Sigma-Aldrich Chemie GmbH · Industriestrasse 25 · Postfach · CH-9471 Buchs / Switzerland
Tel. +41 / 81 755 25 11 · Fax +41 / 81 756 54 49 · flukatec@sial.com

46115 Eupergit® C
46118 Eupergit® C250 L

EUPERGIT®

Matrix Modification of EUPERGIT® C or EUPERGIT® C 250 L after Protein Binding / Removal of Excess of Oxirane Groups

Contents

Sigma-Aldrich Chemie GmbH · Industriestrasse 25 · Postfach · CH-9471 Buchs / Switzerland
Tel. +41 / 81 755 25 11 · Fax +41 / 81 756 54 49 · flukatec@sial.com

1. General Remarks

Subsequent to protein immobilization, the residual oxirane groups of EUPERGIT® C can be removed by treatment with low molecular weight compound's containing mercapto or amino groups. By choosing an appropriate compound for this reaction, a hydrophobic, hydrophilic, anionic or cationic character of the matrix can be generated.

Removal of residual oxirane groups is achieved by incubating the wet beads containing the immobilized protein with a 1.5 molar excess of the appropriate reagent.

Please note:
All the methods described in this brochure for EUPERGIT® C can also be used for the matrix modification of EUPERGIT® C 250 L, with the only difference that the volume of the aqueous phase has to be increased by 50% (i.e. 6 ml instead of 5 ml per 10 grams of wet beads).

2. Reaction with 2-Mercaptoethanol

By treatment with 2-mercaptoethanol the matrix of the beads remains electroneutral.

2.1. Wash 10 grams of wet beads on a sintered-glass filter (porosity 2) with 100 ml of 0.1 M potassium phosphate buffer pH 8.

2.2. Add 4 ml of a 4.2% v/v aqueous 2-mercaptoethanol solution in 0.1 M potassium phosphate buffer adjusted to pH 5 - 8 (whatever pH is best for the stability of the immobilized bioligand) per 10 grams of wet beads.

2.3. Allow to stand for 16 hours at room temperature (20 - 25 °C).

2.4. Wash the product with a total volume of 500 ml of deionized, sterile water. (Divide the total volume of the washing fluid into three aliquots. Use one aliquot for each of three consecutive washing steps. Each single washing step should take a minimum of 20-30 minutes.)

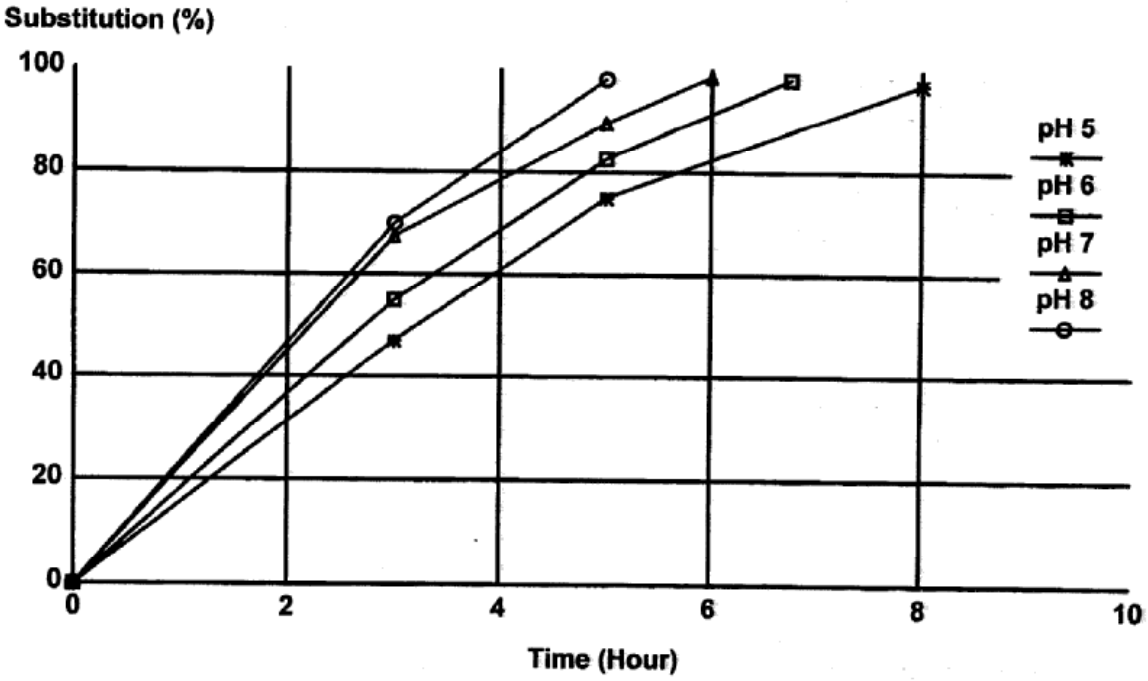
2.5. Equilibrate the product with the same buffer in which it will be used subsequently.

Sigma-Aldrich Chemie GmbH · Industriestrasse 25 · Postfach · CH-9471 Buchs / Switzerland
Tel. +41 / 81 755 25 11 · Fax +41 / 81 756 54 49 · flukatec@sial.com

2.6. The product can be stored in a buffer solution containing a preservative, e.g. 0.1 M potassium phosphate buffer pH 7.5 containing 500 ppm ethyl p-hydroxy benzoate and 2% 2-propanol.

For details of pH and time dependency of reaction of 2-mercaptoethanol with EUPERGIT® C refer to the graph below!

Substitution of Oxirane Groups by Reaction with 2-Mercaptoethanol / pH Dependency, Temperature: 23 °C
1.5 molar excess of SH-compound versus Oxirane Groups



Conclusion: EUPERGIT® C can be easily derivatized with SH-compounds in a pH-range of 5 - 8.

Sigma-Aldrich Chemie GmbH · Industriestrasse 25 · Postfach · CH-9471 Buchs / Switzerland
Tel. +41 / 81 755 25 11 · Fax +41 / 81 756 54 49 · flukatec@sial.com

3. Reaction with Thioacetic Acid

This process results in a weak unionization of the matrix

3.1. All the steps described under 2.1. and 2.3. - 2.6. can be applied to this reaction. Substitute only step 2.2. by the following procedure:

3.2. Add 4 ml of a 4.3% v/v aqueous solution of thioacetic acid in 0.1 M potassium phosphate buffer adjusted to pH 5 - 8 (whatever pH is best for the stability of the immobilized bioligand) per 10 grams of wet beads.

4. Reaction with 2-Mercaptoethanesulfonic Acid Sodium Salt

This process results in a strong anionization of the matrix.

4.1. All the steps described under 2.1. and 2.3. - 2.6. can be applied to this reaction. Substitute only step 2.2. by the following procedure:

4.2. Dissolve 98.5 mg of 2-mercaptoethanesulfonic acid sodium salt per ml of 0.1 M potassium phosphate buffer adjusted to pH 5 - 8 (whatever pH is best for the stability of the immobilized bioligand). Add 4 ml of this solution per 10 grams of wet beads.

5. Reaction with Cysteamine

This process results in a cationization of the matrix.

5.1. All the steps described under 2.1. and 2.3. - 2.6. can be applied to this reaction. Substitute only step 2.2. by the following procedure:

5.2. Dissolve 68 mg of cysteamine hydrochloride per ml of 0.1 M potassium phosphate buffer. Adjust to pH 5 - 8 (whatever pH is best for the stability of the immobilized bioligand). Add 4 ml of this solution per 10 grams of wet beads.

Sigma-Aldrich Chemie GmbH · Industriestrasse 25 · Postfach · CH-9471 Buchs / Switzerland
Tel. +41 / 81 755 25 11 · Fax +41 / 81 756 54 49 · flukatec@sial.com

6. Reaction with Thioglycerol

Modification of the matrix with hydrophilic compounds like thioglycerol leads to a hydro-philization of the matrix, which remarkably reduces the non-specific binding of proteins.

6.1. All the steps described under 2.1. and 2.3. - 2.6. can be applied to this reaction. Substitute only step 2.2. by the following procedure:

6.2. Mix 53µl of thioglycerol (98%) (Sigma) per ml of 0.1 M potassium phosphate buffer. Adjust to pH 5-8 (whatever pH is best for the immobilized bioligand). Add 4 ml of this solution per 10 grams of wet beads.

For hydrophilization of the matrix, thioglucose or amino polyethylene glycol (NH_2-PEG) can be used instead of thiogycerol.

7. Reaction with Thioglucose (1-Thio-ß-D-Glucose sodium salt)

7.1. All the steps described under 2.1. and 2.3. - 2.6. can be applied to this reaction. Substitute only step 2.2. by the following procedure:

7.2. Dissolve 131 mg of thioglucose sodium salt (Sigma) per ml of 0.1 M potassium phosphate buffer. Adjust to pH 5-8 (whatever pH is best for the immobilized bioligand). Add 4 ml of this solution per 10 grams of wet beads.

8. Modification of EUPERGIT® C with Amino Polyethylene Glycol (NH_2-PEG)

8.1. Wash 10 grams of wet beads on a sintered-glass filter (porosity 2) with 100 ml of 1.0 M potassium phosphate buffer pH 8.

8.2. Mix 1 gram of NH_2-PEG per ml of 1.0 M potasssium phosphate buffer. Adjust to pH 5-8 (whatever pH is best for the immobilized bioligand). Add 8 ml of this solution to 10 grams of wet beads.

Sigma-Aldrich Chemie GmbH · Industriestrasse 25 · Postfach · CH-9471 Buchs / Switzerland
Tel. +41 / 81 755 25 11 · Fax +41 / 81 756 54 49 · flukatec@sial.com

8.3. Allow to stand for 18 hours at 4 °C.

8.4. Wash the preparation as described under 2.4.

8.5. For the application of the product proceed as described under 2.5.

8.6. Store the wet beads as described under 2.6.

NH_2-PEG has been used as an efficient reagent for the matrix modification of EUPERGIT® C subsequent to the immobilization of monoclonal antibodies as affinity ligands. An affinity matrix thus prepared, shows reduced non-specific binding of proteins and as a consequence improved elution characteristics and product purity in chromatographic procedures. For further details please refer to the following literature:

Flemminger, G., Wolf, T., Solomon, B., Hadas, E., The Effect of Polyethylene Glycol on the Nonspecific Adsorption of Proteins to EUPERGIT® C and Agarose, J. Chromatography, 510, pp. 310-319 (1990)

Important Note:

Most of the reagents used in the reactions are hazardous. Please refer to the Material Safety Data Sheets provided by the manufacturer.