Koc. J. Sci. Eng., 7(2): (2024) 96-108 https://doi.org/10.34088/kojose.1266492

Kocaeli University

Kocaeli Journal of Science and Engineering

http://dergipark.org.tr/kojose

Fluorescence Glucose Biosensors Assays Analysis and Novel Classifications: Frequency Range Specification for Medical Applications

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Article Info

Research paper

Received : *March 16, 2023*
Accepted : *February 21, 2024*

Keywords

Fluorescence Glucose Sensing
FRET
Competitive Binding
Oxidation
Hydrogels

Abstract

The use of luminous glucose sensing as a potential replacement for more traditional forms of glucose measurement has shown encouraging results. Investigation of the efficiency of fluorescence resonance energy transfer (FRET) in glucose sensing is being conducted, with particular focus on the effect of donor-acceptor arrangement. The findings of the experiments indicated that the FRET efficiency was around 50.4% when FITC was used as the acceptor and TRITC was used as the donor. However, the FRET efficiency increased to over 60% when FITC was employed as the donor and TRITC was utilized as the acceptor in the experiment. The significance of the donor-acceptor configuration for efficient energy transfer has been brought to light by the findings presented here. In the process of glucose sensing, the data suggest that FITC should be utilized as the donor, while TRITC should be employed as the acceptor. The engineering medical application of a FITC-TRICTC biosensor requires an excitation wavelength of 544 nm and an absorption wavelength of 516 nm, respectively. In addition to these requirements, you will also need an antenna for transmission that operates at 580 GHz and a wavelength of 551 for the excitation. This article will be an extremely helpful resource for researchers working in the field of fluorescent glucose sensing. The article elucidates the essential concepts of competitive binding and oxidation, both of which are crucial to the process.

1. Introduction

The dictionary defines fluorescence as the emission of longer-wavelength, either visible or invisible, radiation by a material in the presence of incoming radiation light. When a fluorescent molecule or structure is exposed to light, the entering photons ex-cite their electrons to a higher energy level, and the excited electrons then release photons at a different wavelength. This compound is known as fluorophore [1].

Besides electrochemistry and spectroscopy, fluorescence shows potential as a medically applicable method for accurate glucose measurement. This helps with a wide range of medical problems where glucose is involved, including diabetes [2], HIV [3], and cancer tumors [4-6].

In this context, fluorescent glucose sensing techniques become more relevant. Further, these approaches provide useful benefits. The first distinguishing feature of fluorescent glucose-sensing technologies is their extreme sensitivity to trace amounts of glucose, in contrast to more conventional techniques [7]. Plus, the effort required to tune the response to local tissue responses around the sensor is reduced. The concept of fluorescent glucose sensing is based on the idea the after the binding of the fluorescent material with glucose, the molecule does not only absorb light, but it also emits some fluorescent light which make the prediction of glucose amount available in the medium predictable (Figure 1).

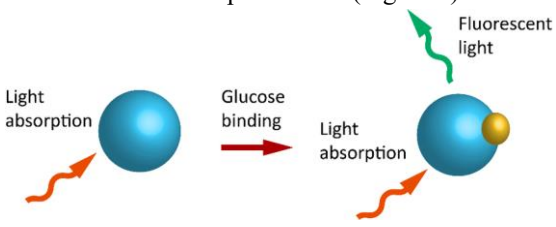

Figure 1. Fluorescence Glucose Sensing Concept [8].

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Fluorescence glucose sensing systems may have occasional faults, as is possible with any technological method. The effects of changes in pH and/or oxygen, for instance, may vary. Long-term use of it might be harmful. Moreover, it requires a special light source to serve as an excitation source [9].

Fluorescence glucose sensing often makes use of the Förster (or fluorescence) resonance energy transfer (FRET) mechanism (Figure 2). Exhausted fluorophores usually chill down by giving off light, dispersing heat, and vibration, or passing on their energy to another fluorophore. In the FRET case study, we focus on the energy transfer itself. Donor fluorophores release photons to transfer their excitation energy to neighboring acceptor fluorophores. An electron is transferred from the donor fluorophore to the acceptor, which then releases a photon of lower energy. In this case, FRET is the evaluation of the shortened fluorescence lifespan, decreased fluorescence intensity, and increased acceptor fluorophore emission caused by the quenching of donor fluorophores.

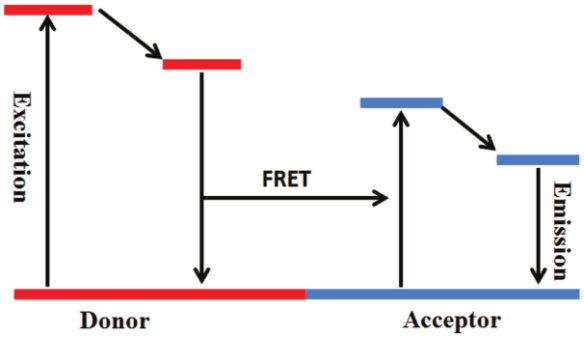

Figure 2. FRET mechanism [10].

Recently, it has become known that using fluorescence for glucose sensing might be a beneficial technique for the accurate research of glucose in many circumstances. Fluorescence-based assays have been the focus of various research projects as an alternative to more conventional methods like electrochemistry and spectroscopy [11-26]. At the core of this technique is fluorescence, the phenomenon where a substance emits light after absorbing it.

One of the most important steps in fluorescence glucose detection is selecting the appropriate donor-acceptor materials for efficient energy transfer. Previous research has examined several fluorophore combinations and their spectrum properties to optimize the FRET process [13-14-26]. For the glucose sensor to work well, suitable materials for the donor and acceptor must be used [11].

Quantum dots have received a lot of attention in fluorescence-based glucose testing because of their potential as sensors and their adaptable fluorescence properties as semiconductor crystals [33]. Because of their fluorescence at acceptable wavelengths or colors, which depend on their size and materials, they are interesting candidates for glucose sensing applications.

It has also been looked at if glucose receptor molecules may be incorporated into carbon nanotubes. Sensitive glucose detection is made possible by attaching glucose to the nanotubes, which causes a shift in fluorescence.

Fluorescence glucose sensors provide several advantages, including continuous glucose monitoring and enhanced sensitivity to low glucose readings. There have been reports of issues such as response to a foreign substance, sensitivity to the pH and oxygen levels in the area, potential toxicity of implanted dyes, and the requirement for specific lighting.

The information presented so far summarizes the potential of fluorescent glucose sensing as a potent technique for measuring glucose levels. Researchers have looked at carbon nanotubes and quantum dots as viable sensor platforms, with an emphasis on enhancing the donor-acceptor materials. These studies seem encouraging, but before fluorescence-based glucose sensors can be employed in clinical settings, biocompatibility and practical implementation concerns need to be addressed.

Over time, several different donor-acceptor fluorophore combinations have been produced for use in glucose sensing. However, methods for specifying the right materials for any application are not reviewed in previous studies.

In this study, a review of different fluorescence glucose biosensors is presented. Biosensor examples are classified based on mechanism of work concepts. The competitive binding concept example is taken into consideration. One of the most famous and successful couples of donors/acceptors is exposed to extract all its fluorescence data to study it is the ability to specify glucose amount for engineering medical applications. Choosing which material will play the role of donor and which one will play the role of acceptor is an issue solved in this study which will help to specify frequency range for further studies.

2. Novel Classification

2.1. Competitive binding

2.1.1. FITC/TRITC con A

Since Con A, glucose, and dextran all bind to their respective targets in a manner that is highly competitive with one another, applications were carried out to assess

physiological glucose concentrations [11]. First, Con A is a carbohydrate-binding lectin that was isolated from jack beans. Jack beans were used as the source. To begin, Con A possesses a wide binding specificity that includes both dextran and glucose as potential targets. There is, however, a challenge associated with the process of agglutination. To create dimeric molecules of the protein, a method was published that involved writing up a process to make "Chemical derivatization of tetrameric concanavalin A (Con A) with succinic anhydride or acetic anhydride converts the protein to a dimeric molecule without altering its carbohydrate-binding specificity" [12]. A glucose assay that is based on Fluorescent Resonance Energy Transfer (FRET) can be created by combining a solution containing fluorescent quenching of Fluorescein Isothiocyanate (FITC) bound dextran with Tetramethylrhodamine Isothiocyanate (TRITC) bound concanavalin A (Con A) as a photon acceptor [13, 14]. This is possible because agglutination is reduced when using this.

To assess glucose levels utilizing FITC/TRITC con A microspheres, a fiber-optic probe and accompanying optical system have been developed by the Diabetes Control and Complications Trial (DCCT) [15]. These developments are based on the aforementioned idea. The findings were positive with regard to both the limitations in flexibility and the long-term effects of dextran.

2.1.2. AlexaFluor family

The resonant transmission of fluorescence energy between fluorescein isothiocyanate and tetramethylrhodamine isothiocyanate was proposed as a new kind of glucose sensor. This sensor would be based on fluorescein isothiocyanate. The research has suggested that the Alexa Fluor family should be used rather than FITC dextran and TRITC Con A. In the past, FITC and TRITC Con A methods relied on the fluorophores AlexaFluor594 and AlexaFluor647 to combat the tendency of the skin to absorb and scatter light. After the set was created using AlexaFluor594 labeled dextran and AlexaFluor647 marked Con A, it was subsequently compressed into a poly (ethylene glycol) hydrogel spherical [16]. By enhancing the signal-to-noise ratio, this method was able to beat the FITC and TRITC Con A approaches in the context of a diabetes monitoring application. Many applications used AlexaFluor family materials [17-20], however it needs more enhancement.

2.1.3. Glucose indicating protein

The existence of protein can be inferred from the concentration of glucose. The Glucose Insight Platform (GIP) is a technological center that monitors glucose levels within cells. When exposed to light of the appropriate wavelength, variants of green fluorescent protein (GFP) and glucose-binding protein combine to generate a molecule that is capable of undergoing fluorescence resonance energy transfer (FRET) [21].

Recent research [22] has suggested using a protein called CoYi, which is pH-independent, as an alternative to GIP and GFP, both of which are sensitive to changes in pH. After a lot of trial and error, they decided that the best use case for their technology would be to measure the glucose levels already present within the cells.

2.1.4. Swelling ratio

A strong connection is formed between boronic acid and glucose. Since boronic acid is present in the polymer solution, glucose works as a cross-linker, which causes the polymer to shrink as a result. Because of this lowering, a glucose test based on the swelling ratio is now feasible. For example, a holographic glucose sensor is constructed out of acrylamide co-polymers that include phenylboronic acid groups (referenced in [23]). They propose utilizing the swelling ratio to account for this, as they predict that lactate causes interference with the original holographic glucose sensor.

Later, as a continuous glucose sensor, the swelling ratio idea was presented over glucose-sensitive hydrogel (which will be examined later) with promising findings [24]. This will be researched more in the future.

2.1.5. S-41

S-41 is a glucose-responsive fluorescent dye that was initially disclosed in 2004 [25] by the Terumo Corporation of Kanagawa, Japan. These dyes are boronic ac-id-based anthracene dyes.

S-41 was tried to be utilized by H. Shibata and his colleagues in a photo-induced electron transfer (PeT) system, which is not too unlike FRET systems. They manufacture their own handmade luminous gel by using S-41 as an ingredient. They were able to do this by establishing glucose response ranges in the glucose density of fluorescent gel beads and fluorescent gel films that ranged from 0-1000 mg/dL [26].

2.2. Oxidation

Glucose oxidase is an essential enzyme that catalyzes a chemical reaction in many different applications. These applications detect glucose levels (1).

$$\text{glucose} + O_2 \xrightarrow{\text{GOX}} \text{gluconolactone} + H_2O_2 \quad (1)$$

In addition to glucose, this equation has a number of other factors, all of which we have discussed and accounted for in order to make it possible for you to use it to calculate your blood sugar levels. A significant amount of research has been conducted with the goal of developing methods for the routine monitoring of diabetes patients' blood glucose levels by their doctors. However, these methods may be applicable to other areas of research, and maybe even to the field of medicine; it is possible that one day we will be able to utilize them to cure a variety of disorders. Several of the most fundamental concepts are discussed in this section.

2.2.1. Oxygen quenching

In a medium containing glucose and GOX, the concentration of oxygen has a direct relation with the concentration of glucose. If the concentration of oxygen decreases means that the reaction of the equation (1) exists and the difference between the initial and the final O_2 concentration express the glucose concentration.

Thus, if we can mark the O_2 variation, we can find glucose concentration. As O_2 has a quenching reaction in the presence of cisDichlorobis (bipyridine) ruthenium (II) $〚\text{Ru}(bpy)_3Cl_2$ (fluorescent indicator), an excitation by compatible wavelength emits a proper fluorescent signal to reflect the glucose concentration. Gels, thin films and microspheres proved that concept using different technologies like lock-in technology [27], electrostatic Layer-by-Layer (LBL) adsorption [28]– [30] and TEOS-derived gel film [31]. Other applications use the PerMX fluidic systems to verify oxygen consumption of the oxidation reaction opening the door for various enzyme-based optical assays as glucose [32].

2.2.2. H_2 O_2 Quenching

Based on the same glucose oxidation and oxygen quenching concepts, another concept came out from the equation (1) which is hydrogen peroxide quenching. The oxidation reaction exports hydrogen peroxide (H_2O_2), thus finding the H_2O_2 concentration ends to find glucose concentration. Hydrogen peroxide had sensitivity to some fluorescent material.

For example, CdTe/CdS Quantum Dots under photoluminescence (PL) are quenched by H_2O_2 . That is why, QDs-based sensor for glucose had the potential to be blood sugar detectors [33].

Similarly, ruthenium complex and europium tetracycline quenched by hydrogen peroxide. Thus, a DLC sputtered slide glass containing FITC, ruthenium complex and europium tetracycline had the role of hydrogen peroxide sensor and glucose sensor [34].

In addition, Kaidi Zhang and team used phosphate-buffered saline (PBS) solution in their research to prove the efficiency a single planar transparent EWOD (electrowetting-on-dielectrics) compact chemiluminescence detector for glucose measurement. HRP in PBS solution play the role of H_2O_2 catalyzer. Thus, the reaction produces oxidized luminol, then produces 3-aminophthalate and emitting fluorescence. Obviously, detecting its strength reflects the glucose concentration [35].

2.2.3. GOX enzyme

GOX or glucose oxidase, which is the main catalyzer of the glucose oxidation re-action, could be an indicator of glucose concentration too. By Immobilization of GOX, N. Thomas and coworkers proved that we could use SU8 within glucose oxidation reaction to find glucose concentration [36].

2.2.4. Oxygen uptakes rate OUR

Oxygen Uptake Rate (OUR) is the rate at which cells consume oxygen. It is important to specify cell metabolic activity. In the presence of alginate pre-cursor solution, described in [37], and measurement chamber done in this work, Binil Starly and Shih Feng Lan assessed the utilization of a fiber-optic ruthenium color-based oxygen sensor to foresee the OUR qualities of HEPG2 liver cells, and they pointed on the ability of using this method to find glucose uptake.

2.2.5. ROS product

Reactive oxygen species (ROS) is a product of the metabolism in cells. There is a relationship between ROS and glucose level. Thus, some applications use ROS level to find glucose level. For example, measurement of the oxidation of H2DCFDA by hydrogen peroxide molecules using real-time fluorescence microscopy to find the intracellular ROS levels, and consecutively, find the glucose level [38].

ROS detection by fluorescent spectrophotometry using DCFH-DA was used with HIT-T15 cells. They proved the effects of LP-1 or LP-2 over glucose-stimulated insulin release from HIT-T15 cells [39].

2.3. Hydrogel

Some research works to prepare glucose-sensitive hydrogels using different materials. Those hydrogels were either on a competitive binding base or on an oxidation base. Pouching it to be more flexible, others create hydrogel fibers. For instance, the glu-cose-responsive fluorescent monomer and PEG-bonded PAAm hydrogels were tested in-vivo on rat animals [40]. In addition, implantable fluorescent hydrogel fibers using fluorescent dye (purchased from TERUMO Corporation) accompanied with a wireless system provide a continuous glucose monitoring system [41]. Moreover, glucose-responsive fluorescent hydrogel was combined with CMOS line sensor to develop an embedded glucose-monitoring mechanism [42]. Again, for a flexible glucose sensor, researchers propose to use parylene-based electrode with glucose-responsive fluorescent hydrogel [41]. Others create a new gel, tetra-PEG gel, and they combined with hydrogel to achieve a continuous glucose monitoring (CGM) system. Tetra-PEG gel is created by polycondensation reactions between NHS glutarate end groups (TNPEG) and amino end groups (TAPEG) of 4-arm PEG [43]. Else, using HepG2-laden hydrogel microfiber, [44] proposed a continuous glucose-monitoring (CGM) system for the monitoring of 3D tissue. Recently, by implanting a glucose receptive hydrogel fiber, researchers developed skeletal muscle tissues which can be a beneficial system for the estimation of glucose absorption [45].

The main advantage of using hydrogel is the long-time sensing ability. That is why, most modern applications of diabetes continuous monitoring were concentrated on hydrogels and hydrogel fibers.

2.4. Other biosensor Methods

Previously, the use of Con A. The research proposed to use apo-glucose oxidase apo-GOx/dextran instead of Con A by enhancing a system on resonance energy transfer (RET) based is discussed. That was to overcome Con A problems like toxicity, aggregation problems and irreversible binding. Results show high precision and sensitive fluorescent biosensors for monitoring glucose [46].

A mixing system between competitive binding and enzyme-base oxidation was demonstrated in [47]. They used Alex-Fluor and GOX in hydrogel form on FRET-base to measure multiple vital data such as glucose.

Smart tattoo biosensor on RET base was developed by a group in Louisiana Tech University [48]. They use the competitive binding concept between the fluorescein isothiocyanate (FITC)-dextran and tetramethylrhodamine isothiocyanate (TRITC)-AG and they capsuled the material into microcapsules suitable for glucose monitoring in diabetic patients.

Wenjun Li and team [49] did another novel in this domain, which is the graphene oxide (GO) low-molecular-weight chitosan (CS) nanomaterial. On the FRET base, GO-CS is used in a simple and rapid biosensor system for monitoring glucose concentrations.

3. FRET Theory

3.1. Concept

The term "fluorescence" refers to a specific type of luminescence that occurs when certain molecules undergo an excitation process that ultimately leads to an electronically excited state as a result of the action of a physical (such as light absorption), mechanical (such as friction), or chemical mechanism [1]. Fluorescence and phosphorescence are the two formal categories of photoluminescence, the process by which a molecule produces light after being activated by ultraviolet or visible light photons, respectively. When a molecule is activated by ultraviolet or visible light photons, a process known as photoluminescence called phosphorescence takes place. Fluorescence is a trait shared by some atoms and molecules, wherein they absorb light at one wavelength and then, after a short period of time (the fluorescence lifetime), emit light at another, longer wavelength. These steps are repeated. In spite of their similarities, phosphorescence and fluorescence have quite different excited-state lifetimes (figure 3).

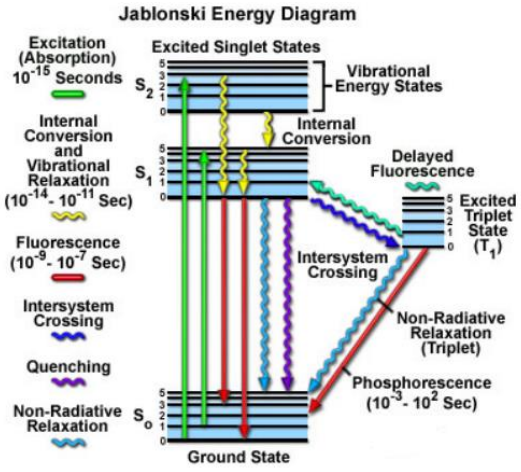

Figure 3. Jablonski Energy Diagram [50]

Three main events control the fluorescence process, each playing a crucial role in the interaction between light and matter (see Figure 4):

- Photon Absorption: In the timescale of femtoseconds (10E-15 seconds), an incoming photon interacts with a sensitive molecule, exciting its electrons to higher energy levels. This process involves the absorption of energy from the photon by the molecule.
- Vibrational Relaxation: After excitation, the excited electrons in the molecule undergo vibrational relaxation, which occurs on the timescale of picoseconds (10E-12 seconds). During this process, the excess energy is dissipated through molecular vibrations, leading to the return of the electrons to their ground state.
- Fluorescence Emission: Within milliseconds (10E-9 seconds) of absorbing a photon, the molecule returns to its ground state by emitting a longer-wavelength photon. This emission is a characteristic feature of fluorescence and allows for the detection and analysis of the molecule.

It is important to note that different molecules can be stimulated by glucose in distinct ways. Among the recognition molecules and fluorophores employed in glucose sensing are enzymes, boronic acid derivatives, and glucose-binding proteins. These chemicals' interactions with glucose change the way in which they glow. By tweaking the excitation and emission properties and changing the chemical system, fluorescence-based glucose sensing techniques may be modified for a variety of applications.

The fluorescence process is a striking example of the dynamic interaction between light and matter, despite its transitory nature. Numerous new fields of research have been made possible by it, including steady-state and time-resolved fluorescence spectroscopy and microscopy, all of which are extensively used in genetics and cell biology. Fluorescence has been widely used as a standard technique because of its high sensitivity, spatial resolution, and specificity. This has tremendously improved the study of biological systems as well as the advancement of our understanding of many processes.

Transition	Process	Rate Constant	Timescale (Seconds)
S(0) => S(1) or S(n)	Absorption (Excitation)	Instantaneous	10^{-15}
S(n) => S(1)	Internal Conversion	k(ic)	10^{-14} to 10^{-10}
S(1) => S(1)	Vibrational Relaxation	k(vr)	10^{-12} to 10^{-10}
S(1) => S(0)	Fluorescence	k(f) or Γ	10^{-9} to 10^{-7}
S(1) => T(1)	Intersystem Crossing	k(pT)	10^{-10} to 10^{-8}
S(1) => S(0)	Non-Radiative Relaxation Quenching	k(nr), k(q)	10^{-7} to 10^{-5}
T(1) => S(0)	Phosphorescence	k(p)	10^{-3} to 100
T(1) => S(0)	Non-Radiative Relaxation Quenching	k(nr), k(qT)	10^{-3} to 100

Figure 4. Timescale Range for Fluorescence Processes [1].

Fluorescence resonance energy transfer is a one-of-a-kind method that uses chromophore pairs made up of donors and acceptors (FRET). FRET is fundamentally inefficient since the donor and acceptor pair must be kept at a distance of less than 10 nanometers for the process to operate. Despite this, FRET has attracted a lot of attention as a promising method for characterizing the nanoscale dynamics of biological molecules' activities.

The acronym FRET is commonly used to refer to Forster (Fluorescence) Resonance Energy Transfer. The Forster energy transfer is a non-radiative process wherein energy (but not an electron) is transferred from an excited donor group to an acceptor group. Forster is honored with the naming of this method. Such a technique relies heavily on spatial separation to enable the study of intricate biological structures. To determine the distance between two specific sites on a large molecule, often a biological macro-molecule, it is necessary to connect the proper donor-acceptor groups to the molecule. If the large molecule's conformation is not altered throughout this process, then the distance between the donor groups and the acceptor groups is straightforward to measure. If the molecule undergoes a drastic conformational shift, then the dynamic activities taking place between two distinct sites on the macromolecule may be measured quantitatively. Protein-protein interactions are one type of this activity. Recently, this method has seen widespread use in many other areas, such as those dealing with single-molecule research, molecular motors, biosensors, and DNA mechanical motions. FRET has so many uses that it is often called the "Spectroscopic Ruler."

The work of Theodore Forster significantly advanced theoretical analysis. It is possible to view a visual representation of this non-radiative transmission technique in Figure 5 which is provided below. When a photon excites a donor group (D), the resulting excited state is the ground state, also known as the lowest singlet state, S1. The Do-nor group might be excited by the electron's kinetic energy if the distance between them is small enough upon the electron's return to the ground state (S0). In both senses of the word, "resonance" refers to this non-radiative phenomenon. If there are no other states that can function as additional quenching states, the excited acceptor will fall back to the ground state by releasing a photon after being excited.

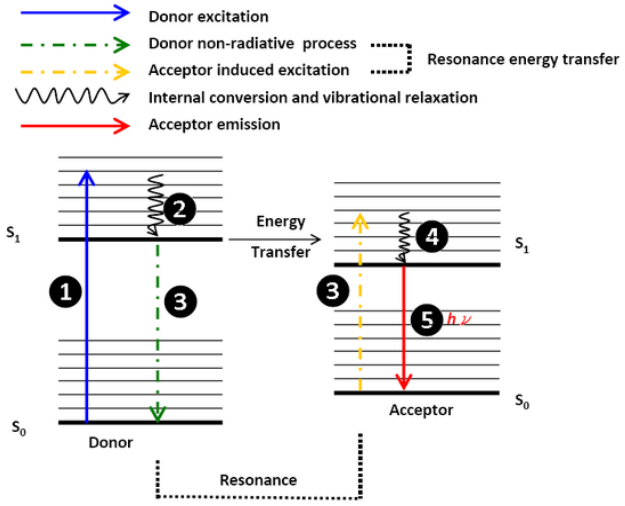

Figure 5. Förster Resonance Energy Transfer: A Schematic Illustration [51].

A crucial part of the process supporting resonance is the ability of one electron to interact resonantly with another electron via the Coulomb force. This suggests that the relative distance of Coulombic contact between the donor and acceptor pair is less important than the overlap of wavefunctions required for the Dexter Energy Transfer, which involves the exchange of electrons. Since electrons are traded during the Dexter Energy Transfer. All that's needed for Coulombic contact to occur is for the spectra to overlap. Since their resonant energies are identical, this is the case. The process of resonance is depicted pictorially in Figure 6. (The energy gap between the ground state and the lowest S1 excited state of the molecule is larger than that between the HOMO and LUMO states).

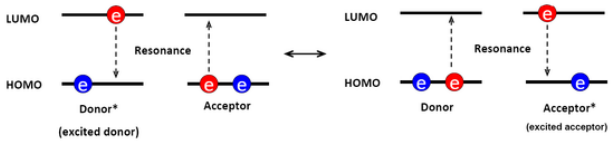

Figure 6: Schematic representation of Coulombic Interactions [51].

3.2. Formulation

The FRET efficiency is measured in terms of the quantum yield of the energy transfer transition, which is defined as the ratio of energy transferred to donor excitation events (E). The FRET efficiency may be calculated using equation 2 if we know the FRET rate, which is denoted by kET, the radiative relaxation rate, which is denoted by kf, and the non-radiative relaxation rates, which is denoted by ki (such as intersystem crossover, internal conversion, and external conversion, among other examples).

$$E = \frac{k_{ET}}{k_f + k_{ET} + \sum ki} \quad (2)$$

The following equation (3), where r is the distance between the donor and acceptor chromophores and Ro is the characteristic distance (the Forster distance or Forster radius) with a 50% transfer efficiency, describes the relationship between the FRET ef-ficiency and the donor-acceptor distance within a point dipole-dipole approximation. Where r is the distance between the donor and acceptor chromophores and Ro is the characteristic distance (the Forster distance or Forster radius).

$$E = \frac{1}{1+(\frac{r}{R0})^6} \quad (3)$$

For FRET to be successful, the absorption and emission characteristics of the donor group are absolutely necessary. To do this, the donor group must have a high extinction coefficient and a substantial quantum yield. Because the emission spectrum of the donor and the absorption spectrum of the acceptor coincide, the energy that is lost by the donor on its way to the ground state can be used to excite the acceptor group. This is because the emission spectrum of the donor coincides with the absorption spectrum of the acceptor. This phenomenon, which is known as resonance, takes place whenever two or more sources of energy are compatible with one another. When this occurs, there is an increase in the amount of energy that may be transferred from a donor to an acceptor. This is due to the fact that the two spectra have overlapped. The donor-acceptor spectrum is seen to overlaps in Figure 7 as a result of the donor-acceptor spectrum overlap integral, denoted by the letter J. Equation 4 describes the overlap integral, and it uses the notation "FD " to represent the normalized emission spectrum of the donor, "A" to represent the standard molar absorption coefficient of the acceptor, and "" to denote the wavelength.

$$J = \int_0^\infty FD(\lambda)\epsilon A(\lambda)\lambda 4 d\lambda \quad (4)$$

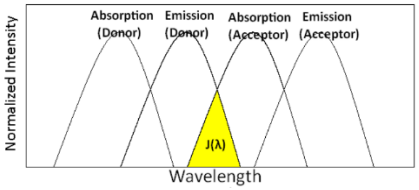

Figure 7. Spectral overlap [51]

In this equation, R is the distance between the donor and acceptor molecules, and 2 is an orientation factor that depends on the spatial orientation of the emission dipole of the donor and the absorption dipole of the acceptor. When the transfer efficiency is 50%, the typical distance between the donor and the acceptor is R 0.

$$R_0 = const\ (J\ k^2\ Q_D\ n^{-4})^{1/6} \quad (5)$$

The quantum efficiency of the donor in the absence of the acceptor is denoted by Q D in this equation. Both the quantum yield of the donor (Q D) and the absorption coefficient of the acceptor (A) need to be sufficiently high (Q D 0.1 and _A 1000 M1cm1) for effective transfer to be observed in the 1- to 10-nm range.

4. Methodology: FRET in Matlab

The proposed method aims to choose the best donor-acceptor pair of materials for a fluorescent glucose sensing system. First, a glucose sensor that is appropriate for the job at hand must be chosen, taking factors like blood diffusion and the surroundings into mind. The excitation and absorption spectra of each material are then calculated using the constants supplied by the manufacturer.

The spectral overlap between the wavelength ranges of the materials is then calculated. It is obvious which material will serve as the donor and which as the acceptor when there is just one spectral overlap. Formula 3 needs to know the wavelength of the peak intensity in order to calculate the FRET efficiency, and from that, Formula 5 is utilized to calculate R0. Acceptance requires a FRET efficiency of at least 50%.

When there are several spectral overlaps, the process is repeated twice, the first time with one material acting as the acceptor and the second as the donor. This allows for the identification of the donor-acceptor pair with the highest FRET efficiency (greater than 50%).

Once the materials have been chosen as the donor or acceptor, it is understood that external stimulation is required for the test. The acceptor material absorbs and reflects the required wavelength while the donor material releases its electrons when stimulated. Engineering applications require an excitation wave that is consistent with the wavelengths of the donor and assay excitation. As a result, data transmission requires an antenna that can transmit the waves produced by the acceptor.

Finally, the systematic technique is used to choose the ideal donor-acceptor pair for glucose sensing through fluorescence. We consider FRET efficiency, spectrum overlap, and the requirement for an appropriate excitation wave and antenna.

Considering competitive binding concepts, especially TRICT/FITC, some calculation was done to get R0. A Matlab tool was used. In order to begin, one must first establish which molecule in TRICT/FITC is the donor and which molecule is the acceptor or absorbent. TRICT is rapidly activated when it is used as a donor cell. The name given to the absorbent dextran is FITC. This analysis relied on information obtained from the database of AAT Bioquest [52], a bioreagents company headquartered in the United States that specializes in assay technologies. This was necessary because the investigators did not have access to a laboratory. The current location of the company's headquarters is 520 Mercury Drive in Sunnyvale, California. The high-performance and durable products offered by AAT Bioquest are beneficial to researchers working in the domains of biochemistry, immunology, cell biology, and molecular biology. Please refer to Figure 8 for the information that they have supplied on TRICT/FITC.

Figure 9 shows a representation of the TRICT emission spectrum, which was computed by making use of the emission spectra of the donor. A web-based tool that exports data from a graph in.png format into an Excel spreadsheet was utilized in the processing and analysis of this figure (Figure 10) [53].

The next thing that needed to be done was to transform the data into a one-dimensional array so that it could be incorporated into the R0 analysis as a variable. Despite this, the outcomes of the search lead to the constants in the table that can be found below Table 1 [54].

The MATLAB code was developed to calculate R0 using formula 5, incorporating data from Table 1 and references [52-54]. The code also includes an intuitive graphical interface, represented by Figure 11.

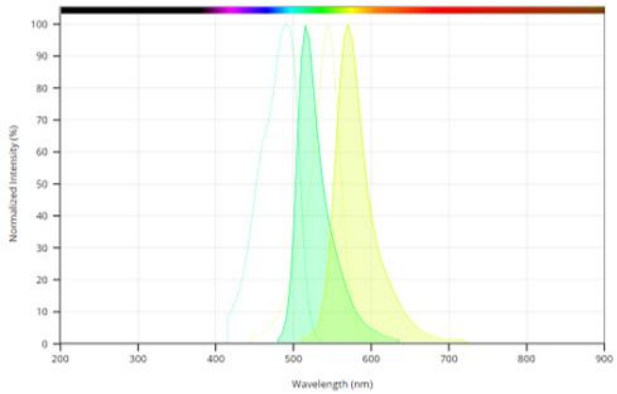

Figure 8. FITC - TRICTC spectrums [52]

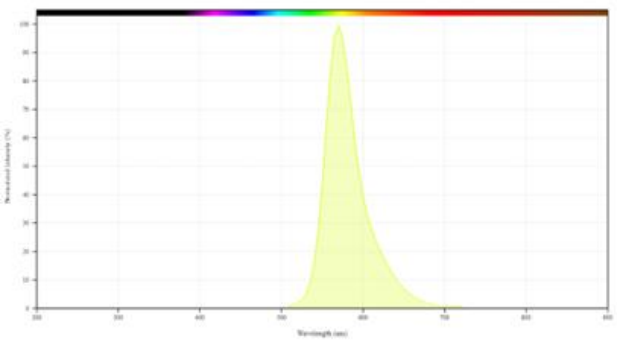

Figure 9. Normalized intensity of TRICT in function of Wavelength [53]

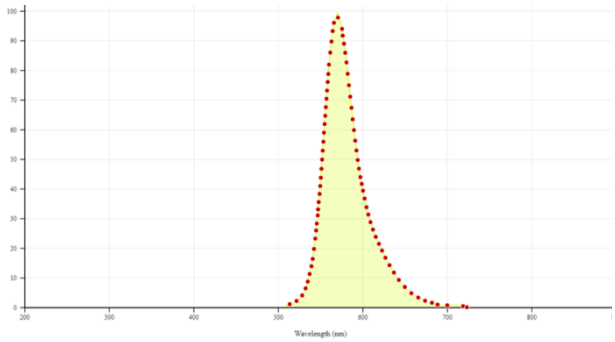

Figure 10. Analysis of TRICTC intensity

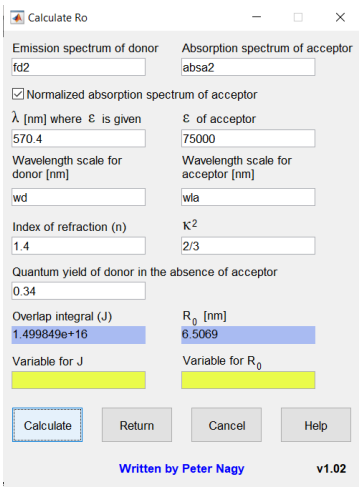

Figure 11. MATLAB interface

5. Results

The implementation of formula 3 in MATLAB serves to draw FRET graphs shown in Figures 12 and 13.

The FRET efficiency of the FRET system is influenced by the arrangement of the donor and acceptor. With TRITC as the donor and FITC as the acceptor, the FRET efficiency is around 50.4%. However, when FITC is the donor and TRITC is the acceptor, the FRET efficiency increases to above 60%. These figures show the amount of energy that is transferred between the donor and acceptor molecules.

According to Figure 12, the TRITC/FITC setup's R0 value is 2.4567 nm. Figure 13 gives us further information about this phenomenon by illustrating how the FRET efficiency fluctuates as a function of the distance (R) between the donor and acceptor chromophores. The estimate accounts for the previously discussed FRET efficiency as well as the distance range of 2–8 nm.

Because the initial outcome was deemed undesirable, a second attempt was conducted, this time using FITC as the donor and TRITC as the acceptor. By adopting this different configuration, FRET's efficiency was considerably increased. Figure 12 shows an increase in FRET efficiency, which is in line with Figure 13's revised R0 value of 6.5069 nm. The R0 number corresponding to the D-A distance at which FRET efficiency is 50% lends credibility to this study.

Figure 13 illustrates how the second arrangement's enhanced biosensing capacity compares to Figure 12. This is due to the fact that as molecules are separated by more space, FRET becomes less effective. Fluorescence-based biosensing produces better findings, particularly when applied to human tissue and cells that are still alive.

The FRET efficiency is roughly 60% better when FITC is used as the donor and TRITC is utilized as the acceptor than it was in the first configuration. This indicates that the donor and acceptor molecules can exchange energy more successfully. The revised R0 value of 6.5069 nm in Figure 11 represents the range of distances where the FRET efficiency is 50%. Figure 13 shows how increasing the distance between the donor and acceptor molecules enhances the FRET mechanism's effectiveness and, consequently, the system's capacity for biosensing.

For technological applications, it is crucial to synchronize the donor (FITC) and assay excitation wavelengths. The wavelengths at which FITC and TRITC are stimulated must thus be matched by an excitation wave. An antenna with the capability of transmitting the waves emitted by the acceptor (TRITC) is also necessary for data tracking in engineering medical applications.

In conclusion, using FITC as the donor and TRITC as the acceptor greatly improves FRET efficiency. This donor-acceptor configuration offers sensitive fluorescent glucose detection in engineering with the appropriate excitation wave and antenna. An antenna for excitation at 544 nm and an antenna for absorption at 516 nm of lambda are required for the usage of a FITC- TRICTC biosensor. That means in engineering medical applications, the system will need an exciter at 551 GHz is needed as well as a transmission antenna of 580 GHz.

Fluorescent glucose sensing techniques are extensively reviewed in this work. Our findings differ from earlier studies in several ways. Our technique considers wavelength ranges, spectrum overlap, and FRET efficiency to find the optimum donor-acceptor combination. Our research focuses on FITC-TRITC FRET efficiency, which is higher than other material combinations. Our fluorescent glucose sensing technique connects theoretical knowledge and practical application, enhancing its applicability and potential advantages.

6. Conclusions

The study's findings demonstrate that a significant impact is made on FRET efficiency in fluorescent glucose sensing by the donor-acceptor combination. The FRET

efficiency was calculated to be at 50.4% when TRITC served as the donor and FITC as the acceptor. The FRET efficiency was more than 60% when FITC was the donor and TRITC was the acceptor, however. These findings offer empirical proof of effective energy transfer between molecules acting as donors and acceptors. The findings of this work justify the use of TRITC as the acceptor and FITC as the donor in experiments using glucose sensing. A FITC-TRICTC biosensor utilized in engineering medical applications must have an excitation wavelength of 544 nm and an absorption wavelength of 516 nm. Consequently, a 551 GHz exciter and a 580 GHz transmission antenna are required. Since it clarifies the foundations of competitive binding and oxidation, the study can be used as a resource for researchers in the field of fluorescent glucose sensing.

Table 1. Constants [54]

Constant definition	symbol	Value
Quantum yield of donor in the absence of acceptor	Q(D)	0.34 [54]
a numeric value which gives the orientation factor defining the relative orientation of the donor and the acceptor	κ2	2/3
Index of refraction	n	1.4
Wavelength scale for donor	wd	1 D array
Wavelength scale for acceptor	wla	1 D array
A numeric value giving the molar absorption coefficient at the wavelength	ε	73,000 cm-1M-1

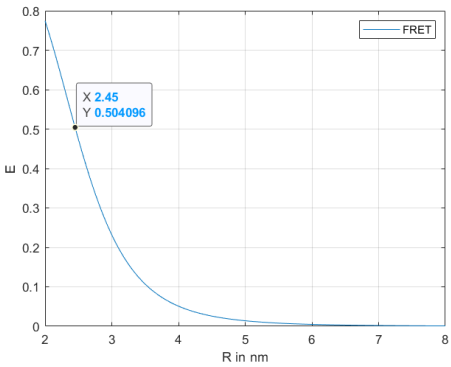

Figure 12. FRET Efficiency in function of R of TRICTC-FITC

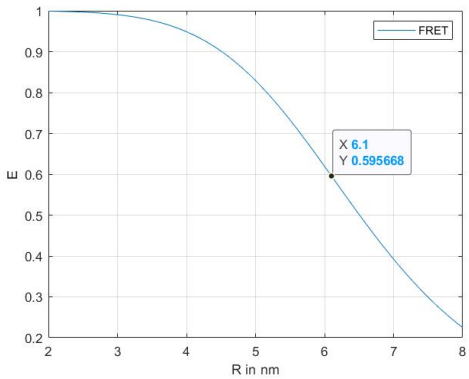

Figure 13. FRET Efficiency of FITC- TRICTC

Declaration of Ethical Standards

The authors of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Conflict of Interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study is a significant component of an ongoing project aimed at developing a fluorescence glucose biosensor for a cancer detection device. The project forms the basis of a doctoral dissertation conducted by a PhD candidate in Graduate School of engineering ECE Department at Altinbas University.

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