Decrease in the Rate of Capsule Dissolution Due to Formaldehyde from Polysorbate 80 Autoxidation

Keyphrases □ Dissolution, capsule—rate decrease, formation of formaldehyde, polysorbate 80 autoxidation □ Polysorbate 80—autoxidation, formation of formaldehyde, decreased dissolution rate, capsules

To the Editor:

Dissolution studies on some stability samples of gemfibrozil capsules showed a significant decrease in dissolution rate with time of storage and exposure to humidity. Observations of these slowly dissolving samples during the tests showed that the capsule contents were held together by a thin, tough, water-insoluble film, the disruption of which was seen to be the dissolution rate-limiting factor for the drug product. A bioavailability study of such film-forming gemfibrozil capsules showed them to be bioequivalent to the readily dissolving product. Thus, the decrease in dissolution rate in the *in vitro* test is an anomaly because it does not correlate with *in vivo* results. This communication describes studies which established that film formation is due to denaturation of the inner surface of the capsule by formaldehyde, formed by trace autoxidation of the polysorbate 80 used as an excipient.

Polysorbate 80 had been useful in our laboratories as a wetting agent in capsule formulations of hydrophobic drugs. It was convenient to prepare a 1:2 dispersion by drying an alcohol solution of polysorbate 80 mixed with colloidal silica. The silica aided in powder flow, facilitating high-speed encapsulation. This dispersion was used in several capsule formulations of investigational drugs, all of which exhibited film formation when stored under high-humidity conditions. Studies on experimental capsule formulations (Table I) made with constituents of the gemfibrozil capsule formula and stored at 37°C, 37°C with 80% relative humidity (RH), and 45°C and tested after 1, 2, and 3 months showed that film formation occurred only when polysorbate 80 was present. All of the capsules containing polysorbate 80 showed film formation during testing after 1 month at 37°C with 80% RH.

The reaction of formaldehyde and other aldehydes with proteins is well known (1, 2). Cross-linking of the amino groups in gelatin results in its denaturation to an insoluble protein (3). Since formaldehyde could result from autoxidation of the end groups in polysorbate 80 and acetaldehyde could be produced by oxidation of alcohol used in preparation of polysorbate 80–silica dispersion, the hypothesis that one or both of these aldehydes was responsible for film formation was tested. Formation of aldehydes would be facilitated by the immense surface area of the silica, so the results in Table I are consistent with the hypothesis.

Six lots of polysorbate 80–silica dispersion were analyzed for formaldehyde by three methods. The HPLC method (4) is based on the reaction of aldehydes with 2-diphenylacetyl-1,3-indanedione-1-hydrazone and afforded a separation of formaldehyde and acetaldehyde derivatives. The fluorometric method (5), based on the Hantzsch reaction, is much more sensitive for formaldehyde than for acetaldehyde. The same sample preparation was used for both; however, the fluorometric method provided significantly higher numbers. (This was determined later to be a result of lower reaction rate for the sample than the standard in the HPLC method.) The colorimetric microdiffusion method (6) was much simpler, with a weighed amount of dispersion in the outer ring of a microdiffusion chamber and chromotropic acid reagent in the inner ring. Table II shows results establishing that formaldehyde is present in all of the samples, half of which showed equivalent amounts of acetaldehyde by the HPLC method. For the purposes of this study, the qualitative results of the tests were much more important than the quantitative results. Obtaining reliable quantitative methods for the lower aldehydes in heterogeneous systems is difficult, owing to their volatility and reactivity; however, further work on this methodology has shown promise and may be reported elsewhere.

The highest estimate of formaldehyde content presented in Table II is <0.08% of the polysorbate 80 content of the dispersion. Thus, elucidation of the film-forming phenomenon provides a further example of the profound effect that trace decomposition may have on a drug product (7). Although the artifact has no bioavailability significance, it requires development of an alternative dissolution method for quality assurance, one where the film is digested or disrupted. Based on these studies, dissolution testing at 1 month of capsules stored at 37°C with 80% RH should provide a reliable indication of potential problems due to aldehydes. Whether film formation occurs in the presence of trace aldehydes likely depends on the reactivity of other constituents of the formulation, the internal moisture content, pH, and other factors. Certainly, the use of aldehyde-generating excipients in capsule formulations should be circumvented wherever possible.

Table I—Experimental Capsule Formulations [a]

Ingredients, mg	Formulation							
---	a	b[b]	c	d[c]	e[d]	f[d]	g[e]	h[d]
Gemfibrozil	171	171	171	171	171	171	—	—
Polysorbate 80 on Silica	5	10	—	—	—	—	150	—
Cornstarch NF	99	94	102	100	97	104	—	—
Polysorbate 80 NF	—	—	2	4	—	—	—	—
Silica Gel NF	—	—	—	—	7	—	—	90
Fill weight, mg	275	275	275	275	275	275	150	90

[a] No. 2 snap-fit white opaque 999 capsules, Lot 162 (Capsugel), were used for all formulations. [b] Questionable film formation at 3 months at 45°C. [c] Film formation 3 months at 37°C. [d] No film formation. [e] Film formation 1 month at 37°C.

Table II—Assay of Formaldehyde in Polysorbate 80 on Silica

Lot	Formaldehyde, $\mu g/g$ of Sample Taken		
	HPLC	Fluorometric	Colorimetric
1	20	163	260
2	13	55	170
3	13	—	75
4	18	—	100
5	27	111	200
6	36	163	110

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Does Cimetidine Impair Nitroreduction?

To the Editor:

A recent report by Ochs *et al.* (1) demonstrated that cimetidine reduced the clearance of nitrazepam in human subjects from a mean of 1.41 mL/min/kg in the control state to 1.17 mL/min/kg during cimetidine treatment. Cimetidine had no effect on the absorption kinetics or on volume of distribution of nitrazepam. These observations, in conjunction with the belief that nitroreduction accounts for most of nitrazepam clearance, prompted Ochs and co-workers to conclude that cimetidine impairs the nitroreduction of nitrazepam. Furthermore, the authors suggested that such metabolic inhibition by cimetidine may interfere with the elimination of other drugs biotransformed by nitroreduction (1).

While the effect of cimetidine on nitrazepam clearance (1) is clear, we suggest that the above conclusion by Ochs *et al.* regarding the inhibition of nitroreduction by cimetidine may be premature. Our doubts are based on a review of published studies (2–5) of the metabolic fate of nitrazepam in humans. These studies reveal that while nitroreduction is an important biotransformation pathway for nitrazepam, it accounts for only a limited amount of the administered dose in humans. For example, Sawada and Shinohara found (2) that 17% of an oral dose of nitrazepam was excreted as nitro-reduced metabolites in the urine of human subjects in 5 d. Unchanged drug (1%) and a ring-opened metabolite (2%) were also detected, but 80% of the administered dose was not recovered (2). Kangas (3) isolated unchanged drug (1%) and nitro-reduced metabolites (52%) from the 7-d urine samples of human subjects administered nitrazepam, but could not account for nearly one-half of the administered dose. Nitro-reduced metabolites recovered from 72-h human urine samples represented only 6% of the administered nitrazepam dose in another study (4). Rieder and

Wendt found (5) that ~7% of an administered oral radioactively labeled nitrazepam dose appeared in the 24-h urine specimen as nitro-reduced metabolites. Ring-opened metabolites accounted for an additional 9%, and traces of unchanged drug were also isolated. Other metabolites, which represented 21% of the administered dose, were also present but could not be identified. Thus, a total of 37% of the administered dose was excreted in the 24-h urine sample, and much of it was not identified (5). Although the recovery of total radioactivity increased to 65–70% after oral dosing and to 94% following intravenous administration during the subsequent 100 h, the proportion of the identified metabolites did not change significantly. Rieder and Wendt also demonstrated the accumulation of large amounts of unidentified metabolites in the plasma of human subjects after oral doses of this drug (5). Quantitative differences among the above-described studies may be due to differences in analytical methods and/or to inclusion *versus* omission of conjugated metabolites. Nevertheless, nitroreduction in humans has been shown to account, at most, for ~50% (3) of total nitrazepam elimination, and for much less in most studies (2, 4, 5). Since negligible amounts of unchanged drug are excreted (2–5), unidentified metabolites probably account (3, 5) for the remainder of nitrazepam elimination.

Cimetidine is known (6) to inhibit oxidative biotransformation of many drugs and, therefore, the following question must be raised: Is the effect of cimetidine on the clearance of nitrazepam due to the inhibition of some unidentified *oxidative* biotransformation rather than to inhibition of nitroreduction? This alternative explanation is supported by the isolation of small amounts of oxidized metabolites of nitrazepam from urine samples after the administration of the drug to humans (2, 5). Furthermore, oxidation may also be involved (7, 8) in the ring-opening biotransformation of benzodiazepines which may be an important metabolic pathway for nitrazepam (5).

Thus, it is clear that previous work has not accounted for a large fraction of the nitrazepam dose, and it appears that oxidative biotransformations may be responsible for a portion of nitrazepam clearance. There is insufficient data to determine whether nitrazepam nitroreduction or some hitherto unidentified oxidative metabolic pathway is impaired by cimetidine. Therefore, we feel that it is premature to suggest (1) that the ability of cimetidine to impair drug oxidation in humans should be extended to include inhibition of nitroreduction. There are several important drugs with a nitro group in their structure (*e.g.*, chloramphenicol, clonazepam, nitrofurantoin, and metronidazole), and some of these compounds have been shown to be metabolized *via* nitroreduction. We are concerned that the suggestion by Ochs *et al.* (1) may foster a general belief that cimetidine—a widely used agent—impairs the elimination of such drugs *via* nitroreduction, a belief for which there is, as yet, no firm evidence.

Perhaps cimetidine will be shown in the future to inhibit one or more of the several distinct nitroreductases known [*e.g.*, cytochrome P_{450}, whose oxidative function is inhibited by cimetidine (9), can also function as a nitroreductase (10)]. However, to demonstrate inhibition of nitroreduction, studies of the effects of cimetidine on specific biotransformations and on metabolite formation will be required. It will be interesting to watch developments in this area.

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BOOKS

Review of Organic Functional Groups: Introduction to Medicinal Organic Chemistry. By THOMAS L. LEMKE. Lea and Febiger, Philadelphia, PA. 1983. 131 pp. 15 × 23 cm. Price $10.50.

The objective of this book is to provide a self-paced review of the nomenclature, physical properties, chemical properties, and metabolism of organic functional groups that are important in medicinal chemistry. The book is designed for use as supplemental material for a pharmacy course in medicinal chemistry as well as a concise reference for students and professional practitioners in pharmacy, medicine, nursing, dentistry, and veterinary medicine. After a general chapter on water solubility and chemical bonding, subsequent chapters focus on specific functional groups. Organic functional groups covered include alkanes, alkenes, aromatic hydrocarbons, halogenated hydrocarbons, alcohols, phenols, ethers, aldehydes, ketones, amines, carboxylic acids and their derivatives, sulfonic acids and sulfonamides, and heterocycles. A chapter on empirical and analytical methods of determining water solubility and an appendix on acidity and basicity are also included. Review questions are placed at the end of chapters to reinforce concepts presented in the text.

Chapters on individual functional groups are concise, and adhere to an outline format where the three major subheadings are Nomenclature, Physical-Chemical Properties, and Metabolism. There are adequate examples and tables of physical data given to illustrate the major points. The sections on nomenclature and physical-chemical properties are especially clearly written and complete. Most of the sections on metabolism are also clear and concise. However, the metabolism sections on aromatic hydrocarbons, halogenated hydrocarbons, and amines may be confusing since they contain several statements which contradict information found in drug metabolism chapters of current medicinal chemistry textbooks.

The major emphasis of this book is in areas of medicinal chemistry which many undergraduate pharmacy students find difficult. The sections on nomenclature and physical-chemical properties should be very helpful to those students who have difficulty extracting information from general organic chemistry textbooks that is directly pertinent to medicinal chemistry. Therefore, this book fills a distinct need in undergraduate medicinal chemistry instruction. As long as weaknesses in some metabolism sections are recognized, this book should be an excellent supplement to most undergraduate pharmacy courses in medicinal chemistry as well as a concise review for students and practitioners of other health professions.

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Applied Clinical Pharmacokinetics. Edited by DENNIS R. MUNGALL. Raven Press, 1140 Avenue of the Americas, New York, NY 10036. 1983. 448 pp.

This book represents the most recent compilation of information related to the discipline of therapeutic drug monitoring. As stated in the preface, the major objective of this text is "to offer students and clinicians in pharmacy, medicine, pharmacology, and clinical chemistry a practical guide to clinical pharmacokinetics." Although a brief introductory chapter discusses general concepts and basic pharmacokinetic principles, students without previous pharmacokinetic course work may have difficulty applying the information presented in the remainder of the textbook. The subsequent chapter which examines protein binding and free drug concentrations is complete, reasonably well referenced, and a good review of the pertinent drug–protein binding literature. However, this chapter cannot be recommended for students because it contains several misleading statements and very minor yet bothersome errors.

The remaining chapters are primarily devoted to the discussion of individual therapeutic agents and include: procainamide, quinidine, digoxin, anticonvulsants, theophylline, aminoglycosides, warfarin, antihypertensives, lithium, tricyclic antidepressants, benzodiazepines, salicylates, and antineoplastics. The absence of a chapter addressing the pharmacokinetics of lidocaine is a limitation of the text and certainly would have proven more useful to the clinician than a chapter discussing antihypertensive agents.

Each of the drugs reviewed has a chapter to itself with the format designed to cover important aspects of clinical pharmacology, pharmacokinetics, plasma concentration and response relationships, dosage regimen design, and assay methods. The book is quite readable in this format and appears to be relatively free from errors. Most chapters contain practice problems along with detailed solutions. The information on each drug is, for the most part, well detailed and referenced. The concluding chapter is devoted to the use of programmable calculators in clinical pharmacokinetics. Included in this chapter is a group of calculator programs that may prove useful to those individuals who utilize such devices in their clinical practice.

On the whole, this book provides a reasonable compilation of the published literature in the areas addressed by the authors. However, this text is not unique in its area of emphasis and a more rigorous and comprehensive examination is available as a reference source. While the practicing clinician may find certain areas of this book of interest, (*e.g.* practice problems, calculator programs) its general appeal is limited; therefore, the student in either introductory or advanced courses will find currently available texts of greater benefit.

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