during e.g. virus inactivation, as well as in the final concentrates of purified proteins.

This need is satisfied by providing the embodiments characterized in the claims.

SUMMARY OF THE INVENTION

The present invention relates to a method for the determination of polysorbate in a protein-containing sample which is based on a pretreatment of the sample by alkaline hydrolysis followed by the colorimetric determination.

In one aspect, there is provided a method for the determination of polysorbate in a protein-containing sample, comprising the steps: (a) subjecting the sample to alkaline hydrolysis with at least 3 N NaOH at a temperature of about 95°C to about 100°C for at least 45 minutes; (b) neutralizing the sample after alkaline hydrolysis; (c) optionally removing denaturated precipitate from the neutralized sample; (d) adding an aqueous mixture of a thiocyanatometal complex to the optionally filtered sample to form a sorbitan polyoxyethylenethiocyanatometal complex; (e) extracting said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d) into a non-water miscible organic solvent; (f) measuring the absorbance of the extract obtained in step (e) to quantify the amount of said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d); and (g) calculating the amount of the polysorbate contained in the sample from the amount of said sorbitan polyoxyethylenethiocyanatometal complex determined in step (f).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for the determination of polysorbate in a protein-containing sample, comprising the steps:

(a) subjecting the sample to alkaline hydrolysis;

(b) neutralizing the sample after alkaline hydrolysis;

(c) optionally removing denaturated precipitate from the neutralized sample;

(d) adding an aqueous mixture of a thiocyanatometal complex to the optionally filtered sample to form a sorbitan polyoxyethylenethiocyanatometal complex;

(e) extracting said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d) into a non-water miscible organic solvent;

(f) measuring the absorbance of the extract obtained in step (e) to quantify the amount of said sorbitan polyoxyethylenethiocyanatometal complex formed in step (d); and

(g) calculating the amount of the polysorbate contained in the sample from the amount of said sorbitan polyoxyethylenethiocyanatometal complex determined in step (f).

The term "polysorbate" as used herein relates to any polyoxyethylene sorbitan fatty acid esters (polysorbates). Preferred polysorbates include polysorbate 20, 40, 60, and 80. Polysorbate 80 is particularly preferred. Said polysorbate 80 may originate from animals or from plants.