

propionamide on the permeability of the *in situ* rat intestine. It appears, therefore, that the nonlinear relationship between the apparent absorption rate constant of prednisolone and the concentration of *N,N*-di-*n*-propylpropionamide in the intestinal drug solution can be explained most readily if it is assumed that the intestine behaves as a series of barriers toward prednisolone and that *N,N*-di-*n*-propylpropionamide increases only the permeability of the first barrier. This could conceivably occur if the permeability of the other barriers to *N,N*-di-*n*-propylpropionamide is very high compared to that of the first barrier so that the concentration of the amide would be effectively zero beyond the first barrier. Alternatively, interaction between the steroid and *N,N*-di-*n*-propylpropionamide may occur only in the first barrier because it may differ in structure or composition from the other barriers.

It must be emphasized that what we have discussed here are functional models, which may or may not have physical reality. Such a functional approach is quite appropriate since this is how most of the present knowledge about biological membranes has been obtained. The studies described here represent a new dimension in this endeavor since they involved the use of two interacting substances, rather than single solutes, as probes to explore the characteristics of biologic barriers.

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## Rates of Hydrolysis of *N*-Chlorinated Molecules

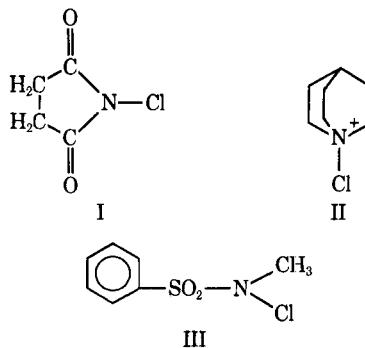
A. HUSSAIN, T. HIGUCHI, A. HURWITZ, and IAN H. PITMAN<sup>▲</sup>

**Abstract** □ Formation and hydrolysis rate constants were calculated for *N*-chlorosuccinimide, *N*-chloroquinuclidinium ion, and *N*-chloro-*N*-methylbenzenesulfonamide in water. The rates of hydrolysis of these compounds were much slower than the rates at which they are known to transfer their active chlorine to nitrogenous bases in water. Hence, the present results confirm an earlier suggestion that chlorine transfer between nitrogenous bases in water is not mediated by hydrolysis of the *N*-chloro compounds to hypochlorous acid but involves a direct reaction between the nitrogenous chlorine donor and acceptor molecules. The kinetic results are also consistent with a postulate that the rate-determining step in the reaction between nitrogenous compounds and hypochlorous acid involves reaction between the amide and imide anions and amine neutral molecules and neutral molecules of hypochlorous acid. The rate-determining step in the hydrolysis reactions appears to involve attack of hydroxide ion on the *N*-chloro compounds.

**Keyphrases** □ *N*-Chlorosuccinimide—formation and hydrolysis rate constants in water □ *N*-Chloroquinuclidinium ion—formation and hydrolysis rate constants in water □ *N*-Chloro-*N*-methylbenzenesulfonamide—formation and hydrolysis rate constants in water □ Hydrolysis rate constants—*N*-chlorinated molecules

The bactericidal and detoxifying action of *N*-chlorinated molecules such as *N*-chloramine results from their abilities to transfer their active chlorine to other molecules. The mechanism of the chlorine transfer was postulated by Higuchi and his coworkers (1, 2) to involve a direct reaction between the organohalogenating agent and another receptor rather than *via* an intermediate forming of hypochlorous acid.

One purpose of this study was to confirm this postulation by showing that the rates of hydrolysis of *N*-chlorosuccinimide (I), *N*-chloroquinuclidinium ion (II), and *N*-chloro-*N*-methylbenzenesulfonamide (III) in water are slower by several orders of magnitude than the rates at which these molecules are known to transfer positive chlorine to other molecules. Another purpose was to obtain information about the mechanism of formation and hydrolysis of *N*-chloro compounds. Weil and Morris (3) previously showed that pH-rate profiles for formation of chloramines from hypochlorous acid and amines can be explained by either ionic ( $\text{OCl}^- + \text{R}_2\text{NH}_2^+$ ) or neutral molecule ( $\text{HOCl} + \text{R}_2\text{NH}$ ) mechanisms. Although these authors favored the neutral molecule mechanism, it was felt that more



**Table I—Rate Constants<sup>a</sup> for Formation<sup>b</sup> and Hydrolysis<sup>c</sup> of I, II, and III**

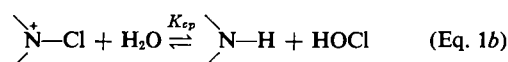
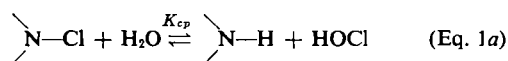
Compound	pH	10 <sup>3</sup> [>NH] <sub>T</sub> , M <sup>d</sup>	10 <sup>3</sup> [HOCl] <sub>T</sub> , M	10 <sup>4</sup> k <sub>obs.</sub> , sec. <sup>-1</sup>	k' <sub>form.</sub> , M <sup>-1</sup> sec. <sup>-1</sup>	10 <sup>7</sup> k' <sub>hyd.</sub> , M <sup>-1</sup> sec. <sup>-1</sup>
I	1.96	8.27	0.80	0.36	0.43	0.084
	2.95	10.00	1.25	5.00	5.0	0.975
	3.41	5.00	1.25	6.50	13.0	2.53
	3.55	2.50	0.50	4.75	19.0	3.71
	3.55	2.50	0.50	4.75	19.0	3.71
	3.92	10.00	2.50	38.0	38.0	7.41
	3.92	5.00	1.25	20.0	40.0	7.80
	4.92	2.50	0.635	115.0	460.0	8.97
	II	3.63	8.16	0.128	0.898	1.10
3.75		8.16	0.128	1.18	1.45	103.0
4.00		8.16	0.128	2.10	2.57	182.0
4.06		8.16	0.128	2.41	2.95	209.0
4.25		8.16	0.128	3.73	4.57	324.0
4.63		8.16	0.128	8.94	10.96	776.0
III	3.55	1.68	0.168	0.447	2.66	0.292
	3.90	1.68	0.168	0.907	5.40	0.592
	4.21	1.68	0.168	1.798	10.70	1.17
	4.70	1.68	0.168	4.956	29.50	3.24

<sup>a</sup> In 0.1 M acetic acid-sodium acetate buffers. <sup>b</sup> Calculated from spectrophotometric measurements. <sup>c</sup> Calculated as described in text. <sup>d</sup> [>NH]<sub>T</sub> in the reactions of II.

systems should be studied in depth before this question could be resolved.

### THEORETICAL

**Calculation of Hydrolysis Rate Constants**—The *N*-chloro compounds examined in this study were extremely stable in water, and their rates of hydrolysis could not be measured directly. However, on the assumption that the hydrolysis and formation reactions in Eq. 1a (for reactions of I and III) and 1b (for II) proceed through the same transition states:



the hydrolysis rate constants could be calculated from the known hydrolytic equilibrium constants (*K<sub>cp</sub>* values in Eq. 1) and the rate constants for formation of the *N*-chloro compound from the amide, imide, or amine in aqueous hypochlorous acid. The *K<sub>cp</sub>* values for many *N*-chloro compounds were measured in this laboratory (2, 4) or were available from other sources (5), and the formation rate constants were readily calculated from measurements of changes in spectra that occurred when solutions of the nitrogen-containing compound and hypochlorous acid were mixed at 25° in sodium acetate-acetic acid buffers. Spectroscopic measurements indicated that the reactions were first order in hypochlorous acid when the initial concentration of amide, imide, or amine was at least 5 times that of hypochlorous acid and that they proceeded essentially to completion in favor of the *N*-chloro compound. Values of the pseudo-first-order rate constants (*k<sub>obs.</sub>* values) were calculated from

**Table II—Acid Dissociation Constants<sup>a</sup> and *K<sub>cp</sub>* Values<sup>a</sup>**

Compound	pKa	<i>K<sub>cp</sub></i>
I	<0 <sup>b</sup>	1.95 × 10 <sup>-8</sup>
II	<0 <sup>b</sup>	7.08 × 10 <sup>-8</sup>
III	<0 <sup>b</sup>	1.10 × 10 <sup>-8</sup>
<i>N</i> -Chlorodimethylamine	0.46 <sup>c</sup>	1.18 × 10 <sup>-18</sup>
Succinamide	9.62	
Quinuclidinium ion	10.95	
<i>N</i> -Methylbenzenesulfonamide	11.35	
Dimethylammonium ion	10.73	
Hypochlorous acid	7.53	

<sup>a</sup> Sources given in Reference 6. <sup>b</sup> pKa value for the conjugate acids of these compounds are not known but are expected to be very low. <sup>c</sup> pKa of the conjugate acid.

plots of log (*D<sub>∞</sub>* - *D*) against time and are listed in Table I together with the second-order rate constants for formation of the *N*-chloro compound (*k'<sub>form.</sub>*) which were calculated from them.

The hydrolysis rate constants for I and III were calculated in the following way. The rate law for the formation reaction at any pH was given by:

$$\text{rate}_{\text{form.}} = k'_{\text{form.}} \left[ \begin{array}{c} \diagdown \\ \text{NH} \\ \diagup \end{array} \right]_T [\text{HOCl}]_T \quad (\text{Eq. 2})$$

where [>NH]<sub>T</sub> and [HOCl]<sub>T</sub> are the total concentrations of all ionic species of the amide or imide and hypochlorous acid, respectively, at a particular pH. On the assumption that the hydrolysis reaction proceeded by the reverse reaction to formation, its rate law would be given by:

$$\text{rate}_{\text{hyd.}} = k_{\text{hyd.}} \left[ \begin{array}{c} \diagdown \\ \text{NCl} \\ \diagup \end{array} \right]_T \quad (\text{Eq. 3})$$

where [>NCl]<sub>T</sub> is the total concentration of all ionic species of the *N*-chloro compound. At equilibrium, the hydrolysis and formation rates would be equal with:

$$k'_{\text{form.}} [\text{HOCl}]_T \left[ \begin{array}{c} \diagdown \\ \text{NH} \\ \diagup \end{array} \right]_T = k_{\text{hyd.}} \left[ \begin{array}{c} \diagdown \\ \text{NCl} \\ \diagup \end{array} \right]_T \quad (\text{Eq. 4})$$

Hence:

$$k_{\text{hyd.}} = k'_{\text{form.}} \frac{[\text{HOCl}]_T \left[ \begin{array}{c} \diagdown \\ \text{NH} \\ \diagup \end{array} \right]_T}{\left[ \begin{array}{c} \diagdown \\ \text{NCl} \\ \diagup \end{array} \right]_T} \quad (\text{Eq. 5})$$

In terms of concentrations of >NCl, >NH, and HOCl, this equation becomes:

$$k_{\text{hyd.}} = k'_{\text{form.}} \frac{[\text{HOCl}] \left[ \begin{array}{c} \diagdown \\ \text{NH} \\ \diagup \end{array} \right]}{\left[ \begin{array}{c} \diagdown \\ \text{NCl} \\ \diagup \end{array} \right]} \frac{([\text{H}^+] + K_{\alpha_{\text{HOCl}}})([\text{H}^+] + K_{\alpha_{>\text{NH}}})K_{\alpha_{>\text{NHCl}}}}{[\text{H}^+]^2([\text{H}^+] + K_{\alpha_{>\text{NHCl}}})} \quad (\text{Eq. 6})$$

$$k_{\text{hyd.}} = k'_{\text{form.}} K_{cp} \frac{([\text{H}^+] + K_{\alpha_{\text{HOCl}}})([\text{H}^+] + K_{\alpha_{>\text{NH}}})K_{\alpha_{>\text{NHCl}}}}{[\text{H}^+]^2([\text{H}^+] + K_{\alpha_{>\text{NHCl}}})} \quad (\text{Eq. 7a})$$

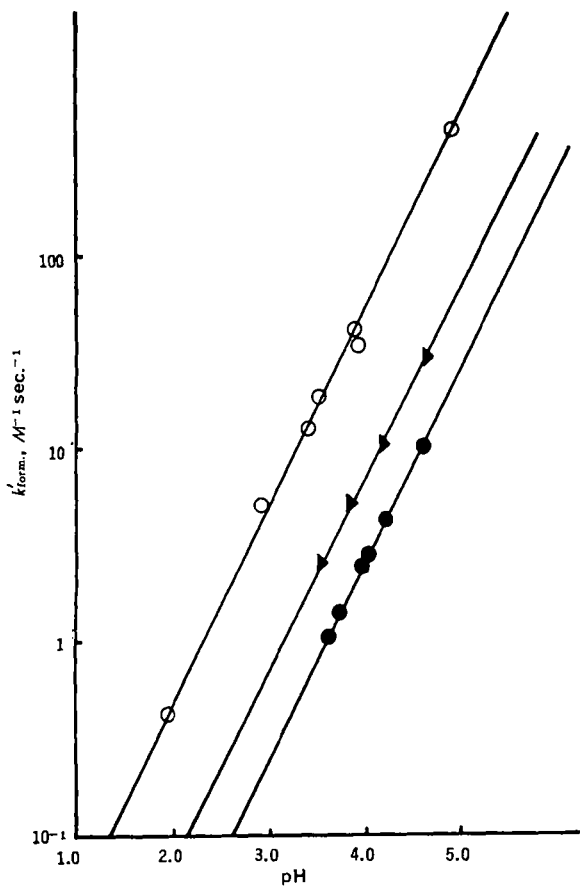


Figure 1—Plot against pH of  $\log k'_{form}$  for I (○), II (●), and III (▲).

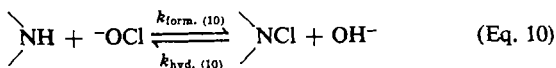
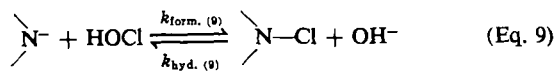
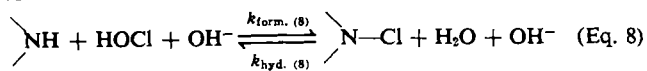
The equivalent expression for reactions of II would be:

$$k_{hyd} = k'_{form} K_{ep} \frac{([H^+] + K_{a_{HOCl}})([H^+] + K_{a_{>NH}})K_{a_{>NHCl}}}{[H^+]^2([H^+] + K_{a_{>NHCl}})} \quad (\text{Eq. 7b})$$

Values used for the acid dissociation constants ( $K_a$  values) of the various acidic species are listed in Table II. Values of  $k'_{hyd}$  calculated in this way are included in Table I.

**pH Dependence of Rates of Formation and Hydrolysis of I, II, and III**—As seen in Figs. 1 and 2, between pH values of 1.95 and 5, plots of  $\log k'_{hyd}$  and  $\log k'_{form}$  against pH were linear with gradients close to 1. This behavior indicates that the transition states for formation and hydrolysis of the *N*-chloro compounds contain one less proton (or one more hydroxide ion) than the reactants or products.

Three mechanisms which are consistent with this pH dependence can be written for each compound. For I and III the mechanisms are:



Equivalent mechanisms can be written for II by replacing  $>NH$  by  $\rightarrow NH$ ,  $>N^-$  by  $\rightarrow N$ , and  $>N^-\text{Cl}$  by  $\rightarrow N\text{Cl}$ .

Although the present study does not conclusively distinguish between these mechanisms, the authors believe that the results support the mechanism of Eq. 9 most strongly. That is, the most basic form of a potential chlorine receptor which can exist in appreciable amounts below pH 14 will be chlorinated more rapidly by a chlorine donor than any less basic species. It is further believed that the

Table III—Calculated<sup>a</sup> Second-Order Rate Constants for the Mechanism of Eq. 9

Compound	$k_{hyd. (9)}, M^{-1} \text{ sec.}^{-1}$	$k'_{form. (9)}, M^{-1} \text{ sec.}^{-1}$
I	$9.80 \times 10^3$	$2.14 \times 10^7$
II	$1.824 \times 10^6$	$2.29 \times 10^7$
III	$7.348 \times 10^2$	$1.50 \times 10^8$

<sup>a</sup>  $k_{hyd. (9)}$  and  $k'_{form. (9)}$  values were calculated from  $k'_{hyd.}$  and  $k'_{form.}$  values by using the following identities:

$$k_{hyd. (9)} = \frac{k'_{hyd.} [H^+]}{K_w} \quad k'_{form. (9)} = \frac{k'_{form.} ([H^+] + K_{a_{>N-H}})}{K_{a_{>NH}}}$$

where  $K_{a_{>NH}}$  is the acid dissociation constant of the imide, amide, or quinuclidinium ion. Values were calculated at each pH value, and the results for each compound were averaged.

most reactive form of the *N*-chloro compound will be the most highly protonated form that can exist in appreciable concentrations above pH 0. This will later be exemplified by the greater reactivity of *N*-chlorodimethylammonium ion as compared to *N*-chlorodimethylamine. Values of second-order rate constants for Eq. 9 [ $k'_{form. (9)}$  and  $k_{hyd. (9)}$ ] calculated from  $k'_{form.}$  and  $k'_{hyd.}$  values are generally consistent with the mechanism. Thus, from the values of  $k_{hyd. (9)}$  and  $k'_{form. (9)}$  in Table III, it can be seen that the rate of chlorination of succinimide, quinuclidinium ion, and *N*-methylbenzenesulfonamide by hypochlorous acid increases with the increasing pKa of the conjugate acid of the acceptor. This is the order expected for a nucleophilic displacement reaction involving the attack of a series of basic nucleophiles on a common substrate. Furthermore, in the hydrolysis of I, II, and III, it can be seen that the attack of the negatively charged hydroxide ion is faster on the positively charged

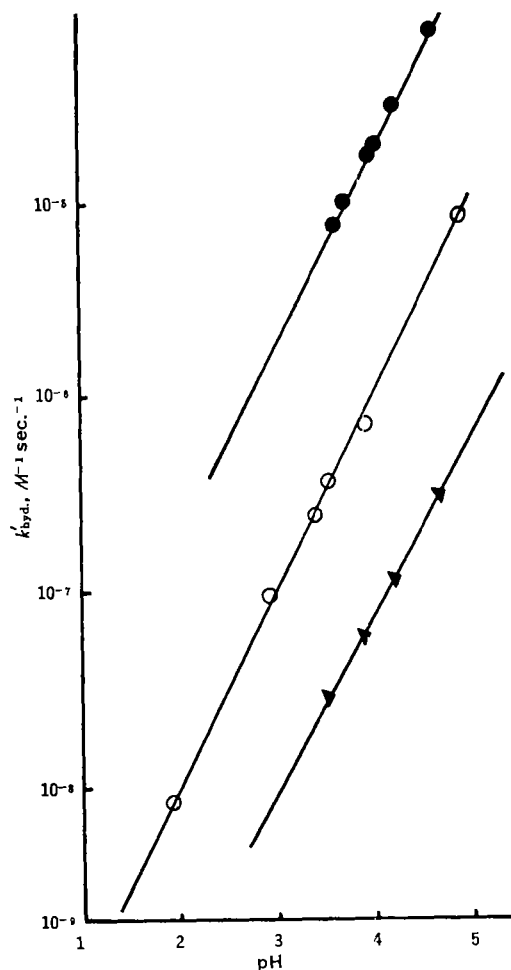


Figure 2—Plot against pH of  $\log k'_{hyd}$  for I (○), II (●), and III (▲).

**Table IV**—Logarithms of Calculated<sup>a</sup> Second-Order Rate Constants ( $M^{-1} \text{ sec.}^{-1}$ ) for the Exchange Reaction:  $\text{ACl} + \text{B} \rightleftharpoons \text{A} + \text{BCl}$

ACl	Succinamide Anion	Me <sub>2</sub> NH	Quinuclidine	<i>N</i> -Methylbenzenesulfonamide Anion
I	—	7.18	7.07	6.47
II	8.26	7.31	—	9.13
III	4.44	5.39	6.02	—
Me <sub>2</sub> NHCl	9.10	—	7.88	9.42

<sup>a</sup> Calculated from pH-dependent "observed" rate constants in Reference 1.

molecule II than on the neutral molecules I and III. The order of reactivity of these molecules toward hydroxide ion also increases with increasing values of the hydrolysis equilibrium constants,  $K_{ep}$  values, for the compounds. Thus, it appears that the more thermodynamically stable these molecules are toward hydrolysis, the slower will be their rate of hydrolysis.

It was found that acetate ion did not catalyze the formation or hydrolysis reactions. This finding is consistent with the mechanism of Eq. 9 but would not be expected if the mechanism of Eq. 8 was operating. In the latter case, it would be expected that general bases such as acetate ion would catalyze the reaction in a similar way to the specific base, hydroxide ion.

Rate constants for the transfer of chlorine between nitrogen-containing molecules in water were published previously from this laboratory (1, 2). The pH dependence of these values is different for different systems. Again, however, pH-independent second-order rate constants can be calculated from them on the basis that the rate-determining step is the attack of the most basic form of the chlorine receptor which can exist to an appreciable extent below pH 14 on the most acidic form of the *N*-chloro compound which can exist to appreciable amounts above pH 0. For example, it is believed that the major reaction in chlorine transfer between *N*-chlorodimethylamine and succinamide involves a reaction between *N*-chlorodimethylammonium ion and succinamide anion rather than between the neutral molecules.

Values of some of these constants are listed in Table IV. Two distinct trends are again clear. First, the attack of anionic receptors on positively charged *N*-chloro compounds is faster than the attack of neutral receptors. Second, in most cases the most basic nucleophile (if the nucleophiles being compared are of the same charge type) reacts fastest with a given *N*-chloro compound. The fact that the strongest base studied ( $\text{OH}^-$ ) reacted more slowly than the other less basic nucleophiles with each *N*-chloro compound may be because of the fact that its reaction involved formation of an O—Cl bond whereas the other reactions involved formation of N—Cl bonds. These different types of reactions would not necessarily be expected to fit simple structure-activity relationships.

**Mechanism of Chlorine Transfer between Nitrogen-Containing Organic Molecules in Water**—If chlorine transfer between nitrogen-containing molecules in water was mediated by hydrolysis of the *N*-chloro reactant to yield hypochlorous acid, then the rate of this latter reaction must be at least as fast as the overall transfer rate. The kinetic data in Tables I–IV clearly show that, at least in aqueous solutions up to pH 5, this is not the case for chlorination of dimethylamine, succinamide, quinuclidinium ion, and *N*-methylbenzenesulfonamide by I, II, or III. For example, the results indicate that the rate of chlorine transfer from *N*-chloroquinuclidinium ion to dimethylamine would occur about  $10^6$  times faster than its hydrolysis at pH values between 1 and 5 under conditions where the initial

total concentration of amine is 1M and considerably in excess of the initial concentration of II. If no change in mechanisms of chlorine transfer or hydrolysis occurred at higher pH values, the rate of chlorine transfer in this system would still be about  $10^2$  times faster than hydrolysis at pH 14. Similar differences in rates of hydrolysis and chlorine transfer to nitrogenous bases are apparent for the other *N*-chloro compounds studied. Thus, it seems likely that, in these systems, the chlorine transfer occurs as a result of a direct reaction between a basic receptor molecule and the *N*-chloro molecule. As implied earlier, it is believed that the mechanism is similar to that shown in Eq. 9.

## EXPERIMENTAL

**Reagents**—Succinamide, hypochlorous acid, and distilled water were obtained or prepared as described in a previous study. Quinuclidine<sup>1</sup> was converted into its perchlorate by dissolving 5 g. in 5 ml. of ether and precipitating with excess perchloric acid. The perchlorate salt was recrystallized from 95% ethanol. This treatment was necessary to eliminate contaminants. *N*-Methylbenzenesulfonamide was prepared from benzenesulfonyl chloride and gaseous methylamine. The benzenesulfonyl chloride was dissolved in ether and gaseous methylamine was bubbled through until nearly all of the acyl chloride had been reacted. The precipitated methylamine hydrochloride was filtered off and the ethereal filtrate was washed with 1 *N* sulfuric acid to remove excess methylamine. The ethereal solution was dried with anhydrous sodium sulfate and the ether was evaporated. The resulting viscous, slightly yellow liquid was purified by distillation under reduced pressure. Crystals were obtained after chilling, m.p. 30°. All other chemicals used were reagent or analytical reagent grade and were used without further purification.

**Procedure**—Fresh solutions of the reactants in water were prepared in the desired concentration range. A concentrated acetate buffer of the desired pH was prepared. After the solutions were brought to  $25.0 \pm 0.1^\circ$ , exact portions of the buffer and amide, imide, or amine were transferred to a 1.0-cm. silica cell, and an exact amount of hypochlorous acid was injected into the cell at time zero. Changes in absorbance were followed in the spectrophotometer<sup>2</sup> at several wavelengths. After the run was completed, the pH of the reaction mixture was rechecked.

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<sup>1</sup> Melpar Inc.

<sup>2</sup> Cary model 15.