Several of the aerobic and anaerobic cultures were found to give high levels of *N*-nitrosodesipramine when incubated with desipramine and sodium nitrite. Though it cannot be stated that the transformation was an entirely enzymatic reaction, the yield of the nitrosamine was far higher than could be obtained by the acid catalyzed reaction between nitrite and desipramine.

Preliminary studies with mixed anaerobic cultures with imipramine and nitrate showed that the nitrosamine was formed more rapidly than the control, but the yield was very low. Additional studies with pure cultures will be needed to determine if the low yield was due to a low rate of *N*-demethylation.

REFERENCES

(1) G. M. Hawksworth and M. J. Hill, *Br. J. Cancer*, **25**, 520 (1971).

(2) D. L. Collins-Thompson, N. P. Sen, B. Aris, and L. Schwinghamer, *Can. J. Microbiol.*, **18**, 1968 (1972).

(3) A. Ayanaba and M. Alexander, *Appl. Microbiol.*, **25**, 862 (1973).

(4) P. J. Coloe and N. J. Hayward, *J. Med. Microbiol.*, **9**, 211 (1976).

(5) A. Ayanaba, W. Verstraete, and M. Alexander, *J. Natl. Cancer Inst.*, **50**, 811 (1973).

(6) C. D. Hufford, G. A. Capiton, A. M. Clark, and J. K. Baker, *J. Pharm. Sci.*, **70**, 151 (1981).

(7) G. S. Rao and G. Krishna, *ibid.*, **64**, 1579 (1975).

(8) G. Scheunig and D. Ziebarth, *Pharmazie*, **33**, 722 (1978).

(9) J. K. Baker, *Anal. Chem.*, **49**, 906 (1977).

(10) G. Hawksworth and M. J. Hill, *Br. J. Cancer*, **29**, 353 (1974).

(11) H. T. Nagasawa, P. S. Fraser, and D. L. Yuzon, *J. Med. Chem.*, **16**, 538 (1973).

(12) V. J. Sander, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 429 (1968).

(13) T. A. Gough, K. S. Webb, and R. F. Coleman, *Nature (London)*, **272**, 161 (1978).

(14) T. Wang, T. Kakizoe, P. Dion, R. Furrer, A. J. Varghese, and W. R. Bruce, *Nature (London)*, **276**, 180 (1978).

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Blood Level Studies of All-*trans*- and 13-*cis*-Retinoic Acids in Rats Using Different Formulations

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Abstract □ Studies to determine the bioavailability of all-*trans*-retinoic acid from a microencapsulated product were carried out using rats as test animals. The microcapsules were tableted in rat food and individual rats given a tablet containing the equivalent of 10 mg of all-*trans*-retinoic acid. Comparisons were made with bioavailability data obtained after intravenous and oral administrations of a solution and a suspension. The elimination of all-*trans*-retinoic acid following intravenous administration of 1- to 5-mg doses occurred by dose-dependent kinetics. The half-lives for the terminal linear portion of the elimination phase after the plateau level were 0.78, 0.74, and 0.93 hr for the 1-, 2.5-, and 5-mg doses, respectively. Based on the doses administered and the relative area under the serum level curves, the all-*trans*-retinoic acid microcapsules were found to be ~34% as bioavailable as the solution dosage form and the microfine suspension 93% as bioavailable. The bioavailability of all-*trans*-retinoic acid in oral solution was ~40% of the intravenous dose. For comparison, rats were also dosed intravenously with 13-*cis*-retinoic acid, and this compound was found not to follow dose-dependent kinetics at similar dosage levels used for all-*trans*-retinoic acid.

Keyphrases □ Bioavailability—Blood level studies of all-*trans*- and 13-*cis*-retinoic acids using different formulations, rats □ Microencapsulation—blood level studies of all-*trans*- and 13-*cis*-retinoic acids using different microencapsulated formulations, rats □ Blood level studies—all-*trans*- and 13-*cis*-retinoic acids using different formulations, rats □ Retinoic acids, all-*trans*- and 13-*cis*- —blood level studies using different formulations, rats

A number of retinoids have been shown to prevent or inhibit the growth of epithelial tumors. The use of these compounds for chemoprevention of tumors has been reviewed previously (1, 2). Although a long-term study with a synthetic retinoid was reported (3), most studies have been relatively short term with manual dosing of the retinoids. For long-term efficacy studies, a more economical and less troublesome mode of drug administration is through the diet of the test animals. However, simple mixing of retinoids with feed is precluded because of the unstable nature of the compounds toward air, light, and moisture. Earlier studies with vitamin A compounds have shown that these chemicals can be protected from environmental hazards by microencapsulation (4, 5).

Requirements of a microcapsule product are that first it must be readily miscible with the feed of the test animals, and second, it must be soluble or digestible in the GI tracts of the animals so that the retinoid is biologically available. In the present study, all-*trans*-retinoic acid was microencapsulated, and the bioavailability of the compound from the finished product was determined and compared with intravenously and orally administered retinoid using rats as test animals. Also, for comparison, rats were dosed intravenously with 13-*cis*-retinoic acid.

EXPERIMENTAL

Chemicals—All-*trans*-retinoic acid[1], 13-*cis*-retinoic acid[2], and all-*trans*-retinol acetate[3] were used as received. All other chemicals and reagents were the highest grade commercially available.

Microcapsules—All-*trans*-retinoic acid was encapsulated in gelatin–dextrose microcapsules by a process similar to that reported for the encapsulation of vitamin A derivatives (6) using a three-phase suspension

[1] Eastman-Kodak, Rochester, N.Y.
[2] Hoffmann-LaRoche, Nutley, N.J.
[3] Sigma Chemical Co., St. Louis, Mo.

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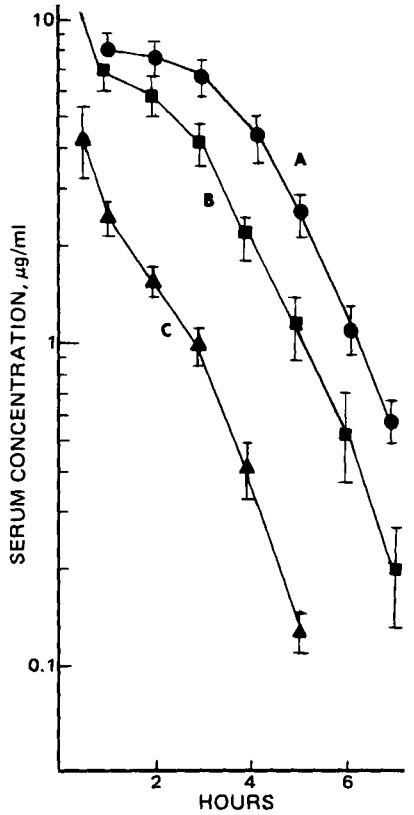

Figure 1—*Average serum concentrations of all-*trans*-retinoic acid following rapid intravenous administration in saline solution. Key: (▲) 1.0-mg dose, six rats; (■) 2.5-mg dose, six rats; (●) 5.0-mg dose, five rats.*

method. Gelatin was chosen as the major encapsulating material because it offers adequate bioavailability and good stability under various storage conditions (5, 7). The geometric mean diameter of the microcapsules was 0.19 mm with a geometric standard deviation of 1.81. The microcapsules were stored at −10° until needed for testing.

The microcapsules were assayed to determine content uniformity and stability using spectrophotometric analysis[4] after dissolution of the microcapsules in water at 65° and extraction with chloroform. The all-*trans*-retinoic acid content determined by assay of 12 samples (50 mg) of microcapsules from a homogenized mixture was found to be 2.83 ± 0.05 mg with a coefficient of variation of 1.77% indicating good uniformity of the formulation. The concentration of all-*trans*-retinoic acid in the microcapsules was 6.03% of the total weight.

Animal Dosing—Adult Sprague-Dawley rats (~360 g) were used. All dosed rats were fasted ~24 hr prior to dosing with retinoid. Water was available *ad libitum*. Orally dosed rats received 5- to 10-mg doses of all-*trans*-retinoic acid. Rats given intravenous injections were dosed with 1–5 mg of either all-*trans*-retinoic acid or 13-*cis*-retinoic acid. Oral and intravenous dosing was staggered ~5–10 min so that blood samples could be collected from each of the rats at approximately the same time intervals after dosing.

Oral doses of all-*trans*-retinoic acid were given in 0.5 ml of 0.3% NaOH–0.9% saline solution and in microfine suspension (<3-μm particles) *via* gastric intubation to lightly anesthetized rats. Intravenous injections for both all-*trans*- and 13-*cis*-retinoic acids were given in a 0.3% NaOH–0.9% saline solution and injected into the tail vein of lightly anesthetized rats.

All-*trans*-retinoic acid microcapsules were fed to fasted rats in the following manner. Ground-up rat food–microcystalline cellulose (2:1) was mixed with all-*trans*-retinoic acid microcapsules and tableted by compression into 12.5-mm disks at 2724 kg for 20 sec. Each tablet contained the equivalent of 10 mg of all-*trans*-retinoic acid. Ten rats were put in individual cages, and each rat was given one food tablet containing the all-*trans*-retinoic acid. Six or more of the rats consumed the tablets completely within 10 min.

Serum Collection—Multiple blood samples were collected from each rat using a modification of a previous method (8). Blood samples of 250–300 μl were collected under reduced pressure from an incision made at the distal end of the tail of unanesthetized rats and collected in serum separation tubes. Immediately following centrifugation, the serum samples were transferred to 15-ml screw-capped test tubes and stored at −10° where they remained for 20–60 hr before they were assayed. Because of the small amount of blood collected per sample, eight or nine blood samples could be taken from each rat over the time-course of 7–10 hr.

[4] Cary 118 Varian Instruments, Palo Alto, Calif.

Table I—Bioavailability Data for All-*trans*-Retinoic Acid Following Oral (5 and 10 mg) and Intravenous (1 mg) Doses

Formulation	*AUC*[a], μg hr/ml	Relative Availability to Oral Solution	Relative Availability to Intravenous Dose
Intravenous Solution, 1 mg	34.7 ± 4[b]	—	100
Oral Solution[c], 5 mg	13.74 ± 1.6	100	39.6
Microfine Suspension[d], 5 mg	12.8 ± 1.4	93	36.9
Microcapsules[e] tableted in rat food, 10 mg	4.6 ± 6[b]	33.5	13.3

[a] Corrected for the body weight. [b] Adjusted to 5 mg by dose ratios. [c] Average 9 rats. [d] Average 11 rats. [e] Average 12 rats.

Serum Assay—Serum samples were assayed by a reversed-phase high-pressure liquid chromatographic method reported previously (9).

A standard curve was obtained by comparing the peak height ratio of all-*trans*-retinoic acid or 13-*cis*-retinoic acid to all-*trans*-retinol acetate and the spiked serum all-*trans*-retinoic acid or 13-*cis*-retinoic acid concentration. Unknown serum sample concentrations were calculated by comparing the peak height ratios of the samples to the processed standards. The lower working limit for the assay was 100 ng/ml of serum

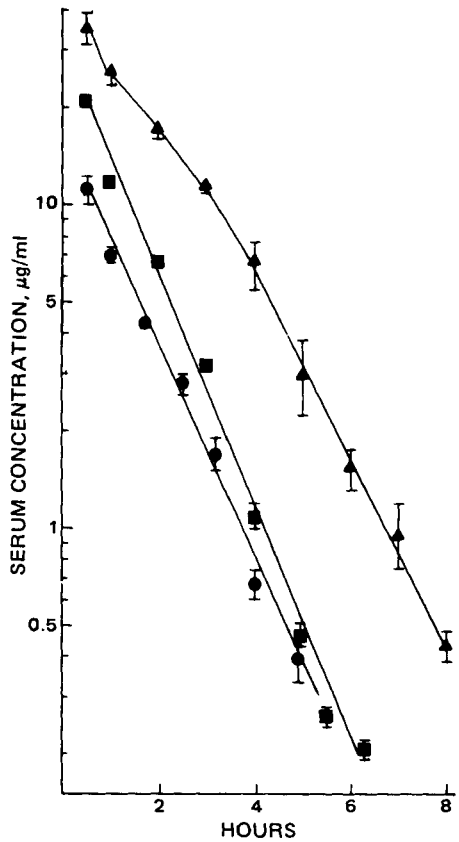

Figure 2—*Average serum concentrations of 13-*cis*-retinoic acid following rapid intravenous administration in saline solution. Key: (●) 1.25-mg dose, eight rats; (■) 2.5-mg dose, six rats; (▲) 5.0-mg dose, eight rats.*

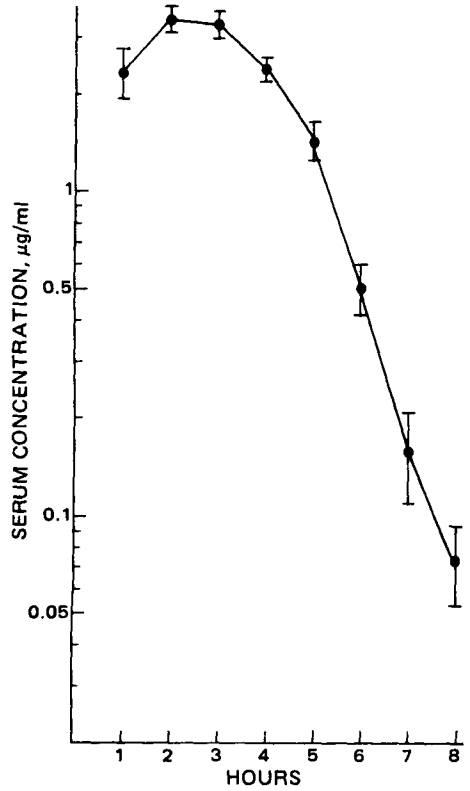

Figure 3—*Average serum concentrations of all-*trans*-retinoic acid following a 5.0-mg oral dose in saline solution to nine rats.*

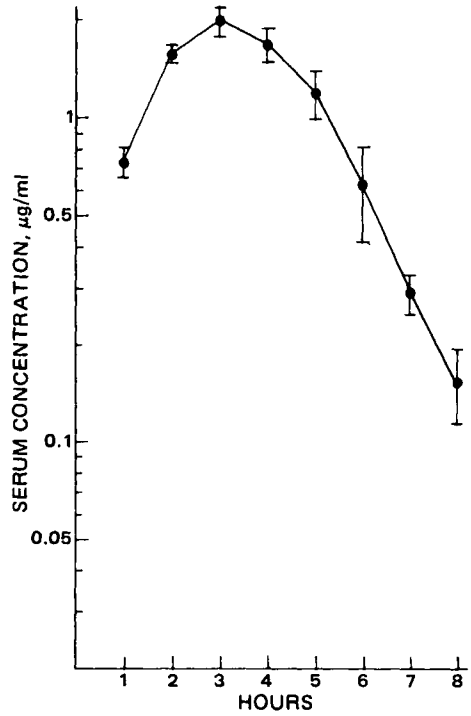

Figure 4—*Average serum concentrations of all-*trans*-retinoic acid following a 10-mg oral dose of encapsulated drug in animal food to 12 rats.*

and the recovery of all-*trans*-retinoic acid and 13-*cis*-retinoic acid was 102.9 ± 5.6 and 91.7 ± 5%, respectively. The areas under the serum level *versus* time curves were determined by the trapezoidal rule. The elimination half-lives were determined from the slope of the elimination curves by using a linear regression program with a programmable calculator.

RESULTS AND DISCUSSION

Serum levels *versus* time profiles following a 1-, 2.5-, and 5-mg iv dose of all-*trans*-retinoic acid were shown in Fig. 1. Serum concentrations showed a rapid decline immediately after injection as the retinoid was distributed into tissues. However, serum levels rapidly reached a plateau and remained stable for several hours before returning to the linear elimination phase. Increasing the dose of all-*trans*-retinoic acid to 2.5 and 5 mg, respectively, resulted in a more pronounced and longer plateau (Figs. 1A and 1B), indicating a possible saturation of elimination pathways at higher doses. The time required for the serum levels to return to the linear elimination phase after the plateau began was ~94, 135, and 190 min for the 1-, 2.5-, and 5-mg doses, respectively. The half-life for the linear elimination phase after the plateau was 0.78, 0.74, and 0.93 hr for the 1-, 2.5-, and 5-mg doses, respectively, and these values were not significantly different ($p > 0.05$).

Recently, after observing nonlinear declining of serum levels of all-*trans*-retinoic acid in the rat, several potential causes for the nonlinear kinetics were investigated (10). Dose-dependent elimination due to the saturation of biliary excretion was ruled out since the same nonlinear behavior in rats with biliary fistulas was observed. It was found that interrupting the enterohepatic recirculation system had no effect on the nonlinear elimination phenomenon. Also discovered was a greater conversion of all-*trans*-retinoic acid to all-*trans*-retinoyl-β-glucuronide, supposedly a minor metabolite, as the intravenous dose was increased. Both results indicated that the dose-dependent elimination was due to saturation of major metabolic pathways instead of saturation of biliary excretion. However, at the present time there is some disagreement as to what the major metabolic pathways might be in the biotransformation of all-*trans*-retinoic acid in rats (11–13).

For comparison, 13-*cis*-retinoic acid was intravenously administered in rats in 1.25-, 2.5-, and 5-mg doses and the results obtained summarized in Fig. 2. The saturation phenomenon was completely absent at lower doses and only a brief nonlinear phase was observed at the 5-mg dosage level. Also, the half-lives of the linear elimination phase were slightly longer than those of all-*trans*-retinoic acid, averaging 0.8–0.9 hr. Similar results were observed (14) when the disposition of all-*trans*- and 13-*cis*-retinoic acids in mice was studied. A plateau phenomenon was found in the serum level *versus* time curve following an intravenous injection of all-*trans*-retinoic acid but not for 13-*cis*-retinoic acid. Since dose-dependent kinetics were observed with all-*trans*-retinoic acid but not with 13-*cis*-retinoic acid, these results would indicate that the metabolic enzymes for these chemicals are probably different. However, currently there is little data available as to the enzymatic reactions of 13-*cis*-retinoic acid in rats.

Since the intravenous dose of all-*trans*-retinoic acid produced dose-dependent kinetics, it was not possible to accurately determine the area under the curve (*AUC*). However, an approximate *AUC* was obtained by using the trapezoidal rule and found to be 6.94 ± 0.8 μg hr/ml for the 1-mg iv dose. After adjusting and comparing the 1-mg dose to the 5-mg dose, the oral solution dosage form was found to be only 40% as bioavailable as the intravenous dose. The low bioavailability of the oral solution could be the result of either a first-pass effect occurring or from the inaccuracy of the *AUC* calculation due to the dose-dependent kinetics. For these reasons, all-*trans*-retinoic acid in oral solution was used as a standard for bioavailability comparisons to the other formulations.

Shown in Fig. 3 are the average serum levels after dosing nine rats orally with saline solutions containing 5 mg of all-*trans*-retinoic acid. Peak serum levels occurred after 2.5 hr with an elimination half-life of 0.69 hr and an *AUC* of 13.74 ± 1.6 μg hr/ml. Average peak serum level was 3.2 μg/ml. Results after oral administration of microcapsules in laboratory animal feed are shown in Fig. 4. The curve represents data averaged from 12 rats each dosed with the equivalent of 10 mg of all-*trans*-retinoic acid. Peak serum concentration occurred at ~3 hr and the *AUC* was 9.21 ± 1.2 μg hr/ml with an elimination half-life of 1.02 hr.

Figure 5 shows the average serum levels achieved when 11 rats were dosed orally with the microfine suspension dosage form. A 5-mg dose was administered which resulted in an average peak serum level time of 3 hr and an *AUC* of 12.8 ± 1.4 μg hr/ml with an elimination half-life of 0.65 hr.

Based on the doses administered and the relative area under the serum level curves (Table I), the all-*trans*-retinoic acid microcapsules were ~34% as bioavailable as the solution dosage form, and the microfine suspension was 93% bioavailable. The low bioavailability of the microcapsules is possible because all-*trans*-retinoic acid was not completely released from the microcapsules in the digestive tract of the rat. The half-life of the elimination phase was 1.5 times greater than that of the solution, indicating that absorption was probably still occurring after the

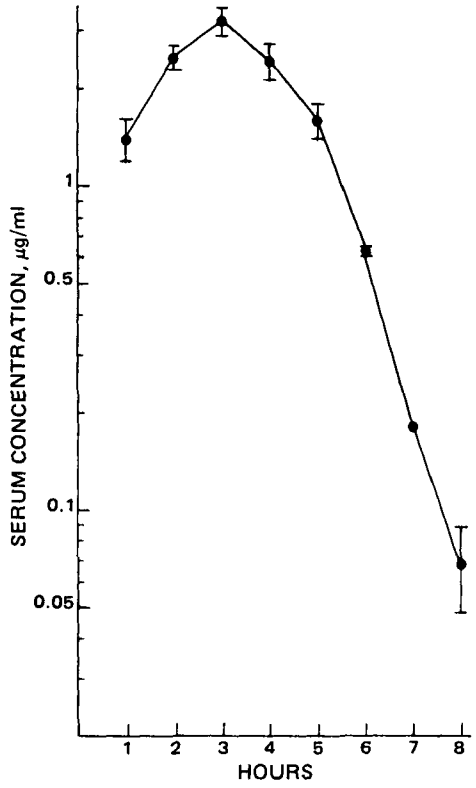

Figure 5—*Average serum concentrations of all-*trans*-retinoic acid following a 5.0-mg dose of a microfine suspension to 11 rats.*

peak concentration thus producing the slowly declining serum concentrations. Another possible cause of the slow release of the chemical from the microcapsules was interaction with the rat food in which the microcapsules were tableted. In a preliminary study, it was found that all-*trans*-retinoic acid crystals tableted in rat food were only 73% bioavailable as the solution, possibly indicating that the rat food in which the crystals were tableted was interfering with absorption.

The microfine suspension was formulated as a possible alternative to microencapsulation. It has the advantage of a greater bioavailability, but the disadvantages of being a less stable formulation and less readily miscible with the rat food as compared to the solid microcapsules. The primary purpose of microencapsulation was to stabilize the retinoids for feeding to rats in their laboratory diets for long-term testing of the compounds for chemo-prevention of tumors.

All-*trans*-retinoic acid was successfully microencapsulated and the bioavailability of the encapsulated and liquid dosage forms compared. Although the microcapsules were only 34% bioavailable as the solution dosage form, this is a good compromise because of the increased stability and ease of handling. Also, all-*trans*-retinoic acid was found to follow dose-dependent kinetics at the doses studied; whereas, 13-*cis*-retinoic acid did not follow dose-dependent kinetics when given at the same dosage levels as the all-*trans*-retinoic acid.

REFERENCES

(1) W. Bollage, *Int. Z. Vitaminforsch*, **40**, 299 (1970).

(2) M. B. Sporn, N. Dunlop, D. Newton, and J. Smith, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **35**, 1332 (1976).

(3) C. D. Port, M. B. Sporn, and D. G. Kaufman, *Proc. Am. Assoc. Cancer Res.*, **16**, 21, (1975).

(4) J. T. Carstensen, *J. Pharm. Sci.*, **53**, 839 (1964).

(5) E. M. Olsen, J. D. Harvey, D. C. Hill, and H. D. Branion, *Poultry Sci.*, **38**, 929 (1959).

(6) E. J. Goett, E. E. MacDonough, and C. J. Salivar (Chas. Pfizer and Co.), U.S. Patent 2, 643, 209 (1953).

(7) J. T. Carstensen, E. S. Aron, D. C. Spera, and J. J. Vance, *J. Pharm. Sci.*, **55**, 561 (1966).

(8) S. T. Nerenberg and P. Zedler, *J. Lab. Clin. Med.*, **85**, 523 (1975).

(9) R. S. Shelley, J. C. Price, H. Won Jun, D. E. Cadwallader, and A. C. Capomacchia, *J. Pharm. Sci.*, **71**, 262 (1982).

(10) B. N. Swanson, C. A. Frolik, D. W. Zaharevitz, P. P. Roller, and M. B. Sporn, *Biochem. Pharmacol.*, **30**, 107 (1981).

(11) A. B. Roberts and H. F. DeLuca, *Biochem. J.*, **102**, 600 (1967).

(12) R. Hanni, F. Bigler, W. Meister, and G. Englert, *Helv. Chim. Acta*, **59**, 2221 (1976).

(13) A. B. Roberts and C. A. Frolik, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **38**, 2524 (1979).

(14) C. Wang, S. Campbell, R. L. Furner, and D. L. Hill, *Drug Metab. Dispos.*, **8**, 8 (1980).

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Tack Behavior of Coating Solutions I

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Abstract □ The tackiness of various tablet coating solutions was determined using a parallel plate technique with a tensile testing machine in conjunction with an oscilloscope where the separation force was displayed as a function of time. Measurements were made at various rates of separation on liquid films of constant thickness. Results showed that the force required to split a liquid film increases with an increase in rate of separation, and that tackiness increases with an increase in viscosity. The relation between tack and viscosity was not linear, and a modified Stefan equation was proposed.

Keyphrases □ Tablet coating solutions—tack behavior, viscosity □ Viscosity—tack behavior of tablet coating solutions □ Tackiness—tack behavior of tablet coating solutions

The effect of coating formulations and coating process variables on the appearance of a coated tablet has been the subject of various investigations (1–3). Much effort has been devoted to the study of the theory of adhesion of film-forming materials to the surface of a tablet (4–6). However, very little is known about the tackiness of coating solutions and its effect on the coating process. Studies on the evaluation of coating formulations have made some reference to the problem of tackiness of hydroxypropyl cellulose solutions during the coating process (7). However,

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