# Dry Powder Therapeutic mAb Formulations with Enhanced Temperature Stability

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Protein Coated Micro Crystal (PCMC) technology was used to process a human therapeutic monoclonal antibody into dry powder formulations, which were studied under accelerated stress conditions. Changes in protein integrity on reconstitution were measured by size exclusion chromatography and turbidity measurements. The effect of glutamic acid (Glu), L-arginine (Arg) and trehalose as precipitation stabilising additives was investigated.

## Abstract

XstalBio have developed platform technologies for stabilizing a wide range of biotherapeutics within a dry powder format. By using a rapid isothermal precipitation process, dry powder products can be prepared for a range of administration strategies including inhalation, high concentration subcutaneous injection (>200 mg/mL) and sustained release.

The aim of this study was to optimise dry powder formulations of a human monoclonal antibody to achieve a highly extended shelf-life.

**Materials & Methods**

Monoclonal human antibody, herein termed PFCP1, was obtained from Pfizer Inc, Chesterfield, St. Louis. PFCP1 dry powders were prepared by coprecipitation of an aqueous mixture containing histidine buffered antibody, concentrated glycine coprecipitant and PSA (L-arginine & L-glutamic acid), into either propan-2-ol or 2-methyl-1-propanol.

An accelerated stress study was then carried out in which PFCP1 dry powders were stored at 40ºC at uncontrolled humidity for ~47 weeks. After temperature stressing, the PFCP1 dry powder was reconstituted back into buffer and monomer content was measured by size-exclusion chromatography.

**Results**

All of the stressed samples retained ≥95% monomer content. Bioactivity was tested in a PFCP1 specific ELISA; the results showed that bioactivity was also not compromised by the solvent coprecipitation process.

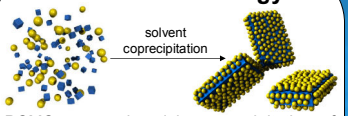
**Conclusions**

Human monoclonal antibodies can be readily formulated using XstalBio technology by incorporating precipitation stabilizing additives (PSA). Coprecipitation leads to finely divided dry powders, which can be rapidly reconstituted back into aqueous. Moreover, conversion to this dry format imparts excellent thermostability to the mAb.

**Applications to Biotherapeutics**

Recent developments of this technology have provided powders that can be rapidly reconstituted to produce very high concentration mAb solutions (>200 mg/mL), deliverable through a 27 gauge needle, with acceptable glide force and osmolality.

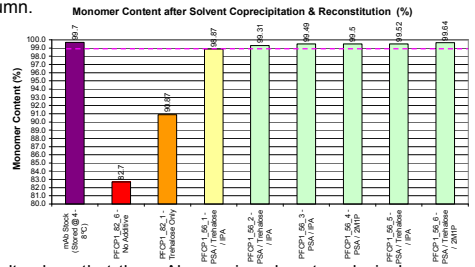
## PCMC Technology




PCMC are produced by coprecipitation of biomolecule and coprecipitant into GRAS solvent. The PCMC are formed by a rapid, self-assembly process, whereby the coprecipitant core *(blue cubes)* forms a support core and the biomolecule *(yellow spheres)* is immobilized on this crystal surface.

## Monomer Content after Coprecipitation

After drying, the PFCP PCMC material was reconstituted into histidine buffer at a target protein concentration of 1 mg/mL, and monomer content was measured by size-exclusion chromatography, using a Tosoh TSKGel G3000 $SW_{XL}$7.8 mm ID x 30 cm column.




These results show that the mAb remains almost exclusively as monomer when PCMC coprecipitation is undertaken with Glu and Arg present. When no additives or trehalose alone were used, significant formation of higher molecular weight species occurred.

## Bioactivity of PFCP

PCMC coprecipitation preserves the activity of the mAb. The bioactivity of the PFCP samples was tested in a PFCP specific ELISA.

| Sample | Theoretical Protein Loading (%w/w) | Measured Protein Loading (%w/w) | % Activity |
|---|---|---|---|
| PFCP1_56_1 | 16.8 | 15.6 | 92 |
| PFCP1_56_2 | 17.2 | 16.8 | 95 |
| PFCP1_56_3 | 32.7 | 30.0 | 108 |
| PFCP1_56_4 | 32.7 | 28.5 | 109 |
| PFCP1_56_5 | 26.6 | 26.1 | 107 |
| PFCP1_56_6 | 26.6 | 23.4 | 96 |

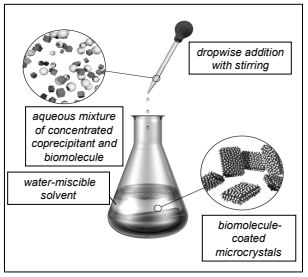
From the results it is clear that bioactivity has not been compromised by the PCMC coprecipitation process. Furthermore the protein loading measured is approximately equivalent to the theoretical composition, demonstrating that protein is not lost in the coprecipitation process, but is fully immobilized on the surface of the microcrystal.

## Human mAb PCMC Formulations

Monoclonal human antibody, PFCP, was obtained from Pfizer Inc, St Louis, MO. PFCP is a fully human monoclonal antibody specific for human cytotoxic T lymphocyte–associated antigen 4.

PFCP PCMC were prepared by coprecipitation of an aqueous mixture of histidine buffered antibody and concentrated glycine coprecipitant into either propan-2-ol or 2-methyl-1-propanol.

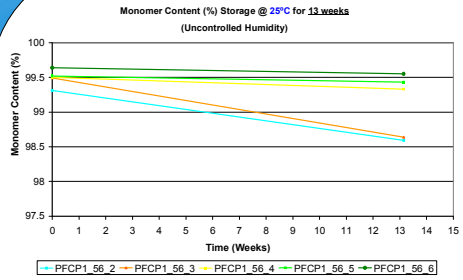
PCMC were prepared in the presence and absence of the precipitation protective additive pair (Glu, Arg) and with and without trehalose.




| Sample | Coprecipitation Solvent | PCMC Composition (%) | | | |
|---|---|---|---|---|---|
| | | Theoretical Protein Loading (%w/w) | Glycine (%w/w) | Precipitation Stabilizing Additive (%w/w) | Trehalose Dihydrate (%w/w) |
| mAb Stock (Stored @ 4-8°C) | | | | | |
| PFCP1_82_6 - No Additive | IPA | 41.1 | 57.0 | 0.0 | 0.0 |
| PFCP1_82_1 - Trehalose Only | IPA | 32.0 | 44.4 | 0.0 | 22.0 |
| PFCP1_56_1 | IPA | 16.8 | 55.7 | 4.5 | 22.3 |
| PFCP1_56_2 | IPA | 17.2 | 56.9 | 13.7 | 11.4 |
| PFCP1_56_3 | IPA | 32.7 | 46.4 | 19.4 | 0.0 |
| PFCP1_56_4 | 2M1P | 32.7 | 46.4 | 19.4 | 0.0 |
| PFCP1_56_5 | IPA | 26.6 | 37.7 | 15.7 | 18.8 |
| PFCP1_56_6 | 2M1P | 26.6 | 37.7 | 15.7 | 18.8 |

The ratio of active mAb to coprecipitant/PSA was varied between 17%w/w and 33 %w/w, as shown in this table (Theoretical Protein Loading (%w/w)).

## Stability Under Accelerated Stress Conditions




PFCP PCMC powders were stored at 25°C in sealed vials at ambient humidity for a standard period of 13 weeks.

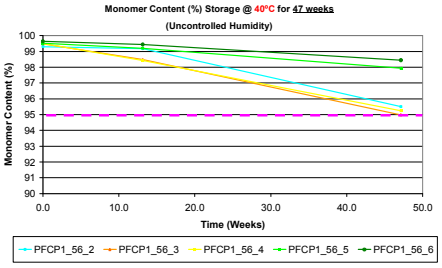
All samples retained >98.5% monomer content.

*(Liquid PFCP stored @ 2-8ºC had a monomer content of 99.6% monomer.)*

PFCP PCMC powders were stored in sealed vials at 40ºC ambient humidity for a standard period of ~47 weeks.

All samples retained ≥95% monomer content. Sample PFCP1_56_6 retained 98.5% monomer - a change of only 1.1% over 47 weeks.

*(Liquid PFCP stored @ 2-8ºC had a monomer content of 99.6% monomer.)*




Dry PCMC mAb powders incorporating Glu and Arg exhibit high stability under accelerated stress conditions. Inclusion of a further neutral additive such as trehalose enhances stability even further.

## Discussion

During the PCMC process, protein molecules are exposed to a very different environment to that arising during lyophilisation or spray-drying. For molecules prone to self-association this can lead to a requirement for novel stabilising excipients. In this work we have demonstrated that a combination of glutamic acid and arginine is able to keep mAbs in a monomeric form during dehydration and precipitation using polar solvents. Lyoprotectants such as trehalose are much less effective.

It is hypothesised that:

- within solvent, protein association is predominately via charge-charge interactions
- neutral additives such as trehalose cannot prevent this
- Glu and Arg additives ion-pair with charged protein side-chains
- a zwitterion-coating minimises intermolecular mAb association in dry-state
- additional neutral additives act synergistically by displacing water molecules

A combination of Glu and Arg has previously been reported to be useful for preventing protein precipitation in highly concentrated aqueous protein solutions with minimal reduction of specific protein-protein interactions (studied by NMR; Golovanov, A. *et al.*, A Simple Method for Improving Protein Solubility and Long-Term Stability, *JACS*, **2004**, 8933-8939). This observation appears contradictory to the above hypothesis. However, this can be explained by the much weaker ion-pairing in water and the importance of hydrophobic interactions for driving protein association.

Recent developments of this technology have provided powders that can be rapidly reconstituted to produce very high concentration mAb solutions (>200 mg/mL), deliverable through a 27 gauge needle, with acceptable glide force and osmolality.

## Conclusion

Human monoclonal antibodies can be readily formulated using PCMC technology by incorporating precipitation stabilizing additives (PSA). PCMC coprecipitation leads to finely-divided dry powders, which can be rapidly reconstituted back into aqueous, to release the monoclonal antibody in monomeric form. Such PCMC mAb dry powders are attractive as a platform for alternate delivery applications.