# A NOTE ON THE RATES OF REACTION OF L-CYSTEINE HYDROCHLORIDE WITH CERTAIN MALEIMIDES[1]

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The effects of maleimide (MI), N-ethylmaleimide (NEMI), and N-phenylmaleimide (NPMI) on mixogram characteristics, on gassing power of yeast, and on loaf volume were studied recently by Lee and Reynolds (3). It was noted that NPMI exerts its sulfhydryl-blocking influence less effectively than NEMI or MI. A steric factor arising from the over-all size of the reagent and its effectiveness in approaching and reacting with sulfhydryls of macromolecules, such as proteins, was suggested as playing a prominent role in determining the observed results. Another factor that has to be considered is the possibility that NPMI may react with sulfhydryls more slowly than does NEMI or MI. It is, therefore, of interest to compare the rates of reaction of these maleimides with a simple sulfhydryl-containing compound such as cysteine.

On the basis of UV absorption at 300 m$\mu$, the reaction of an excess of NEMI with the sulfhydryl group in buffered aqueous solutions has been reported (1,4) to go to completion rapidly. Also the rate of this reaction was found to be greater in a neutral than in an acidic medium (2). With a Cary recording spectrophotometer, we have confirmed these observations. It was further noted that the reaction of NEMI with cysteine hydrochloride proceeds faster in aqueous than in ethanolic solution; that the presence of neutral salts such as sodium chloride also increases the rate; and that the effect of pH is so pronounced that use of cysteine-free base instead of the hydrochloride greatly enhances the rate in either aqueous or ethanolic medium. These findings, together with the second-order kinetic behaviors to be described below, suggest that reaction between the sulfhydryl group and the maleimide type of reagents is one of nucleophilic addition.

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In order to obtain reasonably slow rates so that reaction kinetics may be followed spectrophotometrically, and because NPMI is insoluble in water, the reactions of MI, NEMI, and NPMI with L-cysteine hydrochloride were studied in an ethanolic medium. The UV spectra of MI, NEMI, and NPMI in absolute ethanol show maxima respectively at 275, 294, and 308 mμ. Straight-line calibrations relating concentration to absorbance at these wave lengths were obtained for all three maleimides. In the kinetic runs, 2.0-millimolar (mM) solutions of each of the maleimides were made up in absolute ethanol. L-cysteine hydrochloride was dissolved in a small amount of water and then diluted with absolute ethanol to 2.0-mM concentration. The final water content of this solution was 0.5% by volume. The solutions were maintained at 25°C. An equal volume of the L-cysteine hydrochloride solution was added to each of the three solutions of maleimides, giving reaction mixtures 1.0 mM in L-cysteine hydrochloride and 1.0 mM in MI, NEMI, or NPMI. At various time intervals, aliquots were withdrawn and the concentrations of MI, NEMI, or NPMI determined spectrophotometrically.

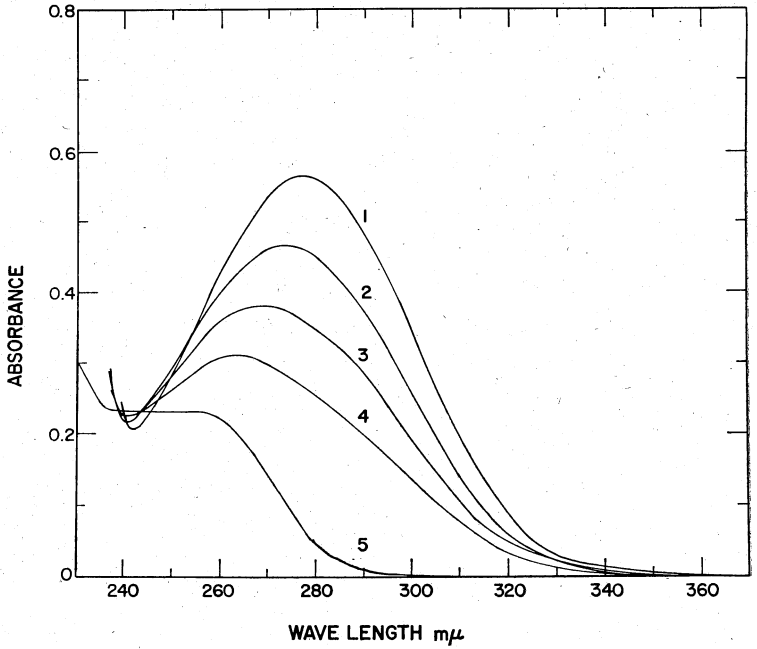


Fig. 1. UV spectra of mixtures of MI and MI-cysteine adduct. 1, 2, 3, 4, 5 represent, respectively: 100% MI; 80% MI-20% adduct; 60% MI-40% adduct; 40% MI-60% adduct; and 100% adduct.

It may be pointed out that during the course of these reactions, λmax for NEMI or NPMI remained unchanged, and the absorbance at λmax decreases as the reaction proceeds according to expectation. On the other hand, during the reaction between MI and L-cysteine hydrochloride, λmax of the reaction mixture shifts from 275 mμ towards shorter wave lengths as the reaction progresses. This results from interference by the appreciable absorption at this region by the MI-cysteine adduct, as illustrated in Fig. 1. Nevertheless, Beer's law is still obeyed at 275 mμ and the concentration of MI in the reaction mixture was measured at 275 mμ, though this wave length no longer corresponded to maximum absorption.

The results from the kinetic studies indicate that these are second-order reactions. A typical set of experimental data is shown graphically in Fig. 2. The specific rate constants derived from Fig. 2 are $0.42 \times 10^{-3}$, $1.25 \times 10^{-3}$, and $4.40 \times 10^{-3}$ l. mole$^{-1}$ sec.$^{-1}$ for the reaction with MI, NEMI, and NPMI, respectively. Thus of the three maleimides, NPMI reacts most rapidly with L-cysteine hydrochloride in

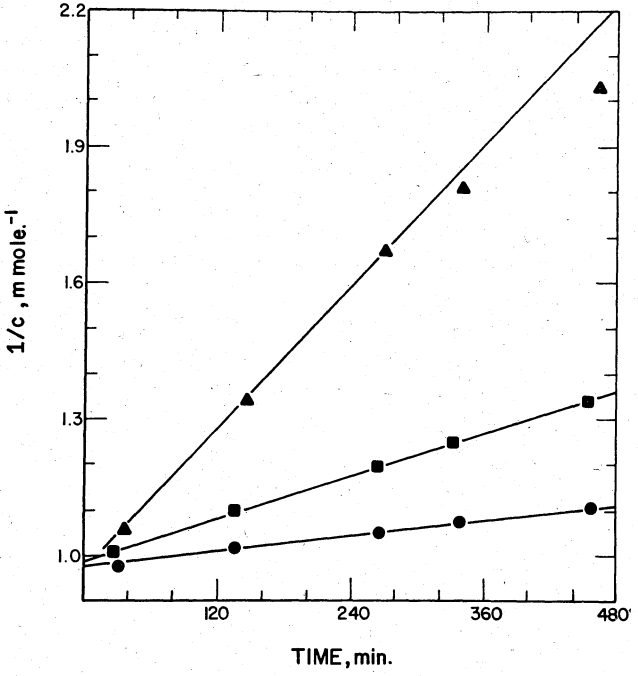


Fig. 2. Kinetic data for reaction of 1.0 mM L-cysteine hydrochloride with 1.0 mM MI, NEMI, or NPMI in ethanol at 25°C. Circles, MI; squares, NEMI; triangles, NPMI.

99.75% ethanol-0.25% water at 25°C. In pure water solution, reactions of MI and NEMI with L-cysteine hydrochloride at 25°C. gave, respectively, second-order specific rate constants of 0.12 and 0.20 l. mole$^{-1}$ sec.$^{-1}$ Hence a change from ethanolic to aqueous media increased the rates of MI and NEMI by about two orders of magnitude. Because of its insolubility, it is, of course, not possible to study the reaction of NPMI in an aqueous medium. In their work on the sulfhydryl-blocking effects of these maleimides, Lee and Reynolds (3) used solutions of MI, NEMI, or NPMI in 25% ethanol-75% water to replace part of the water of absorption. It might, therefore, be of interest to compare the relative rates in a solvent such as 25% ethanol-75% water. However, this again was not possible with NPMI because of the instability of its spectrum in solvents containing a large proportion of water. While the spectra of 1.0 mM MI or NEMI in 25% ethanol-75% water showed no appreciable changes after periods of up to 12 hr., the spectrophotometric behavior of dilute solutions of NPMI in this solvent suggested the occurrence of hydrolysis. The spectrum of 1.0 mM NPMI in 25% ethanol-75% water changed gradually in about 2 hr. to one resembling the spectrum of N-phenylmaleamic acid. This hydrolysis appeared to be slower if the concentration of NPMI was higher.

It is of interest to discuss the above purely chemical results in relation to the sulfhydryl-blocking effects of these maleimides reported earlier (3) on cereal chemical systems. It is realized that the rates of reaction between L-cysteine hydrochloride and the maleimides in ethanol containing very little water would be different from rates of reaction between these maleimides and the sulfhydryls of yeast suspension or dough made up with mostly water and a relatively small proportion of ethanol. Since a change from ethanolic to aqueous media increased the rates for both MI and NEMI, in the yeast suspension or dough system, dissolved MI, NEMI, or NPMI may all react with sulfhydryls at rates greater than the corresponding ones in ethanolic solution. As the relative rates in ethanol show that NPMI reacts most rapidly with the sulfhydryl group, if the changes from ethanolic solution to yeast suspension or dough will affect the rates of all three maleimides by factors of more or less the same magnitude, it is possible that NPMI will maintain its relatively greater reactivity towards sulfhydryls under the cereal chemical environments. Such considerations suggest that differences in rates of reaction with the sulfhydryl group would not be the most probable explanation for the observed lesser effectiveness of NPMI in exerting its sulfhydryl-blocking influence on cereal chemical systems.

The spectophotometric studies also indicated the likelihood of hydrolysis of NPMI. Loss of part of the NPMI through hydrolysis, especially when this reagent was used in very dilute concentrations, may at least be partially responsible for the apparently less effective action of NPMI. In addition, the previously suggested steric factor (3) relating the over-all size of the reagent to its capability of approaching and reacting with sulfhydryls in dough systems may also play a role in determining the relative effectiveness of MI, NEMI, and NPMI in exerting their influence under cereal chemical environments.

**Acknowledgment**

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**Literature Cited**

1. ALEXANDER, N. M. Spectrophotometric assay for sulfhydryl group using N-ethylmaleimide. Anal. Chem. **30:** 1292–1294 (1958).
2. GREGORY, J. D. The stability of N-ethylmaleimide and its reaction with sulfhydryl groups. J. Am. Chem. Soc. **77:** 3922–3923 (1955).
3. LEE, C. C., and REYNOLDS, L. M. Some studies with maleimide-type sulfhydryl-blocking agents. Cereal Chem. **39:** 427–436 (1962).
4. ROBERTS, E., and ROUSER, G. Spectrophotometric assay for reaction of N-ethylmaleimide with sulfhydryl groups. Anal. Chem. **30:** 1291–1292 (1958).