Interference of some ATPS phase-forming components in protein quantification by the Bradford method

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Aqueous Two-Phase Systems (ATPS) are obtained by mixing two aqueous solutions of different constituents that become immiscible under certain critical conditions, like temperature, concentration, etc. Both phases are composed mainly by water (>80%) and each one is enriched in a different component [1]. ATPS formed by two polymers or a polymer and a salt represent the traditional systems. Nevertheless, other alternative biphasic systems can be obtained using surfactants, micellar compounds or ionic liquids. Due to the high percentage of water present in their composition, ATPS can provide a gentle environment for the extraction and recovery of sensitive biological materials, such as proteins.
Different methodologies can be used for protein quantification, such as UV absorbance spectroscopy (280 nm), chemical methods (like Kjeldahl) or colorimetric determination based on dye binding assays (like Bradford, BCA or Lowry). Among them, the Bradford assay is the most currently used for protein quantification in ATPS due to its simplicity, sensitivity and fast response. This assay is based on the absorbance shift observed when the dye Coomassie® Brilliant Blue G-250 binds to protein. The peak absorbance of the acidic solution of Coomassie® changes from 465 to 595 nm when binding occurs. The binding process is fast (5 minutes) and the protein-dye complex formed is stable for about 1 hour [2]. The use of a single reactive and the sensitivity of the dye to small amounts of protein make the Bradford method widely used for protein determination. However, the method can suffer significant interference from some compounds, like detergents and alkaline agents. It is also known that the presence of some salts under certain concentration can compromise the method response. Nevertheless, poor information is found in the literature about interference of specific phase-forming components of ATPS.

In this work, the interference in the Bradford method caused by several salts and polymers usually used in ATPS formation was investigated. For that purpose, calibration curves were obtained for bovine serum albumin (BSA) in the presence of different concentrations of sulfates (Na_2SO_4, $(NH_4)_2SO_4$, $MgSO_4$ and $LiSO_4$), phosphates (Na_2HPO_4, NaH_2PO_4, K_2HPO_4 and KH_2PO_4), citrates (citric acid and trisodium citrate), acetates (acetic acid and sodium acetate) and polymers (PES Mw=100000, UCON Mw=3900, Ficoll Mw=70000 and PVP Mw=40000).

Comparison of these curves with that obtained for BSA in distilled water, allow us to conclude that salts produce a more significant effect in BSA calibration curve than polymers. In all cases, a reduction in the absorbance at 595 nm (A_{595}) was observed.
For most of the salts tested, reduction ≥20% was obtained in A_{595} when a concentration ≥1% (w/w) was present. The only exceptions were observed for the salts NaH_2PO_4, KH_2PO_4, citric acid and acetic acid that, probably due to their acidic pH, presented reductions in the A_{595} lower than 12% for 1% (w/w). For sulfates, the change of the cation also provided a change in the calibration curve and $(NH_4)_2SO_4$ showed the higher interference (A_{595} reduction of 25% for a concentration of 1% (w/w)). Fig. 1 shows the reduction in A_{595} in the presence of different $(NH_4)_2SO_4$ concentrations.

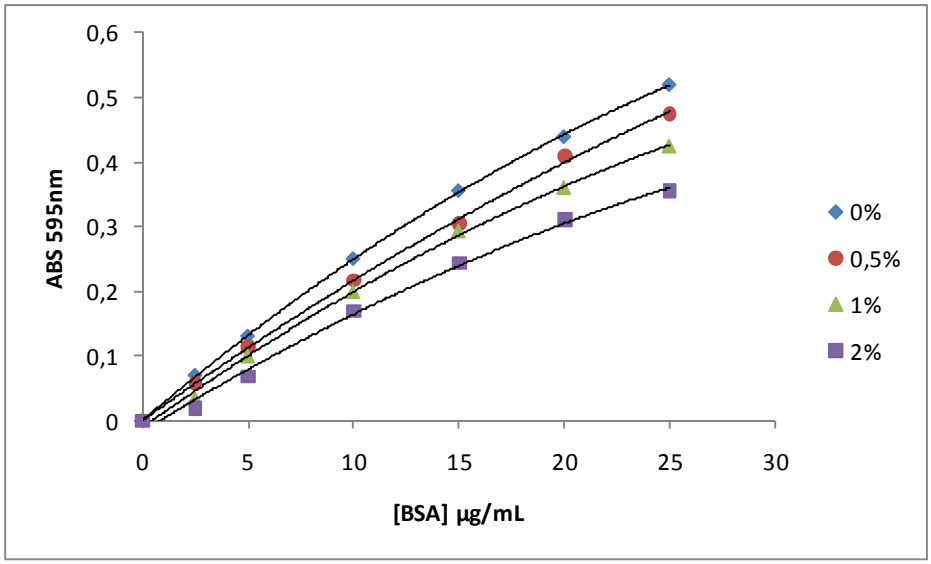

Fig. 1. Calibration curve obtained for BSA in the presence of different concentrations (%w/w) of $(NH_4)_2SO_4$

For all the polymers studied, A_{595} reduction >25% was obtained for concentrations ≥ 5% (w/w). The only exception observed was the Ficoll polymer that showed reductions <6% in the A_{595} values for the different concentrations tested (from 2% up to 10% (w/w)). The interference of the different polymers in a concentration of 5% (w/w) in BSA calibration curve is shown in Fig. 2.

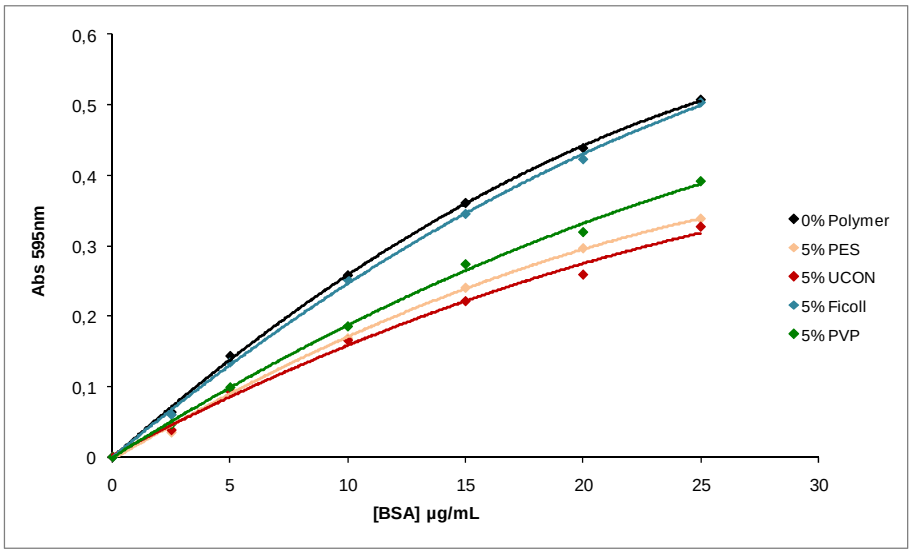

Fig. 2. Calibration curve obtained for BSA in the presence of 5% (w/w) PES, UCON, Ficoll or PVP

These results allowed to conclude that most of the phase-forming components tested in the present study produce significant interference in the BSA calibration curve. Thus, samples have to be conveniently diluted before protein quantification. For almost all the salts, concentration must be below 1% (w/w) to ensure no significant interference (except for citric and acetic acid that produced no interference up to the maximum concentration tested 5% (w/w)). All the polymers, except Ficoll, have to be present in a concentration below 5% (w/w) to avoid significant interference problems.

References

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