COMPLEXATION EQUILIBRIA BETWEEN GALLIUM(III) AND 4-(4'-METHYL-2'-THIAZOLYLAZO)-2-METHYLRESORCINOL. DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF GALLIUM IN BIOLOGICAL SAMPLES

Ana I. JIMÉNEZ, Francisco JIMÉNEZ, Juan P. PÉREZ and Juan J. ARIAS

Department of Analytical Chemistry, University of La Laguna, 38204-La Laguna, Spain

Received April 28, 1989
Accepted August 7, 1989

The reaction of Ga(III) with 4-(4'-methyl-2'-thiazolylazo)-2-methylresorcinol (H_2L) at $I = 0{\cdot}25$ mol l^{-1}, ($NaNO_3$) was investigated spectrophotometrically. Numerical method was used to evaluate the stability constants of the complexes formed GaH_2L_2 ($\log \beta_{122} = 37{\cdot}03 \pm 0{\cdot}09$); GaHL ($\log \beta_{111} = 19{\cdot}08 \pm 0{\cdot}06$); GaL ($\log \beta_{101} = 13{\cdot}65 \pm 0{\cdot}16$). A method is developed for the determination of gallium using first and second derivative spectrophotometry and the effect of interferences has been evaluated. The method has been applied to the determination of gallium in human urine and blood serum samples.

Heteroazo-phenols and naphthols have been used as chromogenic reagents and metallochromic indicators for many metallic ions[1,2]. However, there are few reports on the applications of thiazolyl derivatives as spectrophotometric reagents for gallium[3-6], little attention being given, in general, to studies of equilibria in solution. The study, by spectrophotometric methods, of the complexation equilibria between gallium and 4-(4'-methyl-2'-thiazolylazo)-2-methylresorcinol[7] (MTAMR) is presented in this work, and the complex species in solution are established by numerical calculation analysis of the absorbance–pH curves. Distribution diagrams enable an accurate assessment of the distribution of the individual complexes under various experimental conditions.

The complex species formed in excess of reagent is the basis of a new method of gallium determination applying derivative spectrophotometry. In view of the antitumoral activity of gallium[8] and its toxicity[9,10], it was considered worthwhile to apply the developed method to the determination of gallium in human urine and blood serum.

EXPERIMENTAL

Apparatus

Perkin–Elmer 550S recording spectrophotometer with 1-cm path length glass cells. Radiometer PHM84 pH meter equipped with glass and saturated calomel electrodes. Digital VAS/VMX 11/780 (V.4.0) computer.

Reagents

Solution of MTAMR in methanol (10^{-3} mol l^{-1}). Standard ($2 . 10^{-2}$ mol l^{-1}) solution of gallium nitrate, prepared from the oxide by nitric acid treatment and standardized complexometrically[11]; pH 4·00 and 5·00 (sodium acetate–acetic acid) and pH 6·00 (20% hexamine–HNO_3) buffer solutions were used. The ionic strength was maintained constant at 0·25 mol l^{-1} by addition of a suitable amount of 2·5M-$NaNO_3$.

Analytical reagent grade chemicals and deionized water were used throughout.

RESULTS AND DISCUSSION

MTAMR, whose acid-base and optical characteristics are given in Table I, reacts with gallium(III), at pH above 2, to give a red colour. The absorption spectra of the system Ga(III)–MTAMR, as a function of pH, exhibit an absorption maximum at 510 nm. However, in order to avoid the interference of the reagent which absorbs considerably at 510 nm, the study was performed at 555 nm. The variations of the absorbance with the pH at 555 nm, for solutions with different C_{Ga}/C_L ratios, are given in Fig. 1, and indicate that complexation begins at higher acidities as the C_{Ga}/C_L increases.

The stoichiometry established by mole ratio, at pH 4·00 (λ 530, 555 nm), and pH 6·00 (λ 530, 555 nm), Fig. 2, shows that gallium and MTATR react to form 1 : 1 and 1 : 2 Ga : L complexes, as occurs with other Ga–azo dye systems[12].

In order to calculate the formation constants of the complexes a method of graphical analysis of the A–pH curves derived from that of Sommer et al.[13,14] was used. In accordance with the range of pH in which complexation took place and the pK_a values of the reagent, it could be admitted that the H_2L species of the reagent is the one that took part in the complexation reaction, written as follows

$$Ga^{3+} + m\,H_2L \rightleftarrows GaH_{2m-q}L_m^{(3-q)+} + q\,H^+$$

TABLE I

Optical characteristics of 4-(4′-methyl-2′-thiazolylazo)-2-methylresorcinol

Species	λ_{max} nm	ε l mol^{-1} cm^{-1}	pK_a
H_3L^+	475	21 700	1·21
H_2L	450	10 250	6·49
HL^-	490	33 500	11·07
L^{2-}	520	35 00	

However, the resulting straight line have slopes which are not whole numbers, again showing the presence of several complex species.

The numerical calculations were performed by means of the LETAGROP-SPEFO program[15]. This program determines the best set of constants and complexes minimizing the square sum of residuals, U, defined as $U = \sum_N (A_{cal} - A_{exp})^2$, where N is the number of experimental points introduced into the program, A_{exp} is the absorbance measured A_{cal} is the absorbance calculated by the program on the basis of the experimental parameters provided, assuming a certain set of complexes in solution. The program also calculates the standard deviation, defined as $\sigma(A) = [U/(N - n)]^{1/2}$, where n is the number of parameters estimated. The best model is that for which the lowest values of U and $\sigma(A)$ are obtained.

Some of the models tested are given in Table II. It can be seen that the best fitting model with the experimental data is that formed by species GaH_2L_2, GaHL and GaL, and for which the values of $\log \beta \pm 3\sigma(\log \beta)$ and $\varepsilon(\lambda) \pm 3\sigma(\varepsilon(\lambda))$ are also indicated in the above-mentioned table.

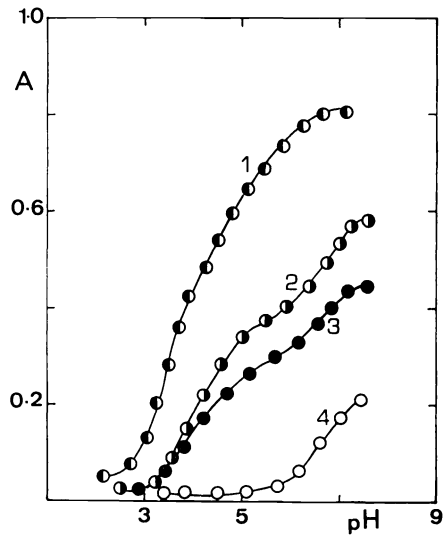

FIG. 1

Absorbance–pH curves for the system Ga(III)–MTAMR. $C_L = 4 \cdot 10^{-5}$ mol l^{-1}, 20% (v/v) methanol–water, $I = 0{\cdot}25$ (mol . l^{-1}, $NaNO_3$), $\lambda = 555$ nm. C_L/C_M: *1* 1; *2* 5; *3* 10; *4* 0

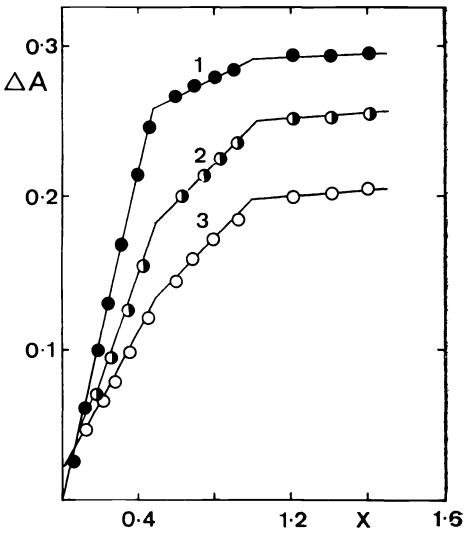

FIG. 2

Stoichiometry of the system: Ga(III)–MTAMR. Mole ratio method: $X = C_M/C_L$, $C_L = 2 \cdot 10^{-5}$ mol l^{-1}. $I = 0{\cdot}25$ (mol l^{-1}, $NaNO_3$). pH 6·0, λ in nm: *1* 555; *2* 530, pH 4·0; *3* 555

Figure 3 shows the diagrams of distribution of species calculated by the HALTAFALL program[16], for solution with different ratios of reagent to metallic ion. In

TABLE II

Values of U and $\sigma(A)$ for the different models tested by the LETAGROP-SPEFO method[a] and log β_{pqr} for the best fit

Species	U	$\sigma(A)$	Species	U	$\sigma(A)$
GaHL/GaL	0·496	0·103	$GaH_2L_2/GaHL_2$/ /GaHL/GaL	$0{\cdot}101 \cdot 10^{-2}$	0·005
GaH_2L_2/GaL_2	0·204	0·066			
GaHL/HaL_2	0·128	0·050			
$GaHL/GaH_2L_2$	0·102	0·030	$GaH_2L_2/GaHL$/ /GaL/GaL_2	$0{\cdot}116 \cdot 10^{-2}$	0·005
$GaHL/GaL/GaL_2$	$0{\cdot}681 \cdot 10^{-5}$	0·038			
$Ga(OH)L/GaL/GaL_2$	$0{\cdot}182 \cdot 10^{-2}$	0·007	$GaH_2L_2/GaHL$/ /GaL	$0{\cdot}889 \cdot 10^{-3}$	0·004

p, q, r[b]	log β_{pqr}	ε, 1 mol^{-1} cm^{-1}
1, 2, 2	37·03 ± 0·094	68 880 ± 311
1, 1, 1	19·08 ± 0·056	5 328 ± 85
1, 0, 1	13·65 ± 0·162	20 320 ± 44

[a] $C_L/C_M = 10/1, 5/1, 1/1$, $\lambda = 555$ nm; [b] $p\,M + q\,H + r\,L \rightleftarrows M_pH_qL_r$.

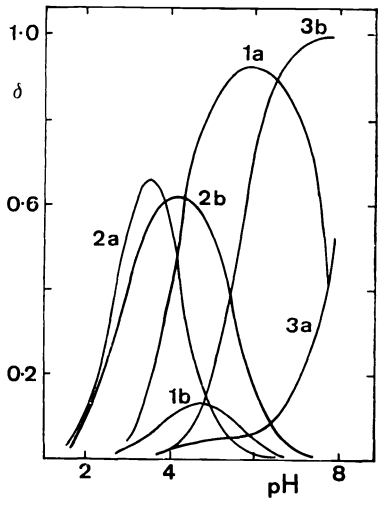

FIG. 3

Distribution diagrams of species. *1* GaH_2L_2; *2* GaHL; *3* GaL; C_L/C_M: *a* 1; *b* 10

this figure, it can be observed that both in the case of the solution with excess of reagent and in equimolar solutions, the different species co-exist in a wide range of pHs, which explains the unsuccessful application of the graphical method.

Spectrophotometric Determination of Gallium with MTAMR

In 20% (v/v) methanol–water medium at pH 6·0 (20% hexamine/HNO_3 buffer solution) the system conforms to Beer's law in the range 0·07–1·95 ppm Ga with molar absorptivity $\varepsilon_{530} = 4{\cdot}03 \cdot 10^4$ l mol^{-1} cm^{-1} and optimal range for the determination of 0·35–1·39 ppm Ga, as determined by Ringbom's method[17] with 1·1% photometric error.

Figure 4 shows the first and second derivative spectra of solution of gallium and reagent excess. From the graphical plot of the peak to peak distance in these spectra (L,cm) vs the gallium content (in ppm), straight lines are obtained, whose equations (fitted by the least-squares method) proved to be: $L_1 = -0{\cdot}240 + 1{\cdot}0 \cdot 10^6 w_{Ga}$ ($r = 0{\cdot}999$) and $L_2 = 0{\cdot}112 + 6{\cdot}61 \cdot 10^6 w_{Ga}$ ($r = 0{\cdot}998$), where w_{Ga} means mass ratio of Ga in ppm, r the correlation coefficient and L_1 is valid for the first and L_2 for the second derivative, respectively.

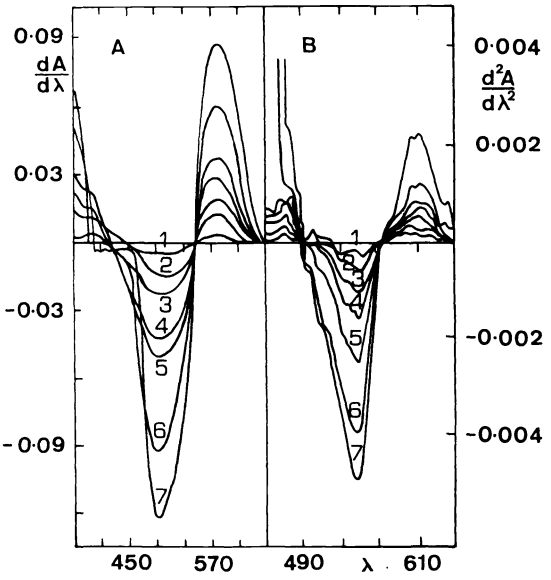

FIG. 4

First (A) and second derivative spectra (B) of gallium(III)–MTAMR complex. $C_L = 2 \cdot 10^{-4}$ mol. l^{-1}, pH 6·0. C_{Ga} (mol l^{-1}): *1* $2 \cdot 10^{-6}$; *2* $4 \cdot 10^{-6}$; *3* $6 \cdot 10^{-6}$; *4* $1 \cdot 10^{-5}$; *5* $1{\cdot}2 \cdot 10^{-5}$; *6* $2{\cdot}0 \cdot 10^{-5}$; *7* $2{\cdot}8 \cdot 10^{-5}$

The statistical study carried out on eleven samples, each containing 0·697 ppm Ga, gave 0·691 and 0·670 ppm Ga as mean values, $2{\cdot}44 \cdot 10^{-3}$ and $5{\cdot}55 \cdot 10^{-3}$ as standard deviations and $\pm 0{\cdot}80$ and $\pm 1{\cdot}87\%$ as relative errors (95% confidence level), for each of them, respectively.

A study of the effect of several ions on the first and second-order derivative spectrophotometric determination of 0·700 ppm Ga was carried out by the first applying the recommended method to solutions with a 500/1 (m/m) ratio of interferent to gallium and, if interference occurred, this ratio was reduced until interference ceased. The criterion for an interference was a deviation of more than $\pm 5\%$ from the value taken. Results are shown in Table III.

The method proposed for the spectrophotometric determination of gallium with MTAMR using the first and the second derivative, proves to be more sensitive

TABLE III

Interference levels of foreign ions on the determination of gallium, 0·700 μg ml^{-1} Ga taken

Ion added	Tolerance ratio	% Relative error	
		1st derivative	2nd derivative
Ca(II)	350	0·3	−4·8
Mg(II)	350	2·3	−3·4
Ba(II)	350	1·3	−0·4
Sr(II)	350	−2·7	−3·2
Li(I)	350	4·3	−5·0
K(I)	350	4·3	4·2
Cl^-	250	2·9	3·4
F^-	100	−1·4	−2·3
Br^-	100	−1·0	2·3
I^-	100	−1·6	1·4
As(III)	50	0·3	2·6
$S_2O_3^{2-}$, NO_3^-	50	2·9	−3·3
$S_2O_8^{2-}$	20	1·0	2·3
Mo(VI)	10	−0·6	−2·1
$C_2O_4^{2-}$	10	−2·4	4·7
Hg(II)	5	−4·7	−3·7
W(VI)	5	3·6	1·3
Sb(III), Pb(II), Al(III), SO_4^{2-}, Fe(III), Ag(I), Bi(III), Cd(II), Cu(II), Ni(II), Fe(II), Mn(II), Pd(II), Au(III), Sn(II), Zn(II), Co(II)	1	>5·0	>5·0

than if 1-(2-thiazolylazo)-2-naphthol-2-carboxylic acid[5] or 4-methyl-2-(2-hydroxy--1-naphthylazo)thiazole[6] were used.

The developed method has been applied to samples of human urine and blood serum to which standard gallium solutions were previously added. The results are shown in Table IV, where it can be seen that the proposed method would be effective for derivative spectrophotometric determination of gallium in this kind of samples.

Procedure of Spectrophotometric Determination of Gallium

Into a 25-ml calibrated flask transfer 5 ml of 10^{-3}M methanolic solution of MTAMR, 3·0 ml of pH 6·0 buffer solution (hexamine–HNO_3), 2·5 ml of 2·5M-$NaNO_3$, the

TABLE IV
Determination of gallium in biological samples

Sample	Ga added (μg/ml)	Ga found (μg/ml)	
		1st derivative	2nd derivative
Urine 1	0·28	0·27	0·28
	0·42	0·44	0·46
	0·56	0·58	0·60
2	0·42	0·43	0·44
	0·52	0·52	0·51
	0·63	0·61	0·63
3	0·69	0·69	0·69
	0·83	0·84	0·84
	0·98	0·96	0·99
4	0·84	0·85	0·81
	1·11	1·05	1·01
	1·39	1·23	1·19
5	1·11	1·11	1·26
	1·25	1·31	1·15
	1·39	1·44	1·60
Serum 1	0·28	0·27	0·26
	0·42	0·40	0·41
	0·56	0·54	0·55
2	0·69	0·70	0·70
	0·84	0·80	0·82
	0·98	0·97	0·97
3	0·42	0·39	0·43
	0·63	0·60	0·61
	0·84	0·82	0·83

sample solution containing between 1·74 and 48·80 μg of gallium, making up with deionized water. Leave for five minutes and record the first and second derivative spectra between 650 and 400 nm using 2 nm slit width, 120 nm min^{-1} scan speed, 30 nm min^{-1} chart speed, and 5 and 7 s response time for the first and the second derivatives, respectively. Measure the distance between the peaks at 495 and 575 nm for the first derivative, and 540 and 605 nm for the second derivative.

Determination of gallium in urine. To 10 ml of urine, containing between 0·69 and 3·48 mg of gallium, are added 0·5 ml of concentrated sulphuric acid and 5·0 ml of concentrated nitric acid, heating for 45 min and slowly adding 5·0 ml of 20% H_2O_2. The mixture is then almost taken to dryness and the treatment is repeated twice. The residue thus obtained in dissolved in deionized water, neutralized with NaOH to approximately pH 5·0 and made up to 100 ml in a calibrated flask with deionized water. Suitable aliquots of this solution are taken and analysed as described above.

Determination of gallium in serum. To 3·0 ml of serum, containing between 0·69 and 2·09 mg of gallium, 0·8 ml of 40% trichloroacetic acid are added; the mixture is shaken for 45 seconds and centrifuged for 10 min. The precipitate is washed twice with deionized water, and the supernatant and washings are treated and analysed following the procedure used for the urine samples.

The authors acknowledge the financial support of this work by the CAYCIT, *Spain, grant No.* PR. 84-0794 *and Prof. F. García Montelongo of this Department for many useful discussions.*

REFERENCES

1. Hovind H. R.: Analyst *100*, 769 (1975).
2. Shibata S. in: *Chelates in Analytical Chemistry* (H. R. Flaschka and A. J. Barnard, Eds), Vol. 4, p. 1. Dekker, New York 1972.
3. Nickless G., Pollard F. H., Samuelson T. J.: Anal. Chim. Acta *39*, 37 (1967).
4. Nazarenko V. A., Biryuk E. A., Ravitskaya R. V.: Zh. Anal. Khim. *33*, 2362 (1978).
5. Busev A. I., Zholondkovskaya T. N., Krysina L. S., Khramtsova T. V.: Zh. Anal. Khim. *32*, 1847 (1977).
6. Pilipenko A. T., Parkhomenko E. A.: Zh. Anal. Khim. *30*, 1101 (1975).
7. García Montelongo F., Arias J. J., Jiménez F.: Microchem. J. *25*, 410 (1980).
8. Hart M. M., Adamson R. H.: Proc. Natl. Acad. Sci. U.S.A. *68*, 1623 (1971).
9. Newman R. A., Brody A. A., Kraloff I. H.: Cancer *44*, 1728 (1979).
10. Kelsen D. P., Alcock N., Yeh S., Brown J., Young C.: Cancer *46*, 2009 (1980).
11. Přibil R.: *Applied Complexometry*, p. 124. Pergamon Press, Oxford 1982.
12. Voznica P., Havel J., Sommer L.: Collect. Czech. Chem. Commun. *45*, 54 (1980).
13. Mushran S. P., Sommer L.: Collect. Czech. Chem. Commun. *34*, 3693 (1969).
14. Sommer L., Langová M., Kubáň V.: Collect. Czech. Chem. Commun. *41*, 1317 (1976).
15. Sillén L. G., Warnqvist B.: Ark. Kemi *31*, 377 (1969).
16. Ingri N., Kakolowicz W., Sillén L. G., Warnqvist B.: Talanta *14*, 1261 (1967).
17. Ringbom A.: Anal. Chem. *115*, 332 (1939).