THE ARSENAZO 2-(2-ARSENOPHENYL)AZO-7-(4-ANTIPYRIL)AZO--1,8-DIHYDROXY-3,6-NAPHTALENEDISULPHONIC ACID AS SPECTROPHOTOMETRIC REAGENT AND METALLOCHROMIC INDICATOR FOR CALCIUM*

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Ions Ca(II) react with the arsenazo [2-(2-arsenophenyl)azo-7-(4-antipyril)azo-1,8-dihydroxy-3,6--naphtalenedisulphonic acid] at pH 10·0 to produce a blue-violet complex with the maximum of absorbance at 578 nm. Its stoichiometry is 1 : 1 and its stability constant has the value of $8·56 . 10^4$ at pH 10·0. Beer's law is obeyed in the range from 0·04 to 0·4 µg ml^{-1} of Ca(II), with a molar absorbance of $1·92 . 10^4$ l mol^{-1} cm^{-1}. The reagent has also been studied as the metallochromic indicator in the complexometric titration of Ca, and the colour change was specified by means of CIE chromaticity diagrams. According to Ringbom's concepts a transition pM of 4·93 is obtained when a $5·0 . 10^{-4}$ mol l^{-1} solution of Ca(II) is titrated.

The bis-azo derivatives of chromotropic acid constitute a very important group of analytical reagents, since they not only react with great sensitivity with the ions of the IIIa and IVa Groups, but also with the ions of the IIa Group, especially calcium, so that the determinations of these elements by spectrophotometry and complexometric volumetry are possible, with these derivatives acting as metallochromic indicators. It is well known that only few reagents yield colour reactions with calcium. Except bromo-oxine[1], TTA (4,4,4-trifluoro-1-(2-thienyl)butane-1,3-dione)[2] and murexide[3,4], the other reagents can be classified in three well-defined groups: derivatives of glyoxal[5], derivatives of complexones (Calcein[6], Metalphtalein[7], Thymolphtalexone[7], Methylthymol Blue[9]) and azo reagents, Eriochrome Black T[10], Calmagite[11], Calcon[12], Acid Chrome Blue K[13], Azo-azoxy BN[14] and Calcichrome[15]. The bis-azo derivatives mentioned above also belong to the last group, of which Arsenazo III[16], Palladiazo[17], Chlorophosphonazo[18] and Antipyrilazo[19] are the most representative. The dis-azo derivatives of chromotropic acid, *i.e.* the non-symmetric derivatives, have been much less studied. However, it can be said that their properties are in this respect similar to the bis-azo derivatives. For this reason and in the course of a systematic study of dis-azo arsenazo derivatives of chromotropic acid[20], we have synthesised the reagent 2-(2-arsenophenyl)azo-7-(4-antipyril)azo-1,8-dihydroxy-3,6-na-

phtalenedisulphonic acid[21] (HL), into which a heterocyclic ring with a high density charge has been introduced. This compound reacts with Ca ions to produce a blue complex that is very suitable for its spectrophotometric determination and, moreover, for its complexometric titration with EDTA.

EXPERIMENTAL

Apparatus and Solutions

Pye–Unicam (Netherlands) SP8—100 spectrophotometer with 1 cm silica cells, Orion Research (U.S.A.) pH-meter (Model 901), Radiometer (Denmark) PHA804, photometric titration unit connected to the Radiometer 51 pH-meter, and the PDP-11/55 computer (U.S.A.) were used. The stock calcium colution $c_{Ca} = 2.0 \cdot 10^{-2}$ mol l^{-1} was prepared by dissolving calcium carbonate in diluted hydrochloric acid, boiling to eliminate CO_2 and diluting appropriately with distilled water. Working calcium solutions were prepared by diluting this stock immediately before use. EDTA (disodium salt) solutions (10^{-2} mol l^{-1} and $2.5 \cdot 10^{-3}$ mol l^{-1}) were obtained according to the recommended methods[22] and standardized complexometrically with standard calcium solution. Tris-nitric acid for pH 7—9, ammonia–ammonium chloride for pH 10—10·5 and borax–sodium hydroxide for pH 11 were used as buffer solutions. Buffer solutions according to Clark and Lubbs were also used. The reagent (HL) solutions ($5.0 \cdot 10^{-3}$ mol l^{-1} and $4.1 \cdot 10^{-4}$ mol l^{-1}) were prepared in distilled water. All other cation solutions were prepared from reagent grade compounds and metals, with the metal concentration 1 g l^{-1}.

Procedures

Spectrophotometric study. The formation of the Ca–HL complex was studied as a function of pH at the ionic strength 0·1 (adjusted with sodium perchlorate) and 25°C. The analytical concentrations of HL and Ca(II) in the final solutions were $2.0 \cdot 10^{-5}$ mol l^{-1} and $2.0 \cdot 10^{-3}$ mol l^{-1}, respectively. The pH value adjusted using sodium hydroxide solutions. The spectra of all solutions were recorded in the range 400—700 nm.

Chromaticity study. 5 ml of the working calcium solution, 2·5 ml of sodium perchlorate solution, 2 ml $4.1 \cdot 10^{-4}$ mol l^{-1} indicator (HL) solution, 5 ml of buffer solution (pH 10·0) and increasing volumes of EDTA solutions were added to 25 ml volumetric flasks, and then diluted to 25 ml with water. The absorbance of each of these solutions was measured at specified wavelengths to determine the chromaticity coordinates. The absorbance values were treated with a regression computer program[23].

Synthesis of the reagent. The organic reagent HL was synthesized by diazotising 4-aminoantipyrine and coupling with Arsenazo I in an alkaline medium[20]. 0·57 g of 4-aminoantipyrine was dissolved in 15 ml of 2M-HCl and the solution was cooled down to 0°—5°C. Then 0·19 g of $NaNO_2$ in the aqueous solution was slowly added. The diazonium salt solution was added, under constant stirring, to 1·4 g of Arzenazo I and 2·6 g of $CaCl_2$ in 45 ml water at pH 9 and at a temperature below 5°C. After being kept for 6 h, the mixture was diluted to 100 ml with distilled water and concentrated HCl was added until full precipitation was achieved. After filtering through sintered glass, the product was purified by dissolving in water and precipitating with conc. HCl. Its purity was verified by TLC on silica gel using 2M-NH_3–ethanol (1 : 1) as eluent. The R_F of HL is 0·88. Chromatography was carried out with 2 µl of 0·5% (m/V) HL solution in water. The visual detection limit of these azo derivatives is 0·09 µg.

The yield of the procedure was 12·5%. For $C_{27}H_{22}O_{12}N_6AsS_2Na$ (782·9) calculated: 42·33% C, 2·81% H, 9·56% As; found: 40·39% C, 2·91% H, 10·08% As. The arsenic was determined according to Tuckerman's procedure[24].

HL was obtained as a dark-red powder. It was readily soluble in water, methanol, ethanol, and it was slightly soluble in hydrochloric acid but very soluble in alkaline solutions. The infrared spectrum (potassium bromide pellet) gave the following peaks: 3 200 and 1 250 cm^{-1} (O—H), 1 710 cm^{-1} (C=O), 1 620 and 1 490 cm^{-1} (aromatic rings), 1 310 cm^{-1} (C—N), 1 100 cm^{-1} (—AsO$_3$H$_2$), 1 060 cm^{-1} (—SO$_3$H), 770 cm^{-1} (aromatic ring with *ortho* subtituent).

RESULTS AND DISCUSSION

Acid dissociation behaviour of HL reagent. HL in concentrated sulphuric acid produces a greenish solution which turns pink with the increase of pH. In alkaline solution its colour is shifted towards the violet. In concentrated sodium hydroxide solution a deep-blue colour is observed. These facts can be explained by the existence of different ionic forms in the solution (Scheme 1) and confirmed by the existence of several isobestic points in the absorption spectra of aqueous solutions of the reagent[20] in the pH interval from 1·5 to 13·0. Six different series of curves appear, from which the values of pK, shown in Table I, are obtained. This table also includes the pK's for Arsenazo III, according to Buděšínský[25]. It is evident that the pK values for the reagent HL are about two pK units lower than those corresponding to Arsenazo III with the same dissociation order of the acid groups. The ranges of existence of each reagent form can be seen in Fig. 1.

Reactivity of HL with different cations. The reactivity of the arsenazo HL with 52 different ions has been studied[26]. The investigation was carried out in aqueous media in the pH range 1·00—10·0. The most interesting reactions were observed

TABLE I
pK values of Arsenazo III and the reagent studied (HL)

Functional group	Arsenazo III[a]	HL[b]
—OH	14·6	12·6
—OH	11·7	9·7
—AsO$_3$H$^-$	9·0	6·9
—AsO$_3$H$_2$	7·1	3·9
—AsO$_3$H$^-$	5·2	—
—AsO$_3$H$_2$	3·8	—
—N$^+$R$_2$H	—	2·1

[a] Ref.[25]; [b] ref.[26].
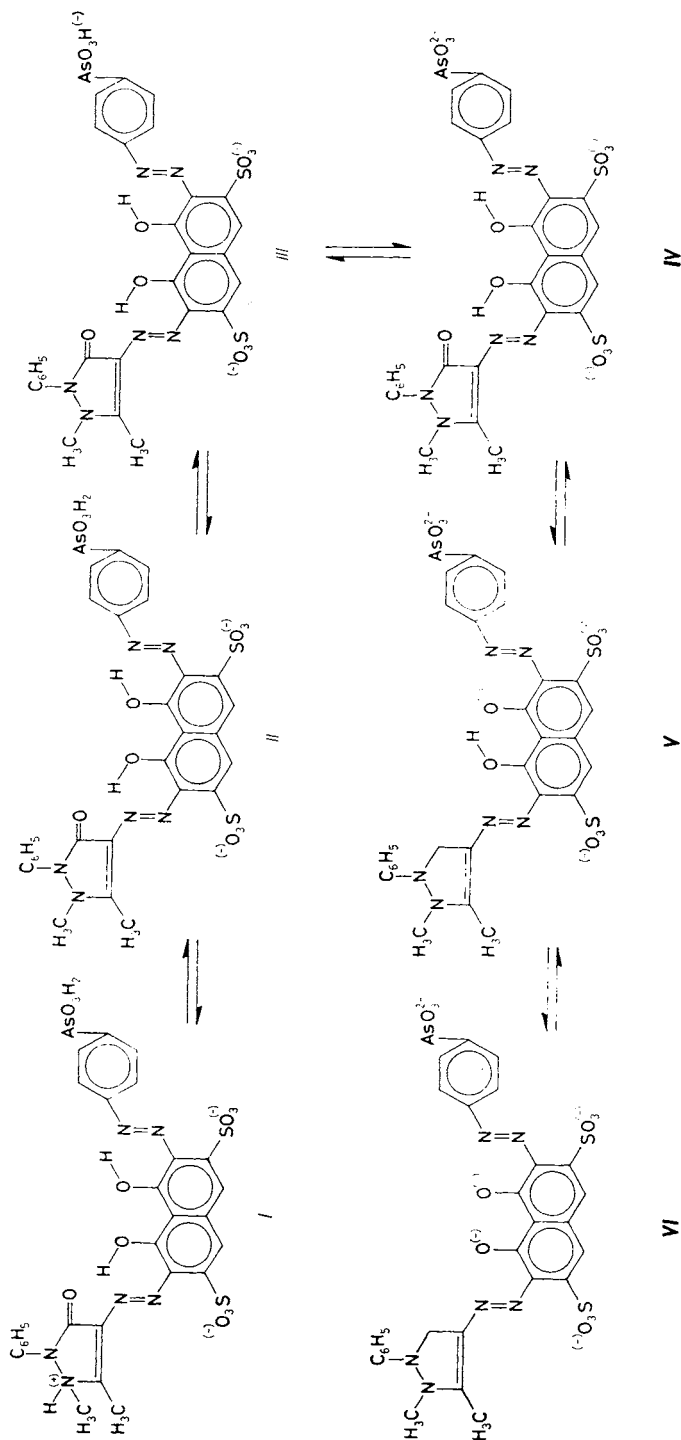
with: Th and Zr (blue solutions with the optimum pH of reaction at 1·5); Bi, Fe (blue-violet), Cu (blue, both at pH 5·0); Co (blue pH 5·5); U (VI) (violet-blue pH 3·5); La and Zn (greenish-blue and blue pH 8·0); Mg, Sr (blue pH 8·5); Cd (violet pH 9·0); and Ca (blue pH 10·0). In comparison with these complexes, the Ca-complex colour is intense. For this reason and since Ca ions have few spectrophotometric reagents, we made a quantitative study of this reaction.

Spectrophotometric studies of the Ca–HL *complexation reaction.* The Ca–HL complex has been studied by Sommer's method[27] at two metal ligand ratios. For the ratio 100 : 1, the complex begins to form at pH 5·00 and the reagent is completely compexed above pH 9·71. From this pH up to at least pH 11 the spectrophotometric curves become superimposed and their absorption values remain constant for over an hour and a half (*i.e.* the complex is stable at these pH values for at least the same period of time).

The absorption − pH curves, (Fig. 2) have two maxima at the wavelengths 578 nm and 528 nm, and according to the shape of the family of curves, with a single isobestic point, the existence of one complex form is supposed.
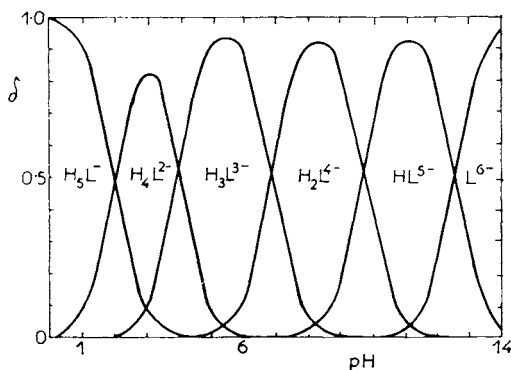

FIG. 1

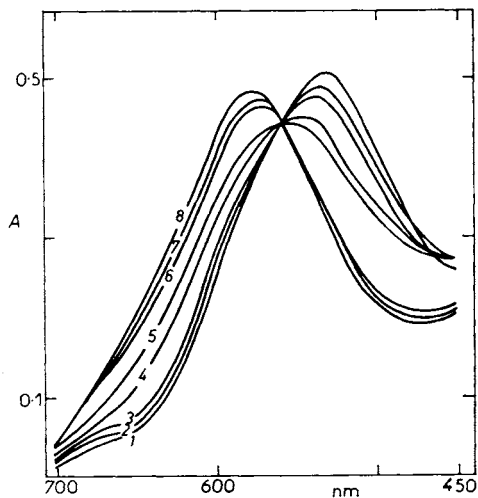Distribution diagram of HL deprotonation forms

FIG. 2

Dependence of absorbance spectra on pH, for calcium(II)–HL complex $c_{Ca} = 2·0 .$ $. 10^{-3} \, mol \, l^{-1}$; $c_{HL} = 2·0 . 10^{-5} \, mol \, l^{-1}$; pH: *1* 2·45; *2* 4·71; *3* 5·26; *4* 6·01; *5* 6·90; *6* 7·84; *7* 8·38; *8* 9·71

When the metal/ligand ratio is 1 : 1, the complex begins to form at pH 7·05 and is completely formed at pH 10·00. The absorbance — pH curves have a similar form to those of the previous case. That is, a great excess of metal does not affect the stoichiometry of the complex. This has been determined by the molar ratio method[28] both for the constant concentration of cation and of the reagent, and by Job's method of continuous variations[29]. In both methods a 1 : 1 composition was obtained. Mathematical treatment of the absorbances of the spectrophotometric A — pH curves at the wavelengths of 573, 578, and 583 nm, shows that in the formation of the complex one proton is split off. The molar absorbance, at the wavelength of the complex maximum absorption (578 nm) has the value of $1·92 . 10^4 \, 1 \, mol^{-1} . cm^{-1}$ and presents a sensitivity index (Sandel's definition) $s = 0·0021 \, \mu g \, cm^{-2}$. The conditional stability constant of the complex formation has been calculated using Job's method, at room temperature, $0·1M$-$NaClO_4$ of ionic strength 0·1 ($NaClO_4$) and pH 10·0, and the value $\beta = 8·56 . 10^4$ was obtained. Beer's law has been verified. It is obeyed up to 0·4 $\mu g \, ml^{-1}$ of calcium, when the concentration of reagent is $4·0 . 10^{-5} \, mol \, 1^{-1}$ (pH 10·0).

HL *as the metallochromic indicator for complexometric determination of calcium with* EDTA. The spectrophotometric curves of HL and its complex with Ca in the pH range, 10−12, (Fig. 3) (a pH range at which the complex is completely formed) show that the greatest bathochromic shift, among the wavelengths of maximum absorption, occurs at pH 10·0 (28 nm). The bathochromic shifts at pH 11·0 and 12·0 are 26 and 22 nm, respectively. At pH 10·0, the reagent solution contains the species H_2L^{-4} and HL^{-5} and has a pink colour. At pH 11·0 the predominant species is HL^{-5}, with a rosy-violet colour, and at pH 12·0 the solution is bluer since it contains not only HL^{-5} but also L^{-6}. The participation of various species at each pH value is demonstrated in Fig. 1. To find the optimum pH for the determination, the colour differences between the free reagent and its complex have been determined at each of the pH values mentioned above by the tristimulus colorimetry, following the CIE-UCS (1960) procedure. Fig. 4 shows the complex-free reagent chromatic transitions at each of the pH values studied. From this figure it can be seen that the colour difference (ΔE) has the value of 48·21 at pH 10·0, 39·32 at pH 11·0, and 34·47 at pH 12·0. Consequently, all further studies were carried out at pH 10·0.

Using titration with $0·01M$-EDTA 4−10 mg of calcium in 50 ml could be determined with both visual and photometric end point estimation. Three series of 10 titrations were carried out for the determination of 4, 6, and 10 mg of Ca under the above--mentioned conditions. The maximum relative error was found to be 0·13% for the 4 mg titration series. The method's precision, expressed as the standard deviation of these 10 titrations, was $s = 4·5 . 10^{-2}$ mg.

The chromatic shift during the titration was determined using the CIE (X, Y) diagram and the more modern CIELAB (1976) diagram. To calculate the chromacity para-

meters, both the methods of 30 selected ordinates and that of $\Delta\lambda = 10$ nm were used. Not only were the initial and end points considered, but also 30 intermediate points, especially in the neighbourhood of point. Table II gives some of these points, and the most representative parameters, according to Reilly and Kotrlý[30,31], *i.e.* the true
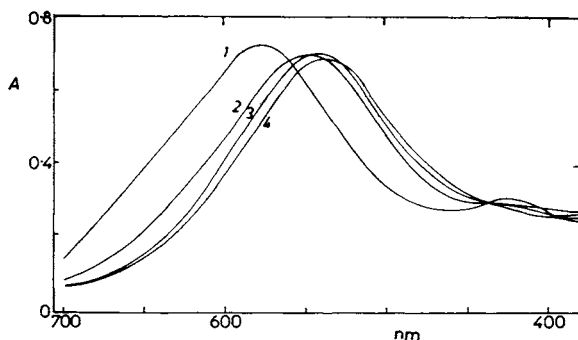

FIG. 3

Absorption spectra of the calcium complex (curve *1*) and of the reagent at different pH values (curves *2—4*). Curve *1*: Calcium(II)–HL complex, $c_{Ca} = 100_{HL}$, $c_{HL} = 3\cdot0 \cdot 10^{-5}$ mol l^{-1}, pH $= 10\cdot02$. Curve *2*: reagent $c_{HL} = 3\cdot0 \cdot 10^{-5}$ mol l^{-1}, pH $= 12\cdot01$; *3* 11·03; *4* 10·01
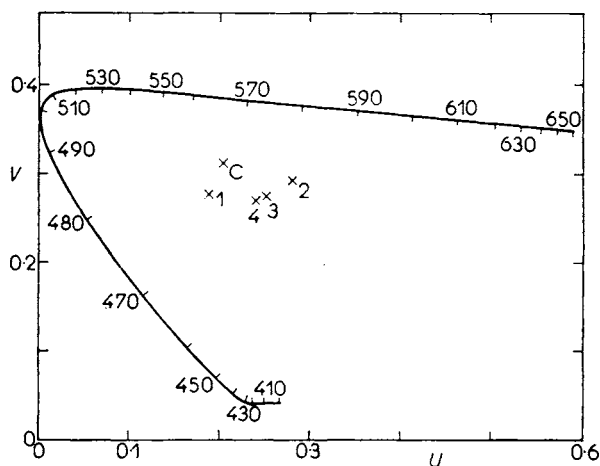

FIG. 4

Colour shift between the complex (1) and the free reagent at different pH's — **2** — pH 10·02, **3** — pH 11·03, and **4** — pH 12·01. $c_{Ca} = 3\cdot24 \cdot 10^{-3}$ mol l^{-1}, $c_{HL} = 3\cdot0 \cdot 10^{-5}$ mol l^{-1}

TABLE II

Chromaticity coordinates for the colour change of HL as metallochromic indicator for calcium

C_L/C_M	Px^a	Py	Y	Qx	Qy	J	D	e	nm
0·000	0·245	0·212	0·2120	0·368	0·406	1·2785	0·011	0·301	465
0·787	0·247	0·211	0·2076	0·364	0·405	1·2902	0·007	—	—
0·945	0·256	0·213	0·2105	0·357	0·403	1·2689	0·004	—	—
0·961	0·259	0·215	0·2131	0·356	0·402	1·2586	0·004	—	—
0·976	0·274	0·226	0·2158	0·340	0·391	1·2671	0·001	—	—
0·984	0·301	0·236	0·2388	0·319	0·388	1·1914	0·002	—	—
0·992	0·362	0·254	0·2928	0·267	0·387	1·0869	0·120	—	—
1·000	0·393	0·262	0·3250	0·240	0·388	1·0550	0·197	0·371	598
1·005	0·393	0·262	0·3250	0·240	0·388	1·0550	0·197	—	—

^{a} Px, Py — true coordinates of the points studied; Y-luminosity; Q_x, Q_y — complementary co-ordinates; Y-optical concentration; D-dichromatism; e-spectral purity and λ-dominant wave-length.
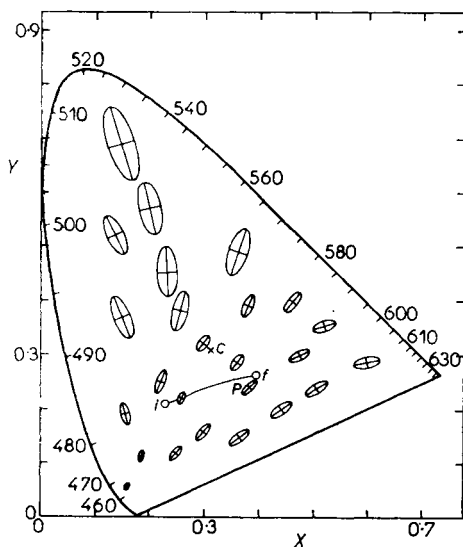

FIG. 5

Chromatic transition of the indicator HL ($c_{HL} = 3\cdot20 \cdot 10^{-5}$ mol l^{-1}) in the complexometric titration of calcium solution ($c_{Ca} = 5\cdot0 \cdot 10^{-4}$ mol l^{-1}) with EDTA ($1\cdot618 \cdot 10^{-2}$ mol l^{-1})
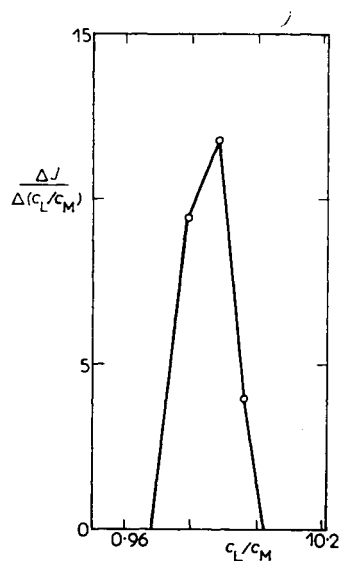
FIG. 6

Progress of colour concentration *vs* EDTA/ /Ca ratio in the vicinity of the end point of the titration (instead of 10·2 read 1·02)

coordinates (P), the luminosity (Y), the complementary coordinates (Q), the optical concentration (J), the dichromatism (D), the dominant wavelength (λ), and the spectral purity (e).

From Fig. 5 it is clear that the chromatic shift is not very large, but that it crosses an area of the chromaticity diagram in which the density of the McAdam's ellipses is high and it is within the blue-purple zone, in which such changes are considered adequate. The colour difference between the titration points corresponding to 98% and 100%, is $\Delta E = 12\cdot40$. The luminosity increases continuously in the course of titration. Nevertheless, it presents a sharp change very close to the end point which facilitates its localization.

The distance between the complementary colour coordinates (which are independent of the reagent concentration) of the colour at the beginning and the end point of the titration is greater than that observed using Arsenazo III as indicator[32].The same occurs with the distance between the points corresponding to 99% and 100% titration. The variation of the luminosity in the transition of the Arsenazo III between these two points is lower than that corresponding to HL.

From the variation of the optical concentration of the solution (ΔJ) during titration $(\Delta c_{L}/c_{M})$, the sharpness of the colour change can be established[23]. Fig. 6 shows this variation. This figure was obtained from 20 experimental points, although only the points which can be deduced from the data in Table II are shown. Clearly, the height/ /width ratio is very large, which confirms the previous reasoning about the sharpness of the end-point colour change. Ringbom's concepts have been applied to this indicator[33] and when a solution of calcium with $c_{Ca} = 5\cdot0 \cdot 10^{-4}$ mol l^{-1} is titrated, a transition pM of $4\cdot93$ is obtained. According to these results, we propose HL as a suitable metallochromic indicator in the complexometric titration of calcium.

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