difference was calculated, and the anti-inflammatory effect was established from the results obtained.

Acute Toxicity—Mice of both sexes (15–20 g) were fasted for 2 hr. Suspensions (1% gum tragacanth) of the test compounds were administered orally, 0.1 ml/10 g of body weight. The LD_{50} was calculated after observation for 1 week (Table III).

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Rapid and Sensitive Colorimetric Determination of Cobalt(II)

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Abstract □ A highly selective and sensitive spectrophotometric determination of cobalt(II) was developed. 7-Nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt was used as the chromogenic reagent for color development. Although other metals form colored chelates with the ligand, it was possible to develop a selective method using McIlvaine's pH 8 citric acid–phosphate buffer. Under these conditions, iron(II), iron(III), copper(II), zinc(II), and manganese(II), minerals likely to be compounded with cobalt(II) in geriatric formulations, do not interfere with the precision of the method or the color development. Calcium(II) and magnesium(II) do not form colored chelates with the used ligand. Hormones, vitamins, and additives likely to be present along with the cobalt ion in pharmaceutical formulations do not interfere. The sensitivity is 0.37 μg of cobalt(II)/ml of sample solution.

Keyphrases □ Cobalt(II)—colorimetric analysis, pharmaceutical formulations □ Colorimetry—analysis, cobalt(II) in pharmaceutical formulations □ Chelating agents—7-nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt used in colorimetric analysis of cobalt(II) in pharmaceutical formulations

Cobalt(II) salts are common ingredients in geriatric preparations along with other mineral salts, vitamins, and hormones. Various analytical procedures for the determination of cobalt(II) are available including volumetric (1), polarographic (2), colorimetric (3–7), fluorometric (8, 9), atomic absorption (10), ion-exchange chromatographic (11), and other miscellaneous (12) methods. Nevertheless, the analysis of cobalt(II) in multicomponent pharmaceutical preparations has not been frequently reported.

In a previous study (13), the chelating properties of 7-nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt were successfully used to develop a highly sensitive method for the determination of iron(II). Accordingly, it was decided to investigate the cobalt(II) complexing properties of this chelating agent to develop an analytical procedure for cobalt(II) that would not necessitate its prior separation from other mineral ions, vitamins, and hormones present in geriatric formulations.

EXPERIMENTAL

Apparatus—A recording spectrophotometer was used to determine the absorbance and obtain the spectra.

Materials—7-Nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt was prepared according to a reported method (14).

Cobalt sulfate, ferrous sulfate, copper sulfate, zinc sulfate, manganese sulfate, magnesium sulfate, calcium chloride, potassium chloride, hydrochloric acid, citric acid, disodium hydrogen phosphate, sodium acetate, acetic acid, and sodium carbonate were analytical grades.

Ethinyl estradiol, methyltestosterone, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, cyanocobalamin, nicotinamide, ascorbic acid, starch, lactose, and glucose were pharmacopeial grades.

Reagents and Solutions—The following solution were used: ligand solution, 0.0025 *M* in double-distilled water; acetic acid–sodium acetate buffer, pH 3.42–5.89 (15); Clark and Lub's potassium chloride–hydrochloric acid buffer, pH 1.0–2.2 (16); McIlvaine's citric acid–phosphate buffer, pH 2.2–8.0 (16); and Sorensen's phosphate buffer, pH 5.0–8.0 (16). Double-distilled water was used throughout.

Standard Solution of Cobalt Sulfate ($CoSO_4 \cdot 7H_2O$, 0.005 M)—About 0.15 g of cobalt sulfate, accurately weighed, was placed in a 100-ml volumetric flask and dissolved and diluted to volume with water. Appropriate dilutions were made from this stock solution.

Table I—Continuous Variation Mixtures[a]

Component	Solution Number										
	1	2	3	4	5	6	7	8	9	10	11
Cobalt(II)[b]	0.0	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
Water	2.0	2.0	2.0	2.0	2.0	2	2.0	2.0	2.0	2.0	2
Reagent[b]	2.0	1.8	1.4	1.4	1.2	1	0.8	0.4	0.4	0.2	0

[a] Values are the volumes in milliliters. [b] Equimolar cobalt(II) and reagent stock solutions were used to prepare each mixture.

Preparation of Assay Solutions—*For Pure Cobalt Sulfate*—Appropriate volumes of the standard cobalt sulfate solution were used as the assay solutions.

For Synthetic Mixtures—An accurately measured volume of the synthetic mixture solution, equivalent to about 0.12 mg of cobalt sulfate, was transferred to a 10-ml volumetric flask and diluted to volume with water. This solution was the assay solution.

For Determination of Cobalt in Cyanocobalamin—About 5 mg of cyanocobalamin, accurately weighed, was placed in a small crucible, 1 drop of sulfuric acid was added, and the mixture was ignited for 30 min and then allowed to cool. One drop of sulfuric acid and 1 drop of nitric acid were added and the mixture was ignited again until no fumes appeared and the residue became violet in color. The residue was dissolved in 3 ml of distilled water and neutralized with solid sodium carbonate until effervescence ceased. It was then transferred quantitatively to a 10-ml volumetric flask, diluted to volume with distilled water, and filtered; the first portion of the filtrate was discarded. The clear filtrate was the assay solution.

Color Development—Into separate stoppered test tubes, each containing 4 ml of McIlvaine's citric acid–phosphate buffer, pH 8, was pipetted 2 ml of the ligand solution. After thorough mixing, 2 ml of the standard solution, 2 ml of the assay solution of appropriate dilution, and 2 ml of distilled water were added. The mixture was shaken thoroughly, and the absorbance of the standard and sample solutions was determined at 525 nm in 1-cm cells against the blank using a suitable spectrophotometer.

The concentration of cobalt(II) in the final sample solution (micrograms per milliliter) was obtained from a previously constructed standard calibration curve.

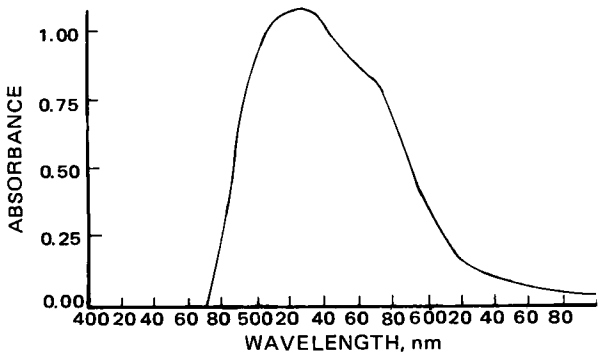

Figure 1—*Absorption spectrum for cobalt(II)–ligand complex in acetic acid–sodium acetate buffer, pH 5 [cobalt(II) = 5.4 μg/ml].*

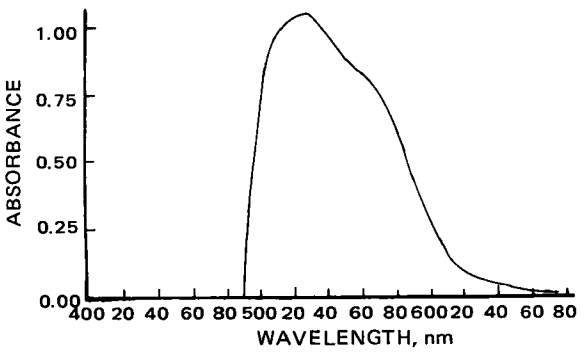

Figure 2—*Absorption spectrum for cobalt(II)–ligand complex in McIlvaine's citric acid–phosphate buffer, pH 8 [cobalt(II) = 3.67 μg/ml].*

Determination of Chelate Combining Ratio—Aqueous solutions of cobalt sulfate (0.001 *M*) and of the reagent (0.001 *M*) were prepared. Various amounts of each component were added to 11 test tubes. The concentrations of water and McIlvaine's citric acid–phosphate buffer, pH 8, remained constant in each mixture (Table I). The total number of moles was also kept constant. The absorbance of the solution in each tube was measured at 525 nm in a 1-cm cell against the blank using a suitable spectrophotometer.

RESULTS AND DISCUSSION

Acetic acid–sodium acetate buffer, pH 5, was used in the early stages of this work for the development of color of cobalt(II) with the ligand. The color developed immediately and exhibited λ_{max} at 525 nm (ϵ = 13,913) (Fig. 1). The relationship between absorbance at 525 nm and concentration was linear up to 5.86 μg/ml of cobalt(II)/ml of sample solution.

When trying to determine cobalt(II) in the presence of other metal ions, the method suffered from serious interferences. Other metal ions, namely zinc(II), manganese(II), iron(II), iron(III), and copper(II), absorbed at wavelengths close to that of the λ_{max} of the cobalt(II) complex. Accordingly, it was decided to investigate the chelation reaction to find out the proper system that would permit selective determination of cobalt(II) without interference from other metal ions. To achieve this objective, a series of buffer systems of various compositions and pH ranges was selected as vehicles for the development of the color reaction.

Variation of the pH of the acetic acid–sodium acetate buffer in the 3.42–5.89 range did not solve the problem due to the partial overlap of the other metal chelates near 525 nm, the λ_{max} of the cobalt chelate. Furthermore, low pH buffers such as that of Clark and Lub's (pH 1.0–2.2) failed to solve this problem.

When the pH of the medium was raised by the use of Sorensen's phosphate buffer, pH 5–8, the problem of interference was solved at pH 8. However, this procedure was hampered by the rather low absorptivity (ϵ = 9328) of the formed chelate, a fact that reflected itself upon the sensitivity of the method.

By using McIlvaine's citric acid–phosphate buffer solutions (pH 2.2–8.0), it was possible to attain proper conditions for the determination of cobalt(II) without interference from other metal ions and with high sensitivity (Table II). When a pH 8 solution of this buffer system was used as the milieu for color development, iron(II) and cobalt(II) formed colored chelates with absorption peaks at 710 and 525 nm, respectively. The other metal ions did not form any colored complexes under these conditions.

The absorption peak of iron(II) was sufficiently far from that of cobalt(II), thus permitting selective determination. The molecular absorptivity of the cobalt(II) complex as calculated from the absorption curve (Fig. 2) under these conditions was 18,478. The re-

Table II—Effect of McIlvaine's Citric Acid–Phosphate Buffer on Chelate Color Product of Cobalt(II)[a]

pH	λ_{max}, nm	*A*	λ_{max}, nm	*A*
2.2	400	0.7625	525	0.912
3	400	0.6750	525	1.112
4	440	0.6500	525	1.062
5	—	—	525	1.012
6	—	—	525	0.987
7	—	—	525	0.800
8	—	—	525	1.037

[a] Cobalt(II) = 3.67 μg/ml.

Table III—Determination of Cobalt(II) in the Presence of Hormones, Vitamins, Minerals, and Excipients Usually Found in Geriatric Preparations

Substances Added (per 0.12 mg Cobalt Sulfate), mg		Cobalt Sulfate Recovered[a]			
		mg	%	*SD*	*CV*
Ethinyl estradiol	0.01	0.1200	100.0 ± 0.2	0.30	0.30
Methyltestosterone	0.12	0.1200	100.0 ± 0.1	0.14	0.14
Thiamine hydrochloride	5.00	0.1199	99.9 ± 0.2	0.26	0.26
Riboflavin	2.50	0.1210	100.0 ± 0.1	0.14	0.13
Pyridoxine hydrochloride	0.50	0.1199	99.9 ± 0.6	0.90	0.90
Folic acid	0.50	0.1199	99.9 ± 0.2	0.26	0.26
Cyanocobalamin	0.001	0.1200	100.0 ± 0.0	0.00	0.00
Nicotinamide	5.00	0.1199	99.9 ± 0.3	0.41	0.41
Ascorbic acid	75.00	0.1210	100.8 ± 0.2	0.50	0.49
Ferrous sulfate	100.00	0.1210	100.8 ± 0.1	0.14	0.13
Zinc sulfate	1.50	0.1990	99.9 ± 0.3	0.26	0.26
Manganese chloride	1.50	0.1200	100.0 ± 0.2	0.30	0.30
Magnesium sulfate	100.00	0.1200	100.0 ± 0.1	0.00	0.00
Starch	100.00	0.1200	100.0 ± 0.0	0.00	0.00
Glucose	100.00	0.1200	100.0 ± 0.0	0.00	0.00
Lactose	100.00	0.1200	100.0 ± 0.0	0.00	0.00

[a] Average of three determinations.

lationship between absorbance and concentration was linear up to 4.44 μg of cobalt(II)/ml of sample solution; the lower limit for detection was 0.37 μg of cobalt(II)/ml of sample solution.

Under the conditions developed for the specific color formation, the combining ratio of the metal to ligand was determined by the method of continuous variation (17) and was found to be 1:3 (Fig. 3). This value is in agreement with the finding of Aly *et al.* (14), who, in a qualitative study, separated the cobalt(II)–ligand complex and determined its structure by elemental microanalysis.

The specificity of the method for the determination of cobalt(II) in the presence of other minerals, hormones, certain vitamins, and excipients likely to be present in geriatric formulations is shown in Table III. Several different amounts of standard solution, or several different weights of each ingredient shown in Table III, either singly or in admixture with all of the others, each approximating sample weights of commercial pharmaceutical dosage forms, were spiked with a known amount of cobalt sulfate and the analysis was performed. The average recovery of cobalt(II) from these synthetic mixtures ranged from 99.5 to 100.2%.

Application of this method for the determination of cobalt in cyanocobalamin gave a percentage recovery of 100.0 ± 0.3. Although the developed method might be suitable for certain formulations of relatively high cyanocobalamin content, it is not applicable to single-dose analysis of formulations with less than 12 μg of cyanocobalamin/dose.

The results demonstrate that the proposed method is applicable for the simultaneous determination of iron and cobalt. Only a single chromogen is used; the method is both simple and rapid. High sensitivity of the ligand chromogenic reagent facilitates the determination of submicrogram amounts of the elements. Moreover, the ligand is easily synthesized. Interferences under the developed condition are nil. The method is strongly recommended over other methods.

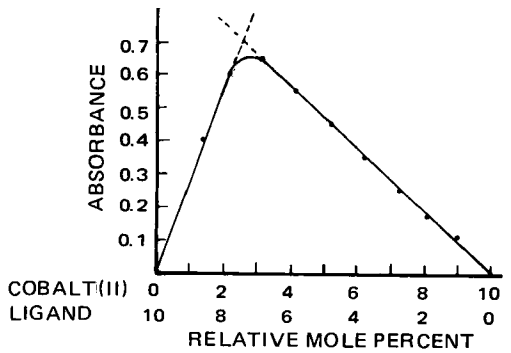

Figure 3—*Continuous variation plot for cobalt(II)–ligand complex in McIlvaine's citric acid–phosphate buffer.*

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