very large number of results and will be reported (21). For the objective of this present work, the product of the two parameters $K_1K_2 = K''$, which can be determined accurately from either type of plot (Eq. 8 or 9), is sufficient to characterize the system. This combined parameter can be regarded as a linear distribution coefficient for a limited range of benzoic acid concentration in cetomacrogol 1000 solutions (Fig. 2, straight-line extrapolation from initial points). The concept of K'' as a distribution coefficient is derived from Eq. 7 which, at low values of the binding constant K_1 or low concentrations of adsorbate ($K_1C \ll 1$), approaches $X = K_1K_2C = K''C$.

Table II shows that the value of 6.05×10^{-2} for K'' by the molecular sieve method is of the same order as values obtained by other methods; the standard deviation, which falls within the range previously obtained, is acceptable. Since the method was found to be rapid and reproducible, it should prove to be suitable for routine solubilization studies, not only for benzoic acid in nonionic surfactants such as cetomacrogol 1000 but also for other unsaturated solubilized systems.

REFERENCES

(1) B. A. Mulley, in "Advances in Pharmaceutical Sciences," vol. I, A. H. Beckett and J. E. Carless, Eds., Academic, London, England, and New York, N. Y., 1964.

(2) J. Swarbrick, *J. Pharm. Sci.*, **54,** 1229(1965).

(3) L. Sjoblom, in "Solvent Properties of Surfactant Solutions," K. Shinoda, Ed., Marcel Dekker, New York, N. Y., 1967.

(4) T. Nakagawa, in "Nonionic Surfactants," N. J. Schick, Ed., Marcel Dekker, New York, N. Y., 1967.

(5) N. H. J. Weiden and L. B. Norton, *J. Colloid Sci.*, **8,** 606 (1953).

(6) T. Higuchi and J. L. Lach, *J. Amer. Pharm. Ass., Sci. Ed.*, **43,** 465(1954).

(7) D. Guttman and T. Higuchi, *ibid.*, **45,** 659(1956).

(8) B. A. Mulley and A. D. Metcalf, *J. Pharm. Pharmacol.*, **8,** 774(1956).

(9) A. G. Mitchell and L. S. C. Wan, *J. Pharm. Sci.*, **53,** 1467(1964).

(10) A. G. Mitchell, *J. Pharm. Pharmacol.*, **15,** 761(1963).

(11) N. K. Patel and H. B. Kostenbauder, *J. Amer. Pharm. Ass., Sci. Ed.*, **47,** 289(1958).

(12) N. K. Patel and N. E. Foss, *J. Pharm. Sci.*, **53,** 94(1964).

(13) H. Matsumoto, H. Matsumura, and S. Iguchi, *Chem. Pharm. Bull.*, **14,** 385(1966).

(14) M. Donbrow, unpublished data.

(15) M. Donbrow and C. T. Rhodes, *J. Pharm. Pharmacol.*, **15,** 233(1963).

(16) *Ibid.*, **17,** 258(1965).

(17) M. Donbrow and C. T. Rhodes, *Int. Kongr. Pharm. Wiss. Vortr. Originalmitt. M.*, **1963,** 397(1964).

(18) A. G. Mitchell and K. F. Brown, *J. Pharm. Pharmacol.*, **18,** 115(1966).

(19) M. Donbrow and C. T. Rhodes, *J. Chem. Soc., Suppl.* 2, **1964,** 6166.

(20) M. Donbrow and J. Jacobs, *J. Pharm. Pharmacol.*, **18,** 92S (1966).

(21) M. Donbrow and E. Azaz, to be published.

(22) M. Donbrow, P. Molyneux, and C. T. Rhodes, *J. Chem. Soc., A.*, **1967,** 561.

(23) W. P. Evans, *J. Pharm. Pharmacol.*, **16,** 323(1964).

(24) E. G. Rippie, D. J. Lamb, and P. W. Romig, *J. Pharm. Sci.*, **53,** 1346(1964).

(25) R. W. Ashworth and D. D. Heard, *J. Pharm. Pharmacol.*, **18,** 98S(1966).

(26) N. K. Patel and N. E. Foss, *J. Pharm. Sci.*, **54,** 1495(1965).

(27) A. T. Florence, *J. Pharm. Pharmacol.*, **18,** 384(1966).

(28) B. J. Gellote, *J. Chromatogr.*, **3,** 330(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 24, 1969, from the *Pharmacy Department, Hebrew University School of Pharmacy, Jerusalem, Israel.*

Accepted for publication June 3, 1970.

Some Factors Affecting Release and Availability of Drugs from Hard Gelatin Capsules

B. J. McGEE, D. R. KENNEDY, and G. C. WALKER

Abstract □ Acetylsalicylic acid, acetylsalicylic acid and lactose, and acetylsalicylic acid and dibasic calcium phosphate were administered orally in No. 3 and No. 4 hard gelatin capsules to rabbits, using a Latin square design. Plasma levels were determined at 1-, 2-, and 3-hr. intervals; an analysis of variance showed that at hour 3, there was a difference between the average for the No. 3 and No. 4 capsules, irrespective of excipient. The significantly better plasma levels obtained with the more tightly compacted No. 4 capsules may be due to the diffusion of gastric juice through the gelatin which created higher pressure within the capsules. Dissolution determinations were made using the Levy beaker method and the oscillating tube method. The mean plasma levels at the end of 1, 2, and 3 hr. were lower than the concentrations obtained at the corresponding hours from the *in vitro* study, and direct correlation of the two sets of data could not be made. Hard gelatin capsules containing acetylsalicylic acid alone broke down slowly *in vitro*.

Keyphrases □ Capsules, hard gelatin—drug release, availability □ *In vivo–in vitro* release rates—drug from capsules □ Aspirin release, capsules—excipient effect □ Gelatin capsule size effect—aspirin release rates

Encapsulation remains a popular method for administering medication because of the general view that capsules readily break down upon ingestion to release the enclosed medicament (1). It was thought desirable to investigate the effects of two different excipients and the effects of two different pressures of fill on the rate of absorption of acetylsalicylic acid (ASA) from hard gelatin capsules in the rabbit. ASA is reported to be absorbed rapidly from all parts of the gastrointestinal tract and may serve as a "marker" to assess the effect of formulation and dosage form characteristics on absorption rate (1). *In vitro* dissolution

Table I—Average Concentrations Obtained in Dissolution Determinations of No. 3 Capsules Containing ASA[a]

Time, min.	Average Concentration,[b] mg./100 ml. Hydrochloric Acid–Water, pH 1.2	Simulated Gastric Fluid	Simulated Intestinal Fluid
10	0	1.0	0
20	0.8	3.3	2.5
30	3.5	10.0	7.8
45	6.8	13.3	13.0
60	10.0	15.3	15.0
90	13.8	16.5	17.3
120	16.0	17.3	18.3
180	18.0	20.0	20.0
240	19.8		

[a] Oscillating tube method, 100-mesh screen. [b] Average of three determinations.

tests were carried out to complement the *in vivo* work to determine if any correlation existed between the two sets of data.

METHODS AND PROCEDURES

ASA BP was used in all cases (British Drug Houses, 20-mesh crystals). The excipients were water-soluble α-lactose and the relatively water-insoluble dibasic calcium phosphate, both of which are used as fillers and diluents in capsules as well as tablets.

The rabbit was used as the test animal. Each rabbit received all four of the following combinations, as well as the control ASA at weekly intervals: ASA alone in a No. 3 capsule, ASA and lactose in No. 3 and No. 4 capsules, ASA and dibasic calcium phosphate in No. 3 and No. 4 capsules. Ten rabbits were used in two 5 × 5 Latin squares, and one rabbit died during the treatment schedule. An analysis of variance was carried out, ignoring the "week" as if the data were from a randomized block experiment and omitting Rabbit 5 from which two observations were missing.

It was determined that 200 mg. of ASA would produce measurable plasma salicylate levels and that 200 mg. of ASA and 66.6 mg. of excipient could be contained in both No. 3 and No. 4 empty gelatin capsules (Parke-Davis), thus affording a relatively greater compaction pressure in the No. 4 capsule. No. 3 capsules containing 200 mg. of ASA alone were also used, because it was felt that the same crystals without excipient or compaction would serve to point out any differences between excipients or compaction pressures. Ten capsules of each combination and size were selected at random and assayed individually according to the method of Routh and Dryer (2). The largest difference between the mean mixture weights of the combination variables was found to be 0.0003 g. For practical purposes the weights may be considered identical.

***In Vitro* Tests**—Dissolution-rate studies were begun using the Levy and Hayes (3) "beaker method," so that the low agitation intensity which Levy regards as important for *in vitro* dissolution-rate measurements to reflect *in vivo* conditions might be achieved (4). This method was found to be unsatisfactory for use with capsules; it was necessary to use the "oscillating tube" method which involves a relatively high agitation intensity as the dissolution fluid is forced through a 100-mesh screen on the bottom of a Plexiglas cylinder (4). Dissolution rates were determined for the No. 3 capsules containing ASA alone using the Levy and Hayes beaker method and the oscillating tube method with distilled water adjusted to pH 1.2 with hydrochloric acid as the dissolution medium. Dissolution rates for each combination variable were determined using the oscillating tube method and either gastric fluid, simulated test solution USP, or intestinal fluid, simulated test solution USP (5) as the dissolution medium. The intrinsic dissolution of the ASA crystals was determined in each simulated test solution using the oscillating tube method. The samples at the end of each time period were assayed by the method of Routh and Dryer (2) (Tables I–IV).

Table II—Average Concentrations Obtained in Dissolution Determinations of No. 3 and No. 4 Capsules Containing ASA and Lactose[a]

Time, min.	Average Concentration,[b] mg./100 ml. No. 3 Capsule SGF	No. 3 Capsule SIF	No. 4 Capsule SGF	No. 4 Capsule SIF
10	5.3	1.0	15.3	8.5
20	10.5	10.0	16.1	16.3
30	13.3	12.0	17.5	19.9
45	14.3	15.5	18.1	
60	16.3	19.8	18.9	
90	18.5		19.1	
120	19.5		19.4	
180	19.8		19.5	

[a] Oscillating tube method, 100-mesh screen. [b] Average of three determinations: SGF, simulated gastric fluid; and SIF, simulated intestinal fluid.

Table III—Average Concentrations Obtained in Dissolution Determinations of No. 3 and No. 4 Capsules Containing ASA and Dibasic Calcium Phosphate[a]

Time, min.	Average Concentration,[b] mg./100 ml. No. 3 Capsule SGF	No. 3 Capsule SIF	No. 4 Capsule SGF	No. 4 Capsule SIF
10	6.8	3.5	7.8	8.8
20	16.5	14.8	19.8	19.6
30	19.0	19.8		
45	19.8			

[a] Oscillating tube method, 100-mesh screen. [b] Average of three determinations: SGF, simulated gastric fluid; and SIF, simulated intestinal fluid.

***In Vivo* Tests**—The rabbit was mildly sedated using halothane. A gag was placed between the teeth, and the capsule was placed well back in the throat and washed down with a little water. Blood samples were withdrawn from the marginal ear vein at 1-, 2-, and 3-hr. intervals after administration of the capsule. The samples were collected in calibrated centrifuge tubes and centrifuged, and the liquid was used for analysis. Plasma blank values were subtracted from the sample values. The plasma was analyzed according to the method of Routh and Dryer (2).

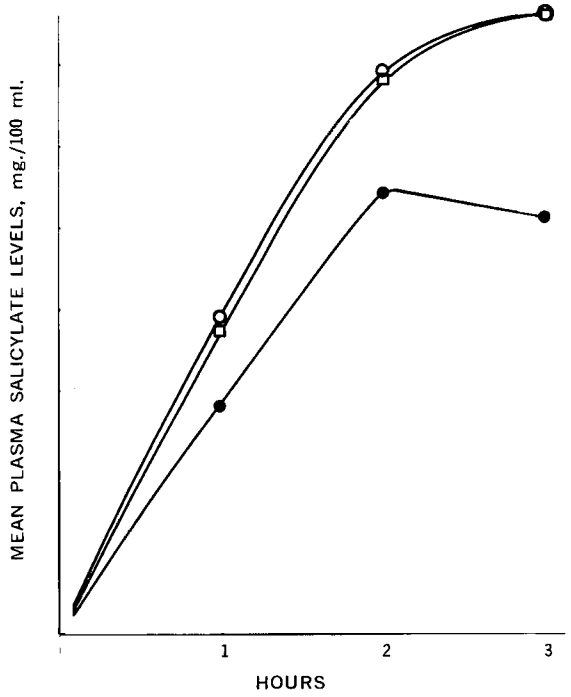

Figure 1—*Mean plasma salicylate levels after administration of ASA in No. 3 and No. 4 hard gelatin capsules. Key:* ○, *ASA + lactose (No. 4);* □, *ASA (No. 3); and* ●, *ASA + lactose (No. 3).*

Table IV—Dissolution of 20-Mesh ASA Crystals in Both Gastric Fluid, Simulated Test Solution and Intestinal Fluid, Simulated Test Solution[a]

Time, min.	Average Concentration,[b] mg./100 ml. SGF	SIF
10	11.5	19.3
20	12.5	20.3
30	13.0	
45	16.3	
60	18.8	
90	19.5	
120	19.8	
180	19.4	

[a] Oscillating tube method, 100-mesh screen. [b] Average of three determinations: SGF, simulated gastric fluid; and SIF, simulated intestinal fluid.

RESULTS AND DISCUSSION

Table V and Figs. 1 and 2 show the mean plasma salicylate levels in mg./100 ml. obtained at 1, 2, and 3 hr. after administration of the capsule. The mean plasma salicylate values were low. A multiple regression analysis[1] (6), in which there was no detailed examination of differences among the five treatment means, showed no suggestion of week differences. An analysis of variance (Table VI) showed that by the 3rd hour, the highly compacted combinations in No. 4 capsules were producing better plasma levels than the No. 3 capsules, with or without excipients.

The better plasma salicylate levels obtained with the tightly compacted No. 4 capsules may be due to the diffusion of gastric juice through the gelatin, which created a higher osmotic pressure within the capsules, thereby leading to more rapid breakdown and absorption. It is difficult to explain the excellent *in vivo* performance of the ASA in a No. 3 capsule.

Under the low agitation conditions of the Levy beaker method, a No. 3 capsule containing ASA alone floated in the hydrochloric acid–water solution for 2 hr. before settling to the bottom of the beaker. Figure 3 presents sketches of the appearance of the capsule at various times. The concentration of ASA was found to be 5.5 mg./100 ml. at the end of 4 hr. A small amount of pepsin was added to the beaker, and the thin gelatin bag broke within 1 min. The beaker method using distilled water, adjusted to pH 1.2 with hydrochloric acid, was felt to be unsatisfactory for dissolution determinations with capsules. A modified Levy–Hayes method, using weighted capsules to prevent floating, has been suggested for overcoming the results experienced in this study (7).

Using the oscillating tube method, the hard gelatin capsules showed distortion and did not appear to break down readily even in the presence of the digestive enzyme used and the relatively high agitation intensities of the oscillating tube method. The average concentration (three determinations) of ASA at the end of 4 hr. was 19.8 mg./100 ml. using hydrochloric acid–water (pH 1.2) as the dissolution medium and 20.0 mg./100 ml. after 3 hr. using gastric fluid, simulated test solution. Intrinsic dissolution determinations showed that ASA dissolves well when unencumbered by gelatin.

The mean plasma salicylate levels at the end of 1, 2, and 3 hr. were far lower than the concentrations obtained at the corresponding hours in the *in vitro* study, so that any attempt to correlate the

Table V—Mean Plasma Salicylate Levels from the Determination of Plasma Salicylate Concentration in Rabbits at 1, 2, and 3 hr. following Administration of ASA Combinations in Hard Gelatin Capsules

Dosage Form	Mean Plasma Salicylate Levels, mg./100 ml. Hour 1	Hour 2	Hour 3
ASA in No. 3 capsules	3.7	6.8	7.6
ASA plus lactose in No. 3 capsules	2.8	5.4	5.1
ASA plus lactose in No. 4 capsules	3.9	6.9	7.6
ASA plus dibasic calcium phosphate in No. 3 capsules	3.9	6.6	6.9
ASA plus dibasic calcium phosphate in No. 4 capsules	4.9	7.0	8.3

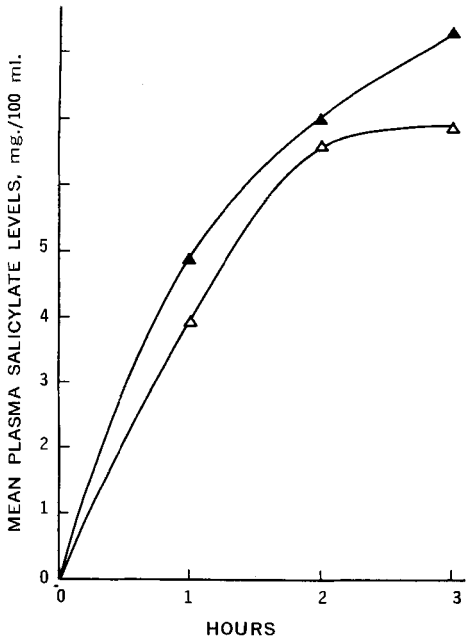

Figure 2—*Mean plasma salicylate levels after administration of ASA in No. 3 and No. 4 hard gelatin capsules. Key: △, ASA + dibasic calcium phosphate (No. 3); and ▲, ASA + dibasic calcium phosphate (No. 4).*

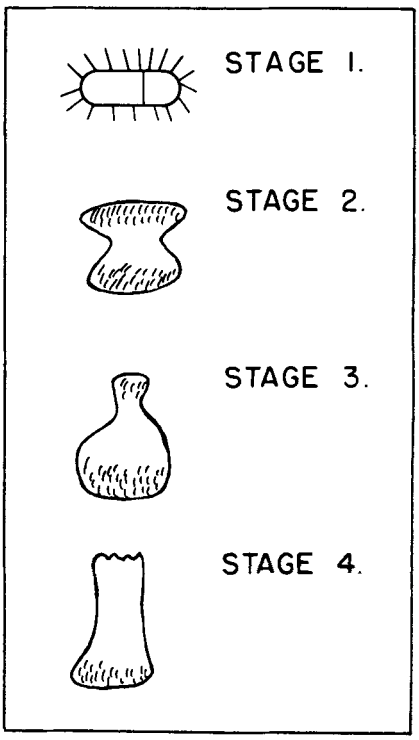

Figure 3—*Appearance of capsule at various stages using the Levy and Hayes beaker method. Stage 1, whiskers or needles, 3 min.; Stage 2, mushroom, 8–10 min.; Stage 3, pycnidium, 25–120 min.; and Stage 4, cylinder, over 120 min.*

[1] Thanks are extended to Professor D. W. Reid and Mrs. Bazoki of the Department of Epidemiology and Biometrics, School of Hygiene, University of Toronto, for their suggestions regarding the experimental design and analysis of the data obtained.

Table VI—Analysis of Data from Plasma Level Determinations

	Hour 1		Hour 2		Hour 3	
	df	*MS*	*df*	*MS*	*df*	*MS*
Control *vs.* average of others	1	0.31	1	0.63	1	2.89
Capsules	1	8.70	1	8.12	1	34.41[a]
Fillers	1	9.71	1	3.93	1	14.95
Capsules + fillers	1	0.01	1	2.94	1	3.00
Rabbits	8	6.70	8	22.18[b]	8	16.03[a]
Error	32	3.86	32	5.83	32	5.50

[a] Significant at the 5% level. [b] Significant at the 1% level.

two sets of data was considered of little value. A rank order correlation may, however, be observed in that the mean plasma salicylate values, as well as dissolution values, are higher for the more highly compacted No. 4 capsules and in the case of dibasic calcium phosphate. The low plasma salicylate levels may be due to a failure to break down *in vivo*. The *in vitro* agitation intensities resulting from the dissolution medium being forced through the 100-mesh screen were reported by Levy (4) to be far greater than the agitation encountered in the stomach, a fact that could account for the widespread difference in concentration levels between the two studies.

In vitro observations showed that the ASA became moist; over a period of time the gelatin stretched and, together with the mechanical action of striking against the 100-mesh screen, assisted in the breakdown. The stretching effect was not as noticeable in the case of capsules containing excipients.

The low plasma salicylate levels obtained *in vivo* together with the *in vitro* behavior of the capsules would seem to indicate that the gelatin in the hard gelatin capsules had been modified in some manner, either by the acidity of the gastric juice, or by the acidity of the ASA (pKa 3.5) within the microenvironment of the capsule, or by a combination of the two. If due to the weakly acidic drug alone or to a combination of drug and gastric juice, this could be of importance for other drugs of similar properties when encapsulated. If due to the acidity of the gastric juice alone, it would appear that the release of any drug enclosed in hard gelatin capsules could be affected adversely. Gelatin is obtained by heat denaturation of collagen and is built of three strands, which are joined primarily by hydrogen bonding between the strands (8). It is possible that the stretching of these strands accounted for the appearance of the hard gelatin capsules in the *in vitro* tests and perhaps for the low plasma salicylate levels.

REFERENCES

(1) G. Levy, *J. Pharm. Sci.*, **50**, 388(1961).

(2) J. I. Routh and R. L. Dryer, "Standard Methods of Clinical Chemistry," vol. 3, Academic, New York, N. Y., 1959, pp. 194–199.

(3) G. Levy and B. A. Hayes, *New Engl. J. Med.*, **262**, 1053(1960).

(4) G. Levy, *J. Pharm. Sci.*, **52**, 1039(1963).

(5) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 1072.

(6) G. W. Snedecor, "Statistical Methods," 5th ed., Iowa State University Press, Ames, Iowa, 1956, p. 237.

(7) J. W. Poole, "Some Experiences in the Evaluation of Formulation Variables on Drug Availability," Proceedings of the DIA Symposium on Formulation Factors Affecting Therapeutic Performance of Drug Products, **3**, 8(1969).

(8) A. White, P. Handler, and E. Smith, "Principles of Biochemistry," 3rd ed., The Blakiston Division, McGraw-Hill, New York, N. Y., 1964, pp. 768–769.

ACKNOWLEDGMENTS AND ADDRESSES

Received February 20, 1969, from the *Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.*

Accepted for publication May 5, 1970.

The authors express their thanks to Smith Kline & French Inter-American Corp. for financial support during this investigation.

Factors Influencing Solvolysis of Corticosteroid-21-phosphate Esters

G. L. FLYNN and D. J. LAMB

Abstract □ The solvolysis of methylprednisolone-21-phosphate in dilute aqueous solution (<0.005 *M*) is qualitatively similar to that observed for methylphosphate and other simple monoalkyl phosphates, particularly in the pH range 3–8. In more concentrated solutions (>0.02 *M*), however, there is an acceleration of reaction velocities and marked deviation from the expected pH dependency. This change in chemical behavior is attributed to association colloid formation. Support for this mechanism is drawn from hydrolysis-rate data obtained as a function of concentration and independently determined critical micelle concentration values.

Keyphrases □ Corticosteroid-21-phosphate esters—solvolysis, micelle formation □ Solvolysis, corticosteroid-21-PO_4 esters—pH profile, activation energy, aggregation effect □ Critical micelle concentration determination—conductivity, surface-tension methods □ Phosphate, inorganic—analysis

The term phosphate ester is extremely ambiguous because it applies to several distinctly different classes of compounds, each characterized by unique chemical behavior. Furthermore, chemistries in each phosphate ester class can be appreciably different both qualitatively and quantitatively, depending on the types and proximities of neighboring atoms within the molecule. Additional complexity arises from the biformity of the carbon–oxygen–phosphorus linkage. Solvolysis, for instance, may entail carbon–oxygen cleavage, phosphorus–oxygen splitting, or both, depending on the reaction conditions (1).

The hydrolyses of monoalkyl phosphates are characterized by these diversities. The prototype of this phosphate ester class is methylphosphate. It is a relatively simple molecule whose aqueous stability has been extensively studied (2). At pH values below zero, the conjugate acid species predominates, and the solvolysis takes place with both carbon–oxygen and phosphorus–oxygen splitting. The neutral molecule is the principal species in pH range 0–2, and it cleaves exclusively at the carbon–oxygen bond. Above pH 3, the monoanion,