CHEMICAL ENGINEERING TRIPOS PART IIB/ENGINEERING TRIPOS PART IIB

MODULE CET IIB / 4G2: BIOSENSORS

COURSEWORK #2

INTRODUCTION: The Lab exercise will introduce the student to one of the most widely distributed biosensor technologies:

- Test-strip biosensors designed for non-expert use requiring no calibration.
- Designing the assay to fit the purpose for which it was intended.
- Ease of use.

One of the principal commercially successful biosensor systems is the blood glucose biosensor and monitor, used by diabetics for the monitoring of blood sugar levels. They offer a convenient and relatively inexpensive method for personal monitoring of blood sugar and are widely available from many sources, in numerous formats.

Glucose is also a core substrate in many fermentation processes: e.g., Brewing, production of biochemicals (pharmaceuticals), etc.

Use of the glucose biosensors will allow students to become familiar with the design of electrochemical biosensors, and how they have been developed for specific applications.

The principle involved in designing these test strips is covered in the lectures. In this assignment we will look at the one-use self-test strips, not the continuous use systems that work for 10-14 days. However, the principle is the same.

Commercially available blood-glucose test strips will be used following manufacturer's instructions. Glucose concentration will be measured in several test solutions that simulate blood samples (note blood should not be used for safety reasons). The data will be collected for analysis and information on accuracy and precision obtained and considered in terms of clinical integrity.

MATERIALS AND METHODS Glucose meters and test-strips are provided together with samples labelled A-D and A*-F* (see identity of samples below). Working as a group use the strips and discuss the results.

Concentration of sugar		Low haematocrit	High haematocrit
mg/dL glucose	mM		
70	3.9	A	A*
90	5.0	B	B*
110	6.1	C	C*
130	7.2	D	D*
1000 (sucrose)	29.2		F*
90 + lemon juice (contains ascorbic acid/ vitamin C)	5.0 + lemon juice		G*

1. You will be provided with test strips from readily available glucose monitors: Accu-Chek, True Metrix and eBWell. These different test strips are based on the same principle but each uses a different enzyme. Have a look at the comparison chart that has been uploaded to Moodle for some of the regularly used devices and familiarise yourself with differences between the manufacturers. Think about how the systems given compare with this table. What are the differences?

2. Each session will test around 40 test strips. These are a mixture of the different manufacturers (see appendix A for the allocation of test strips and the manufacturers). Use the test strips according to the instructions (but substituting blood for the same volume of synthetic plasma sample) to obtain glucose measurements from the samples above. The sampling process will be demonstrated to you within the laboratory.

3. **For solutions A-D and A*-D*, your group must plan a testing regime:**
   - **First, decide on the best combination of tests using just the test-strips available and the given solutions.** (Remember that the data from your group will be uploaded and will be combined with that from other groups. The collective data from the entire class should produce ~270 data points across the 3 manufacturers, so that good choice of the measurements can produce a reasonable set of data to compare. Think about which samples to measure to provide the most useful data, dependent on what the other groups have already done before you). Assume that you are a producing a single data-set from the cohort overall, but eliminate outliers from the data-set if you have an appropriate reason.
   - **When all the data is collected, it is important that you will be able to compare measurements obtained:**
     - With the actual value
     - Repeats of the same concentration
     - Differences between the different makes of instrument/test strip

   So, make sure you use the test-strips wisely and record which tests you did and how many repeats. For example, if you have only a small number of strips of one type, is it better to check the same concentration multiple times or get single results from different concentrations? Remember that you want to obtain data so that you can do some statistically significant analysis. This is the dilemma in every validation study. The way in which the study is undertaken will influence the outcome and thus could influence the legislative approval of the product. Take a look at the tests done by earlier groups, since you may be able to fill in gaps or eliminate some concentrations.

4. For solutions F* and G*, make comparison with C* and discuss the origin of any differences or lack of differences you observe.

During testing consider the functional design of the different instruments and test strips and debate points that you think might be critical in their manufacture. Discuss the design and performance in terms of:

- accuracy
- precision
- clarity of operating instructions,
- ease of use, ease of performing the assay; consider also how this may be easier/more difficult with a 'real sample'.
- display of the result.
- extent to which the design has been developed to be fit for purpose.
- anticipated user acceptance if the User is a diabetic.

After completing the tests, devise a marking system for each of the categories above, with weighting as you see most appropriate, e.g., say mark out of 5 for each category, but if user acceptance is most important category, give this a weighting of 8, resulting in total mark for this category of 40. As a group agree category marks for each system tested and an overall mark.

Collect all your data and give it to your lab Demonstrator, who will put it onto Moodle labelled according to the lab date and session number. Some example comparison plots are shown in the Appendix. This is exemplar data only. Your data may look quite different. When all the data for all the labs is collected, you will analyse it as part of your write up.

REPORT: Individual final reports must be submitted by **Friday 28th March 2025 at 16.00hrs**. All reports must be uploaded to Moodle. The markers will not be able to identify the author. Your report should address all questions posed in this handout clearly justifying your arguments including full references to any additional resources used. Your coursework reports should include a word count and you should not exceed a word count of 3,500 words plus 10 figures/tables overall.

Some indicative guidelines are given below for each section. You may adjust the length of each section

as you wish but the overall length must not exceed the maximum length.

THERE ARE 2 PARTS, A AND B. **YOU NEED TO DO BOTH PARTS**

TOTAL FOR BOTH PARTS: 3,500 WORDS 10 FIGURES/TABLES, INCLUDING APPENDICES

PART A (RECOMMEND NOT TO USE MORE THAN 2,500 WORDS IN THIS SECTION, <10 FIGURES/TABLES):

THE GLUCOSE BIOSENSOR

Present the findings from your lab study under the following headings:

i) **Summary/Abstract**: (~ 100words)
ii) **Introduction**: (<1 page) Introduce the glucose biosensor, how it works, where it is used, any inherent shortcomings and solutions that have been found in the development of the glucose biosensor. Present this as a build up to the lab study undertaken.
iii) **Materials and Methods**: (< ½ page) Describe the methodology and approach used in the lab class.
iv) **Results and Analysis** (key relevant data/plots to be included)
v) **Discussion**

For (iv) and (v):

- **Quantitative evaluation of glucose test-strip output:** Analyse the data you have obtained together with the data collected by the other groups, using statistical methods that you think are appropriate. Use all the data from the entire class (stored on Moodle after the end of the lab), making outlier exclusions only if a justification can be given.

Look at all the data collected and consider:

a. Accuracy of the result.
b. Precision of the result
c. Any curious/unexpected results
d. Compare your conclusions with what you anticipated and deviations, as illustrated from the data present in the appendix, discussing the type of errors you see (if any!) and how they might arise from manufacture and use.

- Discuss what you find and its importance for a diabetic trying to monitor their glucose. Consider the impact of any errors you have found on the clinical care pathway. Decide whether the biosensor is fit for the purpose for which it is manufactured, giving reasons for your conclusions.

- **Qualitative evaluation of glucose biosensor design:** Discuss the design of the biosensors you have used in the context of their ease of use and fitness for purpose. Revisit the marking system that you agreed as a group in (4) above and present the rationalisation for the category weighting. Discuss whether you still support this weighting and the overall performance outcome.

vi) **Conclusions:** (~½ page) Draw a final conclusion, which also considers whether the test-strip technology you have tested is fit for the purpose of glucose testing in a diabetic person, highlighting strengths and weaknesses that you have encountered in their use and explaining the issues that have to be overcome for a reliable glucose test strip for diabetics and suggesting ideas for improvements to the glucose test-strip.

PART B (RECOMMEND <2,000 WORDS, 8 FIGURES/TABLES): THE LACTATE BIOSENSOR

Imagine you are employed by a small company producing glucose test strips; you have been given the task of looking at the market for lactic acid in **(i) blood and (ii) sweat.** The company are investigating 2 different markets:

a) for use in assessing athletic performance. Lactate levels are related to the status of anaerobic metabolism during muscle stimulation.
b) To determine lactate levels in patients in shock or suspected to be in at risk of sepsis or other serious conditions such as meningitis.

You are asked to form an opinion about whether these are viable markets for a lactate sensor, which is based on a direct modification of the company's current glucose test strip. A key factor in the success of this new product range will depend on how far the technology from your glucose test strip can be transferred with minimum manufacturing changes. The assessment requested of you is focussed on the technology match and its limitations (if any)

Think about the glucose biosensors that you used for Part 1 and which parts and components might be able to be used for a lactate sensor and which ones need substitution or change.

- Describe how you would achieve lactate measurement and what range you could measure.
- Include within your answer:

  (i) the mechanism for the biosensor response and what bio-reagents are needed;

  (ii) the range on concentration that those reagents can be used in (remember the shape of the concentration curve)

  (iii) the accuracy **required** versus that expected in the measurement you propose;

  (iv) whether the system you propose be suitable for measurement in blood, CSF and/or in sweat

  (v) key similarities and differences with the glucose biosensor test-strip and

  (vi) the likely advantages and disadvantages of analyses using your biosensor(s).

  (vii) are there any other sample types other than blood or sweat which could be considered for sampling?

You will need to research the required lactate levels in **sweat and blood and CSF** and your answer should include some discussion of the particular challenges you face in achieving an appropriate level of detection that would make your sensor (i) technologically feasible (ii) practically useful.

Give a summary/conclusion <½ page.

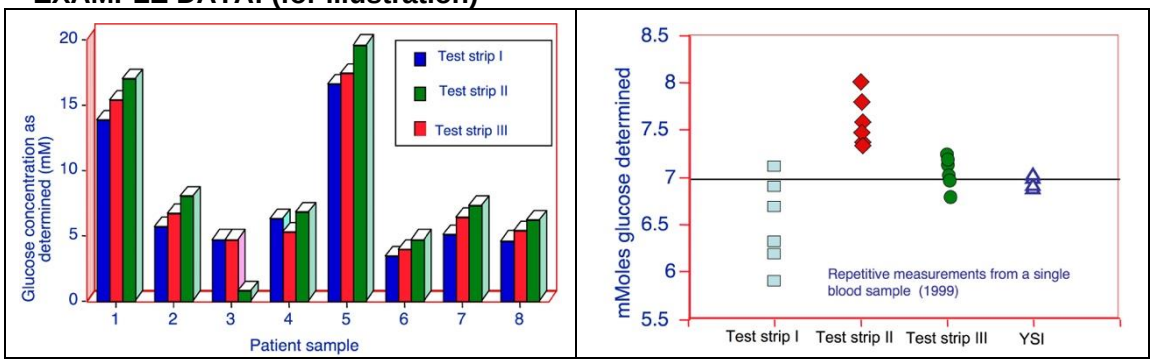
To set you off, there are some links on Moodle to brief you on clinical measurement of lactate and historical literature searches which will provide additional information if you require it.

APPENDIX A; Please sign up to one of the seven sessions

	Date/time		Ebwell	AccuChek	TrueMetrix	TOTALS
1	Mon 10th March	GROUP A	**7**	**8**	**7**	**22**
2	13.30-15.30	GROUP B	**8**	**7**	**8**	**23**
3	Mon 10th March	GROUP A	**7**	**8**	**7**	**22**
4	15.30 – 17.30	GROUP B	**8**	**7**	**8**	**23**
5	Wed 12th March	GROUP A	**7**	**8**	**7**	**22**
6	13.30 – 15.30	GROUP B	**8**	**7**	**8**	**23**
7	Wed 12th March	GROUP A	**7**	**8**	**7**	**22**
8	15.30 – 17.30	GROUP B	**8**	**7**	**8**	**23**
9	Thus 13th March	GROUP A	**7**	**8**	**7**	**22**
10	13.30 – 15.00	GROUP B	**8**	**7**	**8**	**23**
11	Thus 13th March	GROUP A	**7**	**8**	**7**	**22**
12	15.30 – 17.30	GROUP B	**8**	**7**	**8**	**23**
13	Friday 14th March	GROUP A	**7**	**8**	**7**	**22**
14	13.30 – 15.30	GROUP B	**8**	**7**	**8**	**23**
	TOTALS		**105**	**105**	**105**	
	TOTAL:					**315**

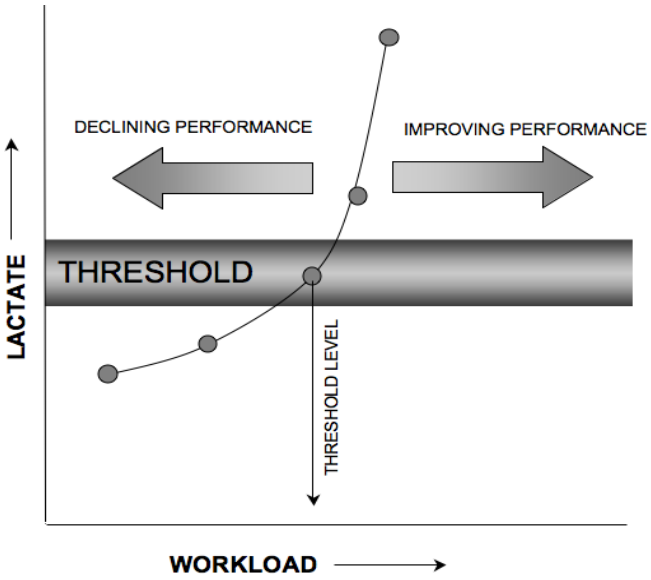
APPENDIX B:

EXAMPLE DATA: (for illustration)



APPENDIX C:

Appendix C has some information and background associated with lactate levels and a reminder about the non-linear relationship between the signal generated and the output from the enzyme pathway that you will should think about. This may influence your write up for Part B and your overall discussion.



At rest the blood level of lactate is low (what are the levels expected?) but as the intensity of exercise increases the lactate level rises. The plot above shows the lactate level against the exercise intensity: it rises gradually at first but then becomes very steep as the exercise intensity (workload) increases. Eventually exhaustion occurs at which time the lactate level might be 10 or 20 times the resting level. For most individuals the exercise level at which the curve becomes very steep is constant. It is referred to as the 'lactate threshold' (what concentration is expected for this threshold? How does in **change** in blood and sweat?). If the curve is plotted during various phases of training how will it change?

In clinical critical care, increased blood lactate levels have been related to increased morbidity and mortality. In emergency departments and in intensive care blood lactate levels can assist in triaging and in resuscitation the immediate lactate level appears to have some relation to outcome prognosis. These indications all point to the potential for a significant but diverse market for lactate measurement.

APPENDIX D

Measurement using enzymes as one of the analytical reagents can be complex because the relationship between the concentration of the analyte that you want to measure and a signal may not be linear. For example as we discussed in the lectures:

The rate of an oxidase enzyme catalysed reaction is given by:

$$-\frac{d[S]}{dt} = \frac{k_2[E_o][S]}{K_M + [S]} \qquad 1$$

[S] is the enzyme substrate concentration, E_0 the total enzyme concentration and K_M is the Michaelis Menten constant (K_M is a characteristic of the enzyme and is the substrate concentration at $V_{max}/2$, where V_{max} is the maximum rate). At the simplest level, the current at an enzyme electrode becomes:

$$I_{enz} = \frac{nFdk_2[E_o][S]}{K_M + [S]} \qquad 2$$

Try, for example, deriving an expression for the maximum current response I_{max} and hence give I_{enz} at K_m and $2K_m$ in terms of I_{max}. If you sketch a plot of I_{enz} versus [S] you can see and comment on the useful analytical range. What does this tell you about one enzyme parameter that you need for a suitable measurement range?