Jungbunzlauer

TEST PROCEDURES FOR XANTHAN GUM
REFERRING TO SPECIFICATIONS AND CERTIFICATES OF ANALYSIS

IDENTITY
Test according to the latest edition of the European Pharmacopoeia monograph for xanthan gum.

ASSAY
Test according to the latest edition of the USP-NF monograph for xanthan gum.

VISCOSITY
Test in accordance with FCC.
1% xanthan gum in 1% KCl, Brookfield viscometer LVDV, spindle 3, 60 rpm at room temperature:
- Prepare a solution of 1% KCl and place 99 ml thereof in a beaker.
- Weigh in 1.000 g of xanthan gum using an analytical scale and slowly add it to the beaker while stirring at 250-300 rpm, using a propeller type stirrer (LRW 10).
- Stir for 1.5 hours at 800 rpm at room temperature
- Pour the solution into centrifuge tubes and centrifuge the solution for 2 minutes at 3500 rpm. Foam formed during the centrifugation is removed subsequently using a spoon.
- Measure the viscosity with a Brookfield LVDV viscometer, spindle 3, at 60 rpm. The steady state viscosity is reached after 3-5 minutes and is shown on the display.

VISCOSITY RATIO V1:V2
Test according to the latest edition of the FCC monograph for xanthan gum.

LOSS ON DRYING
For the determination of the loss on drying, a Halogen Moisture Analyzer is used. Weigh 3.4 – 4.6 g of xanthan gum in the aluminium sample pan for the moisture analysis. After starting the analysis the sample in the Halogen Moisture Analyzer absorbs the infrared radiation from the halogen lamp. As a result, the sample heats up very quickly and is dried. The switch-off criterion (AK) determines the point at which measurement is automatically ended and the result is displayed (AK 4 = 1 mg/90 s).

pH of 1% SOLUTION
Prepare a 1% solution of xanthan gum by adding 1 g xanthan gum to 99 g distilled water. Dissolve with a propeller type stirrer at 800 rpm for 120 minutes. Centrifuge the solution for 2 minutes at 3500 rpm. Foam formed during the centrifugation is removed subsequently using a spoon. Determine the pH-value using a standardized pH-meter according to the method of European Pharmacopoeia (Ph. Eur.).

ISOPROPYL ALCOHOL
Determine the content of isopropyl alcohol with a gas-chromatograph (head-space method).

POWDER COLOUR
Place xanthan gum in a glass dish and determine the powder colour with a Kodak Chromameter (Type CR-410) which is standardized and uses the L*a*b colour space.

TRANSMITTANCE
Prepare a 1% solution of xanthan gum by adding 1 g xanthan gum to 99 g distilled water. Dissolve with a propeller type stirrer at 800 rpm for 90 minutes. Centrifuge the solution for 2 minutes at 3500 rpm. Foam formed during the centrifugation is removed subsequently using a spoon. Transfer approximately 5 ml xanthan solution into a 10 mm cuvette. Measure the transmittance at 600 nm using a spectrophotometer.

PYRUVIC ACID
Preparations: Prepare a buffer solution by dissolving 14.0 g triethanolamine hydrochloride and 0.28 g disodium-EDTA in 80 ml distilled water using a 100 ml volumetric flask. Adjust the pH to 7.6 using NaOH (10%). Add distilled water to 100 ml. Prepare a NADH solution by dissolving 30 mg NADH and 60 mg of $NaHCO_3$ in 6 ml distillied water. Purchase a ready-to-use LDH solution (specific activity ~550 units/mg).
Procedure: in a vial weight out 0.03 g of xanthan gum (W_{XG}) and add 5 ml sulfuric acid (0.5 M). Dissolve the xanthan gum at 105 °C for 30 min on a heating block, prolong if neccesary until xanthan gum is fully dissolved . Cool down and transfer the solution quantitatively to a 100 ml volumetric flask and dilute with distilled water to 100 ml.
Prepare two 10 mm cuvettes and add 1.00 ml of buffer solution and 0.1 ml of NADH solution to each. Add 2.00 mL distillied water to one cuvette (blank) and 2.00 ml of xanthan gum solution to the other cuvette (sample). Mix gently and measure the extinction (E_0) of blank and sample at 340 nm.
Add 0.02 ml LDH solution to each cuvette and mix gently. Wait for 5 min and measure the extinction (E_1) of the blank and sample at 340 nm.
Use the following formula for calculation of pyruvate content:

$$Pyruvate\ content\ [\%] = \left[(E_0 - E_1)_{Sample} - (E_0 - E_1)_{Blank}\right] \cdot \frac{V_t\ [ml]}{V_s\ [ml]} \cdot \frac{M_{Pyruvat}\ [g\ mol^{-1}]}{\varepsilon_{NADH}\ [L\ mol^{-1}\ cm^{-1}] \cdot d\ [cm]} \cdot \frac{1}{\frac{10 \cdot W_{XG}[g]}{10 \cdot 100\ [ml]} \cdot \frac{100\ [\%] - LoD\ [\%]}{100\ [\%]}} \cdot 100\ [\%]$$

with: test volume (V_t = 3.12 ml), sample volume (V_s = 2.00 ml), molar mass of Pyruvate ($M_{Pyruvate}$ = 88.1 g mol^{-1}), molar extinction coefficient of NADH (ε_{NADH} = 6300 L mol^{-1} cm^{-1}), cuvette thickness (d = 1 cm), the amount of xanthan gum (W_{XG}; measured sample weight), and the Loss on Drying (*LoD*; measured separately).

ASH
Test according to the European Pharmacopoeia: Evenly distribute 1.00 g of the substance in a tared crucible. Dry at 100 °C to 105 °C for 1 h and ignite to constant mass in a muffle furnace at 600 °C ± 25 °C, allowing the crucible to cool in a desiccator after each ignition. The weight of the ash is between 6.5% and 16.0%.

NITROGEN
Determine the nitrogen content according to Kjeldahl-Buechi.

ARSENIC
Determination with ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

LEAD
Determination with ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

MERCURY
Determination with ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

CADMIUM
Determination with ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

MICROBIOLOGICAL PARAMETERS

TOTAL AEROBIC MICROBIAL COUNT
Add 1 g xanthan gum to 1 g Tween and 8 g MCT-oil and 90 g TSB broth; homogenize by shaking for at least 5 min. Add 100 µL of the homogenized solution with a spiralplater on a TSA-agar plate. Repeat procedure as required: for ≤1000 cfu/g limit use 2 TSA agar plates (100 µL solution each), for ≤500 cfu/g limit use 4 TSA agar plates (100 µL solution each) and for ≤100 cfu/g limit use 10 TSA agar plates (100 µL solution each). Incubate the plates at 32.5 °C for 72 hours. Determine the total aerobic microbial count.

SALMONELLA SPP.
Add 25 g xanthan gum to 25 g Tween and 200 g MCT-oil and 2450 g TSB broth; homogenize by shaking for at least 5 min. Incubate the solution at 32.5 °C for 24 h. After incubation, add 0.1 ml of the solution to 10 mL RV-bouillon and incubate for further 24 h, 32.5 °C. Streak on XLD-agar plates and incubate for 24 h at 32.5 °C. Lot is released if no growth is visible.

PSEUDOMONAS AERUGINOSA
Add 1 g xanthan gum to 1 g Tween and 8 g MCT-oil and 90 g TSB broth; homogenize by shaking for at least 5 min. Incubate the solution at 32.5 °C for 24 h. Streak on CETA-agar plates and incubate for 24 h at 32.5 °C. Lot is released if no growth is visible.

STAPHYLOCOCCUS AUREUS
Add 1 g xanthan gum to 1 g Tween and 8 g MCT-oil and 90 g TSB broth. Homogenize by shaking for at least 5 min. Incubate the solution at 32.5 °C for 24 h. Streak on MSA-agar plates and incubate for 24 h at 32.5 °C. Lot is released if no growth is visible. If necessary, verify the result by microscopy.

TOTAL YEASTS AND MOULD COUNT
Add 1 g xanthan gum to 1 g Tween and 8 g MCT-oil and 90 g TSB broth. Homogenize by shaking for at least 5 min. Add 200 µL of the homogenized solution with a spiralplater on a SAB-agar plate, repeat the procedure 4 times. Incubate the 5 plates at 23 °C for 5 days. Determine the total yeast and mould count.

XANTHOMONAS CAMPESTRIS
Add 1 g xanthan gum to 1 g Tween and 8 g MCT-oil and 90 g TSB broth. Homogenize by shaking for at least 5 min. Incubate the solution at 32.5 °C for 24 h. Streak on TSA-agar plates and incubate for 72 h at 32.5 °C. Lot is released if no growth is visible. If necessary, verifiy the result by microscopy.

ESCHERICHIA COLI
Add 25 g xanthan gum to 25 g Tween and 200 g MCT-oil and 2450 g TSB broth. Homogenize by shaking for at least 5 min. Incubate the solution at 32.5 °C for 24 h. After incubation, add 1 ml of the solution to 10 mL MacConkey-Broth and incubate for further 24 h at 43 °C. Streak on MCA-agar plates and incubate for 24 h at 32.5 °C. Lot is released if no growth is visible.

BILE-TOLERANT GRAM-NEGATIVE BACTERIA (includes COLIFORMS*)*
Add 1 g xanthan gum to 1 g Tween and 8 g MCT-oil and 90 g TSB broth. Homogenize by shaking for at least 5 min. Incubate the solution at 32.5 °C for 24 h. After incubation, add 1 ml of the solution to 10 mL Mossel-Bouillon and incubate for further 24 h at 32.5 °C. Streak on VRBD-agar plates and incubate for 24 h at 32.5 °C. Lot is released if no growth is visible.

GRANULATION

DIGITAL IMAGING METHOD (standard method)

This method uses Dynamic Image Analysis (ISO 13322-2) as measuring principle. The equipment used is a RETSCH CAMSIZER XT equipped with X-Dry modulus and X-Jet cartridge.
For the analysis, 15 g of xanthan gum are evenly distributed on a feed chute with distribution brush and metal pin. When starting the analysis, the material is transferred into the detector. Before passing the dual camera system for detection, it is further distributed using an air jet. Material remaining on the feed chute is transferred into the detector using an distinct brush. The measurement stops when the sample has passed the detector completely. Based on the pictures taken, the results are calculated by the corresponding software RETSCH CamsizerXT64 and provided with the defined sieving fractions in %.

SIEVING METHOD

Blend :

Place 50 g xanthan gum in a sieve shaker (RETSCH Vibratory Sieve Shaker AS 200 control) and add beads as an auxiliary material. Shake for 5 minutes at an amplitude of 1.300 mm. Weigh each sieve and calculate the sieving fractions in %.

FINE:

Place 10 g xanthan gum in an air jet sieving machine (RETSCH AS 200 jet). The sieves are then one by one sieved by the air jet. Weigh each sieve and calculate the sieving fractions in %.

NORMAL:

Place 50 g xanthan gum in a sieve shaker (RETSCH Vibratory Sieve Shaker AS 200 control) and add beads as an auxiliary material. Shake for 5 minutes at an amplitude of 1.300 mm. Weigh each sieve and calculate the sieving fractions in %.

COARSE:

Place 50 g xanthan gum in a sieve shaker (RETSCH Vibratory Sieve Shaker AS 200 control) and add beads as an auxiliary material. Shake for 5 minutes at an amplitude of 1.300 mm. Weigh each sieve and calculate the sieving fractions in %.