COMPARISON OF SPECTROPHOTOMETRIC METHODS OF DETERMINATION OF ZINC(II) IN BIOLOGICAL MATERIAL AND STUDY OF ITS COMPLEX FORMATION REACTIONS WITH 4-(2-PYRIDYLAZO)RESORCINOL

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The methods of determination of Zn(II) with dithizone, zincone, PAN, and PAR were compared by considering the fundamental parameters of the photometric methods. The compositions, values of the molar absorptivities, and values of the equilibrium and stability constants of the complex species MLH, ML, ML_2H_2, ML_2H, and ML_2 of zinc(II) with PAR were determined graphically by means of the slope-intercept transformations, and numerically from the absorbance-pH curves and from the absorbance dependences on the component concentrations by employing the program PRCEK T200 and from the continual variations curves by using the program JOBCON.

Zinc is an essential trace element of human organism. It has been detected in all organs and body fluids. It occurs predominantly bound to enzymes, proteins, nucleic acids, and other compounds. Its function in metabolic processes is not yet quite clear, and therefore the relevance of its determination in blood serum and urine is so far questionable. Still the importance of the determination is indisputable for malnutrition states and some postoperation states, where the supporting cure with Zn(II) salts and control of its concentration in blood serum and in urine can improve the overall state of the patient and speed up the wound healing[1-7]. A comprehensive account of the behaviour of zinc in human organism and the significance of its determination is given in the publications[5,7].

For the determination of zinc in biological material serve the very sensitive methods of atomic absorption spectrophotometry[8-14], atomic fluorescence spectrophotometry[15-17], or enzymatic methods[18]. In many analytical laboratories, however, the methods of classical molecular absorption spectrophotometry in the UV and visible regions retain their value; the best results have been here obtained with reagents such as dithizone[19-24], zincone[25-27], 1-(2-pyridylazo)-2-naphthol (PAN) (ref.[28]), and the highly sensitive 4-(2-pyridylazo)resorcinol (PAR) (ref.[29-33]).

The present paper gives the results of a comparison of the spectrophotometric methods of zinc determination with the above reagents in biological material from the point of view of their optical characteristics, sensitivity, and selectivity. For the most sensitive method, *viz.* that with PAR, the complexation equilibria in aqueous medium were spectrophotometrically studied in detail. A combination of the graphi-

cal and numerical methods of evaluation of the absorbance curves was employed for the determination of the principal characteristics of the various complex species.

EXPERIMENTAL

Chemicals and Apparatus

4-(2-Pyridylazo)resorcinol (PAR), commercial product of Lachema, was purified by double recrystallization from methanol. The purity was checked by TLC on silica gel (DC-Alufolien, Kieselgel F 254, Merck, Cat. No 5567/6025) in the system benzene–methanol 9 : 1 (v/v) saturated with EDTA. With the purified chemical, only two spots, R_F 0·68 and 0·76 (weak), were detected, whereas the original preparation displayed in addition spots R_F 0·10, 0·19, 0·26, 0·46, and 0·53, apparent on irradiation by continuous UV light. The content of the active component (91·5%) was determined by elemental analysis (C, H, N), that of sodium (11·5%) was determined on the mineralization of the sample. Unless stated otherwise, stock solutions of PAR in 0·002M-NaOH stored at room temperature for at most 48 h were used for the study of the reactions of Zn(II) with PAR. For the Zn(II) determination, $3{\cdot}92 \cdot 10^{-3}$M-PAR was used prepared by dissolving 10 mg of PAR in 10 ml of 0·002M-NaOH.

1-(2-Pyridylazo)-2-naphthol (PAN) ind. (Lachema) was used in the form of $4{\cdot}01 \cdot 10^{-3}$M solution, prepared by dissolving 10 mg of the substance in 2 ml of ethanol for UV spectroscopy and diluting to 10 ml with 20% aqueous solution of Brij 35. The stock solution of dithizone (*p.a.*, Lachema) was prepared by dissolving 50 mg of the substance in 500 ml of CCl_4 for IR spectroscopy (Lachema), extraction of dithizone in 500 ml of water containing 650 mg of EDTA with simultaneous addition of 12% NH_4OH, shaking twice with 50 ml of CCl_4, and successive reextraction in 500 ml of fresh CCl_4 under simultaneous addition of 6M-HCl till the decolouration of the aqueous phase. The resultant concentration was about 100 mg l^{-1}, *i.e.* approximately $3{\cdot}9 \cdot 10^{-4}$ mol l^{-1}. The stock solution stored in a dark bottle at 2—8°C is stable for at least 4 weeks. The stock solution of zincone (BDH, Great Britain) was obtained on dissolving 35 mg of the substance in 1 ml of 0·2M-NaOH and diluting with water to the volume of 10 ml ($7{\cdot}95 \cdot 10^{-3}$ mol l^{-1}). The stock solution of $ZnSO_4$, concentration $1{\cdot}00 \cdot 10^{-2}$ mol l^{-1}, was prepared by dissolving 28·754 g of solid $ZnSO_4.7\,H_2O$ *p.a.* (Merck, FRG) in 1 l of 0·001M-HNO_3. The titre of the solution was determined gravimetrically.

The effect of tensides was examined by employing 0·01—20% aqueous solutions of the compounds as follows: sodium laurylsulfate (SDS) *p.a.* (Merck, FRG); Brij 35 (polyoxyethylene laurylether) (Merck, FRG); Sterox SE (Harleco, USA); Triton X-100 (*p*-isooctylphenoxy-polyethoxyethanol) (Merck, FRG); cetylpyridinium bromide pure (Lachema); and Septonex (N-(1--(ethoxycarbonyl)pentadecyl)trimethylammonium bromide) (Spofa).

All the other chemicals were reagent grade purity, water for the preparation of the solutions was redistilled and deionized.

The acidity of the solutions was measured by means of pH-meters OP-205 with a combined glass electrode OP-800 (Radelkis, Hungary), PHM 72 with a glass electrode G 299 A and a saturated calomel electrode K 172 (Radiometer, Denmark), and PHM 4d with a glass electrode G 202 B and a saturated calomel electrode K 401 (Radiometer, Denmark). The instruments were periodically calibrated by employing a set of aqueous buffer solutions (phthalate, pH 4·01, phosphate, pH 6·48, borate, pH 9·18 at 25°C) and "Buffer Titrisols, pH = 1—13, Merck").

The absorption spectra were recorded on double-beam spectrophotometers Unicam SP 700 (Pye Unicam, Great Britain) and SuperScan 3 (Varian, Switzerland). The other measurements were performed on photometers Chiratic IX with a digital voltmeter Chiratic X (photometric

line Chiratic, Chirana, Research Institute of Medical Instrumentation, Brno) and Spekol with a photomultiplier (Zeiss, Jena, GDR) with a voltmeter ML 21 (Metra, Blansko).

Determination of Zn(II) *with Dithizone*

Most procedures apply extraction of the complex of Zn(II) with dithizone, composition ML_2 (λ_{max} 535 nm, ε_{max} 70000 cm^2 $mmol^{-1}$), from aqueous solution pH ~5–6 into a tetrachloromethane solution of dithizone, the latter being in an at least tenfold excess ($c_L/c_M \geqq 10$) over the zinc[19–23]. Absorption spectra recorded in dependence on the solution acidity for $c_M = 1{\cdot}528 \,.\, 10^{-6}$ mol l^{-1} and $c_L = 3{\cdot}9 \,.\, 10^{-5}$ mol l^{-1} displayed the absorption maximum of the complex at 535 nm in the whole region of pH 3–9. The absorption maxima found at 435 and 618 nm are due to the free ligand. The complex formation is characterized by two isosbestic points at 465 and 565 nm. From the absorbance-pH curve $\Delta A = f(\mathrm{pH})$ for the above concentrations, the optimum pH to be applied to the determination was found to be 5·1–5·5; it was adjusted with the acetate buffer, which in 0·1–2·0M concentrations affected neigher the sensitivity of determination nor the time required for the extraction equilibrium to establish. Using the standard addition method we found that the extraction had to proceed for at least 2 minutes for the Zn(II)–dithizone complex to be extracted quantitatively, and that the complex was stable for at least 2 h. For the procedure of determination we chose the extraction duration 3 minutes.

Under the above conditions, at pH ~ 5 and in the presence of thiosulfate, only Pd, Sn(II), and Zn ions and partly also Cd, Co, and Ni ions are extracted[46] in the solution of dithizone in CCl_4. Since the contents of these elements in human blood serum and in urine are negligible, the extraction of zinc is sufficiently specific. The application of 1 ml of 1M thiosulfate removes in addition the interference of Cu(II) in concentration about 130 μmol l^{-1} and of Fe(III) or Fe(II) in concentration about 360 μmol l^{-1} in the material analyzed, which are approximately 6 times and 10 times, respectively, higher concentrations than as present in average in the serum. Calcium and magnesium do not interfere with the determination of zinc in the serum even in high excess (2·5 and 1·5 mmol l^{-1}, respectively).

The reproducibility of the method was tested by 20 replicate determinations of Zn(II) in a reference sample of urine supplied by Hyland (USA), where the concentration of Zn(II) of 1300 μg l^{-1} was guaranteed for the extraction determination with dithizone, with the allowed interval of 1100–1500 μg l^{-1}. The average concentration obtained from the 20 replicates was 1360 ± 112 μg l^{-1}.

The recovery was tested with the same reference sample by adding 1 μg Zn(II) per ml. The resulting average concentration from 20 determinations was 2200 ± ± 149 μg l^{-1}, which corresponds to the recovery of 95·7% with the interval 87·5 to 104·5%.

The procedure of the determination of Zn(II) with dithizone in the standard solution, serum, and urine, and the preparation of the blank are shown in Table I.

Determination of Zn(II) *with Zincone*

With regard to the considerable instability of the complex of Zn(II) with zincone in nonbuffered solutions or in the medium of phosphate buffer, we measured the absorption spectrum of the complex in borate buffer pH 9·5, where the complex proved to be most stable. The absorption maximum of the ML complex lies at 630 nm. The absorbance-pH curve measured at this wavelength for concentrations $c_M = 5 \cdot 0 . 10^{-6}$ mol l^{-1} and $c_L = 3 \cdot 97 . 10^{-4}$ mol l^{-1} shows the optimum region for the formation of the complex pH 9–10, as compared with the published[25–38] region pH 8·5–9·5.

The sensitivity and the optical characteristics of the complex were affected by the presence of the tensides Sterox SE, Brij 35, and SDS similarly as the optical characteristics of the free ligand. The absorption curves of zincone exhibited a small hypochromic change of A_{max} on adding 0·5% anionogenic SDS, the band position remaining intact. Sterox SE and Brij 35 of the same concentration brought about a mar-

TABLE I
Procedure of the Determination of Zinc with Dithizone

Adding of components, ml	Sample			
	standard	serum	urine	blank
Standard	0·5	—	—	—
Serum	—	1·0	—	—
Urine	—	—	1·0	—
Water	—	—	—	0·5
Deprot. agent	0·5	1·0	1·0	0·5
	shake the components containing proteins, allow to stand for 5 min at room temperature, and centrifugalize at 5000 rpm for 10 min			
Filtrate	—	1·0	1·0	—
Acetate buffer 2M (pH 5·4)	5·0	5·0	5·0	5·0
1M-$Na_2S_2O_3$	1·0	1·0	1·0	1·0
	shake the reaction mixture			
$3 \cdot 9 . 10^{-4}$M-Dithizone in CCl_4	10·0	10·0	10·0	10·0
	extract for 3 min, measure absorbance of the organic phase at 535 nm, l = 10 mm, against CCl_4			

ked hypochromic change, accompanied by a bathochromic shift of λ_{max} from 480 to 500 nm. The absorption curves of the complex of Zn(II) with zincone displayed in the presence of 0·5% tensides hyperchromic changes of A_{max}, in the order Brij 35 > > Sterox SE > SDS, the nonionogenic Brij 35 and Sterox SE causing in addition a bathochromic shift of λ_{max} from 620 to 630 nm.

For the determination of Zn(II) with zincone, the medium of borate buffer with an addition of 0·5% Brij 35 was chosen.

Determination of Zn(II) *with* PAN *and* PAR

Inasmuch as PAN is low soluble in water, a solution of PAN in 20% v/v ethanol containing 16% Brij 35 was used for the determination of zinc(II). The absorption curves measured under the optimum conditions of determination with this solution and with solutions of PAN of the same concentration in pure ethanol, in 20% aqueous solution of Brij 35, and in 20% aqueous solution of Triton X-100 did not differ appreciably. The spectral curves measured for $c_M = 1{\cdot}02 \cdot 10^{-5}$ mol l^{-1} and $c_L = 5{\cdot}35 \cdot 10^{-5}$ mol l^{-1} in dependence on pH show two absorption maxima of the complex of Zn(II) with PAN at 520 and 560 nm and an isosbestic point at 495 nm. From the dependences $\Delta A = A - A_{0L} = f(\text{pH})$ (where A_{0L} is the absorbance of the ligand) for concentrations $c_M = 6{\cdot}95 \cdot 10^{-6}$ mol l^{-1} and $c_L = 3{\cdot}64 \cdot 10^{-4}$ mol l^{-1} measured at 520 and 560 nm, the range pH 8–10 emerges as the optimum one. The above data are in a good agreement with the published results[28,38]. For the determination, the wavelength 560 nm is better suited, as the $A - A_{0L}$ difference is here higher which implies a higher sensitivity of determination.

For the complex of Zn(II) with PAR formed if the ligand is present in excess, the pH 8–10 was found to be the optimum; the λ_{max} value is 495 nm. Other details are given in the section dealing with the complexation equilibria of Zn(II) with PAR.

Since the pH region suitable for the formation of the complexes of Zn(II) with zincone, PAN, and PAR is 8–10, the interfering ions were masked with cyanide. Additions of 20% formaldehyde or 60% aqueous solution of chloral hydrate[26–28,51,52] resulted in the decomposition of the cyano complexes of Zn(II), Cd(II), Mn(II), and UO_2^{2+} only with the same effect. In this arrangement the reaction is sufficiently specific for Zn(II), because the content of Cd, Mn, and U in blood serum and in urine is negligible as compared with that of Zn(II). Cu^{2+}, Fe^{2+}, and Fe^{3+} ions (or Cu^{+} and Fe^{2+} after reduction with ascorbic acid[27,28]) do not interfere in these conditions even in concentrations exceeding several (3–5) times those occurring in human serum and urine. The Ca^{2+}, Mg^{2+}, Cl^{-}, PO_4^{3-}, HPO_4^{3-}, and SO_4^{2-} ions, which may be present in biological fluids in higher concentration, also did not interfere.

The time stability of the complexes of Zn(II) with the various ligands remained preserved under the above conditions in the case of PAN and PAR ($\tau > 30$ min). The time stability of the complex with zincone did not improve in the presence of

ascorbic acid, which is in contrast with the published data[27]. For the determination procedure, 20% formaldehyde was employed for the releasing of the Zn(II) from the cyano complex, because some of the chloral hydrate batches gave milky turbid solutions.

The procedures applied to the measurements of the calibration curves, testing of the reproducibility of the methods, and the analytical determination of Zn(II) in biological material with zincone, PAN, and PAR are given in Table II.

TABLE II

Procedure of Determination of Zinc with Zincone, PAN, and PAR

Buffer: 2M aqueous solution of TRIS for PAN and PAR, 2 parts of saturated solution of $Na_2B_4O_7$ and 1 part of 0·2M-NaOH in 0·5% aqueous solution of Brij 35 for zincone; Reagent A: $3{\cdot}92 \cdot 10^{-3}$M-PAR in 0·002M-NaOH, $4{\cdot}01 \cdot 10^{-3}$M-PAN in 20% ethanol with 16% Brij 35; Reagent B: $7{\cdot}95 \cdot 10^{-3}$M zincone in 0·02M-NaOH. Absorbances measured at 560 nm for PAN, 495 nm for PAR and 630 nm for zincone, $l = 10$ mm, reference liquid: water.

Adding of components, ml	Sample			
	standard	serum	urine	blank
Serum	—	1·0	—	—
Urine	—	—	1·0	—
Deprot. agent	0·5	1·0	1·0	0·5
	shake the components containing proteins, allow to stand at room temperature for 5 min, centrifugalize at 5000 rpm for 10 min			
Filtrate	—	1·0	1·0	—
Standard	0·5	—	—	—
Water	—	—	—	0·5
Ascorbic acid 10%	0·1	0·1	0·1	0·1
	shake and allow to stand for 5 min			
Buffer	2·5	2·5	2·5	2·5
KCN 2% solution	0·1	0·1	0·1	0·1
Reagent A	0·1	0·1	0·1	0·1
Reagent B	0·05	0·05	0·05	0·05
	measure absorbance A_1			
Formaldehyde 20%	0·1	0·1	0·1	0·1
	after the optimum period[a] measure absorbance A_2; calculate the absorbance of zinc as $A = (A_2 - A_1)^{st} - (A_2 - A_1)^{bl}$ [b]			

[a] For zincone up to 1 min after addition of formaldehyde, for PAN and PAR 5—30 min after the addition of formaldehyde; [b] the superscripts st and bl refer to the absorbances of the standard solution and the blank, respectively.

The reproducibility of the individual methods was tested with a sample of mixed serum obtained from patients invited for a preventive medical examination, for which the results of the other analyses fell within the normal region. The average value from 10 determinations was $11{\cdot}3 \pm 0{\cdot}1$ μmol l^{-1} Zn(II) for PAN and $11{\cdot}60 \pm \pm 0{\cdot}15$ μmol l^{-1} Zn(II) for PAR. In the latter case, the recovery was tested by adding 1 μg Zn(II)/ml, hence 15·3 μmol l^{-1}. The average concentration from 10 determinations was 25·4 μmol l^{-1}, which corresponds to the average recovery of 94·4% with the interval 90·5 – 102·3%.

The principal optical characteristics of the various methods and the results of the mathematical processing of the calibration curves for the determination of Zn(II) with dithizone, zincone, PAN, and PAR are summarized in Table III.

From the comparison of the various methods, the method with PAR emerges as the most suitable for the determination of Zn(II) in serum and urine. The high sensitivity allows to reduce the sample volume. As compared with the also very sensitive extraction method with dithizone, the method with PAR is more rapid, is better suited for routine measurements, and avoids handling with organic solvents such as CCl_4.

TABLE III

Comparison of the Principal Parameters of the Methods of Zn(II) Determination

Parameter	Reagent			
	PAR	dithizone	PAN	zincone
pH Optimum	8 – 10	5·1 – 5·5	8 – 10	9 – 10
λ_{max}, nm ligand	412	435, 618	480	500
complex	495	535	520, 560	630
λ_{opt}, nm complex	495	535	560	630
Time stability of ligand solutions	5 days	4 weeks	5 days	5 days
complex solutions	>30 min	>120 min	>30 min	~1 min
Absorbance of the blank[a]	0·19	0·27	0·08	0·01
Molar absorptivity[b]	$9{\cdot}05 \pm 0{\cdot}27$	$7{\cdot}30 \pm 0{\cdot}18$	$4{\cdot}90 \pm 0{\cdot}08$	$2{\cdot}02 \pm 0{\cdot}13$
Limit of determination (μM)	0·110	0·165	0·227	0·843
Sensitivity index for $A = 0{\cdot}01$, ppb	6·7	8·9	13·3	32·4
Reproducibility, %[c]	12·6[d]	8·2[e]	9·2[f]	

[a] For $l = 10$ mm; [b] values $\varepsilon \pm 3\sigma$ (cm^2 mmol^{-1}) under experimental conditions of determination; $c_M \leqq 4 \cdot 10^{-5}$ mol l^{-1}; [c] standard deviation; [d] value for $11{\cdot}6 \pm 0{\cdot}15$ μmol l^{-1}, average of 10 determinations; [e] value for $88{\cdot}9 \pm 7{\cdot}3$ μmol l^{-1} Zn(II), average of 20 determinations; [f] value for $11{\cdot}3 \pm 0{\cdot}1$ μmol l^{-1} Zn(II), average of 10 determinations.

Although the spectrophotometric determination of Zn(II) with PAR is a very sensitive method, it has been seldom used; only Goldstein and coworkers[32] applied it to the determination of Zn(II) in biological material by employing an automatic analyzer GeMSAEC, and Ahrland and Herman[33] worked out a method of determination of trace concentrations of Mn(II) and Zn(II).

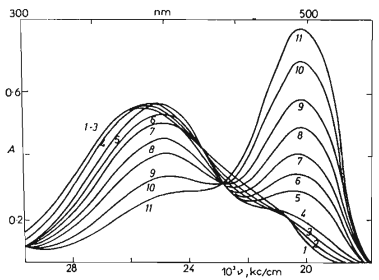

FIG. 1

Absorption Curves of Solutions of Zn(II) with PAR Containing Excess Metal

$c_L = 2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, $c_M = 5{\cdot}0 \cdot 10^{-4}$ mol l^{-1}, $l = 10$ mm, $t = 25°C$, *I* 0·10 (KNO_3 + + HNO_3), pH: *1* 3·64, *2* 3·82, *3* 3·94, *4* 4·14, *5* 4·70, *6* 5·22, *7* 5·70, *8* 6·07, *9* 6·29, *10* 6·60, *11* 6·75.

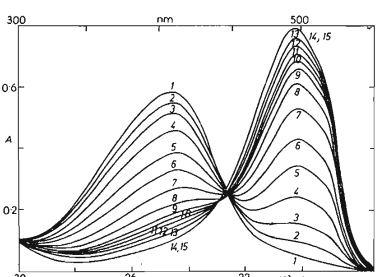

FIG. 2

Absorption Curves of Solutions of Zn(II) with PAR at pH 9·05, with Variable Concentration Excess of the Metal

$c_L = 2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, $c_{CPB} = 5 \cdot 10^{-4}$ mol l^{-1}, *I* 0·10 (KNO_3 + HNO_3), $t = 25°C$, $l = 10$ mm; c_M (10^{-5} mol l^{-1}): *1* 0·0, *2* 0·50, *3* 0·75, *4* 1·50, *5* 2·25, *6* 3·0, *7* 3·75, *8* 4·5, *9* 5·25, *10* 6·0, *11* 6·75, *12* 7·5, *13* 11·25, *14* 26·25, *15* 51·25.

Data concerning the optimum conditions of Zn(II) determination with PAR and the absorptivity values as reported in the literature differ considerably, which with regard to the increasing importance of the method made us undertake detailed spectrophotometric study of the complexation equilibria; this facilitated an explanation of the aforesaid discrepancies.

Complexation Equilibria of Zn(II) *with* 4-(2-*Pyridylazo*)*resorcinol*

The absorption curves of aqueous solutions of Zn(II) with PAR in dependence on the acidity and the concentrations of the components (Table IV, Figs 1 and 2) indicate the formation of at least three complex species in the solution. Absorption maxima appear at 495–520 nm, in dependence on the experimental conditions. A survey of the optical characteristics is given in Table IV. The plots of absorbance versus pH for the wavelengths 490, 500, 510, 520, and 530 nm, obtained for concentrations $c_L = 2{\cdot}50 \cdot 10^{-5}$ mol l^{-1} and $c_M/c_L = 100$, 20, 10, 5, and 1 in the pH region 1–10 and for concentrations $c_L = 7{\cdot}50 \cdot 10^{-5}$ mol l^{-1} and $c_M/c_L = 500$, 100, 10 and 1 in the pH region 1–5·5, indicate at pH < 5 the presence of the first complex, the formation of which is shifted to more acidic value as the concentration of Zn(II) increases. At pH 5–9 a mutual conversion of two complexes occurs, independent of the Zn(II) concentration (Fig. 3 and 4).

TABLE IV

Optical Characteristics of Solutions of Zn(II) with PAR
$c_L = 2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, $t = 25°C$, I 0·10 ($KNO_3 + HNO_3$).

c_L/c_M	pH	λ_{max}, nm	$\lambda_{i.p.}$, nm
20	1·5–4	515, 393[a]	485
	>4	400[a], 495, 515	437
5	2–4·5	515, 395[a]	485
	>4·5	402[a], 495, 515	437
1[b,c]	≧5	498, 518, 406[a]	440
0·2[b]	≧4	498, 518, 406[a]	440
<1[b]	9·05	495, 518, 406[a]	442
>1[b]	9·05	498, 518, 395	435
<1	5·10	498, 518, 406[a]	442
>1	5·10	495, 396[a]	436

[a] Absorption maximum of the free ligand; [b] in the presence of $5 \cdot 10^{-4}$ M cetylpyridinium bromide; [c] $c_L = 2{\cdot}0 \cdot 10^{-5}$ mol l^{-1}.

A direct graphical and graphical-logarithmic analysis of the individual absorbance-pH curves[42,43] as well as their numerical processing by employing the program[44–46] PRCEK T200 evidenced in the first formation region unambiguously the formation of the protonized complex MLH with the detachment of two protons, as described by the equilibrium

$$M + LH_3 \rightleftarrows MLH + 2\,H \quad (*\beta_{111}), \qquad (A)$$

with a simultaneous dissociation of the protonized form of the ligand LH_3^+ to the molecular form LH_2. In the second part of the curves, the two evaluation procedures confirmed unequivocally the conversion of the protonized complex MLH to the unprotonized complex ML accompanied by the detachment of one proton:

$$MLH \rightleftarrows ML + H \quad (*\beta_{110}). \qquad (B)$$

The resulting average values of the molar absorptivities and equilibrium constants for the various sets of curves are given in Tables V and VI. The rather high scatter of the equilibrium constant values is due to the partial overlap of the two equilibria in the range pH 4–6.

The absorbance-pH curves of solutions containing the ligand in excess, $c_L/c_M = 20$, 10, and 5, $c_M = 6{\cdot}25 \cdot 10^{-6}$ mol l^{-1}, indicate in the pH region 1–9·5 three complex

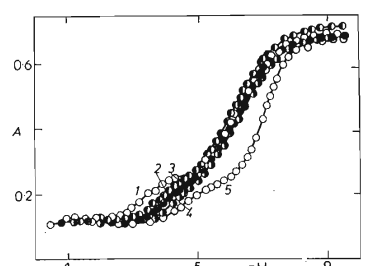

FIG. 3

Absorbance-pH Curves of Solutions of Zn(II) with PAR Containing Excess Metal for the Wavelength 500 nm

$c_L = 2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, I 0·10 (KNO_3 + + HNO_3), $t = 25$°C, $l = 10$ mm; $c_M(10^{-4}$ mol $l^{-1})$ c_M/c_L: *1* 25 100, *2* 5 20, *3* 2·5 10, *4* 1·25 5, *5* 0·25 1.

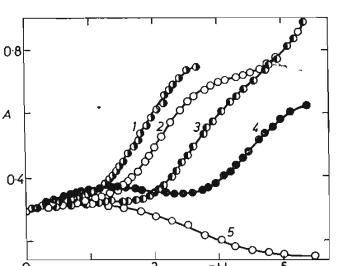

FIG. 4

Absorbance-pH Curves of Solutions of Zn(II) with PAR Containing Excess Metal for the Wavelength 500 nm

$c_L = 7{\cdot}5 \cdot 10^{-5}$ mol l^{-1}, the other parameters as for Fig. 3; $c_M(10^{-4}$ mol $l^{-1})$ c_M/c_L: *1* 375 500, *2* 75 100, *3* 7·5 10, *4* 0·75 1, 0·0.

formation equilibria. Assuming the formation of the complexes MLH_x, ML_2H_x, and ML_3H_x by direct interaction of the molecular form of the ligand with the zinc ions according to the equation

$$M + n\,LH_2 \rightleftarrows ML_nH_x + qH \quad (*\beta_{1nx}) \qquad (C)$$

for $n = 1, 2, 3$ and $q = 1$ to 6, along with the simultaneously considered dissociation equilibrium LH_2/LH^- and the conversions of the protonized complexes to unprotonized or less protonized ones at higher pH values, we proved by the graphical and graphical-logarithmic analysis of the absorbance-pH curves the existence of the protonized complex ML_2H_2 at pH 3·5 – 5·5, formed according to the reaction

$$M + 2\,LH_2 \rightleftarrows ML_2H_2 + 2\,H \quad (*\beta_{122}). \qquad (D)$$

TABLE V

Values of the Molar Absorptivities of the Individual Complexes of Zn(II) with PAR as Determined Graphically (method *A*) and Numerically by Employing the Program PRCEK T200 (method *B*) and the Program JOBCON (method *C*)

Complex	Method	ε_{490}	ε_{500}	ε_{510}	ε_{520}	ε_{530}
MLH^a	*A*	10 000	11 110	10 200	9 910	8 990
	B	10 082 ± 203	11 309 ± 200	10 660 ± 178	10 008 ± 188	9 110 ± 174
ML^b	*A*	29 200	27 230	25 280	21 290	14 000
	B	28 380 ± 313	27 525 ± 315	24 950 ± 306	21 370 ± 262	14 305 ± 232
$M(LH)_2$	A^c	17 900	17 850	15 870	12 220	10 180
ML_2H	A^d	70 765	69 920	60 090	48 975	39 125
	B^e	71 155 ± 586	69 850 ± 607	59 185 ± 557	48 750 ± 587	37 865 ± 514
ML_2	A^d	92 950	92 400	88 010	79 755	54 960
	A^f	82 730	82 500	77 060	—	—
	B^d	92 830 ± 861	92 599 ± 665	87 660 ± 610	79 820 ± 556	55 370 ± 504
	C^g	88 900	87 950	81 600	72 190	50 160
	C^h	89 920	89 540	82 000	73 220	51 760

[a] Average for concentration ratios $c_M/c_L = 500$, 100, and 10, $c_L = 7{\cdot}50 \cdot 10^{-5}$ mol l^{-1}; [b] average for $c_M/c_L = 100$, 20, 10, and 5, $c_L = 2{\cdot}50 \cdot 10^{-5}$ mol l^{-1}; [c] average for $c_L/c_M = 20$, 10, and 5; the program PRCEK T200 did not yield good results as insufficient volume of experimental data were available; [d] average for $c_L/c_M = 20$ and 10, $c_M = 6{\cdot}25 \cdot 10^{-6}$ mol l^{-1}; [e] values for $c_L/c_M = 20$, $c_M = 6{\cdot}25 \cdot 10^{-6}$ mol l^{-1}; [f] curve $A = f(c_L)$ for pH 7·50; [g] continual variations curves for pH 8·91; [h] continual variations curves for pH 10·10.

TABLE VI

Average Values of the Equilibrium Constants as Obtained from the Graphical (method *A*) and Numerical (method *B*) Interpretation of the Absorbance Curves of Zn(II) with PAR

Equilibrium	Method	log $^*\beta_{mnx}$ [a]	Average
$[MLH][H]^2/[M][LH_3]$	*A*	−3·49, −3·46, −3·36	−3·43 [b]
	B	−3·27 ± 0·02, −3·37 ± 0·05, −3·46 ± 0·07, −3·32 ± 0·08, −3·42 ± 0·07, −3·55 ± 0·06	−3·40 ± 0·06 [b]
$[ML][H]/[MLH]$	*A*	6·27, 6·22, 6·30, 6·35	6·29 [c]
	B	6·31 ± 0·08, 6·26 ± 0·06, 6·33 ± 0·04, 6·36 ± 0·05, 6·29 ± 0·07, 6·26 ± 0·05, 6·33 ± 0·02, 6·34 ± 0·06	6·31 ± 0·05 [c]
$[M(LH)_2][H]^2/[M][LH_2]^2$	*A*	−3·70, −3·89, −3·74	−3·80 [d]
$[ML_2][H]/[M(LH_2)]$	*A*	5·65, 5·65	5·65 [e]
	B	5·77 ± 0·09, 5·81 ± 0·06	5·79 ± 0·07 [f]
$[ML_2][H]/[ML_2H]$	*A*	7·42, 7·36	7·39 [e]
	B	7·49 ± 0·04, 7·47 ± 0·05, 7·51 ± 0·03, 7·48 ± 0·03	7·49 ± 0·04 [e]

[a] Average values for the wavelengths 490, 500, 510, 520, and 530 nm; [b] average value for $c_M/c_L = 500$, 100, and 10, $c_L = 7{\cdot}50 \cdot 10^{-5}$ mol l^{-1}; [c] average value for $c_M/c_L = 100$, 20, 10, and 5, $c_L = 2{\cdot}50 \cdot 10^{-5}$ mol l^{-1}; [d] average value for $c_L/c_M = 20$, 10, and 5, $c_M = 6{\cdot}25 \cdot 10^{-6}$ mol l^{-1}; the program PRCEK T200 did not yield good results as insufficient volume of experimental data were available; [e] average for $c_L/c_M = 20$ and 10, $c_M = 6{\cdot}25 \cdot 10^{-6}$ mol l^{-1}; [f] results for $c_L/c_M = 20$, $c_M = 6{\cdot}25 \cdot 10^{-6}$ mol l^{-1}.

TABLE VII

Complexes of Zn(II) with PAR and Their Characteristics
$t = 25°C$, I 0·10 ($KNO_2 + HNO_3$).

Complex	Formation conditions	λ_{max}, nm	$\varepsilon(\lambda)$	$\log \beta_{mnx}$	$\log {}^*\beta_{mnx}$	Ref.
ZnLH	$c_M > c_L$, pH 1–5	515	10 150 (500)	5·2 ± 0·1[a]	−3·40 ± 0·06[b]	
		500	11 000 (500)		0·0 ± 0·1[c]	30, 31
		487	10 700 (490)	12·9	−3·38 ± 0·01[b]	29
ZnL	$c_M > c_L$, pH > 5	495	28 500 (495)	11·5 ± 0·01[d]	6·31 ± 0·05[e]	
		495	31 800 (490)	11·9 ± 0·1[d]	5·9 ± 0·05[d]	30, 31
				10·5, 11·2[d,f]		36
				11·9[d]		39
ZnL_2H_2	$c_L > c_M$, pH 3·5–5·5	498, 518	16 800 (500)	7·3 ± 0·1[g]	−3·80[h]	
		495	17 500 (495)			30, 31
ZnL_2H	$c_L > c_M$, pH 5·5–7·0	498, 518	70 900 (490)	13·0 ± 0·1[i]	5·79 ± 0·07[j]	
		495	67 400 (495)		6·45 ± 0·05[j]	30, 31
ZnL_2	$c_L > c_M$, pH > 7·0	498, 518	92 900 (490)	20·5 ± 0·1[k]	7·49 ± 0·04[m]	
		495	95 800 (495)	22·2 ± 0·2[k]	7·55 ± 0·05[m]	30, 31
					10·3[n]	30, 31
				17·1, 19·0[k,f]	6·6, 7·8[n,f]	36
			63 400 (495)	25·3[k]		29
				22·2[k]		9

[a] $[ZnLH]/[Zn][LH]$; [b] $[ZnLH][H]^2/[Zn][LH_3]$; [b] $[ZnLH][H]/[Zn][LH_2]$; [d] $[ZnL]/[Zn][L]$; [e] $[ZnL][H]/[ZnLH]$; [f] values for aqueous solution or 50% dioxane; [g] $[ZnL_2H_2]/[Zn][LH]^2$; [h] $[ZnL_2H_2][H]^2/[Zn][LH_2]^2$; [i] $[ZnL_2H]/[Zn][LH][L]$; [j] $[ZnL_2H][H]/[ZnL_2H_2]$; [k] $[ZnL_2]/[Zn][L]^2$; [m] $[ZnL_2][H]/[ZnL_2H]$; [n] $[ZnL_2]/[ZnL][L]$.

One proton is detached at pH > 5·5 and the complex ML_2H is formed according to the reaction (*E*); at pH > 7, the complex ML_2 appears according to the reaction (*F*):

$$ML_2H_2 = ML_2H + H \quad (*\beta_{121}), \qquad (E)$$

$$ML_2H = ML_2 + H \quad (*\beta_{120}). \qquad (F)$$

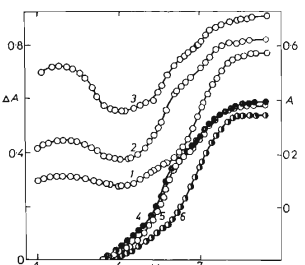

FIG. 5

Absorbance-pH Curves of Solutions of Zn(II) with PAR Containing Excess Ligand for the Wavelength 500 nm

$c_M = 6{\cdot}25 \cdot 10^{-6}$ mol l^{-1}, the other parameters as for Fig. 3; $c_L(10^{-5}$ mol $l^{-1})$ c_L/c_M: *1* 3·125 5, *2* 6·25 10, *3* 12·5 20, *4* 12·5 20, *5* 6·25 10, *6* 3·125 5; curves *1*—*3* $A = f(\mathrm{pH})$, curves 4—6 diffference curves $\Delta A = f(\mathrm{pH})$.

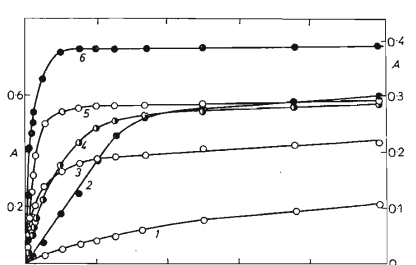

FIG. 6

Dependences of Absorbance of System Zn(II) with PAR at 495 nm on the Concentrations of the Components

l 10 mm, *t* 25°C; pH, c_M $(10^{-5}$ mol $l^{-1})$: *1* 3·75, 1·25; *2* 4·35, 1·25; pH, c_L $(10^{-5}$ mol $l^{-1})$; *3* 6·08, 2·5; *4* 3·75, 5·0; *5* 7·36, 2·5; *6* 4·51, 5·0; the right *A* axis refers to the curves *1*, *2*, *4*, *6*, the left *A* axis to the curves *3* and *5*.

The average values of the molar absorptivities of the various complexes and the equilibrium constants for the equilibria $(D)-(F)$ are summarized in Tables V and VI along with the results of processing of the curves by the program PRCEK T200.

In solutions with a lower concentration excess of the ligand ($c_L/c_M = 1$ and 5) the absorbance-pH curves indicate the formation of a mixture of the complexes ML_2H_2, ML_2H, and ML_2 in the whole region pH 5·5–9·5; as the pH increases, the complexes ML and ML_2H_2 prevail first, while at pH ~ 9 virtually only the complex ML_2 is present. The increase of absorbance of the solutions at pH 1–3 in equimolar solutions is due to the presence of the ML, MLH, and ML_2H_2 species mixture.

The absorbance was measured also in dependence on the concentrations of the components: the dependence on the ligand concentration, $A = f(c_L)$, was investigated with $6{\cdot}25 \cdot 10^{-6}$M-Zn(II) at pH 7·00 and 7·50 and with $c_M = 1{\cdot}25 \cdot 10^{-5}$M Zn(II) at pH 3·75 and 4·35; the dependence on the metal concentration, $A = f(c_M)$, was examined with $2{\cdot}5 \cdot 10^{-5}$M-PAR at pH 6·08 and 7·36 and with $5{\cdot}00 \cdot 10^{-5}$M-PAR at pH 3·75 and 4·51. The graphical as well as numerical interpretation of the curves evidenced the formation of the complex MLH according to the scheme (A) from the $A = f(c_M)$ curve for pH 3·75 and the formation of the complex ML according to the equilibrium

$$M + LH_2 \rightleftarrows ML + 2\,H \quad (*\beta_{112}) \qquad (G)$$

from the dependence $A = f(c_M)$ at pH 7·36. The dependences $A = f(c_L)$ at pH 7·50 to 3·75 did not lead to unique results.

The continual variations curves confirm the presence of a complex with the ratio M : L = 1 : 2 at pH ≧ 7·95 for $c_0 = c_M + c_L = 1{\cdot}0 \cdot 10^{-4}$ mol l^{-1} and $2{\cdot}5 \cdot 10^{-5}$ mol l^{-1}. The concave bending of the curves for pH ≦ 4·55 indicates the presence of the complexes ML and ML_2. The results of processing of some curves by the continual variations method employing the program[47–49] JOBCON are given in Table V.

An account of the complexes and their characteristic parameters together with the available published data is given in Table VII. Our conclusions agree with the results of works of Tanaka and coworkers[30,31], Corsini and coworkers[39], and Nickless and coworkers[36]. Some contradictory conclusions in the papers by Nickless and coworkers[36], Hniličková and Sommer[29], and Iwamoto[30] are probably due to an incorrect interpretation of the insufficient volume of experimental data or to the application of impure PAR preparations.

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