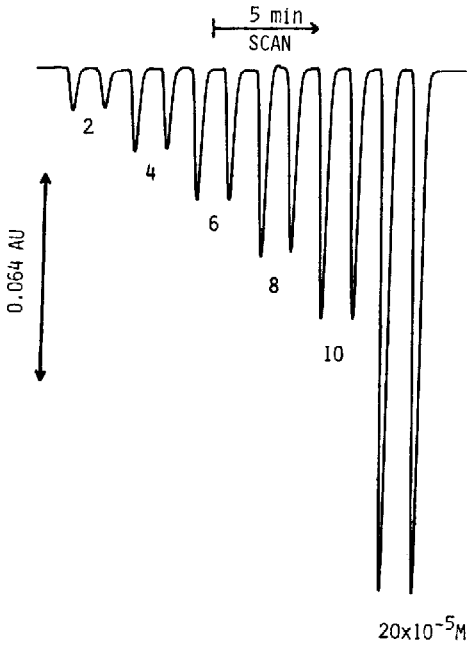

Fig. 6. FIA calibration signals for EDTA in CaMTB system. Sample volume: 100 μl.

Relationship between peak height and stability constants

As mentioned above, the relative peak heights or the relative sensitivities of the ligands in Figs. 3 and 4 are expected to be dependent on the stability constants of the ML complexes, K_{ML}. Log H_L *versus* log K_{ML} plots are shown in Figs. 7 and 8 for the MgMTB and CaMTB system, respectively. In addition to the ligands in Figs. 3 and 4, three cyclic phosphates, *viz.*, trimetaphosphate (P_{3m}), tetrametaphosphate (P_{4m}) and hexametaphosphate (P_{6m}), and two phosphorus oxoacids of lower oxidation numbers, *viz.*, phosphonate (P^{III}) and hypophosphate (P^{IV}–P^{IV}), were plotted.

K_{ML} values for organic ligands were taken from the data book[8] published by the manufacturer of the organic ligands used in this work. For inorganic ligands, various references were available[10–19].

Peak heights were found to be dependent on the ionic strength of the carrier solutions. The log H_L values in Figs. 7 and 8 are based on the FIA signals at an ionic strength of 0.1 (0.1 *M* ammonia–ammonium chloride buffer, pH 10). Therefore, K_{ML} values were also selected from the literature that had been determined at or as close as possible to an ionic strength of 0.1. If K_{ML} values were not given in the literature, such as for P^{III} in Fig. 7 and P^{III} and P^{IV}–P^{IV} in Fig. 8, only log H_L values were plotted (Δ) on the calibrated lines.

A general correlation pattern can be seen in Figs. 7 and 8: log H_L values increase linearly with increasing log K_{ML} values and tend to level off in the region where $K_{ML} > K_{MR}$. However, there are some deviations that cannot be explained satisfactorily at present. In addition to the uncertainty about the log K_{ML} values cited, the incorrectness of C_L values in eqn. 2 and the formation of M_2L complexes, in addition to ML complexes, may be possible sources of errors.

Sample solutions of all inorganic phosphorus compounds were carefully

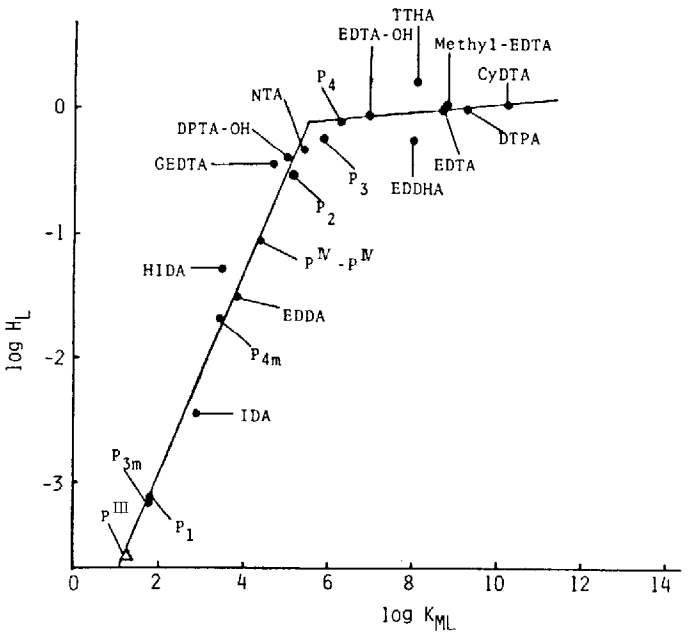

Fig. 7. Plots of stability constants *versus* peak heights in MgMTB system.

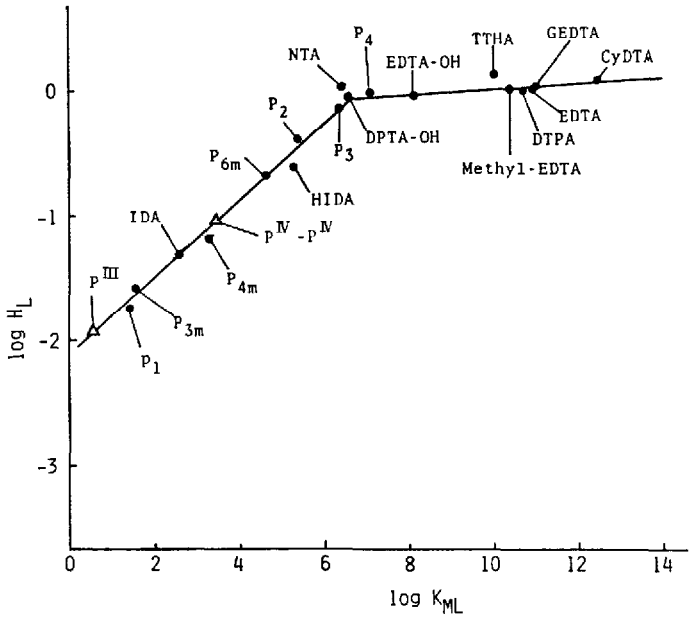

Fig. 8. Plots of stability constants *versus* peak heights in CaMTB system.

checked as pure by a high-performance spectrophotometric method and their concentrations were standardized by a spectrophotometric method[20,21]. On the other hand, the organic aminopolycarboxylic acids were used as received[8] and their sample concentrations were calculated on the basis of weighed amounts and formula weights. Hence there is some uncertainty about the C_L values in eqn. 2, though only C_{EDTA} was carefully determined.

TTHA tends to deviate upwards from the correlation curves in Figs. 7 and 8. This may be ascribed to the well known fact that TTHA binds to two metals to form M_2L complexes[8,9]. The downward deviation of EDDHA is difficult to explain. Careful re-examination is needed concerning the preparation of sample solution and the kinetics of the substitution reaction.

In conclusion, the FIA technique based on substitution reactions is useful not only from an analytical, but also from a physico-chemical viewpoint. It permits the rapid colorimetric determinations of colourless ligands that form colourless metal complexes. The analysis of 60 samples per hour is possible, with a relative standard deviation of measurement of less than 1.5%.

The order of complexing abilities of various ligands can be easily determined. Once calibration graphs had been constructed between measured log H_L values and known log K_{ML} values of some ligands, log K_{ML} values of other ligands can easily be calculated by "*one-point-determination*". For example, an experiment of only 2 min using a 100-μl sample solution is needed in order to establish that the complexing ability of P^{IV} P^{IV} (hypophosphate) for calcium is between those of P_{4m} and P_{6m} and the log K_{ML} value of the calcium hypophosphate complex is *ca.* 3.5.

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