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Determination of total fluorine in blood at trace concentration levels by the Wickbold decomposition method with direct potentiometric detection

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Abstract

The Wickbold decomposition method in combination with differential potentiometric detection via fluoride ion-selective electrode has been applied to analysis of total fluorine in biological matrices. The performance of the method has been evaluated for determination of total fluorine in rat blood. Total mineralization of the biological sample is achieved by combustion of the sample in oxygen/hydrogen flame and subsequent absorption of the resulting fluoride in aqueous absorption medium. The fluoride is then quantified by highly selective automated differential static potentiometry with fluoride ion-selective electrode. Total fluorine determination has been evaluated in terms of sample carryover, reproducibility, precision, as well as feasibility to routine analysis of alternative biological matrices. Our results indicate that, up to 100 ppm fluorine in blood, the method does not suffer from sample carryover. Limits of quantitation of 0.5 ppm and limits of detection of 0.24 ppm fluorine in 0.5 g blood samples were achieved by elimination of inherent limitations of fluoride ion-selective electrode detection via automated differential static potentiometric measurements. The Wickbold decomposition method was found to be suitable for routine total fluorine determination in blood samples despite its relatively low throughput and high operator skill requirements.

Keywords: Total fluorine; Blood concentration; Wickbold decomposition method; Fluoride ion-selective electrode; Potentiometry

1. Introduction

There is increasing interest in the source and fate of fluorinated organic compounds in the environment [1]. Total fluorine measurements are an essential part of the study of the environmental and biological fate of fluorinated materials. Commercial fluorine-containing products such as fluorosurfactants and surface protection products are often complex mixtures containing many fluorinated compounds, and in many cases, different distributions of oligomeric or polymeric compounds. The nature of these materials can make the development of compound-specific analytical methods difficult, impractical or impossible. In addition, the possibility of analyte biotransformation and metabolism further complicates the development of targeted analytical methods. For these reasons the determination of total fluorine is an indispensable part of studies that examine the environmental and biological fate of fluorinated materials. In cases where compound-specific analytical methods are available, total fluorine determination may be a valuable tool for establishing fluorine mass balance in the system under investigation.

Blood can contain two different forms of fluorine [2,3]. These forms referred to by various names, are represented by inorganic fluoride and organic (or covalently-bound) fluorine. Total fluorine represents the sum of inorganic fluoride and organic fluorine.

Methods for the determination of fluoride ion levels in blood, serum, and similar materials have been described. Due to its high selectivity and sensitivity, methods using

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the fluoride ion-selective electrode are most commonly used [4–6]. Other well-described methods include spectrophotometry [6], gas chromatography after derivatization [4,7,8] and furnace molecular absorption spectrometry [9]. Many of these methods are used in combination with reverse extraction [10], analyte addition [11], or flow injection analysis [12].

Methods for determination of total fluorine in blood and other biological materials have been extensively reviewed by Venkateswarlu [13,14]. Most of the methods follow the same general approach consisting of mineralization of fluorine present in the sample to fluoride ion followed by determination of the fluoride ion. Determination of total fluorine in biological samples has been demonstrated using sodium biphenyl reagent [6,9], open ashing [5], oxygen plasma ashing [4], oxygen bomb combustion [7], and pyrohydrolysis using a tube furnace [8]. Inherent in any open ashing technique is the possibility of analyte loss or contamination from extraneous sources. These problems can be avoided in closed thermal decomposition systems if adequate temperature is achieved for complete sample decomposition and complete fluorine release from the sample. A very useful sodium biphenyl method [6,9] requires total extraction of fluorinated compounds into an inert solvent that does not react with sodium metal and back-extraction of fluoride into the aqueous phase after the digestion with the sodium biphenyl reagent and before the fluoride measurement.

Determinations of inorganic fluoride and total fluorine in the same sample have also been demonstrated [4,5,7].

Instrumental techniques for the determination of total fluorine such as nuclear magnetic resonance spectroscopy and X-ray fluorescence spectroscopy are generally suitable for high fluorine concentrations while others such as neutron activation and negative ion mode inductively-coupled plasma-mass spectrometry [15] require equipment which is not readily available. These approaches have not been applied routinely to biological samples such as blood.

Complete recovery of fluorine is a challenge due to the high bond energy of the carbon–fluorine bond. The comparison of the bond energy between F-C_2F_5 (127 kcal mol^{-1}) [16] and H-C_2H_5 (100 kcal mol^{-1}) [17] highlights the difficulty involved in the quantitative recovery of fluorine from a highly fluorinated material. Extremely vigorous conditions are needed for quantitative mineralization of fluorinated compounds due to the exceptional strength of the carbon–fluorine bond. Conventional combustion conditions used for determination of carbon and hydrogen in typical organic compounds are not adequate for total fluorine determination. Sample decomposition in a Wickbold decomposition apparatus where the product of sample vaporization or pyrolysis is swept through an oxy-hydrogen flame is an extremely efficient means of fluorine mineralization even for perfluorinated materials [18–21]. The method was demonstrated to be compound independent [19] for fluorine-containing compounds and has been previously applied to the analysis of total fluorine in relatively “clean” matrices at high concentration levels. The original Wickbold decomposition apparatus was later modified by Sweetser [20] by adding a bypass valve that allows continuous operation as well as modifying the cooling system for air cooling that allows higher mineralization temperatures than the previously used water-cooled version.

To our knowledge, the Wickbold decomposition method has been traditionally applied to analysis of total fluorine at high concentrations, and its application to trace concentration levels in a biological matrix such as blood has not been previously described. This paper demonstrates that the modified original decomposition method first described by Wickbold for analysis of halogens [18,19] and later modified by Sweetser [20] for continuous operation and increased mineralization temperatures can be successfully applied to analysis of total fluorine in blood samples at low concentration levels when combined with fluoride ion determination via static differential potentiometry using fluoride ion-selective electrode.

2. Experimental

2.1. Apparatus and instrumentation

The Wickbold decomposition apparatus was custom made. The quartz and glass components of the system were purchased from H.S. Martin, Inc., Vineland, NJ, USA. The parts that were subjected to high temperatures or high thermal stress were made of quartz (a burner nozzle, a flame chamber, an elbow connector, a hand-held flame probe, and the coil in a coil condenser). The material for the rest of the apparatus was borosilicate glass. Routine consumable glassware (burner nozzle, quartz elbow, and quartz coil condenser) was later supplied by Pesce Lab Sales, Kennett Square, PA, USA. Hydrogen, oxygen, and propane gases were obtained from MG Industries, Malvern, PA, USA. Hydrogen and propane were reagent grade purity and oxygen was ultra high purity. Vacuum in the Wickbold apparatus was maintained via use of a Leybold Trivac® B vacuum pump model D4B equipped with an AF 4–8 exhaust filter both from Leybold Vacuum, Export, PA, USA. Samples were combusted during the mineralization process using 7 mL porcelain combustion boats from CoorsTek, Inc., Golden, CO, USA.

Direct potentiometric determination of fluoride ion in samples mineralized by the Wickbold apparatus was done using a Metrohm Titrino 751 titrator. Automation was possible via a 16-position Metrohm Sample Handler Model 760-16 connected with the Titrino 751 and controlled by a Brinkmann Workcell 4.3 software. The Metrohm 760 sample changer was later replaced with 136-position Metrohm 748 DH Sample Changer due to increased demand on post-mineralization analysis automation. All Metrohm equipment, as well as the Workcell software, were purchased from Brinkmann Instruments, Inc., Westbury, NY, USA. Fluoride potentiometric measurements were done with a combination fluoride ion-selective electrode (F-ISE) model 9409BN from Thermo Orion, Beverly, MA, USA. The electrode was polished as needed using F-ISE polishing paper obtained from Beckman Coulter, Fullerton, CA, USA.

2.2. Chemicals and reagents

All standard stock solution preparation was done using sodium fluoride, >99% purity from Aldrich, Milwaukee, WI, USA. Sodium fluoride was dried to constant weight at 110 °C in a laboratory oven prior to use. Sodium acetate (NaAc) pH 5 buffer was prepared using 30% NaOH solution (prepared from 50% NaOH solution from E. M. Science, Gibbstown, NJ, USA) and glacial acetic acid from J.T. Baker, Phillipsburg, NJ, USA. NaAc buffer is prepared by adding 460 mL of glacial acetic acid to 2800 mL of deionized water and adjusting, the pH to 5.0 with 30% NaOH, and diluting to 4 L with deionized water. All total fluorine method validation was performed using pentadecafluorooctanoic acid ammonium salt (>98% purity, Fluka, Milwaukee, WI, USA) as a sample source of covalently bound fluorine. Water used for any task associated with total fluorine analysis was generated using a NANOpure Diamond UV Ultrapure Water system purchased from Barnstead/Thermolyne, Dubuque, IA, USA, with resistivity values not less than 18.0 MΩ cm (typically 18.2 MΩ cm) resulting in water with less than 0.1 parts per billion (ppb) F^-. All samples for total fluorine determination validation were prepared and diluted on a weight basis and contained 90% of the whole blood matrix and 10% of the spiked solution. Blood for sample preparation was obtained internally from mature Sprague–Dawley strain rats. To minimize sample target fluorine concentration variation whenever possible, extra care was taken to ensure that blood for replicate validation analyses was from the same animal, and the samples were prepared in bulk volumes to ensure spiked F concentration consistency within a series of replicates. Sample size in our study was 0.5 g of spiked rat blood.

2.3. Experimental procedure for a typical sample analysis

The experimental procedure for a total fluorine determination by the Wickbold decomposition method consists of two distinct steps:

1. Total mineralization of the organic biological sample via an oxy-hydrogen flame in a quartz/glass enclosed apparatus under reduced pressure and subsequent collection of the resulting free inorganic fluoride (F^-) in a suitable absorption medium.
2. Determination of fluoride resulting from the mineralization process by a suitable analytical technique. Automated static differential direct potentiometry with F-ISE was used in this case.

2.3.1. Mineralization process

Decomposition of biological samples was done using the apparatus schematically shown in Fig. 1 (Wickbold Torch, WT). The WT can be operated in two modes: a Sample Collection Mode and a Sample Transition Mode.

The Sample Collection Mode is active during the sample mineralization process. In this mode, three-way stopcock valves S-2, S-3, and S-4 direct the flow of gases and combustion products through the collection tower, which is filled with a small amount of deionized water (S-1 closed). A 0.5 g blood sample previously placed in the volatilization chamber is combusted for 30 min. The combustion is initiated by heating the volatilization chamber using a hand-held hydrogen/oxygen probe and then heated with a propane-fueled Bunsen burner. All external heating is stopped 5 min before the system is switched to the Sample Transition Mode.

The Sample Transition Mode becomes active during system preparation for analysis of the next sample. In this mode valve S-1 can be open or closed, the three-way valve S-2 directs the gases and combustion products to waste via a small drain vessel that collects the water then synthesized from oxygen and hydrogen. The air vent valve S-4 is open to atmosphere. The sample collected in the collection tower can be expelled to an appropriate vessel for subsequent analysis by detaching joint J-2 and applying positive pressure with nitrogen. The collection tower is then rinsed with water (the rinses combined), and re-filled with 20 mL of water to serve as an absorption medium for the mineralization of the next sample. In this mode, after detaching joint J-1 and removing the mineralization boat, the volatilization chamber is flame-cleaned using a quartz hand-held hydrogen/oxygen probe. New sample is then inserted and the supply of sweep oxygen re-established. The system is switched to the Sample Collection Mode by re-attaching the joint J-2, switching valves S-3 and S-4 to the Collection Mode positions, opening valve S-1, re-directing valve S-2 to the Collection Mode and, finally, closing valve S-1. The timing is started at this point. To switch from the Sample Collection Mode back to the Sample Transition Mode, the reverse of the above procedure is performed.

Before the apparatus startup, the system exhaust gas flow rate has to be set to approximately 2100 mL min^{-1} (1900–2400 mL min^{-1} depending on the characteristics of the individual mineralization apparatus) via use of valve S-6. The exhaust gas flow rate must be equivalent in the Sample Collection Mode, Sample Transition Mode, and the momentary change-over mode when gases flow via valve S-1. This balance is critical for smooth operation of the apparatus and is achieved by careful adjustment of valves S-1, S-5, and S-6.

To start the apparatus, a small flow of hydrogen is introduced and ignited at the burner nozzle of the volatilization chamber while it is detached from the rest of the WT. Gas flows are established (H_2 ~4000 mL min^{-1}, O_2 ~3000 mL min^{-1}, cooling air 30 psi, 206.8 kPa), volatilization chamber attached to flame chamber, sample inserted,

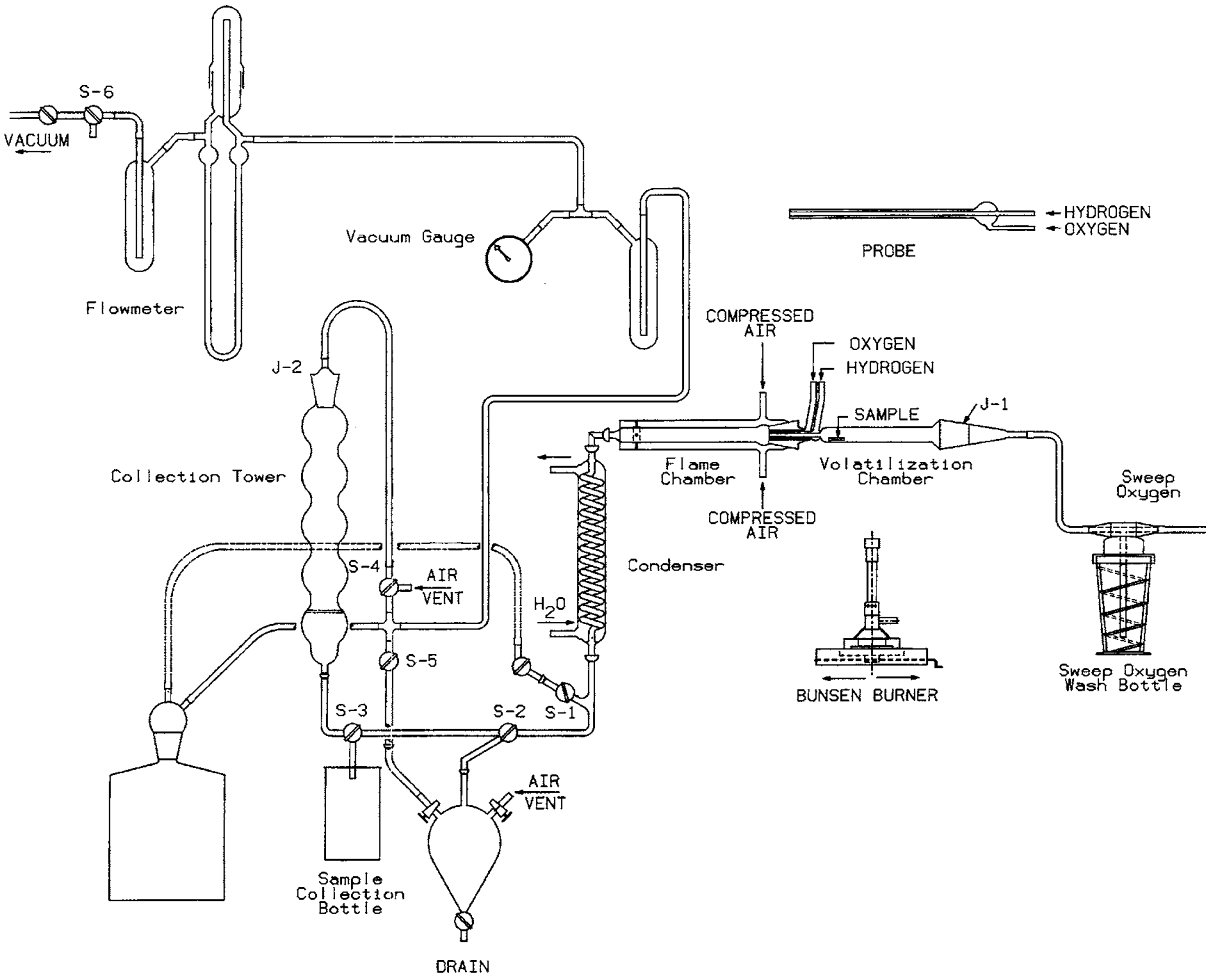

Fig. 1. Wickbold decomposition apparatus (Wickbold Torch, WT).

sweep O_2 adjusted to $\sim$900 mL min^{-1} and the system switched to the Sample Collection mode as described above.

At the beginning of each analysis batch, the background fluorine level has to be established via measurement of 'System Blank' (SB). The SB is measured identically to the sample using an empty combustion boat. If the SB shows higher than typical day-to-day values, the SB has to be re-burned until stable steady-state values are achieved. This provides a stable background fluorine level in the system that will be subtracted from all the samples during that day's measurements.

2.3.2. F^- determination in mineralized samples

After mineralized sample is collected in the Sample Transition mode, a 3 mL standard addition of 1 ppm F^- standard solution is added as well as 7.5 mL of NaAc buffer. Then each sample is filled with water to a constant weight of 150.0 g, which makes the concentration of F^- added by standard addition exactly 20 ppb. This process is followed for each sample and each SB. Quantitation of F^- was done via a calibration curve of dE versus log[F^-], where dE is the difference between the F-ISE potential of 20 ppb reference solution and the F-ISE potential of sample (which has a standard addition of 20 ppb F^-). The standards used for the calibration were 100, 50, and 30 ppb F^- (measured in that order). Each standard solution contained 5 mL of NaAc buffer per 100 mL of solution. Solutions were measured from the highest standard concentration to the lowest, followed by all system blanks (for a batch of measurements), followed by all mineralized sample solutions. A buffered 20 ppb F^- reference solution was measured prior to every measurement of sample, SB, or standard. Measurement of every solution was taken at exactly 10 min after the electrode was equilibrated under constant propeller stirring. Timing and stirring were controlled electronically. After every solution measurement, the electrode was automatically washed using a buffered 10 ppb F^- solution. The F-ISE was polished weekly using a polishing paper supplied by Beckman Coulter, Fullerton, CA, USA. The electrode was stored in 20 ppb F^- reference solution when not in use.

2.3.3. Safety considerations

The WT uses a hydrogen/oxygen (H_2/O_2) flame which when operated improperly can result in dangerous explosive conditions. Extra caution must be exercised while lighting and extinguishing flames. The entire apparatus was located behind a closed safety glass shield which remained closed whenever possible during the operation. In addition, a hydrogen electronic shut-off valve allowed immediate shutdown of hydrogen to the burner nozzle by a remote switch located outside of the apparatus area and within the immediate reach of the operator. This valve also prevented hydrogen flow to the burner nozzle during a power failure in order to avoid pressurization of the apparatus due to loss of vacuum. Individual emergency shut-off valves were also installed for each gas line leading to the apparatus. These valves were located outside of the apparatus area but within immediate reach of the operator, enabling the operator to shut down all gases without reaching into the potentially hazardous apparatus area during a possible malfunction.

It is important to stress the critical nature of the sequence of switching the stopcock valves when switching the apparatus from the Sample Collection mode to the Sample Transition mode and vice versa. It is critical that valve S-1 is always open when manipulating valve S-2. An incorrect switching sequence can cause an imbalance between the system vacuum and the high flow of gases through the system. This may lead to violent back-firing of the sweep oxygen supply attachment and meltdown of the burner nozzle.

The WT apparatus contains hot surfaces that can cause severe burns. It is also important to ensure that the cooling air and water are always on whenever the flame is present to avoid overheating of the apparatus.

Before total F determination by WT was started in our laboratory, we conducted an extensive hazard review. This process was specific to our laboratory and resulted in the safety measures mentioned above. Due to substantial differences between many laboratory facilities, additional precautionary measures might be necessary. Therefore, readers are strongly encouraged to conduct their own facility-specific hazard assessments when trying to set up a total F analysis based on the description in this paper. The discussion above can serve as a valuable source of ideas when trying to do so.

3. Results and discussion

3.1. Determination of F^- in the mineralized samples by F-ISE

Evaluation of total fluorine determination by WT with direct potentiometric detection was accomplished in two steps. In order to understand the extent of experimental and instrumental variability of the F-ISE measurements, the F^- determination in simulated mineralized samples was evaluated first. Limitations of any F-ISE potentiometric method come from the limitations of the F-ISE itself: (a) 20 ppb F^- limit of quantitation as specified by electrode manufacturers, and (b) non-linearity of the electrode response (E versus log concentration, where E is the electrode potential) at concentrations of approx. 800 ppb F^- and less. The non-linearity was compensated by quantifying within a narrow range of calibration standards. The quantitation limits were decreased by utilizing differential potentiometric measurements. This was accomplished by adding 20 ppb F^- to samples and subtracting potentials of 20 ppb F^- reference standards (measured immediately prior to each sample) from the potential of the corresponding samples. The difference in potentials corresponds to the potential due to F^- in the original solution. This difference was then used in calibration curves.

Mineralized samples were simulated by preparing NaF solutions in water with a F^- content at levels expected in biological blood samples. For a blood sample, an aliquot would be weighed out, mineralized, collected at the end of the burning cycle, have standard F^- and NaAc buffer added, and be brought to a constant weight (150.0 g) as described in Section 2. For evaluation of the F-ISE determination, the same aliquots of NaF solutions were weighed, transferred to appropriate containers, and treated the same way as the mineralized solutions (addition of F^- standard, buffer, filling to constant weight). These steps were necessary to account for the variability contribution to the F-ISE analysis from all procedures performed with a blood sample, excluding the actual mineralization. As can be seen from Section 2, the mineralization process results in 300-fold dilution of the original sample (sample size 0.5 g, final weight after mineralization 150 g). The differential measurement was necessary in order to improve the quantitation limits since the 20 ppb F-ISE quantitation limit would only correspond to the total F in a 0.5 g sample at 6 ppm concentration level, and analysis of samples with lower concentration was required.

The potentiometric method for F^- determination in mineralized sample solutions was evaluated in terms of its accuracy and precision as indicated by relative errors and standard deviations of replicate measurements at different initial sample concentrations. The results are presented in Table 1. As indicated by these results, the accuracy and precision of the method can be improved approximately two-fold when more accurate, volumetric glassware is employed for solution preparation and volume transfers during the analysis.

The accuracy and precision of the technique did not improve when higher F^- concentrations were analyzed as indicated by the standard deviation and %CV values for the analysis of 100 ppm simulated samples. This is due to the fact that the F^- concentrations in the final simulated WT solutions resulting form 100 ppm samples had to be diluted 20× to the calibration curve range.

The 100 ppm series of measurements as shown in Table 1 originally contained one significant outlier. This resulted from the electrode resting in water (e.g. during, loading/unloading the sample changer) for an extended period of time (more than 30 s). A similar effect was observed multiple times during our method development. It occurs when the electrode entering the next sample solution (after resting in water) is polarized to very high potentials.

Table 1
Precision and accuracy determination of the differential potentiometric determination of F^- in simulated mineralized solutions for total F determination

Actual F^- concentration (ppm)	Operator I[a]		Operator II[b]	
	Determined F^- concentration (ppm)	Relative error (%)	Determined F^- concentration (ppm)	Relative error (%)
1.0 ($n = 10$)			0.98 ± 0.09 (±9.64%)	7.2 ± 6.0
5.0 ($n = 10$)	5.31 ± 0.18 (±3.47%)	6.1 ± 3.7	4.92 ± 0.09 (±1.87%)	2.2 ± 0.8
100 ($n = 10$)	105 ± 3.86 (±3.66%)	5.4 ± 2.9	104 ± 4.90[c] (±4.73%)	5.5 ± 2.0

[a] Graduated glassware used for volume transfers and solution preparation.
[b] Class A volumetric glassware used for volume transfers and solution preparation.
[c] $n = 9$.

Consequently, the 10 min equilibration time was not sufficient for that solution. In order to minimize this effect, a 10 ppb F^- solution was used as a wash solution in the automated measurements, and 20 ppb solution was used for the electrode storage. We found that the manufacturer's recommended storage conditions would significantly compromise the electrode performance at near-the-limit-of-quantitation concentration levels. The manufacturer-recommended storage conditions were intended for electrode use in the linear portion of its response (above 800 ppb F^-).

A similar effect was observed for the 20 ppb reference measurement measured right after 100 ppb standard. A 100 ppb standard (highest concentration standard) produces relatively low electrode potentials compared to the rest of the standards and samples. Therefore, the next solution might require a slightly longer equilibration time. It is critical for total F analysis that the 20 ppb reference corresponding to the system blank for a sample set is not influenced by this effect because all the results for that sample set depend on the value and accuracy of the measurement of the system blank (and its 20 ppb reference solution). To avoid this situation, the standards should be measured in a descending order, that is 20, 100, 20, 50, and 20, 30 ppb (where the 20 ppb samples were reference solutions measured before each standard, see Section 2). When standards are measured in this order, it is the 20 ppb reference corresponding to the 50 ppb standard which will be slightly affected and not the system blank reference. The effect on the 50 ppb measurement is minimized by means of linear regression performed on all three standards. This is also supported by the fact that the correlation coefficients of our calibration curves from multiple days were higher than 0.999 (typically values higher than 0.9999 were observed). Minimizing the effects described above is critical for the total F measurements at low concentration levels (less than 5 ppm) since the potential differences (20 ppb versus the sample, system blank, etc.) of less than 2 mV need to be measured accurately and reproducibly. For this reason, the electrode was polished weekly, when new, and after extended periods of storage. Electrode-to-electrode variability of new electrodes was so great that they had to be polished prior to initial use. A good indicator of the electrode functionality is the evaluation of the slope of the calibration curve of dE versus log c_F, where dE is the potential difference between the 20 ppb reference and the standard and c_F is the standard concentration. Our criterion for the working electrode was based on the deviation of the actual calibration curve slope from the theoretical one. The working electrode response slope should not deviate more than five units from the theoretical electrode response slope as defined by the Nernst equation at actual room temperature. If all associated solutions were prepared properly, the value of $10^{-b/a}$, where b is the calibration curve intercept, and a is the calibration curve slope, should equal 0.02, because each point of the calibration curve is the potential difference between 20 ppb reference and the actual standard. This value was closely monitored, and, in order to proceed with the analysis and assure day-to-day result reproducibility, it had to be within 4% of the previous day's value. If one of the aforementioned criteria failed, it was an indication of changing electrode integrity or inaccuracy in the solution preparation. In such cases, the electrode needed to be polished, because the accumulated deposits on its surface had an adverse effect on its response time.

Calculations of total F in the original sample before the mineralization process were performed using Eqs. (1) and (2),

$$c_F = \frac{(D \times 10^{(\mathrm{d}E-b)/a} - 0.02) \times \mathrm{Wt_{fin}} - \mathrm{BL}}{\mathrm{Wt}} \quad (1)$$

$$\mathrm{BL} = (10^{(\mathrm{d}E_{\mathrm{bl}}-b)/a} - 0.02) \times \mathrm{Wt_{fin\text{-}bl}} \quad (2)$$

where c_F is the total F concentration in the original sample (in ppm), dE is the potential difference between 20 ppb reference standard and a sample (in mV), dE_{bl} is the potential difference between 20 ppb reference standard and a system blank measurement (in mV), $\mathrm{Wt_{fin}}$ and $\mathrm{Wt_{fin\text{-}bl}}$ is the final weight of the sample and the system blank solution after filling to the constant weight after the mineralization process (typically 150.0 g), D is the dilution factor of the final solution after the mineralization process but before the F-ISE measurement ($D \geqq 1$), BL is the total F contribution from the system blank measurement (in μg), a is the calibration curve intercept, and b is the calibration curve slope. The system blank measurement used in the calculation is reported on an absolute weight basis (in μg of F^-) rather than as a concentration (in ppm) due to inapplicability of sample weight during the system blank measurement. Consequently, the concentration term would be inapplicable.

Table 2
Evaluation of determination of F^- during extended, 24 h automated analysis

Actual F^- concentration (ppm)	Determined F^- concentration (ppm)	Relative error (%)	20 ppb F^-, $E(1)$–$E(60)$ (mV)
1.0 ($n = 60$)	0.91 ± 0.03 (±3.35%)	9.0 ± 3.1	1.3
20 ($n = 60$)	19.7 ± 0.17 (±0.88%)	1.7 ± 0.9	1.3

As clearly indicated by the above discussion, one has to sacrifice sample throughput in order to achieve the best possible accuracy and detection limits. Sample throughput limitations were overcome by performing the mineralization process in parallel by two independent operators each using their own WT apparatus. Due to the throughput limitations of the potentiometric measurement, determination of F^- in mineralized samples was the rate-limiting step in our entire analytical process. For these reasons, a 136-position sample changer was also evaluated for increased sample throughput. The evaluation was done in a similar manner as described above. F^- determination in simulated mineralized solutions was conducted at 20 and 1 ppm concentration levels in the original samples, which corresponds to 66.7 and 3.3 ppb F^- in the mineralized solutions, respectively. Results are summarized in Table 2. These samples were each analyzed 60 times to evaluate instrument stability as well as the influence of sample evaporation on the analytical results during the time course of the analysis sequence. Due to extended sequence run times, solution evaporation during the unattended operation was of a great concern. It can be seen from Table 2 that the potential of 20 ppb F^- reference standard decreased by 1.3 mV from sample 1 to sample 60. This is the indication that the solution evaporation is indeed taking place to a significant degree, however the same is true for sample solutions thus making the potential difference constant. Therefore, the 24 h sequence duration did not compromise the accuracy and precision of the method. An attempt was made to increase the sample throughput by measuring the 20 ppb reference standard just once and using the resulting electrode potential as a reference for all other samples in a sequence of measurements. However, as can be clearly seen from the above discussion and Table 2, this approach would result in approximately 1.3 mV electrode potential error over the 24 h sequence. This would in turn result in a gradual drift in measured concentration up to approximately 50% of the concentration value at 1 ppm total F.

To maximize sample throughput, the total F determination in rat blood by the Wickbold decomposition method was conducted in parallel by two independent operators performing the mineralization step in parallel and the analysis of the resulting samples using one automated potentiometric analyzer as described earlier. The WT mineralization process (described in Section 2) is a crucial step in the entire analysis and involves a number of manual steps and settings that are unique for each individual WT apparatus due to slight differences in their configuration and flow characteristics. Therefore, the evaluation of the technology was conducted individually for each operator and the corresponding apparatus.

The entire total F validation was done using pentadecafluorooctanoic acid ammonium salt (>98% pure) that was spiked into blank rat blood at appropriate concentrations. Samples were then analyzed and the resulting data interpreted for assessment of the method carryover, accuracy, and precision. The compound contains all organically bound, covalent F to demonstrate that the mineralization process can readily and quantitatively disrupt strong C–F bonds. The compound was also selected for is high aqueous solubility, commercial availability with a reasonable purity, and ease of handling. All blood samples were spiked with an aqueous solution of pentadecafluorooctanoic acid ammonium salt on a weight/weight basis. All spiked blood samples contained 90% blood matrix and 10% water from the standard solution.

The extent of possible carryover during the sample mineralization process was studied by comparing the absolute fluorine mass yield from the system blank experiments that were measured right after samples with increasing total fluorine concentrations. The resulting fluorine amounts were compared to the system blank that was established during the routine system startup (as described in Section 2). Results from these experiments are summarized in Table 3. As can be seen in this table, a slight increase in the background fluorine was observed. However, we believe that the extent of this increase is not significant enough to impact 0.5 g samples up to the concentration of 100 ppm F.

As indicated by results from replicate analyses of rat blood samples summarized in Table 4, the results obtained by both

Table 3
Evaluation of carryover of Wickbold mineralization process for rat blood samples

Actual F concentration (ppm)	Determined F concentration (ppm)	Relative error (%)	System blank F content (μg)
System blank 1			0.750
25.42	23.64	−7.0	
System blank 2			0.769
101.36	96.04	−5.2	
System blank 3			0.879

Table 4
Accuracy and precision evaluation for the determination of total F in rat blood, with 0.5 g sample size

Operator I			Operator II		
Actual concentration (ppm)	Measured concentration (ppm)	Relative error (%)	Actual concentration (ppm)	Measured concentration (ppm)	Relative error (%)
23.5 ($n = 3$)	22.5 ± 0.87 (±3.86%)	4.3 ± 3.7	25.6 ($n = 3$)	26.1 ± 1.07 (±4.11%)	3.4 ± 2.5
5.02 ($n = 5$)[a]	4.92 ± 0.09 (±1.88%)	2.9 ± 1.7	4.96 ($n = 5$)	4.86 ± 0.08 (±1.59%)	2.1 ± 1.5
			1.01 ($n = 8$)[b]	0.99 ± 0.08 (±8.20%)	7.1 ± 3.4
			0.52 ($n = 5$)	0.46 ± 0.06 (±12.04%)	12 ± 11

[a] Samples analyzed over 4-day period.
[b] Samples analyzed over 2-day period.

Table 5
Accuracy and precision evaluation for the determination of total F in rat blood at low concentrations for Operator I, with 0.5 g sample size

Average actual concentration (ppm)	Average measured concentration (ppm)	Standard deviation (ppm)	F content corrected for background (ppm)	Relative error (%)
Blank ($n = 13$)[a]	0.24	0.099		
1.03 ($n = 27$)[b]	1.18	0.069	0.94	−8.9
0.52 ($n = 9$)[a]	0.70	0.061	0.46	−11.3

[a] Samples analyzed over 5-day period.
[b] Samples analyzed over 10-day period.

operators at high concentration levels (5 ppm and higher) are relatively equivalent in terms of their accuracy and precision. At low total F blood concentrations (<5 ppm F), the situation was different. The data at the concentrations below 5 ppm F for Operator II are also included in Table 4. It is interesting to note that better reproducibility and accuracy were achieved at 4.96 ppm than at 25.6 ppm. This may be because the 4.96 ppm samples were analyzed at a later time and the operator had the opportunity to gain more experience with the combustion method in general. This would suggest that the operator technique while operating the mineralization apparatus is one of the important factors influencing the accuracy and reproducibility of the method. The operator dependency may be explained by the fact that the operators have great ability to control the rate of the initial combustion and transport of initial combustion products from the burner nozzle to the flame chamber for final combustion. These processes are associated with the degree of external heating relative to linear velocity of the sweep oxygen and burner nozzle gases during the initial sample combustion. It has been previously reported that the mineralization recoveries are greatly dependent on rate of transfer of sample to the flame for combustion [22]. Since each apparatus has its unique operating parameters and gas flow characteristics, the above discussion would suggest that at low concentration levels these validation data should be linked to a specific operator using a specific mineralization apparatus. Data from these experiments was generated using the same apparatus that Operator I used to generate the results in Table 4. The resulting data are summarized in Table 5. Upon careful examination of the average measured concentration values, it can be seen that the results are approximately 0.2 ppm higher than the targeted concentration of F spiked into the samples. The constant nature of this systematic error suggests that the blank blood of these samples already contained some background level of F. If this is true and the operator performed the analysis in a consistent manner, standard deviations of these measurements should be ideally the same. Indeed, the values of standard deviations of the replicate measurements for these levels are equivalent as indicated by Table 5. In order to evaluate the background F level for these samples, blank blood samples were analyzed using the same blood that was used to prepare these samples. As seen from the results shown in this table, the blank blood contained on average about 0.24 ppm F. The reproducibility of this measurement is not as great as for the other results shown, because these results are very close to the method limit of detection (LOD, see Table 6). As shown in Table 5, after the

Table 6
Determination of limit of quantitation and limit of detection of total F determination in rat blood (0.5 g sample size)

	Average actual concentration (ppm)	Average standard deviation of F determination (ppm)
Operator I	Blank ($n = 13$)	0.099
	0.52 ($n = 9$)	0.061
	1.03 ($n = 27$)	0.069
	5.02 ($n = 5$)	0.093
Average		0.081
LOD (3× average S.D.)		0.24
LOQ (6× average S.D.)		0.49
Operator II	0.52 ($n = 5$)	0.055
	1.01 ($n = 8$)	0.085
	4.96 ($n = 5$)	0.077
Average		0.072
LOD (3× average S.D.)		0.22
LOQ (6 × average S.D.)		0.43

correction of the applicable results for the background F in blank blood, the results show acceptable accuracy. The determined blank blood concentration was deemed to be due to background F since the average blank value was at the LOD of the method (Table 6). It should be mentioned, however, that the stated value is only an estimate because the value was under the limit of quantitation (LOQ). It is realistic to expect some background level of F in blank rat blood because of the F content in their diet. The F-ISE method described in this paper was used to determine the concentration of ionizable inorganic F^- in the water that is generally given to rats in our animal facility. The water contained 0.9 ppm F^-. In addition, the standard rodent diet also contains F in the range of 8–15 ppm (data supplied by Purina Mills, diet supplier). Because of the great variation of the F content in this diet, not all blood will contain the same background F concentration. The blood used by Operator I for blank, 0.5 and 1 ppm levels was from the same batch of animals.

Assuming that the total F results produced by the Wickbold decomposition method described in this paper with combination with F-ISE F^- determination behave statistically as normal distribution, the LOD and LOQ can be estimated from the standard deviation of replicate measurements of concentration levels close to the LOQ. LOD was estimated as 3× the average standard deviation at low F concentration levels. LOQ was established taking into account the accuracy needed for the majority of applications for which this method was intended in our laboratory. LOQ was estimated as 6x the average standard deviation at low F concentration levels. LOD and LOQ data for total F determination in blood by both operators are shown in Table 6. As indicated by the data, both operators achieved approximately equivalent LOD (0.24 ppm) and LOQ (0.5 ppm). Furthermore, these results, along with the results presented in Tables 4 and 5, indicate that both operators achieved approximately equivalent accuracy and precision, indicating that the operators performed the analysis in an equivalent manner. Although these data might be equivalent, the data are *not* inter-exchangeable nor applicable to other matrices. As a result, one should apply these validation data to other than blood matrices only with great caution, since these matrices might require adjustment in the burning technique due to their different matrix composition.

It is clear from the discussion above, that the Wickbold decomposition method as described in this paper is operator-dependent, allows for low throughput, and is very skill and experience intensive. All these attributes might be considered method disadvantages; however, when we tried to compare the methodology to other published [6,7,9] or commercially available techniques, we were unable to reach acceptable accuracy, precision, and detection limits for the complicated biological matrices required for our applications. Improvement of detection limits of the current methodology as described here may be further achieved by decreasing the final collection volume of the mineralized solution and/or increasing the sample size being mineralized. According to our experiments to date, the sample size increase is a more viable option for matrices with very low solid content (such as water, serum). On the other hand, the method performance might be improved for matrices with higher solids contents (animal organs), which in turn yield a more controllable burning process.

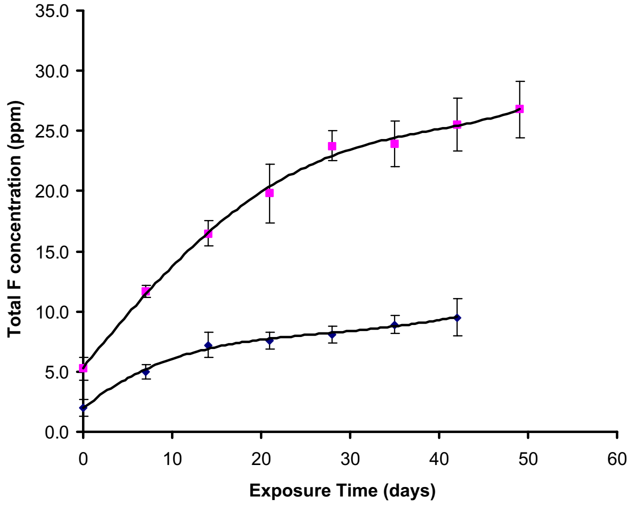

Fig. 2. Time-profile of total F concentration in rat blood from laboratory animals dosed with a complex mixture of fluorinated compounds over a 49-day exposure period at two dose levels (500 and 2000 mg kg^{-1} per day). Each time-point represents concentration measured in five laboratory animals.

The method as described in this paper has been successfully used in toxicological support of various studies with a number of fluorinated compounds. Fig. 2 demonstrates the usefulness of the method when it was used in support of a toxicological study with a complex mixture of fluorinated compounds where the development and application of compound-specific methods was impossible due to complexity of the mixture and the sample matrix relative to the properties of the compounds studied. The complex mixture of fluorinated compounds was dosed to laboratory animals over a period of 49 days at 500 and 2000 mg kg^{-1} per day. Total F concentrations in rat blood as functions of the exposure time for the two dose levels are shown in Fig. 2. Each time-point represents the concentration measured in five different animals. The figure demonstrates that the time-profiles of total F in rat blood for the two dose levels could be readily established and the steady-state blood concentrations and times could be easily estimated.

4. Conclusions

Determination of total F by the Wickbold decomposition method with F-ISE differential potentiometric detection has been evaluated for rat blood samples in terms of its analytical performance. For maximum sensitivity, differential static potentiometric detection was combined with the mineralization process, yielding an LOD of 0.24 ppm F and an LOQ of 0.5 ppm F for 0.5 g blood samples. The mineralization method proved to be time consuming, labor intensive and, at low concentration levels, specific to a particular operator and apparatus. Despite these disadvantages, we were able to achieve acceptable sensitivity and reproducibility in rat blood. We were unable to do so using alternative methods at these concentration levels. Detection of F^- after the mineralization process was fully automated. Because of high temperature during the sample decomposition, the current method is totally destructive and, therefore, compound-independent [19]. The method was successfully used for routine toxicological support.

The Wickbold decomposition method in combination with F-ISE as described here can be easily applied to total F analyses in other biological matrices, such as animal organs, whole fish, and environmental matrices such as water. The method performance might be improved for some of these matrices through improvements in the burning process control (high solid content matrices) or an increase in the sample size for mineralization (highly aqueous matrices). Special attention should be paid to fat-rich samples since fat will serve as a fuel in the mineralization process, resulting in extreme temperatures and thermal stress on the apparatus.

References

[1] M.M. Schultz, D.F. Barofsky, J.A. Field, Environ. Eng. Sci. 20 (2003) 487.
[2] D.R. Taves, Nature 217 (1968) 1050.
[3] P. Venkateswarlu, L. Singer, W.D. Armstrong, Anal. Biochem. 42 (1971) 350.
[4] Y. Bessho, M. Tomita, Y. Kaneko, Stud. Environ. Sci. 27 (1985) 73.
[5] J. Alary, P. Bourbon, C. Balsa, Anal. Chim. Acta 148 (1983) 311.
[6] P. Venkateswarlu, Anal. Chem. 54 (1982) 1132.
[7] J. Belisle, D.F. Hagen, Anal. Biochem. 87 (1978) 545.
[8] M. Tomita, K. Tadokoro, T. Sugimura, S. Tada, Y. Kaneko, J. Dental Health 39 (1989) 767.
[9] P. Venkateswarlu, M.A. LaCroix, G.W. Kirsch, Microchem. J. 48 (1993) 78.
[10] P. Venkateswarlu, Anal. Chem. 46 (1974) 878.
[11] E. Kissa, Anal. Chem. 55 (1983) 1445.
[12] K. Itai, H. Tsunoda, Clin. Chim. Acta 308 (2001) 163.
[13] P. Venkateswarlu, J. Dent. Res. 69 (1990) 514.
[14] P. Venkateswarlu, Adv. Dent. Res. 8 (1994) 80.
[15] G.H. Vickers, D.A. Wilson, G.M. Hieftje, Anal. Chem. 60 (1988) 1808.
[16] D.F. McMillen, D.M. Golden, Ann. Rev. Phys. Chem. 33 (1982) 493.
[17] J. Berkowitz, G.B. Ellison, D. Gutman, J. Phys. Chem. 98 (1994) 2744.
[18] R. Wickbold, Angew. Chem. 64 (1952) 133.
[19] R. Wickbold, Angew. Chem. 66 (1954) 173.
[20] P.B. Sweetser, Anal. Chem. 28 (1956) 1766.
[21] E. Kissa, Fluorinated Surfactants and Repellents, second ed., Marcel Dekker, New York, 2001, pp. 390–439.
[22] D. Erber, J. Roth, K. Cammann, Fresenius J. Anal. Chem. 358 (1997) 585.