Dry powder therapeutic mAb formulations with enhanced temperature stability

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DRUG DELIVERY & PROTEIN STABILISATION

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

Purpose.

There is an increasing demand for differentiated strategies for formulating and delivering mAb in particulate form. The aim of this study was to investigate methods for optimising protein-coated microcrystal (PCMC) formulations of a human monoclonal antibody. PCMC technology provides a novel method of stabilising these important biopharmaceuticals in the form of dry powders

Methods.

Human monoclonal antibody coated microcrystals were prepared by coprecipitation of an aqueous mixture of histidine buffered human monoclonal antibody and concentrated glycine into propan-2-ol. The standard formulation contained mAb, buffer salts and glycine and the effect of including potential precipitation stabilizing additives (PSA) was investigated e.g. Glu, Arg. Following coprecipitation, the PCMC particles were filtered and air-dried to form free-flowing dry powders. Protein integrity was assessed by comparing optical clarity of the reconstituted formulations, protein concentration by UV spectroscopy and monomer content by size-exclusion chromatography, using a Tosoh TSKgel G3000 SW_{XL} 7.8 mm ID x 30 cm column.

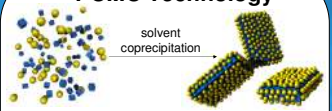
Results.

The measured protein loadings were found to be within 5% of the target protein loading for all formulations. However, the optical clarity of reconstituted PCMC prepared with optimal PSA was significantly better. Protein particulates could be observed in reconstituted standard samples, whilst PCMC utilising PSA, such as Glu, Arg, were optically clear and free of particulates. Consistent with this, measurement of the degree of monomer conservation following coprecipitation showed that material produced without PSA generally had monomer retention of less than 90% whilst optimised samples incorporating PSA resulted in >98% of monomer conservation.

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.

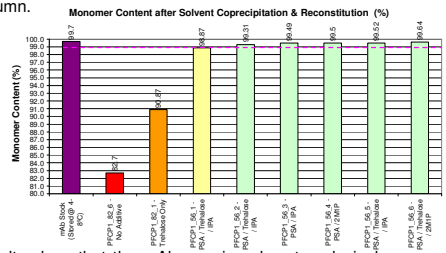
PCMC Technology



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

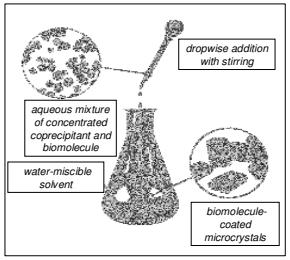
From the results it is clear that bioactivity has not been compromised by the PCMC coprecipitation process. Furthermore the protein loading measured is approximately equivalent to the theoretical composition, demonstrating that protein is not lost in the coprecipitation process, but is fully immobilized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

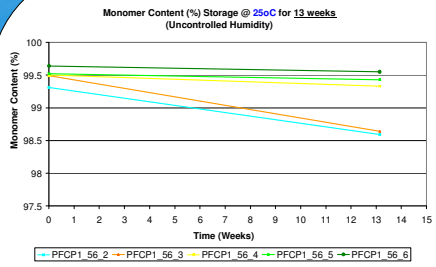
PCMC were prepared in the presence and absence of the precipitation protective additive pair - Glu, Arg, and with and without trehalose.



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.

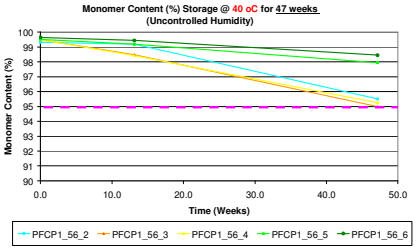
All samples retained >98.5% monomer content.

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

PFCP PCMC powders were stored in sealed vials at 40°C 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 @ 2-8 °C 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 are 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.

These data demonstrate that the choice of best excipients for stabilisation of dry mAb powders is a strong function of the dehydration pathway. The best excipients for preparation of PCMC are not predictable from lyophilisation or spray-drying results.

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.