NEW METHOD FOR ANTIBODY SEPARATION FROM PLASMA AND OTHER BIOLOGICAL RAW MATERIALS

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Introduction

- A method for antibody purification
- Antibody separation directly from biological raw materials like plasma or milk
- The polymer precipitation method suitable for monoclonal and polyclonal antibodies
- Antibody precipitate or crystal harvesting by centrifugation, sedimentation or filtration
- Antibody-polymer precipitate complex dissolution in physiological salt solution
- Antibodies concentrated in small volume
- Pharmaceutical, injectable, high-concentration formulations possible

Unique features of the method

- Cost-effective alternative to chromatographic steps
- Simple and fast to perform
- Easy to scale-up
- Antibodies can be separated effectively using various polyanionic precipitants:
  - biocompatible and biodegradable,
  - natural polysaccharides and their derivatives,
  - polysulfates and polysulfonates,
  - polyamino acids...
  - MW range from < 5 kDa up to 250 kDa
- Developed on the basis of a patented method for crystallization of proteins using polysaccharides (WO2005073245)

- Reversible precipitation or crystallization occurs in low ionic strength buffer typically at pH 4.5 – 6.5
- Very low reagent consumption is needed to achieve high antibody yield and purity
- Polymer concentration range typically 0.05 – 2 mg/ml
- Precipitation efficiency is independent on antibody concentration, precipitation occurs well even if the antibody concentration is 0.1 mg/ml or lower
- Solubility minimum at optimal weight ratio of the antibody and polymer as revealed by solubility curves
- The optimal ratio is dependent on pH and the used polymeric precipitant

Fig. 2. Typical solubility curve for pure gammaglobulins with polymeric precipitants. The curve shows specific interaction between the antibodies and the used anionic polymer. This curve achieved with polyaspartate experiments at pH 4.4-5.1.
Fig. 3. Effect of pH and IgG:polymer weight ratio on precipitation of pure pig gammaglobulins with 0.01 - 0.08 % (w/v) polyanetholsulfonic acid.

**Effect of IgG:polymer weight ratio**

14.8 : 1  9.9 : 1  7.4 : 1  4.4 : 1  2.2 : 1

Bovine colostrum IgG (mg) : polygalacturonic acid (mg)

**Process flow chart for antibody separation from plasma**

**I Pre-precipitation step**

- Process performed at room temperature
- Human plasma 40 ml
- Dilute with 360 ml of water and adjust pH from 7.4 to 5.3 by adding 14 ml of 100 mM succinic acid
- Pre-precipitation 414 ml 1 – 2 hours
- Remove precipitate by centrifugation or filtration
- Filtrate 411 ml
- IgG yield 100 %  IgG purity 19 %
  - 375 mg IgG (1.0 A280 = 1 mg/ml)

**II Polymer precipitation step**

- Add 22 ml of 1 % (w/v) Na-alginate solution to achieve IgG: Na-alginate ratio 1.8 : 1
- Polymer precipitation 433 ml 1 hour – 1 day
- Harvest precipitate by centrifugation and wash with water or dilute buffer at pH 5
- Precipitate harvesting and washing ~ 4 ml
- Remove mother liquor
  - IgG collected in small volume
  - IgG yield 71 %  IgG purity 73 %
  - 265 mg IgG
Antibody separation from mixture of IgG and clarified bovine milk

Experimental procedure
- Caseins removed from skimmed bovine milk with standard methods, then diafiltration in low ionic strength buffer pH 4.8 and filtration to clear solution
- pH adjusted between 4.9 and 5.5 maintaining low ionic strength
- 99% pure human gammaglobulins (Sigma) mixed with this clarified, diafiltered milk at pH 4.9 - 5.5
- Gammaglobulins precipitated by adding polymeric precipitant, polygalacturonic acid (PGA)

Experiment conditions
Assumed for antibodies and other proteins: 1,0 A280 = 1 mg/ml
- Human gammaglobulins conc. 2.00 mg/ml
- Total antibody conc. 2,12 mg/ml, total protein conc. 9,70 mg/ml
- IgG purity 22% in this mixture based on Protein A binding IgG
- IgG:polymer weight ratio 14:1

Results
- IgG yield in precipitate 64 – 79%
- IgG purity in precipitate 62 – 89%
- The best IgG yield at pH 5,3 – 5,4
- The best IgG purity at pH 5,5

Summary
- Antibody precipitation or crystallization with anionic polymer is inexpensive, simple, scaleable and fast method for large scale purification of antibodies from biological raw materials like plasma or milk
- Many polyanionic precipitants are useful
- Important parameters are pH and weight ratio of antibody and the used polymer, optimal conditions can be found empirically for each precipitant
- The method is based on specific interaction between the antibodies and the polymers under optimized conditions
- The method is suitable also for purified antibody drug formulation