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Simultaneous high-resolution pH and spectrophotometric recordings of oxygen binding in blood microvolumes

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Abstract:

Oxygen equilibrium curves have been widely used to understand oxygen transport in numerous organisms. A major challenge has been to monitor oxygen binding characteristics and concomitant pH changes as they occur *in vivo*, in limited sample volumes. Here we report a technique allowing highly resolved and simultaneous monitoring of pH and blood pigment saturation in minute blood volumes. We equipped a gas diffusion chamber with a broad-range fibre-optic spectrophotometer and a micro-pH optode and recorded changes of pigment oxygenation along oxygen partial pressure (P_{02}) and pH gradients to test the setup. Oxygen binding parameters derived from measurements in only 15 µl of haemolymphe from the cephalopod *Octopus vulgaris* showed low instrumental error (0.98 %) and good agreement with published data. Broad-range spectra, each resolving 2048 data points, provided detailed insight into the complex absorbance characteristics of diverse blood types. After consideration of photobleaching and intrinsic fluorescence, pH optodes yielded accurate recordings and resolved a sigmoidal shift of 0.03 pH units in response to changing P_{02} from 0 to 21 kPa. Highly resolved continuous recordings along pH gradients conformed to stepwise measurements at low rates of pH changes. In this study we showed that a diffusion chamber upgraded with a broad-range spectrophotometer and an optical pH sensor accurately characterizes oxygen binding with minimal sample consumption and manipulation. We conclude that the modified diffusion chamber is highly suitable for experimental biologists who demand high flexibility, detailed insight into oxygen binding as well as experimental and biological accuracy combined in a single setup.

Keywords: Diffusion chamber, oxygen equilibrium curve, Hemocyanin, Haemocyanin, Haemoglobin, pH optode, Octopus, Amphipod, Antarctic fish