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फ्लोराइड कॉन्सेंट्रेशन का विश्लेषण

**Dentistry — Analysis of Fluoride
Concentration in Aqueous Solutions
by Use of Fluoride Ion-Selective
Electrode**

ICS 97.170

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NATIONAL FOREWORD

This Indian Standard which is identical to ISO 19448 : 2018 'Dentistry — Analysis of fluoride concentration in aqueous solutions by use of fluoride ion-selective electrode' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Dentistry Sectional Committee and after approval of the Medical Equipment and Hospital Planning Division Council.

The text of ISO standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are however not identical to those used in Indian Standards. Attention is particularly drawn to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and
- b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards which are to be substituted in their respective places are listed below along with their degree of equivalence for the editions indicated:

<i>International Standard</i>	<i>Corresponding Indian Standard</i>	<i>Degree of Equivalence</i>
ISO 835 Laboratory glassware — Graduated pipettes	IS/ISO 835 : 2007 Laboratory glassware — Graduated pipettes	Identical
ISO 1942 Dentistry — Vocabulary	IS 17895 : 2023/ISO 1942 : 2020 Dentistry — Vocabulary	Identical
ISO 3696 Water for analytical laboratory use — Specification and test methods	IS 1070 : 2023 Reagent grade water — Specification (<i>fourth revision</i>)	Technically Equivalent

The Committee has reviewed the provisions of the following International Standards referred in this adopted standard and has decided that they are acceptable for use in conjunction with this standard:

<i>International Standard</i>	<i>Title</i>
ISO 78-2	Chemistry — Layouts for standards — Part 2: Methods of chemical analysis

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

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Introduction

Fluoride is used in dentistry primarily for caries prevention. Fluoride is the active ingredient for caries prevention in many dental products such as dentifrices, gels, oral rinses, and fluoride releasing varnishes. The fluoride concentration in these products is an integral part of other standards where the product is intended to be analysed for fluoride content. This document provides methods based on the use of fluoride ion-selective electrode technology for the analysis of the total fluoride content in aqueous samples. The methods describe uses for fluoride ion-selective electrode technology and anticipate that the sample-specific preparations such as digestion, distillation, etc. have been described in the standards specific to product types.

Indian Standard

DENTISTRY — ANALYSIS OF FLUORIDE CONCENTRATION IN AQUEOUS SOLUTIONS BY USE OF FLUORIDE ION-SELECTIVE ELECTRODE

1 Scope

This document specifies test methods for the quantification of fluoride concentrations in dental products including dentifrices, gels, oral rinses, fluoride releasing varnishes, and other fluoride containing products. The methods are based on fluoride ion-selective electrode technology for the analysis of fluoride in aqueous samples derived from dental products.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 78-2, *Chemistry — Layouts for standards — Part 2: Methods of chemical analysis*

ISO 835, *Laboratory glassware — Graduated pipettes*

ISO 1942, *Dentistry — Vocabulary*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 78-2 and ISO 1942 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

4 Principle

This test method covers the determination of fluoride concentration in aqueous solutions derived from dental products. The following test methods are presupposed to use fluoride ion-selective electrodes (F-ISE).

The sensing element of the fluoride ion-selective electrode consists of a single crystal of europium (II)-doped lanthanum fluoride, LaF₃. When the sensing element is in contact with a solution containing fluoride ions, an electrode potential develops across the sensing element. This potential, which depends on the activity of free fluoride ion in solution {F⁻}, is measured against a constant reference electrode potential with a digital pH/mV meter or ISE (concentration) potentiometer. A potential (E) is developed which is proportional to the logarithm of the activity of the fluoride ion {F⁻} in solution following the Nernst Equation.

$$E = E_0 + S * \log \{F^-\} \quad (1)$$

where

E is the measured electrode potential;

E_0 is the reference potential (a constant);

$\{F^-\}$ is the fluoride ion activity level in solution;

S is the electrode slope (58,2 mV per decade change of $\{F^-\}$ at 20 °C).

The level of fluoride ion, $\{F^-\}$, is the activity or “effective concentration” of free fluoride ion in solution. The fluoride ion activity is related to free fluoride ion concentration, $[F^-]$, by the activity coefficient, γ . The activity coefficient is related to the ionic strength of the solution. For these measurements, the ionic strength of the samples and standards are held constant by the use of total ionic strength adjusting buffer solution (TISAB), which also chelates fluoride-binding cations and establishes the solution pH at the optimal level for the fluoride ion-selective electrode sensitivity. When using TISAB to set the ionic strength of the solution, the electric potential is proportional to the concentration of the fluoride ion in solution. TISAB may come in several variations. Which one to use is specific to the sample preparation procedure. One has to be aware that other interferences may exist and it is up to the user and sample preparation to properly identify them.

A calibration curve is drawn, plotting measured potential versus the log of the fluoride ion concentration of known standard solutions. Then, the potential for the unknown sample is measured and the concentration of F^- in the sample can be calculated from the calibration curve.

The sample will have been pretreated as specified for the particular sample type, and the final composition is an aqueous sample of at least 1 ml volume. Samples that contain more than 0,01 mol/l F (190 mg/l F) shall be diluted to concentrations less than 0,01 mol/l F with deionized water.

5 Reagents and materials

5.1 Chemicals to prepare total ionic strength adjusting buffer (TISAB) solution.

5.1.1 **Cyclohexylene dinitrilotetraacetic acid (CDTA) or 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid.**

5.1.2 **Acetic acid (CH₃COOH) reagent grade.**

5.1.3 **Sodium chloride (NaCl) salt.**

5.1.4 **Sodium hydroxide (NaOH), at 5 mol/l.**

5.1.5 **Deionized water**, in accordance with ISO 3696, grade 2.

5.2 Chemicals to prepare sodium fluoride standards.

5.2.1 **Sodium fluoride (NaF)**, previously dried at least 4 h at 150°C.

5.2.2 **Deionized water**, in accordance with ISO 3696, grade 2.

6 Apparatus

6.1 **Analytical balance**, accurate to 0,000 1 g.

6.2 Magnetic stir bar.

6.3 Magnetic stir plate.

6.4 Pipette, of nominal capacity 5,0 ml to smallest scale division: 0,05 ml; 1,0 ml to 0,01 ml; and 0,10 ml to 0,01 ml, in accordance with ISO 835, Class A.

6.5 Fluoride ion-selective electrode (F-ISE) with separate reference electrode, or combination F-ISE/reference electrode pair.

6.6 Plastic vial (or beaker or small container), 20 or more ml capacity.

NOTE Fluoride does not react with plastic but does react with glass.

6.7 pH/mV electrometer (pH meter), with a sensitivity of $\pm 0,1$ mV.

7 Preparation

7.1 TISAB solution

7.1.1 In a 1 l beaker with about 500 ml deionized H₂O, dissolve 57 ml CH₃COOH, 58 g NaCl, and 4 g CDTA or 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid.

7.1.2 Cool to room temperature and adjust pH to 5,0–5,5 with 5 mol/l NaOH.

7.1.3 Cool to room temperature, transfer to 1 l volumetric flask, and dilute to volume with deionized H₂O.

NOTE Preparation of TISAB solution is described in ASTM D1179–16 [\[1\]](#), 18.1.

TISAB is the buffering system that is used to prepare the sample for analysis via the fluoride ion-selective electrode (F-ISE). The F-ISE has several limitations that can limit its use. It is sensitive only to ionic fluoride in solution, and cannot respond to complexes of fluoride such as calcium-fluoride. Multivalent cations such as calcium, magnesium, iron, aluminium, etc., can form fluoride complexes in solution which reduces the ionic fluoride concentration. Additionally, the F-ISE is sensitive to variations in sample pH and to the ionic strength of the sample. For accurate analyses, the samples and the standards must have the same pH and ionic strength. The fluoride complexes must be disrupted such that all the fluoride in the sample and standard is in its ionic form. To address these limitations, a total ionic strength adjusting buffering system (TISAB) that adjusts the sample pH and ionic strength, and also contains chelators to bind the cations that can form fluoride complexes, is used.

TISAB II® (Orion Research) is available from Thermo Fisher Scientific, Beverly, MA 01915 USA¹⁾.

7.2 TISAB blank solution

Mix equal volumes of TISAB and deionized water for the blank solution.

7.3 Fluoride standard solutions

7.3.1 1×10^{-2} mol/l NaF standard solution: dissolve 209,9 mg accurately weighed NaF into 200 ml deionized H₂O, then quantitatively transfer to a 500 ml volumetric flask, and dilute to volume with deionized H₂O.

1) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

7.3.2 1×10^{-3} mol/l NaF standard solution: pipette 25,0 ml of 10^{-2} mol/l NaF standard solution to 250 ml volumetric flask. Dilute to volume with deionized H₂O.

7.3.3 1×10^{-4} mol/l NaF standard solution: pipette 25,0 ml 10^{-3} mol/l solution to 250 ml volumetric flask. Dilute to volume with deionized H₂O.

7.3.4 1×10^{-5} mol/l NaF standard solution: pipette 25,0 ml 10^{-4} mol/l solution to 250 ml volumetric flask. Dilute to volume with deionized H₂O.

7.4 Calibration curve

7.4.1 Pipette 5,0 ml of each standard solution into a plastic vial (or beaker or small container).

7.4.2 Add 5,0 ml of TISAB buffer solution to each plastic vial.

7.4.3 Add magnetic stir bar and mix thoroughly.

7.4.4 Insert the F-ISE and reference electrode(s) ([6.5](#)) into the standard solution under moderate stirring.

7.4.5 Record the millivolt reading to 0,1 mV at the steady potential difference with the mV electrometer ([6.7](#)).

The F-ISE is considered stable when the drift in millivolts is less than $\pm 0,5$ mV/min. The time required to attain stable millivolt response is typically longer for more dilute samples. It is not unusual for a 1×10^{-5} mol/l solution to take 2 min to become stable; however, it should not take longer than 5 min to reach a stable response.

NOTE When measuring the millivolt response of the standard solutions, determine the response for each standard solution starting with the most dilute and proceed to the most concentrated. These steps prevent contamination of dilute standards from carry over from the concentrated standards, and shortens the response time required to reach stable values.

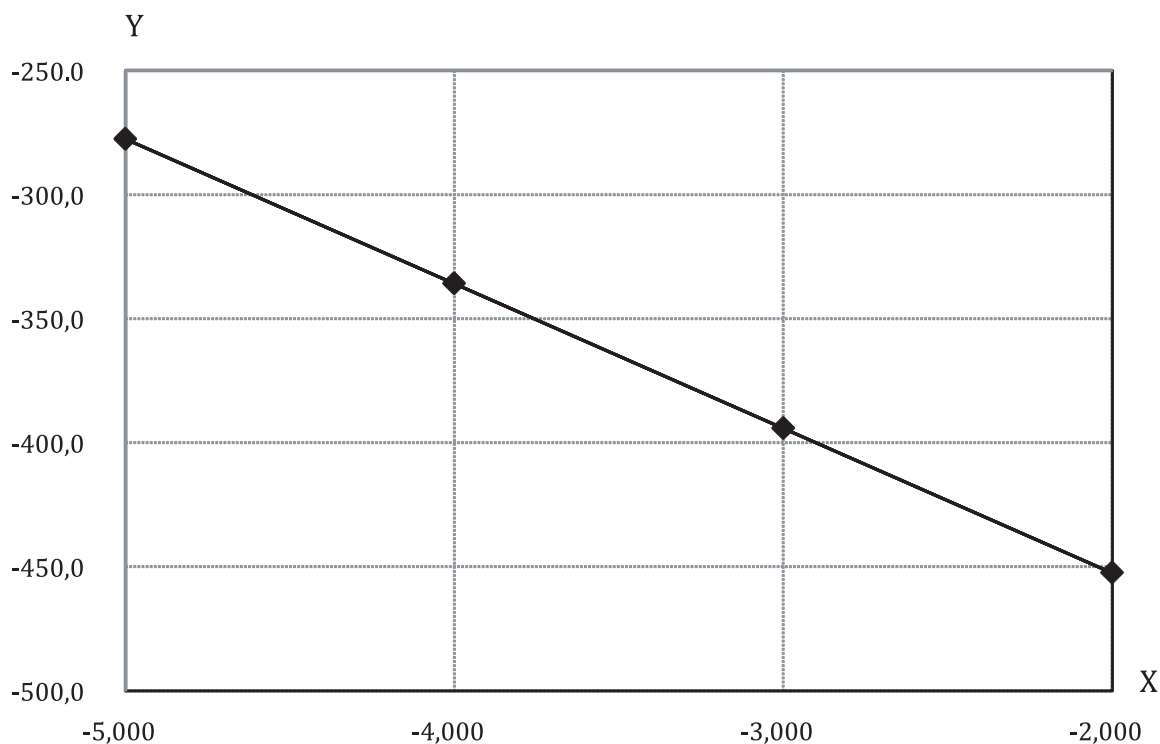
7.4.6 Remove the electrode(s) from the solution, rinse and blot dry and replace into the same solution.

7.4.7 Conduct at least two measurements for millivolt readings until the difference between the two readings is less than 0,2 mV.

7.4.8 Repeat [7.4.1](#) to [7.4.7](#) for each of the other standard solutions.

7.4.9 Construct a calibration curve of electrode potential (millivolts) measured versus the \log_{10} of the fluoride ion concentration of the standard. See [Figure 1](#) where the calibration curve is a straight line

with a coefficient of determination (r^2) of 0,999 90, with a slope of -58,2 mV/log [F] concentration and intercept of -569,1 mV.



Key

X log[F] mol/l
Y potential (mV)

Figure 1 — Fluoride ion-selective electrode calibration curve

The calibration curve shall be linear, with a coefficient of determination (r^2) greater than 0,95, and a slope between -56 mV and -60 mV per decade difference in fluoride concentration.

8 Procedure

8.1 Direct analysis technique

8.1.1 Dilute each sample solution with equal volume of TISAB (50:50) into a separate plastic vial (or beaker or small container) and mix thoroughly.

8.1.2 Insert the F-ISE and reference electrode(s) into the liquid in the plastic vial containing the sample solution while stirring.

8.1.3 Record the millivolt reading to 0,1 mV at the steady potential difference with the mV electrometer.

8.1.4 Remove the electrode(s) from the sample solution, rinse with deionized water and gently blot dry the electrode(s).

8.1.5 Conduct at least two measurements for millivolt readings until the difference between the two readings is less than 0,2 mV.

8.1.6 Repeat [8.1.1](#) to [8.1.5](#) for each of the other sample solutions.

8.1.7 If the apparent fluoride concentration is greater than 1×10^{-2} mol/l, dilute the sample with TISAB blank solution by a factor of 100 and reanalyse the sample.

8.2 Standard addition technique

This technique is suitable for samples that may have bound fluoride ion, or when the unknown sample is contaminated with other substances that alter the assay.

8.2.1 Prepare a standard addition series of four sample solutions of equal and known volume (4,0 ml) in separate plastic vials (or beakers or small containers).

8.2.2 To one sample, add 1,0 ml deionized water.

8.2.3 Add 1,0 ml of NaF standard solution of differing concentrations, such as 1×10^{-3} mol/l, 5×10^{-3} mol/l, or 1×10^{-2} mol/l, to each of the remaining sample solutions.

8.2.4 Dilute each with 5,0 ml of TISAB (50:50) and mix thoroughly.

8.2.5 Insert the F-ISE/reference electrode(s) into the liquid in the first plastic vial containing the sample solution while stirring.

8.2.6 Record the millivolt reading to 0,1 mV at the steady potential difference with the mV electrometer.

8.2.7 Remove the electrode(s) from the sample solution, rinse with deionized water and gently blot dry the electrode(s).

8.2.8 Conduct at least two measurements for millivolt readings until the difference between the two readings is less than 0,2 mV.

8.2.9 If the apparent fluoride concentration of the first sample in the standard addition series is greater than 1×10^{-2} mol/l, dilute the sample with TISAB blank solution by a factor of 100 and return to [8.2.1](#).

8.2.10 Remove the F-ISE/reference electrode(s) from the sample and rinse with deionized water and gently blot dry the electrode(s).

8.2.11 Insert the F-ISE/reference electrode(s) into the liquid in the second sample solution of the series.

8.2.12 Record the millivolt reading to 0,1 mV at the steady potential difference with the mV electrometer.

8.2.13 Remove the electrode(s) from the sample solution, rinse with deionized water and gently blot dry the electrode(s).

8.2.14 Conduct at least two measurements for millivolt readings until the difference between the two readings is less than 0,2 mV.

8.2.15 Repeat [8.2.11](#) to [8.2.14](#) for the third and fourth sample solutions in the series.

8.3 Dilution analysis technique

This technique is suitable for samples of small volume.

8.3.1 Place 4,950 ml TISAB blank solution in a plastic vial with stir bar.

8.3.2 Record the millivolt reading of the TISAB blank solution to within ± 1 mV at the steady potential difference with the mV electrometer.

8.3.3 Add 50 μ l sample to the 4,950 ml blank while stirring.

8.3.4 Determine and record a stable mV potential within $\pm 0,1$ mV.

8.3.5 Repeat [8.3.1](#) to [8.3.4](#) for each of the other sample solutions.

8.4 Multiple sample addition technique

This technique is suitable for samples of complex matrix, and only small amount of samples are available or the samples are of high concentration.

8.4.1 Place 4,950 ml TISAB blank solution in a plastic vial with stir bar.

8.4.2 Record the millivolt reading of the TISAB blank solution to within $\pm 0,1$ mV at the steady potential difference with the mV electrometer.

8.4.3 With the F-ISE/reference electrode(s) in the solution, add 50 μ l sample to the 4,950 ml blank while stirring.

8.4.4 Determine and record a stable mV potential within $\pm 0,1$ mV.

8.4.5 Add an additional 50 μ l sample to the solution while stirring.

8.4.6 Determine and record a stable mV potential within $\pm 0,1$ mV.

8.4.7 Repeat the procedures described in [8.4.5](#) and [8.4.6](#) twice more.

8.5 Internal standards technique

This technique is suitable for samples of complex matrix.

8.5.1 Prepare a set of internal standards where these standards receive the same steps for preparation as the samples. Follow the steps in [8.1](#) to determine the potential of the internal standards and samples.

9 Technical notes about the analytical techniques

9.1 Clean and carefully blot dry the electrode(s) between each measurement.

9.2 Store electrode(s) according to manufactures recommendations.

9.3 Measurements shall be made at a stable temperature.

9.4 Samples and standards shall be at the same temperature.

10 Calculations

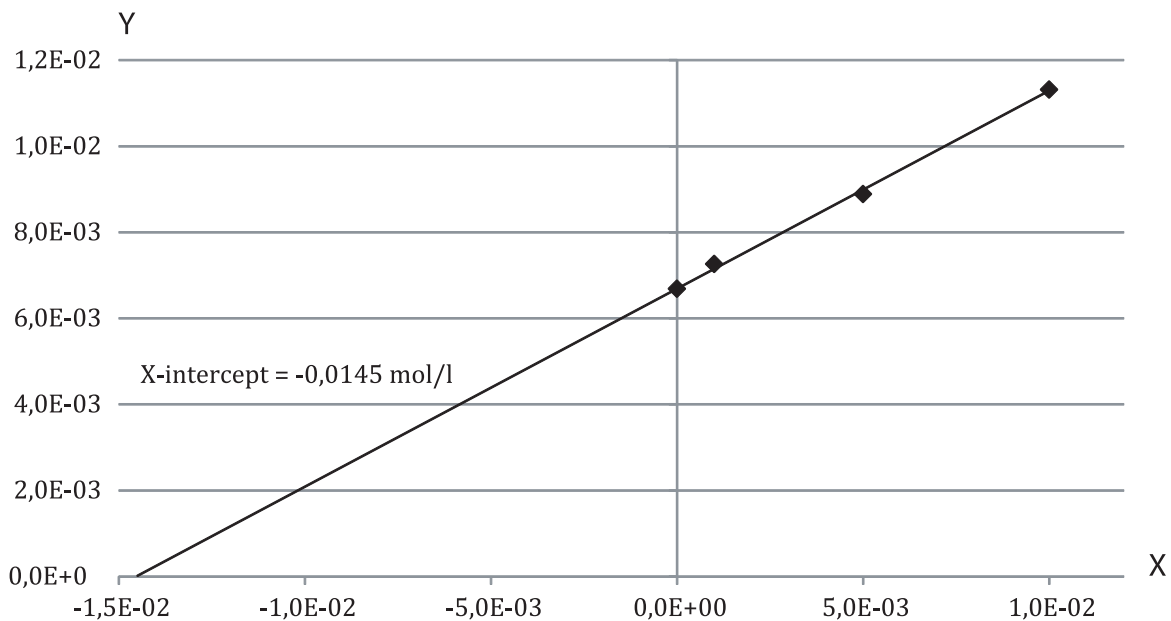
10.1 Direct analysis technique

10.1.1 Use the calibration curve of standard solutions (7.4) to determine the apparent fluoride concentration ($[F_{dil}]$) in the sample solution in mol/l.

10.2 Standard additions technique

10.2.1 Use the calibration curve of standard solutions (7.4) to determine the fluoride ion concentration for the sample solution in the standard addition series in mol/l.

10.2.2 Construct a plot of added fluoride (mol/l) for each sample versus the measured fluoride ion concentration for those samples. The line shall be linear with a coefficient of determination (r^2) greater than 0,95. The X-intercept of the linear regression of this plot is the fluoride ion concentration of the unknown sample in mol/l. Alternatively, the fluoride ion concentration of the unknown can be calculated from the linear regression by dividing the Y-intercept by the slope. See Figure 2. The absolute value of the x intercept is the fluoride concentration in the sample. In this example, the standard addition line is linear with the equation $y = 0,4671x - 0,0068$ and a coefficient of determination (r^2) of 0,9983. The fluoride concentration of the slurry = 0,0145 mol/l (276,3 $\mu\text{g/l}$) and because the dilution factor was 4, to make the slurry, the fluoride concentration of the NaF dentifrice is 4 x the slurry concentration = 0,0582 mol/l (1 105 $\mu\text{g/l}$).



Key

X added F(mol/l)

Y measured F(mol/l)

NOTE Technique for a NaF dentifrice slurry, that was not hydrolyzed or centrifuged, of 1 part dentifrice and 3 parts deionized water. The fluoride concentration of the slurry = 0,0145 mol/l (276,3 $\mu\text{g/l}$) and because the dilution factor was 4 to make the slurry the fluoride concentration of the NaF dentifrice is 4 x the slurry concentration = 0,0582 mol/l (1 105 $\mu\text{g/l}$).

Figure 2 — Fluoride analysis via standard additions

10.3 Dilution analysis technique

Use the calibration curve of standard solutions (7.4) to determine the apparent fluoride concentration ($[F_{dil}]$) in the diluted sample solution in mol/l. Multiply this value by the dilution factor of 100 to calculate the fluoride concentration of the sample in mol/l.

10.4 Multiple sample addition technique

Use the calibration curve of standard solutions (7.4) to determine the fluoride ion concentration in the series of sample solutions in mol/l for each sample. Plot the calculated fluoride concentration of the series of sample solutions versus the number of additions at each addition. Calculate the slope of the curve by linear regression. The concentration of the sample added to the solution is the slope of the regression curve.

10.5 Internal standards technique

10.5.1 Construct a calibration curve of electrode potential (millivolts) measured versus the \log_{10} of the fluoride ion concentration of the internal standard.

10.5.2 The sample concentration can be calculated directly from a standard curve prepared from the internal standards.

11 Test report

Report the mean, standard deviation and number of repeat analyses for each sample in mol/l ($\mu\text{g/g}$) along with the date of the analysis.

12 Trouble shooting

See [Table 1](#).

Table 1 — Troubleshooting

Symptom	Possible causes	Possible solutions
mV readings not stable	a) Electrode(s) not connected to electrometer properly b) Electrode(s) not functioning properly c) Temperature during analysis is not stable	a) Recheck electrode connections b) Replace or clean reference electrode in 1 mol/l HCl for 30 s and retest; if still not stable, gently clean or replace F-ISE c) Run experiments at stable temperature ± 5 °C
mV readings for duplicate samples not the same (drift)	a) Samples and standards are at different temperature b) F-ISE leakage around sensor crystal	a) Equilibrate standards and samples to the same temperature prior to measurement b) Replace F-ISE
Standard curve not linear ($r^2 < 0,95$)	a) Electrometer not in mV mode b) Some standards are contaminated c) Did not use logarithm of fluoride concentration vs. mV measured to calculate standard curve	a) Check electrometer mode setting b) Remake standard solutions c) Use \log_{10} of [F] vs. mV for standard curve
Slope of standard curve outside of -56 to -60 mV/decade range	a) Electrode(s) not functioning properly b) Cross contamination of standards by electrode(s)	a) Inspect electrode(s) for damage, replace b) Remake standard solutions
Standard additions technique plot is not linear	a) Sample matrix binding with sample fluoride ion	a) Dilute sample by factor of 10

Bibliography

- [1] ASTM D1179-16, *Standard Test Methods for Fluoride Ion in Water*

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Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the website-www.bis.gov.in or www.standardsbis.in.

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