2.1 List the 3 types of enzyme immobilization methods with 2 examples each

1). Adsorption: the enzyme is adsorbed to the external surface of the carrier material, which could be mineral (clay) and organic (starch, resin). No permanent bond is formed between the carrier and enzyme.

For example,

(1) lactase could be adsorbed onto phenol-formaldehyde resins to hydrolyze the lactose into glucose and galactose in the milk industry.
https://link.springer.com/chapter/10.1007/3540096868_3#:~:text=Immobilization%20of%20lactase%20for%20continuous,improved%20functional%20and%20nutritional%20properties.

(2) Glucoamylase adsorbed on carbon support including Sibunit and activated carbon was used for hydrolysis of starch dextrin.
https://www.sciencedirect.com/science/article/pii/S0008621508000748
https://bnrc.springeropen.com/articles/10.1186/s42269-019-0148-0

2). Entrapment: the enzyme is physically entrapped inside a porous matrix or a membrane which is a water-insoluble polymer. The pore size of the entrapment material is adjusted to prevent the loss of enzyme and the bonds stabilizing the enzyme to the matrix could be covalent or non-covalent.

(1) Lipase could be entrapped in chitosan to digest triglycerides into fatty acids and glycerol.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3563746/#:~:text=Carrageenan%2C%20a%20linear%20sulfated%20polysaccharide,2007).

(2) Zymase inside the yeast could be trapped using sodium alginate with gelatin to catalyze glucose into ethanol.
https://ijpsr.com/bft-article/immobilization-and-estimation-of-activity-of-yeast-cells-by-entrapment-technique-using-different-matrices/?view=fulltext

3). Covalent bonding: This method involves the formation of covalent bonds between the chemical groups in the enzyme and the chemical groups on the carrier. Hydroxyl groups and amino groups could form covalent bonds more easily.

For example, (1) carbohydrates like cellulose (2) protein carriers like collagen could be used to form covalent bonds between the enzyme and carrier.

(1) glucoamylase could be covalently immobilized onto chemically activated surface of κ-carrageenan for hydrolysis of starch.
https://bnrc.springeropen.com/articles/10.1186/s42269-019-0148-0

(2) Lipase was covalently immobilized on tresyl activated silica to digest triglycerides into fatty acids and glycerol.
https://pubmed.ncbi.nlm.nih.gov/18588186/

2.2 List the physical methods of enzyme entrapment with an example each

1) Lattice/Gel entrapment: The enzymes are trapped in the cross-linked cells of the porous gel formed by water-insoluable polymer. In industry, polyacrylamide is used to trap E. coli to produce L-aspartic acid.
https://reader.elsevier.com/reader/sd/pii/B9780857094155500019?token=E1C504AEE35A9718B7D443776B85651B4BFE0A33E982D43A42577E66DD4BF6B1077927F271DEE29B7CFD8C19F480A655

2) Microcapsule-type entrapment: The enzymes are enclosed by a semipermeable polymer membrane. Enzymes are accumulated inside the membrane and the biochemical products and metabolic wastes are easily released outside the membrane. The key step is the prepare microcapsules could be performed with interfacial polycondensation, liquid drying and phase separation. In interfacial polycondensation, hydrophilic monomers with enzymes are emulsified in water, another hydrophobic monomer is dissolved in a water-immiscible organic solvent. Polycondensation of the monomers then occurs at the interface between the aqueous and organic solvent phases in the emulsion to form a thin membrane of polymer, where enzymes are enclosed. In industry, the microcapsules formed by polyamine and calcium alginate are made via interfacial polycondensation.
https://www.sciencedirect.com/science/article/pii/B9780124104167000112

2.3 What is the purpose of an inhibitor in an enzyme example - give an example

The enzyme inhibitor is a kind of chemical compound that modifies the catalytic properties of the enzyme, in order to slow down the reaction rate or even stop it.
There are 3 kinds of inhibitors: competitive, noncompetitive and uncompetitive. 1) Competitive inhibitor is similar to the substrate of the enzyme which could compete with the real substrate for the catalytic sites of the enzyme, thus slowing down the reaction. For example, malonic acid is the competitive inhibitor of succinic acid for succinate dehydrogenase. 2) Noncompetitive inhibitor binds to the enzyme at sites distinct from the substrate binding site, altering the enzyme configuration and impede the access of the substrate to the active site. For example, Trisodium phosphonoformate (PFA) acts as a non-competitive inhibitor of the pyrophosphate-binding site on the DNA polymerases of hepatitis B and reverse transcriptase of HIV. 3) Uncompetitive inhibitor binds to the enzyme-substrate complex at high concentrations of the substrate, but does not bind at very low concentrations. For example, glyphosate is an uncompetitive inhibitor versus EPSP synthase.
https://www.sciencedirect.com/science/article/pii/B9780128035504000082

2.4 State the effects of cross-link of a substrate on the degree of enzyme-substrate interaction.

Cross-linking is an irreversible method of enzyme immobilization without the need for the support matrix. The enzymes are covalently cross-linked between each other. The reagents like glutaraldehyde are used for creating bridges with the enzymes and form cross linked enzyme aggregate (CLEA). The CLEAs are highly active, easy to handle, and recyclable. However, when catalyzing macromolecular substrates, the cross-linked enzyme is inefficient, because the access of the substrates into the enzyme active site might be occluded by these covalent bridges formed.
https://www.sciencedirect.com/science/article/pii/S0958166999800603

2.5 What are DNA origami's -state the principle and detection mechanism

DNA origami is the nanoscale folding of DNA to create arbitrary two and three-dimensional shapes at the nanoscale. It relies on folding a long ssDNA called scaffold with hundreds of designed short ssDNAs called staples. Each staple has multiple binding domains that bind and bring together otherwise distant regions of the scaffold via crossover base pairing, folding the scaffold in a manner analogous to knitting. The geometries of the resulting structures can be programmed with the staple sequences. The DNA origami technique exhibits higher yield, robustness and the ability to build complex non-periodic shapes, which has various applications on nanofabrication, nanophotonics and nanoelectronics, catalysis, computation, modelcular machines, drug delivery, bioimaging and biophysics.

The principle of DNA origami for detection is due to its attractive optical and electronic properties arising from the DNA origami-templated nanostructures. It has high structural programmability at the nanometre level, allowing tailorable optical or electronic properties, including tuneable conductivity, plasmon coupling, Fano resonances and plasmonic chirality.

3.0 select a classical paper on one of the two topics: implantable biosensor or a wearable biosensor.

Clinical evaluation of the GlucoWatch® biographer: a continual, non-invasive glucose monitor for patients with diabetes

https://www.sciencedirect.com/science/article/pii/S0956566301001890

- **List the key components of a biosensor**

The GlucoWatch biographer is a non-invasive, frequent, and automatic glucose measurement wrist-watch device for diabetes patients. It contains sampling and detection means, electronic circuitry, and a digital display. The key components include: 1) a microprocessor to control the operation of the device and convert the sensor signals into glucose reading; 2) electronic circuitry with two independent potentiostat circuits to operate amperometric biosensors and a galvanostat for iontophoresis function; 3) temperature and skin conductivity sensors for detecting temperature fluctuation and perspiration, respectively; 4) AutoSensor that fits into the skin-side of the biographer and comprises two identical sets of biosensors and iontophoresis electrodes and two hydrogel disks. The biosensor working electrodes consist of a screen-printed layer of Pt/C composite ink and the reference and counter electrodes are made of screen printed Ag and Ag/AgCl layers. The biosensor electrodes also function as the iontophoresis electrode. The hydrogel discs serve as the biosensor electrolyte as well as the reservoirs collecting glucose. The glucose oxidase enzyme (GOx) is dissolved into these discs. 5) data storage memory, a liquid-crystal display, and a serial port for communication with a PC.

- **Discuss the basic principle of sensing - measurand**

First, how to get glucose inside the body across the skin? Reverse iontophoresis utilizes electrical current to extract charged substances outward through the skin. The iontophoretic current occurs with another electro-osmotic flow. Neutral molecules, such as glucose, could be extracted through the skin via the electro-osmotic flow to the iontophoresis electrode along with Na+. The amount of glucose extracted during the one 3-min iontophoresis cycle is about 50-500 picomol.

Second, how to detect the small amount of glucose? The biographer utilizes direct detection of H_2O_2 generated by glucose oxidase-catalyzed reaction on the electrocatalytic surface of the Pt-graphite working electrode:

glucose + O_2 → gluconic acid+H_2O_2 catalyzed by GOx.

- **Discuss the signal processing module, filters, amplifiers etc**

The skin barrier effectively filters out compounds of molecular weight much greater than 500 daltons. To improve sensitivity, measuring glucose only at the iontophoretic cathode while interfering species such as uric and ascorbic acid migrate to the anode. Also, operating biosensor at a relatively low potential for H_2O_2 detection to improve

selectivity towards glucose over other higher potential species such as tyrosine and tryptophan.

- **Discuss the sample processing steps**

1. An iontophoresis current of 0.3mA is delivered for 3 min to collect glucose at the iontophoretic cathode. The concentration of H2O2 produced by the glucose/GOx reaction increases in the hydrogel.
2. Biosensors are biased at 0.42V versus Ag/AgCl and the biosensor current at the iontophoretic cathode is integrated for 7 min during which the concentration of H2O2 in the gel is depleted to near zero. This scheme would increase sensitivity by pre-concentrating the analyte and reduce noise by collecting glucose over 3 min.
3. The polarity of the iontophoresis current is reversed and the process is repeated. The integrated currents of the two biosensors are summed and input to a signal processing signal.
4. A one-point calibration after 3h elapsed time is provided by a single finger-stick blood glucose measurement, to account for the variability in the skin permeability to glucose as well as biosensor sensitivity.
5. The calibration factor is used by the signal processing algorithm to provide a glucose measurement every 20 min for 12h. Since the biosensor is worn on the wrist subjecting to environmental conditions, the raw biosensor data is compared against criteria determined a priori to ensure data integrity before calculating glucose. If low or rapidly changing temperature, excess perspiration and excess noise are detected, the glucose reading is skipped to ensure accuracy.

- **Discuss sensor characteristics - sensitivity, specificity, dynamic range, noise, etc**

The correlation coefficient between biographer readings and the fingerstick show 0.85 and 0.8 in the clinical environment and home environment studies, validating the efficiency of the biographer. Analysis of mean difference, standard deviation, mean absolute relative difference, and root mean square error is performed by comparing the biographer readings to the value predicted by the Deming regression. The result also shows the acceptable amount of noise. The readings are found to be indifferent to demographic factors like ethnicity, gender, type of diabetes, etc.
The biographer accuracy varies at different blood glucose concentrations: hypo-glycemia (<5.56mmol/L), euglycemia (5.56-10 mmol/L), hyperglycemia (10-13.33 mmol/L) and extreme hyperglycemia (> 3.33 mmol/L). A slight positive bias at low glucose levels and a slight negative bias at high glucose levels are observed. In addition, when rapidly decreasing blood glucose happens, more than -6.67 mmol/Lh, the biographer reading exceeds 23% of the mean relative difference, indicating larger bias.

- **Your overall assessment**

The iontophoresis effect enables the non-invasive extraction of interstitial fluid extracted through the skin that contains the glucose for detection. In principle, it's the critical characteristic of this system and reduces the risk of frequent measuring. The measuring mechanism relies on the product of the oxidation reaction of glucose. By taking the glucose outside the body, the oxidation deficiency problem could be eliminated. Also, the utilization of skin to remove interfering species inside the blood could improve measurement accuracy naturally. However, there are a few problems for this system. First, the timing and frequency to extract the body fluid is critical to reflect the real situation, since it's not a continuous monitoring system. Second, the wearable device meets big noise from the surrounding environment that may affect the iontophoresis efficiency, thus causing the calculating results to fluctuate. Also, the GOx enzyme in the hydrogel might suffer denaturation that reduces the reaction activity to further affect measurement accuracy. Actually, the product has been withdrawn from the market due to its skin irritation problem, indicating the safety is the key factor to be considered when designing implantable or wearable biosensors.

Bonus Homework:

Comment on a current device - Dexcom

- What's the working principle

The sensor of the Dexcom G6 system measures the glucose concentration in the interstitial fluid via enzymatic electrochemical reaction using glucose oxidase. It's an amperometric sensor that measures the current generated by the production of hydrogen peroxide, which is catalyzed by the enzyme to oxidize the glucose. The magnitude of the electrical current is proportionate to the interstitial glucose concentration.

- What's the sensor element made of

The sensor consists of the sensor applicator, transmitter holder, and sensor probe. The applicator is a single use, disposable unit that contains an introducer needle holding the sensor probe. The sensor probe has layers of materials. The outermost layer is a semi-permeable membrane that filters the glucose and protects the sensor. Then it's coated with enzyme glucose oxidase that catalyzes the reaction. The innermost layer is the metal electrode to send the current signal to the transmitter, which could be tiny wire made of gold.

- Where is the sensor placed

The sensor is inserted under the skin where people usually wear it in the abdomen. For children ages 2-17 years old, it could be worn in both the abdomen and buttock.

- Dynamic range of measurement

The glucose measurement could be displayed between 40 mg/dL to 400 mg/dL. If exceeding the limits, the results would be displayed as Low and High.

- Cost

The sensor probe is disposable every 10 days and the transmitter lasts for 3 months. The transmitter costs $237 and a box of 3 sensors is $349. The annual cost is around $4500.

- Factors that affect sensor measurement

High concentration of acetaminophen, hydroxyurea, wearing days, calibration, and insertion location that depends on the quality of the interstitial fluid.

Ref: https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170088.pdf