

Evaluation of the interference by ammonium sulphate in the BCA protein quantification

The protein quantification by the BCA assay (Sigma-Aldrich) is prone to interference by several chemical compounds above recognized thresholds. Included in the list is ammonium sulphate, one of the major components of atmospheric PM, where it forms by the neutralization of gaseous H_2SO_4 by NH_3 , the most abundant gaseous base in atmosphere, to form a solid salt. This compound is recognized to induce a noticeable interference on the absorbance of BCA when it is present in the extract at concentrations above 1.5 mM. In order to test the interference in the protein quantification by the presence of this salt in the filter extracts, we prepared blank solutions (containing no aerosol, hence no proteins) of ammonium sulphate at increasing concentrations from 0 to 50 mM, and observed the response of the BCA test as a function of the interfering concentration (Fig. S1).

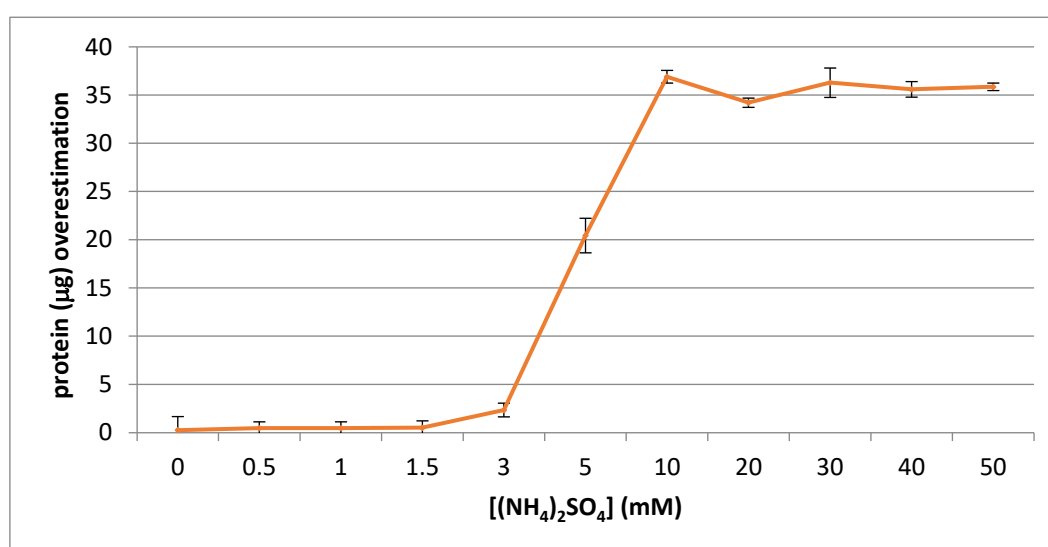


Figure S1. shows the results of this test in terms of amount of protein (µg) overestimated by the presence of ammonium sulphate at different concentrations. From 0 to 3 mM the interference was very low, while it was rapidly increasing from 3 to 10 mM, finally reaching a plateau from 10 to 50 mM, the highest concentration tested.

At the light of such results, protein concentrations were corrected by subtracting 35 µg of proteins for extracts characterized by a concentration of ammonium sulphate above 10 mM, while for extracts with concentration between 3 and 10 mM the amount of protein subtracted was calculated from the equation of the line of the interference as a function of the concentration of ammonium sulphate in the extract. Finally, no correction was applied when ammonium sulphate concentration in the extract was below 3 mM. The correction applied reduced the concentration of proteins from 3 to 33% with respect to the not corrected amount in the coarse ($1 < d < 10$ µm), and from 1 to 13% for the fine fraction. Notwithstanding the highest concentration of ammonium sulphate in the extracts of the fine fraction of aerosol (from 1 to 26 mM) in comparison with the same extracts of the coarse one (from 0.3 to 9 mM), the protein amount per filter was about 5 times higher in the fine than in the coarse fraction, hence the correction was relatively higher for this latter.