866. *Chelating Reactions of 1,2-Dihydroxyanthraquinon-3-ylmethylamine-NN-diacetic Acid with Metal Cations in Aqueous Media.*

By M. A. Leonard and T. S. West.

A systematic study has been made of the variables involved in the synthesis of " alizarin complexan " and related compounds; the identity of the products is confirmed by molecular-weight determinations based on conductometric and radiofrequency titration. An explanation is proposed for the observed similarity between the absorption spectra of metal chelates of the reagents and those of the metal-free reagents of higher pH values. Investigation of the chelates formed by " alizarin complexan " with four selected metal ions by the procedure of continuous variations reveals the formation of 1 : 1 reagent–metal compounds except in the instance of thorium when the ratio is 2 : 1. Mononuclear chelation is favoured on the grounds of observed chemical reactions with cations of tetraco-ordinate habit, etc.

The cerium(III) chelate of alizarin " complexan " gives a specific colour reaction with the fluoride ion. No other anion is found to react similarly, and amongst the rare earths available only lanthanum and praseodymium give the reaction. The mechanism of this reaction which is highly selective towards cation as well as anion is accounted for. The formation of colour by the fluoride ion furnishes a sensitive method for its spectrophotometric determination. The fluoride complex may be extracted into certain amine-containing solvents.

The preparation and analytical properties of *NN*-di(carboxymethyl)aminomethyl derivatives of some hydroxyanthraquinones, particularly those whose trivial names are alizarin complexan (see formulæ on p. 4481) and alizarin bordeaux complexan, have previously been described;[1] so has a submicro-method for the determination of fluorine in organic compounds [2] based on an unusual reaction of the former compound.[3] This paper presents an account of the interaction of the most important members of the group with a range of cations in buffered aqueous media and seeks to elucidate the nature of the reaction between the cerium(III) chelate of alizarin complexan and small amounts of fluoride ion.

RESULTS

Preparation of Reagents.—Since the principal reagent, alizarin complexan, had been obtained in only 13% yield, an attempt was first made to improve its preparation. It was observed that the products obtained by treating various polyhydroxyanthraquinones with iminodiacetic

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[1] Belcher, Leonard, and West, *J.*, 1958, 2390.
[2] Belcher, Leonard, and West, *J.*, 1959, 3577.
[3] Belcher, Leonard, and West, *Talanta*, 1959, **2**, 92.

acid and formaldehyde were formed at greatly different rates. The course of the reaction was followed spectrophotometrically. At intervals aliquot parts of the reaction mixture were diluted and extracted with ether at pH 4·3, to remove unchanged hydroxyanthraquinone, and the aqueous layer was diluted to volume and its absorbancy measured at the appropriate wavelength. The absorption corresponding to 100% reaction was calculated from measurements on known amounts of the appropriate derivative. Curves relating % reaction against time were then prepared.

Preliminary experiments with 1,2-dihydroxyanthraquinone revealed that the reaction proceeded best with a good excess of iminodiacetic acid, and later experiments were conducted with a four-fold excess of this reagent. Variation of time, temperature, and volume revealed that the optimum conditions for the synthesis of alizarin complexan corresponded to the use of: (*a*) a four-fold excess of iminodiacetic acid; (*b*) as small a volume as possible; (*c*) a temperature of 70°; and (*d*) a condensation time of *ca.* 14 hours. These conditions led to 20% reaction.

The optimum conditions for obtaining products from the other polyhydroxyanthraquinones were: 1,2,5-trihydroxyanthraquinone (alizarin bordeaux), 14 hr. at 70° (32% reaction); 1,2,6-trihydroxyanthraquinone (flavopurpurin), 5 hr. at 70° (86% reaction); 1,2,7-trihydroxyanthraquinone (anthrapurpurin), 1·5 hr. at 70° (92% reaction); 1,2,5,8-tetrahydroxyanthraquinone (quinalizarin), 2 hr. at 70° (50% reaction).

Přibil and his co-workers [4,5] have described similar Mannich reactions carried out in glacial acetic acid with excellent yield. Accordingly the above reactions were repeated in this medium with 1,2-dihydroxy-, 1,2,6-trihydroxy-, 1,8-dihydroxy-, and 2-hydroxy-anthraquinone; though the desired products were obtained in the first two instances, the yields were poor, and none was obtained with the other two; this was no doubt partly due to the relatively low solubility of the reagents and the high temperature necessary.

*Molecular Weight of Aminomethyl-*NN-*di(carboxymethyl)-substituted Hydroxyanthraquinones.*—Although the identity of the compounds had previously been established by ultimate analyses and their m. p.s had been recorded,[1] further characterisation was now achieved by molecular-weight determinations. These were effected by conductometric and radiofrequency titration with 0·01M-cerium(III) solution. Previous knowledge of reactions between metal ions and iminodiacetic acid suggests that cerium(III) should react with these reagents to form a 1 : 1 chelate and this point was subsequently confirmed by spectrophotometric measurements. The conductometric titrations were carried out in the 0·01—0·002M-range in a lightly buffered ammonium acetate medium with an instrument operating at 50 c.p.s. Very sharp end-points were obtained in all titrations except with the product from 1,2,7-trihydroxyanthraquinone (later found to be impure). The radiofrequency titrations were carried out with a type I instrument [6] operating in the 15 megacycle/sec. range and similar to that described elsewhere.[7] In this instance, the titration was carried out with cerium(III) acetate, but in an unbuffered solution because of the tendency of electrolytes to load the oscillator. The radiofrequency examination was only applied to alizarin complexan since the results obtained by conventional conductometric titration were satisfactory (cf. Table 1). The results obtained agree well with theoretical requirements and substantiate the identity and purity of the products.

TABLE 1. *Molecular weight by conductometric titration with cerium*(III).

Complexan from	No. of detns.	*M*, Found	*M*, Calc.
1,2-Dihydroxyanthraquinone	6 (6) *	385 (371) *	385
1,2,5-Trihydroxyanthraquinone	5	402	401
1,2,6- ,, ,,	7	395	401
1,2,5,8-Tetrahydroxyanthraquinone	5	418	417

* By radiofrequency technique.

Colour Reactions of Alizarin Complexan.—Alizarin complexan, H_4I, has four ionisable protons, the removal of which produces changes in the resonant system of the molecule. These changes are reflected in the absorption spectra [Fig. 1(*A*) and (*B*)]: the yellow form of alizarin complexan, $\lambda_{max.}$ 423 mμ, predominates at pH < 5, but at pH $\geqslant 6$ the intensity of this band

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[4] Körbl and Přibil, *Chem. and Ind.*, 1957, 233.

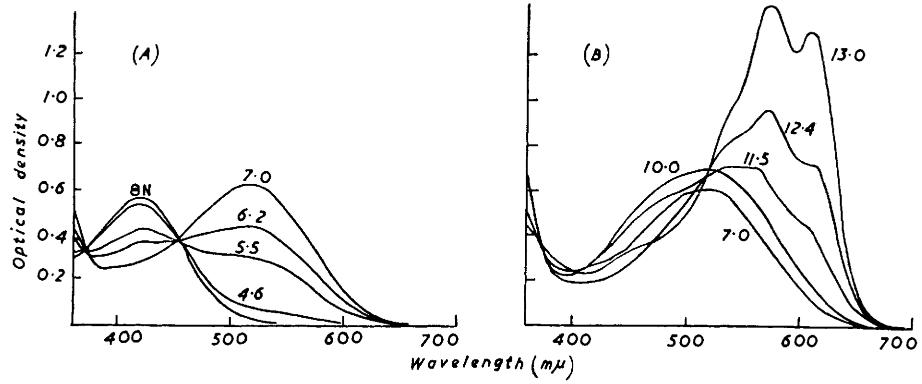
[5] Körbl, *Coll. Czech. Chem. Comm.*, 1957, **22**, 1789.

[6] Cf. Blaedel and Petitjean in Berl's "Physical Methods in Chemical Analysis," Academic Press Inc., New York, Vol. III, 1956, pp. 107 *et seq.*

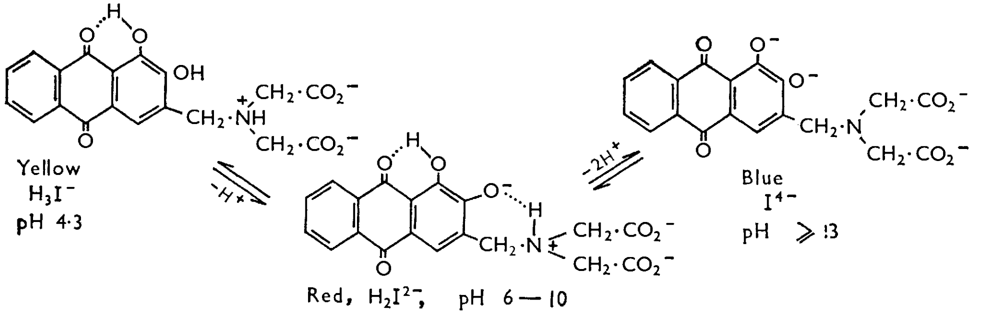
[7] Dowdall, Sinkinson, and Stretch, *Analyst*, 1955, **80**, 491.

falls off and that characteristic of the next higher ionisation form, $\lambda_{max.} \approx 520$ mμ, develops progressively so that the solution develops a red colour over the range 6—10. Beyond this point, the smooth shape of the absorption band is lost and at pH 11·5—12·4 the characteristic fine structure of the spectrum of the fully ionised molecule, pH > 13, begins to emerge. Although the absorption was not traced as far back as desirable into the ultraviolet region it is apparent that the curves between pH 0 and 7 exhibit two well-defined isosbestic points at 370 and 452 mμ, indicating a simple equilibrium between the indicator forms H_4I, H_3I^-, and H_2I^{2-} (where I = the complex ion). The complications introduced by hydrogen bonding between the 1-hydroxyl group and the 9-carbonyl group, and that between the 2-hydroxyl group and the ammonium-nitrogen atom attached make subsequent interpretation difficult though it appears that the next resonant form to appear is the fully deprotonated species, as illustrated.

FIG. 1. pH-*absorption curves for alizarin complexan* (9·63 *μg./ml.*).



(*A*) 8N-H_2SO_4 to pH 7. (*B*) pH 7 to 13·0.



When the reagent is buffered in the region pH 4·3—4·6 (H_3I^-; yellow form), it reacts with many ions, *e.g.*, Al, Cd, Ce(III), rare earths, Co(II), Cu(II), Fe(II), Hg(II), In, Ga, La, Mn(II), Ni, Pb, Th, Ti(III and IV), Zn, and Zr, to form red products. In alkaline solution, with the reagent buffered in the pH region ≈10 (H_2I^{2-}; red form), purple-blue chelates are formed in solution with ions such as Ca, Ba, Sr, Cd, Ni, Mg, Mn(II). In both pH regions chelation produces a bathochromic shift of the wavelength of maximum absorption, typical of the next higher ionisation form of the metal-free reagent, and for further assessment the absorption spectra of four chelates were examined in detail: nickel, which does not react with the quinone-hydroxyl system of 1,2-hydroxyanthraquinone under the experimental conditions; lead and cerium, as bi- and ter-valent metals which were expected to form stable chelates; and thorium as a convenient quadrivalent, octaco-ordinate ion. The spectra obtained (see Fig. 2) show that chelation results in a shift of the wavelength of maximum absorption (490—510 mμ) towards that of the H_2I^{2-} form of the indicator and that the shapes of the curves are closely similar to each other and to those of the metal-free reagent in the pH range 7—10 where $\lambda_{max.}$ varies from 510 to 520 mμ.

Job's method of continuous variations [8] was then applied. This (cf. Fig. 3) revealed that nickel, cerium(III), and lead form stable 1 : 1 chelates with the reagent, whereas thorium forms a chelate in which there are two molecules of alizarin complexan per thorium ion. Since the sharpness of these curves at their maxima is a measure of the stability, the order of stability is Th $>$ Ni $>$ Ce $>$ Pb. This is borne out by the chemical reactivities: of the four, only the lead chelate is broken down by a strong chelating agent such as EDTA at ordinary temperatures; the nickel chelate slowly and only at pH $>$ 7.

Similarly, at pH 4·3 in cold solution, the chelates of alizarin complexan with the following metals, are not broken down by a moderate excess of EDTA: Al, In, Ga, La, Ce(III), Sc, Th, Ti(III and IV), and Zr, though the chelates of the first two, like that of nickel, are broken down on warming. The other di(carboxymethyl)aminomethylhydroxyanthraquinones behave similarly with the cations mentioned, except that the indium chelate of alizarin bordeaux complexan is readily attacked by EDTA and so can be used as a complexometric indicator for indium. 1,2-Dihydroxyanthraquinone-3-sulphonic acid (alizarin S) yields red chelates with Al, Ce(III), La, In, Sc, Th, and Zr at pH 4·3, but these chelates are much more readily broken down by EDTA or fluoride. Zinc and nickel did not form coloured products with this reagent.

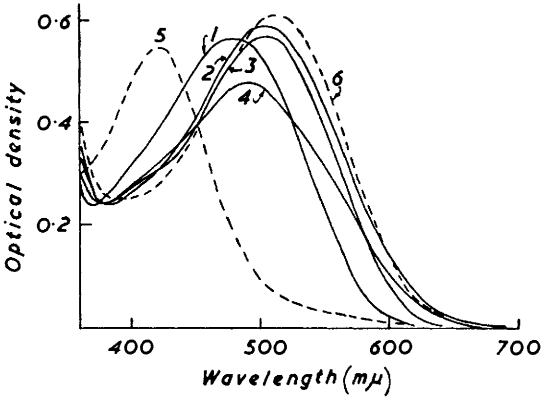
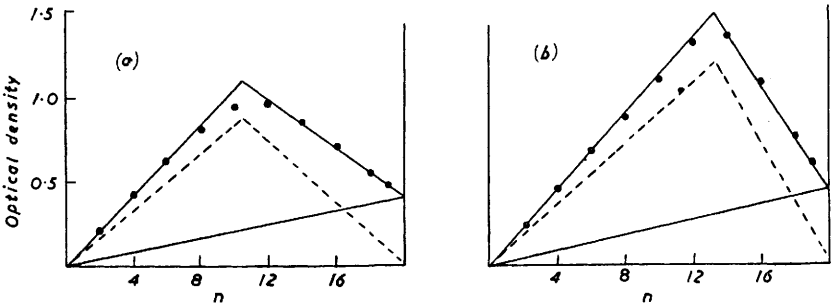


FIG. 2. *Absorption spectra of alizarin complexan chelates of* (1) Pb^{2+}, (2) Th^{4+}, (3) Ni^{2+}, *and* (4) Ce^{3+}. *Broken lines indicate spectra of the metal-free indicator at* (5) pH 4·3 *and* (6) pH 7·0.

FIG. 3. *Job plots for alizarin complexan chelates with* (*a*) Ce^{3+} *and* (*b*) Th^{4+}. *Broken lines are corrected curves.*



(*a*) 1·11 : 1 *and* (*b*) 2 : 1 *alizarin complexan–metal ratio. Measurements at* 495 *m*μ *and* (*a*) pH 4·3, (*b*) pH 4·1. *n* = *ml. of* 5 × 10^{-4}M-*alizarin complexan solution added to* (20 − *n*) *ml. of* 5 × 10^{-4}M-*metal solution.*

DISCUSSION

Nature of the Metal Chelates.—These results reveal that the chelates formed by alizarin complexan are considerably more stable than those formed by alizarin itself, obviously owing to the more powerfully chelating complexan group in the substituted reagent. It

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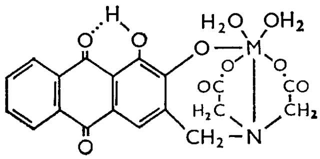
[8] Job, *Ann. Chim.* (*France*), 1928, **9**, **113**.

is obvious that the complexan group is of prime importance in the reagent since metal ions which do not form complexes with the parent hydroxyanthraquinone react well with the complexan-substituted reagent, *e.g.*, zinc, cobalt, nickel, etc.



Typical metal chelate of alizarin complexan

It is not possible for mononuclear chelates to be formed in which the complexan and the 9-carbonyl and the 1-hydroxyl group co-ordinate with the cation, so there can be little doubt but that, with hexaco-ordinate ions, chelation occurs as shown through the two acetic acid groups, the 2-hydroxyl group, and the nitrogen atom.

This attack on the 2-hydroxyl group would explain the close similarity between the absorption spectra of the chelate and the H_2I^{2-} (pH 7) form of the metal-free reagent. The failure of $\lambda_{max.}$ for the chelates to attain the full 510—520 mμ position corresponding to H_2I^{2-} may be attributed, at least in part, to the nature of the metal–oxygen bond. The more ionic the latter the less interference should be caused with the resonant system of the alizarin part of the molecule, and consequently the nearer the approach of $\lambda_{max.}$ for the chelate to $\lambda_{max.}$ for H_2I^{2-}.

Most of the cations that react with alizarin complexan are hexaco-ordinate, but a few can only be tetraco-ordinate, *e.g.*, mercury(II) and zinc (normally regarded as of tetrahedral disposition), and copper(II) (square co-planar). It is certainly impossible for the copper to co-ordinate with the 2-hydroxyl group as well as with the complexan centre, and it may well be that here the colour reaction is caused by a chelation-induced electron-deficiency on the nitrogen atom transferred, to a certain extent, to the vicinal phenolic group, thus weakening the oxygen–hydrogen bond of the latter and causing deprotonation even at pH 4·3—4·6 where normally the H_3I^- form is stable.[9] This idea is supported by an observation that copper(II), in dilute solution, produces a red shade that is brighter than that from most other ions. This colour closely approaches that of the metal-free indicator at pH 6—10 (*i.e.*, the metal ion in this instance causes little interference with the resonant system of the anthraquinone nucleus); equally, in this case an end-point is obtained very easily in complexometric titration with EDTA. Most copper-ion indicators react sluggishly because of their stability.

Stuart and Briegleb * atomic models show that it is possible for a binuclear, *i.e.*, 2 : 2 metal–reagent, chelate to be formed with hexaco-ordinate metals. In such a molecule each metal ion may be bound through the nitrogen atom, the acetic acid groups, and the 2-hydroxyl group of one alizarin complexan molecule and through the 1-hydroxyl and possibly the 9-carbonyl group of the other molecule. This yields a sterically crowded structure in which the metal ion may be fully co-ordinated by the reagent, with complete elimination of the water molecules normally associated with the cation. Certain difficulties are, however, associated with this model. Thus, (*a*) one would expect the fully co-ordinated and unsolvated metal-chelate compound to distribute well into water-immiscible solvents, a behaviour not observed in practice; (*b*) since both the hydroxyl groups are involved one would expect the colour produced to be characteristic of the fully de-protonated form of the reagent (I^{4-}) rather than of H_2I^{2-}; (*c*) ions such as copper(II) nickel, and zinc, which do not form coloured chelates with alizarin itself, still yield characteristic red colours with alizarin complexan.

A 2 : 1 chelate is formed between alizarin complexan and thorium to which we attribute the structure shown, since thorium adopts an octaco-ordinate configuration.

Complex Formation of Chelates with Fluoride Ion.—The remarkable stability of the alizarin complexan chelates of aluminium, cerium(III), thorium, and zirconium against attack

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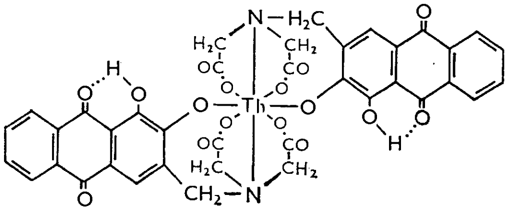
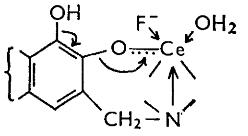
* Supplied by Leybold Nachfolger, Köln-Bayental, Germany.

[9] West, " Recent Developments in Inorganic and Organic Analytical Chemistry," *Roy. Inst. Chem. Monograph*, 1959, p. 21.

by strong complexing agents such as EDTA suggested the use of the compound as a chromogenic reagent for their determination in the presence of other metals which could be masked by EDTA. The effect of fluoride ion was first examined, since it also forms strong complexes with these metals. All chelates proved to be resistant to break-down by proportionate amounts of fluoride, but an unexpected blue colour was formed with the cerium(III) chelate. The absorption spectrum of this solution is compared in Fig. 4 with those of alizarin complexan, the cerium(III)-reagent chelate (all at pH 4·3), and the alizarin complexan at pH 12·4. The wavelength of maximum absorption of the blue fluoride chelate is 567 mμ, which agrees well with that of the metal-free reagent at pH 12·4, and the shape of the curve is similar though the shoulder at 610 mμ is less well developed. The nature of this new fluoride complex was investigated by a continuous variations procedure between the 1 : 1 cerium(III)–alizarin complexan chelate and fluoride ion from which (cf. Fig. 5) it was concluded that a 1 : 1 complex is formed. Adding one molar proportion of fluoride ion to a solution of the 1 : 1 cerium(III) chelate produces maximum colour at 567 mμ; adding a second molar proportion has no further effect, hypso- or hyper-chromic. A large excess of fluoride not only breaks down any existing triple complex but also causes precipitation of cerium(III) as its insoluble fluoride, so that such a system shows only the yellow colour of the metal-free reagent.



On this basis we conclude that the fluoride ion displaces one of the co-ordinated water molecules remaining on the cerium(III) atom; also that the entry of the fluoride ion within the co-ordination sphere of the cerium(III) ion causes deprotonation of the remaining 1-hydroxyl group. The latter result may be explained on the basis that the strong electrophilic effect of the fluoride ion causes withdrawal of electrons from the aromatic ring, with marked deficiency on position 1. Calvin *et al.*[10] have postulated a resonance effect in copper acetylacetone and Kimball,[11] Pauling,[12] and others have discussed the possibility of metal–ligand double bonds; the resonance effect is also known in other systems, *e.g.*, ferrous tris-*o*-phenanthroline.[13] On this basis, if one attributes a partial double-bond character to the cerium(III)–2-oxygen bond, then deprotonation of the 1-hydroxyl group is readily explained as in the annexed diagram.



Since there appears to be no steric factor involved, we conclude that the specificity of the fluoride action is due to the unusually strong electrophilic properties of this atom. The nature of the metal ion is, however, also important, for it was found that all the other rare-earth metals examined (La, Pr, Sm, Sc, Y, Gd, Dy, Er, Yb) formed red chelates with the reagent, but only lanthanum and praseodymium reacted further with fluoride to form similar blue complexes. It is interesting that the only three elements to give this reaction are those of atomic number 57, 58, 59: entry of the first 5*d*-electron in element 57 starts the filling of the 4*f*-level and thus produces the inner transition series of the lanthanons.

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[10] Calvin and Wilson, *J. Amer. Chem. Soc.*, 1945, **67**, 2003; Calvin and Bailes, *ibid.*, 1946, **68**, 949.

[11] Kimball, *J. Chem. Phys.*, 1940, **8**, 188.

[12] Pauling, " The Nature of the Chemical Bond," Cornell Univ. Press, New York, 1939.

[13] Morrison and Freiser, " Solvent Extraction in Analytical Chemistry," Wiley and Sons, Inc., New York, 1957, p. 29; Keller and Parry in Bailar's " Chemistry of the Co-ordination Compounds," Reinhold, New York, 1956, p. 189.

It is also noteworthy that elements 57—62 are those which readily form fluorides when their oxides are heated with chlorine trifluoride. The intermediate lanthanons, 63—68, form fluorides less readily, and the higher members do not do so at all. The shapes of the absorption curves of the lanthanum and the praseodymium fluoride complex were similar to that obtained with cerium(III) ($\lambda_{\text{max.}}$ 565 and 563 mμ respectively), but the effect was not so pronounced; samarium showed a consistently reproducible shift ($<$10 mμ), towards longer wavelengths, but of the other metals examined only zirconium showed a slight activity towards fluoride ion. It should be added that, though the thorium chelate showed no change in $\lambda_{\text{max.}}$ on addition of fluoride, there was a definite hyperchromic effect.

We were unfortunately unable to examine elements 60 and 61, but since samarium (no. 62) showed slight activity it is probable that neodymium and promethium would show intermediate action. Examination of absorption spectra produced in this way may prove useful in identifying this group of rare-earth elements. Whilst we used the purest forms of lanthanum and praseodymium available (" Spec. Pure," Johnson & Matthey) these may well contain some cerium(III), but not sufficient to account alone for the high extinction observed.

FIG. 4. *Absorption curves for* (1) *alizarin complexan,* (2) *alizarin complexan–cerium*(III), *and* (3) *alizarin complexan–cerium*(III)*–fluoride, all at* 4·3, *and* (4) *alizarin complexan at* pH 12·4.

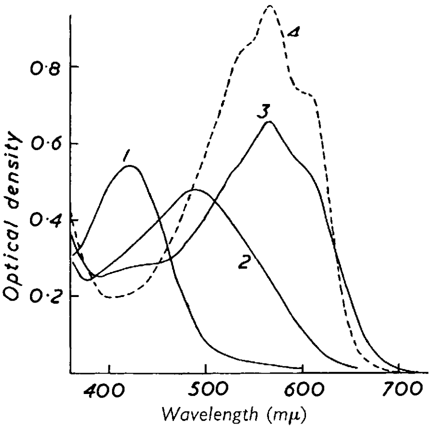
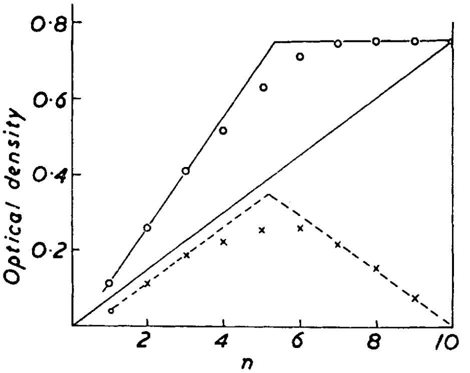


FIG. 5. *Job plot for alizarin complexan–cerium*(III)*-fluoride complex at* pH 4·6 *and* 567 *mμ. The broken line is the corrected curve.*



n = *ml. of* 5 × 10^{-4}M*-alizarin-cerium*(III) *solution mixed with* (10 − *n*) *ml. of* 5 × 10^{-4}M*-fluoride.*

All the other di(carboxymethyl)aminomethyl-hydroxyanthraquinones previously synthesised show a similar action with cerium(III) and fluoride, but the alizarin complexan system provides optimum conditions for detecting and determining fluoride. The formation of the blue complex provides an apparently specific spot test for fluoride ion, with a sensitivity of 0·2 μg. at a dilution limit of 1 in 1,800,000; experimental details of this procedure have been described elsewhere.[3]

Spectrophotometric Determination of Fluoride Ion.—From Fig. 4 it will be seen that there is good separation between the absorption curves for the cerium(III) chelate and the fluoride complex between 560 and 640 mμ. Measurements of optical density showed that maximum divergence occurred at 610 mμ, and consequently this wavelength was adopted for further measurements. The effect of pH value was not studied extensively since at pH 4·3, where the yellow-to-red transition of the metal-free reagent just begins, the results were entirely satisfactory. Equilibrium development of colour is obtained with the control [*i.e.*, formation of the cerium(III) chelate] within 1¼ hr., and with the fluoride complex within 1 hour, and both colours are constant for several hours thereafter. The order of addition of reagents has an important bearing on the speed of formation of the complex: best results are obtained when the alizarin complexan and buffer are added before the cerium(III). It is also important not to add a large excess of the acetate buffer: the concentration of the latter should be kept constant.

Although we have no evidence to suggest photochemical instability of the reagent or complex, solutions were stored as a precaution in diffuse daylight during the development of colour. In other respects normal laboratory conditions obtained during the examination, *e.g.*, no attention was paid to temperature control. A standard procedure was developed for amounts of fluoride from 5 to 30 μg. in which range Beer's law is obeyed. The standard curve was constructed by taking aliquot parts of a standard solution of pure sodium fluoride. A measure of the precision of the procedure was obtained by analysing 23 consecutive " unknown " samples of sodium fluoride weighed on a submicrobalance; the standard deviation was only ±0·27%. The standard solution of alizarin complexan is stable for at least one year (*i.e.*, no significant deviation was observed of the absorbance of known weights of fluoride). The reagent solutions, namely, alizarin complexan, cerium(III) nitrate and buffer, should however be filtered periodically. The effect of other anions and of several cations on the course of colour development by fluoride ion has been noted previously.[3]

Spectrophotometric Determination of Cerium(III) *in Presence of Thorium.*—Alizarin complexan may well have application as a chromogenic reagent for spectrophotometric determination of Ce(III), Th, Ga, La, and Sc in the presence of other metals such as Fe(III), Mn(II), Hg(II), Ni, Pb, etc., all of which can be masked by EDTA against chelate formation with the reagent. The characteristic action of the cerium(III) and lanthanum chelates of alizarin complexan with fluoride ion suggests the further possibility of determining these two metals in the presence of all other metals which do not participate in such a reaction. As a test case, an experiment was designed for the determination of cerium(III) in the presence of thorium.

The separation between $\lambda_{\text{max.}}$ for the thorium chelate and the cerium(III)–reagent–fluoride complex in *ca.* 70 mμ, and the separation between the $\lambda_{\text{max.}}$ values for the thorium chelate and the reagent at pH 4·3 are also *ca.* 70 mμ. There is, however, considerable overlap between the three absorption bands, which is particularly marked at the thorium wavelengths; consequently absorption measurements were made at all three wavelengths of maximum absorption and the data thus obtained were used to solve the equation for the three-colour method thus involved:

$$E^{\text{Total}} = \varepsilon^{\text{R}}C^{\text{R}} + \varepsilon^{\text{X}}C^{\text{X}} + \varepsilon^{\text{Y}}C^{\text{Y}}$$

where ε^{R} denotes the extinction coefficient of the reagent at the wavelength used, C^{R} denotes the concentration of free reagent, etc., and X and Y denote functions of the cerium(III)–reagent–fluoride complex and of thorium respectively. The results provide semiquantitative analysis of a cerium–thorium mixture though the accuracy is not sufficient for a reliable analytical procedure. This criticism appears to be generally applicable to most multi-colour methods, and in this case considerable interaction between the three species is suspected. Nevertheless, it is felt that this particular system is sufficiently promising and important to justify further investigation.

Extraction of Cerium(III)*–Alizarin Complexan–Fluoride Complex.*—Attempts were made to extract the metal chelates of alizarin complexan into water-immiscible solvents without success. This suggests that the binuclear model of chelate formation, with its fully reagent-co-ordinated metal ion, is improbable. However, the fluoride complex of cerium(III)–alizarin complexan distributes well from aqueous solution into a solvent system such as pentyl alcohol containing tributylamine. The presence of the amine appears to be essential for salt formation and/or replacement of the remaining water molecule co-ordinated to the cerium atom in the fluoride complex. Since the cerium(III) chelate itself is not extracted into the organic phase under these conditions, this procedure provides a valuable means of concentrating and separating the fluoride complex from reagent and other metal chelates. Consequently considerable enhancement of sensitivity and range of application may result. Further, this procedure should obviate the need for a three-colour method for resolving cerium and thorium, etc.

EXPERIMENTAL

Apparatus.—A Unicam S.P. 600 spectrophotometer with 4 cm. cuvettes was operated from a 12v heavy-duty accumulator.

Reagents.—5×10^{-4}M-*Alizarin complexan.* Alizarin complexan (Hopkin & Williams, Freshwater Road, Chadwell Heath, Essex) (192·5 mg.) was suspended in water and dissolved by addition of the minimum amount of freshly prepared sodium hydroxide solution. "AnalaR" sodium acetate trihydrate (250 mg.) was added and the pH of the solution adjusted to 5 by dilute hydrochloric acid. The solution was then diluted to 1 l., and was stored away from strong sunlight.

5×10^{-4}M-*Cerium*(III) *nitrate.* A stock solution of pure cerium(III) nitrate was standardised against 0·02M-EDTA with Xylenol Orange as indicator, and an aliquot part was diluted to provide exactly 5×10^{-4}M-reagent.

pH 4·3 *Buffer.* This contained sodium acetate trihydrate (105 g.) in glacial acetic acid (100 ml.), diluted to 1 l. with glass-distilled or de-ionised water.

Standard fluoride solution (5 μg./ml.). B.D.H. extra-pure sodium fluoride (221 mg.) was dissolved and diluted to 1 l., to furnish a standard solution of 100 μg./ml.; 50·00 ml. of this solution were diluted to exactly one l. with distilled water (see above) and transferred immediately to a Polythene bottle for storage.

Preparation of Standard Curve (*Confirmation of Lambert–Beer Law*).—A known volume (1—10 ml.) of the standard fluoride solution was transferred to a 100 ml. graduated flask containing *ca.* 50 ml. of glass-distilled or de-ionised water. 10 ml. of 5×10^{-4} M-alizarin complexan were then pipetted into the flask, and similarly 2·0 ml. of pH 4·3 buffer. The whole was mixed and 10 ml. of 5×10^{-4} M-cerium(III) nitrate were pipetted into it with *constant swirling.* The volume was then made up to 100 ml. and the solution was set aside for 1 hr. in diffuse daylight to allow the colour system to reach equilibrium. A control solution was prepared similar with omission of the fluoride test solution; since equilibrium colour development takes longer in this case, it was prepared first and left for at least 2 hr. before use. Meanwhile, the spectrophotometer was switched to "dark-current" and allowed to stand for 3 hr. with the lamp switched on for the last hour, before measurements were made against the control at 610 mμ. The standard graph of optical density against amounts of fluoride was prepared in the usual way. It is important to add the cerium(III) solution *after* the alizarin complexan reagent and to maintain the two in a 1 : 1 ratio.

Analysis of Fluoride Solutions.—5—50 μg. of fluoride were used in each determination.

Spectrophotometric Determination of Metal Ions: Determination of Thorium.—Spectrophotometric determination of Al, Cd, Co, Cu, Fe, Hg, In, La, Mn, Ni, Pb, Th, Ti, Zr, Ga, and Ce(III) is possible by our reagent. Details are given below for thorium. Variation in time of development, buffering of solution, etc., may be necessary for other metals; *e.g.*, thorium, zirconium, and titanium require a larger excess of reagent because 2 : 1 reagent–metal chelates are probably formed. Optical density should be measured at the wavelength most suitable for each case.

Reagents. (i) Thorium nitrate, $4{\cdot}31 \times 10^{-4}$M (100 μg. per ml. of solution). This was prepared by dilution of 0·02M-thorium nitrate previously standardised against EDTA with Xylenol Orange as indicator at pH $\sim$ 3.

(ii) Alizarin complexan, $4{\cdot}31 \times 10^{-4}$M.

(iii) pH 3 buffer was potassium hydrogen phthalate (10·21 g.) in 5N-hydrochloric acid (4·08 ml.), diluted to 1 l.

Method. An aliquot part (0—5 ml.) of thorium nitrate solution was pipetted into a 100 ml. graduated flask and $4{\cdot}31 \times 10^{-4}$M alizarin complexan (50 ml.) was added, followed by the buffer (20 ml.). The whole was mixed, diluted to 100 ml., and left for 30 min. Then the absorption of the test solution was measured against that of the control at 520 mμ. The calibration curve (0—600 μg.) was a straight line which passes through the origin.

Simultaneous Estimation of Cerium(III) *and Thorium.*—Since the three-colour method for thorium and cerium by means of alizarin complexan and fluoride was only semiquantitative no details are given here except that moderate excesses of alizarin complexan and fluoride were added in that order to the cerium(III)–thorium solution. Measurements of optical density were made at 420, 495, and 567 mμ.

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