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Biomedical Science and Technology

Elixir Biomedical Sci. & Engg. 74 (2014) 27263-27265

ISSN: 2229-712X

Fluorescence based non invasive approach of blood glucose measurement system

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ARTICLE INFO

Article history:
Received: 21 April 2013;
Received in revised form:
15 September 2014;
Accepted: 27 September 2014;

Keywords
Non-invasive,
Blood glucose,
Fluorescence,
Trytophan,

ABSTRACT
This paper gives a non-invasive approach for the measurement of blood glucose concentration in the blood, with the use of fluorescence radiation. By directing the fluorescence radiation at the target trytophan, the reflected radiation from tryptophan is detected at the detector and converted into a measurable signal. This value will correlate with the blood glucose concentration.

Introduction

Diabetes is a chronic life threatening disease for which, at present there is no cure. It is the fourth, leading cause of death by disease in the United States and at least 90 million people worldwide, are estimated to be diabetic. Diabetes is a disease in which the body does not properly produce or respond to insulin. The high glucose levels that can result from this affliction can cause severe damage to vital organs, such as the heart, eyes and the kidney.

Type 1 diabetes, which is otherwise known as insulin-dependent Diabetes Mellitus, is the most severe form of the disease comprising approximately 10% of the diabetes cases. Type 1 diabetes destroys the vast majority of the insulin-producing beta cells in the pancreas, forcing the person to take multiple insulin injections, everyday. Type 2 diabetes, which is insulin independent diabetes mellitus, comprises of the other 90% of the diabetes cases. Type 2 is usually less severe than type 1, as some endogenous insulin production still occurs and, as a result, it is often manageable with dietary modifications and physical exercise, but still requires treatment with insulin or other medications. As the management of glucose to near normal levels can prevent the onset and the progression of complications of diabetes, persons afflicted with either form of the disease are instructed to monitor their blood glucose level in order to assure that the appropriate level is achieved and maintained.

Many attempts have been made to develop painless, non-invasive external device to monitor glucose levels. Various approaches have included electrochemical and spectroscopic technologies, such as near-infrared spectroscopy and Raman spectroscopy. Despite all these extensive efforts, none of these methods yielded a non-invasive method for the invivo measurement of glucose that is accurate, reliable, convenient and cost-effective for routine use.

Traditional methods of monitoring the blood glucose levels of an individual require that blood has to be withdrawn from the ndividual. This method is painful, convenient costly and poses the risk of infection. Another glucose measuring method involves urine analysis, which, aside from being inconvenient, may not reflect the current state of the patient's blood glucose because appears in the urine only after a significant period of elevated levels of blood glucose. An additional inconvenience of these traditional methods is that they require testing supplies as collection receptacles, syringes, glucose measuring devices and test kits. Although disposable supplies have been developed, they are costly and can require specific methods for their disposal.

Basic concept of fluorescence:

Fluorescence spectroscopy aka fluorometry or spectro-fluorometry, is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy, typically, but not necessarily, visible light. A complementary technique is absorption spectroscopy. Devices that measure fluorescence are called fluorometers or fluorimeters.

Molecules have various states referred to as energy levels. Fluorescence spectroscopy is primarily concerned with electronic and vibrational states. Generally, the species being examined has a ground electronic state (a low energy state) of interest, and an excited electronic state of higher energy. Within each of these electronic states are various vibrational states.

In fluorescence spectroscopy, the species is first excited, by absorbing a photon, from its ground electronic state to one of the various vibrational states in the excited electronic state. Collisions with other molecules cause the excited molecule to lose vibrational energy until it reaches the lowest vibrational state of the excited electronic state.

The molecule then drops down to one of the various vibrational levels of the ground electronic state again, emitting a photon in the process. As molecules may drop down into any of several vibrational levels in the ground state, the emitted photons will have different energies, and thus frequencies.

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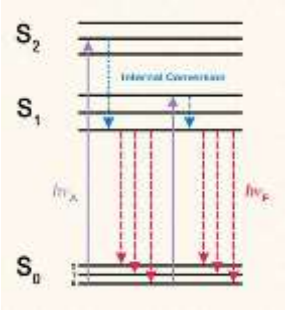

Figure 1.1: A generalized: Jablonski Diagram. Photons are absorbed and re-emitted with different frequencies (wavelengths).

	Absorption		Fluorescence	
	Wavelength	Absorptivity	Wavelength	Quantum
Tryptophan	280	5600	348	0.20
Tyrosine	274	1400	303	0.14
Phenylalanine	257	200	282	0.04

Table 1.1: Fluorescence characteristics of three aromatic amino acids- tryptophan, tyrosine and phenylalanine

Therefore, by analyzing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, the structure of the different vibrational levels can be determined.

In a typical experiment, the different wavelengths of fluorescent light emitted by a sample are measured using a monochromator, holding the excitation light at a constant wavelength. This is called an emission spectrum. An excitation spectrum is the opposite, whereby the emission light is held at a constant wavelength, and the excitation light is scanned through many different wavelengths (via a monochromator).

The fluorescence of a folded protein is a mixture of the fluorescence from individual aromatic residues. Protein fluorescence is generally excited at 280 nm or at longer wavelengths, usually at 295 nm. Most of the emissions are due to excitation of tryptophan residues, with a few emissions due to tyrosine and phenylalanine.

Tryptophan has much stronger fluorescence and higher quantum yield than the other two aromatic amino acids. The intensity, quantum yield, and wavelength of maximum fluorescence emission of tryptophan is very solvent dependent. The fluorescence spectrum shifts to shorter wavelength and the intensity of the fluorescence increases as the polarity of the solvent surrounding the tryptophan residue decreases. Tryptophan residues which are buried in the hydrophobic core of proteins can have spectra which are shifted by 10 to 20 nm compared to tryptophan on the surface of the protein. Tryptophan fluorescence can be quenched by neighbouring protonated acidic groups such as Asp or Glu. Thus, the aromatic amino acid tryptophan is selected as the target for acquiring the blood glucose concentration.

Objective

The main objective of this project is to design an instrument for performing non-invasive measurement of glucose concentrations in the blood. As the existing measures for blood glucose measurement involves painful techniques such as insertion of syringes and withdrawing blood, this approach is provided as a painless and non-invasive solution for the blood glucose measurement. Future advancements in this technique can be done by implementing this technique in a watch, so that the patient can wear it every time of the day and get a continuous update of his/her blood glucose.

Methodology

The instrument design features a non-invasive glucose monitoring instrument useful in-vivo. The instrument comprises of a radiation source, capable of directing radiation to a portion of the exterior or interior surface of a patient. In this design a fluorescence lamp or a fluorescence probe is used as the source of radiation. The surface on which the radiation is directed, can be a mucosal area such as gums or other mucosal area such as the eyeballs and surrounding areas such as the eyelids, but more preferably, the skin. The source emits radiation at a wavelength λ_0 = 295 nm, that excites a target within the patient such that the excited target provides a glucose level indication of the patient.

A glucose level indication is a quantitative or relative measurement that correlates with the blood glucose content or concentration of the patient. The instrument further consists of a radiation detector, kept in a position to receive radiation emitted from the excited target, and a processing circuit connected to the radiation detector that converts the emitted radiation to a measurable signal, to obtain the glucose level indication. The target at which the radiation is directed is not glucose itself, but a molecular component of the glucose such as, for example, a component of skin or other tissue, that reflects or is sensitive to glucose concentration, such as Tryptophan or collagen cross-links. The selected target is to be a component or a compound or a molecule that reflects any environmental alterations in the tissue. The radiation re-emitted by the target will be in the range of 270nm to 1100nm, which is discovered to have correlation with the blood glucose level. The target emits a fluorescence signal and a radiation detector is used to detect the emission band of the target. The radiation emitted from the excited target and signal, correlates with the blood glucose of the patient.

The basic idea of this project, relates to a non-invasive method of detecting or assessing the glucose level by exciting a target which in its excited state, is indicative of the glucose level of a patient, detecting the amount of radiation emitted by the target, and determining the glucose level of the patient, from the amount of radiation detection. Preferred targets are Tryptophan or a matrix target, which are excited by fluorescence rays, and act as bio-amplifiers or bio-reporters.

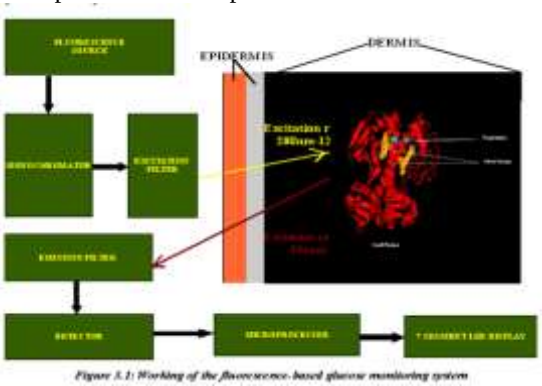

Figure 1.2: Working of the fluorescence-based glucose monitoring system

The radiation source used here is a fluorescence radiation source. The fluorescence from the source is directed to the physiological system (skin). Only a particular wavelength of fluorescence radiation will be able to excite the glucose molecules in the target selected, to give a value that correlates with the blood glucose level. Therefore, before reaching the skin surface, the radiation is passed through a wavelength selector

(monochromator). The monochromator selects a wavelength range 285nm – 1200nm and this wavelength is allowed to fall on the skin. The fluorescence radiation that is directed to the skin, excites the glucose containing components of the blood.

Tryptophan is an important intrinsic fluorescent probe (amino acid), which can be used to estimate the nature of micro environment of the tryptophan. Therefore, the target selected here is Tryptophan (aromatic amino acid), which is a non-glucose target but is indicative of the blood glucose value when it is excited with a specific range of fluorescence. The excited tryptophan target re-emits fluorescence radiation. The wavelength of the re-emitted fluorescence radiation lies in the wavelength range 334nm – 500nm. This re-emitted fluorescence radiation is detected using a radiation detector. The detector is placed at 45^0 with respect to the target, i.e., 90^0 with respect to the incident excitation light. Tryptophan is a relatively rare amino acid; many proteins contain only one or a few tryptophan residues. Therefore, tryptophan fluorescence can be a very sensitive measurement of the conformational state of individual tryptophan residues. The advantage compared to extrinsic probes is that the protein itself is not changed.

As mentioned above, the fluorescence is most often measured at a 90° angle relative to the excitation light. This geometry is used instead of placing the sensor at the line of the excitation light at a 180° angle in order to avoid interference of the transmitted excitation light. No monochromator is perfect and it will transmit some stray light, i.e., light with other wavelengths than the targeted. An ideal monochromator would only transmit light in the specified range and have a high wavelength-independent transmission. When measuring at a 90 angle, only the light scattered by the sample causes stray light. This results in a better signal-to-noise ratio, and lowers the detection limit by approximately a factor 10000, when compared to the 180° geometry.

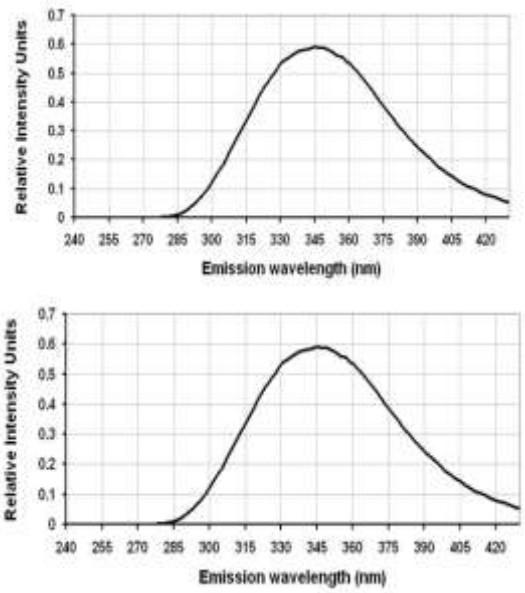

Figure 4.1: The light that is emitted by the amino acid tryptophan when it is illuminated with light that has a wavelength of 230 nm

All wavelengths of light between about 285 NM and more than 420 NM are emitted by the molecule even though the excitation light was at a fixed wavelength of 230 NM This plot is known as the fluorescence emission spectrum for tryptophan. The shape of the emission spectrum generally doesn't depend on the wavelength of the light shined on the fluorophore. If the tryptophan had been illuminated with 240 NM light, the shape would be very similar. At 295 nm, the tryptophan emission spectrum is dominant over the weaker tyrosine and phenylalanine fluorescence.

The output of the fluorescence detector is given to the microprocessor, which converts the emitted radiation into measurable signal. The processor is programmed with a set of template values obtained by passing fluorescence through various blood samples with different glucose concentrations. A PIC 18F series microprocessor is used for this purpose. The value of the signal obtained as the output of the microprocessor, correlates with the blood glucose level of the body.

Type of person	(mg/dl) Fasting value		Post prandial
	Min. value	Max. value	Value 2 hours after consuming glucose
Normal	70	100	< 140
Early diabetes	101	126	140 – 200
Established diabetes	More than 126	-	> 200

Figure 4.1: Table Values For Blood Glucose In The Human Body

Conclusion

Therefore, the fluorescence based non-invasive approach for blood glucose monitoring system, proves to be a painless technique that does not involve any withdrawal of blood from the individual. This procedure reduces patient discomfort, and also eliminates the risk of infection that occurs as a result of invasive methods. As it does not pose any risk of infections, it can be used for mass screening also.

References

1.Intrinsic Fluorescence of Proteins and Peptides
2.Vivian JT, Callis PR (2001). "Mechanisms of tryptophan fluorescence shifts in proteins". Biophys. J. **80** (5): 2093–109. doi:10.1016/S0006-3495(01)76183-8. PMC1301402. PMID11325713.
3.Lakowicz, J. R. (1999). Principles of Fluorescence Spectroscopy. Kluwer Academic / Plenum Publishers
4.Sharma, A. and Schulman, S. G. (1999). Introduction to Fluorescence Spectroscopy. Wiley interscience.
5.Fluorescence & proteins
6.Lakowicz, J. R. (1999). Topics in Fluorescence Spectroscopy, Volume 5. Kluwer Academic / Plenum Publishers
7.Fierabracci V, Novelli M, Ciccarone AM, Masiello P, Benzi L, Navalesi R, Bergamini E, Effects of tryptophan load on amino acid metabolism in type 1 diabetic patients. Istituto di Patologia Generale, Pisa, Italy.