Further studies are needed to assess the potentially adverse effects of drug–nitrite interactions in humans, especially those on chronic medication where readily nitrosatable drugs and high dietary intake of nitrite may be involved.

(1) H. Druckrey, R. Preussmann, S. Ivankovics, and D. Schmahl, *Z. Krebsforsch.*, **69**, 103(1967).
(2) J. Sander and F. Schweinsberg, *Zentralbl. Bakteriol. Hyg.*, **B156**, 299(1972).
(3) W. Lijinsky, E. Conrad, and R. Van de Bogart, *Nature*, **239**, 165(1972).
(4) M. Greenblatt, V. Kommineni, E. Conrad, L. Wallcave, and W. Lijinsky, *Nature New Biol.*, **236**, 25(1972).
(5) W. Lijinsky, *Cancer Res.*, **34**, 255(1974).
(6) R. Montesano, H. Bartsch, and H. Bresil, *J. Nat. Cancer Inst.*, **52**, 907(1974).
(7) G. S. Rao, G. Krishna, and J. R. Gillette, *Fed. Proc.*, **32**, 319A(1973).
(8) G. S. Rao, G. Krishna, and J. R. Gillette, *Pharmacologist*, **15**, 191(1973).
(9) W. Lijinsky and M. Greenblatt, *Nature New Biol.*, **236**, 177(1972).
(10) W. Lijinsky, H. W. Taylor, C. Snyder, and P. Nettesheim, *Nature*, **244**, 176(1973).
(11) M. Greenblatt and S. S. Mirvish, *J. Nat. Cancer Inst.*, **49**, 119(1972).
(12) W. Lijinsky and S. S. Epstein, *Nature*, **225**, 21(1970).
(13) P. N. Magee, *Food Cosmet. Toxicol.*, **9**, 207(1971).
(14) I. A. Wolff and A. E. Wasserman, *Science*, **177**, 15(1972).
(15) R. Hoover and J. F. Fraumeni, *J. Clin. Pharmacol.*, **15**, 16(1975).
(16) G. E. Hein, *J. Chem. Educ.*, **40**, 181(1963).
(17) P. A. S. Smith and R. N. Loeppky, *J. Amer. Chem. Soc.*, **89**, 1147(1967).
(18) W. Lijinsky, L. Keefer, E. Conrad, and R. Van de Bogart, *J. Nat. Cancer Inst.*, **49**, 1239(1972).
(19) J. W. Pensabene, W. Fiddler, C. J. Dooley, R. C. Doerr, and A. E. Wasserman, *J. Agr. Food Chem.*, **20**, 274(1972).
(20) G. S. Rao, *J. Dent. Res.*, **54A**, 172(1975).
(21) G. Schroll, R. G. Cooks, P. Klemmensen, and S. O. Lawesson, *Ark. Kemi*, **28**, 413(1968).
(22) L. Knorr, *Ann.*, **238**, 137(1887).
(23) M. A. Friedman, E. J. Greene, and S. S. Epstein, *J. Pharm. Sci.*, **61**, 1492(1972).
(24) S. S. Mirvish, L. Wallcave, M. Eagan, and P. Shubik, *Science*, **177**, 65(1972).
(25) M. Greenblatt, *J. Nat. Cancer Inst.*, **50**, 1055(1973).
(26) J. J. Kamm, T. Dashman, A. H. Conney, and J. J. Burns, *Proc. Nat. Acad. Sci. USA*, **70**, 747(1973).
(27) J. B. Fox, Jr., and R. A. Nicholas, *J. Agr. Food Chem.*, **22**, 302(1974).
(28) J. Sander, F. Schweinsberg, and H. P. Menz, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 1691(1968).
(29) J. Sander and F. Seif, *Arzneim.-Forsch.*, **19**, 1091(1969).
(30) S. S. Mirvish, *Toxicol. Appl. Pharmacol.*, **31**, 325(1975).
(31) J. Sander, F. Schweinsberg, M. Landenstein, H. Benzing, and S. H. Wahl, *Hoppe-Seyler's Z. Physiol. Chem.*, **354**, 384(1973).

G. Subba Rao [x]
Laboratory of Pharmacology
Division of Biochemistry
Research Institute
American Dental Association
Chicago, IL 60611

Gopal Krishna
Laboratory of Chemical Pharmacology
National Heart and Lung Institute
Bethesda, MD 20014

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[x] To whom inquiries should be directed.

Application of Mixed Electron-Impact–Chemical Ionization High-Resolution Mass Spectrometry to a Medicinal Agent

Keyphrases □ Structure determination—application of mixed electron-impact–chemical ionization high-resolution mass spectrometry □ Mass spectrometry, mixed electron impact–chemical ionization—structure determination

To the Editor:

Chemical ionization mass spectrometry has proven to be a useful technique for molecular structure determination of various classes of medicinal agents, especially for compounds that contain no molecular ion in the electron-impact spectra. Successful identification of drug metabolites and multicomponent drug mixtures, such as those received by forensic laboratories, is an excellent example of the utility of chemical ionization mass spectrometry. Several comprehensive listings of chemical ionization and electron-impact mass spectra are available for comparison with spectra of unknown drugs (1–3). Therefore, it is useful to obtain both types of spectra for structure elucidation of medicinal agents.

Chemical ionization mass spectra are produced *via* gaseous ion–molecule reactions occurring in the ion source of the mass spectrometer. For an ion–molecule reaction to take place, it is necessary to raise the low pressure (10^{-6} torr) which is characteristic of the conventional electron-impact source. "Closing" the source allows the pressure to increase to approximately 0.5 torr when a reagent gas such as isobutane is added. However, this modification, which facilitates ion–molecule reactions in the chemical ionization mode, may precipitate reactions in the source between sample ions and sample molecules in the electron-impact mode (*i.e.*, self-ionization).

Many investigators have noted the occurrence of collision-induced ions in the electron-impact spectra obtained from closed source instruments (4), particularly MH^+ and fragments generally associated with chemical ionization spectra. Collision-induced ions together with those ions resulting directly from electron impact constitute what is referred to here as a mixed electron-impact–chemical ionization spectra. The ratio of collision ions to electron-impact ions contained in a mixed electron-impact–chemical ion-

Table I—Mixed Electron-Impact–Chemical Ionization High-Resolution Mass Spectral Data of Meprobamate

Elemental Composition	Theoretical	Found	Relative Intensity, %
$C_9H_{19}N_2O_4$	219.1344	219.1335	79
$C_8H_{16}NO_2$	158.1180	158.1177	100
$C_7H_{14}NO_2$	144.1024	144.1007	81
$C_7H_{14}O$	114.1044	114.1032	15
C_7H_{12}	96.0938	96.0931	22
C_6H_{11}	83.0860	83.0844	90
CH_4NO_2	62.0241	62.0242	35
C_4H_7	55.0546	55.0550	68
CH_2NO	44.0136	44.0125	51

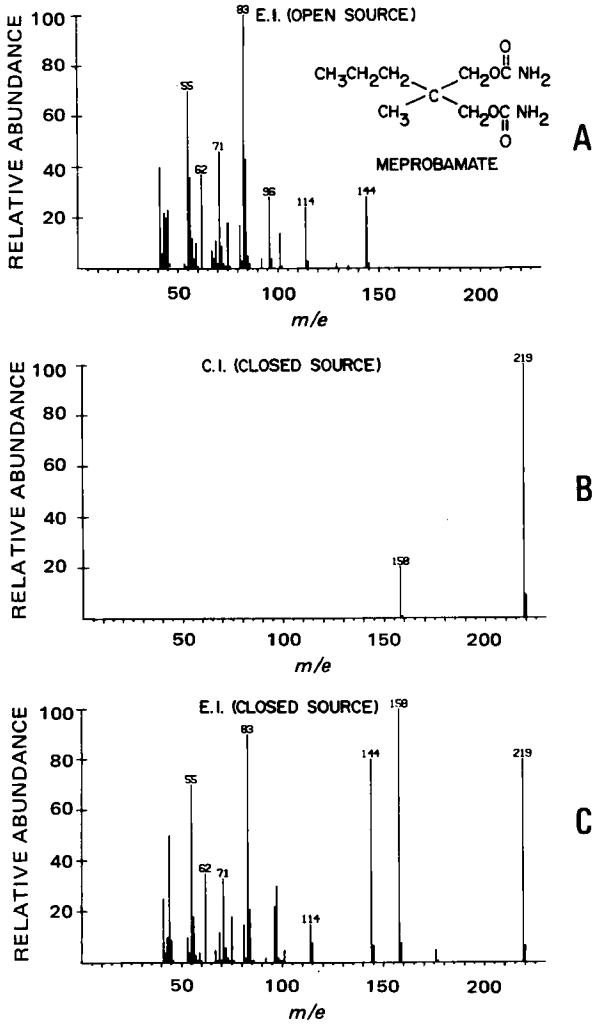

Figure 1—*(A) Mass spectrum of meprobamate obtained prior to chemical ionization modification of the instrument. (B) Chemical ionization (CI) spectrum of meprobamate after installation of a dual electron-impact (EI)–chemical ionization source. (C) Mixed electron-impact–chemical ionization spectrum of meprobamate.*

ization spectra can be altered by varying the pressure in the closed ion source. Higher pressure can easily be attained by either increasing the sample size or reducing the dimensions of the electron entrance and ion exit slit.

Mixed electron-impact–chemical ionization spectra have been particularly useful for compounds, such as meprobamate, that show no molecular ion in the electron-impact spectra (open source). Dicarbamates and many other classes of medicinal agents readily give mixed electron-impact–chemical ionization spectra because of their large cross section for proton capture (5).

All of the mass spectra shown here were obtained on the same high-resolution mass spectrometer coupled with a data processor[1]. Samples were introduced into the source *via* a direct insertion probe. Spectra were obtained at a source temperature of 90°. Figure 1A is a mass spectrum of meprobamate obtained prior to chemical ionization modification of the instrument (open source). Figure 1B shows the chemical ionization spectrum of the same compound after installation of a dual electron-impact–chemical ionization source (closed source). Isobutane was used as the reagent gas. Figure 1C is an illustration of a mixed electron-impact–chemical ionization spectrum obtained from a dual electron-impact–chemical ionization source instrument. This closed source spectrum was obtained in the electron-impact mode by merely increasing the sample pressure. No reagent gas was added.

By adding perfluorokerosene and carefully adjusting the pressure in the source, it is possible to obtain a mixed electron-impact–chemical ionization spectrum suitable for high-resolution mass measurements. While fast scanning the mixed electron-impact–chemical ionization high-resolution spectrum, we were able to measure both electron-impact and collision-induced ions.

Meprobamate shows no molecular ion in the electron-impact spectrum of an unmodified instrument (6, 7). However, additional information was available in the mixed electron-impact–chemical ionization spectra. An MH^+ ion was observed at *m/e* 219, and high-resolution mass spectrometry established its elemental composition as $C_9H_{19}N_2O_4$. The base peak at *m/e* 158 was derived from the MH^+ ion through the loss of carbamic acid. In addition, high-resolution printout data were obtained on all abundant fragment ions usually contained in the electron-impact spectra. In Table I, the exact measured mass values of several major ions are listed and compared with the theoretical mass values.

Simple inspection of the electron-impact spectra obtained from a dual electron-impact–chemical ionization source is usually sufficient to distinguish ions produced by electron impact from collision-induced ions. Mixed electron-impact–chemical ionization spectra cannot be compared with standard electron-impact spectra due to the presence of collision-induced ions. If comparison is desired, however, electron-impact spectra corresponding to a standard can be readily obtained by a simple reduction of the sample pressure in the source.

In conclusion, the mixed electron-impact–chemical ionization high-resolution operation is a simple technique and provides both electron-impact and chemical ionization information on the same spectrum. This technique has proven very useful for the structure determination of medicinal agents in our laboratory.

(1) B. S. Finkle, R. L. Foltz, and D. M. Taylor, *J. Chromatogr. Sci.*, **12**, 304(1974).

(2) G. W. A. Milne, H. M. Fales, and T. Axenrod, *Anal. Chem.*, **43**, 1815(1970).

(3) N. C. Law, V. Aandahl, H. M. Fales, and G. W. A. Milne, *Clin. Chim. Acta*, **32**, 221(1971).

(4) R. L. Foltz, "Abstracts," American Society for Mass Spectrometry, Nineteenth Annual Conference on Mass Spectrometry and Allied Topics, 1971, pp. 142–148.

(5) T. F. Moran and W. H. Hamill, *J. Chem. Phys.*, **39**, 1413(1963).

[1] An A.E.I. MS-902 double-focusing mass spectrometer equipped with a Chemspect CIS-2 chemical ionization–electron-impact source was used, and the high-resolution results were processed by a PDP-8 computer.

(6) G. L. Nelson, C. F. Kuhlman, and T. L. Chang, *J. Pharm. Sci.*, **63**, 1959(1974).
(7) R. T. Coutts, *ibid.*, **62**, 769(1973).

Charles F. Kuhlman[x]
T. L. Chang
Research Division
Wyeth Laboratories, Inc.
Radnor, PA 19087
George L. Nelson
Saint Joseph's College
Philadelphia, PA 19131

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[x] To whom inquiries should be directed.

Methodological Differences in Correlating Digoxin Dissolution with Bioavailability

Keyphrases □ Digoxin—correlating dissolution and bioavailability, differences in methods, paddle-water method and rotating-basket apparatus □ Dissolution—digoxin, methods of determination, correlation with bioavailability □ Bioavailability—digoxin, correlation with dissolution, methods of dissolution determination

To the Editor:

A recent report by Klink *et al.* (1) indicated that the "paddle-water" method for determining dissolution rates for digoxin tablets failed to reflect the comparative bioavailability properties of two commercially available digoxin products. Specifically, large differences in dissolution rates were observed which failed to reflect the similar bioavailabilities of the two brands studied.

A number of reports have appeared indicating successful *in vivo–in vitro* correlation employing different methods (2–8), including the method recently adopted by the USP (9). Thus, the purpose of this communication is to report the results of recent dissolution rate studies carried out on the same batches of tablets employed by Klink *et al.* (1) using the USP dissolution rate test method.

The dissolution properties of six tablets each from Treatment I[1] and Treatment II[2] tablets were determined using the USP rotating-basket apparatus and employing the conditions specified in the USP (9). Samples were withdrawn and assayed by a fluorometric method (10) at 15, 30, 60, and 120 min after commencement of the studies.

The results of these studies are summarized in Table I, which lists the amount of digoxin in solution at various times for both the USP method and the paddle-water method (1). As indicated by these results, the two brands showed fairly similar dissolution properties, especially at the 60- and 120-min sampling times, when the USP method was employed. This finding is in contrast to the large differences observed at all sampling times when the paddle-water method was employed; Treatment I tablets dissolved much more rapidly than did Treatment II tablets.

These results are of significance when the *in vivo* performance of the two brands is considered. As reported by Klink *et al.* (1), the bioavailabilities of Treatment I and Treatment II tablets, as determined by the area under the serum level–time curves from 0 to 48 hr relative to similar areas obtained from digoxin in elixir data, were 106.38 ± 10.27 and 100.75 ± 24.09%, respectively. Similar relationships between the two brands were observed when the 0–5-, 0–12-, and 0–24-hr areas were compared.

Thus, from the dissolution data presented, it appears that, in the case of the specific lots of the two brands of digoxin tablets studied, the USP method for determining dissolution rates is of greater reliability than is the paddle-water method in predicting bioavailability. Based on these findings and the findings of others (2–8), it appears that much work remains to be done before the acceptance of a single *in vitro* dissolution test for the prediction of digoxin bioavailability from compressed tablets.

Table I—Mean Percent (±Standard Error) of Labeled Digoxin in Solution at Various Times following *In Vitro* Dissolution by Two Different Methods

	USP Method[a]		Paddle-Water Method[b]	
Minutes	Treatment I	Treatment II	Treatment I	Treatment II
15	65.1 ± 2.4	29.6 ± 2.7	—[c]	—
30	76.6 ± 1.8	60.0 ± 4.1	34.9 ± 4.3	6.0 ± 1.2
60	83.7 ± 3.0	77.0 ± 1.4	46.9 ± 6.1	8.0 ± 0.8
120	92.2 ± 0.9	86.7 ± 0.9	59.1 ± 3.9	19.4 ± 3.5

[a] Mean of six tablets. [b] Data from Ref. 1; mean of five tablets. [c] Samples not taken.

(1) P. R. Klink, R. I. Poust, J. L. Colaizzi, and R. H. McDonald, Jr., *J. Pharm. Sci.*, **63**, 1231(1974).
(2) J. G. Wagner, M. Christensen, E. Sakmar, D. Blair, J. D. Yates, P. W. Willis, III, A. J. Sedman, and R. G. Stoll, *J. Amer. Med. Ass.*, **222**, 957(1972).
(3) J. Lindenbaum, V. P. Butler, Jr., J. E. Murphy, and R. M. Cresswell, *Lancet*, **1**, 1215(1973).

[1] Lanoxin Tablets, lot 059G, Burroughs Wellcome and Co., Research Triangle Park, NC 27709
[2] Lot 047105, Towne Paulsen and Co., Monrovia, CA 91016