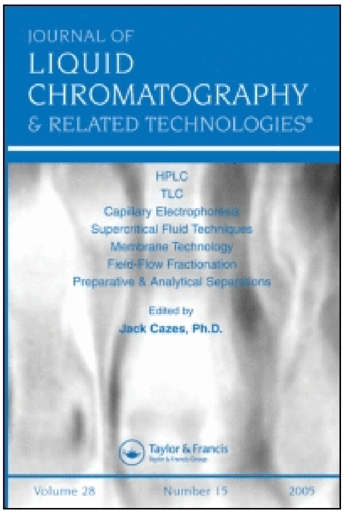

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LC-LC, Low Cost Liquid Chromatography. II. In-Line Flow-Preparation of Eluents of Fixed pHs

Vern Berry[a][b]; Victor Pretorius[c]
[a] SepCon Separations Consultants, Boston, Massachusetts [b] Salem State College, Chemistry, Salem, Massachusetts [c] Institute for Chromatography University of Pretoria, Pretoria, South Africa

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LC-LC, LOW COST LIQUID CHROMATOGRAPHY. II. IN-LINE FLOW-PREPARATION OF ELUENTS OF FIXED pHS

VERN BERRY[1] AND VICTOR PRETORIUS[2]
[1]*SepCon Separations Consultants*
326 Reservoir Road
Boston, Massachusetts 02167 and
Salem State College, Chemistry
Salem, Massachusetts 01970
[2]*Institute for Chromatography*
University of Pretoria
0002 Pretoria, South Africa

ABSTRACT

Conventional preparation of the weak aqueous eluent for reversed phase liquid chromatography by weighing, pipeting, and/or titrating materials can be time consuming, poorly reproducible, and require storage of many containers of final buffer. Stored buffers can become contaminated by vapors, container-leached materials, or microbes.

This paper discribes a novel approach for preparing eluents in which LC-grade water is drawn through lengths of silicone tubing in a small "equilibration-chamber" containing gases, vapors of liquids, or salt-saturated solutions. Besides offering a broad range of pHs from 2 to 13, and saving the time and materials for eluent preparation, other advantages are described, including the possibility of preparing ultra-pure buffered eluents.

INTRODUCTION

Conventional preparation of the weak aqueous eluents for reversed phase liquid chromatography (RPLC) by weighing, pipeting, and/or titrating materials can be time consuming, poorly reproducible, and require storage of many large containers of final buffer. Sometimes stored eluent can become contaminated by laboratory vapors, microbial growth (bacteria, algae, or mold [REF 1], or components can be leached from the containers. For example, Bonfichi showed that even LC-grade acetonitrile in glass containers with Teflon cap liners can produce ghost peaks in RPLC if the acetonitrile is allowed to contact the liner by inverting the bottle [REF 2].

Our earlier work with batch eluent preparation showed the utility of using gases to adjust pH (with carbon dioxide) [REF 3]; or raise the aqueous eluent UV baseline to match that of acetonitrile (with nitrous oxide) [REF 3] for sensitive 190 nm UV detection with full gradients. Flow-through semipermeable membrane "degassers", with a vacuum on one side of the flat fluoropolymer membrane, have been available for over 12 years, first from DuPont and later from Erma [REF 4].

In 1988, ES Industries began marketing a flow-through "deoxygenator" so that oxygen in the eluent could be removed to give a more stable baseline with reductive electrochemical detectors [REF 5]. The ES Industries deoxygenator shows (1) complete disappearance of the signal from oxygen if oxygen is injected as part of the sample, and (2) shows decreases in the baseline noise level by at least 10-fold, by removing oxygen in the eluents. This deoxygenator was used to improve detection for many sample types, such as 40 metal ions (e.g. Ag+, Fe++, Fe+++, and Cu++), 26 organic functional groups (e.g. quinones, halides, and aldehydes), and 7 groups of organometallics (e.g. porphyrins, organotins, and phthalocyanines).

The ES Industries deoxygenator consists of a "knitted tube" of silicone rubber tubing (0.7 feet long knitted, 10 feet long in actual length). The knitted tube is hung from a the top plate in a 300 ml clear plastic container so the outside of the tube can be flushed with an inert gas such as nitrogen, argon, or helium. The original device with 1/16 inch o.d. (0.5 mm i.d.) tubing is intended to be inserted between the column outlet and the reductive electrochemical detector. A later version with larger diameter tubing was intended to be inserted before the pump inlet and the pump, to remove oxygen from the inlet flow stream. The knitted reactor in the ES Industries deoxygenator provides both a time delay and increased surface area so oxygen in the eluent stream can diffuse out. Knitted reactors have the property that a peak shows very little spreading (1) when passed through great lengths of tubing or (2) when passed at various flow rates. [REF 6] The same tube, if not knitted (i.e. straight), shows great peak spreading that increases with both length and flow rate, as described by the Taylor equation [REF 6].

In this paper, we show that it is possible to use a device of silicone tubing like the flow-through deoxygenator, but simpler and not knitted, to add materials to the eluent stream, rather than remove oxygen from the stream.

The novel approach explored here involves pumping LC-grade water through lengths of silicone rubber tubing in a small "equilibration-chamber" containing gas, vapors of liquid, saturated salt solutions, or combinations of these. All approaches require no weighing or volumetric measurements of salts or liquids, a principle advantage to in-line flow-preparation of eluents. Migration of components through the silicone tubing quickly takes the eluent to a fixed "flow-pH", independent of flow rates in the analytical LC range (tested here from 0.5 to 2 ml/min). To change flow-pH, the silicone tubing coil is rinsed in water and placed into another equilibration-chamber containing another gas, vapor, or saturated salt solution.

Besides offering the simple preparation of a wide range of flow-pHs from pH 2 to 13, there are other advantages to in-line flow-preparation of eluents in that the method: is simple; saves time and labor; eliminates weighing or pipeting of components; is reproducible; minimizes storage of large volumes of many different eluents (only 200 ml of saturated salt solutions are stored); and (possibly) permits use of less pure components than might be usable with direct mixing of components with the eluent.

Now that the the in-line flow-preparation of eluent is shown to work, later studies will: establish methods for "drawing" by suction rather than "pushing" by pressure, the water through the silicone tube; determine actual salt concentrations (plus pH); determine if sufficient buffering exists so samples in different pH matrices will give the same retentions and areas; and show chromatographic applications.

EXPERIMENTAL

The apparatus used for this investigation is shown in Figure 1. While initial work was started with the ES Industries deoxygenator, difficulties in keeping the knitted tubing channel open (the initial and final knots sometimes pulled tight closing the channel); the interest in determining if the "knitting" were critical; and the desire to use a thinner-wall silicone tubing, led to our doing all of this work with a simpler system. (The ES system uses a 3,050 mm length of 1.59 mm o.d., and 0.5 mm i.d.).

This work used a 1,000 millimeter length of silcone rubber tubing (1.0 mm o.d. 0.5 mm i.d.)(VEN Products, Ltd., P. O Box 770, Gallo Manor 2052, S. Africa) loosely wound in coils on a 6 mm o.d. glass tube (4 mm i.d.) that was ca. 8 cm long. The loose coiling of this tubing is not expected to have much effect on interrupting laminar flow [REF 6]. For support, the glass tube was inserted into a one-hole stopper that sealed 250 ml Erlenmeyer flasks. The inlet and outlet of the silicone tubing

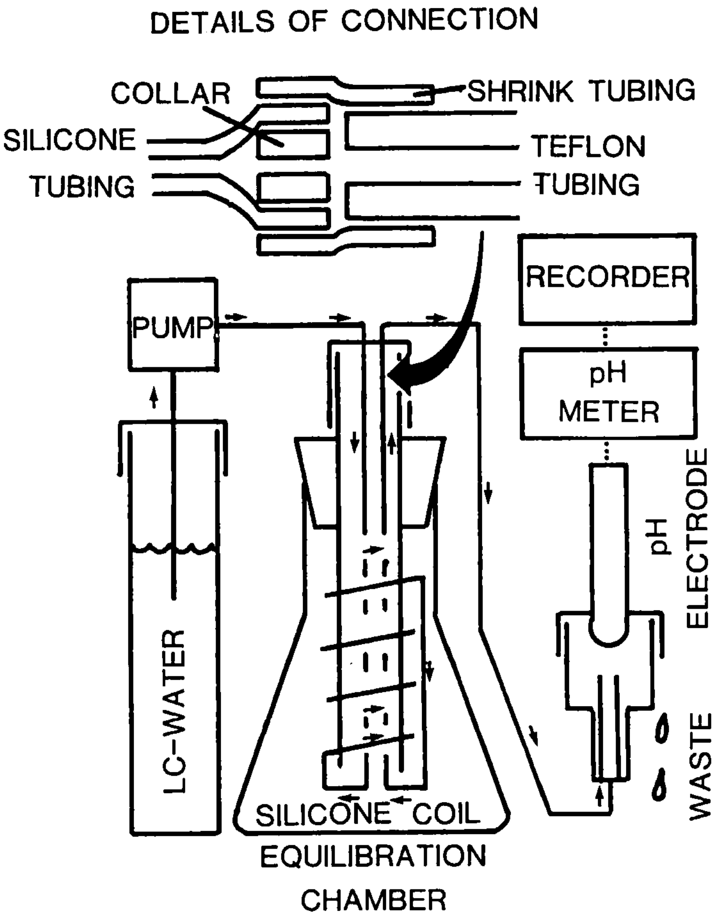

Figure 1. Apparatus for determining the time for equilibrating the pH with the in-line flow-preparation of LC eluents showing (bottom) the equilibration chamber with the coil of silicone rubber tubing and (top) details for the connection of the silicone tubing (over a glass collar) to 1/16 inch o.d. Teflon tubing using a heat-shrink Teflon tubing as a connector. Details in Experimental.

were fed through the middle of the glass tube, and the glass tube capped tightly with aluminum foil to minimize vapor loss, air backflow, etc. Chemicals used in this work were analytical grade (Merck, Darmstadt, Germany).

When saturated salt solutions were used, they were made up in the 250 ml Erlenmeyer flasks, capped when not used, and stirred with a magnet stir bar when used. All saturated salt solutions were made up at least 24 hours before use, and stored in a water trough at ambient temperature to insure that solutions were indeed saturated and at room temperature. (Salts will cool or heat a solution when initally dissolved.) Excess salts were always present, and once made, a saturated salt solution could be used again and again.

When gases were used, such as carbon dioxide or air, the inlet gas line was passed down the center glass tube, and this glass tube tightly capped with aluminum foil. Channels around the aluminum foil acted as the gas vent.

When vapors were used, such as ammonia solution, acetic acid, or diethylamine, the liquid was pipeted into the bottom of the Erlenmeyer flask, and the stopper holding the tubing was re-inserted, with the glass tube for the flow inlet and outlet lines being tightly capped with aluminum foil, so that the chamber became saturated with vapor. The tubing does not contact the solution.

Figure 1 (top) shows details of how the silicone tubing was connected to conventional 1/16 inch o.d. (0.03 inch i.d.) Teflon tubing. The silicone tubing (left) was stretched over a small "glass collar" ca. 4 mm long made from a 1 microliter disposable capillary pipet (ca. 0.1 mm i.d. and 0.6 mm o.d.) (Drummond Scientific, Broomall, PA, USA). The ends of this collar must be fire-polished or sanded so the collar does not cut the silicone tubing. A "connector" of heat shrinkable Teflon tubing (1.0 mm o.d. 0.8 mm i.d., ca. 10 mm long), (Small Parts Incorp., Miami, FL, USA) is then slipped over the silicone and Teflon tubing to be connected and then heat-shrunk into place. This fitting is more pressure resistant, and faster to make than efforts to use silocone cement to make connections (as done by ES Industries).

The 3.8 liter reservoir contained LC-grade Milli-Q water (MilliSep Ltd., Johannesburg, S. Africa) was initially argon-sparged and then tightly capped with aluminum foil to minimize irreproducible levels of atmospheric carbon dioxide. The pump (Millipore #6000A, MilliSep Ltd.) was operated at 0.5 and 2 ml/min, unless otherwise stated.

The combination glass pH electrode (#6.0202-000 Carwyn Instruments, UK) was inserted into tapered Omnifit "universal connector" (#2405, Anatech Ltd., Johannesburg, S. Africa) that just contained the tip of the pH electrode (the large Omnifit cap was left on but the rubber seal removed). Overflow from the eluent-outlet went to waste. The recording pH meter (#501-B, Carwyn Instruments) permitted the flow-pH to be continuously plotted on a chart recorder (#PM8252, Phillips Instruments, Netherlands) set at 2,000 millivolts full scale, so that 1 pH unit gave a 10% full scale movement. The recorder was operated at the slowest speed (2 minutes/centimeter). Periodically, the electrode was transferred to pH 2, 7, and 10 buffers, to calibrate the chart recorder. The apparent pH's of the standards appeared to be linear, and reproducibility of making a measurements was 2-3 percent. Direct measurements of the pHs of saturated salt solutions was made by directly inserting the electrode into the saturated solution and thus these measurements includes a "salt error".

RESULTS AND DISCUSSION

Silicone tubing does not swell in water and the alkane-silicone structure indicates also that silicone rubber is not hydrophilic and does not allow water to readily invade its structure. Thus, ions such as hydroxide, hydronium, ammonium, bicarbonate, carbonate, etc., are not expected to migrate through silicone rubber. However, small non-ionic (hydrophobic) molecular species are expected to dissolve in the structure, such as carbon dioxide and ammonia (NH_3). The experiments below support these hypotheses, and show that some other (more polar) molecular species also migrate through silicone rubber, including HCl, formic acid, acetic acid, and organic amines.

The following discussions of specific results refer to data in Table 1.

Line 1. WATER, as expected, shows a flow-pH drift over a long period of time (5 hours) since it is not buffered. Extensive purging with argon in well-closed container took Millipore Milli-Q LC-grade water to a flow-pH of 6.1, and this is not expected to be reproducible.

Line 2. POTASSIUM CHLORIDE SATURATED SOLUTION produces the equivalent ions as mixing HCl and KOH, a strong acid and strong base, namely potassium and chloride ions, which are not expected to migrate through silicone rubber, nor are they expected to have the components necessary for pH buffering (a weak acid and a salt of a weak acid) [REF 7]. The fact that addition of this salt has no affect on distilled water with flow-pHs ranging from 6.6 to 8.4 reflects that the salt has no important residual pH-active impurities.

Line 3. AMMONIUM ACETATE SATURATED SOLUTION hydrolyzes in water to produce ammonium and acetate ions, as well as ammonium hydroxide molecules (a weak base), and hydrogen acetate molecules (acetic acid, a weak acid). The ammonium hydroxide molecules are in equilibrium with the gas, ammonia molecules. The possiblity of ammonia (NH3) molecules diffusing through the silicone tube

TABLE 1

In-line frow-preparation of eluents of various flow-pHs by means of saturated salt solutions, saturated salt solutions plus gases, and vapors. All flow-pHs were determined at both 0.5 and 2 ml/min, unless otherwise noted. Notes (a), (b) and (c), are the same as in Table 2, bottom.

LINE NUMBER	DESCRIPTION	FINAL FLOW-pH	DIRECT (BULK) pH (a)	MINUTES TO REACH FINAL pH AND COMMENTS
I.	CONTROL			
1.	water (argon sparged)	6.1	--	300, still drifting
2.	water + KCl	6.6-8.4	--	no immed. change, drifted over 120 min
II.	SATURATED SALT SOLUTIONS			
3.	ammonium acetate	9.0	7.8	20
4.	ammonium chloride	7.2	5.4	12
	Ammonium bicarbonate			
5.	saturated	4.3	8.0	8
6.	2.37 Molar	4.1	8.0	8
7.	1.38 Molar	4.5	8.0	40
III.	SATURATED SALT SOLUTIONS (CONTAINER A) PLUS CARBON DIOXIDE (CO2) (CONTAINER B)			
8.	sodium carbonate	6.7	11.3	60, slowly drifting
9.	" " (repeat)	5.8	--	120, slowly drifting
10.	" " + CO2 (b)	4.3	--	24, to 140 min+
11.	sodium bicarbonate	4.7	8.1	40,
12.	" " + CO2 (b)	4.3	7.5	12
13.	ammonium formate	9.4	7.4	40
14.	" " + CO2 (b)	4.3	7.5	20
IV.	VAPORS			
	a. Diethylamine:			
15.	" (1 ml/min)	12.4	---	8
16.	" (0.5 ml/min)	11.3	---	8
	b. Acetic acid:			
17.	vapors of glacial	2.9		8
18.	sol'n 3.2% v/v	4.5		50
19.	sol'n 1.6% v/v	4.7		8
	c. Ammonia:			
20.	sol'n 50 ml/25% (c)	8.9	---	20

faster than hydrogen acetate molecules is reasonable, and explains the alkaline flow-pH of 9.0 (Table 1, line 3). Equilibration is reached with modest rapidity, in 20 min. Since ammonium acetate is equivalent to a mixture of a weak acid (acetic acid) and a weak base (ammonium hydroxide), the mixed solution is expected to be near neutral, and it is (the direct solution pH is 7.8).

Line 4. AMMONIUM CHLORIDE SATURATED SOLUTION hydrolyzes in water to produce ammonium, chloride and hydronium ions. The ammonium chloride is in equilibration with ammonium hydroxide, ammonia, and hydrogen chloride molecules. The flow-pH of 7.2, suggests that ammonia (NH3) diffuses through the silicone tube slightly faster than hydrogen chloride. The steady state flow-pH is achieved quickly (12 min) suggesting that it is the diffusion of the small gas-like molecules of ammonia and hydrogen chloride that determine the final flow-pH. Since ammonium chloride may be thought of as a mixture of a strong acid (hydrochloric acid) and a weak base (ammonium hydroxide), the direct solution is expected to be acidic, and it is, pH = 5.4.

Lines 5.-7. AMMONIUM BICARBONATE SATURATED SOLUTION completely hydrolyzes in water to produce some ammonium cations, some bicarbonate and some carbonate anions, but mostly dihydrogen carbonate molecules (carbonic acid, a weak acid) and ammonium hydroxide molecules (a weak base). The dihydrogen carbonate molecules are in equilibrium with the gas, carbon dioxide, and the ammonium hydroxide molecules are in equilibrium with the gas, ammonia molecules. With these gases involved, it is important to keep the container closed. Since ammonium bicarbonate may be thought of as a mixture of a weak acid (dihydrogen carbonate) and a weak base (ammonium hydroxide), the pH of the solution itself is expected to be near neutral (the direct bulk pH is 8). The fact that the flow-pH comes to equilibrium very quickly (8 minutes) suggests that the ammonia (NH3) and carbon dioxide molecules diffuse rapidly through the silicone tube, with carbon dioxide diffusing faster to give the flow-pH of 4.3.

While the saturated ammonium bicarbonate solution is about 5-10 molar and gives a flow-pH of 4.3 (Line 5), a more dilute solution of 2.37 molar gave a similar flow-pH of 4.1 (Line 6), and gave this pH very quickly (in 8 minutes). However, going to a yet more dilute solution, 1.38 molar, gave a similar flow-pH of 4.5 (Line 7), but required some 40 minutes to reach equilibrium, even though the salt solution was vigorously stirred. This suggests that sufficient concentration of pH determining molecules must be available for equilibrium to be achieved in a reasonable time.

Lines 8. & 9. SODIUM CARBONATE SATURATED SOLUTION completely hydrolyzes in water to produce some monohydrogen carbonate (bicarbonate) ions, and some dihydrogen carbonate molecules, but mostly hydroxide and carbonate ions, and sodium. The dihydrogen carbonate is in equilibrium with the gas, carbon dioxide molecules. The equilibrium between all of these species is usually well defined by the pKs, in a closed container in which the carbon dioxide level in the gas phase can come to equilibrium. However, in a solution saturated in sodium carbonate, or in open containers in which equilibrium with the carbon dioxide in the gas space is not possible, the system is not so well defined.

With sodium carbonate, the acidic flow-pH of 5.8 to 6.7 of the flow stream, suggests that carbon dioxide migrates through the silicone tubing at a slower rate than found with carbon dioxide alone where the flow-pH goes to 4.0 (described below).

It is probable that hydroxide does not migrate through the silicone tubing, and that the sodium carbonate salt level, being very alkaline, has a low level of carbon dioxide molecules, which can migrate though the silicone rubber. The low carbon dioxide level possibly leads to the pH drifting slowly toward pH 4, but this slow drift in flow-pH, continuing beyond 1-2 hours, is unacceptable, (Fig. 2) and the cause was not pursued further.

Line 10. CARBON DIOXIDE BUBBLED THROUGH SATURATED SODIUM CARBONATE creates an entirely new system. The carbon dioxide with water makes dihydrogen carbonate (carbonic acid). The final

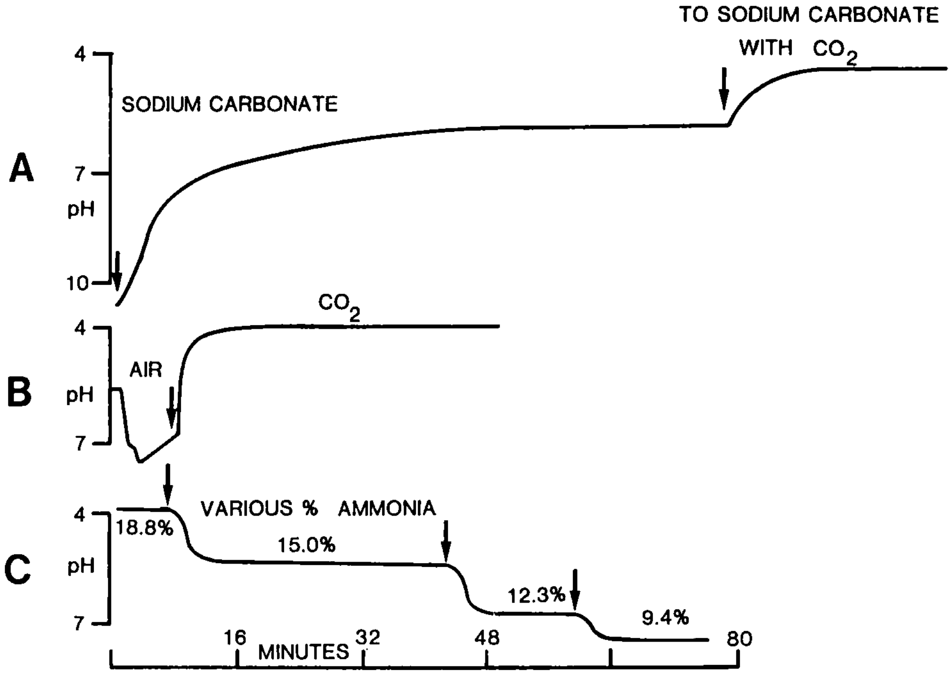

Figure 2. Typical plots of pH vs. time for various changes ranging from (A) a very slow (unacceptable) equilibration of sodium carbonate, and the fast equilibration of sodium carbonate with carbon dioxide bubbled through; (B) fast equilibraton of carbon dioxide alone; and (C) the fast equilibrations of changing from various percents of ammonia in Chamber A (with carbon dioxide in chamber B). Arrows indicate points of change.

system is the equilibrium system of the molecular species (carbon dioxide and dihydrogen carbonate), the cationic species (hydronium and sodium), and the anionic species (monohydrogen carbonate, and carbonate), that results when a water solution is in equilibrium with (saturated with) solid sodium carbonate (or sodium bicarbonate), and gaseous carbon dioxide at one atmosphere pressure. Carbon dioxide bubbled through sodum hydroxide, sodium bicarbonate, or sodium carbonate should reach the same final equilibrium and flow-pH.

Data in Table 1 for the carbon dioxide bubbled through sodium carbonate (Table 1, Line 10) or carbon dioxide bubbled through sodium bicarbonate (Line 12) supports this hypothesis, since both give the same flow-PH of 4.3. Since these systems give a pH different from carbon dioxide alone (pH 4, below), the different pH is probably due to the reduced carbon dioxide solubility in the saturated salt solution vs. plain water (Line 21, described below). These results with various carbonates, and the probability that silicone tubing does not carry ions, suggests that it is the carbon dioxide partial pressure in the saturated salt solution that is determining the pH. A simpler series of salts, such as the sodium, potassium, and cesium chlorides, might allow simpler and more predictable tailoring of the pH, by predictably controlling the carbon dioxide partial pressure. Pre-mixed or flow-mixed carbon dioxide and nitrogen might also give this same controlled partial pressure (as in REF 3).

Equilibrium is reached with modest rapidity, (between 12 and 24 minutes, Line 12 and Line 10) (see also Fig. 2).

Line 13. AMMONIUM FORMATE SATURATED SOLUTION hydrolyzes in water to produce ammonium and formate ions, as well as hydrogen formate molecules (formic acid, a weak acid), and ammonium hydroxide molecules, (a weak base). The ammonium hydroxide molecules are in equilibrium with the gas, ammonia molecules. The possibility of ammonia (NH3) diffusing through the silicone tubing faster than hydrogen acetate molecules seems reasonable, and is consistent with the flow-pH of 9.4.

Since ammonium formate is equivalent to a mixture of a weak acid (formic acid) and a weak base (ammonium hydroxide), the direct pH of the solution is expected to be near neutral, and it is (the direct saturated solution pH is 7.4).

Line 14. CARBON DIOXIDE BUBBLED THROUGH SATURATED AMMONIUM FORMATE, gives the same flow-pH of carbon dioxide bubbled through sodium carbonate and sodium bicarbonate (flow-pH = 4.3). This suggest that the dominating pH effect is from the diffusing ammonia and carbon dioxide alone, and that formic acid plays little role.

Lines 15. & 16. DIETHYLAMINE VAPOR, a weak base, partially hydrolyzes in water once it has diffused through the silicone membrane to produce dihydrogen-diethylammonium cations, and hydroxide anions. Equilibrium is reached very quickly on addition of the liquid (ca. 5 ml) to the vapor chamber. The diethylamane does not contact the membrane. The rate of reaching a steady flow-pH probably depends on the volatility of the amine, which is partially determined by temperature. Unlike other materials tested (flows of 0.5 and 2 ml/min), the diethylamine flow-pH is dependent on the flow rate of the water through the tube, with flows of 0.5 and 1 ml/min showing different, but very steady, flow-pHs of 11.3 and 12.4. In this case, possibly a longer silicone tube would eliminate this flow dependence. The very high flow-pHs of diethylamine solutions (11.3 - 12.4) makes them less useful directly with silica based packings (pH stable from 2 to 7.5). However, the high pH could be very useful with pH-resistant divinylbenzene packings, for use with aromatic amines which often show narrow, non-tailing peaks, with longer retention, and more sensitive UV detection when they are not ionized at pHs above their pKs (pKs of 9-10).

A very important possibility, might be the predictable flow-generation of (very clean) organic amine carbonate buffers of any predictable flow-pH. The principle would be based on the ability to change the migration rate of organic amines through the silicone tubing by changing the alkane moiety on the amine. A first equilibration-chamber would contain the organic amine and a second equilibration-chamber would contain the carbon dioxide (as described for flow-generated ammonium carbonate below (Lines 24 - 37).

Lines 17.-19. ACETIC ACID VAPOR, a weak acid, partially hydrolyzes in water once it has diffused through the silicone membrane to produce acetate ions, and hydrogen ions. The rate of reaching a steady state flow-pH is very fast (8 min) after addition of the glacial acetic acid (ca. 5 ml) to the vapor chamber. The flow-pH of acetic acid is not dependent on the flow rate of the water through the tube, for flows up to 2 ml/min.

The flow-pH reached with the acetic acid vapor (flow-pH = 2.9, Table 1, Line 17) was found to be very different from the flow-pH achieved with dilute solutions of acetic acid contacting the silicone membrane. With the tubing immersed in 1.6 and 3.2 % (v/v) of acetic acid in water, the flow-pHs were 4.7 and 4.5, respectively (Lines 18 and 19). The 3.2% solution took a very long time to reach a steady state (50 minutes), but when this solution was diluted to 1.6%, steady state was reached in only 8 miutes, possibly because the silicone tubing was conditioned with a residual level of acetic acid. These peculiar equilibration times suggests that the immediate history of the silicone tubing is important in determining the time it takes to reach a steady flow-pH.

Line 20. AMMONIA VAPOR FROM ABOVE AN AMMONIUM HYDROXIDE SOLUTION (nominally 25% w/w) a weak base, partially hydrolyzes in water once it has diffused through the membrane to produce some ammonium ions, but mostly ammonium hydroxide molecules and hydroxide ions. Equilibrium is reached with modest rapidly (20 min) on addition of the ammonia solution (ca. 50 ml) to the sealed vapor chamber. (The ammonia solution does not contact the silicone tubing.) The flow-pH of ammonia solutions is not dependent on the flow rate of the water through the tube, for flows up to 2 ml/min.

The following discussions of specific results refer to data in Table 1.

Lines 21. to 23. CARBON DIOXIDE GAS alone takes a modest time (20 min) to reach a stable flow-pH of 4.0 (Fig. 2).

Lines 24.-37. AMMONIA VAPOR PLUS CARBON DIOXIDE GAS, in different equilibration-chambers, provides a method to reach a broad range of flow-pHs (5.5 - 8.6). Ammonia solutions of various concentrations are put in a first equilibration-chamber (not touching the tubing) and carbon dioxide gas is allowed to flow through a second equilibration-chamber. Thus, an ammonium carbonate (or possibly called ammonium bicarbonate) solution is flow-generated.

A problem with flow-generation of ammonium carbonate is the requirement that the ammonia solutions be prepared very carefully

TABLE 2 (continued from Table 1)

In-line frow-preparation of eluents of various flow-pHs by means of gases, and vapors plus gases. All flow-pHs were determined at both 0.5 and 2 ml/min, unless otherwise noted.

LINE NUMBER	DESCRIPTION	FINAL FLOW-pH	DIRECT (BULK) pH (a)	MINUTES TO REACH FINAL pH AND COMMENTS
V. GASES				
21.	carbon dioxide	4.0	---	20
22.	air	5.5	---	100 still drifting
23.	carbon dioxide	4.0	---	20
VI. AMMONIA VAPOR (CONTAINER A) VS. CARBON DIOXIDE (CO2) (CONTAINER B)				
a. Ammonia 5ml of 25% + CO2(b)				
24.	0.5 ml/min	8.6	---	8, held 300+ min
25.	1.0 ml/min	8.6	---	8,
26.	1.5 ml/min	8.6	---	8,
27.	2.0 ml/min	8.6	---	8,
b. Ammonia & water + CO2 (b) (sequential additions of water to ammonia or sequential additions of ammonia to water)				
	ml NH3 & ml H2O (% NH3)	flow-pH		
28.	30 & 10 (18.8%)	8.4		4
29.	30 & 20 (15.0%)	7.0		4
30.	30 & 30 (12.3%)	6.5		4
31.	30 & 50 (9.4%)	5.8		4
c. Water wash				
d. Increasing ammonia concentration				
32.	10 & 30 (6.3%)	5.5		4
33.	20 & 30 (10.0%)	6.7		4
34.	30 & 30 (12.5%)	7.4		4
e. Decreasing ammonia concentration				
35.	30 & 40 (10.7%)	7.0		4
36.	30 & 50 (9.4%)	6.6		4

(a) Determined by placing pH electrode directly in the salt solution, the "salt error" ignored.
(b) The flowing stream of water was passed from a silicone tube in Chamber A and then passed to a second silicone tube in a second chamber, Chamber B. Both silicone tubes were 1000 mm long and 0.5 mm i.d., 1.0 mm o.d.
(c) Concentrated ammonia solution, 25% w/w.

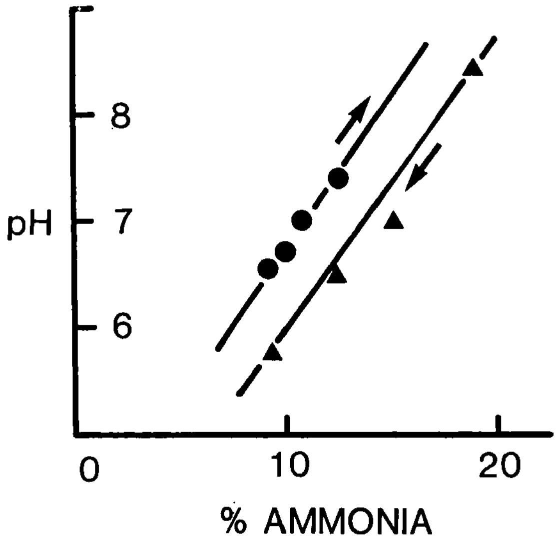

Figure 3. Sequential addition of ammonia to water (top curve) and water to ammonia (bottom curve). The non-overlap of the calibration curves reflects the difficulty in working with the highly volatile ammonia solutions in Chamber A. Carbon dioxide is in Chamber B.

so that losses of the ammonia gas are not important (a difficult task). Table 2, lines 24 - 36 and Figure 3 shows that the sequence of addition of increasing or decreasing the level of water in the ammonia solution gives different calibration lines. The very high volatility of ammonia gas from solutions can be seen also by the refractive index of ammonia solutions changing significantly over seconds on the partially closed Abbe refractometer cell. These data indicate the difficulty in manipulating the ammonia solutions. Careful manipulation of the ammonia solution using syringes, and using large solution volumes could minimize problems.

An important possible advantage of using flow-generated ammonium carbonate is the transfer of vapors from the ammonia solution and through the membrane, as well as the gas phase transfer of carbon dioxide, should produce very pure buffered eluents, with only a fraction of the effort required by other methods. Such eluents might be usable at 190 nm UV detection or below with no ghost peaks. (Carbon dioxide was the only clean enough counter ion for triethylamine for achieving 190 nm UV detection with full gradients in previous work by us. [REF 3])

A possible system that might give an optimally pure eluent might be simple volatile amines in one chamber and carbon dioxide in the second. Untested so far is the hypothesis that the sequence of the chambers (amine then CO2, or CO2 then amine) are predicted to give different flow-pHs.

CONCLUSIONS

A wide variety of saturated salt solutions, gases, vapors, and combinations of these were shown to permit producing a flow-generated aqueous stream with flow-pHs ranging from 2.9 to 12.4 (Table 1), with the primary advantages that no weighing, pipeting, or titrating of eluents is required. Flow-pHs were generally independent of flow from 0.5 to 2 ml/min. An important possible advantage is that flow-preparation of eluents may permit generating extremely clean eluents for very low wavelength UV detection.

It appears that silicone rubber permits transport of small molecular species such as ammonia, carbon dioxide, formic acid, acetic acid, and hydrogen chloride. Ions probably do not migrate through silicone rubber. Saturated salt solutions (such as ammonium acetate, and ammonium chloride) are one way of fixing (low) levels of these molecular species, the the resulting flow-pH. Other, simpler, saturated salt solution might be a way to fix the partial pressures of dissolved gases, such as carbon dioxide, by controlling their solubility. Future work will explore these possiblities.

A series of LC apparatus components that we have developed previously can potentially be put together to permit poorly purified water (possibly even tap water) to be processed, as needed, so as to be: (1) degassed of air, (2) filtered of particles, (3) cleaned of UV absorbing chemicals, and (4) adjusted in pH. Degassing could be done with a membrane (such as the ES Industries deoxygenator), with helium sparging, or with a new sparging method [REF 8]. Filtering can use an in-line absolute membrane filter to 0.22 microns combined with a "gas-ballast" on the low pressure inlet side of the pump. [REF 9] Cleaning of UV absorbing materials in an in-line method was shown previously by us using "photo-oxidation" by drawing the inlet water through a (UV-transparent) Teflon tube wrapped around a 190 nanometer UV light output from a "germicidal" lamp. [REF 10] Finally, adjusting pH might use the in-line flow-preparation approach shown possible in this publication.

We now have combined semipermeabale membrane technology with our gas batch method of eluent preparation to produce a device for in-line flow-preparation of the aqueous eluent of various fixed pHs for RP-LC. The system is not optimized. Now that the concept is shown to work, future investigations will: (1) show how in-line flow-preparation of eluents can be fit on the suction (vs. pressure) side of a pump, (2) determine the actual salt levels (plus pH) in the eluent, (3) determlning if buffering is sufficient to permit samples in matrices of different pHs to give identical retention times and areas, (4) determine if the partial pressure of gases alone (determined by salt levels) is important in establishing the final flow-pHs, and (4) show actual chromatographic applications.

It is worth considering that flow generated pH controlled systems potentially could be used in reverse, with a knitted tube being used after the column with an inert gas or vacuum to remove the volatile buffer components for easier sample isolation. Another possibility is the use of an in-line flow-through system after the chromatography to change the pH to accentuate detection (as mentioned above for amines) or to add reagents for chemical reaction detection.

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