

ON METHYLENE BLUE AND ITS ZINC THIOCYANATE COMPLEX IN CONNECTION WITH A NEW SPECTROPHOTOMETRIC DETERMINATION OF ZINC

Isjrin Noerdin

Department of Chemistry

(Received June 11, 1962)

ICHTISAR

Djika seng direaksikan dengan biru metilena dan tiosianat dan diukur berkurangnya absorpsi, akan terdapat garis kalibrasi untuk suatu penentuan fotometris baru untuk seng, jang, djika dipenuhi sjarat² tertentu, tidak terpengaruhi oleh pH, "ionic strength", temperatur dan djuga tidak oleh selisih waktu jang tidak terlampau besar.

ABSTRACT

By reacting zinc with methylene blue and thiocyanate and measuring the decrease in absorbance, calibration curves for a photometric determination of zinc are found, which under suitable conditions are independent of the pH, the ionic strength, the temperature and also of small time differences.

INTRODUCTION

Presently used methods for the photometric determination of zinc in the microgram range are either complex (dithizone) (4, 15, 21), or, while simple, subject to interference by many foreign ions (zincon) (3, 17, 20).

A number of other methods have been proposed, but none has the sensitivity of either the commonly used dithizone method or the zincon method. The more important among these are those using oxine (21, p. 626), urobilin (26), resorcinol (25, 14, p.620) and, more recently, 1.10-phenanthroline (10) and $\alpha, \beta, \gamma, \delta$ tetraphenylporphine (1).

The discovery that various bivalent metals react with thiocyanate or halide ions and various dyes, among them methylene blue (MB), giving differently colored complexes (11) was used as the basis of a new photometric determination of zinc, in which the zinc is reacted with thiocyanate and MB, thus reducing the amount of MB, the resulting decrease in absorbance of MB corresponding to the amount of zinc present. To develop this method to its fullest possibilities, the factors influencing the absorbance of MB and necessary details on the complex itself were investigated, among these:

1. The influence of pH and electrolytes on the absorption spectrum of MB.
2. The absorption spectrum of MB in organic solvents.
3. The influence of dilution and adherence to Beer's law.
4. The reducing influence of thiocyanate on MB.
5. The composition, solubility product and absorption spectrum of the complex.
6. The kinetics of the reaction.

From the results the most advantageous condition for the photometric determination of zinc can be found.

EXPERIMENTAL DETAILS

Instrumentation. Measurements were made with the Beckman DU spectrophotometer, the Leeds and Northrup potentiometer type K-2, in which the regular galvanometer was replaced by a mirror type galvanometer, Fisher No. 11-506-27, M and with a Beckman pH meter model G, using the regular glass electrode (N.T.L.N. number 290), standardized against a saturated solution of potassium hydrogen tartrate (24, p.868).

Reagents. When available, Reagent Grade Chemicals were used, except when other grades were purposely taken. Buffers were prepared according to (8), except the oxalate buffers which were prepared according to (7).

Methylene blue. The purity of the three available samples (Merck) was determined volumetrically with titanous chloride (24, p.317) in a carbon dioxide atmosphere and concentrated stock solutions shown in Table I were prepared.

TABLE I
MB samples and stock solutions.

Code	Sample (Merck)	Purity as MB.HCl	Conc. mg/l	stock solution F × 10 ²
A	B extra	70.3%	75.2	1.65
B	Redox	70.5%	98.2	1.97
C	Konzentriert	74.9%	—	—

These concentrated stock solution were diluted as required.

Hydrochloric acid. "C.P." acid (sp.gr. 1.20) was redistilled from an all Pyrex glass apparatus with granulated lead as boiling chips and standardized by comparison with standard oxalic acid through sodium hydroxyde titrations and diluted as required.

Sodium tartrate and sodium hydrogen tartrate. Prepared in solution from calculated amounts of tartaric acid and sodium carbonate. Solid sodium hydrogen tartrate was prepared by mixing saturated solutions of "C.P." tartaric acid and "C.P." sodium tartrate, crystallizing, washing and drying the crystals at 50°C.

Sodium thiocyanate. Until it became available, sodium thioyanate (A) was prepared by mixing hot concentrated solutions of sodium nitrate and potassium thiocyanate in equimolar amounts. At 5°C the potassium nitrate crystallized out and was filtered off. The remaining potassium in solution was precipitated as the hydrogen tartrate with sodium hydrogen tartrate.

When it became available, sodium thiocyanate N.F. (B) was used.

The thiocyanate concentration was determined by titration with standard mercuric nitrate solution, using a ferric chloride indicator (9, p. 575).

Zinc solution. A stock solution was prepared from Reagent Grade zinc sulfate and the concentration (1.001 F) determined gravimetrically as zinc pyrophosphate (9). This was diluted as required.

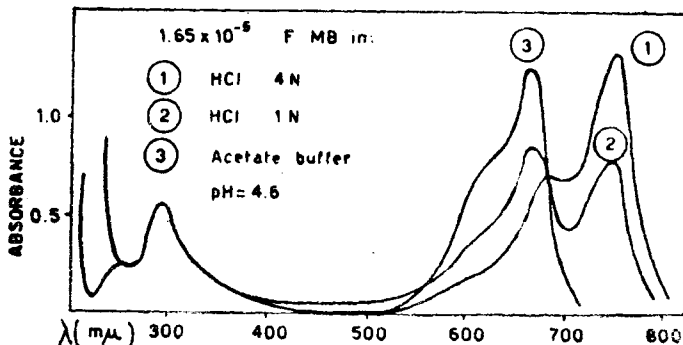
Redistilled water. Laboratory distilled water was redistilled from an all Pyrex glass apparatus, with addition of potassium permanganate and boiling chips of manganese metal.

I. THE INFLUENCE OF pH AND ELECTROLYTES ON THE ABSORPTION SPECTRUM OF METHYLENE BLUE

1. Introduction

Although the absorption spectrum of MB has already been determined (12), no details concerning the relation between spectrum and pH were given.

To investigate this and the effect of pH more fully, absorbance measurements over the range of 200 — 1000 $m\mu$ were made using 1.65×10^{-5} F MB (A) in 4 F and 1 F hydrochloric acid and in an acetate buffer of pH 4.6.

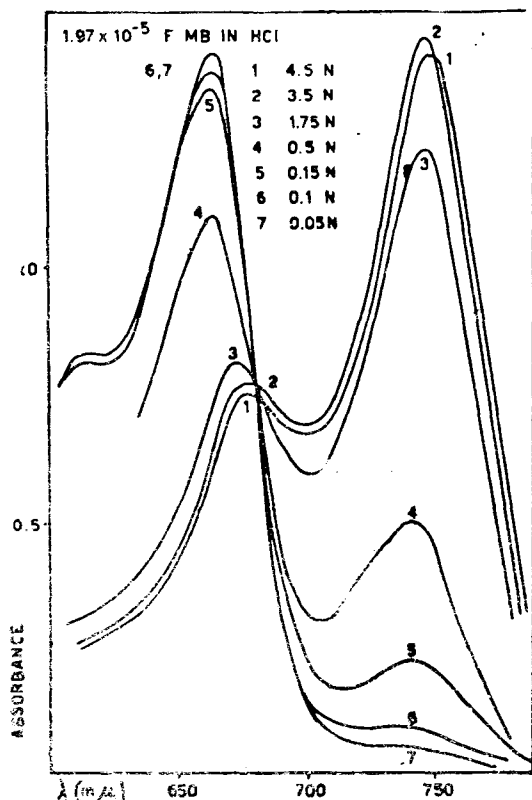


Graph 1. Absorption spectrum of MB.

The resulting spectra in Graph 1 show no peaks beyond 800 $m\mu$. However, between 200 and 800 $m\mu$ three pronounced maxima show in 1.0 F acid at 745, 666 and 290 $m\mu$. The peak at 290 $m\mu$ appears to be insensitive to pH but the absorbance at the peak at 666 $m\mu$ decreases from 1.2 in a buffer of pH 4.6 to 0.85 in 1.0 F acid and the peak at 745 $m\mu$ is reduced almost to zero at a pH of 4.6 from an absorbance of 1.3 in 4 F acid. Also, at pH 4.6 a shoulder is noticed between 610 and 630 $m\mu$, which is barely discernable in 4 F acid. The absorbance increases rapidly below 230 $m\mu$ (250 $m\mu$ in solutions of higher pH).

2. The influence of the pH on the absorption spectrum.

As the peak at 666 $m\mu$ and the shoulder at 625 $m\mu$ appeared to be the most useful, the spectral region between 600 and 800 $m\mu$ was investigated more thoroughly, using solutions of various acidities. The results compiled in

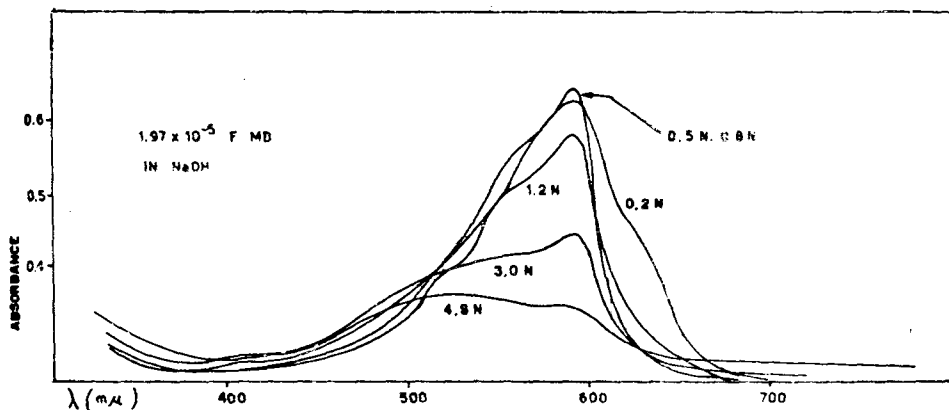
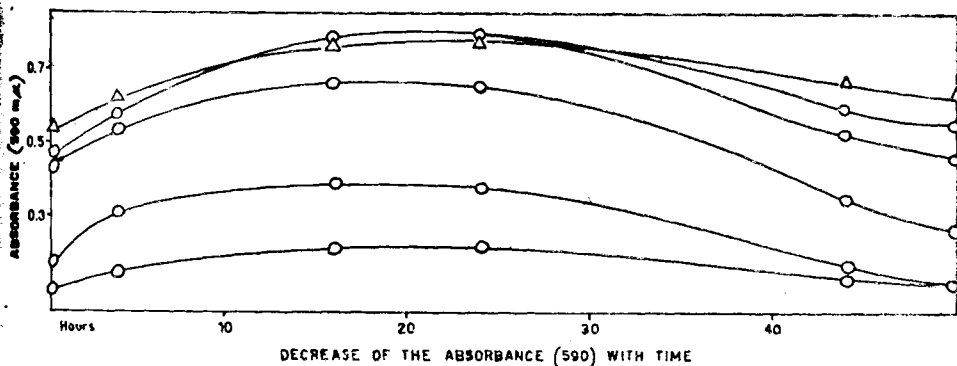


Graph 2. Absorption spectrum of MB in HCl.

Graph 2 show that MB is a pH indicator, its base form having an absorbance peak at 666 $m\mu$ and its acid form at 745 $m\mu$. It appears to be entirely in the base form at pH above 1.3 and entirely in the acid form at pH below -0.5.

The peak at 666 $m\mu$ not only decreases with increasing acid content, but also shifts to the longer wavelengths. The shoulder at 625 $m\mu$ seems to be related to the peak at 666 $m\mu$ insofar as it appears to decrease with the latter, this effect being hardly noticeable at low acidities, but increasing with increasing acidity.

Also were determined absorption spectra of 1.97×10^{-5} F MB (B) in solutions of sodium hydroxide of various concentrations. In these solutions at first the color of MB disappeared, gradually followed by a violet color with an absorption peak at $590 \text{ m}\mu$ which reached its greatest intensity after about 20 hours, after which it decreased again as shown in Graph 3.



Graph 3. Absorption spectrum of MB in NaOH.

3. Calculation of the pK_{Ind}

Graph 2 shows the MB to be entirely in its acid form at acidities above 3.5 F in hydrochloric acid and in both its acid and base form below this acidity.

Assuming that MB reacts with acids according to the equation :

$MB + n H^+ \rightleftharpoons MB^{n+}$, where MB is the base form and MB^{n+} the acid form,

then: $K_{Ind} = \frac{C_{MB}}{C_{MB^{n+}}} \frac{C_{H^+}^n}{C_{H^+}}$ and thus: $n \text{ pH} = \log \frac{C_{MB}}{C_{MB^{n+}}} + pK_{Ind}$

Assuming that with these small concentrations Beer's law is obeyed (or, that at least deviations may be neglected), this ratio $C_{MB}/C_{MB^{n+}}$ equals the ratio

of absorbancies, $\frac{A - A'}{A}$, where A is the absorbance at $C_{H^+} = 3.5$ and A' is the absorbance at that particular acidity.

Results calculated for two samples of MB are given in Table II, where also is given $\log \frac{A - A'}{A} = Q$.

TABLE II.

Relative amounts of the acid and base form, expressed as

$$Q = \log \frac{C_{MB}}{C_{MB^{n+}}} = \log \frac{A - A'}{A}, \text{ at various acidities.}$$

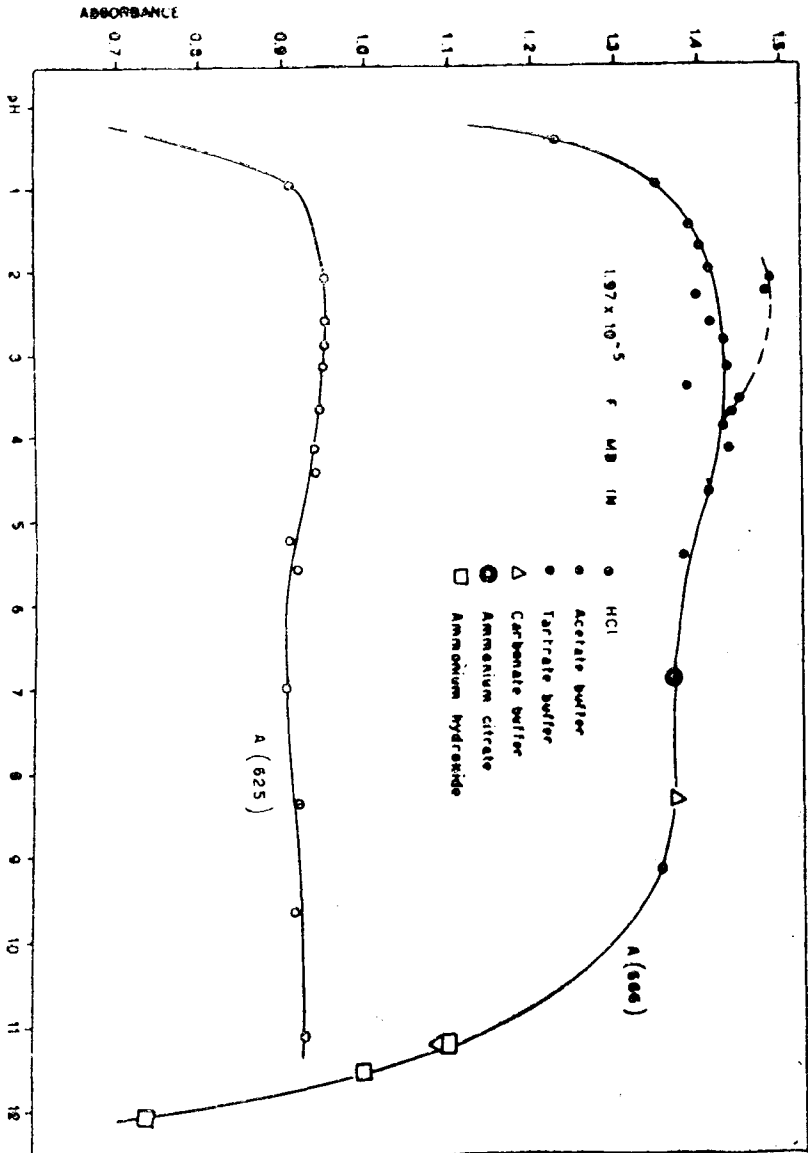
$1.65 \times 10^{-5} \text{ F MB (A)}$				$1.97 \times 10^{-5} \text{ F MB (B)}$			
C_{H^+}	p_{CH}	A	Q	C_{H^+}	p_{CH}	A	Q
5.20	-0.72	1.237	—	4.50	-0.65	1.450	—
3.70	-0.57	1.223	—	2.50	-0.40	1.250	-1.13
2.23	-0.35	1.116	-0.965	1.75	-0.24	1.350	-0.80
1.04	-0.02	0.830	-0.308	1.00	0.00	0.925	-0.25
0.74	+0.13	0.622	-0.005	0.50	0.30	0.510	+0.26
0.30	+0.53	0.250	+0.612	0.25	0.60	0.225	+0.74

Substitution of the values given in Table II in the equation:

$$n \text{ pH} = Q + pK_{Ind},$$

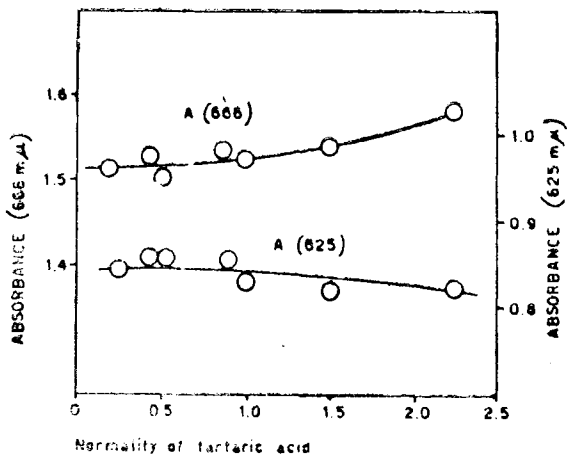
shows that $n = 2$ and $pK_{Ind} = 0.28$.

The absorbancies at the two wavelengths $666 \text{ m}\mu$ and $625 \text{ m}\mu$, being the most useful ones, were investigated over the whole pH range. The resulting pH-absorbance curves at $666 \text{ m}\mu$ and $625 \text{ m}\mu$ shown in Graph 4 have the same general shape, each showing a maximum between pH 3.0 and 3.5, a slow decrease between pH 4 and 6, a level part between pH 6 and 9 and a sharp decrease below pH 1.5 and above pH 9.



Graph 4. Influence of pH on the absorbance of MB.

Tartaric acid also influences the absorbance, in concentrated solutions it markedly increases the absorbance at 666 mμ, although the absorbance at 625 mμ decreases, as shown in Graph 5.



Graph 5. Influence of tartaric acid on the absorbance of MB.

Between a 0.2 — 1 F tartaric acid concentration the change in absorbance is negligibly small.

4. Influence of foreign electrolytes

The salt errors caused by KCl and KNO₃ have been investigated by G.N. Lewis (12) who reported a decrease of 5% in absorbance at 666 mμ in 1 F salt solutions. To obtain more detailed information, the absorbancies at 666 mμ of solutions of 1.97×10^{-5} F MB (B) in a 0.1 F hydrochloric acid and in a 1 F acetate buffer, pH 4.7, with varying amounts of foreign ions, were measured. Results in Table III show that the salt error is much pronounced in 0.1 F HCl.

TABLE III

Salt error of a 1.97×10^{-5} F solution of MB (B), expressed as a difference in absorbance at 666 mμ, ΔA , in:

0.1 F HCl				a 1 F acetate buffer, pH 4.7			
Foreign electrolyte	μ	A_{666}	ΔA	Foreign electrolyte	μ	A_{666}	ΔA
None	0.1	1.365		None	1.0	1.388	
NaCl	0.2 F	0.3	-0.104	NaCl	0.1 F	1.1	-0.010
	0.5 F	0.6	-0.231		0.3 F	1.3	-0.016
KNO ₃	0.2 F	0.3	-0.072		0.5 F	1.5	-0.012
	0.5 F	0.6	-0.198				
NH ₄ Cl	0.4 F	0.5	-0.140				
	1.2 F	1.3	-0.255				

TABLE IV

Salt error in tartrate buffers, pH = 2.8, with varying concentrations of MB and total tartrate content. Expressed as ΔA at 666 and 625 m μ .

MB	tartrate	NaCl	A(666)	$\Delta A(666)$	A(625)	$\Delta A(625)$
1.65×10^{-5} F	0.37 F	0.0 F	1.209		0.708	
—, —	—, —	0.1 F	1.207	-0.002	0.706	-0.002
—, —	—, —	0.2 F	1.201	-0.008	0.699	-0.009
—, —	—, —	0.4 F	1.155	-0.054	0.695	-0.014
—, —	—, —	0.8 F	1.085	-0.124	0.124	-0.032
—, —	0.22 F	0.0 F	1.184		0.680	
—, —	—, —	0.1 F	1.179	-0.005	0.678	-0.002
—, —	—, —	0.2 F	1.168	-0.016	0.680	-0.000
—, —	—, —	0.4 F	1.142	-0.037	0.688	+0.008
—, —	—, —	0.8 F	1.055	-0.129	0.695	+0.017
6.6×10^{-2} F	0.37 F	0.0 F	0.511		0.274	
—, —	—, —	0.1 F	0.506	-0.005	0.275	+0.002
—, —	—, —	0.2 F	0.506	-0.005	0.275	+0.002
—, —	—, —	0.4 F	0.496	-0.015	0.268	-0.007
—, —	—, —	0.8 F	0.493	-0.018	0.273	-0.002
—, —	0.22 F	0.0 F	0.505		0.276	
—, —	—, —	0.1 F	0.505	-0.000	0.276	0.000
—, —	—, —	0.2 F	0.495	-0.010	0.280	+0.004
—, —	—, —	0.4 F	0.495	-0.010	0.280	+0.004
—, —	—, —	0.8 F	0.492	-0.013	0.288	+0.012
13.2×10^{-5} F	0.37 F	0.0 F	0.967	-0.967	0.553	
—, —	—, —	0.1 F	0.693	-0.004	0.550	-0.003
—, —	—, —	0.2 F	0.959	-0.008	0.565	+0.012
—, —	—, —	0.4 F	0.939	-0.028	0.562	+0.009
—, —	—, —	0.8 F	0.298	-0.039	0.562	+0.009
—, —	0.22 F	0.0 F	0.963		0.567	
—, —	—, —	0.1 F	0.959	-0.004	0.567	0.000
—, —	—, —	0.2 F	0.943	-0.020	0.569	+0.002
—, —	—, —	0.4 F	0.932	-0.030	0.553	-0.014
—, —	—, —	0.8 F	0.932	-0.030	0.553	-0.014

than in the acetate buffer, in which it is practically negligible. This may be due to the pH, but also to the fact that the initial μ in the acetate buffer is much higher, thus the small variations caused by the foreign electrolyte have hardly any effect.

Also was investigated the influence of varying the amounts of MB, buffer and electrolyte content, using a 1.65×10^{-5} F solution of MB (B) in tartrate buffers at pH 2.8, giving the results in Table IV.

The influence of different electrolytes was investigated, using a 1.65×10^{-5} F solution of MB (B) and an oxalate buffer, composed of 0.15 F oxalic acid, 0.1 F sodium hydrogen oxalate and 0.5 F tartaric acid; results are compiled in Table V.

These tables show, that the salt error increases with the ionic strength μ , increases with MB content, decreases with tartrate content and that with these buffers the salt error caused by 0.2 F sodium chloride may be neglected.

TABLE V

Salt error, expressed as the difference in absorbance, ΔA , at 666 m μ , with varying electrolytes and 1.65×10^{-5} F MB (B) in an oxalate buffer, pH 1.5.

Absorbance without foreign electrolyte = 1.073.

F for. electr.	KNO ₃		KCl		NaCl	
	A	ΔA	A	ΔA	A	ΔA
0.1	1.064	-0.009	1.080	+0.007	1.081	+0.008
0.2	1.067	-0.006	1.070	-0.001	1.082	+0.009
0.3	1.077	+0.003	1.065	-0.005	1.074	+0.001
0.4	1.053	-0.020	1.058	-0.018	1.069	-0.004
0.5	1.043	-0.030	1.049	-0.025	1.051	-0.022

5. Conclusion.

If MB is to be used in a photometric determination of zinc, conditions must be found where the absorbance of MB is as constant as possible.

From the foregoing it can be concluded that:

- the solution must be buffered, preferably between pH 3.0 and 3.5 or 6 and 9, but if necessary, the whole pH range 1.5 and 9 may be used, where the absorbance of MB varies only slightly with the pH.
- the final tartrate content should be at least 0.2 F.
- the amount of foreign electrolyte should be kept below an ionic strength of 0.2.

II. THE ABSORPTION SPECTRUM OF MB IN ORGANIC SOLVENTS.

1. Introduction

Lewis and co-workers (13) found that in solvents with low dielectric constant like glycerol and ethanol, MB is present as its monomer, the resulting high absorbance of which may well increase the sensitivity. In a zinc determination, an extraction procedure, free from reducing action of thiocyanate, salt errors and the influences of pH and MB concentration might be advantageous and consequently an incentive to investigate other solvents more extensively.

2. Solubility and extractability of MB and the complex

The solubility and extractability of both MB and the complex were determined in 0.1 F hydrochloric acid and in a buffer composed of 0.18 F tartaric acid and 0.18 F sodium hydrogen tartrate. Results compiled in Table VI show that the one promising solvent for the complex, methylsalicylate, unfortunately also extracts uncombined MB in the presence of thiocyanate, although not in its absence. Thus, as no solvents with the right properties were available, the extraction procedure was not feasible.

However, an organic solvent might still be useful; a miscible solvent might increase the sensitivity; an immiscible one might be used to advantage to extract the MB remaining after separation of the complex, thus increasing the sensitivity.

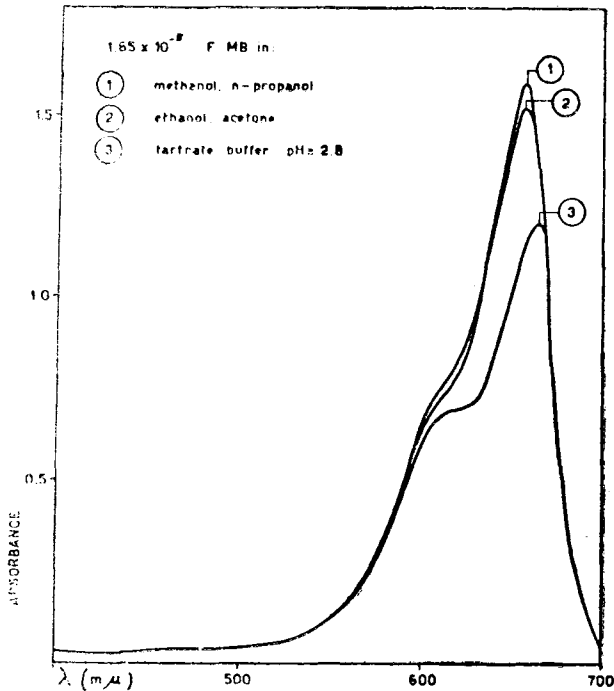
3. Absorption spectrum in miscible solvents

The spectrum of MB in several alcohols and in acetone were investigated with the results shown in Graph 6.

Maximum absorbance occurred at a lower wavelength in the organic solvent and the shoulder at 625 $m\mu$ had almost disappeared. To know the influence of water, several solutions, each 1.65×10^{-5} F in MB (A) were prepared in varying mixtures of water and organic solvents and the absorbance between 650 $m\mu$ and 700 $m\mu$ measured. The results compiled in Table VII show that the peak at 666 $m\mu$ increases with decreasing water content and at the same time shifts to the shorter wavelength, but the increase is only slight, amounting to a few % in a mixture containing 50% organic solvent.

Table VI.
Behaviour of MB and the complex in various organic solvents.

	Solvent	MB (B), 7.52 mg/liter in tartr. buf. pH 2.8		Complex equivalent to 7.52 mg MB per liter in tartrate buffer, pH 2.8	
			0.1 F HCl		0.1 F HCl
Miscible	Acetone	bl. soln	bl. soln.	decompd. bl. soln.	decompd. bl. soln.
	Ethanol	—, —	—, —	—, —	—, —
	Methanol	—, —	—, —	purpl. ppt. light nl. soln.	purpl. ppt. colorless soln.
	Allyl alcohol	—, —	—, —	decompd. bl. soln	brownish soln.
	n-Propyl alcohol	—, —	—, —	—, —	decompd. bl. soln.
	Glycol	—, —	—, —	purpl (ish) ppt.	purplish ppt.
	Ethylene glycol	—, —	—, —	light blue soln + purpl. ppt.	light purpl. ppt.
	Diethylene glycol	—, —	—, —	white ppt.	—, —
	Immiscible	Diethylether	not extd.	extd.	plate out
Di isopropylether		—, —	—, —	—, —	—, —
Petroleum ether 60		—, —	—, —	—, —	—, —
Benzene		—, —	—, —	—, —	—, —
Toluene		—, —	—, —	—, —	—, —
Xylene		—, —	—, —	—, —	—, —
Chloroform		—, —	—, —	—, —	—, —
Carbontetrachloride		—, —	—, —	—, —	—, —
Ethylenebromide		—, —	—, —	—, —	—, —
Trichloro ethylene		—, —	—, —	—, —	—, —
Carbondisulfide		—, —	—, —	—, —	—, —
n-Amyl alcohol		partly extd.	partly extd.	—, —	plate out, partly decompd.
Amylenehydrate		—, —	—, —	—, —	plate out
n-Butyl alcohol		largely extd.	largely extd.	plate out, partly decompd.	plate out, partly decompd.
Benzyl alcohol		compl. extd.	decol.	compl. decompd. extd.	compl. decompd. decol.
Propionic acid		—, —	—, —	—, —	—, — extd.
Nitrobenzene		—, —	—, —	—, —	—, —
Benzaldehyde		decol	—, —	—, —	—, —
Amylacetate		not extd.	not extd.	plate out	plate out, partly decompd.
Ethylacetate		—, —	—, —	largely extd. partly plate out	
Methylsalicytate	—, —	—, —	compl. extd. to a bl. soln.		
Paraldehyde	—, —	—, —	partly plate out, largely extd.		



Graph 6. Absorption spectrum of MB in organic solvents.

4. Absorption spectrum in immiscible solvents

Table VI shows that MB is extracted by nitrobenzene, propionic acid, benzylalcohol and benzaldehyde, the latter two at the same time gradually decolorizing the MB, thus being useless. The absorption spectrum (solvent as blank) was practically the same as for miscible solvents, as shown in the same Graph 6.

Benzylalcohol and benzaldehyde reduced MB and propionic acid is lighter than water, so only nitrobenzene was investigated. Further, as this showed the greatest possibility as an extracting solvent, the distribution coefficient of MB between nitrobenzene and water was determined.

Solutions of MB at various concentrations were prepared, each of which contained the same amount of MB, each of which contained the same amount of MB, by adding varying amounts water to 5.0 ml 1.65×10^{-5} F MB (A). These aqueous solutions were extracted with 5.0 ml nitrobenzene and the absorbance (666 mμ) determined.

TABLE VII

Influence of water on the absorption spectra of 1.65×10^{-5} F MB in organic solvents containing V ml organic solvent per 10 ml solution.

		Absorbance						
		$\lambda(\text{m}\mu)$	V = 9	V = 7	V = 5	V = 3	V = 2	V = 1
n-propenol	700		0.073	0.076	0.085	0.092	0.118	0.122
	675		0.796	0.900	0.983	1.076	1.116	1.126
	665		1.351	1.391	1.387	1.409	1.377	1.345
	660		1.538	1.524	1.467	1.420	1.366	1.282
	665		1.511	1.471	1.397	1.373	1.307	1.221
	650		1.408	1.399	1.270	1.244	1.193	1.123
acetone	700		0.007	0.068	0.094	0.111	0.118	0.116
	675		0.790	0.993	1.041	1.119	1.154	1.111
	665		1.440	1.471	1.472	1.400	1.398	1.364
	660		1.553	1.539	1.498	1.442	1.405	1.346
	655		1.551	1.535	1.462	1.357	1.348	1.294
	650		1.440	1.409	1.330	1.277	1.235	1.190
ethanol	700		0.060	0.071	0.090	0.111	0.118	0.110
	675		0.796	0.903	1.036	1.130	1.112	0.983
	665		1.275	1.399	1.369	1.402	13.28	1.250
	660		1.438	1.431	1.414	1.368	1.291	1.207
	655		1.467	1.452	1.282	1.257	1.229	1.152
	650		1.346	1.329	1.292	1.184	1.102	1.051

Assume that the C_{MB} in the 5 ml nitrobenzene = C and the residual C_{MB} in aqueous solution (volume = V ml), = C'. Assume the molar absorptivity of MB in nitrobenzene = e and the absorbance measured = A. Thus, in the regular 1 cm Beckman cell: $A = e \times C$,

and the amount of MB = $5 \times C = 5 \times \frac{A}{e}$ Mole.

Part of the MB is left in the V ml water. The *total* amount of MB present can be found by extrapolating V to 0 ml, the resulting A is the absorbance,

if all of the MB is present in the nitrobenzene. From Table VIII this is found to be 1.720, giving a total amount of MB = $5 \times \frac{1.720}{e}$ mole and an amount of

MB in water = $5 \times \frac{1.720 - A}{e}$ mole, and as the volume = V ml, the

concentration of MB in water = $C' = \frac{5 \times (1.720 - A)}{e \times V}$ F.

This gives $K_{\text{distr.}} = C/C' = \frac{V \times A}{5 \times (1.720 - A)}$.

Results compiled in Table VIII show, that, except for the first value, agreement is satisfactory and $K_{\text{distr.}} = \text{appr. } 49$, which is large enough for an extraction procedure if desired.

TABLE VIII

Absorbance A of MB extracted by 5 ml nitrobenzene from V ml water containing $5 \times 1,65 \times 10^{-7}$ mole MB (A).

V	A	1.720 - A	$K_{\text{distr.}}$
5	1.680	0.040	42.0
10	1.656	0.064	51.8
20	1.588	0.132	48.1
30	1.541	0.179	51.5
40	1.419	0.254	46.8
50	1.419	0.301	47.2
			Mean: 49.

5. Conclusion

The use of a miscible solvent will be of little value, as it raises the absorbance only a few % and considering the extra trouble and risk to use another reagent, should be dissuaded.

An immiscible solvent could be useful, but the advantage is still questionable.

III. ADHERENCE TO BEER'S LAW

1. Introduction

MB does not obey Beer's law (2, 12, 13, 16, 18), but the problem is, to what extent those deviations will be perceptible in an actual determination when regular 1 cm Beckman cells are used.

2. Organic solvents

Calculated amounts of a 3% solution of MB (A) in water were weighed out, and with miscible solvents these were diluted with the solvent to 50 ml, with an immiscible solvent these were extracted with a single 50 ml portion of solvent. Extraction was practically complete. The resulting were diluted as required and the absorbance determined against the solvent as blank between 650 m μ and 700 m μ . The maximum occurred at about 658 m μ .

TABLE IX

The molar absorptivity $\times 10^4$ of MB at the maximum in various solvents.

F MB $\times 10^6$	1	2	3	4	5	6	7	8	9
3.3	9.58	10.0	10.0	9.27	10.8	8.46	7.73	7.61	8.06
6.6	9.25	9.77	9.85	9.25	10.8	8.35	7.63	7.40	8.34
9.9	9.27	9.70	9.70	9.24	10.3	8.39	8.00	7.68	8.40
13.2	9.24	9.70	9.67	9.24	10.3	8.40	7.99	7.64	7.64
16.5	9.30	9.69	9.68	9.30	10.2	8.21	7.96	7.65	8.22
1 = ethanol 4 = acetone 7 = acetate buffer 2 = methanol 5 = nitrobenzene 8 = 0.1 F hydrochloric acid 3 = n-propanol 6 = tartrate buffer 9 = oxalate buffer.									

The results in Table IX show, that Beer's law practically obeyed in a concentration range up to 1.65×10^{-5} F MB.

3. Aqueous solutions.

From the stock solution MB (A) solutions of varying concentrations were obtained in water, 0.1 F hydrochloric acid and in the following buffers: an acetate buffer of pH 4.7, a 0.18 F hydrogen tartrate buffer of pH 2.8 and an oxalate buffer composed of 0.15 F oxalic acid + 0.1 F sodium hydrogen oxalate + 0.5 F tartaric acid, pH 1.5.

The results are also compiled in Table IX, showing that when the regular 1 cm Beckman cells are used, deviations from Beer's law may be neglected in a concentration range from 3.3 to 16.5×10^{-6} F MB. However, for an extremely diluted solution, 3.3×10^{-7} F in MB, where absorbance was measured in a 15 cm long Lumetron cell, a molar absorptivity of 94000 was found using tartrate buffers. Compared with the values in column 6 of Table IX this shows definitely a deviation from Beer's law.

4. Conclusion

Although the molar absorptivity in organic solvents is larger than in water, the increase is not too great, and consequently the sensitivity will not be increased appreciably by organic solvents.

When regular Beckman cells are used, Beer's law may be assumed to be obeyed.

IV. THE REDUCING INFLUENCE OF THIOCYANATE ON METHYLENE BLUE

1. Introduction

MB is gradually bleached by thiocyanate in dilute hydrochloric acid. The potentials for the MB and thiocyanate systems show that in strongly alkaline solutions MB will be reduced by thiocyanate, but that in acidic solutions reduction of MB by thiocyanate is highly improbable (5) and that bleaching of MB is apparently caused by decomposition products of thiocyanic acid. As thiocyanic acid is commonly regarded as a fairly strong acid (5, 19, 22), the undissociated acid will be present only at a sufficiently low pH. In view of these facts the reagents will be most stable in a weakly acid medium. This was checked experimentally as follows: to 1 ml 1.65×10^{-4} F MB (A) pipetted into a 10 ml volumetric flask, was added the required amount of buffer (or acid) solution and redistilled water to a volume of less than 9 ml. When the remaining reagent, NaCNS, was added (1.0 ml of a 3.8 F solution), the stopwatch was started. The solution was then rapidly diluted to the mark and transferred to the Beckman cell and the absorbance read at $\lambda = 666 \text{ m}\mu$ and slit = 0.03 mm.

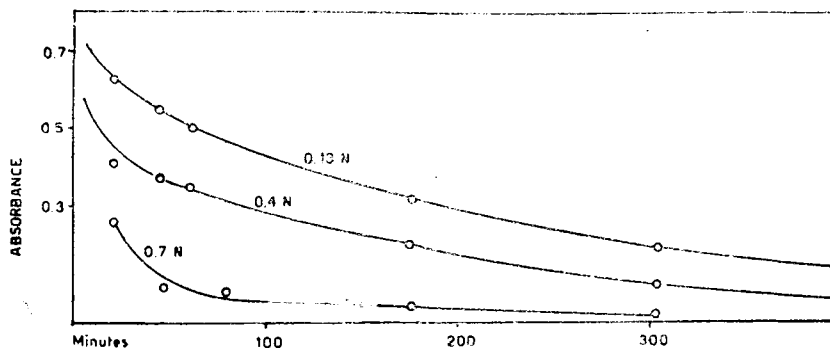
One difficulty was the inevitable contamination by small amounts of zinc, which resulted in an initial decrease of absorbance, thus the influence of the pH on the stability of the MB- thiocyanate mixture was indicated by the gradual decrease of absorbance, *after* the initial drop in the curve of absorbance vs time, which was caused by the zinc impurity.

2. In strongly mineral acid solutions

The samples in 6 F, 3.6 F and 1.2 F hydrochloric acid became almost colorless before they could be transferred to the cells; both peaks at $666 \text{ m}\mu$ and at $745 \text{ m}\mu$ had practically vanished. Apparently reducing action was very fast.

3. In dilute mineral acid solutions

With samples more dilute in hydrochloric acid, resp. 0.7 F, 0.4 F and 0.13 F, the bleaching was much slower and absorbance measurements could be made easily. The sample 0.7 F in acid was decolorized much faster than the other samples, where the decrease in absorbance could be followed easily over a period of five hours, as shown in Graph 7.



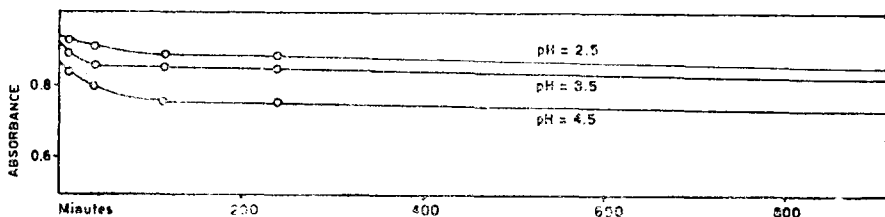
Graph 7. Bleaching of MB by CNS^- in HCl.

4. In weakly acid and buffered solutions

Nine samples were prepared in solutions with pH, varying as shown below.

- a. 2 ml 1 F HAc, pH 2.5
- b. 2.5 ml 1 F HAc + 2.5 ml 1 F NaAc, pH 4.5
- c. 0.5 ml 1 F HAc + 5.0 ml 1 F NaAc, pH 5.5
- d. 5.0 ml 1 F HAc + 0.5 ml 1 F NaAc, pH 3.5
- e. 5 ml saturated potassium hydrogen tartrate, pH 3.5
- f. 5.0 ml 1 F ammonium acetate, pH 7.
- g. 5.0 ml 1 F ammonium chloride, pH 4.5
- h. 5.0 ml 1 F monosodium citrate pH 4.
- i. 5.0 ml 1 F disodium citrate pH 5.

The pH was checked with indicator paper, as exact values were not required. The results are practically the same in all cases and in Graph 8 only representative curves are given because the omitted curves are so similar that their inclusion would serve no purpose. After different initial decreases, caused by differing amounts of zinc contamination, the gradual decrease is practically the same and can be neglected over a period of 1 or 2 hours, thus proving the advantage of low acidities, in the pH range of 2 to 7.

Graph 8. Bleaching of MB by CNS^- in acetate buffers.

5. Influence of elevated temperatures

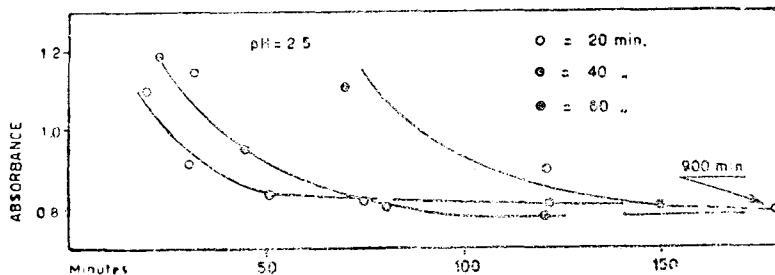
In dilute hydrochloric acid heating caused an almost immediate bleaching of MB, but in weakly acid medium the mixture of MB and thiocyanate proved to be sufficiently stable. Two problems had to be investigated:

1. Does the temperature decrease the stability of MB in thiocyanate. If so, the horizontal part of the curve in Graph 8 will be much lower.
2. Does the temperature increase the reaction. If so, the horizontal part of the curve in Graph 8 will appear much earlier.

As this is not a kinetic problem, a regulated bath is not necessary.

To maintain a reasonably constant temperature, the samples in the volumetric flasks were placed in a 1 liter beaker of water at the desired temperature. This was placed in an empty larger beaker. The temperature dropped from 71° to 64° C during the reaction.

The first three experiments of the preceding section 4 were repeated at these higher temperatures. After intervals of 20, 40 and 60 minutes part of each solution was transferred to Beckman cells and the absorbance measured. The results resembled each other and that at pH 2.5 is given in Graph 9.



Graph 9. Influence of temperature.

This shows, that initially, when the sample was still hot, the absorbance was much higher than in the experiments in the preceding section, but decreased as the solution cooled and finally leveled off, resembling the curve in Graph 8. Apparently at higher temperatures the complex is not

formed, probably being appreciably soluble, as proved by the fact that the initial decrease in absorbance of MB is not observed. Thus increasing the temperature will be useless to speed up the reaction. It is also significant, that the stability of the MB- thiocyanate mixture is not effected by a higher temperature at these pH's, contrary to the results observed with solutions in dilute mineral acids.

6. Mechanism of the reduction

Reduction of MB by the thiocyanate ion itself is highly improbable, as in this case raising the temperature should accelerate the bleaching even at higher pH's, while the results prove the reverse.

If the bleaching of MB is caused by decomposition products of free thiocyanic acid, then raising the acidity should accelerate it, as more free acid is produced. This is indeed shown in section 2. Moreover, raising the temperature in dilute mineral acid solutions will accelerate the bleaching: this was proved by the complete disappearance of the color within 10 minutes in dilute hydrochloric acid solutions at about 100°C. And raising the temperature in weakly acid medium will have no influence on the color, as no free thiocyanic acid can be present; this fact was proved in Section 5.

7. Conclusion

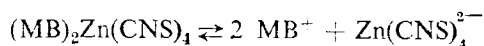
The bleaching of MB is caused by the decomposition products of free thiocyanic acid which is formed only at sufficiently low pH. Consequently, the color of MB is sufficiently stable in weakly acid solutions, but not so in dilute mineral acids.

Raising the temperature will not accelerate the bleaching of MB, but at the same time prohibits the formation of the zinc- MB- thiocyanate complex.

V. COMPOSITION AND SOME PHYSICAL PROPERTIES OF THE COMPLEX

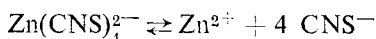
1. Introduction

The complex probably consists of the MB cation and the tetrahedral $Zn(CNS)_4^{2-}$ anion. Suppose for the sake of convenience the formula is $(MB)_2 Zn(CNS)_4$. This should dissociate thus:



with $K_{sp} = C_{MB^+}^2 \times C_{Zn(CNS)_4^{2-}}$.

Assume for the tetra thiocyanate zincate ion that:



with the corresponding $K_{\text{instab.}}$.

The formation of $\text{Zn}(\text{CNS})_4^{2-}$ proceeds in stages from $\text{Zn}(\text{CNS})^+$ to $\text{Zn}(\text{CNS})_2$, but for the complete conversion the $K_{\text{instab}} = K_1 \times K_2 \times K_3 \times K_4$.

Although a mixture of Zn^{2+} and excess CNS^- will contain the various complexes, this relation will hold:

$$K_{\text{instab}} = \frac{C_{\text{Zn}^{2+}} \cdot C_{\text{CNS}^-}^4}{C_{\text{Zn}(\text{CNS})_4^{2-}}}$$

Multiplying by K_{sp} gives:

$$K = K_{\text{sp}} \times K_{\text{instab}} = C_{\text{MB}^+}^2 \times C_{\text{Zn}^{2+}} \times C_{\text{CNS}^-}^4,$$

where $C_{\text{Zn}^{2+}}$ and C_{CNS^-} are the actual concentrations present *as such*, and not the concentration added. However, as K_{instab} is not *very small* (6), so that the complex is not very stable, these actual concentrations will practically equal the concentrations added. Then the relation holds:

$$pK = 2 \text{ pMB} + p\text{Zn} + 4 \text{ pCNS},$$

where the concentrations are the concentrations *added*.

Varying the concentrations of the three reactants will give the lowest limit at which reaction occurs, as indicated by a sharp decrease in absorbance, which can be detected photometrically as well as visually.

If C_{MB^+} is kept constant and $C_{\text{Zn}^{2+}}$ and C_{CNS^-} varied until reaction takes place, then: $p\text{Zn} + 4 \text{ pCNS} = \text{constant}$.

Similarly if $C_{\text{Zn}^{2+}}$ is kept constant and C_{MB^+} and C_{CNS^-} varied until reaction takes place, then: $\text{pMB} + 2\text{pCNS} = \text{constant}$.

Reaction rates will be of no concern, provided enough time is given for the reaction to reach equilibrium.

2. Experimental results

Various sets of solutions were prepared, buffered with 0.16 tartaric acid + 0.18 F sodium hydrogen tartrate. Each set contained a fixed amount of zinc and MB, to which varying amounts of thiocyanate were added. The upper and lower limits of the thiocyanate concentration were found in an orientating experiment by adding widely spaced amounts of thiocyanate. Visual observations located the concentrations between which that of thiocyanate should lie as denoted by an unchanged color and a complete decoloration. The same set was then prepared, but with amounts of thiocyanate spaced at intervals of 0.1 ml. The absorbancies of these solutions were measured after 75 minutes.

It was found that after a certain volume of thiocyanate the transmittance increased suddenly and that the change was so abrupt that it could be noted unambiguously even visually: the set of test-tubes showed a row that was almost identical in color, followed by a row much lighter-and decreasing-in color.

Results are compiled in Table X, where the values of the volumes of thiocyanates are the means of the *last* volume not reacting and the *first* volume reacting. These results prove, that with a constant C_{MB} , the $C_{CNS^-} : C_{Zn^{+2}} = 4 : 1$ and with a constant $C_{Zn^{+2}}$ the $C_{MB} : C_{CNS^-} = 1 : 2$, thus proving the formula $MB_2Zn(CNS)_4$ to be correct.

Substitution of the values found in: $pK = 2 pMB + pZn + 4 pCNS$ will give the value for pK (last column in Table X).

TABLE X
Calculation of the pK.

	pMB	pZn	pCNS	pK
photom.	4.78	3.00	1.83	20.4
		2.00	2.06	19.9
		3.00	1.73	19.5
		2.00	2.03	19.6
		4.00	1.56	19.8
visual	4.78	2.00	2.04	19.7
		2.30	1.94	19.5
		2.61	1.89	19.7
		3.00	1.71	19.4
		3.30	1.67	19.4
photom.	4.78	3.00	1.73	19.5
			1.83	20.4
visual	4.78	3.00	1.71	19.4
			1.59	19.7
			1.99	19.3
			1.79	19.4
			1.95	19.5
			1.85	19.4

The mean value of $pK = 19.6$ and agreement is satisfactory. From the values found for K_{instab} (6, 23), then

$$pK_{sp} = pK - pK_{instab} = \text{approx. } 18.$$

This shows, that K_{SP} is only *moderately small*, so that precipitation will start only at moderately low concentrations, but the zinc can still be precipitated quantitatively if the reagent solutions already contain enough zinc to react with the MB and thiocyanate.

3. Influence of temperature

The variation of K with temperature was determined, using the visual method as described in preceding Section 2, except that the test-tubes were placed in a constant temperature bath. Results compiled in Table XI show the pK to be temperature dependent, but even at 0°C the decrease in K is not enough to increase the sensitivity to such an extent as to justify the additional inconvenience of using an ice bath.

TABLE XI
Influence of temperature on the pK

$t^\circ\text{C}$	pZn	pMB	$pCNS$	pK
$0^\circ \pm 0.1$	3.00	4.78	2.25	20.58
$40^\circ \pm 0.2$	3.00	4.78	1.85	18.95
$55^\circ \pm 0.2$	3.00	4.78	1.70	18.36
$70^\circ \pm 0.2$	3.00	4.78	1.48	17.50
$96^\circ \pm 0.5$	3.00	4.78	1.10	15.97

However, the variation in pK is pronounced enough, that it may be expected that the temperature will effect the accuracy of a determination of zinc based on its complex formation with MB and thiocyanate.

4. Some physical properties of the complex

The complex appeared in dilute solutions as an extremely fine precipitate, clearly visible as an opalescence. When left in the reaction vessel a part was "plated out" on the walls, as a barely visible purplish film. This was more pronounced in the corners as observed when the Beckman cells were soaked in a detergent: the complex decomposed again into MB and the blue color of MB was distinctly deeper in the corners.

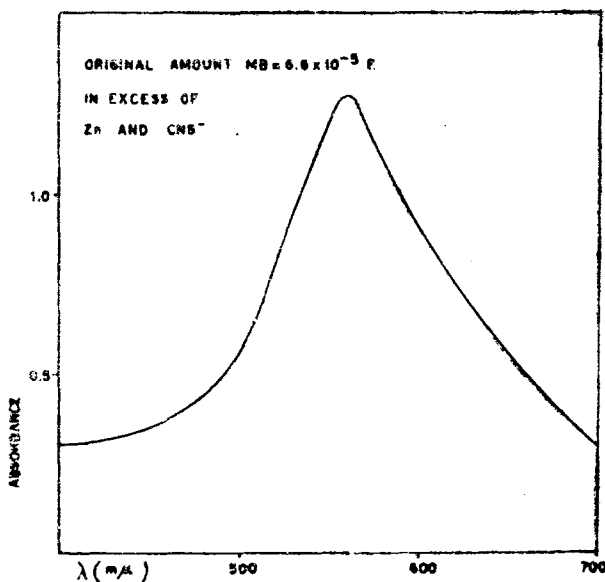
The film was fairly adherent, and the cells had to be scrubbed clean.

Warming the solution caused the complex to coagulate as larger purplish flocks with a density apparently close to that of the solution, as centrifuging with a regular semimicro centrifuge could not separate the flocks from the solution. The complex could be filtered easily, using paper, a cotton plug or a glass wool plug.

5. Absorption spectrum of the complex

A difficulty of this determination was the gradual settling of the precipitate. However, a stable colloidal suspension was obtained with addition of gelatin. Solutions were prepared, containing 0.18 F tartaric acid + 0.18 F sodium hydrogen tartrate, 0.5% gelatine, thiocyanate, zinc (in excess) and MB in varying concentrations.

The absorption spectrum in Graph 10 shows a peak at 560 m μ .



Graph 10. Absorption spectrum of the complex.

The absorbancies of the various solutions at 560 m μ showed a good agreement with Beer's law (Table XII).

These suspensions are quite stable as shown by measurements made at regular intervals and even after standing overnight. Results given in Table XIII show practically no decrease of the absorbance with time.

TABLE XII

Absorbancies of the complex at
560 $m\mu$.

$10^6 \times F$ MB	A	Absorbance per mole MB.
1.65	0.339	20400
3.30	0.625	18900
4.95	0.942	19000
6.60	1.297	19600
8.25	1.601	19500
9.90	1.894	19200

TABLE XIII

Stability of the color of the complex
with time, corresponding to
 6.6×10^{-6} F MB.

Time	A(560 $m\mu$)
1 hours	1.293
2 hours	1.298
4 hours	1.294
24 hours	1.296
28 hours	1.297
30 hours	1.298

The absorbance per mole MB is about 19500 at 560 $m\mu$ and 4150 at 666 $m\mu$. The high absorbance at 560 $m\mu$ would make a determination of zinc via the absorbance of the complex possible, were it not that the MB itself notably absorbs at this wavelength (molar absorptivity = 8500). Correcting the absorbance for the MB present by measuring the absorbance at 666 $m\mu$ is possible, but will make the method more complex.

6. Conclusion

The solubility product of the complex is only moderately small, so to ensure a complete precipitation of zinc as possible, the thiocyanate content should be as high as possible. Preferably the reagent solutions should contain some zinc, enough to cause reaction. Increasing the MB content will not be practical as this will result in a too high absorbance.

The complex itself could be used to determine zinc, but the method would involve some otherwise unnecessary calculation.

VI. KINETICS OF THE REACTION

1. Introduction

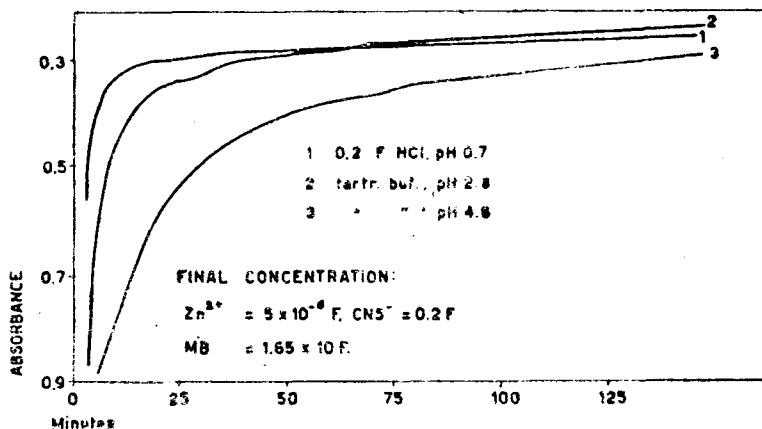
The gradual decrease of the absorbance of a mixture of zinc, MB and thiocyanate shows that the complex formation is a slow reaction and thus the kinetics were investigated insofar as they might influence the determination itself.

As no recording attachment was available, two operators were needed, one of whom operated the spectrophotometer and read the transmittance at the time signalled by the second operator, who also recorded the results.*

In each "run", three samples were started, approximately three minutes apart, by adding the required amounts of thiocyanate to the otherwise completely prepared samples. The exact starting times read on a stopwatch were recorded and subsequent readings were made in the same order and at corresponding times.

2. Influence of pH

The influence of the pH on the reaction rate was found by using several buffers of varying pH's. Results compiled in Graph 11 show clearly that at lower pH's the reaction proceeds considerably slower than at higher pH's.

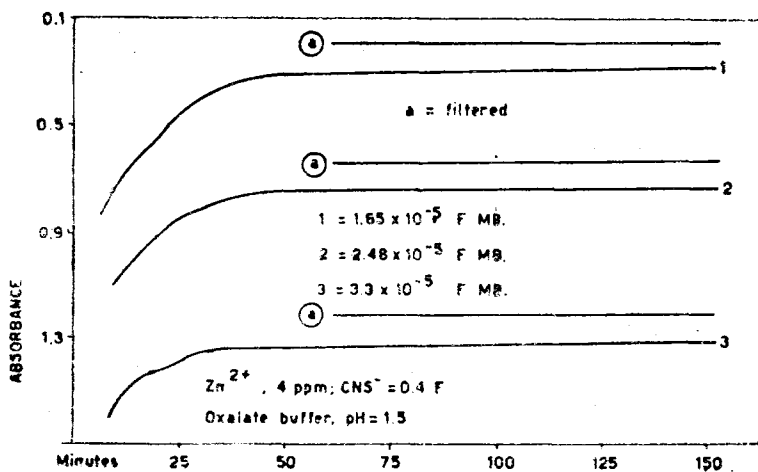


Graph 11. Influence of pH.

In 0.2 F acid the reaction is apparently complete after 30 minutes, as the curve shows only a slight regular decrease of absorbance after this. In the tartrate buffers however, apparently the reaction was not entirely complete after 60 minutes as shown by a steady increase of transmittance, the increase being larger with higher pH's. From this viewpoint a low pH would be advisable and oxalic acid buffers are apparently the most suitable, their only disadvantage being the low solubility of sodium hydrogen oxalate. But although with these buffers the reaction is much faster than with tartrate buffers, a small residual decrease in absorbance still persists, which should be taken into account in a zinc determination.

* Pratiwi and Ang Tjoan Liem.

This phenomenon may be due to a slow interaction between thiocyanate and MB in the weakly acid solution, but also may be due to absorption phenomena. To check this, three samples were prepared, with the same final concentrations of 4 ppm zinc, 0.4 F thiocyanate and buffered with 0.15 F oxalic acid + 0.15 F sodium hydrogen oxalate, but with different amounts of MB, resp. 1.65×10^{-5} and 3.3×10^{-5} F in MB (A). Part of each solution was filtered and the transmittance of both the filtered and unfiltered solutions measured at regular time intervals. (Graph 12). If the increase in transmittance is due to absorption, the increase in solutions with larger MB content would be larger and filtered solutions should not show any increase at all.



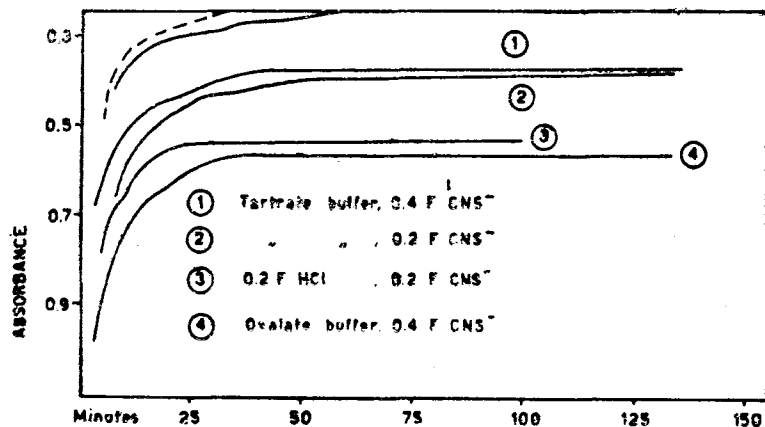
Graph 12. Decrease in absorbance.

However, practically the same increase is observed for *all* solutions, thus any absorption may be neglected.

3. Influence of thiocyanate concentration

This was investigated by adding different amounts of thiocyanate to various samples and determining the transmittance vs time curve.

Results in Graph 13 show clearly the advantage of a large excess of thiocyanate. Although in dilute (0.2 F) hydrochloric acid the reaction is rapid enough with final a concentration of 0.2 F in thiocyanate, in oxalate buffers about the double amount (0.4 F) is required to get approximately the same rate. Of course more concentrated solutions are possible, but this has its practical limitations.



Graph 13. Influence of CNS^- .

4. Calibration curves.

Reliable calibration curves for a zinc determination will be obtained only if the absorbancies are taken from the part of the transmittance vs. time curve that is as level as possible and the small decrease in absorbance still present makes it necessary to read the absorbance after a fixed time (about 60 minutes), but differences up to 5 minutes will cause no appreciable error. Plotting the absorbancies observed against the zinc concentrations in the usual way will give calibration curves with a downward slope and although there is no particular objection against this, a curve with an upward slope would probably be easier to use. These will be obtained if semilog paper is used or regular graph paper where the absorbance is plotted with the smallest A at the top.

5. The influence of temperature

As the temperature influence the solubility product of the complex, it can be expected to influence the calibration curve also.

To check this, 4 sets of solutions were prepared, all containing the same final concentration of a buffer at a certain pH, approximately 4×10^{-5} F MB (A), 0.4 F sodium thiocyanate and different amounts of zinc. Each set was kept at a chosen temperature and the absorbance read after 60 minutes. Results are compiled in Table XIV and a representative calibration curve is given in Graph 14.

Set I uses a buffer composed of 0.5 tartaric acid + 0.25 F sodium hydrogen tartrate, with a pH = 2.8. Apparently the curve at 20°C will lie a trifle higher, but between 20° and 40°C the deviations are so small that they may

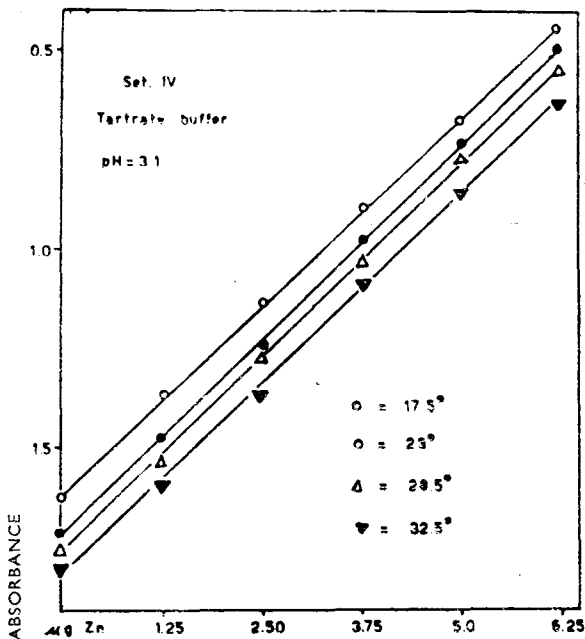
TABLE XIV

Absorbancies of the residual MB after reacting with different amounts of zinc in various buffers.

	Temp. (°C)	amounts of zinc per 10 ml in μg						
		0.0	2.50	3.75	5.00	6.25	7.50	8.75
Set I. pH=2.8	20	1.897	1.452	1.210	0.939	0.703	0.455	0.235
	26	1.901	1.482	1.253	0.941	0.751	0.483	0.280
	30	2.000	1.492	1.239	0.960	0.721	0.475	0.291
	40	1.892	1.495	1.242	0.918	0.733	0.495	0.306
Set II. pH=1.5	17	1.648	1.145	0.918	0.688	0.450	0.315	0.298
	22	1.683	1.195	0.984	0.742	0.452	0.321	0.285
	27	1.703	1.223	0.989	0.755	0.492	0.301	0.290
	32	1.693	1.229	1.002	0.757	0.480	0.315	0.283
	40	1.688	1.201	1.002	0.767	0.512	0.321	0.292
	50	1.357	0.921	0.741	0.551	0.343	0.218	0.169
Set III. pH=0.7	20	1.812	1.333	1.107	0.893	0.678	0.480	0.291
	28	1.785	1.350	1.136	0.914	0.710	0.498	0.295
	35	1.807	1.414	1.206	0.974	0.732	0.540	0.282
	45	1.650	1.328	1.126	0.939	0.726	0.529	0.291
Set IV. pH=3.1	17.5	1.628	1.139	0.896	0.678	0.446	1.25 μg : 1.354	
	23	1.724	1.245	0.975	0.735	0.498	1.483	
	28.5	1.755	1.281	1.041	0.775	0.559	1.548	
	32.5	1.801	1.376	1.086	0.851	0.642	1.602	

well be due to experimental errors and it can be concluded that between 20°C and 40°C with buffers of pH 2.8 the temperature has no influence on the results. In Set II an oxalic acid buffer is used, composed of 0.5 F tartaric acid + 0.15 oxalic acid + 0.10 sodium hydrogen oxalate, with a pH of 1.5. Again practically the same results are obtained within a considerable temperature range, this time between 22° and 32°C. Beyond these limits however, marked changes are noted. At 17°C the absorbancies have decreased markedly and at higher temperatures of 40° and 50°C also a marked change in slope occurs.

In Set III a mixture of 0.2 F hydrochloric acid and 0.5 F tartaric acid, pH 0.7, is used. The deviations here are much more pronounced and the changes in slopes at higher temperatures are much more perceptible.



Graph 14. Calibration curves.

two opposing effects of the temperature: an increase of temperature will increase the solubility of the complex, which increases the amount of free MB and thus increases the absorbance, but at the same time it will also probably increase the liberation of free thiocyanic acid, resulting in an increase in the bleaching of MB and thus decreases the absorbance.

Apparently these factors at pH 1.5—2.5 cancel out within a wide temperature range and no correction is necessary. Beyond these limits appropriate corrections can be applied.

It is remarkable, that at pH 3.1 the absorbance increases with decreasing temperature. This can be expected, as at this high pH value liberation of free thiocyanic acid is highly improbable, thus no counterbalancing decrease in absorbance is to be expected.

6. Conclusion

From the viewpoint of reaction rate a low pH is advantageous and buffers with a pH between 1.5 and 2.5 are desirable, as with these the calibration curves are temperature independent within a wide temperature range.

The thiocyanate content should be as high as conveniently possible; a final concentration of about 0.4 F is adequate.

In Set IV a 1 F solution of tartaric acid was used, neutralized with solid sodium carbonate to a pH of 3.1. The calibration curves have about the same slope, their location is different: those at lower temperatures lying higher than at higher temperatures. Corrections can be applied of 0.05 μg zinc for each decrease in temperature of 1°C.

The unexpected temperature independence with buffers of pH 1.5—2.5 is probably due to

The calibration curve found shows that a difference in absorbance of 1 corresponds to 0.5 ppm of zinc, thus giving an extremely high sensitivity.

DISCUSSION AND CONCLUSION

The formation of a complex by zinc with thiocyanate with MB can be made the basis of a new spectrophotometric determination of zinc, where the decrease in absorbance corresponds to the amount of zinc present. As one mole of zinc ties two moles of MB and MB itself has already a very high molar absorptivity, this method can be a very sensitive one. The absorbance of MB is practically pH independent over a wide pH range of 1.5—9 and with an ionic strength below 0.2 also free of salt errors. Measuring the decrease in absorbance of MB provides a method already sensitive enough and the use of organic solvents to increase the sensitivity will be of little use. Extraction with immiscible solvents offers too many difficulties and not enough advantages.

As the complex formation is a slow reaction, conditions which offers the greatest speed should be found: the pH should be low (1.5—3) and the thiocyanate content should be high, about 0.4 F. Absorbance measurements should be taken after a fixed time (60 minutes), but differences of about 5 minutes will cause no appreciable errors. Convenient calibration curves are obtained when absorbancies are plotted with the smallest value *above*. These are practically straight and temperature independent over a wide temperature range.

REFERENCES

1. BANKS, C.V. and BISQUE, R.E., *Anal. Chem.* 29, 552 (1957).
2. BHAGWATT, W.V., *J. Indian Chem. Soc.* 17, 53 (1940), cf. *CA* 40, 5346 (1946).
3. MCCALL, J.T. and DAVIS, G.K., *Anal. Chem.* 30, 1345 (1958).
4. CHARLOT, G. and BEZIER, D., "Quantitative Inorganic Analysis" p. 634 John Wiley & Sons Inc., New York, 1957.
5. CHARLOT, G. et BEZIER, D., "Theorie et methode nouvelles d'analyse qualitative", 3me ed., p. 298. Masson et Cie., Paris, 1949.
6. FRANK, R.E. and HUME, D.N., *J. Am. Chem. Soc.* 75, 1736 (1953).

7. ISJRIN, NOERDIN, *Buffer solutions and their electrometric control Unpublished results (I.T.B., 1956), Bandung, Indonesia.*
8. KOLTHOFF, I.M. and ROSENBLUM, C., "Acid-base indicators", p. 239 *The Macmillan Company, New York, 1937.*
9. KOLTHOFF, I.M. and SANDELL, E.B., "Textbook of Quantitative Inorganic Analysis", revised ed., p. 703 *The Macmillan Company, New York, 1949.*
10. KRUSE, J.M. and BRANDT, W.W., *Anal. Chem.* **24**, 1306 (1952).
11. KUTZNETSOW, V.I., *Doklady Akad. Nauk. S.S.R.* **41**, 113 (1943), *of. CA* **38**, 3925 (1944).
12. LEWIS, G.N. and BIGELEISEN, J., *J. Am. Chem. Soc.*, **65**, 1144 (1943).
13. LEWIS, G.N., GOLDSCHMIDT, Magel and Bigeleisen, *J. Am. Chem. Soc.*, **65**, 1150 (1943).
14. MELLAN, "Organic reagents in Inorganic analysis" p. 617 *The Blakiston Company, Philadelphia, 1941.*
15. MELLON, M.G. and BOLTZ, D.F., *Anal. Chem.* **32**, 632 (1960).
16. MICHAELS, L. and GRENICK, S., *J. Am. Chem. Soc.* **67**, 1212 (1945).
17. PLATTE, J.A. and MARCY, V.M., *Anal. Chem.* **31**, 1227 (1959).
18. RABINOVITCH, E and EPSTEIN, L.F., *J. Am. Chem. Soc.* **63**, 69 (1941).
19. REMY, H., "Treatise on Inorganic Chemistry" Vol. II, p. 465. *Elsevier Publishing House, Amsterdam 1955.*
20. RUSH, R.M. and YOE, J.M., *Anal. Chem.* **26**, 1345 (1954).
21. SANDELL, E.B., "Colorimetric determination of traces of metals" 2nd. ed., p. 616 *Interscience, New York, 1950.*
22. SCHWARZENBACH, G., "Allgemeine und Anorganische Chemie" 4te. auflage, p. 358 *George Thieme Verlag, stuttgart, 1950.*
23. SOESANTO IMAN RAHAJOE, *Unpublished results I.T.B. 1961, Bandung, Indonesia.*
24. VOGEL, A.I., "A textbook of Quantitative Inorganic Analysis" 2nd. ed., p.92 *Longmans Green & Co., London, 1958.*

25. WELCHER, "*Organic Analytical reagents*", Vol. I, 2nd. ed., p. 281 Van Nostrand, New York, 1948.
 26. WELCHER, "*Organic Analytical reagents*", Vol. IV, p. 310 Van Nostrand, New York, 1948.
-