

Appendix to

Directional freezing and thawing of biologics in drug substance bottles

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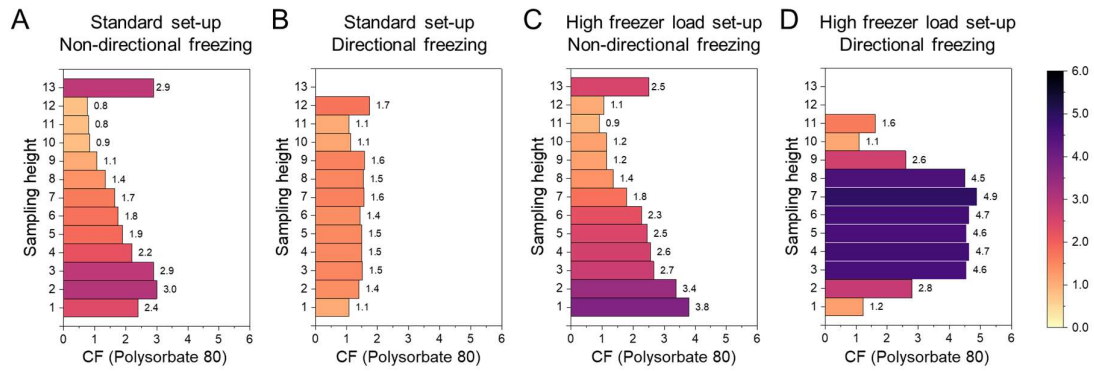


Figure A-1: Cryo-concentrations presented as concentration factors (CF) of polysorbate 80 (PS80) analyzed in ice core samples along the axial center in 2 L bottles after freezing: with the standard set-up (in an empty -80°C freezer) for (A) non-directional freezing (without device) versus (B) directional freezing (with device); and with the high freezer load set-up (with 10 additional bottles loaded in a -80°C freezer) for (C) non-directional freezing (without device) versus (D) directional freezing (with device). Bar charts show respective numerical values and are color coded for better visibility.

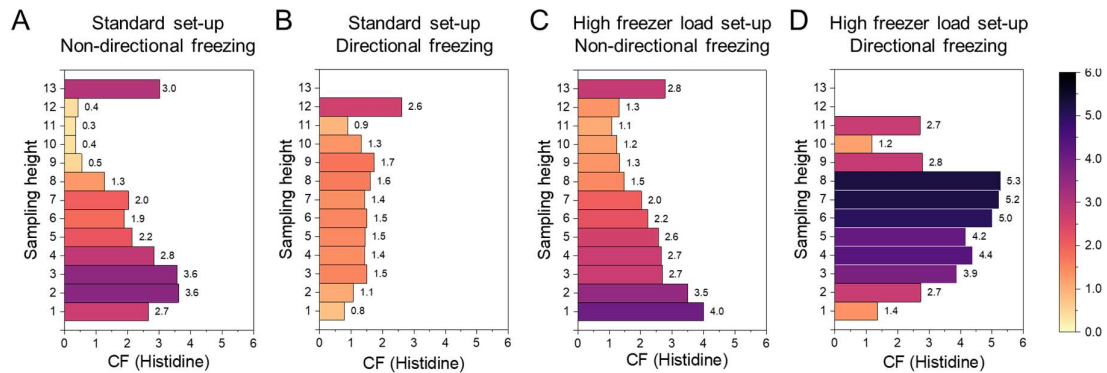


Figure A-2: Cryo-concentrations presented as concentration factors (CF) of Histidine analyzed in ice core samples along the axial center in 2 L bottles after freezing: with the standard set-up (in an empty -80°C freezer) for (A) non-directional freezing (without device) versus (B) directional freezing (with device); and with the high freezer load set-up (with 10 additional bottles loaded in a -80°C freezer) for (C) non-directional freezing (without device) versus (D) directional freezing (with device). Bar charts show respective numerical values and are color coded for better visibility.

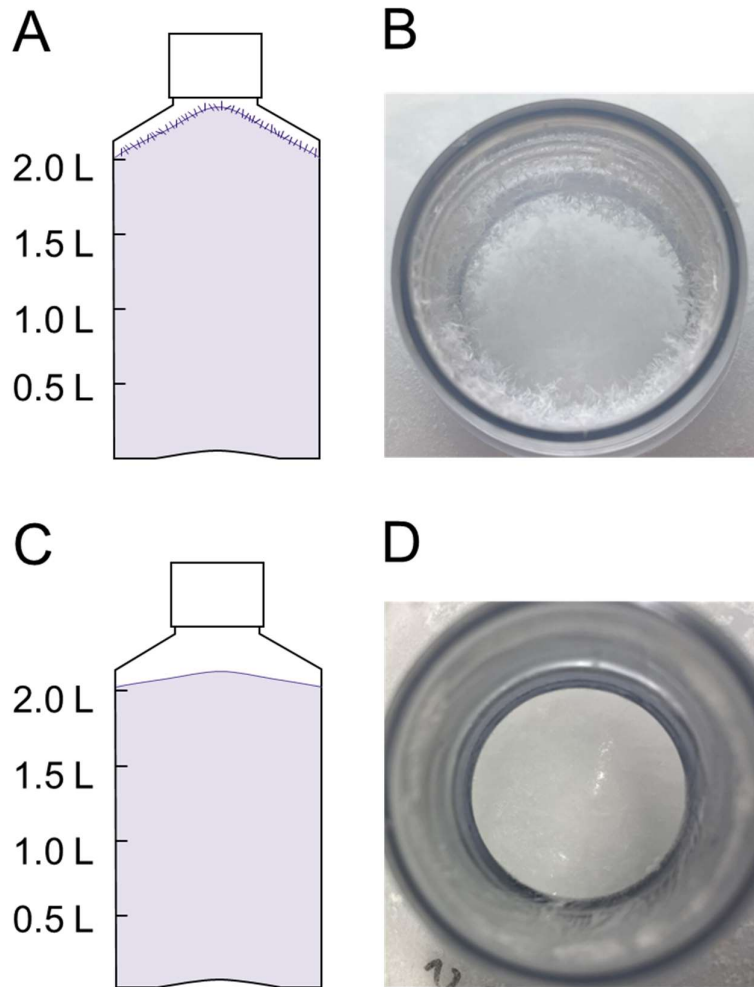


Figure A-3: Schematic drawings indicate volume expansion and ice mounds after freezing to -80°C (standard set-up) (A) non-directional freezing (without device) and (C) directional freezing (with device). Pictures were taken from the top through the bottle opening prior ice core sampling and show different appearance of ice surfaces after freezing to -80°C (standard set-up) (B) without the device and (D) with the device.

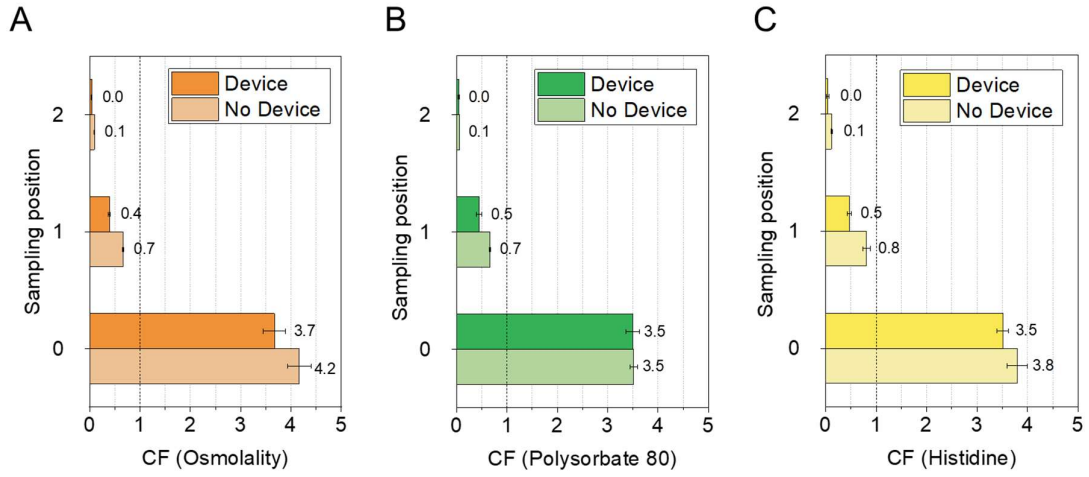


Figure A-4: Analytical data from samples collected after thawing at positions in the edge of a 2 L bottle at the top (E2), middle (E1) and bottom (E0). Data shown as mean \pm SD (of $n=3$ independent experiments) as CF compared to the initial concentration of the solution before F/T, which is also indicated as dashed line.