the oscillator is at the point of going out of oscillation.

The polarograph is adjusted so that a constant voltage of the same polarity as the bias voltage developed by the oscillator is impressed across the 1000-ohm resistor, R_2, in series with the grid-leak resistor. Any voltage from zero up to the span voltage setting of the polarograph may be selected by adjustment of the polarograph bridge control. Better control of the voltage is attained with lower values of span voltage. Thus, if a voltage of 0.1 volt is to be applied to the resistor, R_2, and the span voltage is 1 volt, the bridge is set to 10% of the span. If a 0.4-volt span is used, the bridge setting is 40%. It is easier to adjust the bridge to the proper value at the lower span voltage because the increment per dial division is considerably less, and the backlash and overtravel in the adjustment of the bridge control produce correspondingly smaller variations in applied voltage.

The polarograph bridge control setting depends upon the type of system being titrated. For a strong acid, the per cent of span voltage is so selected that at the beginning of a titration the recorder pointer is near the 280-mm. limit. If a weak acid is titrated, the applied voltage is such that the pointer is near the zero limit.

A current measuring sensitivity of 0.06 μa. per mm. is satisfactory for most titrations. The range used in this study was 0.06 to 1.5 μa. per mm.

TITRATIONS

To check the performance of the wide-range high-frequency titration apparatus, various acidimetric titrations were carried out. The results for the titration of aliquots of standardized hydrochloric acid diluted to 150 ml. and titrated with standardized sodium hydroxide are shown in Table II. The most noteworthy result listed is the one for the direct titration of 100.3 ml. of 3N hydrochloric acid solution with 4N sodium hydroxide. For this titration, the titration vessel was raised 2 cm. above the normal position. The normal oscillator circuit was altered by installing a 100-$\mu\mu$f. capacitor between the cathode of the 955 tube and ground (shown as C_7 in the circuit diagram). The titration curve is shown in Figure 6. The greatest concentration previously titrated directly was 1.15N hydrochloric acid (*9*).

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Colorimetric Determination of Carboxylic Acid Derivatives as Hydroxamic Acids

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▶Aqueous alkaline hydroxylamine is widely used to form hydroxamic acids which may be estimated by their color reaction with ferric ion, in the analytical determination of carboxylic acid derivatives. This study determined the influence of substituents on the ease of hydroxamic acid formation by monocarboxylic acid derivatives. The esters tested required 2 to 120 minutes for maximum hydroxamic acid formation. The data show a correlation beween structure and rate of hydroxamic acid formation. Bulky and electron-donating groups inhibit hydroxamic acid formation by substituted benzoic acid esters. Replacement of the hydrogen atoms of formamide by a methyl or phenyl group results in decreased rate of hydroxamic acid formation. In setting up the conditions for the determination of α-hydroxy, α-amino, and α-chloro aliphatic esters as hydroxamic acids, the hypochromic effect of these substituents must be considered.

CARBOXYLIC acid derivatives react with hydroxylamine to form hydroxamic acids which may be estimated by their color reaction with ferric iron. This reaction is used extensively in analytical chemistry. In 1934, Feigl and coworkers (*4*, *5*) introduced the use of the ferric–hydroxamic acid reaction as a spot test for carboxylic esters. Lipmann and Tuttle (*14*) based a specific quantitative method for acyl phosphates on their reaction with hydroxylamine in water at pH 6. Hill (*11*, *12*) applied the ferric–hydroxamate reaction to the quantitative determination of long-chain fatty acids and esters. In 1949, Hestrin (*10*) developed a rapid colorimetric micromethod for the quantitative estimation of short-chain carboxylic acid esters, lactones, and anhydrides based on their ready conversion to hydroxamic acids when added to alkaline hydroxylamine in aqueous medium. He concluded that the hydroxylamine reaction of soluble carboxylic acid esters was completed within 1 minute and predicated his quantitative test on this short reaction time. The hydroxylamine reaction has also been used for the determination of carboxylic acid amides (*3*, *6*).

Bergmann (*2*) employed essentially Hestrin's procedure (*10*) with longer intervals of reaction at various temperatures. He studied the comparative

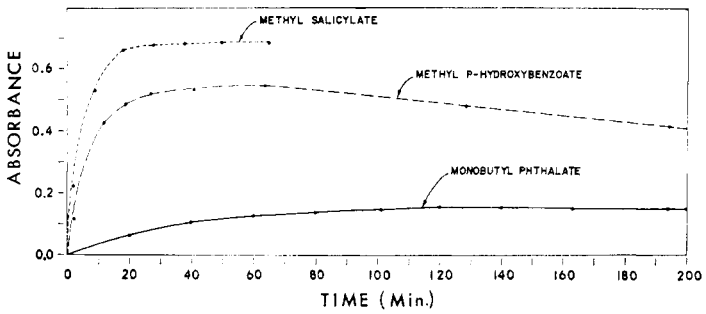


Figure 1. Comparative rates of hydroxamic acid formation by several carboxylic acid esters ($2.5 \times 10^{-3}M$) at 25° C.

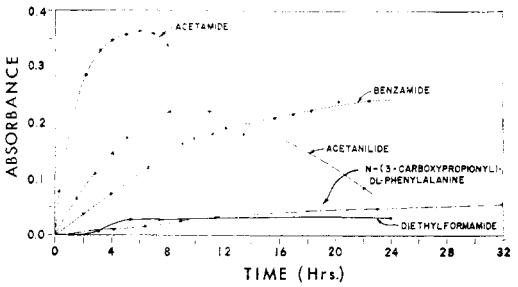


Figure 2. Comparative rates of hydroxamic acid formation by monocarboxylic acid amides ($2.5 \times 10^{-3}M$) at 25° C.

rates of hydroxamic acid formation by several amides and observed that they react much more slowly than the corresponding esters. The influence of such variables as temperature, pH, and concentration of reactants on the ease of hydroxamic acid formation by carboxylic acid derivatives was studied by Hestrin (*10*), Bergmann (*2*), and Wollish and Schmall (*17*). They also studied the effect of acidity and iron concentration on the color reaction between hydroxamic acids and ferric ion, and the absorption maxima of the ferric complex of several hydroxamic acids.

Further study of the hydroxylamine reactions of monocarboxylic acid derivatives seemed desirable when it was observed that several soluble esters react very slowly with aqueous alkaline hydroxylamine. The present study was undertaken to determine the effect of substituents on the ease of hydroxamic acid formation by monocarboxylic acid derivatives and the potential application of these findings to analytical chemistry.

Table I. Hydroxylamine Reactions

	Reaction Time for Maximum Hydroxamic Acid Formation, Minutes	Corresponding Iron-Complex Absorbance[a] at 540 Mμ
Monocarboxylic Acid Esters[b] (25.0° C.)		
Ethyl acetate	2	0.38[c]
Methyl propionate	2	0.37[c]
Methyl butyrate	2	0.37
Monomethyl succinate	2	0.39
Ethyl benzoate	2	0.45
Methyl *o*-methoxybenzoate	2	0.61[d]
n-Butyl *o*-methoxybenzoate	15	0.54
Methyl *p*-hydroxybenzoate	60	0.55
Methyl salicylate	40	0.68[d]
n-Butyl salicylate	70	0.53[d]
Monomethyl phthalate	28	0.25
Mono-*n*-butyl phthalate	120	0.15
Methyl *o*-toluate	10	0.40
Methyl *m*-toluate	2	0.49
Methyl *p*-toluate	2	0.54
Methyl anthranilate	9	0.35
Methyl *p*-aminobenzoate	7	0.54
Acetylsalicylic acid	2	0.71[e]
Alkyl and Phenylalkyl Carboxylic Acid Esters[f] (25.0° C.)		
Ethyl acetate	2	0.38
Methyl propionate	2	0.37
Methyl butyrate	2	0.37
Monomethyl succinate	2	0.39
Glycine ethyl ester	2	0.16
l-Phenylalanyl ethyl ester	2	0.13
Acetyl-DL-phenylalanyl ethyl ester	2	0.17
l-Tyrosine ethyl ester	2	0.14
Ethyl lactate	2	0.23
Ethyl 1-β-phenylacetate	2	0.23
Ethyl DL-β-hydroxy-*n*-butyrate	2	0.37
Methyl methoxyacetate	2	0.30
Ethyl β-ethoxypropionate	2	0.39
Ethyl chloroacetate	2	0.23

[a] Zero time blanks subtracted for monocarboxylic acid esters.
[b] Ester solutions (prior to addition of hydroxylamine reagent) were $0.0025M$ with respect to ester.
[c] Hestrin obtained similar results (*10*).
[d] *o*- and *p*-Hydroxy and methoxy groups seem to exert hyperchromic effects on these absorbances.
[e] High absorbance due to production of salicylic acid as one end product of reaction.
[f] Carboxylic acid esters were $0.0025M$ before they were mixed with hydroxylamine reagent.

EXPERIMENTAL

Alkaline Hydroxylamine Reaction. The procedure and reagents were similar to those described by Hestrin (*10*) and Bergmann (*2*). The reagents included $2N$ hydroxylamine hydrochloride, $3.5N$ sodium hydroxide, $4.0N$ hydrochloric acid, and acid-iron solution ($0.74M$ ferric chloride and $0.1N$ hydrochloric acid). Solutions of carboxylic acid derivative to be tested for reaction with hydroxylamine were generally made $0.0025M$ with respect to carboxylic acid derivative and 10% (volume) with respect to methanol; they were tested shortly after preparation.

To increase the sensitivity and reproducibility of the test, the following modifications were made: The hydroxylamine hydrochloride reagent was stored in the refrigerator after preparation, and discarded after 48 hours. Immediately prior to a hydroxylamine reaction, alkaline hydroxylamine reagent was prepared by mixing equal volumes of refrigerated sodium hydroxide and hydroxylamine hydrochloride solutions. The cold solution was rapidly brought to room temperature by immersion in a water bath. Two volumes of this reagent were added to one volume of ester or amide solution. The reaction was allowed to proceed in a constant temperature bath at 25.0° C. or at room temperature. At periodic intervals a 3-ml. aliquot was transferred to a test tube and 1 ml. of the hydrochloric acid reagent added. Exactly 1 minute later, 1 ml. of acid-iron reagent was introduced. The absorbances of the resulting solution which corresponded to 1.50, 1.75, 2.0, and 2.25 minutes' standing in acidic iron solution were read with the Coleman spectrophotometer (Model 6B) at 540 mμ, and the initial absorbance was obtained by interpolation. All readings were arbitrarily taken at 540 mμ for convenience.

Organic Preparations. While a substantial number of the compounds tested could be purchased at a high grade of purity, some were prepared: hydrogen methyl succinate (*16*), monomethyl phthalate (*16*), phthalamidic

acid (*1*), succinamic acid (*13*), l-tyrosine ethyl ester hydrochloride (*15*), l-phenylalanyl ethyl ester hydrochloride (*9*), ethyl l-β-phenyllactate (*9*), and acetyl phenylalanyl ethyl ester (*8*). The melting points of these preparations agreed with those reported in the literature. *N*-(*o*-Carboxybenzoyl)-DL-phenylalanine and *N*-maleyl-DL-phenylalanine were prepared for the first time; experimental analyses were in good agreement with the theoretical analyses (*7*).

MONOCARBOXYLIC ACID ESTERS

The effect of time on hydroxamic acid formation by monocarboxylic acid esters is summarized in Table I and Figure 1. For a considerable number of esters, hydroxamic acid formation is not completed within 2 minutes. The esters tested in this study require from 2 to 120 minutes for maximum hydroxamic acid formation. Contrary to the impression of previous workers, this indicates that the period required for maximum hydroxamic acid formation by the ester under consideration must be taken into account—e.g., when Hestrin obtained a low color yield for the hydroxylamine reaction of methyl *p*-hydroxybenzoate he assumed that the abnormal absorbance was caused by the hypochromic effect of the hydroxy group (*10*). However, he ran his test for no more than 4 minutes, whereas the present study has shown that this compound requires 60 minutes for maximum hydroxamic acid formation (Figure 1). Moreover, when the hydroxylamine reactions of the hydroxybenzoate esters, methyl *p*-hydroxybenzoate, and methyl and *n*-butyl salicylate were allowed to run to completion, normal or high color values were obtained. Because a normal color yield for methyl *p*-aminobenzoate was obtained, it is likely that the low chromogenic value obtained by Hestrin for the *p*-aminobenzoic acid ester of 2,2′-dimethyl-3-dimethylaminopropanol was caused by incomplete reaction with hydroxylamine rather than the color depressant effect of the *p*-amino group.

A correlation exists between the structure of the ester under consideration and ease of hydroxamic acid formation. The speed of the hydroxylamine reaction of a series of ortho-substituted and para-substituted methyl benzoate esters varied approximately inversely with the electron releasing strengths of these substituents:

$$\text{Para substituted } O^- \ll NH_2 < CH_3$$

$$\text{Ortho substituted } O^- < COO^- \ll NH_2, CH_3 < OMe$$

The fact that methyl *p*-hydroxybenzoate requires 60 minutes for maximum hydroxamic acid formation, while ethyl benzoate requires only 2 minutes, suggests that in the case of the *p*-hydroxy ester the presence of a strategically located electron-donating group inhibits reaction with hydroxylamine. Steric hindrance by bulky groups is indicated by the slower rates of hydroxamic acid formation of *n*-butyl *o*-substituted benzoate esters as compared to that of the analogous methyl ester—e.g., monomethyl phthalate requires 28 minutes while mono-*n*-butyl phthalate requires 120 minutes for maximum hydroxamic acid formation. Benzoic acid esters which undergo hydroxamic acid formation least readily have both bulky and electron releasing groups. Acetylsalicylic acid, which is a phenol rather than a benzoic acid ester, reaches maximum hydroxamic acid formation within 2 minutes in spite of the vicinal negative carboxylate ion. In view of the fact that the presence of strategically located bulky and/or electron donating groups inhibits hydroxamic acid formation, it appears that the reaction between monocarboxylic acid esters and the hydroxlamine reagent proceeds according to a nucleophilic displacement mechanism.

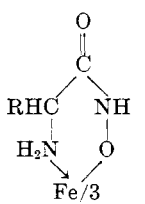
Table II. Hydroxylamine Reaction of Monocarboxylic Amides

	Temp. of Reaction, ° C.	Reaction Time for Maximum Hydroxamic Acid Formation, Hours	Corresponding Iron-Complex Absorbance (at 540 Mμ)
Formamide (*2*)[a]	26	1	...
Fluoroacetamide (*2*)[a]	26	1	...
N-Methylacetamide (*2*)[a]	26	24	...
Acetamide (*2*)[a]	26	6, 8	...
Acetamide[b]	25.0	6	0.36
Acetanilide[b]	25.0	9	0.22
N-Maleyl-DL-phenylalanine[b]	25.0	8[c]	0.05
Phthalamidic acid[b]	25.0	19[c]	0.06
N-(*o*-carboxybenzoyl)-DL-phenylalanine[b]	25.0	18[c]	0.04
Succinamic acid[b]	25.0	6.5	0.25

[a] Concentration of amides tested by Bergmann (*2*) prior to addition of hydroxylamine reagent was 0.005 to 0.01*M*.
[b] Concentration of amide solution (prior to addition of hydroxylamine reagent) was 0.0025*M* with respect to amide.
[c] No maximum hydroxamic acid formation discerned by end of this period.

α-SUBSTITUTED ALIPHATIC CARBOXYLIC ACID ESTERS

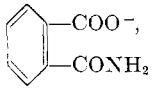
The results pertaining to hydroxamic acid formation by α-substituted aliphatic carboxylic acid esters are summarized in Table I. Although the esters tested complete maximum hydroxamic acid formation within 2 minutes, they yield abnormally low absorbances. Whereas 0.0025*M* acetate, propionate, and butyrate solutions yield ferric-hydroxamate absorbances in the range 0.38, 0.0025*M* α-aminoacid ester solutions yield absorbances 0.13 to 0.16. The hypochromic effects of the substituents increased in the following order: α-methoxy < α-chloro = α-hydroxy < α-NHCOR < α-amino. The abnormally low color yields that are obtained with α-substituted aliphatic esters must be considered when procedures are being set up for their determination as hydroxamic acids. Ethyl β-ethoxypropionate and ethyl DL-β-hydroxy-*n*-butyrate yield normal absorbances. The following type iron-hydroxamate chelated structure is suggested as an explanation for the abnormally low color yields produced by the α-amino and α-hydroxy aliphatic esters tested in this series:



MONOCARBOXYLIC ACID AMIDES

The results pertaining to hydroxamic acid formation by monocarboxylic acid amides are summarized in Table II and Figure 2. They agree with Bergmann's findings (*2*) that monocarboxylic acid amides which have no electron-withdrawing substituents react very slowly with the hydroxylamine reagent. Many amides react so slowly that their quantitative determination as hydroxamic acids is not feasible. When the amides tested are arranged in decreasing order of speed of reaction, it becomes apparent that the replacement of one hydrogen of formamide by methyl, higher alkyl, or a phenyl group results in retardation of the hydroxylamine reaction:

$$HCONH_2 = FCH_2CONH_2 > CH_3CONH_2 = {}^-OOCCH_2CH_2CONH_2 > CH_3CONHC_6H_5 > CH_3CONHCH_3,$$

$$C_6H_5CONH_2 > \text{  }$$

$^{-}OOCCH_2CH_2CONHCHCOO^{-}$

$\quad\quad\quad\quad\quad |$

$\quad\quad\quad\quad\quad CH_2C_6H_5$

$\quad\quad\quad\quad\quad\quad HCON(CH_2CH_3)_2$

The inhibitory influence of the methyl or alkyl group may be attributed to their greater electron-releasing properties over that of hydrogen. Conversely, the inductive effect of the fluorine atom in fluoracetamide accelerates nucleophilic attack by hydroxylamine. The influence of the methyl and phenyl groups on the acid strengths of formic acid, and the influence of fluorine on the acid strength of acetic acid, run parallel to their effects on the velocities of the hydroxylamine reactions of the respective amides. In the case of bulky neighboring groups, inhibition due to steric hindrance must also be noted. The reaction between monocarboxylic acid amides and alkaline hydroxylamine is impeded by bulky and electron-donating groups, which is in agreement with a nucleophilic mechanism for the reaction.

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Spectrochemical Determination of Fluorine in Porcelain Enamel Frits

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▶ Rapid comparison of porcelain enamel frits is facilitated by a spectrochemical determination of fluorine. Calcium carbonate and graphite are mixed with the sample to produce calcium fluoride bands. The intensities of the band head at 5291.00 A., corrected for background, are compared with those of chemically analyzed standards. The range of fluorine covered is from a few tenths to 6% in frits. Some factors considered are the emulsion, amperage, time of exposure, and band components. Various amounts and kinds of additives are evaluated.

Numerous articles indicate the inadequacy of many methods for determining fluorine. Classical wet and photometric methods are time-consuming. An attractive approach to fluorine determination is by emission spectroscopy. Frits containing fluorine from a few tenths to 6% can be analyzed by this fast, convenient method.

The spectrographic method is based on the emission of calcium fluoride bands in the direct current arc. Ahrens (*2*), Harrison, Lord, and Loofbourow (*6*), and many others, show these molecules as diatomic with no indication of valence. The molecular bands of calcium fluoride (CaF) have many heads, including ones at 5291.00, 6036.92, 6064.40, and 6086.91 A. The individual components of these bands are often distinct enough for densitometry. In the spectrochemical determination of fluorine, the emphasis has been on water, rock, and slag samples (*1–3*, *5*, *7*), using the calcium fluoride bands and the bands of barium fluoride (BaF) and strontium fluoride (SrF) to a lesser extent (*2*). Suitable methods for fluorine determination in enamel frits may be established after considering the important variables.

Porcelain enamel frits, which are the basic material for porcelain enameling, are selected glasses of widely varying composition. These molten glasses are fritted by air or water quenching to form brittle flakes or granules. One of these frits has the composition shown in Table I.

Fluorine may be added to a glass batch as an alkali silicofluoride or fluorspar. Fluoride aids in smelting the raw batch. In zircon enamels, it increases the opacity. The firing temperature of porcelain enamel is sometimes lowered by using glasses high in fluorine. Fluorine losses occur during smelting as silicon tetrafluoride, boron trifluoride, and alkali fluorides.

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Table I. Typical Frit Composition, Zircon Cover Coat

	%		%
SiO_2	26.6	ZnO	3.0
B_2O_3	22.2	CaO	7.7
Na_2O	10.1	F_2	6.0
Al_2O_3	5.7	K_2O	1.9
BaO	0.4	P_2O_5	2.0
ZrO_2	16.8		

PHOTOGRAPHIC EMULSIONS AND APPARATUS

Because the bands of calcium fluoride are in the visible region, the usual ultraviolet emulsions were not used. The Eastman I-L emulsion (Eastman Kodak Co., Rochester, N. Y.) was preferred over the slower but finer-grained IV-L plate and the faster 103-F plate, which has a background too high for densitometry.

A Bausch & Lomb dual-grating spectrograph was used with the standard optics. As the primary aperture setting was 2.5 mm., the entire analytical gap is used. The grating of 15,000 lines per inch with a reciprocal dispersion of 8 A. per mm. (blazed at 6000 A.) was considerably more sensitive than the grat-