

EXAMPLES ILLUSTRATING THE USE

Isoelectric focusing

EXAMPLE I

Isoelectric focusing using media synthesized according to Examples 1A-1C and 1:1 (w/w) mixtures of the media according to Examples 1B and 1C was carried out employing Flat Bed Apparatus FBE 3000 (Pharmacia Fine Chemicals AB, Uppsala, Sweden). The solutions used in the cathode and anode strips were 1 M NaOH and 0.05 M H₂SO₄ respectively. Test proteins (e.g. myoglobin, β -lactoglobuline, carbonic anhydrase, haemoglobin and plasminogen) were applied in the usual way. The focusing was carried out at a maximal power of 30 W and a maximal voltage of 1000 V during 2 h. A surface electrode was used to measure the pH in the gel after 1 h and 2 h and the pH gradient was plotted in a diagram with pH as a function of the distance in cm from the cathode. From the result it could be concluded that the products obtained in Examples 1A and 1B were overcompensated with respect to the negatively charged groups in the agarose and that the product according to example 1C was undercompensated whereas the mixture of the products of Examples 1B and 1C in the ratio 1:1 gave a stable pH gradient.

After focusing the proteins were fixed by immersing the gel into a bath of ethanol containing 10% trichloroacetic acid (w/w) and 5% 5-sulphosalicylic acid (w/w) for 30 min and then into ethanol for 5 min. The gel was then covered with 10 layers of paper towelling and a piece of glass upon which a weight of 2 kg was placed. The paper towelling was removed after 15 min and the gel was dried completely in a hot air stream.

The dried agarose gel was stained by immersion for 15 min in a 0.2% solution of Comassie-Brillantblau R 250 (Colour Index No. 42660; E Merck, Darmstadt, Germany) in a mixture of ethanol, acetic acid and water, 7:1:2. Excess dye was removed by washing in a mixture of ethanol, acetic acid and water, 7:1:2. After complete drying of the gel in a hot air stream the focused proteins appeared as distinct blue lines on a transparent, colourless film which could easily be filed.

EXAMPLE II

The focusing was carried out as in Example I except that products according to Examples 2A and 2B and a mixture of those products in the ratio 3:2 were used.

The results showed that the product according to Example 2A was overcompensated and that the one

according to Example 2B was undercompensated whereas the mixture of the products gave a completely stable gradient.

EXAMPLE III

The focusing was carried out as in Example I except that an agarose medium according to Example 3 was used.

A completely stable pH gradient was obtained.

EXAMPLE IV

The focusing was carried out as in Example I except that an agarose medium according to Example 4 was used.

A completely stable pH gradient was obtained.

EXAMPLE V

The focusing was carried out as in Example I except that a medium according to Example 5 was used.

A completely stable pH gradient was obtained.

EXAMPLE VI

The focusing was carried out as in Example I except that products according to Examples 6A and 6B and a mixture of these products in the ratio 41:9 were used.

The results showed that the product according to Example 6A was undercompensated and that the product according to Example 6B was overcompensated whereas the mixture gave a stable pH gradient.

We claim:

1. A medium for isoelectric focusing based on agarose, wherein said medium consists of or comprises agarose into which positively charged substituents have been introduced to the neutralisation of negatively charged groups present in the medium, said substituents containing as the only charged group a quaternary amino group and the charge of said substituents being independent of pH at least in the range 2-12.

2. A medium according to claim 1, wherein said substituents are bound to the agarose via an ether or carboxylic acid ester linkage involving the oxygen atom of a hydroxyl group of the agarose.

3. A medium according to claim 1 or 2, wherein said medium consists of a mixture of at least two agarose preparations of different degrees of substitution with regard to the introduced substituents.

4. A medium according to claim 1 or 2, wherein said medium also comprises unmodified agarose.

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