Claims

1. A method for the determination of the hyaluronic acid content of a hydrogel, the method comprising the following steps:
   a. preparing, as reagent A, a solution of sodium tetraborate in sulfuric acid;
   b. preparing reagent B by dissolving carbazole in ethanol;
   c. preparing test solutions by dissolving the hydrogel in an aqueous solution, wherein the test solutions comprise hydrogel dispersions;
   d. treating the test solutions with ultrasounds for a period of time sufficient to obtain a macroscopically homogeneous solution;
   e. preparing a reference stock solution by dissolving glucuronic acid, or a glucuronic acid-containing substance in an aqueous solution;
   f. preparing at least 3 reference solutions by dilution of the reference stock solution in aqueous solution, preferably at concentration comprised between 0.0005% w/v and 0.0100% w/v, preferably between 0.0010 % w/v and 0.0050 % w/v;
   g. preparing the test tubes by admixing reagent A, reagent B and one of the following: reference solution, test solution, aqueous solution (blank), and optionally solution for interference (crosslinker sample or additive sample); place each test tube on a water bath for at least 5 min, then cool them to room temperature;
   h. reading the absorbance at a wavelength comprised between 500 and 580 nm, preferably at about 530 nm, against the blank and optionally the sample for interference.
2. The method according to claim 1 further comprising, after step c, the following step:
   c2. heating the test solutions to a temperature higher than room temperature, preferably between 75 and 85°C for at least 15 minutes, preferably at least 2 hours, more preferably about 3 hours, preferably while continuously stirring.
3. The method according to claim 1 or 2 wherein in step g, the water bath is a hot bath and the time is preferably about 15 minutes.
4. The method according to any one of claims 1-3, wherein in step d. sonication is performed at a specific power between 0.5 W/kg and 50 W/kg.
5. The method according to any one of claims 1-4 wherein sonication is performed at a specific energy comprised between 500 J/kg and 30 kJ/kg.
6. The method according to any one of claims 1-5, wherein reagent A is a solution 0.95 % w/v of disodium tetraborate in sulfuric acid, and reagent B is a solution 0.125 % w/v of carbazole in ethanol.

7. The method according to any one of claims 1-6, wherein in step c. the test solution is prepared at least in double.
8. The method according to any one of claims 1-7, wherein step g. is performed as follows: adding 5 parts of reagent A to each test tube, preferably previously cooled; layer each sample onto the reagent A in the test tube, as follows: Blank: 1 part of water solution; Reference solutions: 1 part of each dilution of the Reference stock solution; Samples: 1 part of each Test solution; shake the tubes to dissolve the double-phase created by the sample; put each sample in a water bath for at least 5 minutes, preferably in a boiling water bath for 15 minutes, then cool them down to room temperature; add to each tube 0.2 parts of reagent B, shake the tubes to dissolve the double-phase and put each tube in a warm water bath for at least 5 minutes, preferably in a boiling water bath for 15 minutes.
9. The method according to any one of claims 1-8, wherein, after step h., the percentage content of sodium hyaluronate is calculated by interpolating the experimental absorbance values with the reference standard curve.