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RESEARCH ARTICLES

Interaction of Isoniazid with Magnesium Oxide and Lactose

WEN-HUNG WU, TING-FONG CHIN, and JOHN L. LACH

Abstract □ Interactions of isoniazid with magnesium oxide and with lactose were investigated in the solid state. In the isoniazid–magnesium oxide system, chemisorption as well as physical adsorption of isoniazid molecules onto the magnesium oxide surface was confirmed by diffuse reflectance spectroscopic data. An absorption maximum due to chemisorbed isoniazid molecules was found to occur at 325 mμ, whereas physical adsorption was detected at 268 mμ. The mechanism of surface chemisorption is different from that of the formation of the isoniazid–metal-ion complex in solution. The browning reaction of the isoniazid–lactose system in solid state was also studied using diffuse reflectance spectroscopy. The rates of browning at 95, 100, 105, and 110° were followed by measuring the reflectance at 450 mμ. From the data obtained, the approximate time needed for browning to be perceptible has been predicted. TLC separations of the reaction products confirmed the presence of isonicotinoyl hydrazones of lactose and hydroxymethylfurfural.

Keyphrases □ Isoniazid interaction—magnesium oxide, lactose □ Lactose–isoniazid systems—browning rates □ Magnesium oxide, chemisorption—isoniazid □ Diffuse reflectance spectroscopy—analysis □ IR spectrophotometry—identity

Difficulties in formulating a new pharmaceutical dosage form have often been experienced because of the interactions between the supposed inert adjuvants and the active ingredient itself. Although the nature and intensity of these interactions vary, such interactions may alter the stability, dissolution rate, and, consequently, the absorption of the drug. A literature survey indicates that such interactions involving the formation of complexes have been studied extensively in aqueous solution; relatively few studies have been carried out in the solid state.

Stearic acid and calcium stearate have been shown by Kornblum and Zoglio (1) to catalyze the degradation of aspirin. Ribeiro *et al.* (2) studied the influence of lubri-

cant type and concentration of the lubricant in the decomposition of aspirin in APC tablets. In most cases, an increase in lubricant concentration caused a corresponding increase in decomposition. The most striking effect was observed with calcium stearate, stearic acid, and magnesium stearate. It is conceivable that the metallic salts of these weak organic acids catalyze aspirin degradation: however, the catalytic effect brought about by stearic acid alone suggests that some other mechanism(s) may also be operative. The influence of antacid compounds and tablet diluents on the stability of aspirin has also been investigated by Bandelin and Malesh (3). The authors reported that there is no relationship between the solubility of the adjuvants and the decomposition of aspirin. For example, a highly insoluble magnesium trisilicate produced a high rate of decomposition, while calcium gluconate, a very soluble salt, was less reactive.

Adsorption of antibiotics on antacid materials has been studied by several authors (4, 5). Blaug and Gross (6) showed that the adsorption of some anticholinergic drugs on magnesium trisilicate was so strong that complete desorption was difficult. Chulski and Forist (7), studying the effects of some solid buffering agents on prednisolone, found that the steroid was adsorbed by magnesium trisilicate and that magnesium oxide produced a first-order degradation of the steroid.

Although these studies dealing with drug–adjuvant interactions covered different types of drugs and adjuvants, the nature of the interactions involved and their reaction mechanisms were not fully discussed. This is probably attributable to the fact that reactions in the solid state are usually complex, complicated by numerous parallel and consecutive reactions. However, more important has been the lack of available effective and dependable instrumentation for the study of such interactions in the solid state.

Guillory *et al.* (8) reported on thermal methods using differential thermal analysis (DTA) for the detection of interactions occurring between solid components of pharmaceuticals. Drug–adjuvant interactions in the solid state have been investigated by Lach and Bornstein (9–11), Bighley (12), and McCallister (13), using diffuse reflectance spectroscopy (DRS). The authors covered a wide range of active medicinal agents including antibiotics, analgesics, and anticoagulants. The adjuvants investigated included both metallic and nonmetallic compounds. The degree of strength of the interactions between drugs and adjuvants was interpreted from the spectral shifts observed.

Since the clinical response of any solid dosage form depends not only on the accurate labeled amount of medicament present but also on its actual availability to the patient once administered, a study of these solid-state interactions is of prime importance in an understanding of the formulation of therapeutically effective dosage forms. The purpose of this investigation was to study drug–adjuvant interactions, since drug availability might be significantly altered as a result of these solid–solid interactions. Since isoniazid (INH) does form chelates in solution with various metallic ions (14–16), it is highly probable that similar type interactions would occur on the surface of metallic-containing adjuvants commonly employed in the preparation of solid dosage forms. It is also probable that INH would undergo other types of surface interactions. An investigation dealing with these aspects was therefore undertaken.

EXPERIMENTAL

Reagents—The following were used: isoniazid,[1] recrystallized from 95% ethanol, m.p. 171–172°; MgO USP,[2] heavy, average size 30 μ; KBr,[3] spectroscopic grade; lactose monohydrate,[2] A.R., m.p. 201–202°; silica gel G[4] for TLC; deuterium oxide[4] for spectroscopy, 99.75%; 5-hydroxymethylfurfural,[5] recrystallized from a mixed solvent consisting of equal volumes of ether and petroleum ether, m.p. 31–32°; methanol,[2] A.R.; and methylene chloride,[6] A.R.

Apparatus—All diffuse reflectances were measured using a spectrophotometer[7] equipped with reflectance attachment. The cell consists of a square aluminum block, an aluminum back cover, and two metal bolts. A piece of circular quartz,[8] 3.17 cm. (1.25 in.) in diameter and 0.16 cm. (0.064 in.) in thickness, is fitted into the front side of the block. The detailed dimensions are shown in Fig. 1. The powder compartment is shown as the shaded area. The other apparatus used were: recording spectrophotometer;[9] IR spectrophotometer;[10] vacuum oven;[11] dry oven,[12] analytical, low gradient; heater and circulator,[12] equipped with microset thermoregulator;[13] submersion rotator;[14] and TLC apparatus.[15]

General Procedure for Sample Equilibration—Specified quantities of a drug and an adjuvant were accurately weighed on an analytical balance and transferred into a suitable size amber-colored bottle, which held enough void volume for sample mixing after the powders and solvent (10 ml. solvent/1 g. adjuvant) were added. After the cap was replaced, the bottle was fixed on the rotator and tumbled for 24 hr. at room temperature (25°). The content was poured into a mortar and dried at 25° in a vacuum oven. After the bulk of the solvent was driven off, the powder was thoroughly triturated and then dried again at 35° under vacuum. Finally, the sample was cooled in a vacuum desiccator over calcium sulfate.

Reflectance Measurement—The prepared sample was packed into a cell. Since the process of packing has been reported (17) to cause fluctuation in reflectance reading, it is essential to maintain the same condition for every sample packed. In all cases, pure adjuvant itself was used as a white standard (18).

Preparation of Deuterated INH—One gram of INH was dissolved in 10 ml. of D_2O, and the clear solution was transferred to an ampul which was sealed after the air was replaced by nitrogen. The ampul was allowed to stand in the dark for 24 hr., and the D_2O was evaporated under vacuum until completely dry.

Browning of INH–Lactose Solid System—According to the previously stated procedure, a sample containing 15 mg. INH/1 g. lactose was equilibrated in CH_2Cl_2. For purposes of comparison, lactose containing no INH was treated in the same manner. After the reflectance spectra were taken, both samples were heated at 100 ±0.5° in a dry oven. At various time intervals, the samples were transferred to a desiccator containing calcium sulfate and allowed to cool for 15 min.; the reflectance spectra were then taken. The same experiment was carried out also at 95, 105, and 110°.

Browning of INH–Lactose Mixture in Solution—Three solutions were prepared by dissolving: (*a*) 150 mg. INH, (*b*) 150 mg. INH and 10 g. lactose, and (*c*) 10 g. lactose, respectively, in small amount of water and made to 100-ml. volume. Five milliliters each of the solutions was filled into 10-ml. ampuls and sealed. These ampuls were heated at 100 ± 0.1° in an oil bath; at various time intervals, one ampul containing the respective solution was removed. The reaction was quenched by quick cooling, 1 ml. each of the content was diluted

[1] Conray Products Co., New York, N. Y.
[2] Mallinckrodt Chemical Works, St. Louis, Mo.
[3] Matheson Coleman & Bell.
[4] E. Merck (Germany).
[5] Aldrich Chemical Co., Milwaukee, Wis.
[6] J. T. Baker, Phillipsburg, N. J.
[7] Beckman model DB-G.
[8] Suprasil, Amersil Inc.
[9] Beckman model DK-2.
[10] Beckman IR-10.
[11] Precision Scientific, model 524.
[12] E. H. Sargent & Co.
[13] Precision Scientific.
[14] Scientific Industries, Inc., model SR-250-V.
[15] Desaga/Brinkmann.

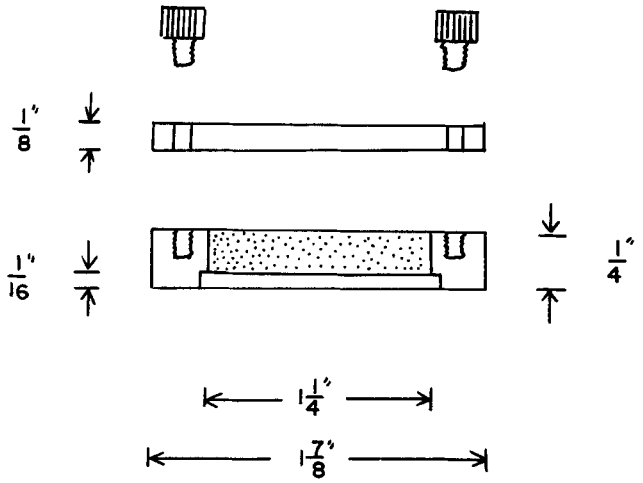


Figure 1—*Cell diagram for diffuse reflectance measurement.*

to 100 ml., and the transmittance spectra were taken from 220–340 mμ.

Preparation of Isonicotinoyl Hydrazone of Hydroxymethylfurfural (INH–HMF)—One gram HMF was dissolved in 8 ml. of methanol and diluted with an equal volume of water. To this solution was added 1 g. of INH and diluted with 5 ml. of water. The clear solution was heated to boiling and then chilled in a refrigerator. The precipitate was filtered and recrystallized from 45 ml. of 50% ethanol, using charcoal as a decolorizing agent. The light-yellow needles obtained were again recrystallized and finally dried at 30° under vacuum, m.p. 216–218° (corrected). $\lambda_{max.}^{H_2O}$ = 320 mμ, $a_{320}^{H_2O}$ = 2697.0. Elemental analysis showed the following:

	C	H	N
Theoretical	58.77	4.52	17.14
Determined	58.86	4.63	17.09

TLC—Silica gel G plates (0.25 mm.) were developed by a solvent mixture made by diluting 60 ml. methanol with $CHCl_3$ to 125 ml. Air-dried plates were then examined under UV light and also exposed to iodine vapor.

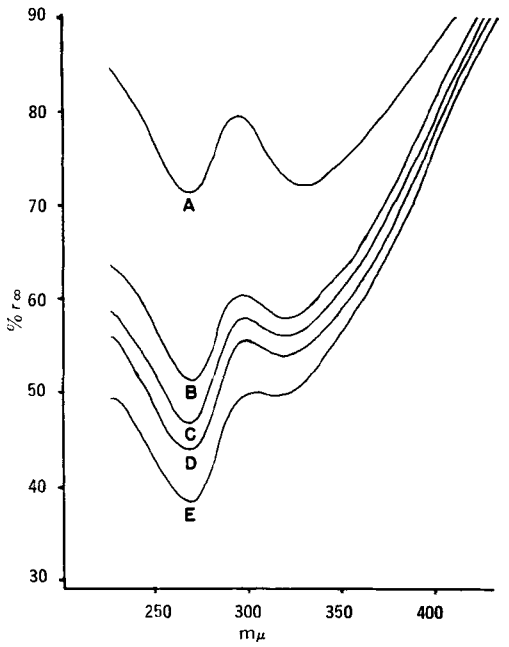


Figure 2—*DRS showing the effects of varying isoniazid concentrations on equilibrated samples using 1.00 g. of magnesium oxide as the adsorbent. Key: A, 3 mg.; B, 7 mg.; C, 10 mg.; D, 13 mg.; and E, 16 mg.*

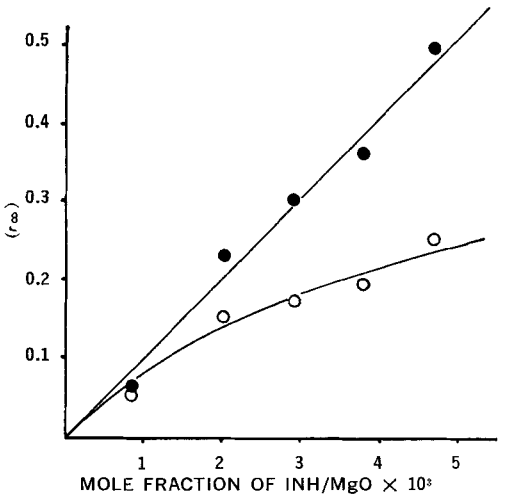


Figure 3—*Relationship between reflectance and concentration of isoniazid. Key: ●, at 268 mμ; and ○, at 325 mμ.*

RESULTS AND DISCUSSION

INH–MgO System—DRS of various samples, containing 3, 7, 10, 13, and 16 mg. INH/1 g. MgO and equilibrated in methanol according to the general procedure previously described, are shown in Fig. 2. In each spectrum, two absorption maxima were observed, one at 268 mμ and the other at 325 mμ. However, the transmittance spectrum of INH in aqueous solution showed only one absorption maximum at 262 mμ. The influence of solvent polarity on this maximum was small, since $\lambda_{max.}$ in $CHCl_3$ was also found at the same wavelength. It was also observed (Fig. 2) that the intensity increase of the maximum at 268 mμ as a function of drug concentration was much greater than that for the maximum at 325 mμ.

These intensity relationships (Table I) are represented by the observed reflectances and expressed as a remission function, which is also referred to as the Kubelka-Munk equation (19):

$$f(r\infty) = \frac{(1 - r\infty)^2}{2r_\infty}$$

where $r\infty$ is a measured reflectance. As seen in the last column of Table I, a comparison of the ratio $f(r\infty)_{268}/(r\infty)_{325}$ indicates that this

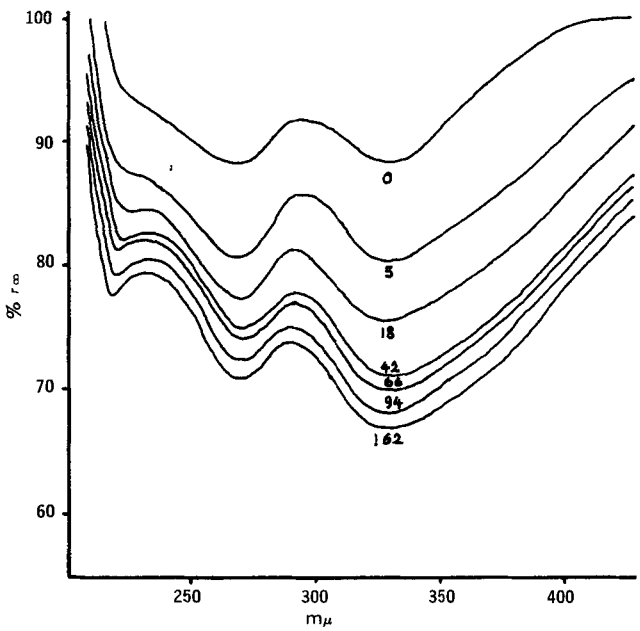


Figure 4—*DRS of a sample prepared by triturating 15 mg. isoniazid with 1 g. magnesium oxide, showing the effects of time on reflectance intensity change. (The numbers shown are time in hours.)*

Table I—Relationship between Concentration of Isoniazid and Reflectances at Two Different Wavelengths

Concentration		Reflectance				
mg. INH/g. MgO	Mole Fraction	$r_{\infty 268}$, %	$f(r_\infty)_{268}$	$r_{\infty 325}$, %	$f(r_\infty)_{325}$	$f(r_\infty)_{268}/f(r_\infty)_{325}$
3	8.82×10^{-4}	71.3	0.05776	72.0	0.05444	1.06
7	2.03×10^{-3}	51.2	9.2326	58.0	0.1521	1.53
10	2.94×10^{-3}	46.8	0.3024	56.0	0.1729	1.75
13	3.82×10^{-3}	43.7	0.3627	54.0	0.1959	1.85
16	4.71×10^{-3}	38.3	0.4970	49.8	0.2530	1.97

concentration effect was considerably more significant for the 268 mμ maximum thanfor the other at 325 mμ. If one assumes that the appearance of these two maxima is due solely to the INH molecules adsorbed onto the MgO surface and that the forces involved in this interaction for these adsorbed molecules are comparable, then this ratio for these two maxima should not change. Theoretically, the ratio of absorbances as well as remission functions at two different wavelengths should be equal to the ratio of the molar absorptivity at the respective wavelengths and should be a constant. Since this is not the case here, it is more likely that the maxima at 268 and 325 mμ are the results of INH molecules being adsorbed by several different mechanisms.

Figure 3 shows a plot of remission function, $f(r_\infty)$, *versus* concentration expressed in mole fraction, *i.e.*, mole INH/mole MgO. Although fluctuation of data was rather significant, it was possible to observe that an approximately linear relationship did exist between the remission function and concentration at 268 mμ, whereas at 325 mμ the remission function approached a plateau. Since in physical adsorption, multiple-layer adsorption may occur, this intensity increase for the 268-mμ maximum with an increase in concentration may be assigned to physical adsorption of INH molecules. This assignment might well be supported by the bathochromic effect observed, which has also been reported by several workers (9, 20) in such adsorption. The second maximum at 325 mμ may be due to chemisorbed INH molecules. Since chemisorption is known to occur only to the extent of formation of a monolayer on the adsorbent surface, the adsorbed molecules in excess of this amount (multilayer) no longer contribute to the intensity of a maximum due to chemisorption, as is seen in the maximum at 325 mμ which gradually shows a plateau at higher concentration and a decrease in the clarity of the peak.

From the molar absorptivity of INH, $a_{262}^{H_2O} = 518.0$, the maximum at 262 mμ can be assigned to a $\pi \rightarrow \pi^*$ transition of the conjugated system in the INH molecule. There is then a possibility of assigning the weaker maximum at 325 mμ in the reflectance spectrum to the $n \rightarrow \pi^*$ transition of the carbonyl group. This is not possible, however, since the intensity of the $n \rightarrow \pi^*$ transition can never exceed that of the $\pi \rightarrow \pi^*$, so the 325-mμ maximum should be always weaker than the 268-mμ maximum. However, this was not observed when the experimental conditions were altered.

To study further the nature of these maxima, 15 mg. of INH/1 g. of MgO was triturated in a mortar for 10 min. and packed into a cell; the reflectance spectrum was then taken at different time intervals. The results are shown in Fig. 4. As is evident from this figure and contrary to Fig. 2, the intensity ratios, $f(r_\infty)_{268}/f(r_\infty)_{325}$, determined within the time interval studied were smaller than 1; as time increased, these ratios gradually decreased and then slowly increased. These relationships between ratios and time calculated from Fig. 4 are shown in Fig. 5, and they may be explained in the following manner. Immediately after the trituration, both chemisorption and physical adsorption have occurred. However, the extent of adsorption in both cases is far from the equilibrium state, as may be observed by a comparison of Fig. 2 and Fig. 4. Consequently, at this stage, except for some of the INH molecules that have been removed from the surface of the INH crystals and chemisorbed onto MgO surface, the rest of the INH molecules remain intact in the crystalline form. However, as the time interval lengthens, some INH molecules become separated from the INH crystalline surface due to particle collision or surface diffusion, and they undergo chemisorption with the large unsaturated MgO surface. This is reflected in the increase in the intensity of the 325-mμ maximum in the diffuse reflectance spectrum. Statistically speaking, it is probable that some of these free INH molecules may also be adsorbed physically to other already chemisorbed molecules, so that the intensity of the maximum at 268 mμ increases but at a much slower rate. This is indicated in Fig. 4, which shows this ratio decrease during the first 66 hr. However, beyond this period the ratio begins to increase. This is probably due to the fact that the unsaturated MgO surface sites are, for the most part, satisfied by the chemisorbed layer of INH molecules and that additional molecules liberated from the INH crystal surface are now physically adsorbed to this layer. If the system just discussed is allowed to attain equilibrium by storage for a sufficient period of time, the reflectance values would be comparable to that of the sample equilibrated in a solvent. Zeitlin *et al.* (18) have confirmed this aspect in their study.

The assignment of the two maxima was further supported by a study that involved equilibration and solvent elution of the drug from the adjuvant surface. Each sample was prepared by equilibrating 5 g. of MgO with 200 mg. of INH dissolved in 50 ml. of methanol and CH_2Cl_2, respectively. The sample was then filtered with gentle

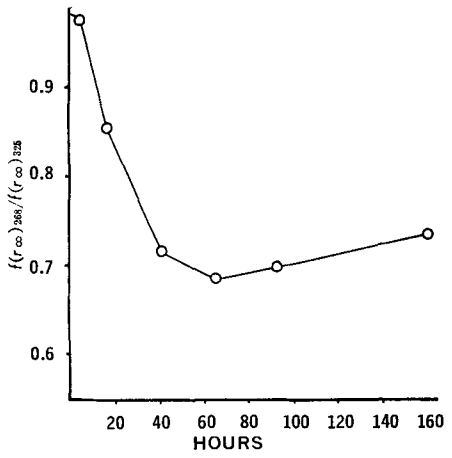


Figure 5—*Relationship between the ratio of two reflectances at different wavelengths and time of a sample prepared by triturating 15 mg. isoniazid with 1 g. magnesium oxide.*

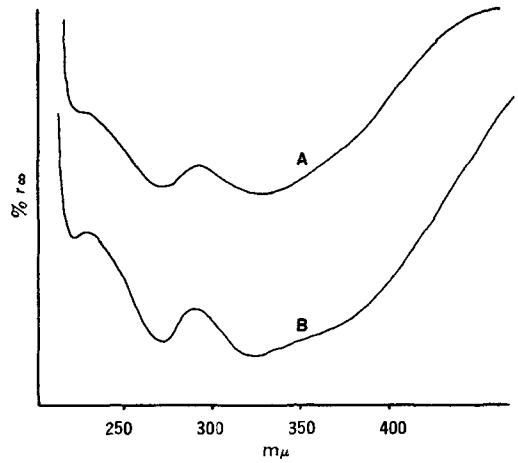


Figure 6—*DRS showing the effects of washing equilibrated dried samples containing 200 mg. isoniazid and 5 g. magnesium oxide. Key: A, equilibrated in methanol and washed with the same solvent; and B, equilibrated in methylene chloride and washed with the same solvent.*

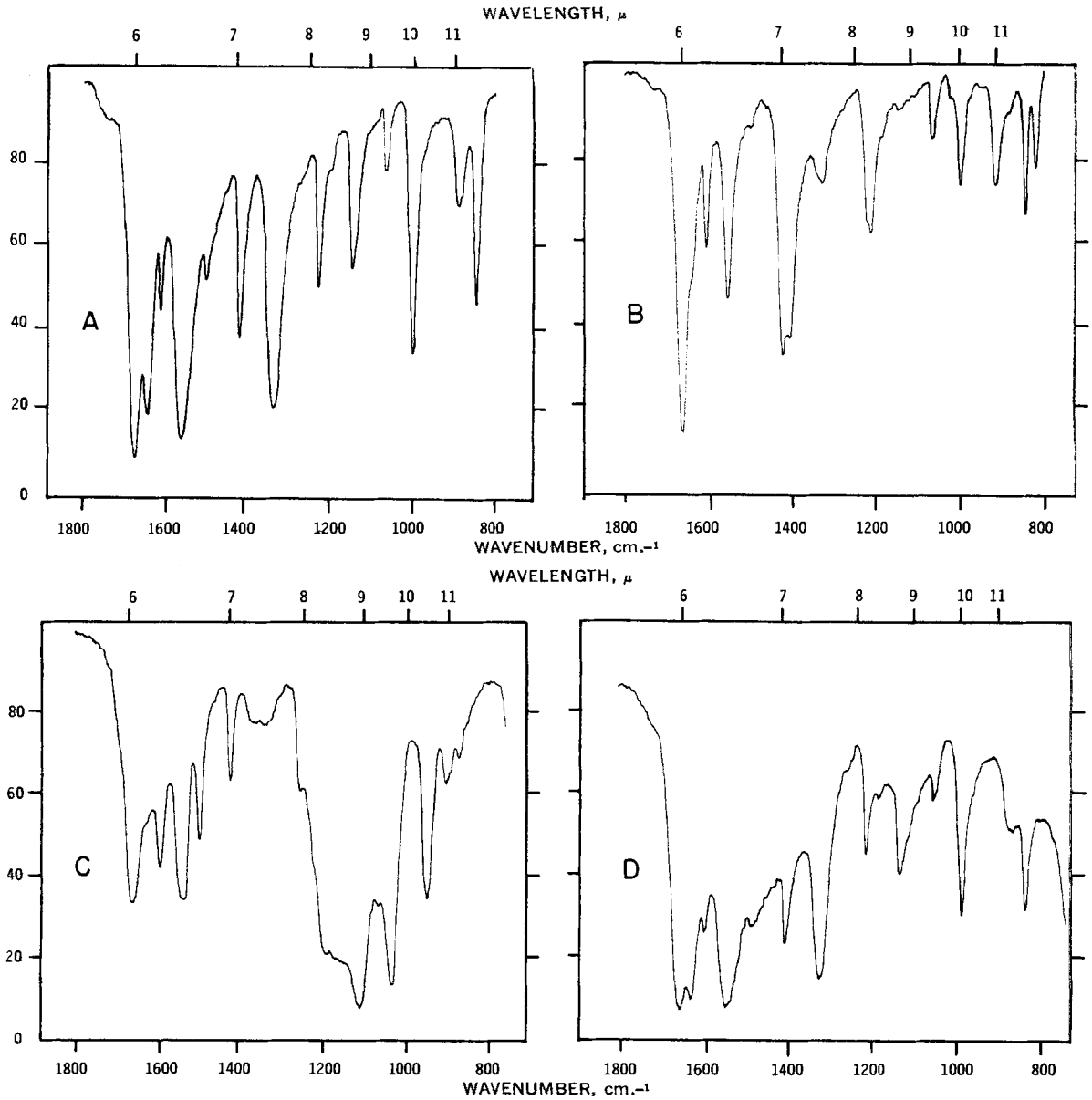
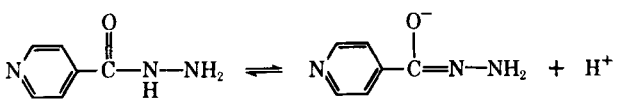


Figure 7—*IR spectra of isoniazid (A), deuterated isoniazid (B), isoniazid–copper complex (C), and isoniazid adsorbed on magnesium oxide (D).*

suction, followed by slow elution with 200 ml. of the respective solvent used in equilibration, and dried at 35° in a vacuum drying oven. Differential thermal spectrograms of these samples indicated that no solvent residue remained. DRS of these samples are shown in Fig. 6. As can be seen, the absorption intensities for the 268-mμ maxima are weaker than for the 325-mμ maxima, and the ratios, $f(r\infty)_{268}/f(r\infty)_{325}$, are less than 1. This is contrary to the ratios calculated from Fig. 2 and listed in Table I. This finding is significant and is due to the fact that in this elution process, physically adsorbed INH molecules are more easily removed than those that are due to chemisorption.

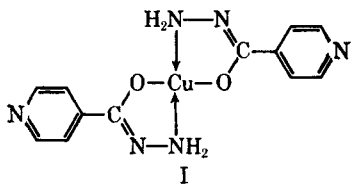
The possibility that this 325-mμ maximum is due to the existence of inter- or intramolecular complexes within the crystal lattice of the INH particle is unlikely in view of the fact that these equilibrated samples were thoroughly eluted with the respective solvents. Under such conditions the presence of crystalline INH particles is very limited.



Scheme I

In their studies dealing with the determination of dissociation or formation constants of INH complexes, various workers (21–24) have reported that spectral shifts of INH solutions do occur with a change in pH. At a pH above 11.6, the 262-mμ maximum is shifted to 300 mμ and is due to ionization of the INH tautomer (Scheme I).

No strong maximum at 325 mμ was observed even at very high pH values. In view of the fact that the pH of a saturated MgO aqueous solution is approximately 10.3, it is highly improbable that the 325-mμ maximum observed in the solid INH–MgO system discussed is due solely to the pH effect. It is interesting to note here that the diffuse reflectance spectrum of a mixture of MgO and INH–Cu complex [the complex prepared according to the procedure of Foye and Duvall (15)] did show a small hump in this 300-mμ region. This would be expected since, in the postulated copper complex structure,



I

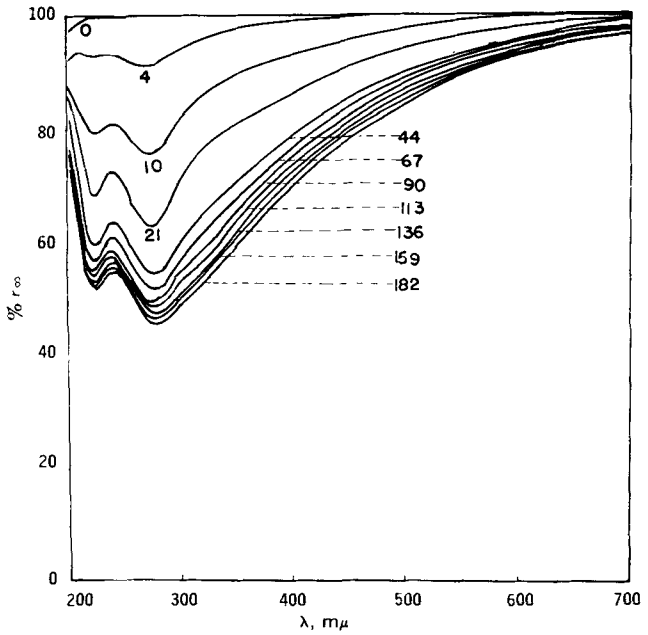


Figure 8—*DRS showing the browning of lactose at 100°. (The numbers shown are time in hours.)*

ionization of the INH tautomer is necessary prior to its formation. The absence of this 300-mμ hump, as seen in Figs. 2, 4, and 6, does suggest that a mechanism or mechanisms other than that involved in the formation of the INH–Cu complex in solution is operative in the INH–MgO equilibrated system. The formation of an INH–Mg complex in solution has not been reported.

These observations are also supported by the IR spectra shown in Fig. 7. Spectra A, B, and C were taken after mixing 1 mg. of INH, deuterated INH, and INH–Cu complex, respectively, with 150 mg. of KBr. Spectrum D was taken of a thoroughly triturated mixture of 1 mg. INH and 3 mg. MgO. This sample was placed in a vacuum desiccator over calcium sulfate for 30 hr. and mixed with 150 mg. of KBr. Spectra A, B, and D portray a strong carbonyl stretching band, νC=O at 1665 cm.$^{-1}$; C exhibits only a weaker band at 1655 cm.$^{-1}$, which is probably due to νC=N rather than the νC$_{-1}$=O. Comparison of A and B at 1636 cm.$^{-1}$ reveals the fact that the 1636-cm.$^{-1}$ band in A is responsible for the bending vibration of an amino group, δ—NH$_2$. Because this amino group bending vibration disappears

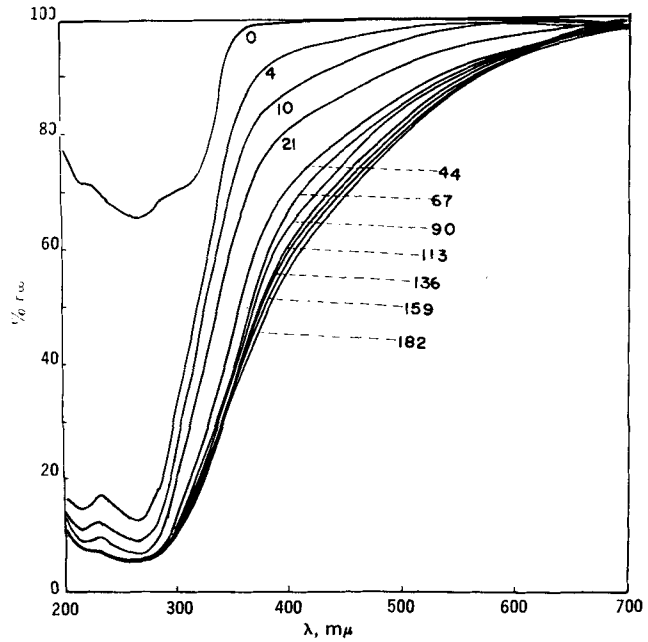


Figure 9—*DRS showing the browning of isoniazid–lactose system at 100°. (The numbers shown are time in hours.)*

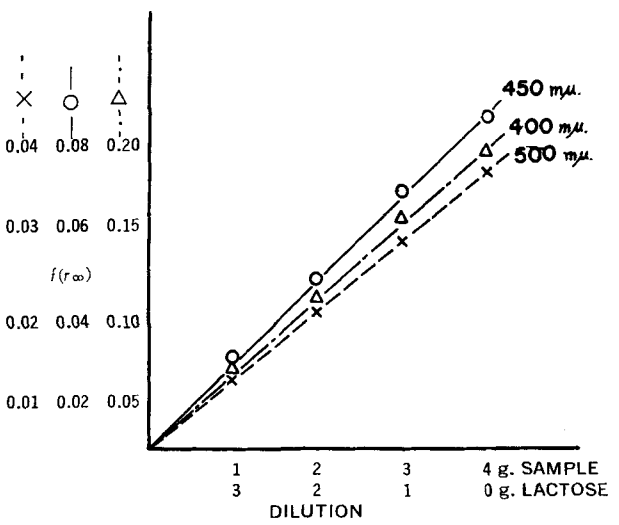


Figure 10—*Relationship between reflectance and dilution of browned substance obtained from heating the isoniazid–lactose system at 100° for 182 hr.*

upon deuteration, it is absent in B. The same band is still present in D but not C. Therefore, it may be assumed that the —NH$_2$ groups in A and D exist in the same free form, while in C it is quite different, probably because of the formation of a coordination bond between cupric ion and the lone pair electrons on the amino nitrogen. However, the most confirmatory evidence is obtained at 1330 and 1110 cm.$^{-1}$, which may be assigned to νC—N and νC—O bands, respectively. Again A and D give the same spectra while C is variant. It is quite natural that Spectrum D shows the 1330-cm.$^{-1}$ band, even if INH has reacted with MgO in a manner similar to that of the INH–Cu complex, because the 1 mg. of INH used is in excess of the amount needed to interact with the surface of the 3 mg. of MgO present. However, the complete absence of a band at 1110 cm.$^{-1}$ indicates that the carbonyl double bond in Spectrum D is still intact. On the other hand, Spectrum C displays a strong νC—O band at 1110 cm.$^{-1}$, which coincides well with the postulated structure of this INH–Cu complex. Hence, it may be concluded that, contrary to that of the INH–Cu complex, the interaction of INH with MgO does not induce significant alteration in the molecular structure of INH. Thus the interaction, as well as the formation of the new UV maximum at 325 mμ in the diffuse reflectance spectrum, is the result of a mechanism different from that involved in this INH–Cu complex.

One possibility is that a donor–acceptor mechanism is operative in this INH–MgO interaction. It is known that the pyridine ring itself is a good donor. This donor capacity, however, might be somewhat reduced by the presence of a carbonyl group that exhibits a negative inductive effect. This effect would not be too significant if one considers the counterbalancing effect of the hydrazide group which may supply enough flow of electrons into the carbonyl group. Electron affinities of many compounds are not well known. However, if the energy level of the antibonding molecular orbital of MgO lies lower

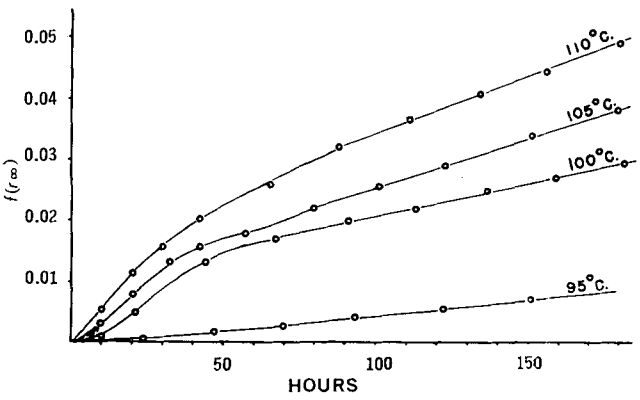


Figure 11—*The rates of browning of lactose at 95, 100, 105, and 110°.*

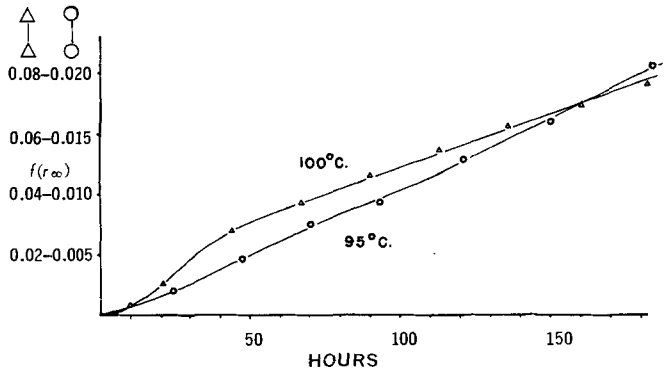


Figure 12—*The rates of browning of the isoniazid–lactose system at 95 and 100°.*

than the antibonding orbital of the conjugated system in the INH molecule, there is a possibility that a π-electron of INH will be transferred to the low-lying antibonding orbital of MgO instead of to the higher energy antibonding orbital of its own. This could happen when the two molecular orbitals approach close proximity to each other so that the overlapping of molecular orbitals actually occurs. Such a situation would require strong adsorption of a substrate onto an adsorbent surface, that is, chemisorption rather than physical adsorption. The immediate consequence of this type of electron transfer is the appearance of a resultant two-charged species which, in turn, would attract each other much more than the orginal two polar molecules. This does indicate the complexity of surface interactions since both chemisorption and physical adsorption are operative.

INH–Lactose System—The reflectance spectra of both lactose and the INH–lactose system are shown in Fig. 8 (Curve O) and Fig. 9 (Curve O). As is evident in Fig. 8 (Curve O), lactose has not been influenced by this equilibration process. Figure 9 (Curve O), which represents the spectrum of the equilibrated INH–lactose sample, shows a broad maximum at 265 mμ and shoulders at both sides of the maximum. Because of the lack of well-separated maxima in the spectrum of the INH–lactose system as compared to that of MgO, a study dealing with concentration effects with respect to intensity changes and spectral shifts was not successful.

Heating of the equilibrated lactose and INH–lactose samples in an oven at 100 ± 0.5° did produce changes in the DRS spectra, as shown in Figs. 8 and 9, as well as browning of the samples. Although no absorption maximum was initially seen in the equilibrated lactose system containing no drug, a maximum did appear at about 275 mμ after heating. In the INH–lactose system, this thermal effect broadened the maximum at 265 mμ. Since no maximum appeared in the visible region of the spectra in Figs. 8 and 9, selection of a proper wavelength at which the extent of browning was to be measured was next undertaken. For this purpose, the darkened sample was subjected to serial dilutions with lactose, and the reflectance spectra were taken. When the remission function, $f(r\infty)$, *versus* extent of dilution was plotted at 400, 450, and 500 mμ, respectively, all

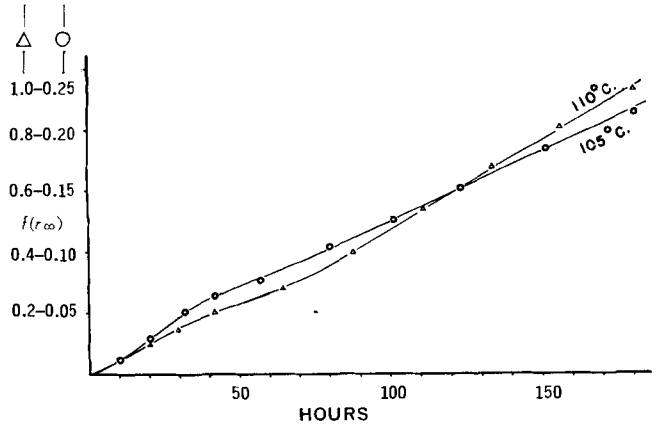


Figure 13—*The rates of browning of the isoniazid–lactose system at 105 and 110°.*

Table II—Apparent Zero-Order Rate Constant, k, of Browning of Lactose and Isoniazid–Lactose Systems

Temperature	Lactose		Isoniazid–Lactose	
	k	log k	k	log k
95°	5.305×10^{-5}	−4.2753	1.238×10^{-4}	−3.9073
100°	1.093×10^{-4}	−3.9614	3.560×10^{-4}	−3.4486
105°	1.574×10^{-4}	−3.8030	1.150×10^{-3}	−2.9393
110°	1.890×10^{-4}	−3.7235	5.860×10^{-3}	−2.2321

showed straight-line relationships as illustrated in Fig. 10, showing the exact conformity with an equation described by Wendlandt and Hecht (25):

$$f(r\infty) = \frac{2.303 \cdot a \cdot C}{S} \qquad \text{(Eq. 2)}$$

where a is the molar absorptivity, C is the molar concentration, and S is the scattering coefficient.

The reflectance at 450 mμ was arbitrarily chosen and employed in the subsequent reflectance measurements for the investigation of the rate of browning in the solid systems. The results are shown in Figs. 11–13. Careful examination of each curve revealed that it consists of two portions. In the first stage of the browning reaction, which in most cases occurred between 0–50 hr., a sigmoidal curve was obtained; in the second stage, an almost straight-line relationship with time was observed. From these two distinct patterns in the reflectance data, it may be assumed that two fundamentally different mechanisms are probably operative. Since each lactose molecule contains 1 molecule of water which may have a direct relationship in the first stage of this browning reaction, a study of the dehydration aspect of lactose was carried out at the same temperature ranges. The results showed that, except at 95°, the complete dehydration of lactose occurred within about 50 hr., which coincided with the time necessary for the completion of the sigmoidal portion of the curve. During this stage of browning, it appears that a true solution phase is formed locally between the water liberated from lactose monohydrate, and that this dehydrated lactose and INH then proceed to react to form: (*a*) a condensation product of lactose and INH, and (*b*) various carbonyl compounds through stepwise degradation of lactose. This initial reaction can be regarded as being in a solution phase and not the so-called solid–solid reaction in the true sense of the meaning.

During the second stage of browning, the reaction may be considered essentially in the solid state. From the straight-line relationship obtained between remission function and time, the browning reaction may be assumed to follow an apparent zero-order rate law; from the slope, the rate constants, k, were calculated and are listed in Table II. However, since the actual reaction is very complicated and many different types of reaction nuclei may be formed during the

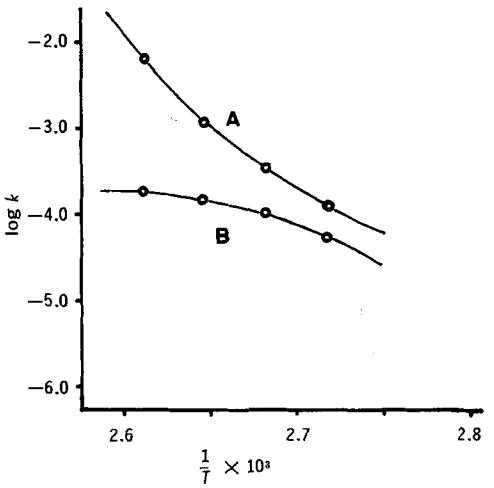


Figure 14—*Temperature dependence of apparent zero-order rate constant for browning reactions of the isoniazid–lactose system (A) and lactose alone (B).*

Table III—Comparison of the Rate of Formation of Hydroxymethylfurfural and Isonicotinoyl Hydrazone of Hydroxymethylfurfural

Time	HMF, *M*	INH–HMF, *M*
0	0	0
5	5.16×10^{-5}	1.63×10^{-4}
10	2.98×10^{-4}	3.15×10^{-4}
16	4.76×10^{-4}	4.63×10^{-4}
21.5	5.67×10^{-4}	5.67×10^{-4}
27	7.16×10^{-4}	7.08×10^{-4}
34	9.74×10^{-4}	7.86×10^{-4}
41	1.03×10^{-3}	1.06×10^{-3}
46	1.22×10^{-3}	1.03×10^{-3}
53	1.21×10^{-3}	1.16×10^{-3}
64	1.34×10^{-3}	1.34×10^{-3}

first stage of the browning reaction, these, along with other nuclei generated in this second stage, may then proceed to form various products. Therefore, the apparent rate constant, k, in this case does not have the usual kinetical significance. It does, however, represent a total overall reaction and does enable the approximate estimation of the rate of reaction at the other temperatures. From the data in Table II, an Arrhenius plot was attempted (Fig. 14). From the curves obtained instead of the usual straight lines, it is obvious that the reaction mechanisms are not consistent throughout the temperature range studied, and this reflects the complexity of the browning reaction. When the curves are extrapolated to room temperature, assuming that the curves do not change their curvature, it is possible to estimate the approximate time required for the extent of browning to be perceptible. Since lactose showed a convex curve that does not intersect with a vertical line down through a point at 25°, it would appear that this browning of lactose would require an infinite time to occur. Brownley and Lachman (26) reported that lactose remained white after storage for 36 months under ambient conditions. On the other hand, the equilibrated mixture of INH and lactose showed a concave curve. From the intersect with the vertical line at 25°, log k was determined and the time required for initial browning to be perceptible, assuming $r\infty = 90\%$, was calculated as approximately 4 years. In this calculation, it is assumed that the straight-line portion of the curve passes through the origin. This 4-year stability of the INH–lactose system will certainly be reduced to some extent when the sample is exposed to excessive moisture. Ritschel and Rahman (27) and Horikoshi and Himuro (28) drew attention to the fact that atmospheric moisture did accelerate the browning of INH tablets.

Since it is well known that browning of many compounds, such as food and food products, is also faster in the presence of moisture, the browning of the INH–lactose system was carried out in solution for purposes of comparison and to isolate reaction products where possible. Prior to heating, Solutions *a* and *b* showed absorption at 262 mμ, which is due to INH as mentioned before (see *Browning of INH–Lactose Mixture in Solution*). Solution *c* did not show any absorbance. After heating, Solution *a* showed a shoulder at about 300 mμ. Solution *b*, which is a mixture of INH and lactose, showed a new strong absorption at 320 mμ. Solution *c* exhibited an intense maximum at 283 mμ, which was identified by TLC as 5-hydroxymethylfurfural (HMF). The new maximum at 320 mμ in Solution *b* was believed to be the result of a reaction between INH and HMF, which was confirmed by TLC using prepared INH–HMF as a standard. The compound INH–HMF has been prepared differently by Knotz (29). Since both HMF and INH–HMF were found to obey Beer's law within 0–0.6 mg. HMF/100 ml. H_2O and 0–0.8 mg. INH–HMF/100 ml. H_2O,[16] the quantities of INH–HMF and HMF produced in Solutions *b* and *c* were determined from the spectra and are listed in Table III. This table indicates that the rates of appearance of both INH–HMF and HMF were approximately equal. From these results, it is assumed that HMF, which is formed from the degradation of lactose in aqueous solution, reacts instantly with INH to form INH–HMF. The high reactivity of both species was substantiated by the fact that swift precipitation of INH–HMF was observable as moderately concentrated solutions of both species were mixed together. Also, an almost theoretical yield was obtained in the preparation of INH–HMF.

[16] With the aid of small amount of methanol.

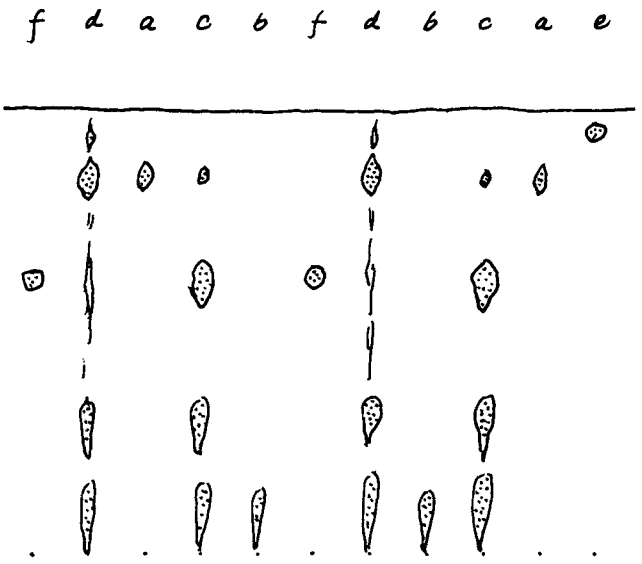
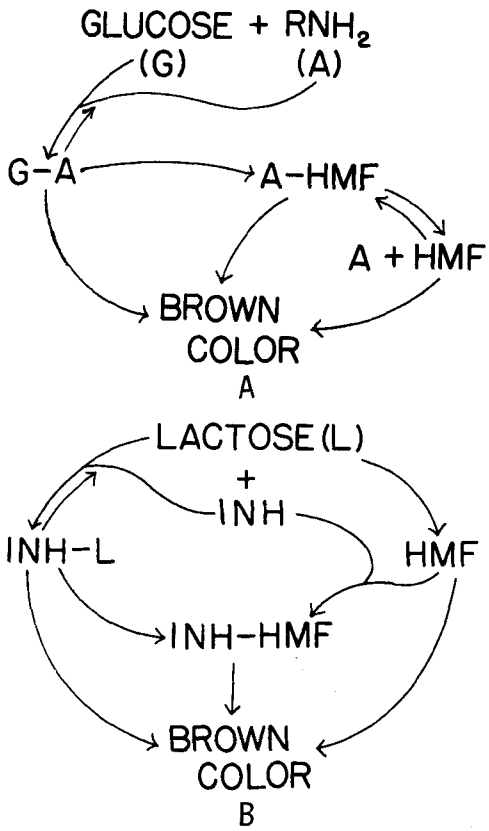


Figure 15—*Thin-layer chromatogram showing thermal degradation products of the isoniazid–lactose system. Key: a, INH–HMF, R_f = 0.85, pink spot under UV light, brown spot by I_2 vapor; b, INH–L, R_f = 0.12, brown spot by I_2 vapor; c, methanol extract of degradation products in solid state; d, methanol extract of degradation products in solution; e, HMF, R_f = 0.96, black spot under UV light, brown spot by I_2 vapor; and f, INH, R_f = 0.62, brown spot by I_2 vapor.*

Since INH–HMF was found in solution degradation, it was interesting to know whether the same compound was also formed in the solid system. Identification of the reaction products in the solid state was carried out using TLC. The highly darkened solid sample was first extracted with anhydrous methanol and then spotted on a plate. Simultaneously, methanol solutions of INH, INH–HMF, HMF, and isonicotinoyl hydrazone of lactose (INH–L) were also spotted. INH-L was tested since Yamamoto and Tanaka (30) reported its formation in INH tablets. The compound was prepared according to a method described by the same authors. The results of TLC are shown in Fig. 15. The presence of INH–HMF and INH–L was evident in this figure. The isolation and confirmation of INH–HMF were carried out first by concentration of a large quantity of methanol extract. This concentrate was then streaked onto a 3.0-mm. thick preparative plate and developed as before. Using UV light as a guide, the pink-color band was scraped off and extracted with anhydrous methanol. The extract was concentrated, diluted with an equal volume of water, and then purified; it showed the same UV and IR spectra when compared with the prepared INH–HMF. Since the quantity of INH–HMF formed in the solid system was very small, many difficulties were encountered in its quantitative determination; consequently, attempts to correlate the rate of browning and the rate of formation of INH–HMF in the solid system were unsuccessful.

The mechanisms of the Maillard-type browning reactions involving glucose and glycine have been discussed in detail by several authors (31, 32); a stepwise reaction scheme was proposed. A simplified reaction scheme is shown in Scheme II A. Although the mechanisms involved in the reactions between sugars and amino acids might resemble the present system, the results from the present studies showed some discrepancies. The degradative mechanisms have been modified and are given in Scheme II B. In the reaction of the INH–lactose system, the formation of INH–HMF may not completely rely on INH–L as a source. Since the formation of INH–L and HMF needs approximately the same conditions, *i.e.*, high temperature and humidity, and since the reactivity of HMF is high, the formation of INH–HMF from INH and HMF is also possible. As shown in Scheme II A, most workers have considered that an equilibrium exists between A − HMF and A + HMF. However, this is not conceivable, except in the presence of catalyst which may promote the hydrolysis of INH–HMF. In fact, the whole process of the browning reaction may be expected to be much more complicated than the proposed scheme, since many parallel pathways may exist in this process. Recently, Mitsuda *et al.* (33) reported that electron spin



Scheme II—Mechanisms af browning reactions. Key: Scheme A, described by Keeney and Bassette (32); and Scheme B, modified

resonance spectroscopy revealed that a free radical reaction was found operative in the aminocarbonyl reaction of food browning.

Although the interaction of INH and lactose does not proceed to an appreciable degree under ambient conditions, the situation may become significant under moderate temperature and moisture conditions as those found in tropical or subtropical climates. It should also be recognized here that during wet granulation procedures, in which both INH and lactose are exposed to higher temperatures and excessive moisture, these degradation aspects cannot be overlooked.

As has been pointed out by Bernstein *et al.* (34), none of the INH derivatives, including hydrazones, is more active on a molar basis than INH itself. It is also interesting to speculate here as to the tuberculostatic activity of darkened tablets of INH–HMF and INH–L itself.

The INH–HMF prepared in this study has been evaluated for its antitubercular activity.[17] Preliminary *in vitro* results using the standard NCBC plate method indicate that INH–HMF and INH have approximately comparable activity. However, due to the fact that their physicochemical properties are sufficiently different (for example, aqueous solubility) and that even if the *in vitro* tests appear to be comparable, it does not necessarily mean that they are clinically equivalent. For example, Kakemi *et al.* (35) studied absorption of INH and its derivatives from the gastrointestinal tract and found that the INH–L is hardly absorbed. A recognition of these INH–lactose reactions and their effects on drug availability and therapeutic response is therefore necessary in the formulation of satisfactory INH dosage forms.

[17] At the State of Iowa Hygienic Laboratory.

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