

# Continuous Flow Coprecipitation of IgG-coated Microcrystals Using a Novel Three-line System

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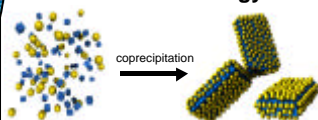
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## Introduction

Protein coated microcrystal technology offers a platform for formulating biomolecules with full retention of bioactivity and high stability towards temperature and humidity. Free-flowing powders with a specific particle size and morphology are obtained and can be used to provide a range of delivery options for protein therapeutics<sup>1,2</sup>.

## PCMC Technology



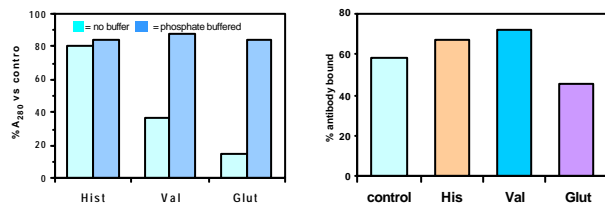
Protein-coated microcrystals (PCMC) may be prepared by dissolving biomolecules in a concentrated aqueous solution of an amino-acid or sugar and rapidly mixing the solution with water miscible solvent. During precipitation the protein molecules are found to spontaneously self-assemble over the surface of the resultant microcrystals<sup>3,4</sup>.

## Aims of Study

- Prepare IgG-coated microcrystals using a novel three-line coprecipitation system
- Assess quality relative to IgG-coated microcrystals prepared using standard two-line coprecipitation system
- Identify any advantages of producing PCMCs by three line coprecipitation

## PCMC Antibody Formulations

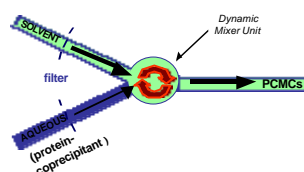
Previously we reported bovine IgG-coated microcrystals prepared using three different carrier amino-acids, histidine, valine and glutamine. Coprecipitation from concentrated amino-acid solution followed by filtering and air drying provided formulations with significantly different levels of soluble protein depending on the carrier used. Upon reconstitution His led to clear solutions but Val and Glu contained aggregates. When the same solutions were buffered to pH 7.2 with phosphate, increased soluble protein was achieved for all formulations. Protein integrity of reconstituted PCMC formulations was assessed by measuring binding of the polyclonal IgG to Protein G which recognises intact Fc. For His and Val the protein bound was higher than the supplied control. For Glu there appeared to be some loss of activity.



Following on from this we decided to investigate if similarly high levels of soluble protein could be obtained at low buffer concentrations. It was envisaged that this might be achieved by using a 3-line system whereby the protein stock is kept separate from the ionisable carrier solution prior to mixing with the solvent.

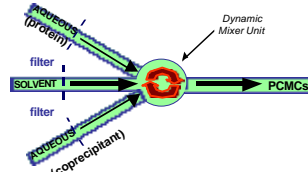
## PCMC Coprecipitation Systems

### Standard Two Line Coprecipitation



In the standard two line coprecipitation system aqueous protein-coprecipitant mixture and the solvent stream are sterile filtered and pumped continuously into the dynamic mixer unit. PCMCs are produced spontaneously, and typically 50 g/hr of PCMCs particles may be produced on a simple bench top machine.

### Novel Three Line Coprecipitation



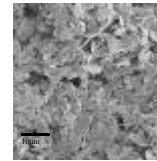
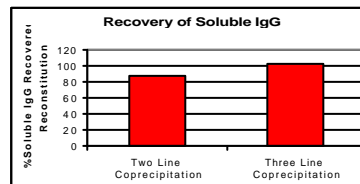
In the novel three line coprecipitation system, the three components of PCMC coprecipitation, namely protein, coprecipitant and solvent, are pumped independently via three lines<sup>5</sup>. Consequently, protein and coprecipitant are kept separate until mixing and coprecipitation in the dynamic mixer unit.

In both cases, the resultant fine suspension of PCMC particles may be harvested by filtration and left to air-dry, to produce a fine, free-flowing powder.

## 3-Line vs 2-Line

In the conventional 2-line precipitation significant amounts of phosphate buffer needed to be added to raise the pH of the IgG-valine solution to a value suitable for obtaining high levels of soluble protein. This is because of the buffering action of the concentrated Val solution.

Using the 3-line system, it was envisaged that optimum formulations could perhaps be produced without adding additional buffer because precipitation in solvent might proceed faster than ion-exchange. To study this possibility, bovine IgG was coprecipitated with valine, in the absence of additional buffer salts, using the 2-line and 3-line coprecipitation systems. IgG-valine PCMC crystals were harvested, filtered and allowed to air dry. Thereafter, dry IgG-valine PCMCs were reconstituted into buffer and soluble IgG measured by UV. (theoretical IgG loading = 7.5 %w/w).



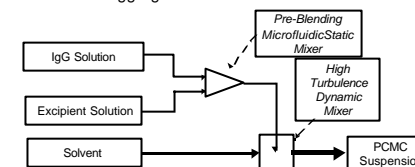
SEM images of a typical IgG-valine PCMC formulation.

The results demonstrate that for similar buffer compositions coprecipitating IgG with valine using a three-line system produces formulations with significantly more soluble protein than the two-line system.

This is because the three-line system offers the possibility of controlling excipient and protein protonation state independently during the coprecipitation step.

## Advantages of Continuous Three Line Coprecipitation

- No need to pre-blend IgG stock with excipient prior to coprecipitation
- Possible to maintain pure IgG stock solution at 4 °C
- Possible to control temperature of excipient stock solution as required
- Feasible to control the pH of IgG solution independently from excipient, using significantly less buffer
- Easy to control amount of protein loaded onto crystal surface, simply by altering the constituent flow rates
- Possible to construct a sterile, closed loop system, for producing sterile PCMCs
- Possible to produce > 50 g PCMC / hr
- Protein loading varied between 2.5 – 35 % w/w
- Production of insoluble aggregates inhibited



If no change in pH of the excipient is required it is also possible to achieve many of the above advantages by placing two, 2-line mixing systems, in series (see above). Again the excipient and protein may be prepared and stored independently of each other but here, just prior to addition to solvent, they are blended in a low shear mixer.

## Preparation of PCMC

The protein coated microcrystals in this study were prepared by a continuous flow coprecipitation process. For the two-line process, a pre-blended solution containing 3.25 mg/ml IgG and 40 mg/ml valine flowing at 1.25 ml/min was mixed with solvent (propan-2-ol) flowing at 23.75 ml/min. Total flow rate = 25 ml/min

For the three-line process, IgG (9.72mg/ml) was delivered at 0.42 ml/min; valine (60mg/ml) was delivered at 0.83 ml/min and solvent (propan-2-ol) was delivered at 23.75 ml/min. Total flow rate = 25 ml/min.

Soluble protein recovery was measured by UV spectroscopy @ 280 nm. IgG binding was measured with a Pierce protein-G spin purification kit.

## References

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- Enzyme-coated Micro-crystals: A 1-step Method for High Activity Biocatalyst Preparations. M. Kreiner, B.D. Moore and M.C. Parker. *Chem. Commun* 2001, 1096-1097
- DNA Coated Microcrystals: M. Kreiner, G. Fuglevand, B. D. Moore, M. C. Parker *Chem. Commun.* 2005, 2675-2676
- Process for preparing microcrystals. B.D. Moore, J. Vos GB Patent Application 0416694.8

## Conclusions

- In this work a three-line PCMC coprecipitation system has been demonstrated
- Using this system, it is possible to produce high quality PCMC particles without having to add additional buffer
- Upon reconstitution, the IgG-valine PCMCs rapidly dissolve to leave soluble, native IgG monomer