Solution Phase Interaction of Nicotinamide with Ascorbic Acid

By DAVID E. GUTTMAN and DANA BROOKE†

A study was made of what appears to be a charge-transfer interaction that is responsible for the instantaneous generation of a yellow color in aqueous solutions containing nicotinamide and ascorbic acid. Spectral studies were utilized to elucidate some of the factors that influence the interaction. Analysis, by the method of continuous variation, showed that a 1:1 complex was formed. The extent of association was pH dependent and exhibited a maximum at a pH of approximately 3.8. The pH dependency was compared with that of the 3-carbamyl-1-methyl-pyridinium chloride–ascorbic acid system and indicated that the interactants were protonated nicotinamide and ascorbate anion. Association constants, at a number of different temperatures, were determined by using the Benesi-Hildebrand treatment of spectral data. The standard enthalpy change that resulted from association was estimated to be −1,500 cal.

COMPLEX OR ADDITION compound formation in systems containing ascorbic acid and nicotinamide was first reported in 1944 by Milhorat (1). Since that time a limited number of publications have appeared which have described in greater or lesser detail some of the properties of this combination. Bailey, Bright, and Jasper (2), for example, constructed temperature-composition diagrams for nicotinamide-ascorbic acid and nicotinic acid–ascorbic acid systems and demonstrated in this way that the reaction products had 1:1 stoichiometric compositions. Molecular weight determinations revealed that a high degree of dissociation occurred in aqueous and alcoholic solutions. Wenner (3) also studied the reaction and observed that it proceeded at a perceptible rate. His observation contrasts with those of others who reported that an almost instantaneous formation occurred upon mixing the interactants. Najer and Guépet (4), on the basis of studies in the U.V. and I.R. regions, concluded that the isolatable product was essentially the ascorbate of nicotinamide, but that some interaction occurred between the amide grouping and a secondary hydroxyl group of the acid. Investigations of the antiscorbutic properties of the complex were conducted and reported by Chalopin (5) and Nigeon-Dureuil (6). In addition, at least two patents were issued covering the product (7, 8).

The present investigation was undertaken to provide more definitive information about the nature of the interaction between the two vitamins, the extent of their association in aqueous solution, and the factors that influence such an occurrence. As will be seen, finite concentrations of a 1:1 complex were formed when the vitamins were combined in solution. The interaction was markedly dependent on the pH of the solution and appeared to be because of a charge-transfer interaction between protonated nicotinamide and ascorbate ion.

EXPERIMENTAL

Reagents.—Nicotinamide and ascorbic acid were U.S.P. grade. 3-Carbamyl-1-methyl-pyridinium chloride was synthesized by the method of Huff and Perlzweig (9), m.p. 237–239° (uncorrected). All solutions were freshly prepared in double-distilled, deionized water that had been thoroughly flushed with nitrogen. Disodium ethylenediaminetetraacetic acid (0.1%) was added to all solutions. Precautions were taken in all mixing procedures to prevent the

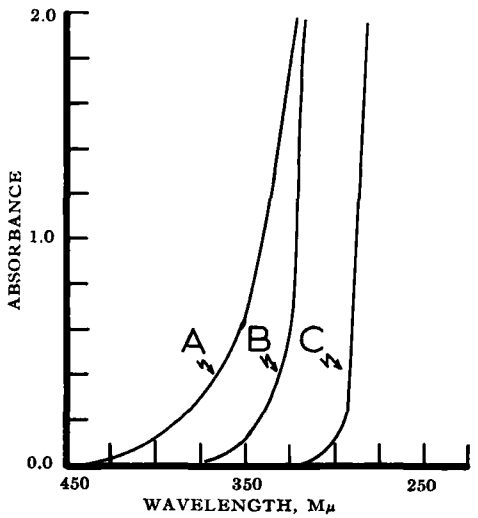

Fig. 1.—A plot showing the spectral characteristics of nicotinamide, ascorbic acid, and a mixture of the two. Curve A is the spectrum of a solution that was 0.02 *M* with respect to nicotinamide and 1.8 *M* with respect to ascorbic acid. Curve B is the spectrum of a 1.8 *M* solution of ascorbic acid. Curve C is the spectrum of a 0.02 *M* solution of nicotinamide.

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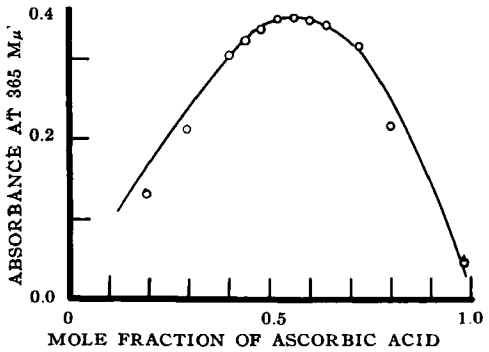

Fig. 2.—A plot illustrating the method of continuous variation used to investigate the stoichiometry of the ascorbic acid–nicotinamide interaction. The total solute concentration was 0.4 *M*.

introduction of air. Such precautions were necessary to preclude rapid autoxidation of ascorbic acid.

Methods.—Spectral studies were based on the fact that the addition of ascorbic acid to solutions of nicotinamide or its N^1-methyl derivative resulted in the instantaneous generation of a faint yellow color. Spectrophotometric examination, which is illustrated in Fig. 1, revealed the appearance of a new band as a long tail to the longer wavelength side of the ascorbic acid spectrum. Absorbance values of solutions were determined at two arbitrarily chosen wavelengths—345 and 365 mμ—and these served as quantitative indications of the concentration of complex in solution. The absorbance of each sample containing ascorbic acid and nicotinamide was corrected for the absorbance contribution of ascorbic acid by using as a solvent blank an ascorbic acid solution having the same concentration as the sample. This contribution was found to be small at lower pH values but became increasingly significant as the pH was increased. All spectrophotometric determinations were made with a Beckman model DU spectrophotometer, which was equipped with thermostatic plates, through which constant-temperature water was circulated to maintain the cell compartment at the desired temperature. Prior to absorbance measurements, the solutions were cooled or heated in an external bath to a temperature close to that of the compartment.

Analysis by Continuous Variation.—Equimolar solutions (0.4 *M*) of ascorbic acid and nicotinamide were prepared. Aliquots of the two solutions were mixed in various proportions to yield mixtures having a total volume of 25 ml. The absorbance at 365 mμ was determined for each solution using an appropriate solvent blank.

Influence of pH.—One-milliliter aliquots of a stock solution of nicotinamide or its N^1-methyl derivative were placed in a series of 25-ml. volumetric flasks. A 20-ml. volume of a 1.25 *M* solution of ascorbic acid was added to each flask. Volumes of concentrated aqueous sodium hydroxide were added; each flask was made up to volume with water. Absorbance values were determined at 365 mμ using solutions prepared in the same manner as solvent blanks, but which did not contain the amide. The pH of each solution was determined with a Beckman model G pH meter immediately after spectrophotometric examination.

Association Constant Determination.—One milliliter of a stock solution of nicotinamide was placed in each of a series of 10-ml. volumetric flasks. A volume of a 1.8 *M* solution of ascorbic acid, which had previously been adjusted to a pH of 4.0 by the addition of aqueous sodium hydroxide, was placed in each flask. Solutions were then made up to volume by the addition of water. An appropriate blank was formulated for each sample. Absorbances at 365 and 345 mμ were determined for each solution. Initially, the pH of each solution was measured. Repeated trials showed that dilution did not markedly change the pH from that of the original stock solution.

RESULTS

The analyses by continuous variation experiments are summarized in Fig. 2. The maximum absorbance appeared to correspond to a solution that was approximately equimolar with respect to the two vitamins and thus indicated the reversible formation of a complex of 1:1 stoichiometry. The high degree of dissociation of the complex was suggested by the absence of a well defined maximum in Fig. 2.

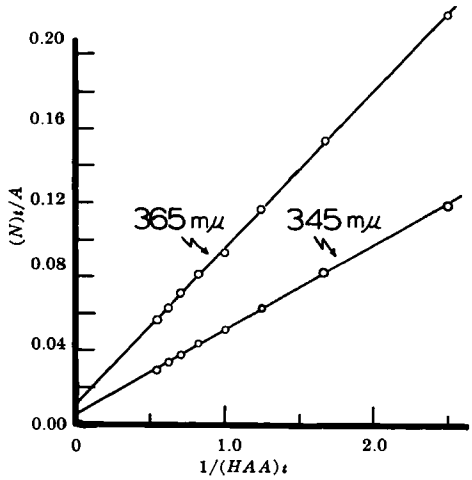

Fig 3.—A plot illustrating the Benesi Hildebrand treatment used to investigate the interaction between ascorbic acid and nicotinamide at 25°C. and at a pH of 4.0. The nicotinamide concentration was 0.04 *M*.

The stoichiometry was substantially confirmed by the plots shown in Fig. 3, where the effect of ascorbic acid concentration on the absorbances of solutions containing the two vitamins at constant pH was summarized. The treatment illustrated by the figure is analogous to that first proposed by Benesi and Hildebrand (10) and which is commonly used to determine complex association constants from spectral data. Here it was assumed that nicotinamide and ascorbic acid reversibly interacted to form a 1:1 complex, that the interaction obeyed the law of mass action, that the absorbance at the chosen wavelength was entirely due to the complex and followed Beer's law, and that the concentration of complex was insignificantly small compared to that of ascorbic acid. With these assumptions, it can be shown that

$$(N)_t/A = 1/a_c + 1/a_cK(HAA)_t \quad \text{(Eq. 1)}$$

where $(N)_t$ = analytical concentration of nicotinamide, A = absorbance at a particular wavelength, a_c = molar absorptivity of the complex at that wavelength, K = apparent association constant, and $(HAA)_t$ = analytical concentration of ascorbic acid.

Equation 1 predicts a linear relationship between $(N)_t/A$ and $1/(HAA)_t$ with a slope value of $1/a_cK$ and an intercept value of $1/a_c$. Excellent linearity of the curves of Fig. 3, over a wide range of ascorbic acid concentrations and at two different wavelengths, served to confirm the assumptions that were basic to the derivation of Eq. 1. Similar linearity was observed at three other temperatures.

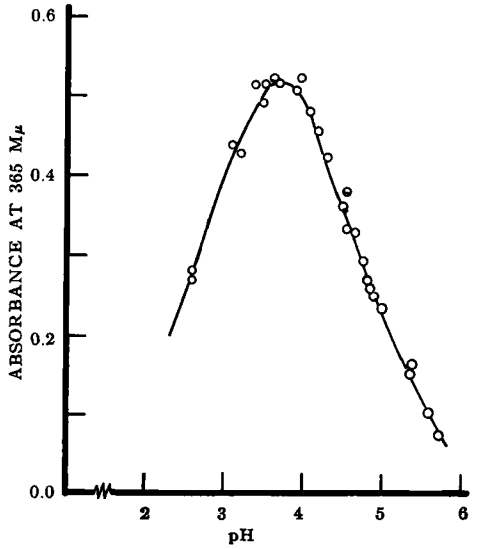

Fig. 4.—A plot illustrating the effect of pH variation on the interaction between ascorbic acid and nicotinamide at room temperature. Each solution was 0.05 M with respect to nicotinamide and 1.0 M with respect to ascorbic acid.

The influence of pH on the system and on a similar system containing 3-carbamyl-1-methyl-pyridinium chloride is depicted in Figs. 4 and 5. Here absorbance values of solutions of identical composition, except for hydronium-ion concentration, were plotted as a function of pH. The ascorbic acid–nicotinamide system exhibited the maximum degree of interaction at a pH of approximately 3.8. In the case of the N^1-methyl derivative, the degree of interaction increased with an increase in pH in a manner that is characteristic of the degree of ionization behavior of a weak acid. It will be seen that these results could be reasonably interpreted to mean that the species that underwent association to form the complex were ascorbate anion and the quaternary form of the pyridine derivative.

DISCUSSION

In the pH region which was investigated in the study illustrated by Fig. 4, nicotinamide existed in solution as the free base (N) and/or as the protonated base (NH^+). Similarly, ascorbic acid was present as the free acid (HAA) and/or as ascorbate ion (AA^-). The relative distribution of species in a particular solution would, of course, be dependent on the pH of that solution. This consideration leads to the possibility that one or more of four equilibria were operative and responsible for the formation of a colored 1:1 complex. These include

$$NH^+ + HAA = NH^+{:}HAA \quad \text{(Eq. 2)}$$

$$NH^+ + AA^- = NH^+{:}AA^- \quad \text{(Eq. 3)}$$

$$N + HAA = N{:}HAA \quad \text{(Eq. 4)}$$

$$N + AA^- = N{:}AA^- \quad \text{(Eq. 5)}$$

The pH profile of the interaction, as illustrated in Fig. 4, indicated, however, that equilibria of Eqs. 2 and 5 were not significantly responsible for the formation of a colored complex. It can be seen that at the pH extreme where the species NH^+ and HAA predominated and at the other extreme where N and AA^- predominated, the degree of interaction was low. This behavior can be verified visually by noting that the yellow color of a solution containing the vitamins can be instantaneously discharged by strong acidification or alkalinization. The pH of solutions which exhibited maximum absorbance and, therefore, maximum concentration of complex fell in the region where the interactants of equilibria of Eqs. 3 or 4 predominated.

The equilibrium expressed in Eq. 3 can be characterized by the association constant $K' = (NH^+{:}AA^-)/(NH^+)(AA^-)$, or in terms of the analytical concentrations of the vitamins as

$$K' = \left\{\frac{(NH^+{:}AA^-)}{(N)_t(HAA)_t}\right\}\left\{\frac{(H^+) + K_{NH^+}}{H^+}\right\}\left\{\frac{(H^+) + K_{HAA}}{K_{HAA}}\right\} \quad \text{(Eq. 6)}$$

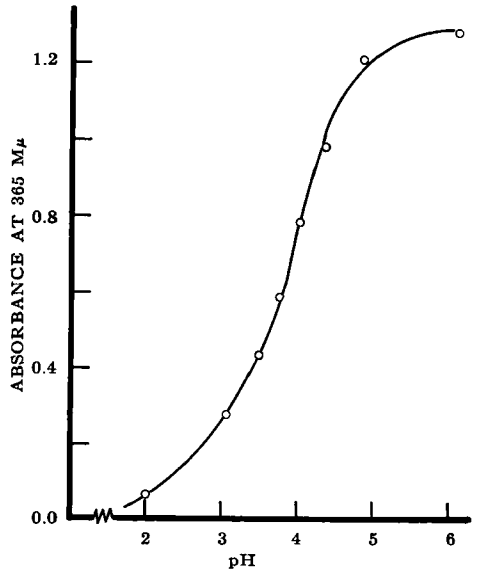

Fig. 5.—A plot illustrating the effect of pH variation on the interaction between ascorbic acid and N^1-methyl nicotinamide at room temperature. Each solution was 0.015 M with respect to N^1-methyl nicotinamide and 1.0 M with respect to ascorbic acid.

where K_{NH^+} and K_{HAA} = acidity constants of protonated nicotinamide and ascorbic acid, respectively.

Similarly, it can be shown that equilibrium of Eq. 4, involving free nicotinamide and free ascorbic acid, can be described by the association constant

$$K' = \left\{\frac{(N{:}HAA)}{(N)_f(HAA)_f}\right\}\left\{\frac{K_{NH^+} + (H^+)}{K_{NH^+}}\right\}\left\{\frac{K_{HAA} + (H^+)}{(H^+)}\right\} \quad \text{(Eq. 7)}$$

Differentiation of Eqs. 6 and 7 to obtain $d(\text{complex})/d(H^+)$, setting the resulting differentials equal to zero, and solving for the hydronium-ion concentrations at which the concentration of complex would be expected to maximize, yielded an identical result for both possible equilibria, that

$$(H^+)_{max.} = (K_{NH^+} \cdot K_{HAA})^{1/2}$$

or

$$pH_{max.} = {}^1/_2 pK_{NH^+} + {}^1/_2 pK_{HAA} \quad \text{(Eq. 8)}$$

Using a value of 3.42 for the pKa of protonated nicotinamide (11) and that of 4.17 for ascorbic acid (12), the pH of maximum concentration of complex was calculated to be 3.80. The experimental curve exhibited a maximum which agreed closely with this theoretically expected value. The close agreement between theory and experiment confirmed that one or both of the equilibria were operative but did not permit a differentiation between the possibilities.

Since it was impossible to differentiate in this way between the equilibria which, on the one hand, involved the ionized, and on the other, the unionized forms of the vitamins, the influence of pH on a similar, previously unreported interaction between ascorbic acid and 3-carbamyl-1-methyl-pyridinium chloride was studied. Here, the degree of ionization of the N^1-methyl nicotinamide was pH independent. It was therefore felt that if a similar interaction occurred and was influenced by pH, then the nature of the influence would point to either unionized ascorbic acid or ascorbate ion and the cationic form of nicotinamide as the interacting species. Interaction did occur and the pH profile shown in Fig. 5 unequivocally implicated ascorbate ion as the species which was involved. This evidence, although indirect, strongly suggests that the predominant equilibrium which resulted in the generation of the yellow color in solutions of ascorbic acid and nicotinamide was that described by Eq. 3.

On the basis of this evidence, the association constants which were calculated from the Benesi-Hildebrand plots by regression analysis and which were based on the analytical concentrations of the vitamins were multiplied by the factor

$$\left\{\frac{(H^+) + K_{NH^+}}{(H^+)}\right\}\left\{\frac{(H^+) + K_{HAA}}{K_{HAA}}\right\}$$

to yield constants which were consistent with the implicated equilibrium. These are presented for the temperatures and wavelengths investigated in Table I. The effect of temperature on the equilibrium was summarized in Fig. 6, where log K' was plotted as a function of the reciprocal of absolute temperature. The enthalpy change which resulted from association was estimated from this graph to be −1,500 cal.

TABLE I.—ASSOCIATION CONSTANTS FOR THE COMPLEX FORMED BY THE INTERACTION OF NH^+ AND AA^- IN AQUEOUS SOLUTION

Temp., °C.	Wavelength Used for Determination, mμ	K' (with 95% confidence range), L./Mole
10	345	1.64 ± 0.038
21	345	1.52 ± 0.024
25	345	1.45 ± 0.035
25	365	1.43 ± 0.020
29	345	1.40 ± 0.020

A number of considerations indicate that charge-transfer forces were responsible for the formation of the ascorbic acid–nicotinamide complex in solution. That the interaction involved ascorbate ion and the cationic form of nicotinamide immediately suggests such a possibility because of the well-known ability of pyridinium compounds to function as electron acceptors in charge-transfer interactions with negatively charged species. In addition, the bathochromic shift which occurred as a manifestation of the interaction is similar to those observed in a number of charge-transfer systems. Furthermore, the association constants determined in the present study are of the same order of magnitude as those reported by Kosower (13) for the charge-transfer complexes of iodide and substituted pyridinium compounds. The observed enthalpy change is approximately the same as that reported by Negoro, *et al.* (14), for the interaction of nicotinamide with *p*-aminosalicylic acid which they attributed to a charge-transfer mechanism.

Evidence has been presented which permits a more quantitative understanding of the interaction between ascorbic acid and nicotinamide, an interaction which has been variously referred to as a pharmaceutical incompatibility and a means of providing a more efficient therapeutic combination. In view of the low association constant of the complex, and the fact that the physiological pH does not favor the interaction, it is doubtful that the activity of the vitamin combination is in any way altered from that of the individual components. In addition to its pharmaceutical significance, the interaction is of interest because it focuses attention on ascorbate

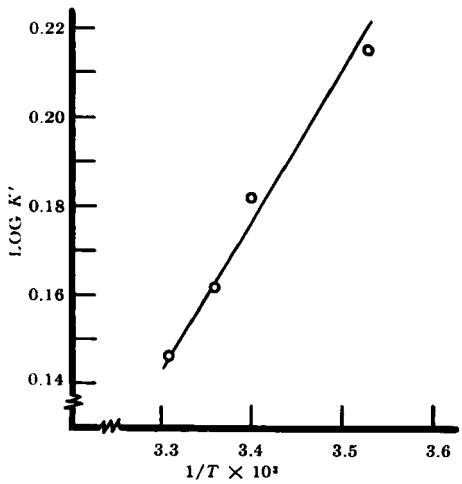

Fig. 6:—A plot illustrating the temperature dependency of the interaction between ascorbic acid and nicotinamide.

ion as a participant in a charge-transfer interaction and points to the possibility that it can interact similarly with enzyme or coenzyme systems in the body. It is conceivable that such an occurrence might be involved in metabolic processes which are dependent on this vitamin.

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Determination of Ephedrine Salts in Liquid Dosage Forms

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Analytical procedures are presented for the determination of ephedrine salts in syrups, elixirs, solutions, and injections. The ephedrine is extracted with the strong cation exchange resin, Dowex 50 X-8, and is subsequently eluted with 2 normal hydrochloric acid. After evaporation of the eluate to dryness, the residue is titrated nonaqueously with perchloric acid in dioxane in the presence of mercuric acetate. In formulations containing sodium chloride and other salts, the ephedrine is eluted with an alcoholic solution of ammonia. The eluate is aerated until all the ammonia is expelled. The ephedrine is determined by titration with hydrochloric acid. Comparison is made with the official assay for ephedrine sulfate solution.

EPHEDRINE, ephedrine salts, and their dosage forms have been official since U.S.P. XI and N.F. VI. The official assay procedure has usually involved ether extraction of the alkaloid liberated from the salt combination by addition of base. The ephedrine is then determined by residual alkalimetry. Ephedrine sulfate and phenobarbital capsules (1) are analyzed for ephedrine by a distillation method developed by Hilty (2) and further applied by Hilty and Wilson (3). Until recently ephedrine sulfate capsules (4) were also analyzed by this method. There is no official assay for N.F. XI ephedrine sulfate syrup.

Colorimetric procedures have been developed for the estimation of ephedrine. These have been reviewed by Higuchi and Bodin (5) and Snell and Snell (6).

Ephedrine and ephedrine salts have been determined by nonaqueous titration. Auerbach (7) titrated ephedrine in acetic acid with acetous perchloric acid. Salts of organic bases were titrated with perchloric acid in dioxane by Pifer and Wollish (8). Salts of alkaloids including ephedrine were studied. Titrations were effected visually and potentiometrically. Since chloride ion is too weakly basic to react quantitatively with the perchloric acid, the authors found that by adding mercuric acetate to the titration mixture, the chloride is tied up as undissociated mercuric chloride. The liberated acetate ion is then titratable with perchloric acid. Mercuric acetate itself is essentially undissociated in acetic acid and, therefore, does not titrate as a base. Rink and Lux (9) and Ekeblad (10) determined ephedrine hydrochloride with acetous perchloric acid by applying the method of Pifer and Wollish. "The British Pharmacopoeia" (11) recognizes this procedure for the assay of ephedrine hydrochloride tablets. Chatten and Pernarowski (12) analyzed for ephedrine in oily nasal sprays with acetous perchloric acid. Aqueous sprays were extracted with chloroform prior to nonaqueous titration.

Ion-exchange resins have been employed in the determination of alkaloidal salts including ephedrine hydrochloride and ephedrine sulfate. Amberlite IR-4B, a weak anion-exchange resin, was used by Jindra (13) for ephedrine sulfate but was found unsuitable (14) for ephedrine hydrochloride. The resin removes the acid component of the sul-

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