

# E18 HYDROXYETHYLCELLULOSE

Stage 4, Revision 3,

CP:EP

## BRIEFING NOTE

Compared to the Stage 4, revision 2 document the following changes are proposed:

1. Apparent viscosity: the test has been replaced by a very similar test as in the signed-off monographs for Hypromellose and Methylcellulose.
2. Assay: a determination of the molar substitution has been added based on extensive validation work. The validation report is attached for your information.  
This assay applies the same general principle for determination of alkoxy groups in substituted celluloses (Zeissel-reaction followed by gas chromatography) which has already been proposed for Ethylcellulose (collaborative trial with TriPEC on-going) and presented by JPEC for Hydroxypropylcellulose and Hydroxypropylcellulose, low substituted at the last PDG-TriPEC meeting.  
EP considers it to be advantageous for the users to apply a consistent approach for assays of all substituted celluloses.
3. In addition, the text has been converted to "global style".

## HYDROXYETHYLCELLULOSE

### DEFINITION

Partly *O*-(2-hydroxyethylated) cellulose.

Content: 30.0 per cent to 70.0 per cent of hydroxyethoxy (-OC<sub>2</sub>H<sub>4</sub>OH) groups (dried substance).

### CHARACTERS

*Appearance*: white, yellowish-white or greyish-white powder or granules.

*Solubility*: soluble in hot and cold water giving a colloidal solution, practically insoluble in acetone, in alcohol and in toluene.

### IDENTIFICATION

A. Heat 10 ml of solution S (see Tests) to boiling. The solution remains clear.

B. To 10 ml of solution S add 0.3 ml of a 120 g/l solution of acetic acid and 2.5 ml of a 100 g/l solution of tannic acid. A yellowish-white, flocculent precipitate is formed which dissolves in a 140 g/l solution of concentrated ammonia.

C. In a test-tube about 160 mm in length, thoroughly mix 1 g with 2 g of finely powdered manganese sulphate. Introduce to a depth of 2 cm into the upper part of the tube a strip of filter paper impregnated with a freshly prepared mixture of 1 volume of a 200 g/l solution of diethanolamine and 11 volumes of a 50 g/l solution of sodium nitroprusside, adjusted to about pH 9.8 with 1 M hydrochloric acid. Insert the tube 8 cm into a silicone-oil bath and heat at 190-200 °C. The filter paper becomes blue within 10 min. Carry out a blank test.

D. Dissolve 0.2 g completely, without heating, in 15 ml of a 700 g/l solution of sulphuric acid. Pour the solution with stirring into 100 ml of iced water and dilute to 250 ml with iced water. In a test-tube, mix thoroughly while cooling in iced water 1 ml of the solution with 8 ml of sulphuric acid, added dropwise. Heat on a water-bath for exactly 3 min and immediately cool in iced water. While the mixture is cold, carefully add 0.6 ml of ninhydrin solution and mix well. Allow to stand at 25 °C. A pink colour is produced immediately and does not become violet within 100 min.

### TESTS

**Solution S.** Disperse a quantity of the substance to be examined equivalent to 1.0 g of the dried substance in 50 ml of carbon dioxide-free water. After 10 min, dilute to 100 ml with carbon dioxide-free water and stir until dissolution is complete.

**pH:** 5.5 to 8.5 for solution S.

~~**Apparent viscosity (2.2.10):** 75 per cent to 140 per cent of the value stated on the label. While stirring, introduce a quantity of the substance into 50 g of water R. Dilute to 100.0 g with water R and stir until dissolution is complete. Determine the viscosity using a rotation viscometer at 25 °C and at a shear rate of 100 s<sup>-1</sup> for substances with an expected viscosity up to 100 mPa·s, at a shear rate of 10 s<sup>-1</sup> for substances with an expected viscosity between 100 mPa·s and 20 000 mPa·s and at a shear rate of 1 s<sup>-1</sup> for substances with an expected viscosity above 20 000 mPa·s. If it is impossible to obtain a shear rate of exactly 1 s<sup>-1</sup>, 10 s<sup>-1</sup> or 100 s<sup>-1</sup> respectively, use a rate slightly higher and a rate slightly lower and interpolate.~~

1  
2 **Apparent viscosity:** minimum 80 per cent and maximum 120 per cent of the nominal value for  
3 samples with a viscosity less than 600 mPa·s (Method 1); minimum 75 per cent and maximum  
4 140 per cent of the nominal value for samples with a viscosity of 600 mPa·s or higher (Method 2).  
5 Method 1, to be applied to samples with a viscosity of less than 600 mPa·s. Weigh accurately a  
6 quantity of the substance to be examined equivalent to 4.000 g of the dried substance. Transfer  
7 into a wide-mouthed bottle, and adjust the mass to 200.0 g with water. Capping the bottle, stir by  
8 mechanical means at  $400 \pm 50$  r/min for 10-20 min until the particles are thoroughly dispersed  
9 and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there  
10 is no undissolved material on the sides of the bottle, and continue the stirring for another 20-  
11 40 min. Adjust the solution mass if necessary to 200.0 g using water. Centrifuge the solution if  
12 necessary to expel any entrapped air bubbles. Using a spatula, remove any foam, if present.  
13 Determine the viscosity of this solution using the capillary viscometer method to obtain the  
14 kinematic viscosity ( $\nu$ ). Separately, determine the density ( $\rho$ ) of the solution and calculate the  
15 dynamic viscosity ( $\eta$ ), as  $\eta = \rho\nu$ .

16  
17 Method 2, to be applied to samples with a viscosity of 600 mPa·s or higher. Weigh accurately a  
18 quantity of the substance to be examined equivalent to 10.00 g of the dried substance. Transfer  
19 into a wide-mouthed bottle, and adjust the mass to 500.0 g with water. Capping the bottle, stir by  
20 mechanical means at  $400 \pm 50$  r/min for 10-20 min until the particles are thoroughly dispersed  
21 and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there  
22 is no undissolved material on the sides of the bottle, and continue the stirring for another 20-  
23 40 min. Adjust the solution mass if necessary to 500.0 g using water. Centrifuge the solution if  
24 necessary to expel any entrapped air bubbles. Using a spatula, remove any foam, if present.  
25 Determine the viscosity of this solution at  $20 \pm 0.1$  °C using a rotating viscometer.

26  
27 Apparatus: single-cylinder type spindle viscometer Brookfield type LV (low viscosity) model is  
28 suitable.

29  
30 Rotor number, revolution and calculation multiplier: apply the conditions specified in Table  
31 0348.-1.

32  
33 Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of 2 min  
34 between subsequent measurements. Repeat the measurement twice and determine the mean of the  
35 3 readings.

36 Table 0348.-1.

<u>Labelled viscosity*</u> <u>(mPa·s)</u>	<u>Rotor</u> <u>number</u>	<u>Revolution</u> <u>(r/min)</u>	<u>Calculation</u> <u>multiplier</u>
<u>600 to less than 1400</u>	<u>3</u>	<u>60</u>	<u>20</u>
<u>1400 to less than 3500</u>	<u>3</u>	<u>12</u>	<u>100</u>
<u>3500 to less than 9500</u>	<u>4</u>	<u>60</u>	<u>100</u>
<u>9500 to less than</u> <u>99 500</u>	<u>4</u>	<u>6</u>	<u>1000</u>
<u>99 500 or more</u>	<u>4</u>	<u>3</u>	<u>2000</u>

\* the nominal viscosity is based on the manufacturer's specifications.

1  
2 **Chlorides:** maximum 1.0 per cent.

3 Dilute 1 ml of solution S to 30 ml with water. To 15 ml of the solution add 1 ml of a 200 g/l  
4 solution of nitric acid and pour the mixture as a single addition into a test-tube containing 1 ml of  
5 a 17 g/l solution of silver nitrate . Prepare a standard in the same manner using 10 ml of chloride  
6 standard solution (5 ppm Cl) and 5 ml of water. Examine the tubes laterally against a black  
7 background.

8 After standing for 5 min protected from light, any opalescence in the test solution is not more  
9 intense than that in the standard.

10  
11 **Nitrates:** maximum 3.0 per cent (dried substance), if hydroxyethylcellulose has an apparent  
12 viscosity of 1000 mPa·s or less and maximum 0.2 per cent (dried substance), if  
13 hydroxyethylcellulose has an apparent viscosity of more than 1000 mPa·s.

14 Determine potentiometrically using as indicator a nitrate selective electrode and a silver-silver  
15 chloride electrode with 0.1 M ammonium sulphate as reference electrolyte.

16  
17 *Prepare the solutions immediately before use.*

18  
19 *Buffer solution.* To a mixture of 50 ml of 1 M sulphuric acid and 800 ml of water, add 135 g of  
20 potassium dihydrogen phosphate and dilute to 1000 ml with water.

21  
22 *Buffered water.* Dilute 80 ml of buffer solution to 2000 ml with water.

23 *Nitrate standard solution (500 ppm NO<sub>3</sub>).* Dissolve 0.8154 g of potassium nitrate in 500 ml of  
24 buffered water and dilute to 1000.0 ml with the same solvent.

25  
26 *Test solution.* Dissolve 0.50 g of the substance to be examined in buffered water and dilute to  
27 100.0 ml with the same solvent.

28  
29 *Reference solutions.* If hydroxyethylcellulose has an apparent viscosity of 1000 mPa·s or less,  
30 dilute 10.0 ml, 20.0 ml and 40.0 ml of nitrate standard solution (500 ppm NO<sub>3</sub>) to 100.0 ml with  
31 buffered water and mix.

32  
33 If hydroxyethylcellulose has an apparent viscosity of more than 1000 mPa·s, dilute 1.0 ml, 2.0 ml  
and 4.0 ml of nitrate standard solution (500 ppm NO<sub>3</sub>) to 100.0 ml with buffered water and mix.  
35 Carry out the measurements for each solution. Calculate the concentration of nitrates using the  
36 calibration curve.

37  
38 **Glyoxal:** maximum 20 ppm.

39 Introduce 1.0 g into a test tube with a ground-glass stopper and add 10.0 ml of anhydrous ethanol.  
40 Stopper the tube and stir mechanically for 30 min. Centrifuge. To 2.0 ml of the supernatant liquid  
41 add 5.0 ml of a 4 g/l solution of methylbenzothiazolone hydrazone hydrochloride in an 80 per  
42 cent V/V solution of glacial acetic acid in water. Shake to homogenise. After 2 h, the solution is  
43 not more intensely coloured than a standard prepared at the same time and in the same manner  
44 using 2.0 ml of glyoxal standard solution (2 ppm C<sub>2</sub>H<sub>2</sub>O<sub>2</sub>) instead of the 2.0 ml of supernatant  
45 liquid.

46  
47 **Ethylene oxide.** Head-space gas chromatography: maximum 1 ppm.

48 *Test preparation.* Place 1.00 g of the substance to be examined in a 5 ml vial (other sizes may be  
49 used depending on the operating conditions) and add 1 ml of water. It swells in water but does not  
50 dissolve.

1  
2 *Reference preparation (a).* Place 1.00 g of the substance to be examined into an identical 5 ml  
3 vial. Add 0.2 ml of cooled ethylene oxide solution and 0.8 ml of water. It swells in water but does  
4 not dissolve.

5  
6 *Reference preparation (b).* To 0.1 ml of ethylene oxide solution in a 5 ml vial add 0.1 ml of a  
7 freshly prepared 10 mg/l solution of acetaldehyde.

8  
9 *Close the vials immediately with a butyl rubber membrane stopper, coated with aluminium or*  
10 *polytetrafluoroethylene and secured with an aluminium crimped cap.*

11  
12 The following static head-space injection conditions may be used:

- 13 — equilibration temperature: 70 °C,  
14 — equilibration time: 45 min,  
15 — transfer-line temperature: 75 °C,  
16 — carrier gas: helium for chromatography or nitrogen for chromatography,  
17 — pressurisation time: 30 s,  
18 injection volume: 1 ml.

19  
20 The chromatographic procedure may be carried out using:

- 21 — a glass or fused-silica column 30 m long and 0.32 mm in internal diameter, the inner surface  
22 of which is coated with a 1.0 µm layer of poly(dimethyl)siloxane,  
23 — helium for chromatography or nitrogen for chromatography as the carrier gas with a linear  
24 velocity of about 20 cm/s and a split ratio of 1:20,  
25 — a flame-ionisation detector, maintaining the temperature of the column at 50 °C for 5 min,  
26 then raising the temperature at a rate of 30 °C per minute to 230 °C and maintaining at 230 °C for  
27 5 min; that of the injection port at 150 °C and that of the detector at 250 °C.

28  
29 Inject 1.0 ml of the gaseous phase of reference solution (b). Adjust the sensitivity of the system so  
30 that the heights of the 2 principal peaks in the chromatogram obtained are not less than 15 per  
31 cent of the full scale of the recorder. The test is not valid unless the resolution between the peaks  
32 corresponding to acetaldehyde and ethylene oxide is at least 2.0.

33  
34 Inject separately 1.0 ml of the gaseous phases of the test solution and reference solution (a). In the  
35 chromatogram obtained with the test solution, the area of any peak corresponding to ethylene  
36 oxide is not greater than half the area of the peak due to ethylene oxide in the chromatogram  
37 obtained with reference solution (a).

38  
39 Alternatively the content of ethylene oxide in parts per million can be calculated from the  
40 expression:

$$\frac{A_T \times M_{EO} \times C}{0.25(A_R \times M_T - A_T \times M_R)}$$

41

$$\text{where } C = \frac{C_{EO}}{M_{EO} \times 10}$$

42

$A_T$  = area of the peak corresponding to ethylene oxide in the chromatogram obtained  
with the test solution,

$A_R$  = area of the peak corresponding to ethylene oxide in the chromatogram obtained  
with reference solution (a),

$M_{EO}$  = mass of absorbed ethylene oxide (used for preparing ethylene oxide solution) in

grams,

- $M_T$  = mass of the substance to be examined in grams in the test solution,  
 $M_R$  = mass of the substance to be examined in grams in the reference solution,  
 $C$  = correction factor to be determined from the formula,  
 $C_{EO}$  = ethylene oxide content in milligrams per millilitre, determined by titration.

**2-Chloroethanol.** Head-space gas chromatography.

*Test preparation.* To 50 mg of the substance to be examined in a 10 ml vial (other size may be used depending on the operating conditions), add 2  $\mu$ l of 2-propanol. Seal the flask and mix.

*Reference preparation (a).* Dissolve 0.125 g of 2-chloroethanol and dilute to 50.0 ml with 2-propanol. Dilute 1.0 ml of the solution to 10.0 ml with 2-propanol.

*Reference preparation (b).* To 50 mg of the substance to be examined into an identical 10 ml vial, add 2  $\mu$ l of reference solution (a). Seal the flask and mix.

*Close the vials immediately with a butyl rubber membrane stopper, coated with aluminium or polytetrafluoroethylene and secured with an aluminium crimped cap.*

*Column:*

— size:  $l = 50$  m,  $\varnothing = 0.32$  mm,

— stationary phase: poly(dimethyl)siloxane (1.2  $\mu$ m)CP SIL 5CB (CHROMPACK) is suitable..

*Carrier gas:* helium for chromatography.

*Flow rate:* 25-35 cm/s.

*Split ratio:* 1:10.

*Static head-space conditions which may be used:*

— *equilibration temperature:* 110 °C,

— *equilibration time:* 20 min,

— *temperature of injection system:* 115 °C.

*Temperature:*

	Time (min)	Temperature (°C)
Column	0 - 6	60
	6 - 16	60 → 110
	16 - 31	110 → 230
	31 - 36	230
Injection port		150
Detector		250

*Detection:* flame ionisation.

*Injection:* 2 ml.

*Retention time:* 2-chloroethanol = about 7.8 min.

*Limit:*

1 — *2-chloroethanol*: not more than 0.5 times the area of the peak due to 2-chloroethanol in  
2 reference solution (b) (10 ppm).

3  
4 **Heavy metals**: maximum 20 ppm.

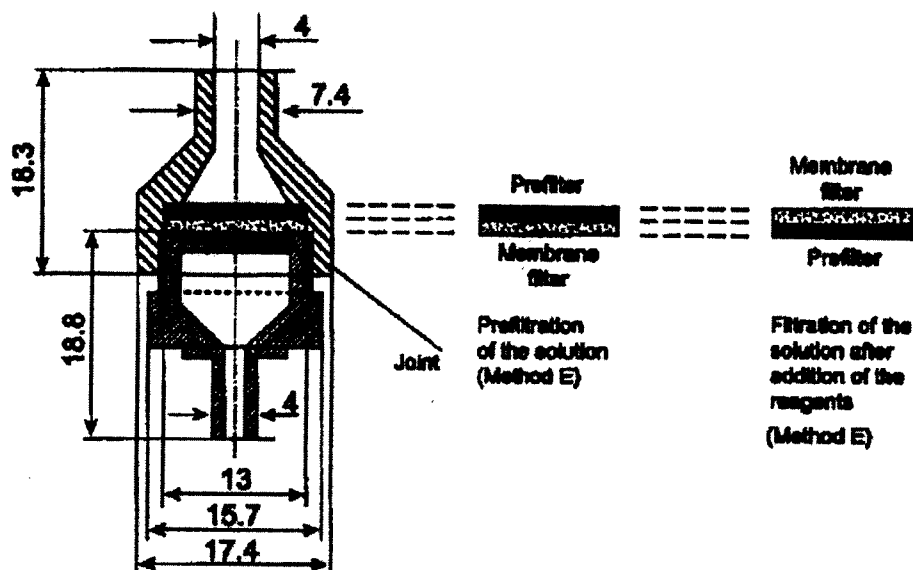
5  
6 *Test solution*. Place the 1.0 g of the substance to be examined in a silica crucible with 4 ml of a  
7 250 g/l solution of magnesium sulphate in a 5.5 V/V per cent solution of sulphuric acid. Mix using  
8 a fine glass rod. Heat cautiously. If the mixture is liquid, evaporate gently to dryness on a water-  
9 bath. Progressively heat to ignition and continue heating until an almost white or at most greyish  
10 residue is obtained. Carry out the ignition at a temperature not exceeding 800 °C. Allow to cool.  
11 Moisten the residue with a few drops of dilute sulphuric acid. Evaporate, ignite again and allow to  
12 cool. The total period of ignition must not exceed 2 h. Take up the residue in 2 quantities, each of  
13 5 ml, of a 200 g/l solution of hydrochloric acid. Add 0.1 ml of phenolphthalein solution, then  
14 concentrated ammonia until a pink colour is obtained. Cool, add glacial acetic acid until the  
15 solution is decolorised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to  
16 20 ml with water.

17  
18 *Reference solution (standard)*. Prepare as described for the test solution, using 2 ml of *lead*  
19 *standard solution (10 ppm Pb) R* instead of the substance to be examined. To 10 ml of the  
20 solution obtained add 2 ml of the test solution.

21  
22 *Monitor solution*. Prepare as described for the test solution, adding to the substance to be  
23 examined the volume of *lead standard solution (10 ppm Pb) R* prescribed for preparation of the  
24 reference solution. To 10 ml of the solution obtained add 2 ml of the test solution.

25  
26 *Blank solution*. A mixture of 10 ml of water and 2 ml of the test solution.  
27 To 12 ml of each solution, add 2 ml of buffer solution pH 3.5. Mix and add to 1.2 ml of  
28 thioacetamide reagent. Mix immediately. Examine the solutions after 2 min. The test is invalid if  
29 the reference solution does not show a slight brown colour compared to the blank solution or if  
30 the monitor solution is not at least as intense as the reference solution. The substance to be  
31 examined complies with the test if any brown colour in the test solution is not more intense than  
32 that in the reference solution.

33  
34 If the result is difficult to judge, filter the solutions through a membrane filter (pore size 3 µm; see  
35 Figure 2.4.8.-1, without the prefilter). Carry out the filtration slowly and uniformly, applying  
36 moderate and constant pressure to the piston. Compare the spots on the filters obtained with the  
37 different solutions.



1  
2 Figure 2.4.8.-1. - Apparatus for the test for heavy metals. Dimensions in millimetres

3  
4 **Loss on drying:** maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C  
5 for 3 h.

6  
7 **Sulphated ash:** maximum 4.0 per cent, determined on 1.0 g.

8  
9 **ASSAY**

10 Gas chromatography. Prepare the solutions immediately before use.

11 Carry out the test under a ventilated hood and behind a safety shield (reaction at 165 °C), wear  
12 acid-resistant gloves and safety glasses.

13 Internal standard solution. To 10 ml of o-xylene add 0.5 ml of octane and dilute to 100.0 ml with  
14 o-xylene.

15 Blank solution. In a 10 ml vial, add 60 mg of adipic acid, 4.0 ml of o-xylene, 4.0 ml of hydroiodic  
16 acid close the vial with a septum and mix. After phase separation, pierce through the septum of  
17 the vial with a syringe and extract 1 ml of the upper phase. Use this solution as the blank.

18 Test solution. Prepare the solution twice on the day of analysis. To 30 mg, add 60 mg of adipic  
19 acid in a 5 ml reaction vial. Add 2.00 ml of internal standard solution and 2.0 ml of hydroiodic  
20 acid and close immediately and weigh exactly the reaction vial (total weight before heat up).  
21 Place the vial in a oven at about 165 °C for 2.5 h. Cool about 45 min and weigh exactly the  
22 reaction vial (total weight after heat up). If the difference of the total weight before heating to the  
23 total weight after heating is more than 10 mg, prepare a new test solution. After phase separation,  
24 open the cooled reaction vial, pierce through the septum of the vial with a cooled syringe and  
25 extract 1 ml of the upper phase.

26  
27  
28 Reference solution. Prepare the solution twice on the day of analysis. Place 4.00 ml of internal  
29 standard solution in a 10 ml vial and close immediately with a septa. Inject 55 µl of iodoethane  
30 through the septum in the vial, weigh exactly each time (with difference weighing) and mix.  
31 Pierce through the septum of the vial with a syringe and extract 1 ml of the solution.



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Column:

- material: fused-silica.
- size:  $l = 30\text{ m}$ ,  $\varnothing = 0.53\text{ mm}$ .
- stationary phase: poly(dimethyl)siloxane ( $3\ \mu\text{m}$ ) RTX-1 Restek.
- Carrier gas: helium for chromatography.
- Flow rate: 4.2 ml/min (at start temperature).
- Split ratio: 1:40.

Temperature:

— temperature programme as follows:

	<u>Time (min)</u>	<u>Temperature (°C)</u>
<u>Column</u>	<u>0-3</u>	<u>50</u>
	<u>3-8</u>	<u>50 → 100</u>
	<u>8-12.3</u>	<u>100 → 250</u>
	<u>12.3-20.3</u>	<u>250</u>
<u>Injection port</u>		<u>250</u>
<u>Detector</u>		<u>270</u>

11  
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21

Detection: flame-ionisation.

Injection: 1  $\mu\text{l}$  of the test solution, of the blank and of the reference solution. Inject the first reference solution 6 times and the second 3 times.

Relative retention with reference to octane (retention time = about 9 min): iodoethane = about 0.5.

System suitability: reference solution:

— resolution: minimum of 2.0 between the peaks due to octane and ethyl iodide in the chromatogram obtained with the reference solution:

— the relative standard deviation of the response factor of the principal peak in the chromatogram obtained after 6 injections of the first reference solution is not more than 2.0 per cent.

Calculate the response factor (R) from the following expression:

$$\frac{A_1 \times W_1 \times C}{A_2 \times 100}$$

22

$A_1$  ≡ peak area of the internal standard peak in the chromatogram of the reference solution;

$A_2$  ≡ peak area of iodoethane peak in the chromatogram of the reference solution;

$W_1$  ≡ weight of iodoethane in the reference solution, in milligrams;

$C$  ≡ percentage content of iodoethane from the certificate of the manufacturer;

23 Calculate the percentage content  $m/m$  of the ethoxy groups from the following expression:

$$\frac{A_4 \times R_2 \times M_1 \times 100}{A_3 \times W_2 \times M_2 \times 2}$$

24

$A_3$  ≡ peak area of the internal standard peak in the chromatogram of the test solution;

$A_4$  ≡ peak area of iodoethane peak in the chromatogram of the test solution;

$R_2$  ≡ average value of the response factors of all reference solutions;

$M_1$  ≡ molar mass of iodoethane: hydroxyethoxy group: 61.1.;

$M_2$  ≡ molar mass of iodoethane: 156.0.;

1  $W_2$  = weight of the sample in the test solution, in milligrams:

2 If necessary: calculate the percentage content of the alkyloxy groups with reference to the dried  
3 salt and/or silica free substance.

4  
5 LABELLING

6 The label states the apparent viscosity, in millipascal seconds for a 2 per cent *m/m* solution.

7  
8 **Reagents**

9 **Ninhydrin solution. XXXXXXXX.**

10 Dissolve 3 g of ninhydrin in 100 ml of a 45.5 g/l solution of sodium metabisulphite.

11  
12 **Ethylene oxide solution. XXXXXXXX.**

13 Macrogol 200: introduce 500 ml of *macrogol 200 R* into a 1000 ml round bottom flask. Using a  
14 rotation evaporation remove any volatile components applying for 6 h a temperature of 60 °C and  
15 a vacuum with a pressure of 1.5 kPa to 2.5 kPa.

16 Weigh 1.00 g of cold ethylene oxide stock solution (equivalent to 2.5 mg of ethylene oxide) into a  
17 cold flask containing 40.0 g of cold macrogol 200. Mix and determine the extract mass and dilute  
18 to a calculated mass to obtain a solution containing 50 µg of ethylene oxide per gram of solution.  
19 Weigh 10.00 g into a flask containing about 30 ml of water, mix and dilute to 50.0 ml with water  
20 (10 µg/ml of ethylene oxide). Prepare immediately before use.

21  
22 **Phenolphthalein solution. 1063702.**

23 Dissolve 0.1 g of phenolphthalein in 80 ml of ethanol 96 per cent *V/V* and dilute to 100 ml with  
24 water.

25 *Test for sensitivity.* To 0.1 ml of the phenolphthalein solution add 100 ml of carbon dioxide-free  
26 water. The solution is colourless. Not more than 0.2 ml of 0.02 *M sodium hydroxide* is required to  
27 change the colour to pink.

28 *Colour change:* pH 8.2 (colourless) to pH 10.0 (red).

29  
30 **Buffer solution pH 3.5. XXXXXXXX.**

31 Dissolve 68.0 g of potassium dihydrogen phosphate in water and dilute to 1000.0 ml with the  
32 same solvent. Adjust the pH with phosphoric acid.

33  
34 **Thioacetamide reagent. XXXXXXXX.**

35 To 0.2 ml of a 40 g/l solution of thioacetamide add 1 ml of a mixture of 5 ml of water, 15 ml of  
36 1 *M sodium hydroxide* and 20 ml of glycerol (85 per cent). Heat in a water-bath for 20 s. Prepare  
37 immediately before use.

38  
39 **Sulphuric acid, dilute. XXXXXXXX.**

40 Contains 98 g/l of H<sub>2</sub>SO<sub>4</sub>.

41 Add 5.5 ml of sulphuric acid to 60 ml of water, allow to cool and dilute to 100 ml with the same  
42 solvent.

43  
44 **Lead standard solution (0.1 per cent Pb). XXXXXXXX.**

45 Dissolve *lead nitrate R* equivalent to 0.400 g of Pb(NO<sub>3</sub>)<sub>2</sub> in *water R* and dilute to 250.0 ml with  
46 the same solvent.

47  
48 **Lead standard solution (10 ppm Pb). XXXXXXXX.**

1 Immediately before use, dilute *lead standard solution (0.1 per cent Pb) R* to 100 times its volume  
2 with water.

3  
4 **Chloride standard solution (5 ppm Cl). XXXXXXXX.**

5 Immediately before use, dilute with water to 100 times its volume a solution containing sodium  
6 chloride equivalent to 0.824 g of NaCl in 1000.0 ml.

7  
8 **Glyoxal standard solution (20 ppm C<sub>2</sub>H<sub>2</sub>O<sub>2</sub>). XXXXXXXX.**

9 In a 100 ml graduated flask weigh a quantity of a 40 per cent *m/m* solution of glyoxal  
10 corresponding to 0.200 g of C<sub>2</sub>H<sub>2</sub>O<sub>2</sub> and make up to volume with ethanol. Immediately before use  
11 dilute the solution to 100 times its volume with the same solvent.

12  
13 **Glyoxal standard solution (2 ppm C<sub>2</sub>H<sub>2</sub>O<sub>2</sub>). .**

14 Immediately before use, dilute *glyoxal standard solution (20 ppm C<sub>2</sub>H<sub>2</sub>O<sub>2</sub>) R* to 10 times its  
15 volume with *ethanol R*.

1 Replace the text in Rotavirus (15/T (06) 41 ANP) pE2 line 12 – pE3 line 33 with the  
2 following text.

3  
4 *VIRUS PROPAGATION, SINGLE HARVEST, MONOVALENT POOLED HARVEST*

5 All processing of the cell banks and subsequent cell cultures is done under aseptic conditions  
6 in an area where no other cells are being handled. Suitable animal (but not human) serum may  
7 be used in the culture media, but the final medium for maintaining cell growth during virus  
8 multiplication does not contain animal serum. Serum and trypsin used in the preparation of  
9 cell suspensions and culture media are shown to be free from extraneous agents. The cell  
10 culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the  
11 lowest effective concentration. It is preferable to have a substrate free from antibiotics during  
12 production.

13  
14 **STORED VIRUS INTERMEDIATE CULTURE (where applicable)**

15 On the day of inoculation with the virus working seed lot, not less than 5 per cent or 500 ml  
16 of the cell cultures employed for stored virus intermediate, whichever is greater are set aside  
17 as uninfected cell cultures (control cells). Stored virus intermediate cultures are harvested at a  
18 time appropriate to the strain of virus and stored at temperatures below – 60°C.

19  
20 Where justified and authorised, only a stored virus intermediate culture that complies with the  
21 following requirements may be used for virus propagation.

22  
23  
24 **Identification.** Each stored virus intermediate culture is identified by rotavirus type by an  
25 immunological assay using specific antibodies or by a molecular identity test such as NAT  
26 (2.6.21).

27 **Bacterial and fungal contamination.** Each stored virus intermediate culture complies with  
28 the test for sterility (2.6.1), carried out using 10 ml for each medium.

29  
30 **Virus concentration.** Each stored virus intermediate culture is determined as prescribed  
31 under Assay to monitor consistency of production. Both direct cellculture based methods and  
32 NAT (2.6.21) such as PCR quantification of virus replication in cell-culture system may be  
33 used.

34 **Extraneous agents (2.6.16).** Each stored virus intermediate culture complies with the tests  
35 for extraneous agents.

36 **Control cells.** The control cells of the production cell culture from which each stored virus  
37 intermediate is derived comply with a test for identity and with the requirements for  
38 extraneous agents (2.6.16).

39  
40  
41 **VIRUS PROPAGATION AND SINGLE HARVEST**

42 On the day of inoculation with the virus working seed lot or stored virus intermediate culture,  
43 cell cultures employed for vaccine production are set aside as uninfected cell cultures (control  
44 cells). If bioreactor technology is used, the size and handling of the cell sample to be  
45 examined is approved by the competent authority. The virus suspensions are harvested at a  
46 time appropriate to the strain of virus being used.

47  
48 Only a single virus harvest that complies with the following requirements may be used for  
49 further processing.

1 **Bacterial and fungal contamination.** Each single virus harvest complies with the test for  
2 sterility (2.6.1), carried out using 10 ml for each medium.

3 **Control cells.** The control cells of the production cell culture from which each single harvest  
4 is derived comply with a test for identity and with the requirements for extraneous agents  
5 (2.6.16).  
6  
7

#### 8 MONOVALENT POOLED HARVEST

9 Monovalent pooled harvests may be prepared by pooling a number of single harvests of the  
10 same virus type. If no monovalent pooled harvest is prepared, the tests below are carried out  
11 on each single harvest. Only a single harvest or a monovalent pooled harvest that complies  
12 with the following requirements may be used in the preparation of monovalent pooled  
13 purified harvest.

14 **Identification.** Each single or monovalent pooled harvest is identified by rotavirus type by an  
15 immunological assay using specific antibodies or by a molecular identity test such as NAT  
16 (2.6.21).

17 **Bacterial and fungal contamination.** Each single or monovalent pooled harvest complies  
18 with the test for sterility (2.6.1), carried out using 10 ml for each medium.

19 **Virus concentration.** The virus concentration of each single or monovalent pooled harvest is  
20 determined as prescribed under Assay to monitor consistency of production. Both direct cell-  
21 culture based methods and NAT (2.6.21) such as PCR quantification of virus replication in  
22 cell-culture system may be used.

23 **Extraneous agents (2.6.16).** Each single harvest or monovalent pooled harvest complies with  
24 the tests for extraneous agents.  
25  
26

#### 27 PURIFIED MONOVALENT HARVEST

28 The purified monovalent harvest may be prepared from a single or pooled monovalent  
29 harvest. The single or pooled monovalent harvest is clarified to remove cell debris and may be  
30 further purified.  
31  
32

33 Only a purified monovalent harvest that complies with the following requirements may be  
34 used in the preparation of the final bulk vaccine.  
35

36 **Bacterial and fungal contamination.** The purified monovalent harvest complies with the test  
37 for sterility (2.6.1), carried out using 10 ml for each medium.

38 **Virus concentration.** The virus concentration of the purified monovalent harvest is  
39 determined as prescribed under Assay to monitor consistency of production. Both direct cell-  
40 culture based methods and NAT (2.6.21) such as PCR quantification of virus replication in  
41 cell-culture system may be used.