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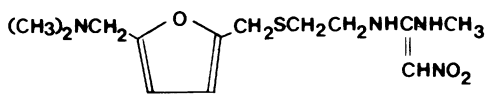
Ion-Selective Electrodes for the H_2-Receptor Antagonists Cimetidine and Ranitidine

ANASTASIA MITSANA-PAPAZOGLOU, ELEFTHERIOS P. DIAMANDIS[x], AND THEMISTOCLES P. HADJIIOANNOU

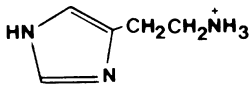
Received January 5, 1987, from the *Laboratory of Analytical Chemistry, University of Athens, Athens 106 80, Greece.* Accepted for publication April 16, 1987. Present address: Cyber-Fluor, Inc., 179 John Street, Toronto, Ontario, Canada M5T 1X4.

Abstract □ Liquid-membrane and polyvinyl chloride (PVC)-matrix ion-selective electrodes (ISE) that respond to the cationic forms of cimetidine and ranitidine are described. The ion-exchangers were the salts of cimetidine and ranitidine with tetrakis(*m*-chlorophenyl)borate dissolved in *p*-nitrocumene or entrapped in PVC polymer in the presence of 2-nitrophenyl octyl ether as plasticizer. The electrodes exhibited a near-Nernstian response in the range 10^{-2}–10^{-6} M (working pH range 2–7) for ranitidine, and 10^{-2}–2×10^{-5} M (pH 2–6) for cimetidine. Very small PVC-matrix ISE with internal diameters as small as 0.035 inches were constructed and used in combination with small cuvettes, so that measurements could be carried out in 250 μL of stirred solution. The electrodes were applied successfully for the determination of the pK_a of the protonated bases and for the determination of the drugs in pharmaceutical preparations. New selective and effective solid-state extraction procedures are described for the extraction of ranitidine from urine and serum samples. Potentiometric methods were developed for the determination of ranitidine in urine and serum samples during a pharmacokinetic experiment.

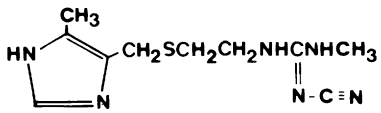
RANITIDINE



HISTAMINE



CIMETIDINE



Cimetidine (*N''*-cyano-*N*-methyl-*N'*-[2-[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]-guanidine) is a potent H_2-receptor antagonist which inhibits gastric acid secretion in humans and has been proven to be highly effective in the treatment of duodenal ulcer.[1–4] Cimetidine, like histamine, contains an imidazole ring. The newer H_2-receptor antagonist ranitidine (*N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-*N'*-methyl-2-nitro-1,1-ethenediamine) does not contain the histamine imidazole nucleus, but is an aminomethylfuran derivative. Ranitidine is ~5–10 times more potent than cimetidine, on a molar basis, in inhibiting histamine-induced gastric acid secretion.[5–9] In addition, it has fewer side effects and is therefore considered to be the drug of choice for the treatment of duodenal ulcer patients.

Ion-selective electrodes (ISE) for these two important drugs have not yet been reported. In this paper we describe liquid-membrane and polyvinyl chloride (PVC)-matrix ISE for the cationic forms of cimetidine and ranitidine. The ion-exchangers used were cimetidine hydrogen tetra(*m*-chlorophenyl)borate [(Cim H^+)(TCPB$^-$)] and ranitidine hydrogen tetra(*m*-chlorophenyl)borate [(Ran H^+)(TCPB$^-$)], dissolved either in *p*-nitrocumene (liquid ion-exchangers) or entrapped in PVC polymer in the presence of 2-nitrophenyl octyl ether (NPOE) as plasticizer. The electrodes are very sensitive (linear response down to 10^{-6} M for ranitidine and to 2×10^{-5} M for cimetidine) and sufficiently selective. Both electrodes were used for the potentiometric determination of the ionization constants of the protonated bases and for the assay of the active compounds in pharmaceutical preparations by using simple procedures.

Polyvinyl chloride (PVC)-matrix microelectrodes were constructed which are suitable for potentiometric measurements in 250 μL of stirred solutions. New methods were devised for the selective extraction of ranitidine from urine and serum samples by using reversed-phase octadecylsilane-bonded silica. Very sensitive potentiometric methods are proposed for the determination of ranitidine in urine and serum samples. The methods have been applied successfully for the determination of ranitidine in urine in the range 2.5×10^{-5}–5×10^{-4} M, and in serum in the range 1×10^{-6}–1.5×10^{-5} M, in a preliminary pharmacokinetic experiment.

Experimental Section

Apparatus—The electrodes were used with a double-junction calomel electrode (Radiometer) as the reference. The outer chamber of the reference electrode was filled with a 100-g/L $NaNO_3$ solution. Potential readings were obtained with a Corning Research pH/mV meter (model 12) and recorded simultaneously on a strip-chart recorder. The pH measurements were carried out with a combination glass electrode and a Metrohm pH meter (model E350B). All measurements were carried out at room temperature (22 ± 2 °C) with constant magnetic stirring.

Chemicals—All chemicals used were of reagent grade. All solutions were prepared in distilled deionized water.

Stock solutions of cimetidine hydrochloride and ranitidine hydrochloride (0.100 M) were prepared in water. The pure substances used were of the highest quality available and were gifts from Glaxo Laboratories, Athens, Greece. Pharmaceutical preparations were obtained from local drugstores. Sodium tetra(*m*-chlorophenyl)borate was a gift from Dr. C. Moore and it can be synthesized as described by Jarzembowski et al.[10] It is also commercially available from Fluka. 2-Nitrophenyl octyl ether (NPOE), *p*-nitrocumene (4-isopropylnitrobenzene), and polyvinyl chloride (high molecular weight for ion-selective electrodes) were obtained from Fluka. Sep-Pak C_{18} cartridges were purchased from Waters Associates, Inc. (Milford, MA).

Liquid Ion-Exchangers—Cimetidine and ranitidine were precipitated by mixing 0.5 mL of a 0.1 M cimetidine hydrochloride or

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ranitidine hydrochloride solution with 5 mL of a 0.01 M sodium tetrakis(*m*-chlorophenyl)borate solution. The precipitate was extracted with 5 mL of *p*-nitrocumene and the organic phase was washed three times with water. The organic solution was dried by adding 0.5 g of Na_2SO_4 which was subsequently removed by centrifugation. The liquid ion-exchangers were $\sim 10^{-2}$ M in $(CimH^+)(TCPB^-)$ or $(RanH^+)(TCPB^-)$.

The ion-exchanger for the PVC-matrix electrodes was prepared exactly as described above with NPOE as the organic solvent instead of *p*-nitrocumene. For the PVC membrane, 0.085 g of PVC was dissolved in 3 mL of tetrahydrofuran (THF). In this solution, 0.25 mL of the liquid ion-exchanger in NPOE was added, and the mixture was kept in a well-capped glass vial until use.

Electrode Construction—*Liquid-Membrane Electrodes*—An Orion liquid-membrane electrode body (model 92) was used as the electrode assembly with a Millipore LCWPO 1300 PTFE membrane; the PTFE membranes were cut to the appropriate size, and a stack of four was used to avoid any leakage of the liquid ion-exchanger. All the internal aqueous reference solutions were 0.010 M in cimetidine or ranitidine and 0.10 M in NaCl, and they were saturated with AgCl. The operating life of the electrodes was ~2 months.

Polyvinyl Chloride (PVC)-Matrix Electrodes—A new procedure for the preparation of dip-type microelectrodes was developed as follows. An autoanalyzer plastic tube (e.g., the color code purple–black, i.d. 0.090 inches, was generally used in this study) was cut to a length of ~2 cm (Figure 1). A metal wire was inserted into the tube and positioned ~1 mm below the tube ending. The tube with the wire was set in an upright position, and one drop of the mixture of the liquid ion-exchanger in NPOE and PVC in THF was added. After the THF was evaporated (~1 h), another drop was added. The procedure was repeated until the gap at the top of the tube was filled with PVC (~6–10 drops are required). For PVC tubing of narrower diameter (e.g., 0.035-inch i.d.), the internal supporting metal wire was not required. The drops of the ion-exchanger were placed directly at the top of one end of the tube. The constructed ion-selective electrode modules were either left overnight to dry and then stored in a well-capped glass vial or used to construct the ion-selective electrode as follows. The metal wire was removed, and the module was filled with internal reference solution and fitted to the end of a glass tube which surrounds an Ag/AgCl wire as shown in Figure 1. The PVC-matrix electrodes were ready to use just after preparation. When stored in 10^{-2} M cimetidine or ranitidine solutions, their operating life was ~1 week.

Construction of the Measuring Cells—A measuring cell with a capacity of ~1.5 mL was constructed by embedding the bottom part of a round plastic tube in Epofix resin, a casting plastic obtained from H. S. Struers, Scientific Instruments, Copenhagen. The dimensions of the cell are shown in Figure 1. This cell was used for the determination of ranitidine in urine. A smaller cell, with maximum capacity of ~300 μL, was constructed by embedding the flat bottom of a round glass tube in Epofix resin (Figure 1). This cell was used for the determination of ranitidine in serum.

Preparation of Calibration Graphs—A 20.00-mL volume of water or other appropriate solution was pipetted into a 50-mL beaker, the electrodes were immersed in it, and, after the potential had stabilized, various increments of a 0.0100 or 0.100 M solution of cimetidine hydrochloride or ranitidine hydrochloride were added. The electromotive force (emf) readings were recorded after stabilization following each addition, and the potential (E) versus log [cation] plot was constructed. The slope of the electrode response was found by regression analysis of the linear part of the graph.

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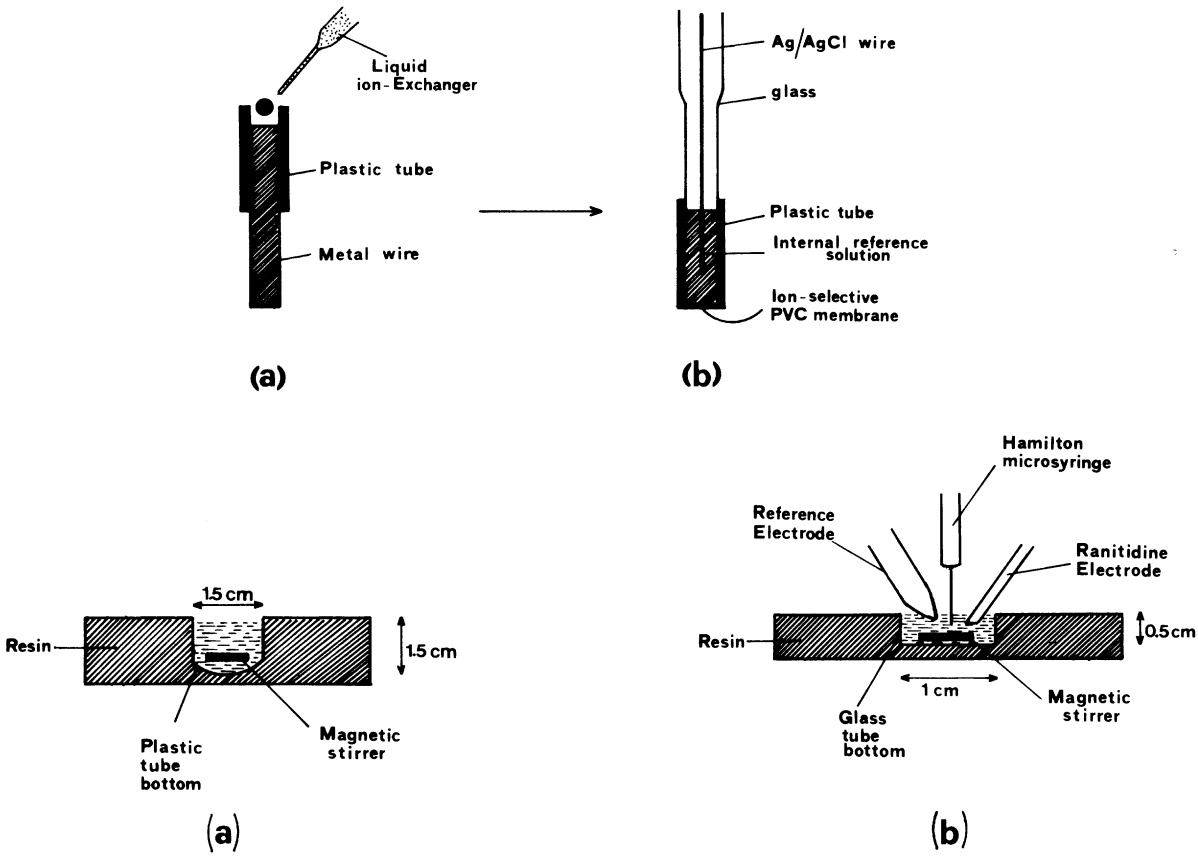

Figure 1—*Upper panel:* (a) *preparation of an ion-selective module;* (b) *fitting the module to the end of a Ag/AgCl wire. Lower panel: measuring cells with total capacity of ~1.5 mL for urine analysis* (a) *and ~300 μL for serum analysis* (b).

stabilized, various increments of a 0.0100 or 0.0100 M solution of cimetidine hydrochloride or ranitidine hydrochloride were added. The electromotive force (emf) readings were recorded after stabilization following each addition, and the potential (E) versus log [cation] plot was constructed. The slope of the electrode response was found by regression analysis of the linear part of the graph.

Effect of pH—A 25.00-mL aliquot of a solution consisting of 3×10^{-3} or 3×10^{-4} M in cimetidine or ranitidine, respectively, 10^{-2} M in HCl, and 10^{-3} M in H_3PO_4, was pipetted into a 50-mL beaker. The electrodes (ISE, reference, and a combination glass electrode) were immersed in the solution and, after the potential had stabilized, the emf and pH readings were recorded. The pH was altered by adding small volumes of a 2 M NaOH solution. The emf and pH readings were recorded after stabilization following each addition. The E versus pH plot was constructed for each concentration.

Direct Potentiometric Assay of Pharmaceutical Preparations (Standard Addition Method)—*Cimetidine for Injection*—A 2.00-mL aliquot of the commercial product was diluted with 0.1 M acetate buffer (pH 5.2) to a final volume of 500 mL; 20.00 mL of the resulting solution (V_x) was used for analysis. A first potential reading was recorded for this solution. Subsequently, a second potential reading was obtained after the addition of a small volume (V_s) of concentrated standard drug solution of concentration C_s. The initial concentration, C_x, of the sample was calculated from[10]

$$C_x = C_s V_s / [10^{\Delta E/S}(V_x + V_s) - V_x] \quad (1)$$

where ΔE is the change in potential and S is the slope of the electrode response.

Cimetidine Tablets—At least five tablets were made into a powder. An appropriate amount of the powder was weighed and dissolved in 500 mL of a 0.1 M HCl solution by stirring for 1 h. The amount weighed was selected so that the final solution was $\sim 3 \times 10^{-4}$–3×10^{-3} M in cimetidine. Fifteen milliliters of the cimetidine unknown solution was mixed with 3.00 mL of a 1 M acetate buffer (pH 5.2), and the resulting solution was analyzed as described above by the standard addition method.

Ranitidine Tablets—The procedure was the same as described for the cimetidine tablets, but the powder was dissolved in 0.07 M phosphate buffer (pH 6.0). A 20.00-mL aliquot of the resulting solution was analyzed as described above by the standard addition method.

Determination of Ranitidine in Urine—A 20.00-mL aliquot of urine was pipetted into a 100-mL beaker and mixed with 2.00 mL of a 1 M acetate buffer (pH 4.6). The Sep-Pak cartridge was mounted in a 20-mL syringe barrel and was prepared for use as follows. The cartridge was washed with 5 mL of methanol followed by 10 mL of water. The 22.00-mL sample was applied to the Sep-Pak and passed through slowly by using the syringe plunger (flow rate ~1 drop/s). The Sep-Pak was washed with 4 mL of a 0.1 M acetate buffer (pH 4.6) followed by 1 mL of water. Ranitidine was eluted with 5.00 mL of a 20% (v/v) acetonitrile solution in a 5×10^{-3} M phosphate buffer (pH 6.5). For the measurement, 1.00 mL of the eluant was pipetted into the measurement cell shown in Figure 2A, the electrodes were immersed (reference and PVC-matrix ranitidine electrode), and stirring was started. The potential reading was obtained for this solution. Subsequently, a second potential reading was obtained after the addition of a small volume (10–50 μL) of a concentrated standard ranitidine solution (10^{-2} or 10^{-1} M). The initial concentration was calculated as described above.

Determination of Ranitidine in Serum—A 5.00-mL aliquot of serum was mixed with 0.5 mL of a 1 M acetate buffer (pH 4.6) in a glass tube. The Sep-Pak cartridge was prepared as described above, and the 5.5-mL sample was applied. The Sep-Pak was washed exactly as described above and ranitidine was eluted with 3.00 mL of methanol. The eluant was evaporated to dryness under a stream of nitrogen at 60 °C. The residue was redissolved in 0.6 mL of 5×10^{-3}M acetate buffer (pH 4.6) by thorough vortexing. For the measurement, 250 μL of the eluant was pipetted into the microcell shown in Figure 1. For the standard addition technique, 10–25 μL of a 10^{-4} or 10^{-3} M standard ranitidine solution was added.

Results and Discussion

Electrode Construction—Two types of ion-selective electrode configurations were used for these studies: the conventional Orion series 92 liquid-membrane electrodes and new dip-type PVC-matrix electrodes. The PVC-matrix electrodes were constructed in a manner similar to that described by Spaciani and Fowler[11] and by Meyerhoff and Kovach[12] for flow-through electrodes. In all configurations, the ion-selective membrane becomes part of a PVC tube. Our technique

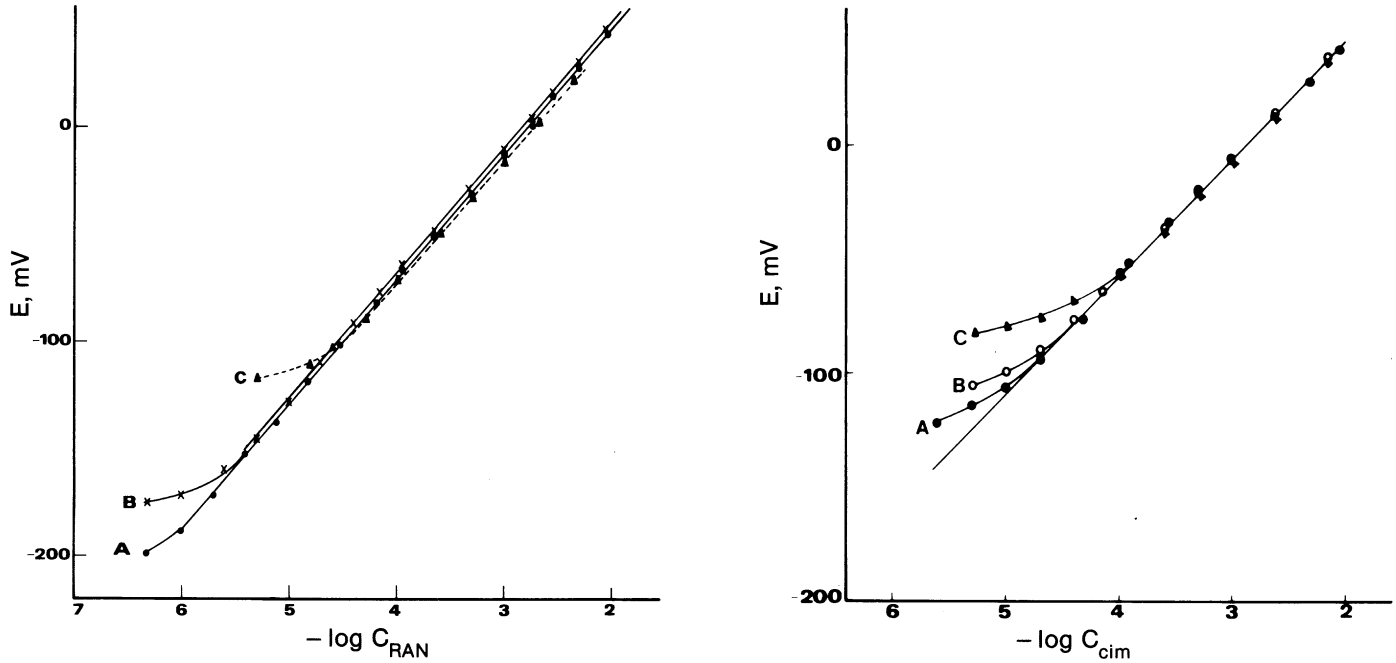

Figure 2—*Left panel: calibration curves for the ranitidine electrode in water* (A), 5×10^{-3} *M acetate buffer of pH 4.6* (B), *and 20% acetonitrile in* 5×10^{-3} *M phosphate buffer of pH 6.50* (C). *Regression analysis:* (A) *slope* = 58.40 ± 0.18, $E°$ = 162.2 ± 0.6; (B) *slope* = 58.57 ± 0.17, $E°$ = 164.9 ± 0.6; (C) *slope* = 57.26 ± 0.31, $E°$ = 156.2 ± 1.0. *Right panel: calibration curves for the cimetidine electrode in water* (A), 5×10^{-3} *M acetate buffer of pH 4.6* (B), *and at pH 2.1 HCl* (C). *Regression analysis:* (A) *slope* = 50.7 ± 0.3, $E°$ = 145.7 ± 0.9; (B) *slope* = 50.8 ± 0.8, $E°$ = 147.2 ± 2.5; (C) *slope* = 50.7 ± 0.7, $E°$ = 144.5 ± 2.1.

for preparing dip-type PVC-matrix electrodes has the following advantages over the classical technique described by Craggs et al.[13] First, there is no need to attach the PVC membrane at the end of the tubing, which is time-consuming and may present the problem of leakage. Second, microelectrodes can be constructed easily (we were able to construct modules with i.d. of 0.035 inches with the same technique). Third, many modules can be constructed simultaneously and stored for future use, and the modules can be replaced in a few minutes. Finally, the liquid-membrane and PVC-matrix electrodes have similar response characteristics.

Calibration Curves—Typical calibration curves of the electrodes under different experimental conditions are shown in Figure 2. For the ranitidine electrode, the response in water was linear down to a concentration of 10^{-6} M. Most liquid-membrane ISE for anions and cations described in the literature have lowest linear response ranges down to 10^{-5} M. In 5×10^{-3} M acetate buffer (pH 4.6), the linear response was evident down to a concentration of 2.5×10^{-6} M due to a slight interference by Na^+ cations. The limit of linear response in 20% acetonitrile solution in 5×10^{-3} M phosphate buffer of pH 6.50 (measurements in urine are carried out in this medium) was 5×10^{-5} M. The slope of the calibration curve was (in mV per decade) 58.4 for water, 58.6 for acetate buffer, and 57.3 for 20% acetonitrile.

The limit of linear response of the cimetidine electrode was 2×10^{-5} M in water and 4×10^{-5} M in 5×10^{-3} M acetate buffer of pH 4.6. The slopes of the calibration curves were 50.7 mV for water and 50.8 mV for acetate buffer.

Effect of pH—To check the pH dependence of the potential of the ranitidine and cimetidine electrodes, potential–pH curves at two concentrations were constructed. The plots (Figure 3) show that the potential is practically unaffected by changes in pH over the ranges 3–7 for ranitidine and 3–5.5 for cimetidine. At higher pH values, there is a gradual decrease in potential because of the gradual increase in the concentration of the unprotonated drug. For ranitidine, below pH 3 and especially at higher concentrations, there is a gradual decrease in potential which is probably due to the response of the electrode of the biprotonated cation of the drug. A similar phenomenon has been observed with the nicotine-selective electrode.[14]

The potential–pH plots can be used to calculate the dissociation constant, K_a, of the cationic acid[15] which, for ranitidine, is equal to

$$K_a = \frac{[\text{Ran}][\text{H}^+]}{[\text{Ran H}^+]} \tag{2}$$

The plot of [Ran]/[Ran H^+] versus 1/[H^+] is a straight line which passes through the origin and has a slope of K_a. The ratio [Ran]/[Ran H^+] can be calculated at each pH value from

$$[\text{Ran}]/[\text{Ran H}^+] = \text{antilog}(\Delta E/S) - 1 \tag{3}$$

where ΔE is the potential difference, $E_1 - E_2$, between the potential E_1 at the plateau of the E versus pH plot and the potential E_2 which corresponds to a certain pH value. The results for the calculation of K_a for ranitidine are shown in Figure 4.

The pK_a of ranitidine was found to be 8.37. A value of 8.2 has been reported in the literature.[16] The pK_a of cimetidine was found with the same technique to be 6.91 ([Cim] = 3×10^{-4} M) or 7.05 ([Cim] = 3×10^{-3}M); the mean was 6.98. A value of 6.80 has been reported in the literature.[4]

Selectivity—The interference of various cations was studied by the mixed-solution method. Negligible interference was found by the common cations Na^+, Ca^{2+}, and Mg^{2+}. Weak interference was detected for the K^+ and NH_4^+ cations. The potentiometric selectivity coefficients, K^{pot}, were 7×10^{-3} and 5×10^{-3} (cimetidine electrode), and 5×10^{-4} and 7×10^{-4} (ranitidine electrode) for K^+ and NH_4^+, respectively. The interference from K^+ becomes a problem when measuring in the microcell (Figure 1) with a single-junction, saturated calomel electrode as reference. For this reason, all measurements were carried out with a double-junction refer-

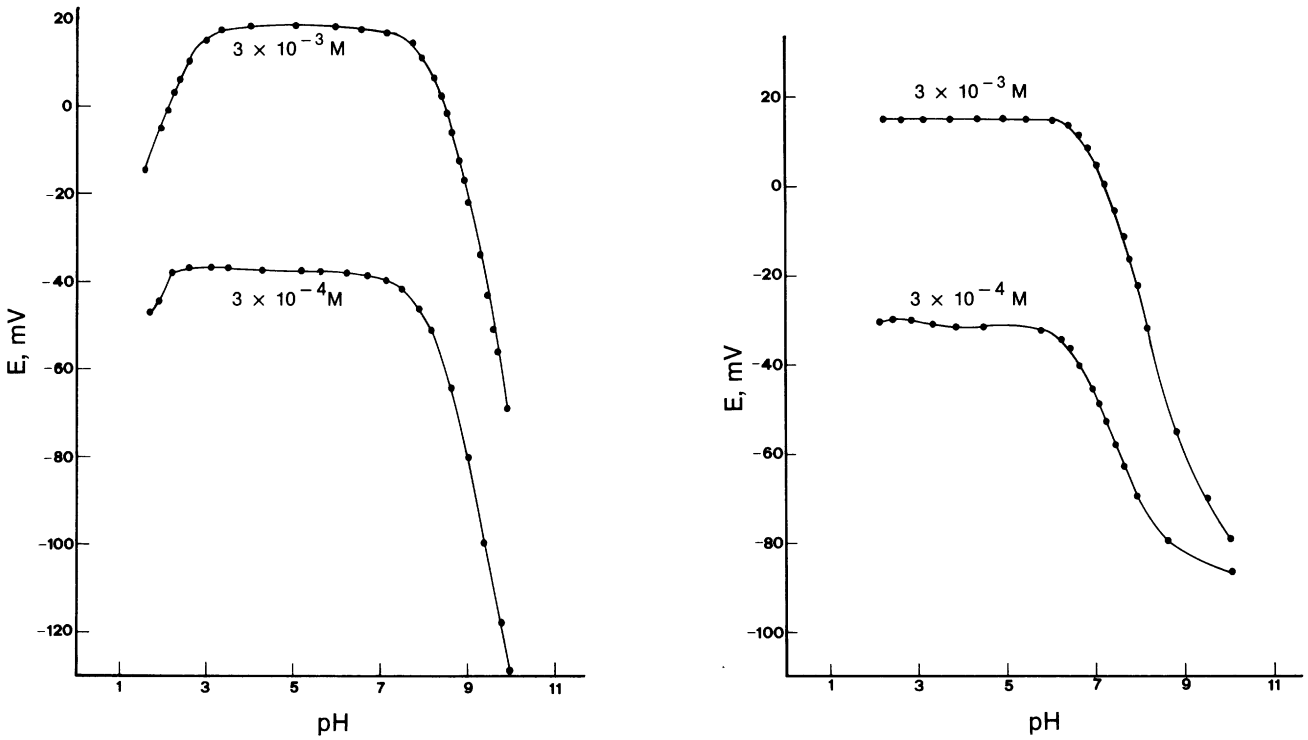

Figure 3—*Left panel: effect of pH on the potential of the ranitidine-sensitive electrode. Right panel: effect of pH on the potential of the cimetidine-sensitive electrode.*

ence electrode which contained a 10% $NaNO_3$ solution as the salt bridge, instead of saturated KCl. Organic cations which form insoluble ion pairs with $TCPB^-$ may interfere with the response of the two electrodes. For applications different from those reported, targeted interference studies may be useful.

Assay of Cimetidine and Ranitidine in Pharmaceutical Preparations—Ion-selective electrodes have already been applied successfully to the assay of drugs in pharmaceutical preparations.[17–19] In Table I, results are presented for the assay of cimetidine and ranitidine in injection solutions and tablets. It can be seen that the electrodes are suitable for application in pharmaceutical analysis.

Extraction of Cimetidine and Ranitidine with Sep-Pak Cartridges—Solid-phase extraction techniques are an effective tool for the separation of drugs and other substances from aqueous and biological fluids.[20–22] They are rapid, reproducible, and give recoveries which are comparable to those expected from classical liquid–liquid extraction. We used the C_{18} Sep-Pak reversed-phase cartridge to extract cimetidine and ranitidine from aqueous solutions. By using 3 mL of methanol as eluant, we obtained a recovery of 102 ± 5% for additions of 2–20 μmol of the drug in a 20-mL sample size. We conclude that the solid-state technique is a simple and effective way of extracting the drugs from aqueous solutions. Based on this observation, we devised methods for the determination of ranitidine in urine and serum.

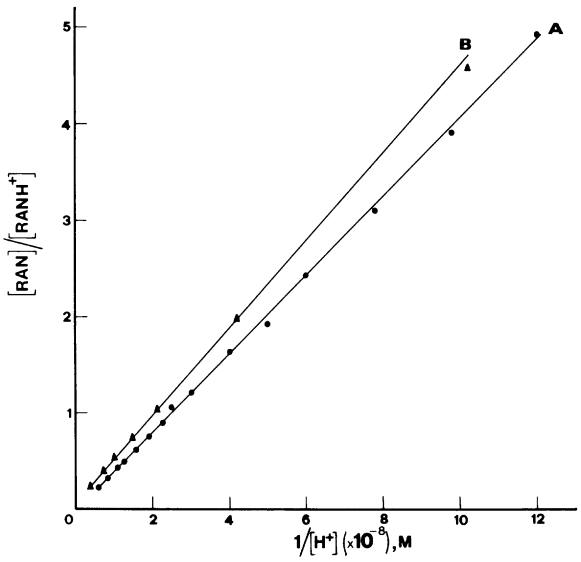

Figure 4—*Calculation of the K_a of ranitidine at $C_{Ran} = 3 \times 10^{-3}$ M* (A) *or 3×10^{-4} M* (B). *Regression analysis:* (A) *slope = $4.06 \times 10^{-9} \pm 3.3 \times 10^{-11}$, y intercept = $-2.47 \times 10^{-2} \pm 1.72 \times 10^{-2}$, r = 0.9996;* (B) *slope = $4.43 \times 10^{-9} \pm 4.62 \times 10^{-11}$, y intercept = $8.18 \times 10^{-2} \pm 1.99 \times 10^{-2}$, r = 0.9997. The pK_a values are 8.39 for* (A) *and 8.35 for* (B).

Table I—Assay of Cimetidine and Ranitidine in Pharmaceutical Preparations by Direct Potentiometry (Standard Addition Method)

Compound	Content, mg/tablet or mg/mL				
	Nominal	Found	CV, % (n = 5)	Reference Method[a]	Recovery, %[b]
Tamper (cimetidine injection)	100	100	3.1	—	99 ± 3
Tagamet (cimetidine tablet)	200	203	2.3	194	102 ± 4
Baroxal (ranitidine tablet)	150	157	1.6	155	106 ± 2
Zantac (ranitidine tablet)	150	160	2.3	153	105 ± 1

[a] High-performance liquid chromatography (HPLC). [b] Addition of a known amount of the drug in the unknown solution after the dissolution of the tablet in the appropriate medium as described in the *Experimental Section.*

Determination of Ranitidine in Urine—Most of the procedures for the determination of ranitidine and its metabolites in urine and serum are based on high-performance liquid chromatography[16,23–25] and radioimmunoassay.[26] In all HPLC procedures, ranitidine and/or its metabolites are extracted from the biological fluid with various organic solvents.

It is now established in the literature that in urine, ranitidine is the main species present and its metabolites represent only a minor fraction.[16] For this reason, no effort was made to study the possible interference of ranitidine metabolites in the urine analysis.

After the application of the urine sample to the Sep-Pak, ranitidine and other unidentified substances are retained on the cartridge. Our purpose was to elute ranitidine selectively, leaving the interfering substances on the Sep-Pak. For this reason, we tried the eluants methanol, acetone, ether, and acetonitrile, and mixtures of the organic solvents with water (where possible) or buffers of various pH values. The best results were obtained by using a 20% (v/v) mixture of acetonitrile with 0.1 M acetate buffer of pH 4.6. The optimum elution volume was 5.00 mL. With this eluant, the blank determination of normal urine samples (analysis of urine of individuals not receiving the drug) is minimal and can be neglected without serious error if urine of individuals who take the drug is analyzed (see results of the pharmacokinetic experiment below). Another advantage of using this eluant is that measurements can be carried out directly, without the need of evaporating and reconstituting the eluant in a suitable solvent. As we have already shown (Figure 2), the ranitidine electrode response is satisfactory in this medium.

We assessed our method for urine by carrying out recovery experiments (Table II). For urine containing 0.5–10 μmol of ranitidine per 20 mL of sample, the mean recovery was 102.7%.

Determination of Ranitidine in Serum—The therapeutic concentration of ranitidine in serum is very low ($<2 \times 10^{-6}$ M). For a successful potentiometric determination, ranitidine has to be extracted selectively and preconcentrated. For this reason, we have eluted ranitidine from the Sep-Pak with methanol. The eluant was then evaporated, and the residue was redissolved in 0.6 mL of 0.1 M acetate buffer of pH 4.6. With this procedure, the sample is preconcentrated 8.33-fold. In the reconstituting medium, the electrode response is linear down to 2.5×10^{-6} M (Figure 2) so that the minimum theoretical concentration which can be detected is 3×10^{-7} M. For accurate results, the blank value of the serum must be

Table II—Recovery of Ranitidine from Urine after Extraction with Sep-Pak

Amount of Ranitidine per 20-mL Sample, μmol		Percent Recovery
Added	Found ± SD (n = 3)	
0	0.61 ± 0.04[a]	—
0.50	0.50 ± 0.09[b]	100
1.00	1.11 ± 0.10[b]	111
5.0	4.81 ± 0.16[b]	96
10.0	10.3 ± 0.3[b]	103
20.0	16.2 ± 0.4[b]	81[c]

[a] This is the blank reading of a urine sample. [b] After blank subtraction. [c] Ranitidine is not eluted quantitatively at this concentration level.

determined and subtracted from the measurements. The final eluant (0.60 mL) is sufficient for duplicate analysis.

We assessed our method for serum by carrying out recovery experiments (Table III), and the mean recovery was 103.2%.

Pharmacokinetic Experiment—A 36-year-old female (57 kg) collected a 1-h timed urine sample for the determination of the blank. At time zero, blood was withdrawn for the determination of blank and two tablets of ranitidine (Baroxal, 2 × 150 mg) were taken. Blood samples were withdrawn every 1 h (for 5 consecutive hours). Urine samples were collected every 1 h for 8 h. Ranitidine was determined in serum and urine, and the results are presented in Table IV and Figure 5.

Detailed pharmacokinetic data on ranitidine have been published.[5,27] We found peak values in serum somewhat later than those described in the literature (2–3 h)[24] because of the higher dose administered. The ranitidine renal clearance, RRC, calculated by the formula RRC, mL/min = (urine flow, mL/min)(urinary ranitidine, M)/(serum ranitidine, M), where serum ranitidine is the mean concentration of the drug during the interval period, was calculated for the interval periods 2–3, 3–4, and 4–5 h. The results were 411, 342, and 339 mL/min, respectively, with a mean of 364 mL/min or 6.38 mL/min·kg. This value is close to that reported in the literature (7.2 mL/min·kg for an intravenous dose and 5.6 mL/min·kg for an oral dose).[5] The total excretion of ranitidine at 8 h (190 μmol or 22% of the oral dose) is in close agreement with values given in the literature.[5,16]

In conclusion, we have described ion-selective electrodes for the H_2-receptor antagonists cimetidine and ranitidine. We have studied their response characteristics and applied them for the determination of the pK_a of the protonated bases and the assay of the drugs in pharmaceutical preparations. For the more difficult assays of ranitidine in urine, and especially in serum, we had to combine the excellent response characteristics of the ranitidine electrode, the microelectrode configuration, and the effective and selective solid-state extraction. We hope that further development of new and of high quality potentiometric sensors will lead to their more frequent application to the analysis of drugs in biological fluids, an important part of biochemical analysis.

Table III—Recovery of Ranitidine from Serum after Extraction with Sep-Pak

Amount of Ranitidine per 5-mL Sample, nmol		Percent Recovery
Added	Found	
0	7.82[a]	—
12.5	11.4[b]	91
25.0	26.1[b]	104
50	57.7[b]	115
75	77.3[b]	103

[a] This is the blank reading of a serum sample. [b] Average of two measurements; after blank subtraction.

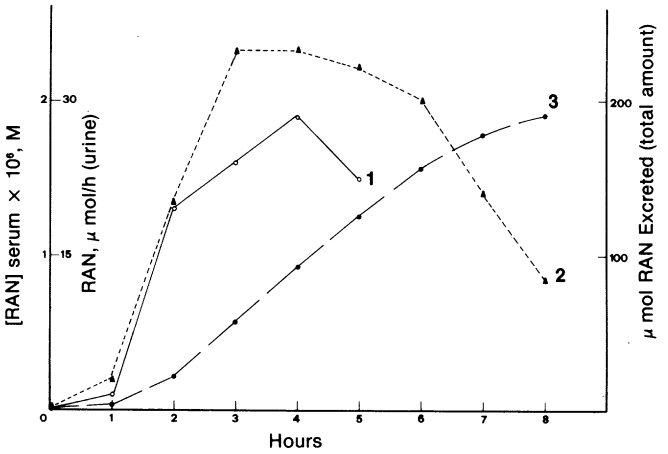

Figure 5—*Results of the pharmacokinetic experiment. Key:* (1) *concentration of ranitidine in serum versus time after a 300-mg oral dose;* (2) *rate of excretion of ranitidine in urine (μmol per h) versus time;* (3) *cumulative excretion of ranitidine (μmol) versus time.*

References and Notes

1. Brimblecombe, R. W.; Duncan, W. A. M.; Duran, G. J.; Emmett, J. C.; Ganellin, C. R.; Parsons, M. E. *J. Int. Med. Res.* **1975**, *3*, 86–92.
2. Winship, D. H. *Gastroenterology* **1978**, *74*, 402–406.
3. Hirschowitz, B. I. *Ann. Rev. Pharmacol. Toxicol.* **1979**, *19*, 203–244.
4. *Drugs of the Future*; Prous, J. R., Ed., 1976; Vol. 1, pp 13–18.
5. *Drugs of the Future*; Prous, J. R., Ed., 1981; Vol. VI, pp 583–586, and 1982; Vol. VII, pp 697–700.
6. Domschke, W.; Lux, G.; Domschke, S. *Gastroenterology* **1980**, *79*, 1267–1271.
7. Daly, M. J.; Humphray, J. M.; Stables, R. *Gut* **1980**, *21*, 408–412.
8. Walt, R. P.; Male, P. J.; Raulings, J.; Hunt, R. H.; Milton-Thompson, G. J.; Misiewisz, J. J. *Gut* **1981**, *22*, 49–54.
9. Hirschowitz, B. I.; Danillwitz, M.; Molina, E. *Gastroenterology* **1982**, *82*, 1314–1326.
10. Jarzembowski, F.; Cassareto, F.; Posvic, H.; Moore, C. E. *Anal. Chim. Acta* **1974**, *73*, 409–412.
11. Spaciani, F. F.; Fowler, J. E. Br. Patent 2 065 629, Jan. 23, 1980, Appl. 925, 518, Jul. 17, 1978; *Chem. Abstr.* **1980**, *92*, 226093U.

Table IV—Results of the Pharmacokinetic Experiment[a]

Time, h	Serum Concentration of Ranitidine, M × 10^{-6}	Urine Volume, mL	Urine Concentration of Ranitidine, M	Rate of Ranitidine Excretion, μmol/h	Total Excretion, μmol
1	0.1[b]	31	1.29×10^{-4}	2.87	2.87
2	1.28	141	1.48×10^{-4}	19.8	22.7
3	1.62	308	1.16×10^{-4}	34.6	57
4	1.91	209	1.73×10^{-4}	35.1	92
5	1.52	70	5.0×10^{-4}	33.9	126
6	—	52	5.9×10^{-4}	29.8	156
7	—	125	1.78×10^{-4}	21.1	177
8	—	422	3.25×10^{-5}	12.6	190

[a] The serum blank reading at time zero was 1.40×10^{-6} M; this reading was subtracted from all subsequent values of the second row of the table; the urine blank reading for a 1-h timed urine collection was 3.15×10^{-5} M (urine volume 36 mL), corresponding to a blank excretion rate of 1.13 μmol/h; this value was subtracted from all subsequent values on the fifth row of the table. [b] This value is only an approximation.

12. Meyerhoff, M. E.; Kovach, P. M. *J. Chem. Educ.* **1983**, *60*, 766–768.
13. Craggs, A.; Moody, G. J.; Thomas, J. D. R. *J. Chem. Educ.* **1974**, *51*, 541–544.
14. Efstathiou, C. E.; Diamandis, E. P.; Hadjiioannou, T. P. *Anal. Chim. Acta* **1981**, *127*, 173–180.
15. Christopoulos, T. K.; Mitsana-Papazoglou, A.; Diamandis, E. P. *Analyst* **1985**, *110*, 1497–1500.
16. Carey, P. F.; Martin, L. E.; Owen, P. E. *J. Chromatogr.* **1981**, *225*, 161–168.
17. Cosofret, V. V. *Membrane Electrodes in Drug Substances Analysis*; Pergamon: Oxford, NY, 1982.
18. Cosofret, V. V. *Ion Select. Electrode Rev.* **1980**, *2*, 159–218.
19. Cosofret, V. V.; Buck, R. P. *Ion Select. Electrode Rev.* **1984**, *6*, 59–121.
20. Stewart, J. T.; Reeves, T. S.; Honigberg, I. L. *Anal. Lett.* **1984**, *17(B16)*, 1811–1826.
21. Zief, M.; Crane, L. J.; Horvath, J. *Amer. Lab.* **1982**, *14*, 102–112.
22. Shackleton, C. H. L.; Whitney, J. O. *Clin. Chim. Acta* **1980**, *107*, 231–243.
23. Misciewicz, J. J.; Sewing, K. F., Eds.; *Proceedings First International Symposium on Ranitidine; Scand. J. Gastroenterol.* **1981**, *16(Suppl. 69)*, 1–130.
24. Vandenberghe, H. M.; MacLeod, S. M.; Mahon, W. A.; Lebert, P. A.; Soldin, S. J. *Therap. Drug Monit.* **1980**, *2*, 379–384.
25. Mihaly, G. W.; Drummer, O. H.; Marshall, A.; Smallwood, R. A.; Louis, W. J. *J. Pharm. Sci.* **1980**, *69*, 1155–1157.
26. Jenner, W. N.; Martin, L. E.; Willoughby, B. A.; Fellows, I. *Life Sci.* **1981**, *28*, 1323–1329.
27. Miller, R. *J. Pharm. Sci.* **1984**, *73*, 1376–1379.