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## Practical Aspects of the Automated Preparation of Aqueous Two Phase Systems for the Analysis of Biological Macromolecules

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### Highlights:

- Robotic liquid handling methods are developed for aqueous two-phase systems.
- The importance of control of aspiration/dispense speeds and delay times is stressed.
- The % bias accuracy of system preparation is assessed by gravimetric methods.
- The critical effect of the geometry of the binodal on this accuracy is reported.
- The methods are demonstrated as applied to the analysis of ovalbumin isoforms.

### Abstract:

A robust strategy for the automated preparation of aqueous two-phase systems (ATPS) using a liquid handling sample processor was developed using gravimetric methods to determine the accuracy of preparation. The major robotic control parameters requiring adjustment were; speed of aspiration and dispense; delay times following aspiration and dispense alongside measures to control cross-contamination during phase sampling. In general mixture compositions of both polymer / polymer and polymer / salt mixtures could be prepared with a target bias accuracy of less than 5%. However, we found that the bias accuracy with which systems of defined TLL and MR could be constructed was highly dependent on the tie line length of the ATPS and the geometrical form of the ATPS co-existence curve. For systems with a very low degree of curvature (PEG / salt systems here) increases in bias (accuracy) are appreciable at relatively long tie line lengths. Where the degree of curvature is more pronounced (PEG/dextran systems) closer approach to the critical point was possible without

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