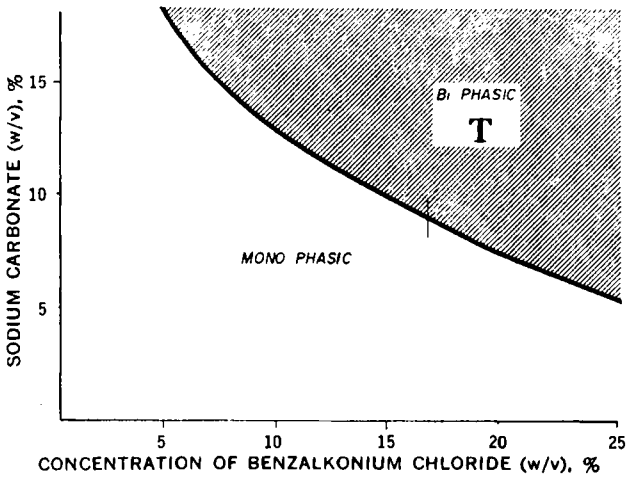

Figure 2—*Phase transition diagram of sodium carbonate–benzalkonium chloride coacervate system at 25°. Shaded area [T] represents the region in which biphasic coacervate system can be formed with lighter coacervate phase.*

bivalent anions, *i.e.*, sodium chloride and sodium carbonate, with benzalkonium chloride. Also reported is the effect of sodium carbonate concentration on the coacervate volume and refractive index of the coacervate phase.

The coacervate systems were obtained by mixing the indicated concentrations (Fig. 1) of salt and benzalkonium chloride in water. Figure 1 shows the phase transition diagram of the sodium chloride and benzalkonium chloride coacervate system at the points of coacervate formation. Figure 2 shows the phase transition diagram of the sodium carbonate and benzalkonium chloride coacervate system. These diagrams illustrate two main regions: (*a*) biphasic coacervate system, represented by the shaded area; and (*b*) monophasic solution, represented by nonshaded area. In both coacervate systems, the formed coacervate phase was lighter than the equilibrium liquid phase.

Figure 3 represents the effect of the sodium carbonate concentration on the refractive index and coacervate volume. For this study, 100 ml. of coacervate systems, containing 25% (w/v) benzalkonium chloride with 5.6, 6.0, 7.0, 8.0, and 10.0% sodium carbonate, was prepared. The systems were allowed to equilibrate for 24 hr. at room temperature (25°). The volume of coacervate was noted as the percent of the whole system. The refractive index of the coacervate phase was measured at 25° using a refractometer[1].

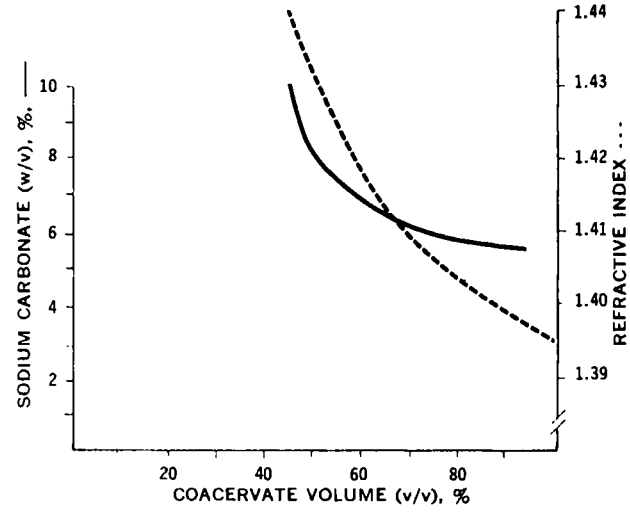

Figure 3—*Volume and refractive index of coacervate phase of sodium carbonate–25% benzalkonium chloride coacervate system at 25°.*

(1) D. G. Dervichian, *Discuss. Faraday Soc.*, **18**, 231(1954).
(2) G. Ram Mohan and M. Eisenberg, *J. Phys. Chem.*, **63**, 671 (1959).
(3) D. Shah, B. Ecanow, M. Sadove, and F. Shieh, *J. Pharm. Sci.*, **62**, 1210(1973).
(4) H. G. Bungenberg de Jong and H. R. Kruyt, *Kolloid-Z.*, **50**, 39(1930).
(5) I. Cohen and T. Vassiliades, *J. Phys. Chem.*, **65**, 1774(1961).
(6) N. N. Shurygira, A. I. Agatova, and T. N. Evreinova, *Nauch. Dokl. Shk. Biol. Nauki*, **2**, 110(1967).
(7) P. Ekwall, L. Mandell, and K. Fontell, *J. Colloid Interface Sci.*, **33**, 215(1970).
(8) R. Acharya, B. Ecanow, and R. Balagot, *ibid.*, **40**, 125 (1972).
(9) B. Ecanow, B. Gold, and P. Tunkunas, *Psychother. Psychosom.*, **21**, 125(1972).

DEVENDRA SHAH
BERNARD ECANOW▲
College of Pharmacy
University of Illinois
Chicago, IL 60612

REUBEN BALAGOT
Hines V. A. Hospital
Surgical Service
Hines, IL 60612

Received June 11, 1973.
Accepted for publication August 2, 1973.
▲ To whom inquiries should be directed.

[1] Abbe.

Possible Errors and Role of Mercuric Chloride in Using Trinder's Reagent for Assay of Salicylates in Urine Specimens

Keyphrases ☐ Salicylates in urine—analysis, Trinder's reagent with and without mercuric chloride ☐ Mercuric chloride in Trinder's reagent—effect on salicylate analysis in urine ☐ Trinder's reagent, with and without mercuric chloride—effect on salicylate analysis in urine ☐ Colorimetry—analysis, salicylates in urine, Trinder's reagent with and without mercuric chloride

Sir:

Trinder's reagent has been used in many colleges of pharmacy as a colorimetric agent to assay urinary salicylate and thereby to estimate the bioavailability of compounds such as aspirin, sodium salicylate, and *p*-aminosalicylic acid (1–4). The assay procedure is simple. One milliliter of the urine sample is usually diluted with 5 ml. of the reagent, and the absorbance of the resultant solution at 540 nm. is measured using the properly diluted (with water or urine blank) reagent as a reference solution. The absorbances of the urine samples

Table I—Absorbances at 540 nm. of Various Urine Blank Samples

Urine Samples	Subjects 1	2	3	4	5	6
Before breakfast	0.05	0.10	0.23	0.05	0.17	0.06
Breakfast–lunch	0.22	0.08	0.20	0.13	0.14	0.05
Lunch–supper	0.22	0.09	0.19	0.17	0.12	0.06
Supper–bedtime	0.39	0.12	0.21	0.22	0.15	0.06

Table II—Absorbances of Sodium Salicylate in Urine after Adding Trinder's Reagent Prepared with or without Mercuric Chloride (an Average of Two Studies)

Concentrations of Sodium Salicylate, mg./ml.	With Mercuric Chloride	Without Mercuric Chloride
0.125	0.180	0.179
0.500	0.728	0.723
1.000	1.439	1.424

collected after dosing are corrected for the absorbance of the blank urine sample usually taken before dosing in the calculation of the salicylate concentrations in the urine. In doing so, it is assumed that the basic blank absorbances of the urine samples not due to ingested salicylates remain essentially unchanged during the period of study. To our knowledge, no data have been published to support this assumption.

In the bioavailability study of aspirin dosage forms using Trinder's reagent, we have occasionally found that the subjects excreted much more salicylate than ingested. We were also often puzzled by the fact that the absorbances of the urine samples after dosing were much lower than the absorbance of the blank sample. We thought that these observations might result from the intrasubject variation of the urinary blank contribution, and the present study was undertaken to explore this possibility. Urine specimens of six normal adult subjects who had not taken salicylates or other drugs during the previous week and the day of study were collected at various times during the day: before breakfast, between breakfast and lunch, between lunch and supper, and between supper and bedtime. Normal food and fluid uptake were allowed. Specimen absorbances at 540 nm., measured with a spectrophotometer[1] after adding the colorimetric agent, are shown in Table I. An absorbance of 0.1 corresponds to a urinary concentration of 0.07 mg./ml. of sodium salicylate. The possibility of marked intersubject and intrasubject blank variations is clearly demonstrated from these data. The highest blank reading found in the supper-to-bedtime urine of the Subject 1 corresponded to 0.273 mg./ml. of sodium salicylate. If one uses the urine of Subject 1 collected before breakfast as a blank sample, this subject can be calculated to excrete 159 mg. of sodium salicylate during the day even when he ingested no salicylate. This calculation was based on the average absorbance of three samples and a urine output of 1 l. An error of this magnitude is obviously unacceptable when one is evaluating the bioavailability of 324–648 mg. (5–10 grains) of an aspirin preparation administered as a single dose. In Subjects 2, 3, and 5, the excretion of salicylates can sometimes be underestimated if one also uses the urine sample collected before breakfast as a blank. For Subject 6, all blank readings were quite low and consistent.

The inclusion of mercuric chloride (4% w/v) in Trinder's reagent was used primarily to precipitate protein during salicylate analysis of serum or plasma samples (1). Since urine samples normally contain only a negligible amount of protein, it was interesting to determine whether its inclusion is necessary. Such a study seemed especially significant in light of the pollution problem of the mercury compound and its relatively high cost. One milliliter of various standard solutions of sodium salicylate in urine was diluted with 5 ml. of the Trinder reagent prepared with or without mercuric chloride. Their absorbances at 540 nm. were measured using suitable blanks as references. As shown in Table II, absorbances of solutions were essentially not affected by the absence of mercuric chloride and all followed Beer's law in the range studied. A similar phenomenon was also observed for salicylate dissolved in distilled water. Therefore, it is concluded that the mercuric chloride included can be deleted from Trinder's reagent for the assay of salicylates in urine or other aqueous media not containing proteins.

(1) D. Trinder, *Biochem. J.*, **57**, 301(1954).
(2) E. L. Parrott, *J. Pharm. Sci.*, **60**, 867(1971).
(3) E. L. Parrott and W. Saski, "Experimental Pharmaceutical Technology," 3rd ed., Burgess, Minneapolis, Minn., 1971, p. 63.
(4) E. L. Parrott, *Amer. J. Pharm. Educ.*, **37**, 41(1972).

WIN L. CHIOU▲
IJEOMA ONYEMELUKWE
Department of Pharmacy
College of Pharmacy
University of Illinois
Chicago, IL 60612

Received April 9, 1973.
Accepted for publication June 25, 1973.
Supported in part by Public Health Service General Research Support Grant 1-501-RR05735-01.
▲ To whom inquiries should be directed.

[1] Beckman DBGT.