



# Optimized Protein Recovery During Solvent Precipitation Depends on Salt Type

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## HIGHLIGHTS

Salt type and salt concentration influence not only precipitation but also resolubilization recoveries of proteins.  
Both cation and anion play important roles.

## INTRODUCTION

Precipitation in organic solvent is a classic approach to purify and concentrate proteome samples ahead of mass spectrometry analysis. Our lab has shown that salt ions are essential to facilitate quantitative proteome precipitation [1]. The goal of the current study is to examine the precipitation efficiency of proteins in organic solvent (i.e. acetone), as influenced by the presence of different types of cations and anions. This work will lead to an optimized proteome purification and preconcentration protocol to maximize top-down and bottom-up proteoform characterization by mass spectrometry.

## METHODS

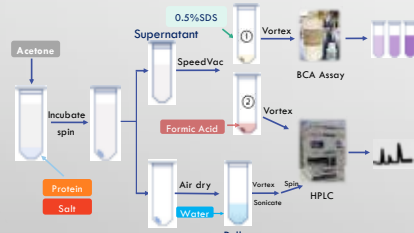


Figure 1

- ① Supernatant with NaCl are redissolved by SDS and analyzed by BCA assay.
- ② Supernatant with other salts, e.g., ZnSO<sub>4</sub>, MgCl<sub>2</sub>, are redissolved by formic acid and analyzed by HPLC, for many metal ions are not compatible with BCA assay.

## RESULTS

### Salt Type & Concentration

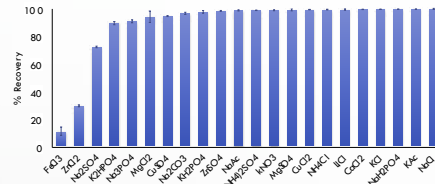


Figure 2

Precipitation recovery: 1 g/L of BSA with 100 mM of salts precipitated by 80% acetone for 5 min

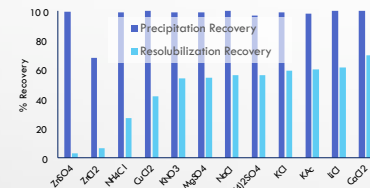


Figure 4

Resolubilization recovery: 1 g/L of BSA with 100 mM of salts, precipitated by 80% acetone for 5 min, pellet redissolved by water

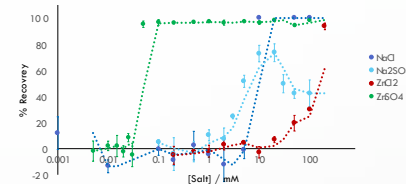


Figure 3

Salt curves: 1 g/L of BSA with different concentrations of 4 salts precipitated by 80% acetone for 5 min

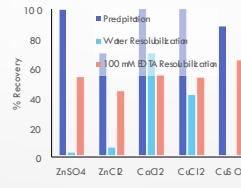


Figure 5

1 g/L BSA with 100mM of salts. EDTA can help to redissolve some insoluble pellets indicating that Zn<sup>2+</sup> and Cu<sup>2+</sup> may combine with protein

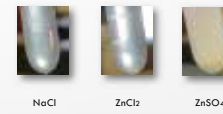


Figure 6

Salt type affects the form of pellets

### Acetone Concentration

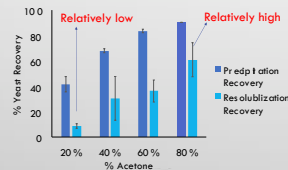


Figure 7

1 g/L of yeast with 100 mM of NaCl, precipitated by acetone for 5 min

### Precipitation Time

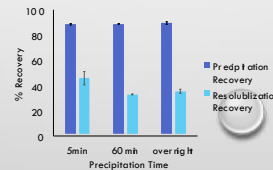


Figure 8

1 g/L of yeast with 100 mM of NaCl, precipitated by 80% acetone

### Centrifuge Time

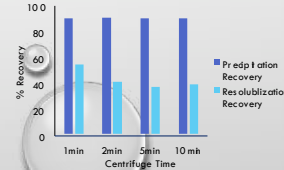


Figure 9

1 g/L of yeast with 100 mM of NaCl, precipitated by 80% acetone for 5 min

## CONCLUSIONS

Conditions	Influence on Precipitation Efficiency	Influence on Resolubilization Efficiency	Recommend Condition
Salt Type	Yes	Yes	ZnSO <sub>4</sub> or CaCl <sub>2</sub> , (add EDTA when zinc salts are employed)
Salt Concentration	Yes	Not sure*	Higher, but depend on salt type
Acetone Concentration	Yes, positive correlation	Yes, positive correlation	Higher, e.g. 80%
Precipitation Time	No	No	2~5 min
Centrifuge Time	No	Yes, negative correlation	1 min

## FUTURE WORK

- Do more salt curves on different proteins, e.g. pepsine, lysozyme, and yeast protein. Compare the precipitation recoveries to Hofmeister series and discuss the mechanisms.
- Digest precipitated yeast pellet:
  - a) Run SDS-PAGE to see protein MW distribution.
  - b) Analyze pellet protein by LC-MS/MS to see if salt type influence the precipitated proteomes.

## REFERENCES

[1] Nickerson, J. L. & Doucette, A. A. (2020). *J Prot Res*, 19(5), 2035–2042.

## ACKNOWLEDGEMENTS

