Appendix H Sensor calibration

Calibration method

An experimental sensor was built to measure the fluorescein concentration in the gravel column elution experiment. While this sensor allowed much more frequent sampling of the elutant and automatic data logging (every five seconds) it also introduced a number of other variables, and as this sensor served as the primary data source, it needed to be calibrated.

The sensor used a blue light emitting diode (LED) as a light source with a peak emission at a wavelength of 430nm which is different from the spectrophotometer wavelength setting of 492nm. This had the effect of making the sensor more responsive to the low pH fluorescein species that absorb more strongly at shorter wavelengths. Furthermore the sensor LED produces a spectrum of wavelengths rather than the relatively monochromatic light of the spectrophotometer. The interaction of polychromatic light source with the different fluorescein ionic forms was tested at the pH of each experimental run to determine how the sensor readings could be converted to its equivalent spectrophotometer absorbance value.

Two sets of sensor/spectrophotometer comparison data were collected. The first used the elutant samples collected during experimental runs. At the end of a test run these 100 ml samples were passed through the sensor and then tested on the spectrophotometer. Figure 15 shows the different responses at the different test pH as well as a fitted result based on a combination of two linear responses. A two gradient response was tested because there appeared to be two distinct gradients in the pH 7.1 and pH 7.8 samples, and because the predominant fluorescein species at the test pH would be the dianion and monoanion. The fluorescence of fluorescein at 512 nm was also expected to influence the sensor response. However despite these apparently plausible justifications for a two gradient sensor response, this fitting approach remains empirical. This should not be a problem here as the most important requirement is to calculate an equivalent absorbance value for each sensor measurement. Figure 15 suggests that the two gradient linear response can fit the pH 7.1 and pH 7.8 data but may not be adequate for the pH 5.1 and pH 6.7 data.

The second sensor/spectrophotometer comparison test used buffer solutions that had not passed through the gravel column. These data are the "calibration" readings shown in Figures 16 and 17. This approach was used because the elutant samples from the first test runs at pH 7.1 and pH 7.8 each developed a small quantity of precipitate (these first test data were excluded from the calibration curve fitting exercises). Figure 16 shows that there was a

large difference between the calibration readings and the experimental readings and this difference was thought to be related to the precipitate. The method selected to convert these sensor readings to their equivalent absorbance values for the pH 7.1 and pH 7.8 runs was based on a least-squares fitted two gradient line using the experimental data. This is labelled as "Avg" in Figure 16.

Figure 17 shows that the sensor and absorbance relationship for the pH 5.1 and pH 6.2 experimental runs was not simple, so a series of average values were calculated for each run, and these data were used in a look-up table. As the calibration data did not differ from the experimental data they were included in this calculation.

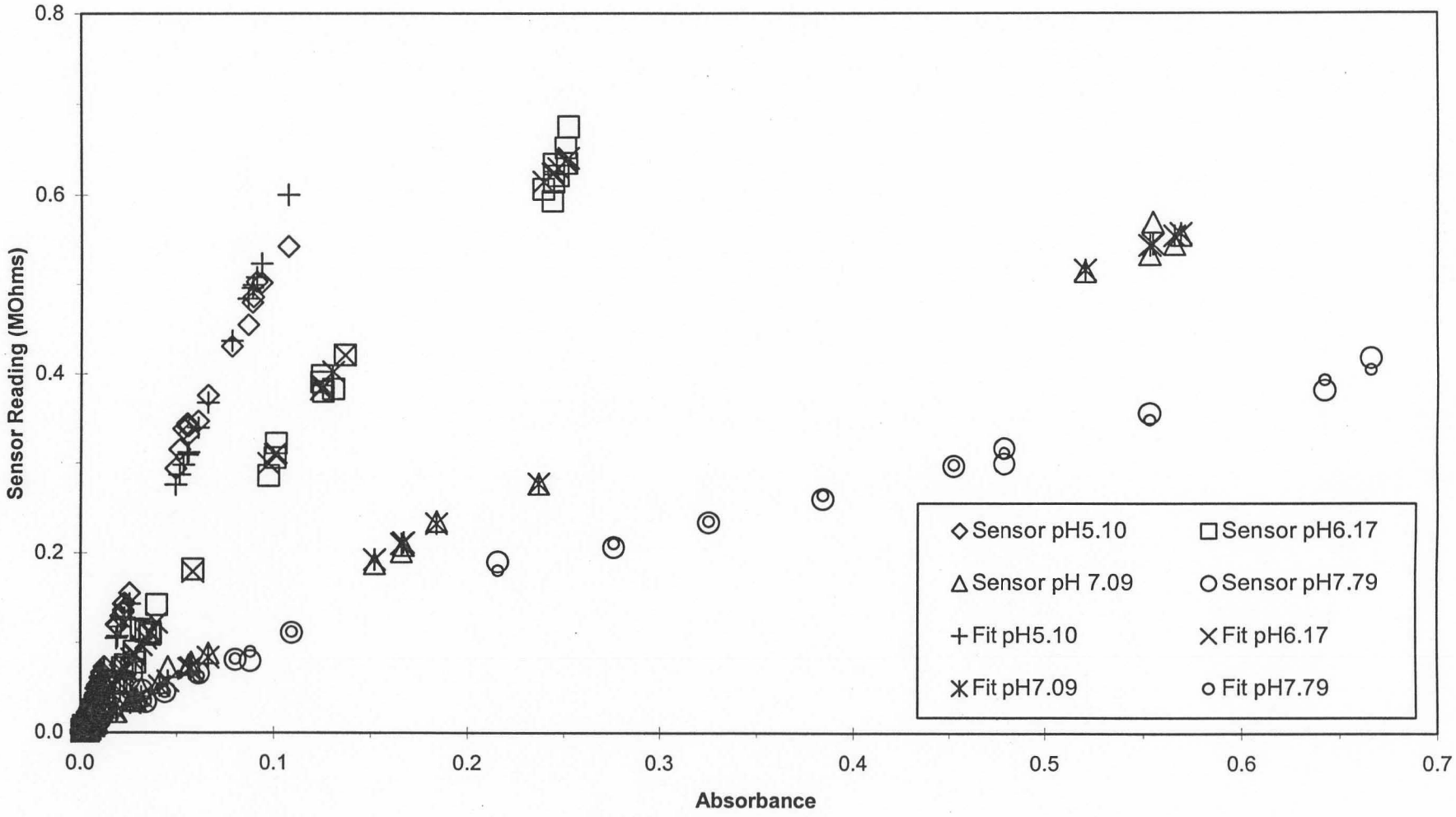

Figure 15 Sensor and absorbance measurements (actual and fitted) at different pH

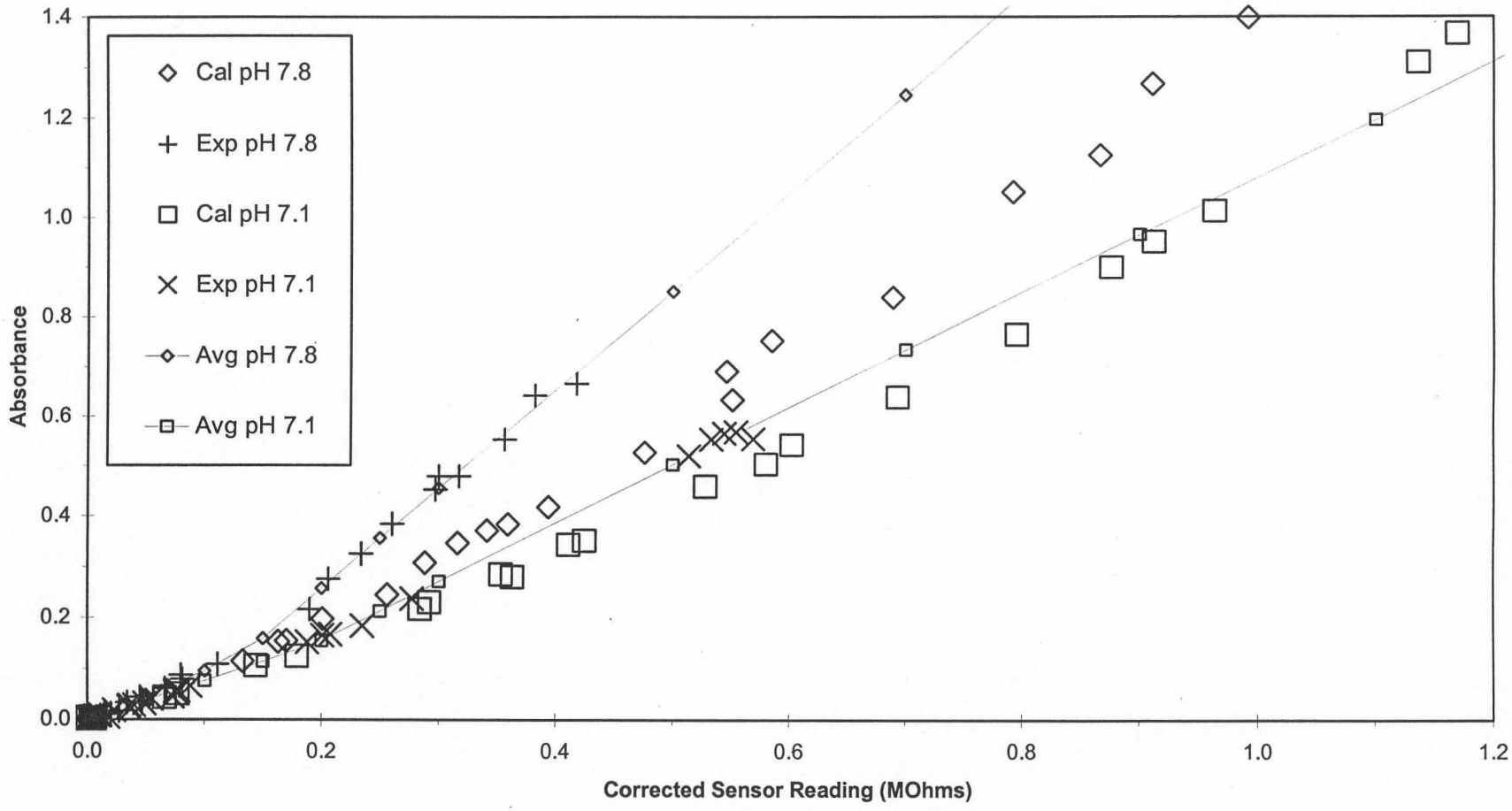

Figure 16 Experimental, calibration and average absorbance values for pH 7.1 and pH 7.8 sensor readings

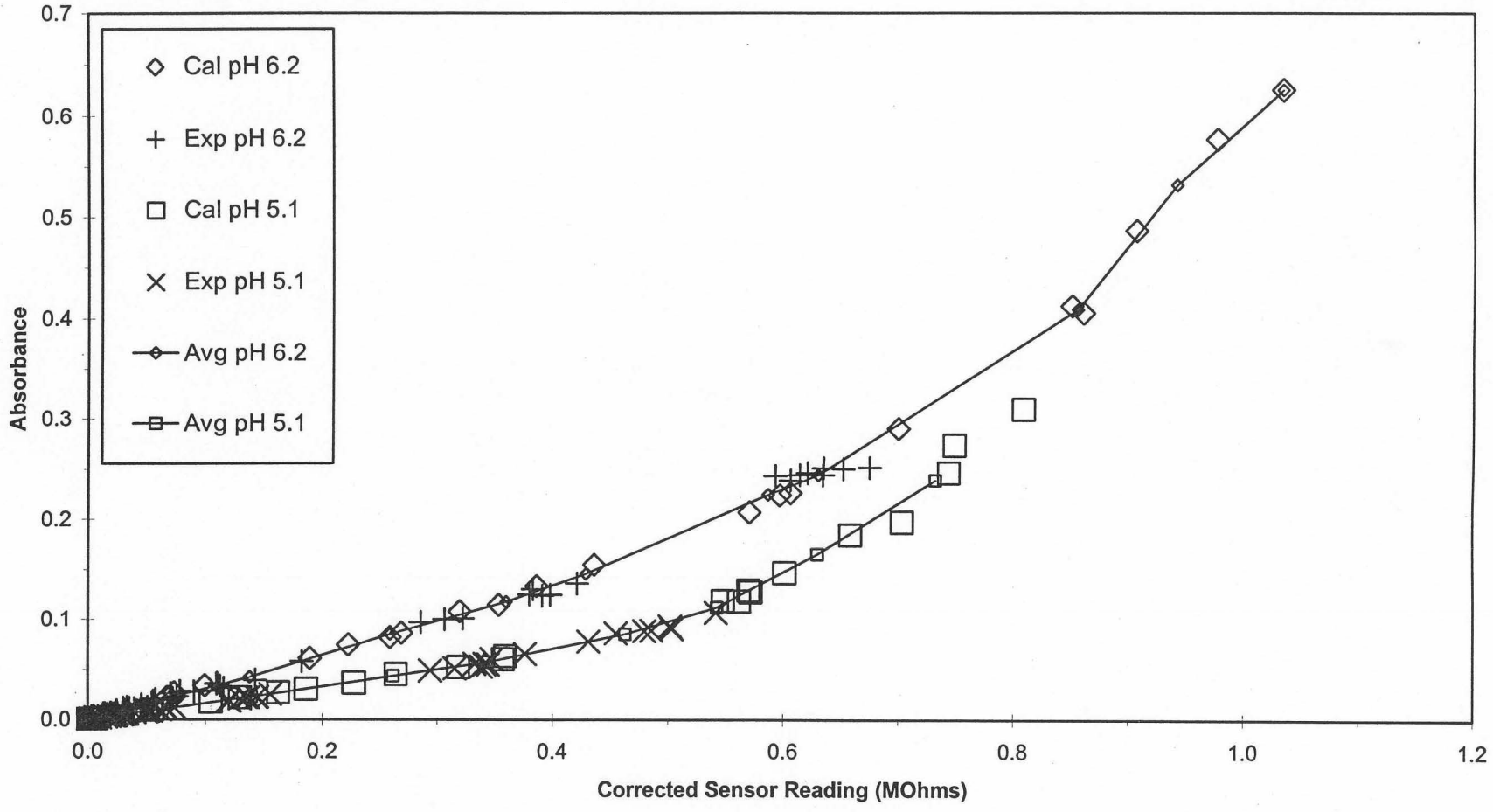

Figure 17 Experimental, calibration and average absorbance values for pH 5.1 and pH 6.2 sensor readings