The shifts in $-E_{1/2}$ values observed in the presence of itaconate and oxalate ions is greater than that in oxalate ions alone. This proves the formation of Cd(II)-oxalate-itaconate mixed complexes. The relationships between $-(E_{1/2})_c$ and log C_L, at two concentrations of itaconate, were represented by three distinct segments.

With the help of Schaap and McMasters method[9], the values of coefficients A, B, C and D in both the sets were obtained. These are given below:

At $0\cdot08M$ [itaconate]: log $A = 1\cdot18$, log $B = 3\cdot04$ $\pm 0\cdot02$, log $C = 4\cdot14 \pm 0\cdot06$ and log $D = 5\cdot10 \pm 0\cdot06$.

At $0\cdot24M$ [itaconate]: log $A = 1\cdot78$, log $B = 3\cdot51$ $\pm 0\cdot02$, log $C = 4\cdot23 \pm 0\cdot05$ and log $D = 5\cdot23 \pm 0\cdot08$.

Complete replacement of itaconate by oxalate is further confirmed from the mean value of coefficient D coinciding exactly with the stability constant β_{30} for $[Cd(OX)_3]^{4-}$ complex (log $\beta_{30} = 5\cdot14$ and mean log $D = 5\cdot17$).

From the coefficients A, B, C and D, the stability constants for mixed complex species existing in solutions, $[Cd(Ox) (itaconate)]^{2-}$, $[Cd(Ox) (itaconate)_2]^{4-}$ and $[Cd(Ox) (itaconate)]^{4-}$ were calculated by applying the method of Schaap and McMasters. These are: log $\beta_{11} = 3\cdot75$ $(3\cdot52)$, log $\beta_{12} = 4\cdot37$ $(3\cdot75)$ and log $\beta_{21} = 4\cdot36$ $(4\cdot23)$, values in parentheses were calculated according to the procedure of Watters et al.[13], which is elaborated in our previous papers[3,4].

In all the three mixed complex species, the experimentally observed stability constants are greater than those predicted on statistical considerations. The observed trend in the enhancement values is $\beta_{12} > \beta_{11} > \beta_{21}$. Mixed complex is formed when both the ligands are attached to the central metal ion simultaneously. It is possible that simultaneous π-bonding between the metal ion and ligands accounts for the higher values. The simultaneous displacement of four water molecules, during the mixed ligand complexation, will also lead to a favourable entropy factor.

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TLC Separation of Closely Related Amino Acids

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A method for TLC separation of sixteen closely related amino acids on silica gel-calcium oxalate mixed adsorbent system has been developed and the probable mechanism of separation has been indicated. The Martin relationship for R_m is found to be obeyed by similarly constituted amino acids.

MIXED adsorbents for TLC separation of closely related phenols[1], aromatic amines[2] and aliphatic amines[3] were developed in this laboratory. It offers promise for a successful TLC separation of other class of compounds. The present paper deals with the TLC separation of closely related amino acids on silica gel-calcium oxalate plates.

TLC plates (0·5 mm thick) were prepared from silica gel (40 g) and calcium oxalate (20 g, mesh size 200). The plates were activated at 60° for 24 hr. Solutions of amino acids (1%, w/v) in distilled water were used for spotting.

The chromoplates after development (180 min) were sprayed with 1% ninhydrin in 50% aq. methanol. Violet spots appeared after heating at 60° for 15 min. Various adsorbents and developers were tried and it was found that a mixture of silica gel-calcium oxalate (40: 20) was most suitable for the separation of all the amino acids studied in the presence of a mixed developer (a) butanol-acetic acid-ethyl acetate-water (50: 20: 30: 20) or (b) butanol-acetic acid-chloroform-water (50: 20: 30: 40). The results are presented in Table 1.

It is seen that the R_M values of the similarly constituted amino acids, viz. glycine, alanine, valine and leucine, in the solvent systems (a) and (b)

TABLE 1 — R_F VALUES OF THE VARIOUS AMINO ACIDS ON SILICA GEL-CALCIUM OXALATE CHROMOPLATES

Amino acid	$R_F \times 100$	
	a	b
Glycine	22	42
Aspartic acid	28	46
Glutamic acid	34	53
Serine	24	40
Leucine	53	67
Methionine	48	60
Asparagine	20	37
Alanine	29	45
Valine	42	56
Threonine	26	35
Cysteine	39	50
Lysine	14	30
Phenylalanine	57	70
Histidine	17	26
Tyrosine	62	73
Tryptophane	67	76

(a) Butanol-acetic acid-ethyl acetate-water (50: 20: 30: 20).
(b) Butanol-acetic acid-chloroform-water (50: 20: 30: 40) (chloroform layer).

bear a linear relationship with the number of carbon atoms. This shows that the additive character of R_M is exhibited by these amino acids. The mechanism of separation of amino acids on silica gel-calcium oxalate layer is probably an adsorption process which is influenced mainly by (i) reversible hydrogen bond formation between the hydrogen atom of the amino group or carboxylic group and the oxygen atom of the adsorbent (silica gel and calcium oxalate), as has been proposed by Bark and Graham[4] for the TLC separation of phenols on polyamide impregnated layers of cellulose and (ii) a weak bond formation by the donation of lone pair of electrons by N of NH_2 group to the calcium ion, as suggested by Yasuda[5] for the TLC separation of aromatic amines on $CdSO_4$ impregnated silica gel plates. Besides the above two factors, the extent of adsorption is also influenced by steric and electronic effects of the substituent on the amino acid molecule. Further the possibility of hydrogen bonding between the N of NH_2 and the H of the hydrated silica in the same manner as proposed by Pires and Roseira[6] cannot be ruled out. Hence the movement of spots, i.e. R_F values depend upon the above adsorption factors and the solvation factor.

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Chlorpromazine Hydrochloride as a Spectrophotometric Reagent for Os(VIII) & Os(VI)

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Chlorpromazine hydrochloride forms a red coloured species with Os(VIII) and Os(VI) in hydrochloric acid medium. The red species exhibits absorption maximum at 530 nm. A twelve-fold molar excess of the reagent is necessary for the full development of colour intensity. The sensitivity of the reaction is 0·0152 μg/cm² for Os(VIII) and 0·0134 μg/cm² for Os(VI). Beer's law is valid over the concentration range 0·08-7·2 ppm for Os(VIII) and 0·04-8·6 ppm for Os(VI). The effects of acidity, time, order of addition of reagents, temperature, reagent concentration and diverse ions are reported.

CHLORPROMAZINE hydrochloride (CPH) was proposed for the spectrophotometric determination of Pd(II)[1]. A survey of chemical literature showed that no attempt has been made to study the reaction between CPH and osmium. In the present investigation, the reaction of CPH with Os(VIII) and Os(VI) has been studied. On the basis of the results CPH is proposed as a sensitive reagent for the rapid spectrophotometric determination of Os(VIII) and Os(VI).

Standard solution of Os(VIII) was prepared from osmium tetroxide (Johnson Matthey) in 0·2M sodium hydroxide. Standard solution of Os(VI) was prepared from potassium osmate (Johnson Matthey) in doubly distilled water. The stock solutions were further diluted to give standard solutions containing 30 μg/ml of Os(VIII) or Os(VI). A 0·2% aqueous solution of CPH (May & Baker) was prepared and stored in the refrigerator. Solutions of acids and diverse ions of suitable concentrations were prepared using analytical grade reagents.

Procedure for the determination of Os(VIII) *or* Os(VI) — To an aliquot of the stock solution containing 2 to 180 μg of Os(VIII) or 1 to 215 μg of Os(VI) were added 12·5 ml of 6M hydrochloric acid and 3 ml of 0·2% CPH solution and the volume made up to 25 ml by adding doubly distilled water. The solution was mixed well and the absorbance was measured at 530 nm against a reagent blank prepared in the same way. The amount of osmium was then deduced from the standard calibration curve.

CPH is oxidized to red-coloured radical cation[2,3] by Os(VIII) or Os(VI) instantaneously in hydrochloric acid medium at room temperature (27°). Os(IV) does not react with CPH. The red radical cation formed in sulphuric acid medium is stable for only 10 min. Nitric acid medium cannot be used as it slowly oxidizes CPH. Maximum absorbance is achieved in 2·0-3·5M hydrochloric acid. The absorbance values remain constant for 1 hr in hydrochloric acid medium. Hence, a hydrochloric acid strength of 3M was chosen for all subsequent studies.

The red species exhibit maximum absorbance at 526-532 nm. The CPH and Os(VIII) or Os(VI) do not absorb around this wavelength. A 12-fold molar excess of the reagent is required to obtain the maximum intensity of colour. The absorbance readings are constant in the temperature range 4-50°. No change in the absorbance is observed when the order of addition of reactants was changed. Beer's law is valid over the concentration range 0·08-7·2 ppm of Os(VIII) or 0·04-8·6 ppm of Os(VI). The optimum concentration ranges as evaluated by Ringbom's method[4,5] are 0·8-7·0 ppm for Os(VIII) and 0·8-8·0 ppm for Os(VI). According to Sandell's expression, the sensitivities of the reaction are 0·0152 μg/cm² for Os(VIII) and 0·0134 μg/cm² for Os(VI). Sample solutions containing 4 μg/ml of Os(VIII) or Os(VI) prepared by the standard procedure gave a relative error of ±0·5%.

The following amounts (μg/ml) of diverse ions which commonly accompany Os(VIII) are found to give less than ±2% error in the determination of 4 μg/ml of osmium: rhodium(III) 14, iridium(III) 13, platinum(IV) 12, gold(III) 0·5, ruthenium(III) 0·4, palladium(II) 0·4, nickel (II) 575, cobalt(II)