ELSEVIER

Carbohydrate Research 267 (1995) 115–126

CARBOHYDRATE RESEARCH

A multichambered apparatus for HF solvolysis experiments: reaction of cellulose HF solvolysis products with acetic acid and acetic anhydride

Brian A. Bergamaschi *, John I. Hedges

School of Oceanography, WB-10, University of Washington, Seattle 98195, WA, USA

Received 16 February 1994; accepted in revised form 15 August 1994

Abstract

Solvolysis of polysaccharides in anhydrous HF has not gained wide acceptance as an analytical tool despite superior reaction characteristics as compared to conventional hydrolytic agents. This reluctance seems primarily related to difficulties in handling HF. However, these difficulties are substantially reduced by an inexpensive, fully-enclosed, many-chambered reactor suitable for use in HF experiments as described in this article. The reactor has the advantages of multiple independent reaction vessels, means to precisely measure liquid HF, means to add measured volumes of reagents directly to the closed reaction vessels during the course of the solvolysis, and provision for rapid temperature changes and control. A series of experiments exploring the direct transition from HF solvolysis to acetolysis conditions by the addition of acetic acid and acetic anhydride, as an alternative to previously published solvolysis termination schemes, is also described.

Keywords: Apparatus, multichambered; HF solvolysis experiments; Cellulose HF solvolysis products; Acetic acid; Acetic anhydride

1. Introduction

Solvolysis in anhydrous hydrogen fluoride is an effective but underutilized technique for the analysis of plant and bacterial polysaccharides [1] and for use in carbohydrate synthesis [2]. Its effectiveness is related to the high efficiency with which HF cleaves *O*-glycosidic linkages, the high solubility of large polysaccharides in liquid HF, and the high relative stability of the released reducing-end termini in liquid HF solution [3]. However, routine laboratory use of anhydrous HF is difficult because HF is a gas at room temperature, the

* Corresponding author.

SSDI 0008-6215(94)00279-7

liquid having a boiling point of 19.5°C. Experiments must be performed in sealed reaction vessels and at reduced temperatures. Furthermore, HF is highly toxic as well as corrosive to most common laboratory ware. Apparatus made of glass, most metals, and many plastics is unsuitable for use with HF.

These difficulties are overcome by using a fully enclosed system constructed entirely of HF-resistant materials, such as that described by Sanger and Lamport [4]. These authors constructed a microapparatus suitable for HF solvolysis experiments out of commercially available components and materials. The apparatus was essentially a sample vial connected through a series of fittings to an HF cylinder. In order to conduct an experiment, a sample was placed within the vial, and it was immersed in a cold bath (liquid nitrogen or dry ice–acetone) until sufficient HF was condensed. The solvolysis reaction was terminated by evaporation of the HF into a calcium oxide trap by vacuum aspiration. The configuration of this apparatus, however, does not permit precise timing of solvolytic reactions because HF is distilled directly into the reaction vial. Exploration of other experimental parameters is difficult because no provision is made for reagent addition subsequent to solvolysis, or for sample removal except after the evaporation of HF.

Mort [5] modified a commercially available apparatus (manufactured for HF deprotection of proteins) for use with HF solvolysis reactions. The modified apparatus permitted precise timing of reactions by dispensing liquid HF into the reaction vessel to begin solvolysis, and expelling the contents of the reaction vessel into a quenching solution to terminate it. Furthermore, the apparatus provided means for temperature control and stirring of the mixture. The system functioned by accumulating liquid HF from the gas cylinder into a dispensing vessel and then pressurizing the dispensing chamber to force the liquid HF into a reaction vessel. Solvolysis was terminated by forcing the contents of the reaction vessel through a cooling coil into a slurry of calcium carbonate in dry ice–dichloromethane, or directly into cold diethyl ether. However, even with modification this system is impractical for routine analytical use because of its inability to measure liquid HF, lack of means for adding reagents to the reaction vessel, and limitation to a single sample.

We became interested in exploring the advantages of experiments with liquid anhydrous HF may provide for analyzing hydrolysis-resistant polymers encountered in environmental samples [6–8]. In particular, we wished to explore new means of terminating the solvolysis reaction, and the possibility of smooth transitions to other depolymerization schemes. Also, we wanted to determine the reaction rates of different known polymers, the effect of changing such parameters as reaction temperature, salt concentrations, and solution volume, as well as the effect of a variety of reagents added both during and following HF solvolysis. Consequently we desired an apparatus that accommodated multiple simultaneous experiments, allowed reagent additions to the reaction vessel at any time, and provided greater control of the experimental parameters.

On the basis of these previous designs [4,5], and aided by a wide selection of commercially available Teflon laboratory ware, we developed an inexpensive and easily constructed apparatus that offers a high degree of flexibility in reaction parameters. The apparatus is assembled almost entirely from commercially available components and offers several unique advantages over previous designs. The principal advantage is that it permits measurement of the volume of liquid HF introduced into a reaction vessel. Like the apparatus described by Mort [5], our apparatus handles HF in liquid form by distillation from its

shipping cylinder into a separate dispensing reservoir. From this reservoir HF is measured in a metering loop and dispensed into a reaction vessel while still in liquid form. Precise measurement of HF volumes and precise timing of reactions are consequently attainable. Another important advantage is the addition of multiple reaction vessels, all connected to the HF dispensing circuit. With six individual reaction vessels, the existing apparatus can accommodate multiple simultaneous experiments. The reaction vessels are clustered on a single plate and attached by flexible tubing to the network of valves that control HF and reagent additions. This arrangement permits the clustered vessels to be easily moved between temperature-controlled baths, facilitating rapid and precisely timed temperature changes. The final advantage is the provision of a separate reagent addition circuit with which auxiliary reagents may be measured in the same way as HF, and added to an otherwise closed reaction vessel without opening or removing it from the temperature-controlled bath.

2. Equipment and materials

Design of the apparatus.—The HF solvolysis apparatus was assembled according to the schematic in Fig. 1, with symbols corresponding to the list of principal components and their manufacturers presented in Table 1. Universal characteristics of the apparatus were that all tubing was of 0.125″ o.d. heavy-wall Teflon pressure rated to 500 psi, all valves and fittings were either T-316 stainless steel or pure Teflon, and all swage-type connections used Teflon ferrules. Valves were mounted conveniently on a single board to form the fluid control panel. Sufficient scope was left in the flexible tubing from the fluid control panel to the HF and reagent reservoirs, as well as from the fluid control panel to the reaction vessels, to permit free movement of the reaction vessels or reagent reservoirs into and out of temperature control baths. The chemical trap was made from a polyethylene bottle modified to admit the requisite waste tubes and filled with calcium sulfate (Drierite). The waste bottle was also polyethylene. The entire apparatus, with the exception of the constant temperature refrigerant circulator, was located within a fume hood.

The reaction vessels were assembled in a cluster to form the reactor. In order to ensure a reliable gas-tight seal and to provide an added measure of safety, each reaction vessel was partially enclosed within a custom-made T-316 stainless steel cylindrical capture, and the captures were assembled collectively on a base plate. The reactor is simply seven pieces of threaded pipe of suitable size to accommodate a sample vial and cap (listed in Table 1), all welded to the base plate. The seven-place assembly and an exploded view of one of the capture sub-assemblies is shown in Fig. 2. The custom-made components are the base, vial capture, cap capture, and the washer below the snap ring. The sample vials and caps are commercially available as listed in Table 1, and the remaining components may be purchased from a hardware or machinery supply store. The base was made from a 6.375″ diameter disk of 0.125″ thick T-316 plate, with 0.5″ holes drilled under each capture location to permit cooling fluid to drain. The vial captures were made from T-316 stainless pipe 2.25″ long, machined to 1.47″ o.d. by 1.20″ i.d. and threaded externally 0.5″ from the end. The cap captures were similarly made from T-316 pipe, but were 1.25″ long and machined to 1.67″ o.d. by 1.42″ i.d. The cap captures were threaded internally to mate with the vial captures on one end and an internal snap ring groove was cut 0.125″ from the other end.

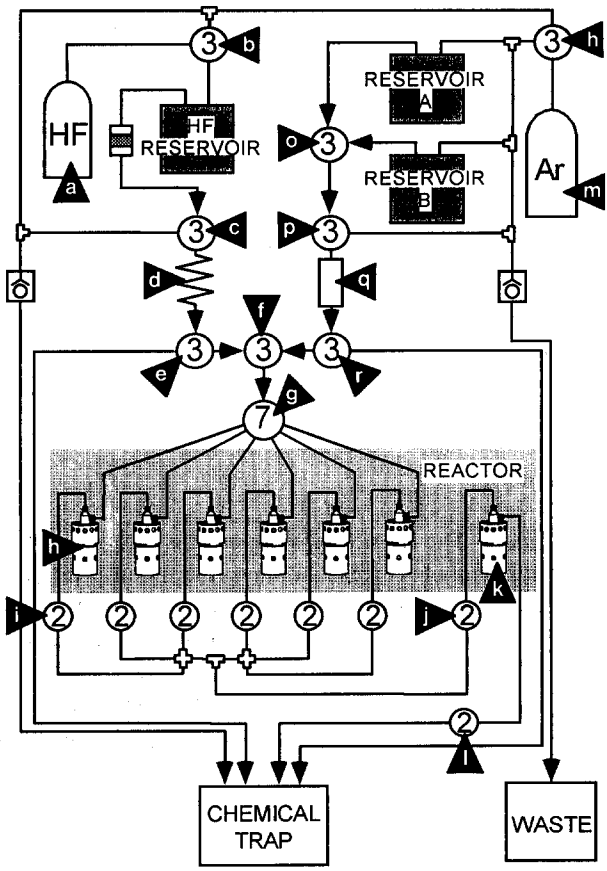

Fig. 1. Schematic of the HF solvolysis apparatus. Reagent flows are indicated by arrows. The symbols are defined in Table 1. Flags indicate valves or assemblies referred to in the text: (a) HF tank and valve, (b) HF pressure/distillation selector valve, (c) HF/pressure selector valve, (d) metering loop, (e) HF reactor/waste valve, (f) circuit selector valve, (g) distribution valve, (h) reaction vessel and capture assembly (6 total), (i) reaction vessel vent valve (6 total), (j) trap inlet valve, (k) reactor trap assembly, (l) trap outlet arm, (m) argon tank, tank valve, and pressure regulator, (n) circuit pressure selector valve, (o) reagent reservoir selector valve, (p) reagent/pressure selector valve, (q) metering vial, and (r) reagent reactor/waste selector valve.

Holes were drilled in both the cap and vial captures to permit cooling bath fluids to enter and drain. The custom-made washer was 1.11″ i.d. by 1.40″ o.d. by 0.03″ thick. The vial captures were welded around the perimeter of the base, as is shown in the top view in Fig. 2, and the components were assembled as indicated.

Testing.—Pressure testing of the empty solvolysis system was done at ca. 40 psi. This is eight times the operating pressure of the system and so offers a considerable safety margin. The network of valves and tubing comprising the HF metering and dispensing circuit, the valves and tubing in the reagent metering and dispensing circuit, the tubing to each reaction vessel, and the reactor trap were all independently tested. The sequence of operations for each of these tests is outlined below.

In order to check the integrity of the HF circuit, pressure was applied sequentially to the individual components of the circuit and all fittings were checked for leaks with liquid leak

Table 1
System components

Item	Quantity	Description	Manufacturer	Part number	Symbol
1	8	Valve, 2-way	Whitey	SS-41S2	
2	7	Valve, 3-way	Whitey	SS-41XS2	
3	1	Valve, 7-way	Whitey	SS-43ZFS2	
4	2 (both optional)	Pressure relief valve	Nupro	SS-2C-TR-25	
5	3	Union cross	Swagelok	SS-200-4	
6	4 (2 optional)	Union tee	Swagelok	SS-200-3	
7	3	Pressure vessel	Savillex	573R2	
8	8	Sample vial	Savillex	0201C	
9	8	Two port cap	Savillex	633-2-2	
10	1	In-line filter holder	Savillex	2-25-2 w/ 1115 filters	
11	1	0.125″ o.d. Tubing	Savillex	1200	
12	1	Reactor base assembly	Custom fabrication		

detector. (Each of the italicized valve names below corresponds to one of the labeled valves in the system schematic, Fig. 1.) First, the HF reservoir was checked to ensure it was completely empty and tightly closed. Then the argon pressure regulator was adjusted to 40 psi, the *circuit pressure selector valve* directed to the HF circuit, the *HF pressure/distillation selector valve* moved to select the argon tank, and the *HF/pressure selector valve* was positioned to select the HF reservoir, thereby pressurizing the HF reservoir and the metering loop. Next, the *HF reactor/waste selector valve* was positioned towards the metering loop which pressurized the circuit to the *circuit selector valve*. In order to keep pressure in the HF line, the *circuit selector valve* was turned to select the reagent circuit. If no leaks were indicated with the liquid leak detector, the *argon tank valve* was closed and the pressure was monitored for 5 min. Anything less than a 5% pressure drop was considered acceptable.

To test the integrity of the reaction lines, the *argon tank valve* was then reopened and a reaction vessel was installed at each position and securely closed. The reactor circuits were sequentially pressurized and leak tested by first moving the *circuit selector valve* to the HF circuit position. This pressurized whichever vessel was selected by the *distribution valve* as far as the corresponding *reaction vessel vent valve*. For only the first vessel tested, the *trap inlet valve* was opened and the *trap outlet valve* closed in order to pressurize the reactor trap. The presence of leaks was then determined by both a liquid leak detector and by

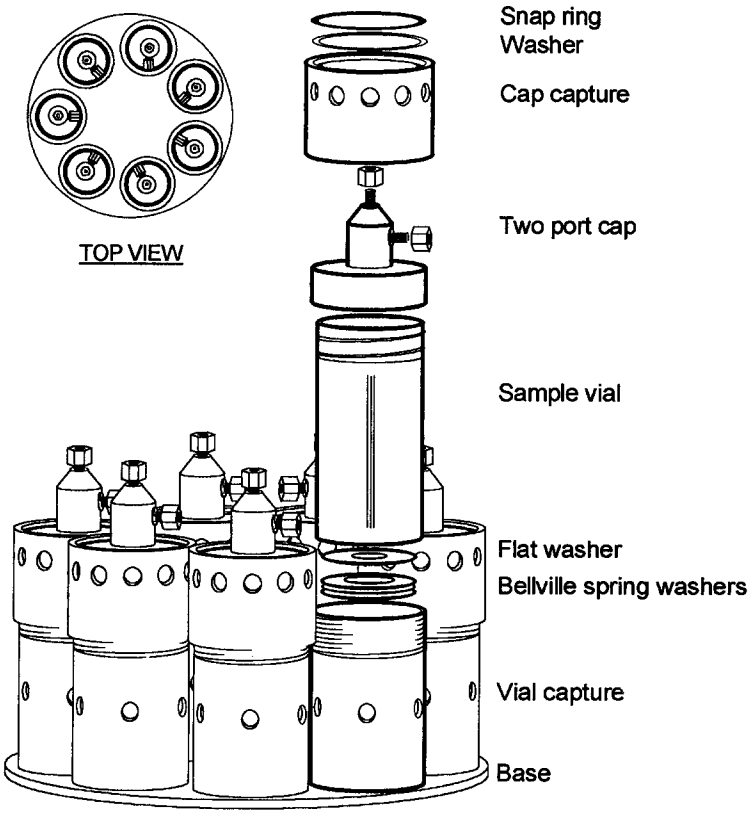

Fig. 2. Reactor assembly. An exploded view of a typical reaction vessel (or trap) is shown. A description of the individual items and their arrangement is in the text.

closing the *argon tank valve* and checking the pressure drop after 5 min. Again, anything less than a 5% pressure drop was considered acceptable. The remaining reaction vessels were then sequentially tested by turning the *argon tank valve* back on, indexing the *distribution valve* to the next position and repeating the leak test for that circuit.

The reagent circuit was similarly pressure tested and leak checked by first ensuring that all reagent reservoirs were completely empty and securely closed, and then moving the *reagent/pressure selector valve* to the pressure position. The *reagent reservoir selector valve* may be in either position because both reservoirs are pressurized.

The remainder of the circuit was pressurized by first selecting the reagent reservoirs with the *reagent/pressure selector valve*, then selecting the metering vial with the *reagent reactor/waste selector valve*. The reagent circuit was isolated from the downstream components by moving the *circuit selector valve* to the HF circuit position. The system pressure was increased to 40 psi with the argon pressure regulator, and leak and pressure tests were performed. Following testing, the argon pressure regulator was reset to 5 psi.

Caution.—Any users of this or similar apparatus for handling HF under pressure should make themselves familiar with the hazards involved, and the symptoms and treatment of burns from anhydrous HF. The toxic properties of HF should be well understood. First aid

materials appropriate for use on HF burns should be immediately available and laboratory personnel should be familiar with their use. A lab coat, face shield, elbow-length gloves, and acid-proof lab apron rated for use with HF should always be worn while HF is in the unit. Always replace valves, tubing, and fittings at the first sign of wear.

Chemicals.—HF was obtained from Air Products (Allentown, PA) in a 2.3 kg tank (size D). Glacial acetic acid, pyridine, acetic anhydride, and cobalt fluoride were purchased from the Aldrich Chemical Co. (Milwaukee, WI) in the purest grades available.

3. Experimental

Solvolysis reactions.—At the start of each day's runs, the entire apparatus except the reaction vessels was pressure tested (but not leak tested) and sufficient HF for that day's experiments was distilled from the HF storage tank into the HF reservoir. If it was necessary to dry the HF, cobalt(III) fluoride was placed in the reservoir prior to distillation. To accomplish the distillation, the *HF/pressure selector valve* was positioned on pressure and the *HF pressure/distillation selector valve* directed toward the HF reservoir. The HF reservoir was immersed in either liquid nitrogen or a dry ice–ethanol bath to condense the gaseous HF, the *HF tank valve* was opened, and the amount of HF accumulating in the reservoir was closely monitored. Once sufficient HF was transferred, the *HF tank valve* was closed. The HF reservoir was not pressurized until HF transfers were begun. Since a total of only 10–20 mL of HF was required, only ca. 30 min was necessary for charging the reservoir.

In a typical experiment, 1–2 mg of sample was weighed directly into each of six Teflon sample vials, a Teflon micro stir bar was added to each, the sample vials were attached to the two-port caps, and the capped vials installed in the reactor. The reactor vessel cap captures were securely attached taking care not to stress the inlet and outlet tubing. For safety purposes, vials were always installed at all reactor positions, even if unused. The reactors were placed directly into a dewar serving as a temperature-controlled bath. The dewar was equipped with an integral magnetic stirrer (Agitainer, Neslab Instruments, Portsmouth, NH). The bath temperature was maintained by pumping 1:1 MeOH–water through the dewar from a refrigerated constant-temperature circulator (Neslab Instruments RTE-210) equipped with a digital controller. The reaction vessels were allowed to equilibrate for ca. 15 min, and the integrity of each vessel tested.

To begin the experiment, all *reaction vessel vent valves* and the *trap inlet valve* were closed, the reactor trap was vented by briefly opening the *trap outlet valve*, the *HF/pressure selector valve* was positioned on pressure, the *HF reactor/waste selector valve* was positioned towards the reactor, the *circuit selector valve* was opened to the HF circuit, and the *distillation selector valve* was moved to the pressure position. At this point the reaction vessel selected by the *distribution valve*, usually vessel one, was pressurized to the system pressure.

To measure liquid HF, the *HF reactor/waste selector valve* was moved to the off position and the *HF/pressure selector valve* opened to the HF reservoir. The *HF reactor/waste selector valve* was then cracked *slowly* to waste to admit liquid HF slowly into the graduated metering loop. Once the loop contained the desired amount of HF, typically 0.5 mL, the

reactor/waste selector valve was closed, the *HF/pressure selector valve* moved to pressure, and the *HF reactor/waste valve* moved to select the reactor. The measured aliquot of HF was transferred into the reaction vessel by opening the *reaction vessel vent valve* and then the *trap inlet valve*. This decreases the pressure in the reaction vessel sufficiently to transfer the liquid HF from the metering loop into the reactor. The *distribution valve* was then indexed to the next reactor vial and the process was repeated.

For safety purposes, the HF reservoir was charged to system pressure only prior to the first injection and then the *HF/pressure selector valve* was placed in the off position. The head space in the reservoir provided sufficient residual pressure to make the entire series of HF transfers. Limiting the pressure head on the HF circuit in this way significantly reduced the risk of ejecting the contents of the HF reservoir in the event of a tube, valve, or fitting failure.

Termination of reactions.—Samples were solvolyzed at 0°C for 2–3 h. Following solvolysis, the reaction was terminated by adding acetic acid followed by acetic anhydride, acetic anhydride alone, or a 1:1 mixture of the two while they were still in the cold bath. The acetic anhydride was chilled to −70°C prior to addition by immersing the appropriate reagent reservoir in a dry ice–ethanol bath. Acetic acid was cooled to 0°C prior to addition. The reagent was added by first venting any excess pressure in the reactor trap by briefly opening the *trap outlet valve*, selecting the appropriate reagent reservoir with the *reagent reservoir selector valve*, selecting the appropriate reaction vessel with the *distribution valve*, moving the *circuit selector valve* to the reagent circuit, selecting the reactor on the *reagent reactor/waste selector valve*, and selecting pressure on the *reagent/pressure selector valve*. This pressurized the reaction vessel. To measure the amount of reagent, the *reagent/pressure selector valve* was turned to the off position, and the *reactor/waste selector valve* vented to waste. The reagent was admitted to the graduated metering vial by slowly opening the *reagent/pressure selector valve* to the reagent circuit. Once the requisite amount of reagent was in the vial, the *reagent/pressure selector valve* was moved to pressure and the *reagent reactor/waste selector valve* opened to the reaction vessel. The reagent was passed to the reaction vessel by opening the *trap inlet valve*, which partially depressurized the reaction vessel and then, if necessary, briefly opening the *trap outlet valve*. The process was repeated until appropriate additions were made to each reaction vessel. Reagent additions were typically completed within less than 2 min.

Once all the reagent additions were made, the reactor was removed from the cold bath and placed directly either into a warm water bath, onto a hot plate equipped with a magnetic stirrer, or on a stand-alone magnetic stirrer at room temperature. Typically, samples were heated to 80°C for 60 min following the addition of acetic anyhdride. At the end of the reaction time the reaction vials were taken to room temperature, removed from the reactor, and placed into an evacuating centrifuge (SpeedVac, Savant Instruments, Farmingdale, NY) equipped with both calcium sulfate (Drierite) and liquid nitrogen traps. The vacuum pump vent was plumbed into an exhaust hood. The samples were dried completely to form light beige, syrupy residues. To ensure complete conversion to the per-*O*-acetate derivatives, the syrups were dissolved in a mixture of 0.5 mL pyridine and 0.5 mL acetic anhydride, and the vials were securely capped and placed into a heating/stirring module at 80°C for 1 h. The samples were then dried a second time under vacuum to remove the acetic anhydride and taken up in 0.5 mL of pyridine.

Aliquots of ca. 50 μL were transferred into crimp-top autoinjector vials fitted with 100 μL glass inserts. The vials were placed in a Shimadzu AOC-14 autoinjector (Shimadzu Instruments, Columbia, MD) modified to mount on a Hewlett–Packard 5890a gas chromatograph (Hewlett–Packard Scientific Instruments, Santa Clara, CA). The gas chromatograph was equipped with a split/splitless injector operating in the split mode with a split ratio of 10:1 and fitted with a 30 meter bonded-phase methyl/cyanopropyl 0.25 mm i.d. capillary column (DB-1701, J&W Scientific, Folsom, CA). The chromatograph was connected to a mass-selective detector (Hewlett–Packard 5970) operating at 70 eV and coupled to a computerized data acquisition system (Hewlett–Packard 59970 operating PASCAL Chemstation v. 3.0). Data were collected from scans of the mass range 50–550 Da and presented as relative abundances determined from peak area integrations of the total ion chromatogram. Peaks were identified by comparison of retention times and mass spectral characteristics with authentic standards.

4. Results and discussion

The experiments presented here utilized the above-described multichambered apparatus to study the reactivity of HF solvolysis products during transition to acetolysis conditions, i.e., acetic acid in acetic anhydride. An analytical scheme allowing such a transition would permit HF depolymerization of polysaccharides into oligomers to be combined with more gentle acetolysis of those oligomers into monomers (or dimers). One of the benefits common to both HF solvolysis and acetolysis is the formation of stable intermediates during depolymerization and therefore protection of the reducing-terminal ends of the cleavage products during the course of the solvolysis reaction [9]. In order to study this transition, a series of experiments was performed which exposed HF solvolysis products to a variety of concentrations of acetic acid alone or in concert with acetic anhydride, under a range of conditions. Cellulose (1–2 mg, Sigma Chemical, St. Louis, MO) was chosen for this series of experiments in order to simplify the interpretation of the reaction products and because of its well characterized reactivity in HF [1].

This study illustrates the type of experiment which may easily be performed with the multichambered HF solvolysis apparatus. Such experiments, where additional reagents are measured into an otherwise sealed HF solvolysis reaction chamber, were not possible with HF solvolysis apparatus previously described in the literature [2,5]. Also, the availability of six reaction chambers permitted multiple simultaneous, experiments which greatly facilitated the study.

In these experiments, cellulose was dissolved in liquid HF for 2–3 h at 0°C. Under these conditions, cellulose is completely depolymerized into an equilibrium mixture of monomeric glucosyl fluorides and the corresponding oxonium ions, which have been shown to have good stability in liquid HF [1]. The effect of acetic acid on the degree of formation of glucose pentaacetate relative to glucosyl fluoride tetraacetate was tested by terminating the reaction in one of three ways (Table 2): (*a*) reaction with acetic acid alone followed by treatment with acetic anhydride, (*b*) direct reaction with acetic acid in acetic anhydride, or (*c*) reaction with with acetic anhydride alone. Controls were performed both by adding 10 mL of pyridine to some samples prior to heat treatment, and by not heating some samples

Table 2
Description of individual experiments

Sample number	Time of solvolysis (min) [a]	Post-solvolysis temperature (°C)	Post-solvolysis time (min)	Acetic anhydride (mL)	Acetic acid [b]/ anhydride (molar ratio)	Post-termination temperature (°C)	Post-termination time (min)	Glucose pentaacetate formed (%) [c]
Dried immediately after termination with acetic anhydride								
1	180			20	0.08	N/A	N/A	10.7
Heated after termination with acetic anhydride								
2	180			20	0.08	100	60	2.5
3	180			10	0.31	80	120	6.4
4	120			5	0.89	80	90	17.1
5	120			10	0.31	80	90	19.6
Pyridine (10 mL) added to reactor immediately after termination								
6	120			20	0.13	80	90	6.1
7	120			5	0.89	80	90	9.5
8	120			10	0.31	80	90	10.1
Added 1:1 v/v mixture of acetic acid and acetic anhydride to terminate								
9	165			5	4.0	100	120	15.9
10	165			5	4.0	100	120	35.4
Reacted with acetic acid (10 mL) prior to termination with acetic anhydride								
11	165	25	120	10	2.5	80	60	7.6
12	165	25	120	10	2.5	80	60	11.1
13	165	25	120	10	2.5	80	60	15.6
14	165	100	120	10	2.5	80	60	71.0
15	120	80	60	10	3.9	80	60	85.4
16	165	100	120	10	2.5	80	60	87.8
17	165	100	120	10	2.5	80	60	90.0

[a] All samples were treated with 0.5 mL of HF with the exception of 1 and 2, which received 0.3 mL, and 15, which received 1 mL.

[b] Including that formed by reaction of the acetic anhydride with HF (see text).

[c] Expressed as mol percent of the detectable monomeric products, the other being glucosyl fluoride tetraacetate.

subsequent to acid addition. In any case the analytes were glucosyl fluoride tetraacetate and glucose pentaacetate, and the values in Table 2 represent the per-*O*-acetate fraction of these two products. The anomeric configuration of the glucosyl fluoride was not determined, but it was presumed to be the more thermodynamically favored α form. The glucose pentaacetate was a mixture of anomers.

The introduction of acetic acid results in a nucleophilic displacement of fluoride by acetate at the anomeric carbon to form glucosyl acetates. Acetic anhydride added to the mixture either simultaneously with or subsequent to the addition of acetic acid reacts rapidly and completely with HF to form acetyl fluoride and acetic acid [10]. The acetyl fluoride then reacts with unacetylated hydroxyls to form per-*O*-acetates [11]. Thus, the effect of the three termination treatments is to remove all HF, and at the same time expose the solvolysis products to various concentrations of acetic acid in acetic anhydride, i.e., acetolysis conditions.

The reaction of the HF solvolysis products with glacial acetic acid under conditions of vigorous heating resulted in conversion of 71–90% of the glucosyl fluoride into the corre-

sponding acetate. Increasing the treatment time had no effect on the degree of conversion. Conversely, low concentrations of acetic acid ($<10\%$, formed from the reaction between acetic anhydride and HF) in surplus acetic anhydride gave only minor amounts of the acetate derivative (2.5–19%) even after vigorous heating. Increasing the acid concentration to 50% did not appreciably improve the yield of the glucosyl acetate; conversions were 16 and 35% in two experiments. The presence of pyridine in the system with acetic acid had no discernible effect on the formation of the acetate even after heat treatment. Glucosyl acetate yields ranged from 6 to 10%, similar to those obtained with small concentrations of acid. Also, treatment of the solvolysis products with excess acetic acid without heating resulted in yields of the peracetate of 6–15%, similar to those from low concentrations of acid.

Clearly, the greater the acetic acid concentration, the higher the yield of per-*O*-acetate, provided the system was heated. Even under vigorous conditions and with an excess of acid, however, the reaction did not go to completion. The probable reason for this is two-fold. First, the stability of the glucosyl fluoride is increased by introduction of acyl groups onto the hydroxyl positions under the conditions of acetolysis, because formation of the requisite oxonium ion intermediate is suppressed. Second, the direct effect of introducing acid or acid anhydride solvent is to destabilize the oxonium ion and force the equilibrium towards the fluoride.

5. Conclusions

The fully enclosed, multichambered solvolysis apparatus described here permitted a great variety of experiments to be executed in a rapid, safe, and convenient manner. The measurement of both anhydrous HF and subsequent reagents as liquids was precise and safe, and the integrity of the reaction vessels was maintained over a wide range of temperatures. Following HF solvolysis of cellulose to its monomer, transition to mild acetolysis conditions by the addition of acetic anhydride did not result in the formation of appreciable amounts of glucose pentaacetate, while treatment with glacial acetic acid at elevated temperatures prior to acetylation displaced the fluoride so that greater than 85% conversion to the pentaacetate was achieved.

Acknowledgements

The authors wish to thank David Thorsen for assistance in manufacturing the stainless steel components. The manuscript benefited from careful reviews by the UW MOG group and two anonymous reviewers. This research was supported by National Science Foundation Grants OCE9401903 and OCE9101908, and the Royalty Research Fund of the University of Washington. This is contribution number 2008 from the School of Oceanography, University of Washington, Seattle, WA.

References

[1] Yu.A. Knirel, E.V. Vinogradov, and A.J. Mort, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 167–202.
[2] K. Bock, C. Pedersen, J. Defaye, and A. Gadelle, *Carbohydr. Res.*, 216 (1991) 141–148.
[3] E.V. Vinogradov, A.S. Shashkov, Yu.A. Knirel, N.K. Kochetkov, N.V. Tochtamysheva, S.F. Averin, O.V. Goncharova, and V.S. Khlebnikov, *Carbohydr. Res.*, 214 (1991) 289–297.
[4] M.P. Sanger and D.T.A. Lamport, *Anal. Biochem.*, 129 (1983) 66–70.
[5] A.J. Mort, *Carbohydr. Res.*, 122 (1983) 315–321.
[6] R. Benner, K. Weliky, and J.I. Hedges, *Geochim. Cosmochim. Acta*, 54 (1991) 1991–2001.
[7] G.L. Cowie and J.I. Hedges, *Geochim. Cosmochim. Acta*, 48 (1984) 2075–2087.
[8] M.D. McCarthy, J.I. Hedges, and R. Benner, *Chem. Geol.*, 107 (1993) 503–507.
[9] C.J. Biermann and G.D. McGinnis, *Analysis of carbohydrates by GLC and MS*, CRC Press, Boca Raton, FL, 1989.
[10] T.A. Curtis, T.R. Ryan, and D.D. Lindley, U.S. Patent 4 990 681, 1991; *Chem. Abstr.*, 114 (1991) 209 563c.
[11] J. March, *Advanced Organic Chemistry*, Wiley, New York, 1985.