

- (2) "Biochemical Applications of Mass Spectrometry", G. R. Waller, Ed., Wiley-Interscience, New York, 1972, pp 51-132.
 (3) R. A. Hites and K. Biemann, *Anal. Chem.*, **39**, 965-970 (1967).
 (4) M. S. B. Munson and F. H. Field, *J. Am. Chem. Soc.*, **88**, 2621-2630 (1966).
 (5) J. R. Plattner and S. P. Markey, *Org. Mass. Spectrom.*, **5**, 463-471 (1971).
 (6) T. Aczel, *Anal. Chem.*, **40**, 1917-1918 (1968).
 (7) T. H. Risby, P. C. Jurs, F. W. Lampe, and A. L. Yergey, *Anal. Chem.*, **46**, 726-728 (1974).

- (8) I. Dzidic, D. M. Desiderio, M. S. Wilson, P. F. Crain, and J. A. McCloskey, *Anal. Chem.*, **43**, 1877-1879 (1971).

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Deviation from Beer's Law Caused by Change in Bathochromic Shift of Absorption Maximum

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Metallochroic indicators such as Xylenol Orange, Catechol Violet, or Chromeazurol S exhibit a bathochromic shift often accompanied by a considerable increase in sensitivity when used together with micelle-forming reagents such as cetylpyridinium bromide and cetyltrimethylammonium bromide or chloride for the determination of metals (1-3). Attempting to determine beryllium by the method described by Nishida (4) using Chromeazurol S as color reagent, but substituting benzyldimethylhexadecylammonium chloride for zephiramine (benzyldimethyltetradecylammonium chloride), because the first was available as a chemically pure reagent, we observed a reproducible change in the slope of the standard working curve at 610 nm. In an attempt to discover the origin of this change, we prepared absorption spectra of the color complex using varying beryllium concentrations. The results of this investigation showed that the bathochromic shift caused by the presence of benzyldimethylhexadecylammonium chloride varies with beryllium concentration.

EXPERIMENTAL

Reagents. Beryllium standard: A solution containing 1 μg of beryllium per ml was always freshly prepared by appropriate dilution of a more concentrated stock solution which had been obtained by dissolving beryllium nitrate (Merck extra pure) in deionized water containing 0.1M hydrochloric acid and standardizing gravimetrically as beryllium cupferate. Other reagents used are as follows: Chromeazurol S: A 0.06% solution of the reagent (Merck) in deionized water. Benzyldimethylhexadecylammonium chloride (BDHA): A 10mM solution of the reagent (Fluka purum) in deionized water. Hexamethylene tetramine (HMT): A 1.0M solution of the reagent in deionized water. Ca-EDTA: A solution 50mM in Ca-EDTA (Merck, Calcium-Titriplex III, rein) and 10mM in CaCl_2 (Merck "pro analysi") in deionized water.

All common reagents were of analytical reagent grade purity. Dilutions were carried out with distilled water further purified by passing through an Elgastat deionizer. Glassware was cleaned by boiling in 1:1 hydrochloric acid and rinsing with deionized water.

Apparatus. A Metrohm E300 pH meter calibrated at pH 7.00 with a guaranteed buffer was used for pH measurements. The calibration was checked after each series of measurements.

A Zeiss PMQII spectrophotometer connected to a stabilized power supply was used for spectrophotometric measurements.

Absorption Spectra for Various Amounts of Beryllium. Amounts of the beryllium standard solution containing from 0.5 to 5.0 μg of beryllium were measured out accurately and added to 5 ml 1M ammonium chloride solution in deionized water. Five ml of the Ca-EDTA solution were added followed by 5 ml of the BDHA and 5 ml of the chromeazurol S solution. The solutions were mixed thoroughly after each addition. Finally 5 ml of HMT solution were added and the pH was measured with a pH meter. It always was

within 6.65 ± 0.05 . The solutions then were transferred into volumetric flasks of 50-ml volume and made up to volume with deionized water. After about 2 hours, the absorbance of the solutions was measured vs. a reagent blank. The results are presented in Figure 1. Figure 2 shows two standard working curves, one at 620 nm, which was the wavelength of maximum absorbance of the standard with the highest beryllium concentration, the other one at 611 nm, the wavelength which on inspection of Figure 1 seemed to give the best approximation to a straight line.

DISCUSSION

Figure 1 shows a very definite change in the wavelength of the position of the absorbance maximum with decreasing

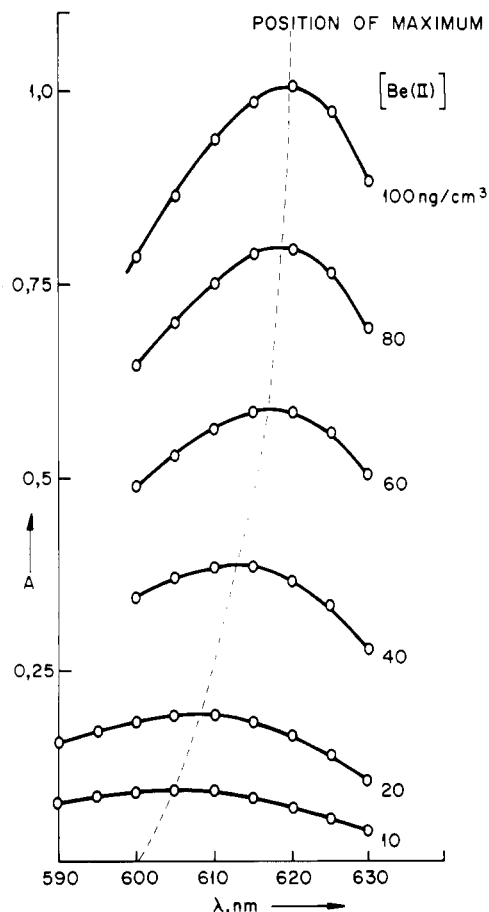


Figure 1. Absorption spectra for various amounts of beryllium with Chromeazurol S (0.1 mM), BDHA (1.0 mM), hexamethylene tetramine (0.1 M), and ammonium chloride (0.1 M) at pH 6.65 ± 0.05

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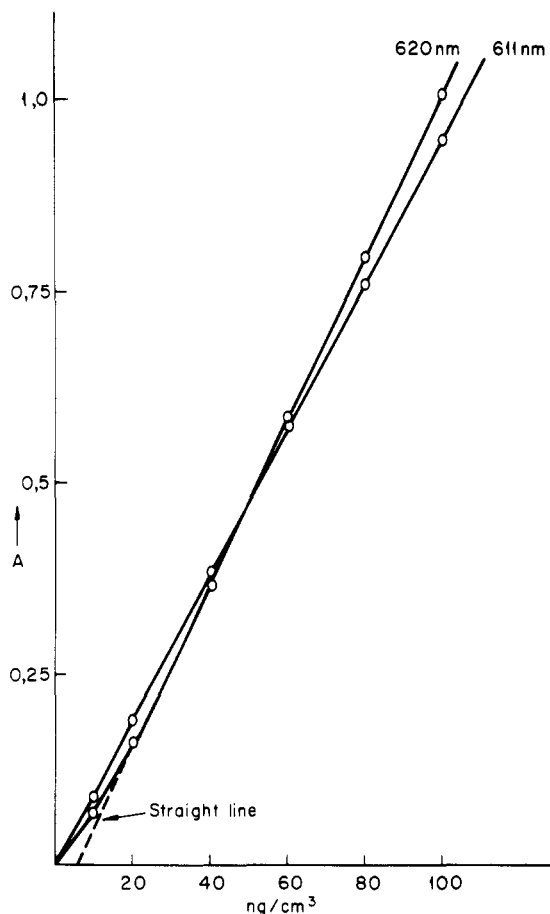


Figure 2. Standard working curves for beryllium as Chromeazurol S complex at 611 and 620 nm. Conditions similar to Figure 1

beryllium concentration. The change is only small at higher beryllium concentrations, but becomes quite appreciable at low concentrations. In addition, there is also a slight non-linearity when absorbance values at the absorbance maximum are plotted vs. beryllium concentration. As a result, the absorbances of a series of standards measured at the wavelength of the absorbance maximum of the standard with the highest beryllium concentration (620 nm) will not produce a straight line through the origin when plotted vs. beryllium concentration (Figure 2). Beer's law, therefore, is not valid for the whole concentration range. The divergence is mainly in the lowest concentration range where the

line shows a bend. This can easily be overlooked, especially when the analyst assumes that his lowest point on the standard working curve may be faulty because of experimental factors when it is not perfectly on the line. Measurement at 611 nm, a wavelength arbitrarily chosen by inspection of the absorbance curves on Figure 1, produces a considerably better straight line going through the origin, though a small divergence is still present for the lowest beryllium concentration measured.

A bend in the standard working curve similar to that shown for the measurements at 620 nm on Figure 2 was also found by us when determining tin spectrophotometrically as its complex with pyrocatechol in the presence of cetyltrimethylammonium bromide in tartaric acid medium, adapting the method described by Dagnall et al. (2) and H. B. Corbin (5). Furthermore, Sommer et al. (3) also show a bent Beer's plot for beryllium determined with Chromeazurol S using polyvinyl alcohol as micelle-forming agent and triethanolamine as buffer. We therefore suspect that changes in bathochromic shift with changing metal ion concentration similar to that described on Figure 1 may be fairly general when using micelle-forming agents to obtain increased sensitivities during spectrophotometric determination of metals. This has to be taken into account when attempting accurate analytical determinations.

In addition, other electrolytes present also can cause a slight change in the bathochromic shift, and the intensity of the color of the beryllium-Chromeazurol S complex in the presence of BDHA changes quite appreciably with foreign electrolyte concentration. It also is dependent on the kind of electrolyte. Using exactly the same conditions as given for Figure 1, but omitting the 0.1M ammonium chloride will decrease the adsorbance for 100 nanograms of beryllium at the adsorption maximum by about 15%. Experimental conditions, therefore, should be carefully controlled and reagents used for preparing the standard working curve should match those in the samples when micelle-forming reagents are employed for obtaining increased sensitivities in the spectrophotometric determination of metals.

LITERATURE CITED

- (1) V. Swoboda and V. Chromy, *Talanta*, **12**, 431 (1965).
- (2) R. M. Dagnall, T. S. West, and P. Young, *Analyst*, **92**, 27 (1967).
- (3) L. Sommer and V. Kuban, *Anal. Chim. Acta*, **44**, 333 (1969).
- (4) H. Nishida, *Bunseki Kagaku (Jpn Anal.)*, **20**, 1080 (1971).
- (5) H. B. Corbin, *Anal. Chem.*, **45**, 534 (1973).

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Colorimetric Estimation of Phenols and Tyrosine

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The most commonly used method for the colorimetric estimation of phenols is the color reaction with aminoantipyrine (1-4). This method, based on the oxidation by potassium ferricyanate, is fast and accurate but many ring compounds give the test, and many aromatic hydroxy compounds, especially with an occupied para position, do not react. Several other nonspecific methods are based mostly on coupling of the estimated phenols with diazonium salts. The diazonium salts of sulfanilic acid (5), *p*-nitroaniline

(6-8), *m*-nitroaniline (9), 1-nitroso-2-naphthol (10), and hydrazinobenzene sulfonic acid (11) were used. The color reactions of phenols with FeCl_3 (12, 13), HNO_2 (14, 15), titanium sulfate (16), Millon's reagent (17) and others have also been used.

Several methods have been described for the colorimetric estimation of tyrosine. Millon's mercury reagent (18-20), Folin-Ciocalteu phenol reagent (21-23) or 1-nitroso-2-naphthol were used for the estimation of tyrosine (24-