# STANDARDISED METHODS FOR THE TESTING OF EDIBLE GELATINE

## GELATINE MONOGRAPH

This is only the short version of the GME monograph

Version 15 – October 2020

GME

GELATINE MANUFACTURERS OF EUROPE

# NOTICE

The information, methods and recommendations herein are presented in good faith and are believed to be accurate and reliable, but may well be incomplete and/or not applicable to all conditions or situations that may exist or occur. No guarantee or warranty is made as to the accuracy, reliability or completeness of the information, methods and recommendations or that the application or use of any of the same will avoid hazards, accidents, losses, damages or injury of any kind to persons or property. Readers are cautioned to satisfy themselves as to the suitability of said information, methods and recommendations for the purposes intended prior to use. By necessity, the authors disclaim their liability with regard to misinterpretations or misuses of the information, methods and recommendations.

*All GME members are obliged to use the GME methods as they are written in the GME monograph. Alternative methods may be used, only in case if they have been validated against the GME method.



**Enquiries must be addressed to the secretariat of the Gelatine Manufacturers of Europe**
**Tel : (32) 2 436 94 59**
**E-mail : lje@cefic.be**

# Contents

# 1. FOOD GRADE GELATINE – EDIBLE GELATINE

## *1.1. Definition*

Gelatine is a natural, water-soluble protein, gelling or non-gelling, obtained by the partial hydrolysis of collagen produced from bones, hides and skins, tendons and sinews of animals (including fish and poultry).

In relation to the production process, two basic types are recognized:

Type A: produced by the acid processing of collagenous raw materials, having an isoelectric point of pH 6.0-9.5.

Type B: produced by the alkaline processing of collagenous raw materials, having an isoelectric point of pH 4.7-5.6.

Mixtures of types A and B, as well as gelatines produced by modifications of the above-mentioned processes, may exhibit isoelectric points outside the stated ranges.

In relation to the degree of hydrolysis, two different types of gelatine are obtained:

Gelling (type G) : mainly composed of high molecular weight components.

Non-gelling (type F) : mainly composed of low molecular weight components.

## *1.2. Description*

Gelatine is a vitreous, brittle solid that is faintly yellow to white and nearly tasteless and odourless. It is supplied in various physical forms such as coarse granules, fine powders and leaves.

## *1.3. Stability*

Gelatine is generally recognised as stable for at least 5 years, when suitably stored in sealed containers, under typical warehouse conditions, to prevent ingress or loss of moisture.

Small changes in moisture could result in consequent small changes in Bloom value. However, any such Bloom change is directly calculable from the moisture content and does not reflect any change in intrinsic Bloom gel strength or loss in quality.

“Stability” refers to physical, chemical and microbiological properties. There is no evidence for any statistically significant change in Bloom value for gelatine properly stored for five years. It is suggested that after this period the Bloom and moisture be re-tested to re-establish these values. The gelatine remains entirely safe and suitable for use.

## *1.4. Quality Standards*

### 1.4.1. Description

Specific health conditions for gelatine intended for human consumption are laid down in Regulation (EC) No 853/2004 of the European Parliament and of the Council (See Annex to this document) Annex III, section XIV.

This decision specifies requirements for raw materials, their transport and storage, gelatine manufacturing processes, gelatine quality, packaging, storage and transport.

The chemical and microbiological quality requirements for finished gelatine are listed in 1.5.2 and 1.5.3.

Additional specifications regarding characteristics and purity of pharmaceutical gelatine are defined in the Gelatine Monograph of the European Pharmacopoeia (current edition).

## 1.4.2. Chemical quality

Maximum values for gelatine:

| | | Food regulation EC/853/2004 | European Pharmacopoeia | GME requirements | Ref. |
|---|---|---|---|---|---|
| | | **Edible** | **Pharma** | | |
| Moisture | % | | ≤ 15 | ≤ 15 | 2.2.1 |
| Ash | % | | | ≤ 2 | 2.2.2 |
| pH (1%, 55°C) | | | 3.8 - 7.6 | | 2.4.7 |
| Conductivity (1% -30°) | mS/cm | | ≤ 1 | | 2.4.8 |
| $SO_2$ | ppm; mg/kg | ≤ 50 | ≤ 50 | ≤ 50(*) | 2.2.3 |
| $H_2O_2$ | ppm; mg/kg | ≤ 10 | ≤ 10 | ≤ 10 | 2.2.4 |
| As | ppm; mg/kg | ≤ 1 | | ≤ 1 | 2.2.5 |
| Pb | ppm; mg/kg | ≤ 5 | | ≤ 5 | 2.2.5 |
| Cu | ppm; mg/kg | ≤ 30 | | ≤ 30 | 2.2.5 |
| Zn | ppm; mg/kg | ≤ 50 | ≤ 30 | ≤ 30 | 2.2.5 |
| Cr | ppm; mg/kg | ≤ 10 | ≤ 10 | ≤ 10 | 2.2.5 |
| Hg | ppm; mg/kg | ≤ 0.15 | | ≤ 0.15 | 2.2.6 |
| Cd | ppm; mg/kg | ≤ 0.5 | | ≤ 0.5 | 2.2.5 |
| Fe | ppm; mg/kg | | ≤ 30 | ≤ 30 | 2.2.5 |

(*)

Edible gelatine must contain neither preservatives nor inhibitors.

## 1.4.3. Microbiological quality

Maximum values for edible gelatine are described in Regulation (EC) No 2073/2005, Annex I, Chapter 1, point 1.10. Only Salmonella testing is required for food grade gelatine.
The GME Members agreed to extend the bacteriological testing for edible gelatine with Total Aerobic Microbial Count, E. coli and Anaerobic sulphite-reducing spores.

| | **Food regulation EC/2073/2005** | **European Pharmacopoeia** | **GME requirements for edible gelatine** |
|---|---|---|---|
| Salmonella | (n=5) Absence/25g | Absence/10g | Absence/25g |
| Total aerobic microbial count | - | max1000 CFU/g | <1000 CFU/g |
| E.coli | - | Absence/g | Absence/10g |
| Anaerobic sulphite-reducing spores | - | - | <10 CFU/g |
| Yeast and moulds | - | max100 CFU/g | - |

# 2. TEST METHODS

**REMARK: For all tests purified water should be used, unless the quality of the water is defined differently in the respective test description. Purified water is prepared by distillation, ion exchange, reverse osmosis or by any other suitable method that complies with the regulations on water intended for human consumption laid down by the competent authority.**

## *2.1. CHEMICAL CHARACTERISTICS*

*(Other methods may be used provided that correlation has been established with the reference methods. In case of disagreement, the reference method is the one described in this document)*

## 2.1.1. Loss on drying (moisture)

### 1. Principle

A weighed sample of gelatine is maintained for 16 to 18 hours at 105 +/- 2°C and is then reweighed. The moisture content is defined as the percentage loss in weight of the sample.

### 2. Reference(s)

AFNOR NF V 59-003
European Pharmacopoeia (current edition)

### 3. Apparatus

- E.g.Pyrex evaporating dishes, 45 mm in diameter and 30 mm high
- Drying oven, set at 105 +/- 2°C
- Dessicator containing $CaCl_2$ or silicagel
- Analytical balance

### 4. Procedure

*4.1. SAMPLE PREPARATION*

Wash the evaporating dish very carefully in hot water, place it in the drying oven at 105 +/- 2°C for at least one hour, then leave it to cool in the dessicator until room temperature is reached. Weigh approximately 5 g of the sample to the nearest milligram and note the weight of the test sample (m0) and the weight of the sample together with the evaporating dish (m1).

*4.2. DETERMINATION*

Place the evaporating dish containing the sample in the drying oven at 105 +/- 2°C and leave for 16 to 18 hours. Leave to cool in the dessicator until room temperature is reached and weigh to the nearest milligram (m2).

## 5. Result

**THE MOISTURE CONTENT, EXPRESSED AS A PERCENTAGE BY WEIGHT, IS EQUAL TO :**

$$\frac{m_1 - m_2}{m_0} \times 100$$

where:

$m_0$ is the weight in grams of the test sample

$m_1$ is the weight in grams of the test sample and the evaporating dish before drying

$m_2$ is the weight in grams of the test sample and the evaporating dish after drying

## 2.1.2. Ash (Residue on ignition)

### 1. Principle

Gelatine is incinerated in a crucible and ashing is finished in a muffle furnace at 550°C. The residue is determined by differential weighing and the result expressed as a weight percentage of the sample.

### 2. Reference(s)

USP 35 (The United States Pharmacopoeia 35); The National Formula (30) = USP35/NF30.

### 3. Reagents and Solutions

Paraffin

### 4. Apparatus

- Muffle furnace 550°C (± 25°C)
- Analytical balance

### 5. Procedure

*5.1. SAMPLE PREPARATION*

Weigh approx. 5.0 g of gelatine to 0.001 g in a suitable crucible that previously
has been ignited, cooled and weighed. Add 1.5 to 2.0 g of paraffin to avoid loss due to swelling.

Heat, gently at first on a low flame, electric hot plate or muffle furnace, until the substance is thoroughly charred and finish ashing in a muffle furnace at 550°C for 15 to 20 hours.

*5.2. DETERMINATION*

Cool in a desiccator, weigh and calculate the percentage of residue.

(*Do not touch dried crucibles without gloves or crucible tongs*).

## 6. Result

**THE ASH CONTENT, EXPRESSED AS % ASH, IS EQUAL TO:**

$$= \frac{m_1}{m_0} \times 100$$

Where: $m_0$ is the quantity weighed in

$m_1$ is the quantity weighed out

## 2.1.3. Sulfur Dioxide

## 2.1.3.1. Distillation - Method

### 1. Principle

Sulfur dioxide is set free from the gelatine sample by boiling it with the hydrochloric acid. The created sulphur dioxide is distilled into a hydrogen peroxide solution with a stream of carbon dioxide.
Sulfuric acid is formed that can be titrated with sodium hydroxide.

### 2. Reference(s)

European Pharmacopoeia (current edition)

### 3. Reagents and solutions (for additional details see also Eur.Pharm.)

- Carbon dioxide : CO2 > 99,5 % V/V
- Dilute Hydrogen peroxide solution : H2O2, 3 % in water (+/- 0,5 %)
- Bromophenol blue solution : 1 g/L in ethanol (20 % V/V) Ethanol: 96 % C2H5OH (V/V)
- Dilute hydrochloric acid : HCl, 2 mol/l (73 g / l)
- Sodium hydroxide : NaOH, 0.1 mol / l or 0.01 mol / l
- Water: Purified water

### 4. Apparatus

Apparatus for the determination of sulfur dioxide.

### 5. Procedure

*5.1. SAFETY INSTRUCTIONS*

When working with HCl, NaOH or $H_2O_2$, avoid contact with eyes and skin; do not breathe vapours.

Wear protective glasses and gloves.

*5.2. PREPARATION OF APPARATUS*

Introduce 150 ml of water into the flask (A, see figure) and pass carbon dioxide through the whole system for 15 min. at a rate of 100 ml +/- 5 ml/min

To 10 ml of dilute hydrogen peroxide solution add 0,15 ml of a 1 g/L solution of bromophenol blue in ethanol (20 % V/V). Add 0,1M sodium hydroxide until a violet-blue colour is obtained, without exceeding the end-point. Place the solution in the test-tube (D).

*5.3. SAMPLE PREPARATION*

Without interrupting the stream of carbon dioxide, remove the funnel (B) and introduce through the opening into the flask 25.0 g (m in g) of the gelatine sample with the aid of 100 ml water. Replace the funnel.

Close the tap of the funnel and add 80 ml of dilute hydrochloric acid to the funnel. Open the tap of the funnel to allow the hydrochloric acid solution to flow into the flask, making sure that no sulfur dioxide escapes into the funnel by closing the tap before the last few millilitres of hydrochloric acid solution drain out. Boil for 1 h.

Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little water to a 200 ml wide-necked, conical flask. Heat on a water-bath for 15 min and allow to cool.

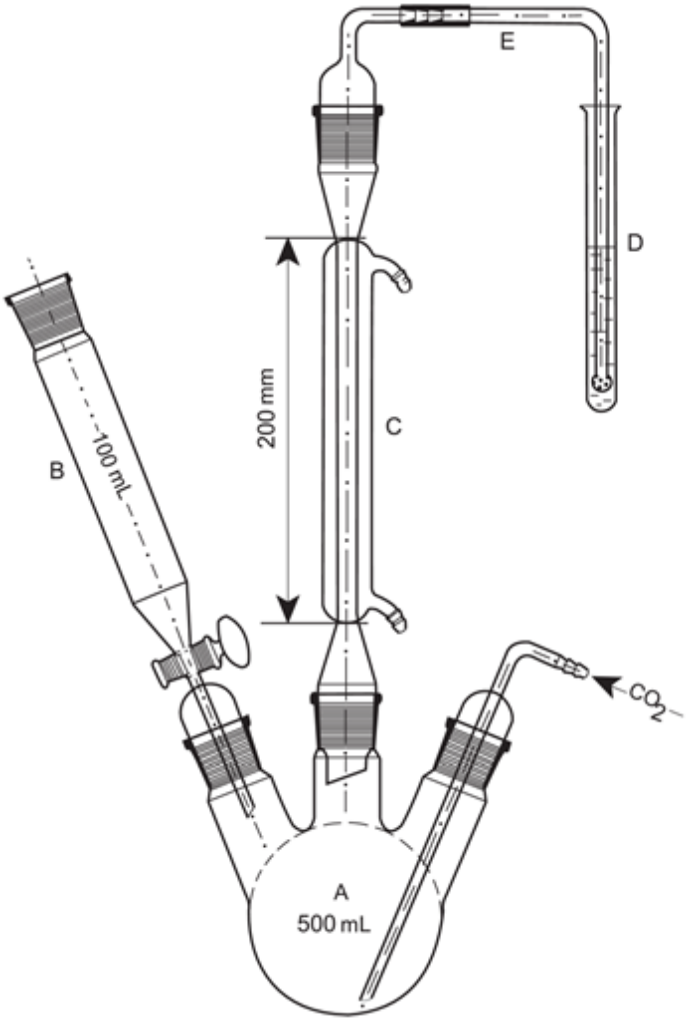
*5.4. DETERMINATION*

Add 0.1 ml of a 1 g/L solution of bromophenol blue R in ethanol (20 per cent V/V) R and titrate with 0.1 M sodium hydroxide until the colour changes from yellow to violet-blue (V1 mL). Carry out a blank titration (V2 mL).

## 6. Result

6.1. Calculate the content of sulfur dioxide in mg / kg gelatine using the following expression:

32030 x (V1 – V2) x n / m
n = molarity of the sodium hydroxide solution used as titrant.




Apparatus for the determination of sulfur dioxide

6.2 Remarks: The sensitivity of the described method is improved by increasing the sample weight to 50 g and the use of a 0,01 mol/L NaOH as the titrant.
The official EP - Method 2.5.29 defines 25.0 g sample amount and 0,1 M NaOH as a titrant.

## 2.1.3.2. Annex I - EU Regulatory limits

- Max. 50ppm: for edible (food hygiene regulation) and pharma (European Pharmacopoeia (current edition))
- >10ppm: mandatory allergen labelling on final consumer products in accordance with regulation EC N° 1169/2011.

## *2.2. PHYSICAL PROPERTIES*

*(Physical properties are of importance for fitness for use. Other methods may be used if officially recognized in other standards. In case of disagreement, the reference methods are the ones described in this document).*

## 2.2.1 Gel Strength (Jelly strength, Bloom)

### 1. Principle

A 6.67 % solution of the gelatine sample is prepared in a wide-mouthed test bottle at 60°C, cooled to 10.0°C and kept for 17 h for maturation at this temperature. The resulting gel is tested using a gelometer.

### 2. Definition

The gel strength (Bloom) is the mass in grams necessary to depress a standard plunger 4 mm into the gel having a concentration of 6.67 % and matured at 10.0°C for 17 h.

### 3. References

| | |
|---|---|
| GMIA | Gelatine Manufacturers Institute of America, Standard Methods for the Sampling and Testing of Gelatines, 1986 |
| BSI | British Standards Institution, BS 757:1975 |
| AOAC | Ass. of Official Analytical Chemists, Off. Meth. of Analysis, 23.007 |
| Eur. Ph. | European Pharmacopoeia (current edition) |
| AFNOR | Norme Francaise, NF V 59-001 |
| JIS | Japanese Industrial Standard, K 6503-1970 |
| PAGI | Methods for Testing Photographic Gelatine; Photographic and Gelatine Industries, Japan; Seventh Edition, 1992 |

### 4. Apparatus

*4.1. FOR MEASUREMENT OF GEL STRENGTH THE FOLLOWING GELOMETERS CAN BE USED* **(ANNEX 1) :**

- Lloyd TA Plus
- LFRA Texture Analyser (Brookfield)
- LFRA Texture Analyse CT3 (Brookfield)
- Texture Analyser TA-XTplus (Stable Micro Systems)
- Texture Analyser TA-XT2i (Stable Micro Systems)
- Zwick /Roell

(The manufacturers' procedure for calibrating and operating the instruments should be followed).

Recommendation for distance resolution: minimum 1% of the measuring distance;

***4.2 Plunger:***

AOAC plunger, with 12.70 mm (0.500 inches) diameter, plane surface and sharp edge, no measurable radius (annex 2)

*4.3. BLOOM JARS :*

The standard Bloom jar has a capacity of approximately 155 ml, internal diameter of 59 mm +/- 1 mm, overall height 85 mm and a flat bottom to ensure it does not rock on a flat surface.

- References: Schott, Brookfield (Type: TA-GBB-2) and Avitec Art et Technique (Fr)

*4.4. THERMOSTATIC WATER BATH, HELD AT 65°C (± 2).*

***4.5 Refrigerated water bath***

Refrigerated water bath capable of maintaining the water at 10.0°C (± 0.1), throughout the bath. It is important to have sufficient cooling capacity to limit increase of temperature when Bloom bottles are introduced.

The bath should be provided with a platform that is rigid and horizontal at such a height that the water level is approximately 1 cm above the surface of the gelatine solution in the sample bottles.

This bath should be provided with a precision thermometer for checking the temperature or with a device to record the temperature at regular intervals.

***4.6. Balance with a sensitivity of 0.01 g.***

***4.7 Dummy Bloom device***

Spring steel that can be used to check the instrument regularly.

## 5. Procedure

5.1. Weigh 7.50 g (± 0.01) gelatine into the Bloom bottles (usually two Bloom bottles are prepared for each sample).

Add 105 ml (± 0.2) water (deionised quality), stir so that all the gelatine is moistened, cover the bottles with a rubber stopper or a watch glass and allow the sample to stand at room temperature for 1 - 4 h.

Place the bottles in the 65°C water bath for about 20 min. (± 5) for dissolving the samples. To dissolve the gelatine completely and to achieve a homogeneous solution the bottles should be shaken (when covered with stoppers) or stirred gently with a glass rod (when covered with watch glasses) occasionally. Take care not to build up too much froth and check that all of the gelatine has dissolved before removing from the water bath. The temperature of the solution should not exceed 60°C.

5.2. Allow the bottles to cool for about 15 min. at room temperature, and then place in the refrigerated water bath for 17 h (± 1). Ensure that the platform in the bath is horizontal and that the bottles stand evenly on it.

5.3. Adjust the gelometer according to the manufacturers manual.
Settings are: Distance 4 mm, Speed 0.5 mm/sec.

Remove the sample bottles from the bath and quickly wipe the water from the exterior of the bottle. Centre the bottle on the platform of the gelometer so that the plunger contacts the sample as nearly at its midpoint as possible and start the measurement.

## 6. Results

The value given by the gelometer is the gel strength (grams Bloom) for the test gelatine (not corrected for moisture).

## 7. Remarks

### *7.1 Standard gelatines*

To ensure that the equipment and procedure is satisfactory it is recommended that standard gelatines of established Bloom strength are prepared and tested daily as reference gelatines.

### *7.2 Correction of the results with respect to moisture*

Moisture content of the samples influences the Bloom result, so that with all Bloom results also the moisture content should be indicated.

The Bloom value that corresponds to a certain moisture content can be calculated approximately using a graph (annex 4) or the following formula:

$$B2 = B1 + B1 \times 0.02 \times (M1 - M2)$$

(B2 = Bloom at moisture M2, B1 = Bloom at moisture M1)

### *7.3 Dummy Bloom:*

The instrument should be checked regularly using the Dummy Bloom device, utilising the elastic deformation of spring steel to check the instrument.

The instrument could be calibrated using weights according to the manufacturers' instructions, where appropriate.

### *7.4 Plunger:*

Up until 1997 in Europe the BS plunger has mainly been used. This has the same diameter (12.70 mm) as the AOAC plunger but has a rounded lower edge (0.35 – 0.43 mm radius).

The move to using the AOAC plunger as the "standard" reflects the desirability of world-wide standardisation of gelatine Bloom testing.

### *7.5 Repeatability of Bloom measurement:*

Within one laboratory for a given sample a relative standard deviation (repeatability coefficient of variation) of 1.5 % is realistic.

### *7.6 Reproducibility of Bloom measurement*:

Results of ring tests have shown that between different laboratories using the same procedure a relative standard deviation (reproducibility coefficient of variation) of 3 % or better should be achievable.

### *7.7. Design of thermostatic bath:*

The design of the chill bath should be such that when the Bloom jars are placed in the bath the temperature, which will rise, recovers to 10.0°C ± 0.1 within about 1 h.

This may limit the number of Bloom jars, which can be placed in the water bath.

### *7.8 Test report*:

In addition to the Bloom value and the moisture also details of the method used should be given:

Type of instrument
Type of plunger
Type of Bloom bottle
Any other parameters different form this procedure

## 8. Annexes

Annex 1: Gelometers
Annex 2: Bloom plunger
Annex 3: Bloom bottles
Annex 4: Sliding scale for correction of Bloom as a function of humidity

**ANNEX I**


LLOYD TEXTURE ANALYSER

## ANNEX I


| MODEL | Load Range | Load Resolution | Load Accuracy | Repeatability | Speed Range | Position Range | Setting Resolution | *Maximum Cycles | Position Accuracy |
|---|---|---|---|---|---|---|---|---|---|
| TALFRA1500 | 0-1500g | 1g | ±0.2FS | 0.15FS | 6-120mm/min | 1-75mm | 1mm | Multi-Cycle | ±0.02mm |
| TALFRA1000 | 0-1000g | 1g | ±0.2FS | 0.15FS | 6-120mm/min | 1-75mm | 1mm | Multi-Cycle | ±0.02mm |
| TALFRA100 | 0-100g | 0.1g | ±0.2FS | 0.15FS | 6-120mm/min | 1-75mm | 1mm | Multi-Cycle | ±0.02mm |

mm = millimeter g = grams FS = Full Scale min = minute

*with cycle until reset

**ANNEX I**


LFRA TEXTURE ANALYSER CT3

## ANNEX I


TA XTplus

## ANNEX I

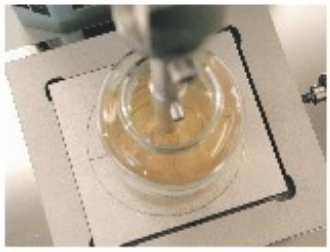

TA XT2i

**ANNEX I**

Zwick / Roell

Zwick
Materials Testing

# Gel strength (Bloom) measurement with zwicki-Line Z0.5 - Zwick materials testing machine



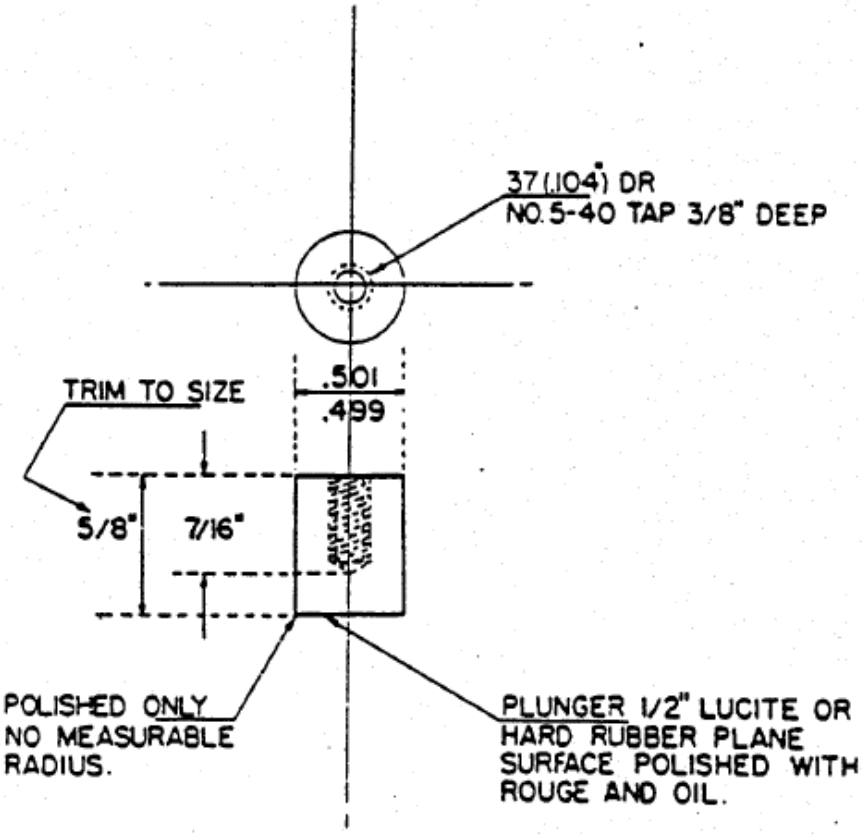
**Advantages/characteristics**

- The Zwick testing machines use a digital controller, *testControl*, which is vertically mounted on the machine frame to prevent ingress of liquids, and conductive particles from test specimens coming into contact with the electronic systems.
- *testControl* technology allows the crosshead positioning of the testing machine to be corrected during the test to compensate for the system compliance.
- *testControl* incorporates an adaptive drive control system. This ensures that force or extension control is correctly maintained during the test.
- The Zwick testing machines operate with standard PCs or laptops as they require no special interface cards.
- *testXpert*® II testing software provides a flexible configuration whilst maintaining remarkable ease of use.
- High quality industrial drive systems ensure the highest reliability and availability.
- Smart sensor technology recognises & calibrates all corrected sensors including their defined force limits.

| **zwicki-Line testing machine Z0.5** | |
|---|---|
| Conformity | to ISO 9000 and CE |
| Test load $F_N$ in tensile/compression direction | 500 N |
| Crosshead speed $v_{min}$ ... $v_{nom}$ | 0.001...2000 mm/min |
| Accuracy of the set speed | 0.02 % of $v_{nom}$ |
| Drive system's travel resolution | 0.2453 µm |
| Positioning, repetition accuracy | ± 2 µm |
| Accuracy grade of load cell Xforce HP 0.5 | 1 from 1 N, resp. 0.5 from 5 N |

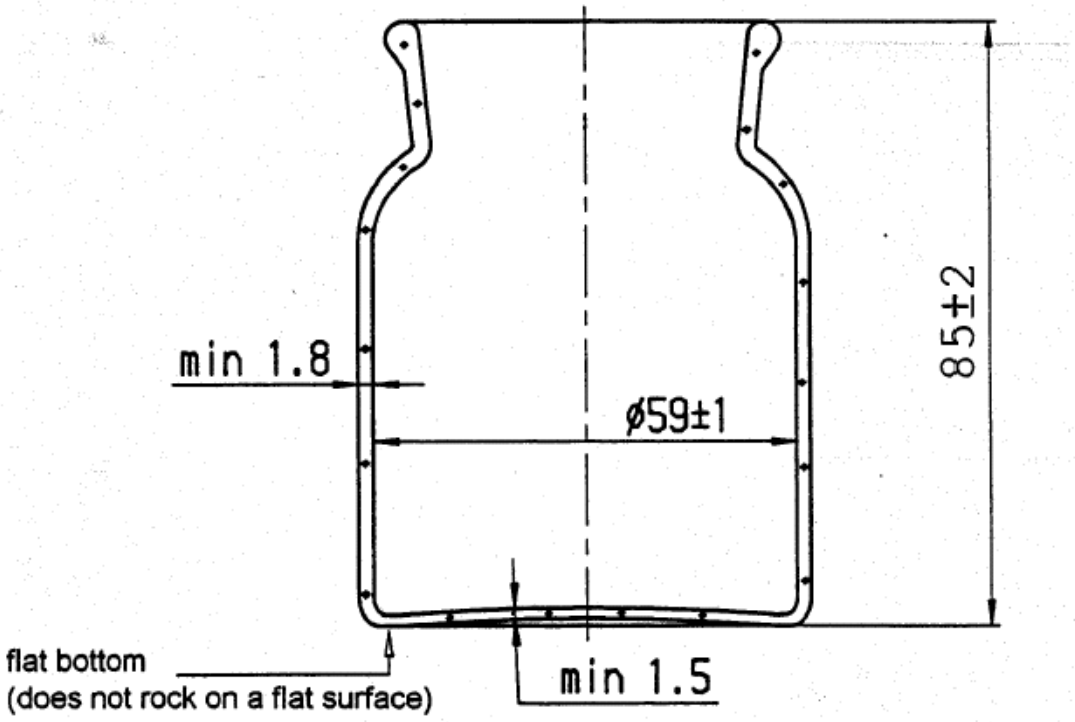
**Annex 2**

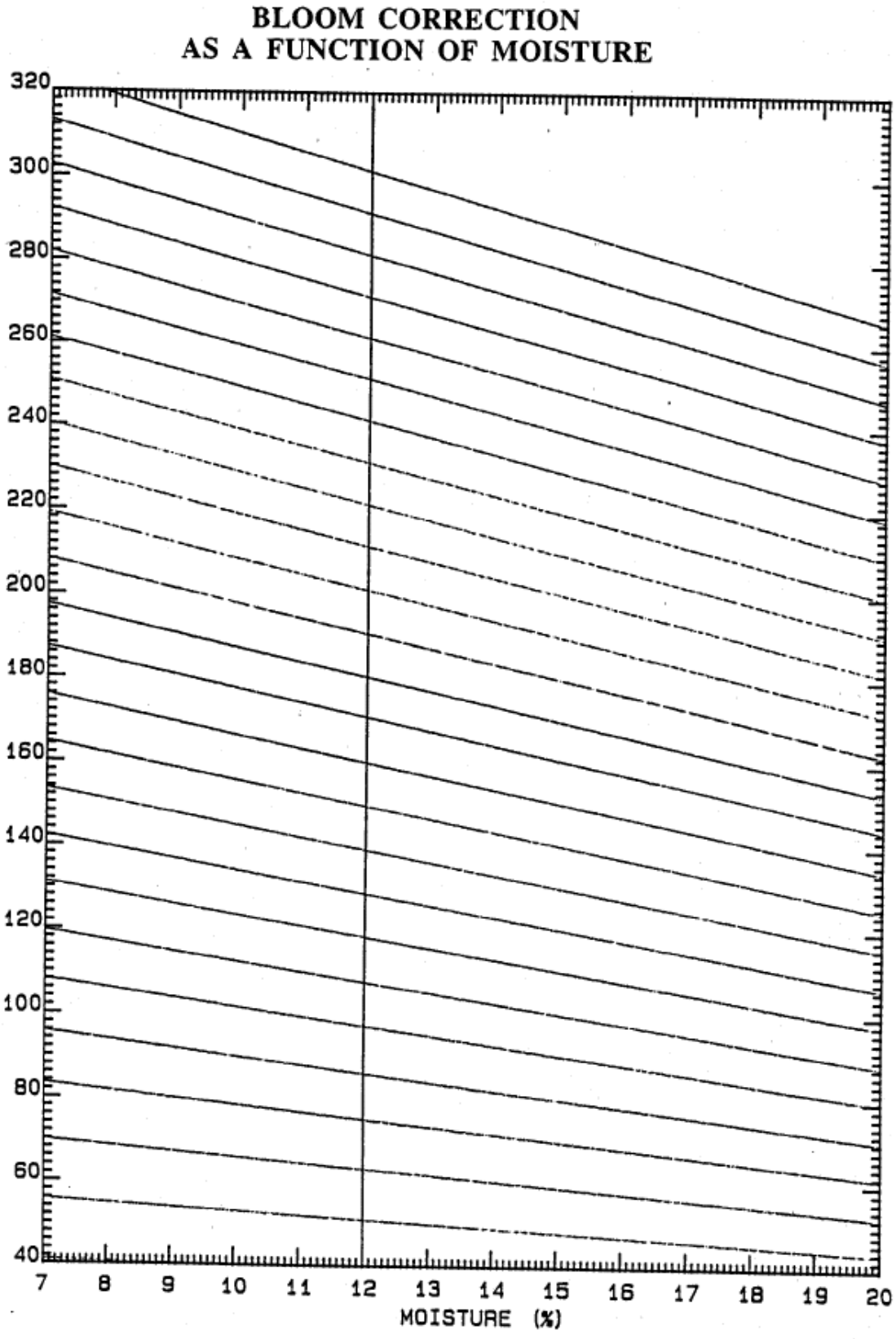
## FIG. 23:01—0.5 in. Bloom Gelometer plunger



**Annex 3**

## BLOOM TEST JAR




BLOOM CORRECTION
AS A FUNCTION OF MOISTURE
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
7
8
9
10
11
12
13
14
15
16
17
18
19
20
MOISTURE (%)

## 2.2.2. Viscosity

### 1. Principle

The viscosity of a 6.67 % solution of gelatine is determined at 60°C by measuring the flow time of 100 ml of the solution through a standard pipette.

### 2. Definition

The viscosity of a gelatine is defined as its dynamic viscosity, expressed in m Pa.s when tested as a 6.67% solution in water at 60°C.

### 3. References

| | |
|---|---|
| GMIA | Gelatine Manufacturers Institute of America, Standard Methods for the Sampling and Testing of Gelatines, 1986 |
| JIS | Japanese Industrial Standard, K 6503-1970 |
| PAGI | Methods for Testing Photographic Gelatine; Photographic and Gelatine Industries, Japan; Seventh Edition, 1992 |
| BSI | British Standards Institution, BS 757:1975 (U-tube method) |

### 4. Calibrations oils

Two standard (reference) viscosity oils calibrated at 60° C, having viscosities within the approximate range 2-10 cS. One oil should have a viscosity at least twice that of the other oil.

### 5. Apparatus

5.1. Pipette (Bloom pipette, see Annex 1):
100 ml pipette with a precision capillary outlet and an upper and lower mark on the glass.

5.2. Thermostatic bath for the pipette with a thermostatic device for stirring and heating (60°C ± 0.1°C).

5.3. Precision thermometer (mercury or electronic thermometer), graduated in 0.1°C, with a long slim stem for measuring temperature inside the pipette.

5.4. Stop watch, accurate to 0.1 sec.

5.5. Thermostatic water bath for sample preparation, held at 65°C ± 2 .

5.6. Balance with a sensitivity of 0.01 g

## 6. Procedure (Gelatine)

6.1. Weigh 7.50 g (± 0.01) gelatine into a 150 ml bottle, add 105 ml (± 0.2) water, stir, so that all the gelatine is moistened, cover the bottles with a rubber stopper or a watch glass and allow the sample to stand at room temperature for 1 - 4 h. Place the bottles in the 65°C water bath for about 20 min. for dissolving the samples.

To dissolve the gelatine completely and to achieve a homogeneous solution the bottles should be shaken (when covered with stoppers) or stirred gently with a glass rod (when covered with watch glasses) occasionally. Take care not to build up too much froth.

6.2. When the temperature of the solution reaches 61 – 62° C and the sample is completely dissolved, remove the thermometer and transfer the solution to the viscosity pipette as quickly as possible without entrapping air.

6.3. Sufficient solution is poured into the pipette to bring its level about 1 cm above the upper mark while a finger closes the capillary end. The bottle with the residual solution is placed directly under the exit tube.

The temperature of the solution in the pipette can be checked with the thermometer and when it has reached 60°C the measurement can be started by removing the finger and determining the flow time between upper mark and
lower mark.

6.4. Read and record the time required for the 100 ml of solution to pass through the capillary tube of the pipette.

6.5. After each determination and also before use the pipette is washed out with about 25 ml of water heated to about 62°C and allowed to drain thoroughly.

## 7. Results

The viscosity of any sample (V) with the efflux time t may be calculated from the equation:

$$V = (A \times t - B / t) \times d$$

where V = dynamic viscosity in mPa.s

For gelatine at 6,67 %, 60°C $d = 1.001$

## 8. Calibration

8.1. Pipettes can be calibrated using two standard oils of different viscosity (determination of constants A and B).

8.2. The pipette must be thoroughly cleaned before use and dried with reagent grade acetone.

8.3. Preheat each oil in a constant temperature bath set at 63 – 64°C by stirring and transfer to the pipette, holding a finger over the pipette outlet until the oil temperature is exactly 60°C as read on the immersed thermometer.

8.4. Measure the efflux time of the oil between the upper and lower graduation of the pipette using the stopwatch. Repeat the measurement at least three times with each oil.

8.5. Clean the pipette thoroughly between different oils using suitable organic solvents for removing the oil, wash out the solvent with acetone and dry.

8.6. Calculation of the constants A and B:
(t = efflux time in sec; d = oil density at 60°C; A and B = pipette constants)

$$V = (A\,t - B / t) \times d$$

$$B = \frac{t1 \times t2\,(V2 \times t1 - V1 \times t2)}{t2^2 - t1^2}$$

$$A = \frac{V1 + B / t1}{t1} = \frac{V2 + B / t2}{t2}$$

V1 = kinematic viscosity of lower viscosity oil in cSt
V2 = kinematic viscosity of higher viscosity oil in cSt
t1 = average efflux time of lower viscosity oil in sec

t2 = average efflux time of higher viscosity oil in sec

## 9. Remarks

### *9.1. Standard gelatines:*

To ensure that the equipment and procedure is satisfactory it is recommended that standard gelatines of established viscosity are prepared and tested regularly as reference gelatines.

The standard gelatines must be stored under suitable conditions so that the moisture does not change with the time.

### *9.2. Correction of the results regarding to the moisture:*

Moisture content of the samples influences the viscosity result for a 6.67 % solution, so that with all viscosity results also the moisture content should be indicated.

The viscosity value that corresponds to a certain moisture content can be calclated approximately using a graph (annex 3) or the following formula:

$$V2 = V1 + V1 \times 0.02 \times (M1 - M2)$$

(V1 = viscosity at moisture M1, V2 = viscosity at moisture M2)

### *9.3. Test report:*

In addition to the viscosity value and the moisture also details of the method used should be given, if any parameters are different form this procedure.

### *9.4. Other viscometers:*

If other viscometers are used, e.g. U-tube viscometer (BSI), Ostwald viscometer or Ubbelohde viscometer, this must be indicated in the results.

### *9.5. Repeatability of viscosity measurement:*

Within one laboratory for a given sample a relative standard deviation (repeatability coefficient of variation) of 1.0 % is realistic.

### *9.6. Reproducibility of viscosity measurement:*

Results of ring tests have shown that between different laboratories using the same procedure a relative standard deviation (reproducibility coefficient of variation) of 5 % or better should be achievable.

### *9.7. Cleaning of pipette:*

The pipette must be thoroughly cleaned before use, preferably by washing with soap and hot water, and rinsed with distilled water. When not in use it is best to keep the pipette filled with distilled water.

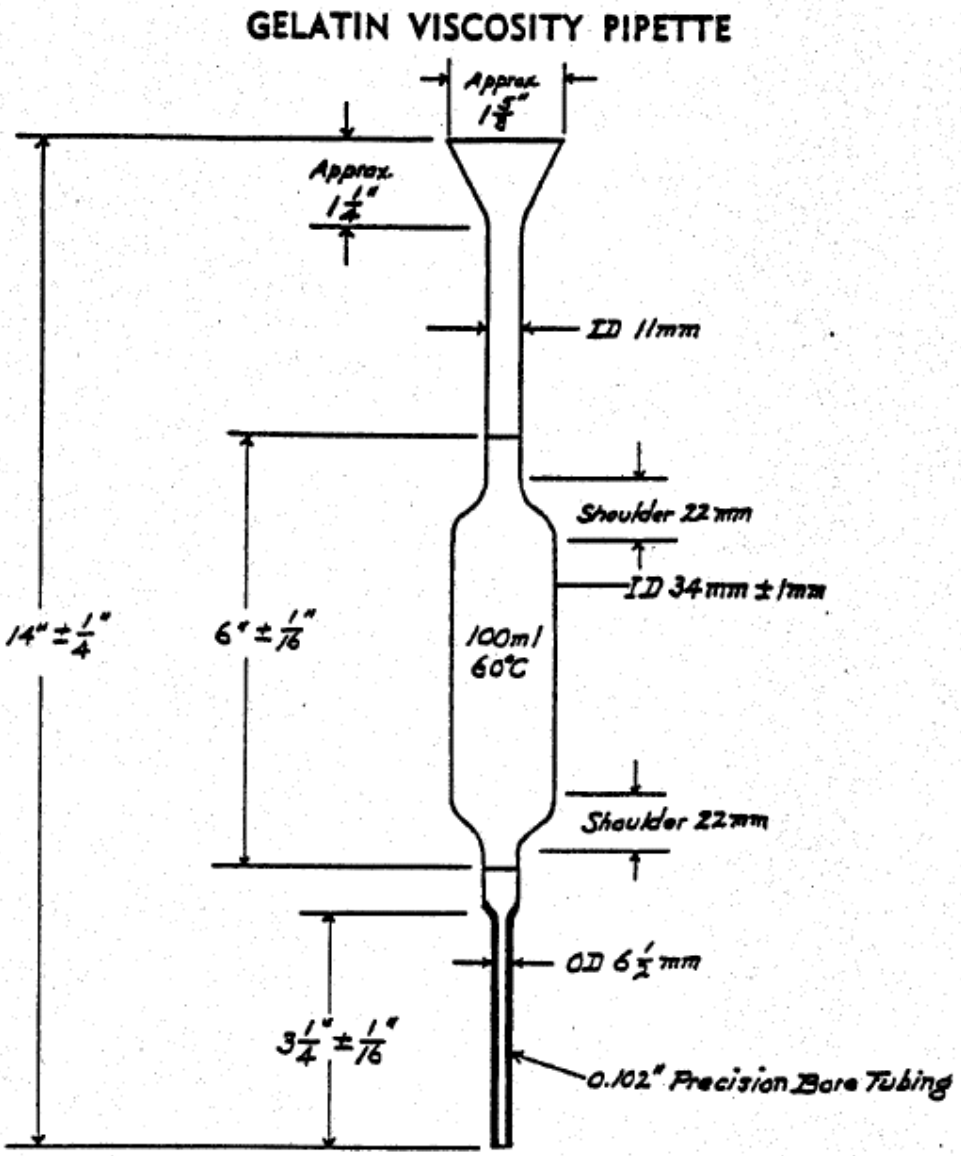
### *9.8. Thermal viscosity breakdown:*

Due to the possibility of thermal viscosity breakdown samples should not be held longer than 60 min at 60°C.

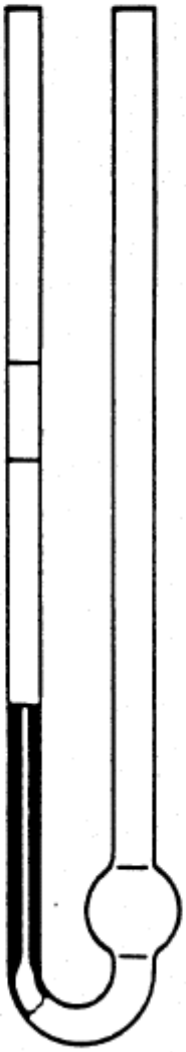
## 10. Annexes

| | |
|---|---|
| Annex 1: | Viscosity pipette (Bloom pipette) |
| Annex 2: | U-tube viscometer (BSI) |
| Annex 3: | Sliding scale for the correction of viscosity as a function of moisture |

**Annex 1**

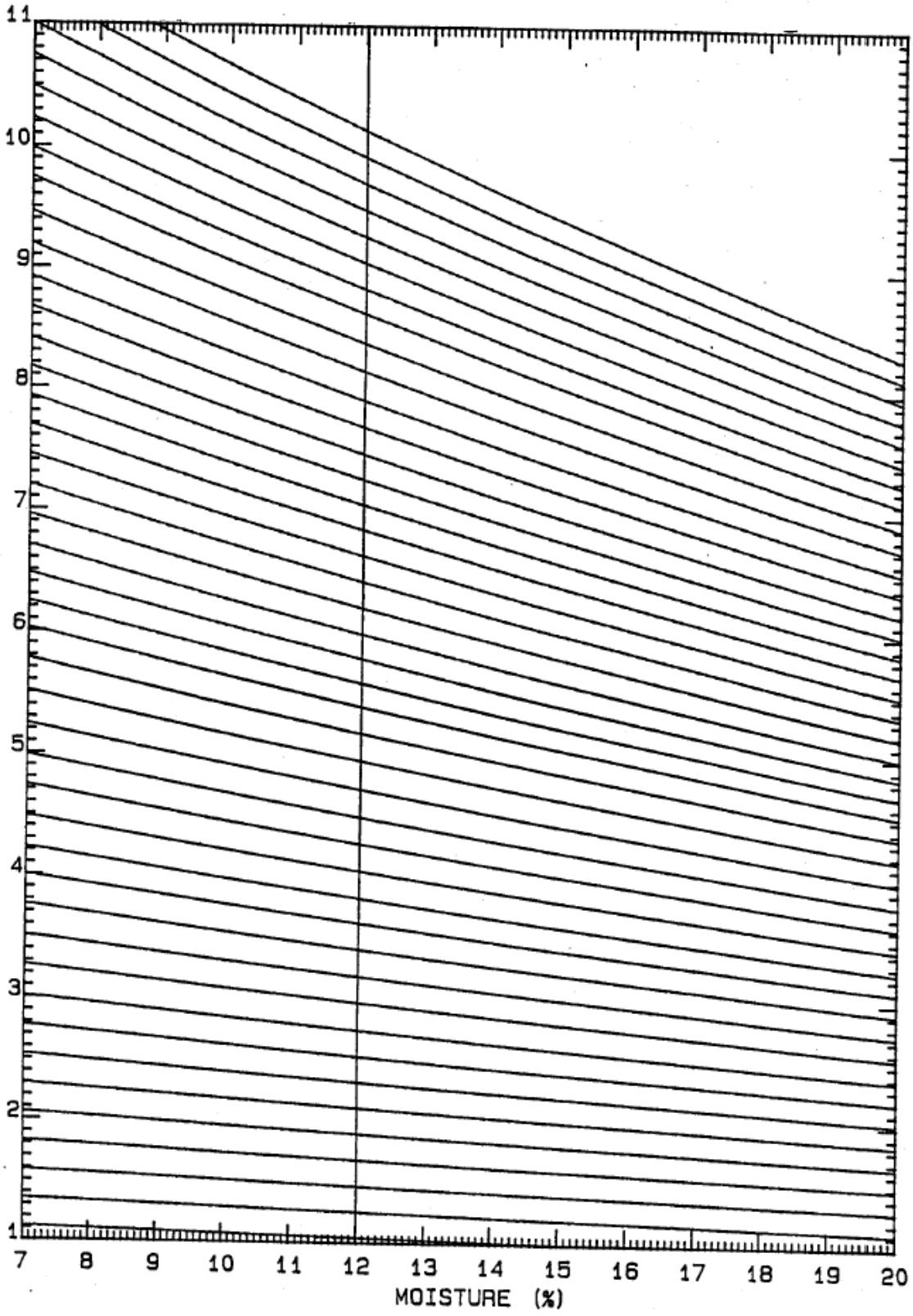

GELATIN VISCOSITY PIPETTE
Approx. 1 5/8"
Approx. 1 1/4"
ID 11mm
Shoulder 22mm
ID 34mm ± 1mm
14" ± 1/4"
6" ± 1/16"
100ml 60°C
Shoulder 22mm
OD 6 1/2 mm
3 1/4" ± 1/16"
0.102" Precision Bore Tubing

**Annex 2**



**Annex 3**

# VISCOSITY CORRECTION AS A FUNCTION OF MOISTURE



## 2.2.3. pH

### 1. Principle

The pH is a number, which represents conventionally the hydrogen ion concentration of an aqueous solution. The pH of a 6.67 % gelatine solution is determined by potentiometry at a temperature of 55-60°C using a pH meter. The pH may also be determined on a 1 % solution at 55°C like described in the European pharmacopoeia.

### 2. Reference(s)

- European Pharmacopoeia (current edition)
- GMIA, Gelatine Manufacturers Institute of America, Standard Methods for the Sampling and Testing of Gelatines, 1986; (1.5% solution at 25°C)
- PAGI, Methods for Testing Photographic Gelatine; Photographic and Gelatine Industries, Japan, Seventh Edition 1992; (5% solution at 35°C)

### 3. Reagents and solutions

Carbon dioxide free water i.e. distilled water or equivalent
pH 4 reference solution
pH 7 reference solution

### 4. Apparatus

Analytical balance
Glasswork
pH meter:

The measuring apparatus is a voltmeter with an input resistance of at least 100 times that of the electrodes used. The sensitivity must allow discrimination of at least 0.05 pH units (better 0.01) within a region of 0 - 14. The pH meter consists of a thin glass $H^+$-selective membrane, an internal reference electrode, an external reference electrode and a meter with control electronics and display. Commercial pH electrodes usually combine all electrodes into one unit that are then attached to the pH meter. An automatic temperature compensation is nowadays available as standard.

### 5. Calibration

Calibration should be done at the same temperature as the pH measurement.

The calibration is performed by using the 2 reference solutions at a pH of 7 (i.e. potassium dihydrogen phosphate 0.025 M + disodium hydrogen phosphate 0.025 M, pH 6.87 at 55-60°C) and a pH of 4 (i.e. Potassium hydrogen phthalate 0.05 M, pH 4.01 at 55-60°C).

Calibration is performed according the pH meter-instructions. Swirl well the solution to fully saturate the electrode with buffer and rinse well with distilled water or equivalent afterwards. Electrodes are regularly cleaned by using an enzyme solution.

## 6. Procedure

6.1 Weigh 7.50 g (± 0.01) gelatine into a 150 ml bottle, add 105 ml (± 0.2) water, stir so that all the gelatine is moistened, cover the bottles with a rubber stopper or a watch glass and allow the sample to stand at room temperature for 1-4 h.

Place the bottles in the 65°C water bath for about 20 min. for dissolving the samples. To dissolve the gelatine completely and to achieve a homogeneous solution the bottles should be shaken (when covered with stoppers) or stirred gently with a glass rod (when covered with watch glasses) occasionally. (The solution prepared for bloom or for clarity determination could also be used)

6.2 Determine the pH of the gelatine solution according the pH-meter instructions.
Swirl well the solution to fully saturate the electrode and rinse well with warm distilled water or equivalent afterwards.

## 7. Result

The pH result is determined with an accuracy of ± 0.1.

# ANNEXES:

*Annex 1: Regulation (EC) No 853/2004 of the European Parliament and of the Council*

Link: http://ec.europa.eu/food/safety/biosafety/food_hygiene/legislation_en

*Annex 2: Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuff*