



CERTIFIED REFERENCE MATERIAL IRMM – 435

CERTIFICATE OF ANALYSIS

PHARMACEUTICAL GLASS CONTAINERS		
	Alkali leaching and release	
	Certified value ²⁾ [mL]	Uncertainty ³⁾ [mL]
Volume of titration solution 0.01 mol/L HCl per 50 mL of leachate ¹⁾	0.38	0.04
	Certified value ²⁾ [mg/L]	Uncertainty ³⁾ [mg/L]
Sodium release per volume of leachate ¹⁾	1.41	0.14
Release of Na ₂ O per volume of leachate ¹⁾	1.91	0.19
1) As determined according to the adapted method for alkali release based on the European Pharmacopoeia method and ISO 4802		
2) Unweighted mean of 5 accepted mean values, independently obtained by 5 laboratories. The value is traceable the International System of Units (SI) as far as gravimetrically prepared calibrants are concerned.		
3) Expanded uncertainty with a coverage factor k = 2, corresponding to a level of confidence of about 95 %.		

This certificate is valid for five years after purchase.

Sales date:

The minimum amount of sample to be used is 9 vials

Geel, February 2007
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Signed: _____

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DESCRIPTION OF THE MATERIAL

Each unit of reference material consists of 20 vials of 18.9 mL brimful capacity, made of a semi-durable type of glass, with screw caps (holes to be drilled by the user). Details of the testing procedures are included with this certificate. Since the certified values are operationally defined, these procedures must be strictly followed.

ANALYTICAL METHODS USED FOR CERTIFICATION

Atomic absorption spectrometry (AAS)

Titration with 0.01 mol/L HCl solution

PARTICIPANTS

- British Glass Manufacturers Confederation, Sheffield (GB)
- BSN – Centre de Recherche, Givors (FR)
- Forschungsgemeinschaft für technisches Glas, Wertheim (DE)
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- Kimble Italiana, Pisa (IT)
- Schott Glas (previously: Schott Glaswerke), Mainz (DE)
- Schott Rohrglas, Mitterteich (DE)
- Stazione Sperimentale del Vetro, Venezia-Murano (IT)
- Turkish Glass Research Centre, ŞİŞECAM, Istanbul (TR)
- Vetreria Parmense Bormioli Rocco, Parma (IT)

SAFETY INFORMATION

The usual laboratory safety precautions apply.

INSTRUCTIONS FOR USE

This material is intended for performance checks of the complete procedure of autoclave leaching of the inner walls of glass containers and subsequent determination of alkali and/or sodium release by titration and/or flame spectrometry. Since the certified values are operationally defined, the testing protocol (see Annexes I to III) must strictly be followed also in the normal usage of the CRM. At least 10 vials are to be used when the sodium release determinations are carried out with flame spectrometry, at least 9 vials with titration for alkali release.

The vials can be reconditioned by etching (Annex II) and re-used for the determination of sodium and alkali release at least 19 times without change in the release rate.

Since small differences in autoclave temperature result in large differences in alkali release, the use of calibrated thermometers is required. The temperature as measured in the actual vials is not always the same as that in the autoclave atmosphere around them. It is, therefore, necessary to carry out temperature measurements in one of the actual vials (e.g. with a calibrated thermocouple - see Annex III) and to continuously record the temperature so as to verify that the conditions of the test procedure are fulfilled.

Note – The pre-etching step (Annex II) of the procedure for use of this CRM shall not be applied to the routine testing of unknown glass containers.

STORAGE

This material may be stored at room temperature away from damp and corrosive vapours.

However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

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NOTE

A technical report on the production of IRMM-435 is available on the internet (<http://www.irmm.jrc.be>). A paper copy can be obtained from IRMM on request.

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Full procedure for the determination of hydrolytic resistance of the interior surfaces of the reference glass containers IRMM-435

In principle, the testing methods comply with the International Standard ISO 4802 for titration and flame spectrometry (titration also identical with the European Pharmacopoeia, Glass Monograph) but are **deviating in some points**. Therefore, for the purpose of the certification of the test containers intended to serve as a certified reference material, the following procedure was **applied strictly**. Also in the normal usage of the CRM, this procedure must strictly be followed in order to establish comparable leaching and measurement conditions.

The deviations concern especially the closure of the vials, the traceability of standard solutions, the acidification of the extract solutions prior to the flame measurements, the accurate time-temperature control of the autoclave, and the exact documentation of all steps concerning calibration. Therefore, all clauses have to be applied strictly.

1. Reagents

Unless otherwise stated, use only reagents of recognized analytical grade.

1.1 Test Water

The water shall be double distilled, or de-ionized and then distilled (grade 2 water according to ISO 3696). This water shall be boiled for at least 15 minutes in a boiling flask of fused silica or borosilicate glass. The boiling flask shall be pretreated by subjecting it to the autoclave conditions described in clause 5.1, before it is used for the first time.

Such test water can normally be stored for 24 h in a stoppered flask without change of the pH value.

When tested immediately before use, the water shall be neutral to methyl red, i.e. it shall produce an orange-red (not a violet-red or yellow) colour corresponding to $\text{pH } 5.5 \pm 0.1$ when four drops of the methyl red indicator solution (1.5) are added to 50 mL of the test water.

Note – The water, so coloured may also be used as the reference solution.

For flame spectrometric purposes only, also water of lower conductivity, e.g. grade 1 water according to ISO 3696, may be used. This water does not comply with the pH requirements for the waters as specified before, and shall therefore **not be used for determinations by titration**.

1.2 Hydrochloric acid, standard volumetric solution,

$c(\text{HCl}) = 0.01 \text{ mol/L}$. This must be standardized against sodium hydroxide, which in turn is standardized against potassium hydrogen phthalate.

1.3 Hydrochloric acid solution, $c(\text{HCl}) \approx 2 \text{ mol/L}$

1.4 Hydrofluoric acid, $c(\text{HF}) \approx 22 \text{ mol/L}$

(i.e. = 400 g HF/L solution).

1.5 Methyl red, indicator solution

Dissolve 25 mg of the sodium salt of methyl red ($C_{15}H_{14}N_3NaO_2$) in 100 mL of the test water.

1.6 Distilled water

or water of equivalent purity (grade 3 water complying with the requirements specified in ISO 3696).

1.7 Ionisation buffer solution

Dissolve 10 g of cesium chloride CsCl in 0.01 mol/L HCl (1.2) contained in a 250 mL volumetric flask (2.5), make up the volume with 0.01 mol/L HCl and mix. Store the ionisation buffer solution in a pretreated borosilicate glass bottle.

1.8 Stock solution

Dry the chloride of sodium at $(110 \pm 5)^\circ\text{C}$ for 2 h. Prepare gravimetrically an aqueous stock solution, using the test water (1.1) directly from the chloride such that the solution contains a concentration of 1000 mg/L, calculated as sodium oxide.

Note – take care whether the concentration of the standard solution is given as Na or Na_2O for later calculations!

Note – for the preparation of analytical solutions the test water (grade 2 water) can be used without further pre-boiling!

Note – **In usage of the CRM:** A commercially available standard solution may also be used.

1.9 Standard solution

Prepare a standard solution by transferring 20 mL of the stock solution 1.8 into a 1 L volumetric flask and make up the volume with the test water (1.1, but see Note 2 above).

The concentration of Na_2O is 20 mg/L.

Note – **In usage of the CRM:** A commercially available standard solution of appropriate concentration may also be used.

1.10 Reference solutions and establishment of the calibration curve

Transfer by means of a pipette (2.6) 10 mL of the ionisation buffer solution (1.7) and then by a burette of 10 mL capacity (2.2) the following volumes of the standard solution (1.9) into 100 mL volumetric flasks (2.5).

0 – 2 – 5 – 7.5 – 10 mL, corresponding to

0 – 0.4 – 1.0 – 1.5 – 2.0 mg/L Na_2O (after making up the volume with test water)

Measure the extinctions by spraying the solutions into the flame of the atomic absorption instrument, following the instructions of the producer's manual. Start with the zero solution, then continue stepwise up to the 2.0 mg/L solution.

Measure each solution three times, calculate the mean and establish the calibration graph.

2. Apparatus

Ordinary laboratory apparatus, glassware – if not otherwise stated – are made of borosilicate glass 3.3.

2.1 Autoclave or steam sterilizer,

capable of withstanding pressure of at least $2.5 \times 10^5 \text{ N/m}^2$ ⁽¹⁾ and of carrying out the heating cycle specified in 5.1. It should preferably be equipped with a constant-pressure regulator or other means of maintaining the temperature at $(121 \pm 1) ^\circ\text{C}$. The vessel shall have an internal diameter of at least 300 mm and shall be equipped with a heating device, a possibility to lead a calibrated thermocouple (2.9) from the inner autoclave to an external measuring instrument (2.10), a pressure gauge, a pressure-release safety device, a vent-cock, and a rack for supporting the samples.

The autoclave vessel and ancillary equipment shall be thoroughly cleaned before use.

2.2 Burettes, having a suitable capacity of 2 mL or 10 mL

complying with the requirements specified for class A burettes in ISO 385/2 and made of glass of hydrolytic resistance grain class HGA 1 as specified in ISO 720⁽²⁾.

2.3 Beakers, having a suitable capacity

and complying with the requirements specified in ISO 3819.

Before use, each new beaker shall be pretreated by subjecting it to the autoclaving conditions described in 5.1.

2.4 Conical flasks, having a capacity of 100 mL or 250 mL

and complying with the requirements of ISO 1773. Before use, each new flask shall be pretreated by subjecting it to the autoclaving conditions described in 5.1.

2.5 One-mark volumetric flasks having a capacity of 100 mL and 1000 mL

and complying with the requirements specified for class A one-mark volumetric flasks in ISO 1042.

2.6 Pipettes, having a suitable capacity

and complying with the requirements specified for class A pipettes in ISO 648.

2.7 Water bath, capable of being heated to approximately 80 °C

2.8 Flame atomic absorption (AAS) or Flame atomic emission (AES) spectrometer.

AAS instruments shall be equipped with a line source, gas supply and burner for measuring sodium.

AES instruments of older generation shall be equipped with an air/propane or air/acetylene gas supply and burner for measuring sodium.

Table 1 gives the conditions for measurement.

¹ $2.5 \times 10^5 \text{ N/m}^2 = 0.25 \text{ MPa} = 2.5 \text{ bar}$

² Glass of hydrolytic resistance grain class ISO 719-HGB 1 adequately meets the requirements of class HGA 1 specified in ISO 720.

Table 1: Instrument conditions for measuring sodium (Na)

Element	Instrument	Typical working range	Gas mixture	Detected line
Na	AES	up to 10 µg/mL	Air/propane or air/acetylene	Filters
Na	AAS	up to 3 µg/mL	Air/acetylene	589.0 nm

- 2.9 **Thermo couple of NiCr-Ni (Type K),**
or resistance thermometer integrated in the autoclave.
- 2.10 **Suitable measuring device (millivolt meter),**
with recorder for documentation. Calibrate the whole system thermocouple/resistance thermometer and measuring system, for instance millivolt meter and recorder (see Annex III).
- 2.11 **Pipette of suitable capacity,**
made of plastic.

3. Preparation of the closures (stoppers)

- 3.1 **Apply a small hole (before first use)**
of maximum 0.3 mm diameter, e.g. by drilling or by perforating with a needle.
- 3.2 **Cleaning of the closures**
Put the closures in a suitable glass beaker, fill with test water (1.1), boil for 5 minutes, empty, fill with water again and boil, empty and boil with water a last time as before. Then empty quantitatively, and allow to dry in an oven at about 110 °C, store in a desiccator, and **avoid any contamination** (also during later handling).

4. Preliminary etching

Mark 10 of the set of 20 containers with a number (scratching by glass writer for instance) and fill all containers up to the rim with a mixture of 1 volume of hydrofluoric acid (1.4) and 9 volumes of hydrochloric acid (1.3) using the pipette (2.11). Allow the filled samples to stand at ambient temperature for 10 minutes, then empty the solution very carefully (HF is dangerously etching!). Rinse the containers once with distilled water (1.6), then fill them again with the same mixture of acids, and allow to stand for further 10 minutes. Repeat the etching a third time. Then empty all solutions very carefully, rinse the containers 3 times with the distilled water (1.6), then at least twice with the test water (1.1). Dry in an oven at about 110 °C and store in a desiccator to cool.

5. Procedure

5.1 Autoclave process

After etching (4) fill all 20 dried containers by means of a burette (2.2) exactly with the prescribed volume of 17 mL = weight of the test water. Cap each container with a cleaned closure (3) (avoid contamination!).

Place all samples on the rack of the autoclave, containing distilled water (1.6) at ambient temperature, and ensure that they are held above the level of the water in the vessel. Insert into one of the filled containers the end of the thermocouple or resistance thermometer (2.9) through a hole of the closure of a not marked vial and lead it out of the autoclave. Connect it with the measuring device (2.10) as described in Annex III. Close the autoclave lid or door securely, but leave the vent-cock open. Start automatical plotting of the temperature on the recorder (2.10) versus time and heat the autoclave at a regular rate such that steam escapes vigorously from the vent-cock after 20 to 30 minutes, and maintain a vigorous evolution of steam for a further 10 minutes. Close the vent-cock and increase the temperature at a rate of 1 °C/min (= within 20 minutes) to (121 ± 1) °C, maintain this temperature for (60 ± 1) minutes from the time when the holding temperature is reached, then cool at a rate of 0.5 °C/min (= within 40 minutes) to 100 °C, venting to prevent formation of a vacuum. Open the autoclave carefully and note this point on the time-temperature record.

Note – The rate of heating to (121 ± 1) °C and the rate of cooling to 100 °C are critical. Variations from the specified conditions can produce variable results even to the extent of invalidating them.

Remove the hot samples from the autoclave, place in the water bath (2.7) heated to about 80 °C, and run cold water into and out of the bath at a rate which will cool the samples to ambient temperature as quickly as possible (maximum 15 minutes).

Note – Make sure that during cooling the edge of caps does not touch the cooling water, otherwise water could be sucked into the container and heavily contaminate the extraction solution.

The temperature-time process of the autoclave shall be from the start:

- (autoclave open) 20-30 minutes from room temperature to steam escape
10 minutes of vigorous steaming
- (autoclave closed) 20 minutes from 100 °C to 121 °C
60 minutes maintaining 121 °C
40 minutes decreasing from 121 °C to 100 °C
- (autoclave possibly slightly opened)
- (autoclave open) removing the containers as quickly as possible from the autoclave
maximum 15 minutes cooling down from 100 °C to room temperature.

Note – Recent safe operation practices (EN 61010-2-041 point 7.101.2) require that autoclaves are opened at 95 °C. With the necessary precautions against injuries due to burning with hot steam, however, all autoclaves *can be* opened at 100 °C. A collaborative study of TC2 of the International Commission on Glass has shown that the prolongation of the cooling time down to 95 °C increases the extracted alkali by measurable amounts. The certified values of IRMM-435 are valid only for opening temperatures of the autoclave at 100 °C. This is in accordance with ISO 4802 and European Pharmacopoeia.

Carry out the analytical determinations immediately, i.e. the same day.

6. Analysis of the extraction solutions

6.1 By titration

Remove the closures from 9 unmarked containers, excluding the one with the thermometric device. Combine the extraction solutions of 5 or 4 containers, respectively, in two dry 250 mL conical flasks (2.4) and pipette by means of a pipette (2.6) a volume of 50.0 mL from each combined solution into new separate conical flasks (2.4) of 100 mL.

Prepare a reference solution by pipetting 50 mL of the test water into a third flask (2.4) of 100 mL.

Add four drops of methyl red indicator solution (1.5) to each flask, then titrate the extraction solutions with hydrochloric acid (1.2) until the colour matches exactly that of the coloured test water in the third conical flask.

Express the titration values to two decimal places in the unit mL of 0.01 mol/L HCl per 50 mL of leachate.

6.2 By flame atomic absorption spectrometry

Remove the closures from the 10 marked containers. Add to each, by means of a 2-mL burette (2.2) 1.7 mL of the ionisation buffer (1.7) which is equal to 10 % of the filling volume. Shake. Aspirate the extraction solutions immediately from each container directly into the flame of the atomic absorption instrument (2.8) and determine the concentrations of **sodium oxide** by reference to the calibration graph, produced according to clause 1.10.

Express the measurement results to two decimal places in the unit mg Na₂O per L of leachate, corrected for the dilution with the ionisation buffer.

7. Repetition of the measurements

After having finished the first run of the tests, rinse the containers 2 times with test water, allow to drain and dry.

For a new test run start at another day (autoclave cooled down to room temperature), as described from clause 3.2 onwards with a new cleaning of the closures, new etching, followed by a new autoclave process and analyses. Repeating the whole procedure for a third time is usually not necessary.

Annex II THE ETCHING PROTOCOL FOR RECONDITIONING THE VIALS

Fill all containers up to the rim with a mixture of 1 volume of hydrofluoric acid (1.4) and 9 volumes of hydrochloric acid (1.3) using the pipette (2.11). Allow the filled samples to stand at ambient temperature for 10 minutes, then empty the solution very carefully (HF is dangerously etching!). Rinse the containers once with distilled water (1.6), then fill them again with the same mixture of acids, and allow to stand for further 10 minutes. Repeat the etching a third time. Then empty all solutions very carefully, rinse the containers 3 times with the distilled water (1.6), then at least twice with the test water (1.1). Dry in an oven at about 110 °C and store in a desiccator to cool.

Note – Unknown containers, which are to be tested, shall not be etched, of course.

Annex III TEMPERATURE MEASUREMENTS WITHIN VIALS IN AN AUTOCLAVE

It is not sufficient to calibrate only the thermocouple, but the whole measuring system needs calibration. It is therefore essential to calibrate the temperature scale on the recordings using a thermometer, calibrated within $\pm 0.1^\circ\text{C}$ and, for example, an oil bath in which the thermocouple/resistance thermometer is inserted.

Possible positions and feed-throughs of the thermometric devices in an autoclave vessel are shown in Figure 1. To avoid by-pass currents, the thermoelement should be insulated. A possible solution is shown in Figure 2a. The connection from the thermocouple to the measuring device shall be made via cold-junction compensation (automatic compensation integrated in the measuring instrument) or by an external compensation facility (for instance an ice-water mixture) to obtain defined temperature measurements (Figure 2).

These conditions must be identical during calibration and later measurements.

Some modern autoclaves are already equipped by the producers with thermometric devices, mostly resistance thermometers. Such measurement apparatus can also be used, but must be calibrated as well.

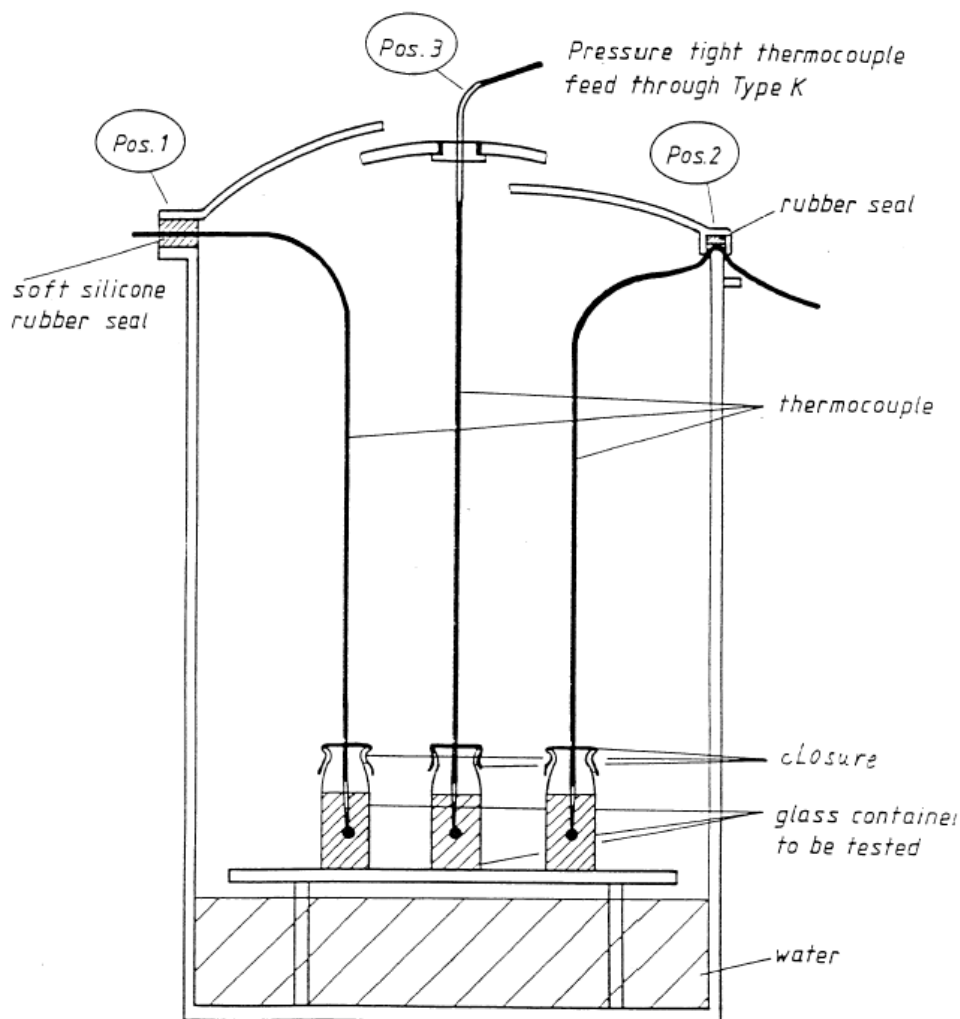


Figure 1: Temperature measurement in an autoclave by thermocouples or resistance thermometers, using 3 different autoclave lid configurations.

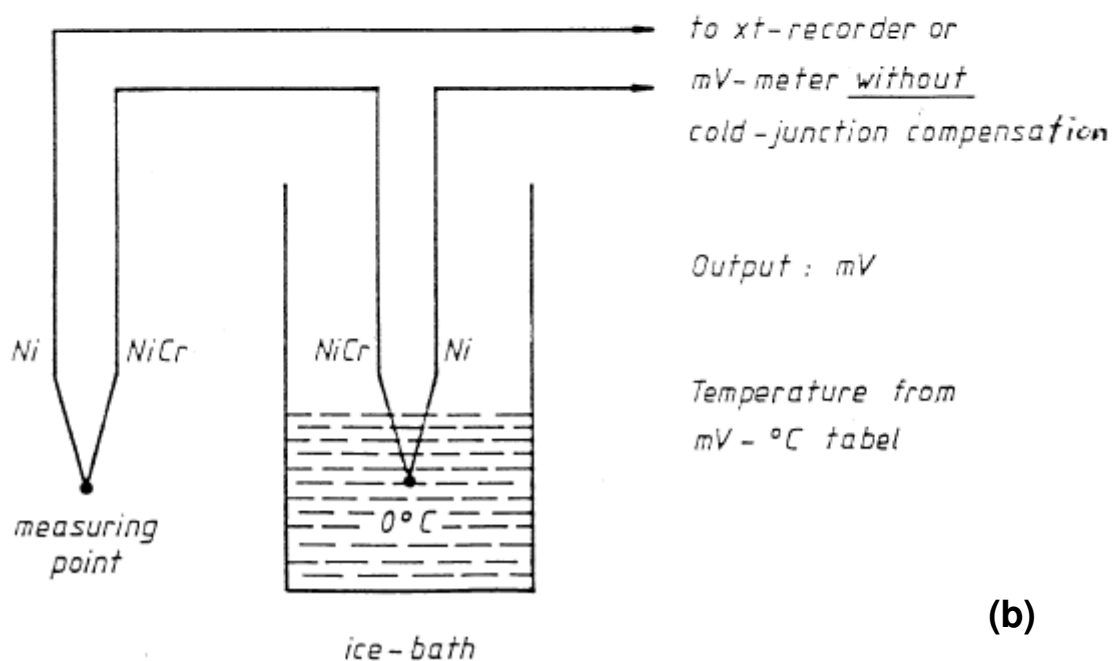
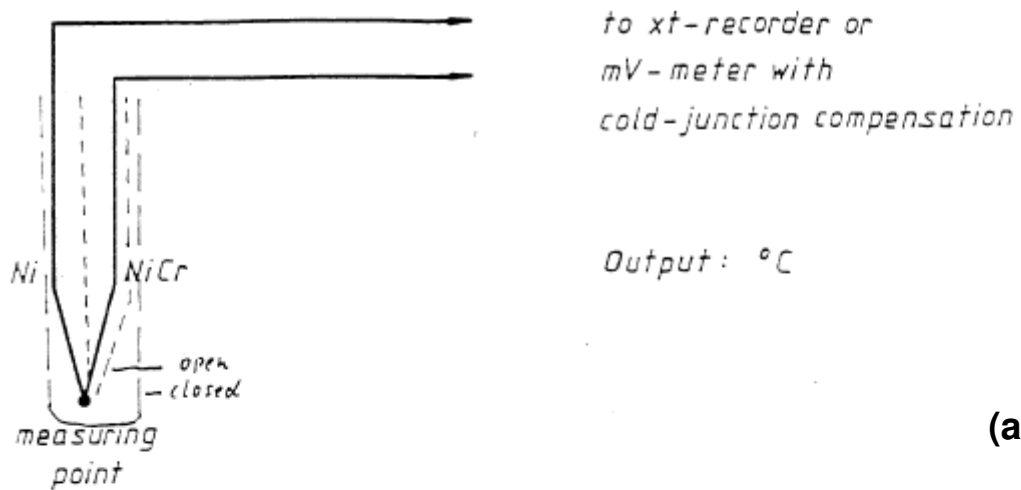


Figure 2: Connection of thermocouple with measuring instrument

a) with cold-junction compensation

b) with external compensation (ice-bath).

a) also shows the necessary insulation: open Al_2O_3 tube over one lead, both thermocouple leads in a closed Al_2O_3 tube.