Development of Printed-Ion Selective Electrodes for the Measurement of Calcium in Bovine Blood

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ABSTRACT

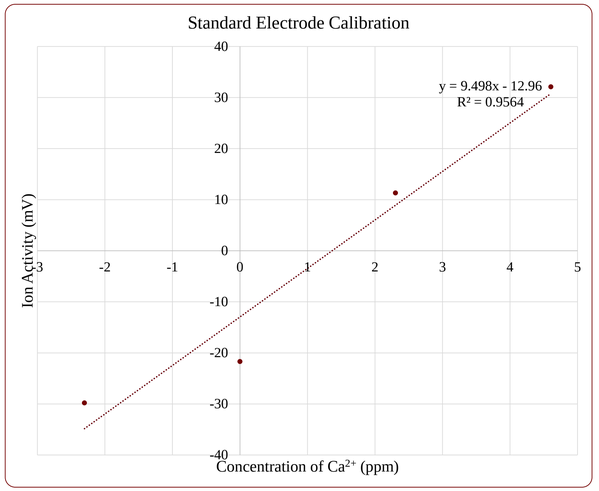
Hypocalcemia, or milk fever, is characterized by a low level of calcium in cows. It is one of the most significant disorders that afflicts dairy cows; almost a quarter of all dairy cows will develop this condition. A single case of hypocalcemia can cost up to $500 to treat, and the cost can rise from the problems that develop after recovery. The death of the cow can cost even more since thousands of dollars have been spent to raise it to maturity. At present there is no cow-side test that exists for a farmer to establish that a cow is at the first stage of milk fever. It can be time-consuming to send blood samples to a laboratory for testing. Printed ion-selective electrodes have the potential to solve this problem and create a portable and efficient way for dairy farmers to test their cows right at the farm. In this study, the development of printed calcium ion-selective electrodes to measure calcium levels in bovine blood was the major focus. The method of acidification of the blood to a pH of 3.5 using acetate buffer was used to unbind calcium from its complexes in blood so total calcium levels could be measured. A standard calcium ion-selective electrode and printed calcium ion-selective electrodes were used to measure the calcium concentrations in the blood samples obtained to compare results. Both types of electrodes produced varying results.

INTRODUCTION

Ion-selective electrodes are widely used to measure the concentration, specifically, the activity, of ions in solution. Printed ion-selective electrodes serve the same purpose but are cheap, disposable, and more portable. This is what makes them ideal for the diagnosis of hypocalcemia, a potentially deadly disorder, in dairy cows. Immediately after a cow calves, the farmer needs to know whether the blood calcium levels of the cow are too low and if she needs to be given calcium. Sending blood samples out to a laboratory is time consuming and a cow-side test is needed as milk fever can be fatal within 24 hours if left untreated. The loss of a single cow can cost a dairy farmer thousands of dollars, the money they spent raising the cow and the money that is lost during milk production. Printed ion-selective electrodes are an ideal basis for a cow-side test; the farmer can draw blood from the cow and test it right away with little preparation necessary. The main goal of this research was to develop a printed electrode and establish test conditions that would successfully measure total calcium levels in whole cow blood. Most previous work in clinical chemistry with ion-selective electrodes has focused on the use of serum rather than whole blood and was also primarily measuring ionized calcium. This project used acidification of whole blood as a means of measuring total calcium and used printed electrodes prepared with a calcium-selective membrane. Overall, these methods were chosen because they are inexpensive and require little sample preparation. This is ideal because the electrodes are meant to be brought out into the field instead of being used in the lab. With the development of successful printed electrodes for measuring calcium in cow blood, this aspect of electrochemistry can be expanded to measuring different ions or drugs and to using these electrodes everywhere, including crime scenes.

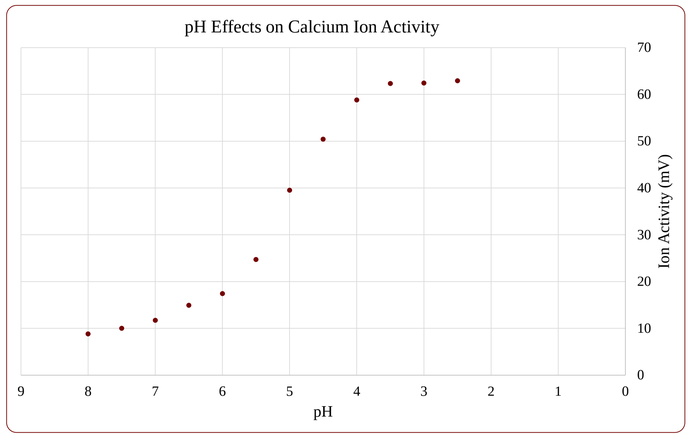
MATERIALS & METHODS

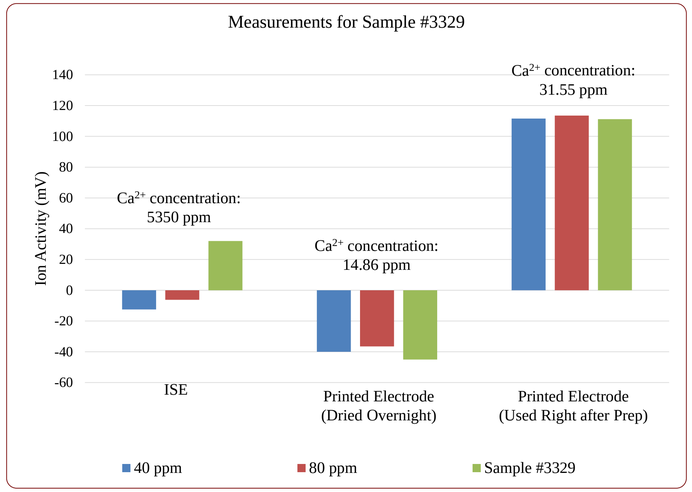
The standard ion-selective electrode used was a Cole-Parmer Combination Ca^{2+} Ion-Selective Electrode. When connected to a multi-meter, the activity of the ions, in mV, can be measured and then converted to the concentration of the ions, in ppm. The standard electrode was calibrated every 1-2 hours using 0.1 ppm, 1 ppm, 10 ppm, and 100 ppm Ca^{2+} standard solutions, made from calcium chloride, $CaCl_2$, and water. The printed electrodes used were Zensor screen-printed electrodes. Each electrode prepared had a membrane drop-cast onto the working electrode. This membrane was made of calcium ionophore I, bis(1-butylpentyl)decan-1, 10-diyl diglutarate, potassium tetrakis(4-cholorphenyl)borate, poly(vinyl chloride), and THF as a solvent. About 12.5 µL were dropped onto each electrode starting with a 2 µL drop and then 1.5 µL drops until 12.5 µL were deposited. Initially, alligator clips were used to connect the printed electrodes to the multi-meter but, eventually, an adapter was obtained into which the electrodes can simply be inserted. The printed electrodes were calibrated using a two-point calibration. Diluted 40 ppm and 80 ppm Ca^{2+} solutions were measured. A diluted blood sample was then measured, the ion activity, in mV, was substituted into the equation acquired, and the concentration of calcium in the blood was obtained. The equation acquired from a calibration is in the form of $y = mx + b$. Each equation is the Nernst equation for that particular electrode, with the original Nernst equation being expressed as $E_{cell} = E^0 - \frac{RT}{nF} lnQ$. In the case of measuring ion activity in blood, E_{cell} is the ion activity, E^0 is the y-intercept, $\frac{RT}{nF}$ is the slope, lnQ is x, and Q is the concentration of calcium. The blood sample itself was prepared by diluting the blood by a factor of 20 with acetate buffer to a pH of 3.5 .[1] This was determined to be the ideal pH at which the calcium concentration in blood became constant. The experiment for this determination was done by adding 0.2 µL of concentrated HCl, 37% w/w, dropwise to blood and measuring the calcium ion activity at pH levels at a 0.5 interval.



RESULTS

When calcium levels are measured in blood with the stepwise-addition of acid to lower the pH from neutral to pH 3, it can be seen that the concentration of calcium increases until it reaches a plateau at approximately pH 3.5. Because maximum calcium levels were observed at this pH, all future experiments were conducted at pH 3.5, unless otherwise stated. Initial experiments were performed in which undiluted blood at a normal pH of approximately 7 was measured. The concentrations measured were 32.6 ppm, 27 ppm, 45 ppm, and 21.2 ppm for blood samples #3703, #3853, #3329, and #3858 respectively. In order to measure the *total* calcium concentration in the acidified blood, the procedure with the two-point calibration was followed. In the graph below, the measurements made with sample #3329 are shown. The difference between the two printed electrodes is in the drying time of the ion selective membrane after deposition; one was left to dry overnight while the other was used right after deposition. The standard ion-selective electrode showed that the calcium concentration is 5350 ppm, the printed electrode with the membrane that dried overnight showed a concentration of 14.86 ppm, and the printed electrode with the membrane just deposited showed a concentration of 31.55 ppm. A different measurement made by the standard electrode showed a concentration of 6.55×10^6 ppm for sample #3331. Another measurement made by the printed electrode with the overnight dried membrane showed a concentration of 1.61×10^{-5} ppm for sample #3703. Other measurements made by the printed electrode with the membrane just deposited include concentrations of 207.9 ppm for sample #3331 and 1.06 ppm for sample #3703.





DISCUSSION & CONCLUSION

Many measurements made did not fall within the normal calcium concentration range in cow blood, 80 ppm – 100 ppm. The method for acidification of the blood to free bound calcium proved to be simple, but both the standard ion-selective electrode and printed electrodes prepared did not measure ion activity properly at a low pH. The reasons for these anomalies include that the low pH of the acidified blood adversely affects the operation of both the standard ion-selective electrode, as well as, the printed electrodes, and, morel likely, that the low pH might be affecting the cells in the blood themselves and releasing interferences into the blood.[1] The printed electrodes are affected in a similar way because the membrane deposited onto the working electrode is similar in composition to the membrane built into the standard electrode. When the blood was not diluted and acidified, however, both types of electrodes were able to correctly measure ionized calcium between the normal range of 30 ppm – 50 ppm. It can be concluded that the best way to utilize a printed ion-selective electrode is to use it right after the membrane is deposited and to use one electrode per three samples at the most, preferably one electrode per sample. In conclusion, a new method for blood preparation that would unbind calcium from its complexes while not interfering with the electrode operation needs to be developed. Printed electrodes are, in theory, a very convenient way to measure calcium concentrations in cow blood cow-side, but the methods have not yet been perfected to do this correctly and efficiently.

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