in connection with a suitable electronic circuit. Delay in coming to equilibrium is not a disadvantage for control purposes because the control circuit tends immediately to correct humidity, leaving the unit always in a region of substantially constant humidity, and also because materials may be selected from Fig. 1 so that operation occurs where the curve is so steep that the unit responds with almost triggerlike action.

References and Notes

1. Made by Microcircuits Co., New Buffalo, Mich.
2. H. J. Reich, *Theory and Applications of Electron Tubes* (McGraw-Hill, New York, ed. 2, 1944), p. 620.
3. Victoreen Instrument Co., Cleveland, Ohio.
4. A. Wexler and W. G. Bromberger, *Methods of Measuring Humidity and Testing Hygrometers* (National Bureau of Standards, Circular 512, 1951), p. 13; W. E. Windsor *et al., Rev. Sci. Inst.* **24**, 334 (1953).

4 October 1954.

Estimation of Basicity with a Novel Thermochromic Indicator

Saul Soloway and Perry Rosen

Department of Chemistry,
The City College, College of the City of New York

In the course of extending the analytic utility of iron-III compounds many striking cases of solvochromism (*1*) and thermochromism have been discovered (*2*). These phenomena have been observed particularly with the iron-III chelates of hydroxamic acids, amidoximes, and a variety of orthosubstituted phenols. For example, solutions of ferric chloride, propyl gallate, and *m*-chloroaniline may be blue, green, or yellow at room temperature, depending on the solvent.

Water, hydrocarbons and their halogen derivatives, alcohols, mercaptans, carboxylic acids, and diaryl ethers yield blue solutions; some arylalkyl ethers, acetals, and orthoesters give green solutions; aliphatic and heterocyclic ethers, esters, aldehydes, ketones, nitriles, and nitro compounds yield yellow solutions (*3*). These solutions change color reversibly with temperature in the order blue $\rightleftharpoons$ green $\rightleftharpoons$ yellow. Substitution of progressively weaker or stronger bases for *m*-chloroaniline in a given solvent produces the same chromic order as increasing or decreasing the temperature respectively. These findings may be summarized by the equation

$$\text{Propyl gallate} + \underset{\text{(yellow)}}{\text{ferric chloride}} + \text{base} \underset{\text{increasing temperature}}{\overset{\text{increasing strength of base; increasing "basicity" of solvent}}{\rightleftharpoons}} \underset{\text{(blue)}}{\text{chelate}} + (\text{H base})^+$$

For the purpose of indicating the function of the base, the products are shown as separate entities. In reality they are probably linked as an ion pair or a hydrogen bonded complex in the low dielectric mediums in which this system was studied.

A quantitative study of this observed interdependence of temperature, solvent, and strength of added base on the extent of chelate formation led to the finding that the classical ionization constant of aromatic amines could be estimated to ± 0.4 pK_b unit at worst. It was observed that the temperature at which the blue color of the chelate just reappeared on allowing a momentarily boiled solution to cool slowly in the atmosphere could be reproduced to ± 2°C. Using bromobenzene as a solvent, we studied the critical thermochromic temperature as a function of the base strength of aromatic amines. A plot of these temperatures against the aqueous pK_b values resulted in an approximately linear relationship. The analytic expression for the straight line passed among the points is given by the equation $pK_b = 13.1 - (T/30)$. Table 1 gives the experimental data and the deviations of the measured values from the calculated pK_b values.

Table 1. Basicity constants of aromatic amines from thermochromic measurements.

Aromatic amine	Critical thermochromic temp. (°C)	Calculated pK_b	Literature pK_b*	ΔpK_b
Diphenylamine	−12	13.5	13.1	0.4
2,5-Dichloraniline	5	12.9		
o-Bromoaniline	26	12.2		
o-Chloroaniline	34	12.0	12.0	.0
m-Bromoaniline	79	10.5		
m-Chloroaniline	81	10.4	10.4	.0
Dimethylaniline	98	9.8	9.6	.2
p-Chloroaniline	102	9.7	9.8	.1
p-Bromoaniline	102	9.7	10.0	.3
p-Toluidine	105	9.6	9.7	.1
m-Toluidine	105	9.6	9.3	.3
Aniline	105	9.6	9.3	.3
o-Toluidine	110	9.4	9.5	.1
Pyridine†	110	9.4	8.6	.8
6-Methyl quinoline	113	9.3		

* These pK_b values were calculated from tables of dissociation constants in the *Handbook of Physics and Chemistry* (ed. 34), p. 1560 and in various works on organic chemistry.

† The temperature value for pyridine is relatively lower than the others because of an appreciable loss through evaporation during measurement. This was the only base tried whose boiling point was lower (by 40°C) than that of the solvent. Hence the statement in the text that this method can give pK_b values to ± 0.4 unit refers to those bases whose boiling points exceed that of the solvent.

The measurements were carried out in the following manner. To 7.5 ml of bromobenzene were added 5 drops (0.05 ml/drop) of ferric chloride-propyl gallate indicator, and 5 drops or 150–170 mg of base in a test tube (o.d., 29 mm). The indicator solution consists of 2 g anhydrous ferric chloride and 4 g propyl gallate dissolved in 100 ml glacial acetic acid and 1 ml

acetyl chloride (*4*). A thermometer was placed in the tube and the solution was heated to boiling. The resulting yellow solution was then allowed to cool in the atmosphere while stirring gently, and the temperature noted at the moment when the solution turned blue.

The critical thermochromic temperature was unaffected by considerable variation in base concentration. Such a large excess is present relative to the indicator components that its concentration remains practically constant regardless of the extent of the chelate formation. The mole ratios of ferric chloride : propyl gallate : various bases were 1 : 1.5 : 28 to 58.

The aliphatic amines and 2-aminopyridine were strong enough bases to keep the bromobenzene solution of the indicator blue at its boiling point. Attempts to use solvents possessing higher boiling points and/or greater intrinsic acidities to extend the range to strong bases proved futile. However, in view of the wide variation in stability constants of various chelates, it should be possible to find different chelate-solvent combinations for the estimation of other ranges of basicity and acidity.

References and Notes

1. A term coined to indicate change of color with solvent.
2. S. Soloway and A. Lipschitz, *Anal. Chem.* **24**, 898 (1952); S. Soloway and S. Wilen, *ibid.* **24**, 979 (1952); S. Soloway and P. Rosen, *ibid.* **25**, 595 (1953).
3. The detailed presentation of these data, their meaning, and their use as a basis for classifying organic compounds will be the subject of a future publication.
4. The utility of this indicator for the determination of water in alcohols will be described in a later paper. The determination depends on the observation that the addition of water to an alcoholic solution of this indicator causes a color change from yellow or green to blue. This change results from the fact that water functions as a base in dilute alcoholic solution.

6 October 1954.

Action of Cortisone on Disseminated Tumor Cells after Removal of the Primary Growth

Renato Baserga and Philippe Shubik with the assistance of Joseph Baum

Division of Oncology, Chicago Medical School, Chicago, Illinois

It has been reported by several workers (*1*) that repeated injections of cortisone give rise to an increased metastatic spread of a variety of transplanted and induced tumors. This finding has been disputed by Kaliss using a transplanted tumor (*2*). From the sum of evidence available it would seem clear that cortisone does have an effect in metastasis, but that it is one that may well operate only with specific tumors and not with others.

In attempting to analyze this phenomenon Pomeroy (*3*) has found that cortisone will increase the number of "takes" following an intravenous injection of a suspension of transplantable tumor cells. This would seem to indicate that cortisone exerts an action on the phase of metastasis occurring after vascular penetration, and that it might well be primarily concerned with the growth of cells that have already been disseminated systemically; under these conditions it would be expected that different tumors might well respond differently. The use of the intravenous injection technique for the study of metastasis introduces several additional factors not seen when spontaneous metastasis occurs, such as the introduction of many dead as well as living cells, and the introduction of material from another host. In the present experiments (*4*) use has been made of a technique of implantation into the tail, subsequent complete removal of the tumor, and study of cells disseminated previous to excision.

The tumor used was a transplantable carcinoma originally from the bladder epithelium of a C57 black mouse, designated as T150, and subsequently carried in the C57 black strain. The tumor was ground in saline, and the suspension diluted to provide a concentration of 10^7 cells/cm^3; 0.05 ml of this suspension was injected subcutaneously into the tail with a 22-gage needle. The injection was directed caudally to avoid spread toward the base of the tail. Under these conditions a viable tumor growth appears on the 14th to 19th day following injection, reaches a size of 1 cm in diameter in 2 days and then spreads rapidly, invading the subcutaneous tissue of the caudal region of the body. There are three possible mishaps that may occur with this procedure: (i) in some 10 percent of cases the tumor may fail to grow at all; (ii) in some 8 percent of this material the tumor grew diffusely down the tail, instead of as a localized mass; (iii) in scattered instances the injection was made directly into the tail vein, noted at first by an absence of resistance to the plunger of the syringe, and often by distress and even death of the animal. All these occurrences were taken as indications to discard these particular animals.

Tumor T150 is a spontaneously metastasizing neoplasm, inducing multiple metastases in the lungs that have been described elsewhere in detail (*5*). When the growth in the tail has reached a diameter of 1 cm some tumor emboli are released, and preliminary studies have revealed that a small number of metastatic lesions are noted if the tail is amputated at this stage.

For the present experiment 78 C57 black mice from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me. were used. They were housed in plastic cages and fed Rockland mouse diet with water ad libitum. The mice were implanted with the tumor in the tail as previously described, and after the appearance of the growth were divided into pairs having tumors of the same size and duration of growth. When the tumors had reached a size of 1 cm the tail was amputated as near the root as possible, and of the pair, one animal was then maintained as a control