not available. However, a small peak with a retention time of 252 sec appeared occasionally in extracts of serum from volunteers but did not interfere with the assay; it was attributed to a metabolite.

A typical standard graph of peak height ratio (I/II) *versus* weight ratio (I/II) is presented in Fig. 3. A straight line with a slope varying between 1.31 and 1.35 and an intercept varying between 0.04 and 0.06 on the weight ratio axis was obtained by linear regression analysis over 1 month during which the analyses were performed. The intercept on the weight ratio axis corresponds to a loss of 0.8–1.2 ng of I on the GLC column during chromatography, thus imposing a lower limit of detection of I in serum by this method at about 5 ng/ml. A peak for I just begins to appear in the chromatogram at this concentration. A summary of the recovery results of I obtained with human serum when 20–80 ng/ml was added to the serum is presented in Table I.

This GLC method was applied to a large number of samples of human serum during a trial comparing serum levels attained after administration of a standard formulation with levels attained after administration of a timed-release formulation of I.

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Colorimetric Determination of Formaldehyde *via* Free Radical Formation

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Abstract □ Aliphatic aldehydes react with 2,3-dimethyl-2,3-bis(hydroxylamino)butane and sodium periodate to form colored free radicals. These radicals were stabilized with pyridine in aqueous solution. Low levels of formaldehyde in aqueous solutions were determined utilizing this reaction.

Keyphrases □ Formaldehyde—stabilized colorimetric analysis in aqueous solution *via* reaction with 2,3-dimethyl-2,3-bis(hydroxylamino)butane and sodium periodate □ 2,3-Dimethyl-2,3-bis(hydroxylamino)butane—colorimetric reagent for determination of formaldehyde in aqueous solution □ Colorimetry—analysis, formaldehyde in aqueous solution using 2,3-dimethyl-2,3-bis(hydroxylamino)butane

Aldehydes have been shown to form stable free radicals when reacted with 2,3-dimethyl-2,3-bis(hydroxylamino)butane (I) (1) (Scheme I). The anhydro product (II) can be isolated directly or may be converted to the free radical (III) by the action of lead dioxide or sodium periodate. The free radicals are red or blue in solution. The color of these free radicals suggests that this reaction may be suitable for colorimetric determination of aldehydes.

Preliminary data suggested that only aliphatic aldehydes and ketones react with I to form a colored product in aqueous solution. A spot test based on this reaction was developed that allows detection of as little as 0.2 μg of aliphatic aldehydes (2). Aliphatic ketones gave a much smaller response, with 100–200 μg being the minimum detectable quantity.

Initial attempts to utilize this reaction in a quantitative manner were plagued by the instability of the color of the free radical. The half-life for the disappearance of color was about 7 min at 25°. This paper reports the stabilization of the color and the adaptation of this reaction to the determination of formaldehyde at low concentrations in aqueous solution.

EXPERIMENTAL

Materials—Formaldehyde stock solutions were analyzed by peroxide oxidation (3). Acetaldehyde was redistilled and chilled. A portion of acetaldehyde was added to a tared flask, sealed, and reweighed. Water was then added to give a known concentration. All other aldehyde stock solutions were prepared by dissolving weighed amounts of the redistilled aldehyde in water. 2,3-Dimethyl-2,3-bis(hydroxylamino)butane sulfate[1] was recrystallized from a 2-propanol–water mixture, mp 185° dec.

Anal.—Calc. for $C_6H_{18}N_2O_6S$: C, 29.27; H, 7.32; N, 11.38. Found: C, 27.78; H, 7.54; N, 11.12.

Sodium periodate[2] and the organic solvents were reagent grade and were used directly.

Effect of Solvent on Stability of Color—An aqueous reaction mixture containing formaldehyde (1.34×10^{-3} *M*) and 2,3-dimethyl-2,3-bis(hydroxylamino)butane sulfate (Ia) (1.76×10^{-2} *M*) was prepared and allowed to react for 1 hr at 25°. One-milliliter aliquots of the reaction mixture were added to a tube containing 1 ml of the solvent being studied and 1 ml of sodium periodate (0.094 *M*).

[1] Eastman Organic Chemicals

[2] J. T. Baker Chemical Co.

Table I—Effect of Solvent on Stability of Color of Free Radical

Solvent	$10^3 k_{obs}$, sec^{-1}	Concentration of Added Solvent, M	k_{obs} (solvent)/k_{obs} (water)
Water	1.63	—	1.00
Methanol	1.70	8.2	1.04
2-Propanol	1.35	4.3	0.83
Hydrogen peroxide (30% w/v)	2.0	3.3	1.23
Dioxane	1.61	3.9	0.99
Tetrahydrofuran	0.60	4.1	0.37
Pyridine	0.028	8.2	0.017
Acetonitrile	0.85	6.3	0.52
Dimethyl sulfoxide	1.61	4.7	0.99
Dimethylformamide	1.36	4.3	0.83

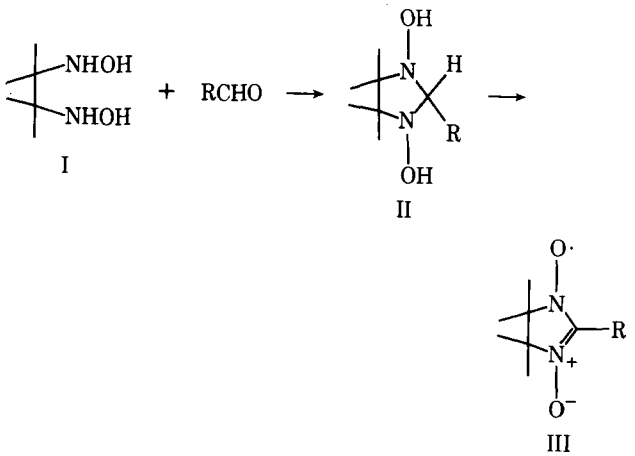
The absorbance[3] at 515 nm was measured as a function of time at 25°. Plots of $A_t - A_\infty$ *versus* time were linear for at least three half-lives of the color disappearance. Pseudo-first-order rate constants for this process were calculated from the slopes of these plots.

Effect of Pyridine Concentration on Stability—Reaction mixtures were prepared in the same manner as described in the previous section. The final solutions contained formaldehyde (4.47×10^{-4} *M*), Ia (8.22×10^{-3} *M*), and pyridine ranging from 0.41 to 8.24 *M*. Absorbance was measured as a function of time at 25°, and pseudo-first-order rate constants were calculated from plots of $A_t - A_\infty$ *versus* time.

Effect of Reaction Time on Extent of Color Formation—A reaction mixture containing Ia (1.32×10^{-2} *M*) and formaldehyde (2.76×10^{-3} *M*) was prepared and allowed to react at 25°. Aliquots (0.5 ml) were removed as a function of time and mixed with 0.5 ml of sodium periodate (3.7×10^{-2} *M*) and 2.0 ml of pyridine. The absorbance at 515 nm was measured at 1500 sec after the mixing of the final solution.

Effect of Ratio of Sodium Periodate to Formaldehyde on Extent of Color Formation—A reaction mixture was prepared containing Ia (1.32×10^{-2} *M*) and formaldehyde (2.68×10^{-3} *M*) and allowed to react for 5 min at 25°. Then 0.5 ml of the reaction mixture was added to 2.0 ml of pyridine and 0.5 ml of a sodium periodate solution. The absorbance was measured at 515 nm after 25 min.

Calibration Curve for Formaldehyde—Reaction mixtures were prepared containing Ia (1.32×10^{-2} *M*) and varying amounts of formaldehyde. The reaction mixture was allowed to stand at 25° for 5 min, and 0.5 ml was added to a cell containing 0.5 ml of sodium periodate (0.037 *M*) and 2.0 ml of pyridine. Absorbance at 515 nm was measured 25 min after the addition of the sodium periodate and pyridine.



I II III

Scheme I

[3] Absorbance measurements were made with a Cary 15 recording spectrophotometer equipped with a thermostatted cell compartment or a Gilford model 240 spectrophotometer.

Table II—Effect of Reaction Time on Extent of Color Formation[a]

Reaction Time, sec[b]	A_{515}[c]
10	0.525
20	0.540
80	0.561
120	0.590
300	0.594
600	0.588

[a] Formaldehyde substrate. [b] Time of reaction of Ia and formaldehyde before the addition of sodium periodate. [c] Absorbance measured 1500 sec after the addition of sodium periodate.

Reaction of Other Compounds—Reaction mixtures were prepared containing Ia (1.32×10^{-2} *M*) and some aldehydes and ketones. As a function of time, 0.5 ml of the reaction mixture was added to 0.5 ml of sodium periodate (0.037 *M*) and 2.0 ml of pyridine. Absorbance was measured at the appropriate wavelength after 25 min.

RESULTS AND DISCUSSION

Of the solvents tested (Table I), pyridine, acetonitrile, and tetrahydrofuran stabilized the color of the free radical. Only pyridine gave sufficient stability for analytical procedures. It was observed that the color increased immediately after the addition of the sodium periodate and then stabilized after 15 min. The color was quite stable after this time for at least 1 hr. During this span, the absorbance decreased at a rate of 0.8%/hr.

While these free radicals have been found to be stable in organic solvents, such as cyclohexane (1), their stability in primarily aqueous solution has not been reported. Although the solvents were not added in equimolar concentrations, an examination of the concentrations (Table I) does not suggest that any of the solvents approach the ability of pyridine to stabilize the color when the stability is compared on an equimolar basis.

Formaldehyde reacted very rapidly, the reaction being complete within 5 min at room temperature (Table II). Other aliphatic aldehydes reacted more slowly than formaldehyde under the same conditions (Table III). Acetone and benzaldehyde failed to react within 30 min under the same conditions and equivalent concentrations.

Increasing pyridine concentration in the final solution led to greater color stability (Table IV). A concentration of 8.2 *M* was chosen for convenience of sample handling. Furthermore, this concentration gave sufficient stability for the analytical procedure. The rate constants obtained for pyridine concentrations of 2.06 and 4.12 *M* were redetermined to check the apparent decrease in stability upon going from 2.06 to 4.12 *M* pyridine. These data are apparently real and at this time unexplained. All rate constants were determined at a single formaldehyde concentration.

The ratio of sodium periodate to formaldehyde (and by inference the anhydro product II) in the final solution was found to be

Table III—Rate and Extent of Reaction of Aldehydes and Ketones

Compound	Wavelength of Maximum Absorbance, nm	t_∞[a], sec	Log ϵ[b]
Formaldehyde	515	300	3.06
Acetaldehyde	538	600	3.28
Propionaldehyde	543	1000	3.20
Butyraldehyde	546	1000	3.16
Isobutyraldehyde	547	1400	3.13
Acetone		N.R.[c]	
Benzaldehyde		N.R.[c]	

[a] Time to reach maximum absorbance. [b] Molar absorptivity calculated on basis of formaldehyde concentration in sample (and by inference the concentration of free radical). [c] No reaction within 30 min.

Table IV—Effect of Pyridine Concentration on Color Stability[a]

Pyridine, M	$10^5 k_{obs}$, sec^{-1}
0.41	9.8
2.06	0.92
4.12	1.7
8.24	0.45

[a] Formaldehyde, 4.47×10^{-4} M; Ia, 8.22×10^{-4} M; sodium periodate, 3.12×10^{-2} M; 25°.

critical in that sodium periodate must be in at least a twofold excess for maximal color development (Table V). Therefore, a relatively high concentration of sodium periodate should be used when a wide concentration range of aldehyde is to be studied.

A calibration curve for the determination of formaldehyde gave excellent results. The slope of the line was 1156 liters $mole^{-1}$ cm^{-1} (1-cm cell used) with an intercept of 0.008 when calculated by linear regression analysis. The sample correlation coefficient was 0.999 with a standard error of the estimate of 0.009 ($S_{y \cdot x}$). Formaldehyde concentrations down to 4×10^{-5} M can be determined using a 1-cm cell and a spectrophotometer[4] (no scale expansion). It is quite conceivable that lower concentrations could be determined if enough sample were available to allow for the use of longer path length cells. Above concentrations of 10^{-3} M, deviation from Beer's law was observed.

Although other aliphatic aldehydes reacted more slowly than formaldehyde, each gave a similar color yield. It is not unexpected that acetone and benzaldehyde did not react since much higher concentrations of these compounds must be present to give a positive spot test using a variation of this reaction (2). Furthermore, on the basis of spot test data, no interference from a variety of carboxylic acid derivatives is expected (2).

[4] Cary 15.

Table V—Effect of Ratio of Sodium Periodate to Formaldehyde on Color Formation

Sodium Periodate: Formaldehyde[a]	A_{515}[b]
0.70	0
1.40	0.133
1.75	0.362
2.10	0.563
3.49	0.553
6.99	0.563
13.67	0.572

[a] Molar concentrations. [b] Absorbance measured 25 min after the addition of sodium periodate and pyridine.

This method represents a rapid and sensitive means for the determination of formaldehyde in aqueous solutions. Total analysis time for a single determination is 30 min, with as many as 30 samples being analyzed per hour.

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Nidation Inhibition by Simple Ergoline Derivatives

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Abstract □ The ability of four ergoline-type compounds (elymoclavine, its *O*-benzoate and *O*-carbamate, and *N*-methyl-6,7-seco-elymoclavine) to inhibit nidation in rats was determined and found to parallel their prolactin-inhibiting activity.

Keyphrases □ Nidation—inhibition by four ergoline-type compounds, rats, relationship to prolactin-inhibiting ability □ Ergoline-related compounds—inhibition of nidation, relationship to prolactin-inhibiting ability □ Elymoclavine and related compounds—inhibition of nidation, rats

Research interest in the pharmacology of ergot alkaloids is currently focusing on two areas: their antihypertensive activity and their effect on hormone release from the anterior pituitary gland. The latter effect, primarily due to a direct inhibition of the synthesis and release of prolactin, manifests itself in the inhibition of some prolactin-dependent processes, *e.g.*, lactation in various animals and in humans, development of certain types of mammary tumors, and implantation of the fertilized egg (nidation) in rats and mice (1). While nidation inhibition in rats is generally thought to be predominantly or exclusively the result of prolactin inhibition, Flückiger and Wagner (2) obtained results that led them to conclude that 2-bromo-α-ergokryptine, a modified peptide ergot alkaloid which is undergoing clinical evaluation, has a specific nidation-inhibiting effect.

Since it has been shown (1, 3–5) that the peptide portion of these alkaloids is not required for activity, it was of interest to compare the ability of some simple ergolenes to inhibit prolactin release and nidation. For this comparison, four compounds were se-