Table II—Analytical and Physical Data for New Compounds

Compound	Melting Point	Yield, %	Recrystallization Solvent	Formula	Analysis, % Calc.	Found
V	239–240°	90	C_2H_5OH	$C_{21}H_{17}BrN_4$	C 62.23	62.07
					H 4.23	4.29
					Br 19.72	19.67
XXX	166–168°	60	C_2H_5OH	$C_{12}H_{15}BrN_2O_2$	C 48.17	48.42
					H 5.05	5.01
					Br 26.71	26.64
XXXIX	209–210°	92	C_2H_5OH	$C_{12}H_{16}BrN_3O$	C 48.33	48.48
					H 5.41	5.28
					Br 26.80	26.81

EXPERIMENTAL[2]

2-(*m*-Trifluoromethylanilino)quinolizinium bromide (XXXIV) was prepared as follows. To a solution of 2-bromoquinolizinium bromide (I) (1) (45 g, 0.15 mole) in ethanol (600 ml) was added *m*-trifluoromethylaniline (50 g, 0.30 mole). The stirred mixture was boiled under reflux for 5 hr, then treated with charcoal, and filtered. The product was precipitated from the filtrate by the addition of anhydrous ether.

The crude product weighed 42 g (76%). Recrystallization from isopropanol–ether provided analytically pure material, mp 229–230°.

Anal.—Calc. for $C_{10}H_{12}BrF_3N_2$: C, 52.03; H, 3.28; N, 7.59. Found: C, 51.96; H, 3.25; N, 7.61.

All other 2-(substituted amino)quinolizinium bromides were prepared similarly. Compounds V, XXX, XXXIV, and XXXIX have not been reported previously; the analytical and physical data for V, XXX, and XXXIX are shown in Table II. Analytical and physical data for all other compounds (Table I) were reported previously (1).

[2] Melting points were determined in open capillary tubes using a Mel-Temp melting-point apparatus and are uncorrected.

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Halothane Uptake by Coacervate Systems

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Abstract □ Two aggregated coacervae systems (acacia–gelatin and gelatin–benzalkonium chloride) were analyzed for gas sorption of halothane with a gas chromatograph, using a modified tonometer as an absorption chamber. Similar studies were performed on each system after the coacervate had been broken or "dissolved." Differences in absorption or solubilizing effect between coacervates and dissolved coacervates were noted. A significant halothane gas uptake was observed in the highly structured coacervate system.

Keyphrases □ Halothane—uptake by acacia–gelatin and gelatin–benzalkonium chloride aggregated coacervate systems, uptake compared to broken coacervate systems □ Coacervate systems—halothane uptake by acacia–gelatin and gelatin–benzalkonium chloride systems and similar broken systems □ Acacia–gelatin coacervate system—halothane uptake, compared to similar broken system □ Gelatin–benzalkonium chloride system—halothane uptake, compared to similar broken system

The importance of solvent abilities in structured systems as well as a renewed interest in liquid crystalline states suggested that it would be of value to investigate the solvent abilities of a coacervate in contrast to the solvent abilities of an analogous "broken" coacervate. Coacervates may be considered to be coagulated systems, while "dissolved" or broken coacervates can be considered noncoagulated systems. In this study, a unique ability of coacervate systems to attract and solubilize nonpolar gases in a polar medium is discussed.

The term coagulation, as used here, refers to a type of aggregate characterized by film–film bonded particulates. The term coacervation denotes the formation of a liquid precipitate by the mutual coagulation of hydrophilic colloids (1). The definition of coacervation has been more elaborately discussed (2) as a phenomenon of the separation of colloidal solutions into two or more immiscible liquid phases. The electrostatic and thermodynamic properties of a colloidal solution can be varied in such a way that the system separates into two amorphous liquid layers. One layer contains most of the colloid (the coacervate), while the second layer is colloid poor (the equilibrium liquid). The coacervate appears in the form of

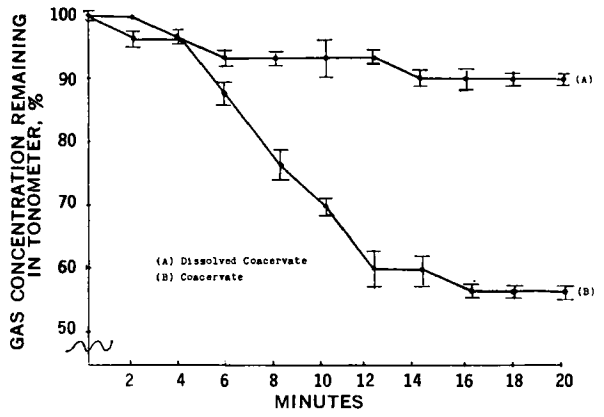

Figure 1—*Halothane gas sorption by gelatin–benzalkonium preparations.*

droplets which coalesce rapidly to form one homogeneous, colloid-rich layer (2).

EXPERIMENTAL

Preparation—Two aggregated coacervate systems (acacia–gelatin and gelatin–benzalkonium chloride) were analyzed for their ability to attract halothane vapor. Similar studies were performed on each system after the coacervate had been broken or dissolved.

A solution of 6.0% (w/v) acacia and 5% (w/v) gelatin comprised the acacia–gelatin coacervates[1]. Three milliliters of 0.2 *N* NaOH, pH 9.95, was added to a portion of the coacervate which had the effect of breaking or dissolving the structure.

The gelatin–benzalkonium chloride coacervate was a solution of 2% gelatin and 0.6% benzalkonium chloride at a pH > 9. To break the coacervate, 1.7 ml of concentrated hydrochloric acid was added to a portion of the coacervate. The major difference between a coacervate and a "dissolved coacervate" is that the coacervate, if mixed with the equilibrium liquid, will maintain its immiscibility and volume, whereas when the dissolved coacervate is mixed with the equilibrium liquid, a clear single-phase system is obtained.

Halothane was originally in the liquid state. In preparation for the desired vapor volume, the following formula was used:

$$\frac{(A)(B)(C)}{(D)(E)} = \text{volume of liquid anesthetic (milliliters)} \quad \text{(Eq. 1)}$$

where A = percent of gas concentration, B = volume (milliliters) of tonometer, C = molecular weight of anesthetic agent, D = specific gravity of anesthetic agent, and E = gas constant (22,400 ml).

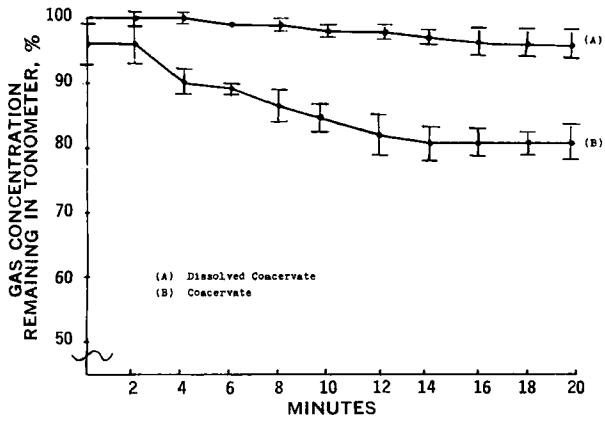

Figure 2—*Halothane gas sorption by acacia–gelatin preparations.*

[1] Acacia–gelatin and gelatin–benzalkonium chloride preparations were obtained through the courtesy of Dr. Harun Takkuri, College of Pharmacy, University of Illinois.

Table I—Statistical Statement

Acacia–Gelatin System: Dissolved Coacervate *versus* Coacervate Halothane Sorption		Gelatin–Benzalkonium Chloride System: Dissolved Coacervate *versus* Coacervate Halothane Sorption	
Minutes	$t_{[4]}$	Minutes	$t_{[4]}$
0	1.98[a]	0	1.03[a]
2	2.34[a]	2	5.51[a]
4	9.91[b]	4	2.45[a]
6	29.16[b]	6	5.07[a]
8	8.91[b]	8	13.10[b]
10	13.60[b]	10	34.03[b]
12	9.19[b]	12	21.11[b]
14	10.56[b]	14	28.85[b]
16	10.61[b]	16	9.71[b]
18	11.36[b]	18	68.93[b]
20	8.98[b]	20	49.05[b]

[a] Not significant. [b] $p < 0.001$.

Equipment[2]—Gas uptake was followed by GLC analysis. A modified tonometer (3), constructed of glass and fitted with rubber septums, was used as an absorption chamber. The tonometer was designed in both 50- and 150-ml sizes for enclosing the gas samples and in a 300-ml size for use as the preparation and storage facility.

Ten-milliliter samples of each coacervate and dissolved coacervate were placed individually in a 50-ml modified tonometer. A 10-ml aliquot of halothane vapor at ambient temperature and pressure was added for gas uptake observations. Since the gelatin–benzalkonium chloride coacervate was a gel at room temperature, it was necessary to heat it in a water bath to 40° before transfer to the tonometer. Once transferred, it was allowed to reach room temperature before the gas absorption experiments.

Gas syringes were used to transfer the gas volume from the preparation tonometer to that containing the coacervate or broken coacervate. These syringes were fitted with 22-gauge, 6.4-cm (2.5-in.) needles. Gastight syringes[3] (100 μl), fitted with Teflon hub needles [24 gauge, 3.8 cm (1.5 in.)], were used for removal of halothane vapor samples from the second tonometer and for injection of samples into the gas chromatograph.

A gas chromatograph with dual hydrogen flame-ionization detectors was utilized to follow gas uptake. The hydrogen flow rate was maintained at 10 ml/min, air at 350 ml/min, and nitrogen at 60 ml/min. The stainless steel columns used for analysis of gas uptake were 1.8 m × 0.64 cm (6 ft × 0.25 in.) and were packed with 25% silicone gum rubber (SE-30) on Anakron (70–80 mesh).

Operating temperatures used in the gas chromatograph were: injection port, 165°; column, 125°; and internal detector, 130°. Gas analysis samples were removed at approximately 1-min intervals for 20 min.

RESULTS AND DISCUSSION

The tonometer gas concentration (microliters per milliliter) in the vapor phase above the liquid samples *versus* the time (minutes) was followed graphically. It was determined during experimentation that 20 min was sufficient for equilibrium to occur. For presentation purposes, graphic data have been arranged to indicate the percentage of gas concentration remaining in the tonometer *versus* time (minutes). Figure 1 represents the gelatin–benzalkonium chloride coacervate system, and Fig. 2 represents the acacia–gelatin coacervate system.

Results (Table I) indicate that a significant uptake occurred with the intact coacervate system, considerably greater than the degree of solubilization observed in the dissolved or broken system. This significant uptake of halothane is probably indicative of

[2] Sources of the materials were as follows: WCLID 1670 model gas chromatograph, Warner-Chilcott Laboratories, Richmond, Calif.; columns, HCL Scientific Inc., Rockford, Ill.; and halothane (Fluothane), Ayerst Laboratories, New York, N.Y.

[3] Hamilton.

the presence of a highly structured, nonpolar system, similar to surfactant micelles in a polar medium.

Further ramifications are currently under study in these laboratories.

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Electron-Capture GLC Determination of Blood Levels of 7-Chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one in Humans and Its Urinary Excretion as Lorazepam Determined by Differential Pulse Polarography

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Abstract □ This report presents data to demonstrate the clinical utility of a previously published electron-capture GLC assay in the measurement of blood levels of 7-chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one in humans following a single oral 4-mg dose. It also describes a differential pulse polarographic assay for the quantitation of its major urinary metabolite, lorazepam, in humans. This assay has greater sensitivity and specificity than the previously published Bratton–Marshall spectrophotometric assay.

Keyphrases □ 7-Chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one—electron-capture GLC determination of human blood levels □ Lorazepam—urinary metabolite of 7-chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one, determined by differential pulse polarography □ GLC, electron capture—determination, 7-chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one in human blood samples □ Differential pulse polarography—determination, lorazepam as urinary metabolite of 7-chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one in humans

The 1,4-benzodiazepine class of compounds are clinically important as tranquilizers, hypnotics, and muscle relaxants (1). 7-Chloro-1,3-dihydro-5-(2′-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (I) was synthesized by Sternbach *et al.* (2) and is currently undergoing clinical evaluation as an antianxiety agent. A sensitive and specific electron-capture GLC assay for the determination of I and its major metabolite, lorazepam (II), was published previously (3)[1].

This report presents data to demonstrate the clinical utility of the electron-capture GLC assay in the measurement of blood levels of I in humans following a single oral 4-mg dose. It also describes a differential pulse polarographic assay for the quantitation of lorazepam. The polarographic analysis of oxazepam and lorazepam in pharmaceutical formulations was described previously (4, 5).

EXPERIMENTAL

Clinical Protocol—A pilot blood level study was conducted in three subjects[2] following the administration of a single 4-mg oral dose (as 2 × 2-mg tablets), and whole blood specimens were collected as indicated in Table I. Urine specimens were collected as indicated in Table II. The oxalated blood specimens and urine specimens were stored frozen until analyzed.

Analysis of Specimens—Blood and urine levels of I were determined by the published electron-capture GLC assay (3), which has sensitivity limits of 0.5–1.0 ng/ml for I and of 4–5 ng/ml for II in blood or urine using a 15-mCi nickel-63 electron-capture detector. The major urinary metabolite in humans (lorazepam) is present mainly as a glucuronide conjugate (6). It is extracted from urine following deconjugation with glucuronidase–sulfatase and is analyzed by differential pulse polarography (Fig. 1), with a sensitivity limit of 50 ng/ml for II in urine using a 5-ml specimen/analysis.

Differential Pulse Polarographic Analysis of II—Lorazepam (II) is present in the urine mainly as a glucuronide and/or sulfate conjugate; small amounts of I are also excreted. The urine specimen is first extracted at pH 9.0 with ether[3], which quantitatively removes I and any unconjugated lorazepam, and is processed for electron-capture GLC analysis (3). The specimen is then titrated to pH 5.3, incubated with glusulase enzyme[4] (2% by volume) to deconjugate II, and then extracted into ether after adjusting the pH to 9.0 as previously described (3).

Internal standards of 200, 400, or 1000 ng of II are added to the control urine specimens prior to incubation and are carried through the entire procedure. After incubation, the samples are cooled to room temperature and the pH is adjusted to 9.0 using a pH meter by titrating the sample dropwise with 6 *N* NaOH. This

[1] Consult Fig. 1 and Table I in this reference for the chemical structures and nomenclature of Compounds I, II, and V.

[2] Conducted under the supervision of Dr. James D. Moore at the Deer Lodge Research Unit, Deer Lodge, Mont.

[3] Diethyl, absolute, analytical reagent grade, Mallinckrodt.

[4] Endo Laboratories, Garden City, Long Island, N.Y.