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Research Article

New Method of Quality and Quantity Control of the Insulin Glulisine Pharmaceuticals Based on Intrinsic Radiothermal Emission

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Abstract

According to the WHO, the prevalence of type I and type II diabetes in the world exceeds 800 million people (14% of the adult population). Insulin pharmaceuticals are therapeutically applied in various configurations with different pharmacokinetic characteristics. There exist highly effective, validated methods for their quality control. However, each method exhibits a number of disadvantages, including long-term sample preparation, significant expense, and the inability to analyze the sample without opening the primary packaging.

Objective: The aim of current work is to develop a new approach to control the qualitative and quantitative characteristics of a drugs based on insulin glulisine without opening the primary packaging.

Materials and methods: Insulin glulisine; TES-92 for estimating the intensity of the flux density of its intrinsic radiothermal emission; Zetasizer Nano ZSP for determining the dimensional characteristics of the samples.

Results: The heating of the samples to 37^{0}C effectively activates the emission activity (9.5 ± 0.5 μW/m^2). The proposed method enables the differentiation of drugs that possess divergent qualitative characteristics. The effect of stress factors on the emission activity of insulin glulisine has been studied: heating, freezing and UV irradiation reduce the values of the flux density (1.2 ± 0.1; 1.7 ± 0.2; 3.2 ± 0.7 μW/m^2). The feasibility of employing the proposed method for the quantitative determination of insulin samples is demonstrated.

Conclusion: Based on the results obtained, the radiothermal emission detection method can be applied to control the qualitative and quantitative characteristics of biologically active drugs without opening the primary packaging.

Keywords: *insulin, quality control, quantity control, non-invasive method, intrinsic radiothermal emission, diabetes*

INTRODUCTION

The earliest documented mention of diabetes in the scientific literature dates back to the 18th century [1], although it remains a significant problem in the 21st century [2]. *Diabetes mellitus* is currently categorized into two distinct types: type 1 (classically known as insulin-dependent *diabetes mellitus*) [3] and type 2 (formerly known as non-insulin-dependent *diabetes mellitus*) [4]. The fundamental distinction between these two forms of diabetes lies in the underlying pathophysiology. In type I diabetes, there is an autoimmune reaction that results in the destruction of pancreatic beta cells [5,6]. In contrast, type II diabetes is characterized by a loss of sensitivity of the cell receptors to insulin [7].

The primary medicinal biomolecule employed in the treatment of both type I [8], as well as for the advanced stage of type II diabetes [9] is the polypeptide hormone insulin [10]. The term "insulin" was introduced by J. de Meyer in 1909 to denote the secretion of the pancreas, which was discovered to regulate blood glucose levels [11]. The successful utilization of ethanol as an insulin extractant from the pancreas rendered it possible to employ this extract as a medicinal product. The first patient to receive the injection was Leonard Thompson, a 14-year-old male. The injection of the solution resulted in immediate alleviation of his condition, thus enabling him to resume a normal life following therapy. Consequently, the development of a life-saving medicine led to the awarding of the Nobel Prize in Physiology and Medicine to the developers [12].

Pharmacologically active types of insulin in prepared dosage forms are diverse: monomers – for the rapid onset of therapeutic effect [13]; hexamers – insulins of this form are the longest to show their pharmacological effect

[14]; and dimers – the time-averaged form of activity [15]. The configuration of insulin is subject to variation depending on the set of an excipients. For example, zinc salts have been shown to stabilize insulin in the form of a hexamer [16]. It is evident that, in certain instances, the utilization of such methodologies is not a prerequisite. The modification of the insulin molecule to yield the requisite characteristics is sufficient, as evidenced by the manufacturers of insulin glulisine [17]. In this instance, biotechnologists substituted the amino acid asparagine in position B3 with lysine, and lysine in position B29 with glutamic acid.

The approach to quality control of biomolecules is quite diverse, ranging from polyacrylamide gel electrophoresis in sodium dodecyl sulfate [18] to high-performance liquid chromatography with a reversed phase [19] with tandem mass spectrometry [20]. Contemporary methods facilitate the analysis of drugs with high reproducibility, accuracy and reliability. However, it should be noted that all of these approaches have a limitation in common, namely the destruction of the sample. The primary objective of this research is to propose a novel non-invasive approach to the quality control of pharmaceuticals containing insulin, with the aim of empowering patients to personally verify the quality of their medication.

MATERIALS AND METHODS

Insulin Glulisine

A dose of insulin glulisine in the form of a solution for subcutaneous administration of 100 units (3.49 mg)/1 ml (reg. No.: LP-(000072)-(RG-RU)) was selected as samples for research of proper and improper (expired for more than six months) quality, which is a recombinant analogue of human insulin. In addition to the active pharmaceutical ingredient, this pharmaceutical compound also contains a number of excipients (Table 1).

Table 1: List of excipients in the pharmaceutical of insulin glulisine.

Excipients	Weight, mg	Application
Metacresol (m-cresol)	3.15	Preservative
Trometamol (trometamine)	6.0	Buffer solution
Polysorbate 20	0.01	Surfactant
Sodium hydroxide	Up to pH 7.4	The pH regulator
Hydrochloric acid	Up to pH 7.4	The pH regulator
Water for injection	Up to 1000	Solvent

The substances must be stored in a dark place at a temperature from +2 to +8 ^{0}C prior to use. Following the initial application, they should be stored at a temperature not exceeding +25 ^{0}C, without freezing.

During sample preparation, high-resistance water (18.2 MΩ×cm, Milli-Q) was used for the dynamic light scattering method.

Dynamic Light Scattering (DLS)

The dimensional spectra of the samples were analyzed using the Zetasizer Nano ZSP DLS method (MALVERN Instruments, Malvern, UK). The Zetasizer Nano is an instrument that utilizes non-invasive backscattering technology, thereby enabling the determination of the particle size in dilute samples ranging from 1 nm to 10 μm. The method is based on photon correlation spectroscopy, which is itself based on the analysis of Brownian motion of particles of a dispersed phase in a dispersed medium [21]. The particle size is calculated using the Stokes-Einstein equation. Measurements of each sample were carried out at least 7 times.

Determination of Intrinsic Radiothermal Emission

The study of the emission characteristics of the insulin samples was carried out using the electromagnetic radiation flux density detector TES-92 (TES Electrical Electronic Corp., Taipei, Taiwan), the sensor of which was configured for anisotropic measurement along the Z axis (i.e. directed strictly at the sample). The configuration of the experimental setup is illustrated in Figure 1.

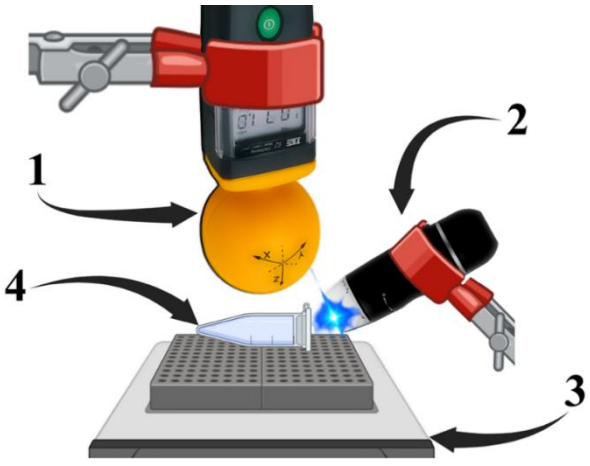

Figure 1: An installation for detecting intrinsic radiothermal emission in the millimeter wavelength range, where 1 – TES–92 radiothermal emission flux density detector with a sensor configured for anisotropic measurement along the Z axis, 2 – emitter with high spectral power LEDs (λ = 412 nm), 3 – solid-state thermostat based on Peltier elements, 4 – sample.

The maximum average value of the emission flux density from the sample was detected. To study a possible technique for activating the intrinsic radiothermal emission of insulin samples, heating up to 37^{0}C and irradiation with light were used. The subject of this study was heated using a solid-state thermostat with Peltier elements (MK200-2, Hangzhou Allsheng Instruments Co. Ltd., China). The temperature control of the samples was carried out using a non-contact laser infrared thermometer (Bentech GM320, China). In order to study the possibility of activating drugs by light irradiation, LEDs with a power of up to 50 MW/cm^2 in the range of 412 nm with a spectral line width of up to 2-4 nm (AA3528LVBS/D, type C503B–BCN-CV0Z0461, CreeLED, Durham, North Carolina, USA) were used. Prior to conducting the measurements, background emission values were meticulously monitored. The background values did not exceed 1.0 ± 0.1 μW/m^2. The geometry of the installation was observed throughout all measurements. Each measurement was performed at least 7 times.

Stress Factors

A detailed study was conducted to examine the impact of stress factors, such as elevated and low temperatures, and direct UV irradiation, on the dimensions and emission characteristics of insulin pharmaceuticals. The experimental setup involved the meticulous preparation of samples to facilitate analysis. Four dilutions of proper quality insulin glulisine with a concentration of 1.75 mg/ml were prepared. Subsequently, three samples were exposed to extreme conditions.

Sample No. 1 was a native sample of an insulin preparation of proper quality, which did not undergo physicochemical changes.

For sample No. 2, the process of heat treatment was conducted at a temperature of 100 °C for a duration of 1 hour. The heating system was operated using a solid-state thermostat. The temperature was monitored using a remote thermometer.

The freezing process was conducted on sample No. 3 in a -18 °C freezer for a duration of 24 hours. Prior to conducting the research, the frozen sample was extracted from the chamber and thawed at ambient temperature.

Direct irradiation of sample No. 4 with UV light was conducted using a UV lamp with a wavelength of 365 nm and a luminescence excitation range of 320-400 nm (NV-Med, Russia) for a duration of 1 hour.

For the expired sample No. 5, sample preparation was also conducted by preparing dilutions with a concentration of 1.75 mg/ml, without the application of extreme conditions.

Statistics

The results of the analyses are presented as mean ± standard deviation (n=7). All calculations and statistical processing were conducted using OriginPro 21 software (OriginLab, USA).

RESULTS AND DISCUSSION

Phenomenon of Intrinsic Radiothermal Emission from Drugs Containing Biologically Active Configurations of Insulin

Intrinsic radiothermal emission of pharmaceuticals containing nanostructures is a physicochemical characteristic that has been described in a number of publications [22,23]. The discovery of this phenomenon is of great significance to the pharmaceutical industry, as it has enabled the development of a novel non-invasive method for the quality control of drugs from various pharmacological groups [21].

The physics of this phenomenon is contingent on a number of factors. Firstly, it is important to acknowledge the diversity of shapes of contemporary nanosubstances [24]. There are two categories of shapes of nano objects: spherical and non-spherical. It has been demonstrated that insulin monomers can be attributed to the last one [13, 25]. It is evident that, upon closer inspection of the structure of insulin, specifically its monomeric configuration, the observation is made that the surface of the protein is characterized by heterogeneity (Figure 2). The size of such monomers varies from 1 to 4 nm [26,27].

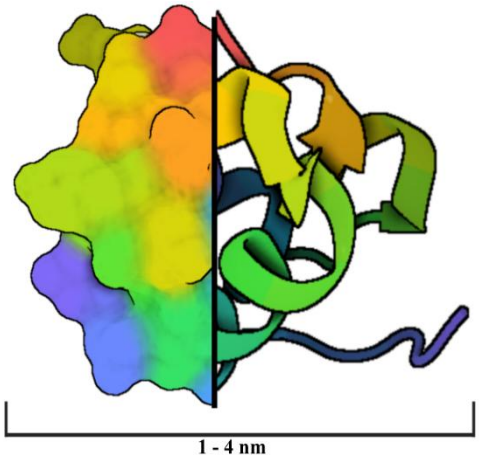

Figure 2: Monomeric configuration of insulin, which is a nanostructure of complex non-spherical shape. (The illustration presented here is an original work, based on data obtained from the Protein Data Bank).

The phenomenon of intrinsic radiothermal emission is dependent on the morphology of the particle, thus giving rise to the formation of an electric potential gradient and an ultrahigh electric field strength of greater than 10^5 V/cm on the surface of nanostructures with intricate shapes [22]. This process contributes to the appearance of a plasma-like structure on the surface of nanopharmaceuticals, which is capable of emitting in the range of 75 GHz (EHF, W-band), that is, in the millimeter range [28,29]. A variety of external energy sources, including temperature and the excitation of protein particles by light within the visible spectrum, have been demonstrated to contribute to an enhancement in the flux density of radiothermal emission from nanostructures [22]. In this case, the particle will undergo reversible conformational transitions, a phenomenon documented in the case of polypeptides and oligomeric protein molecules [31], and experimental data indicate that

insulin monomers exhibit significant conformational heterogeneity [13]. The process of formation of radiating plasma-like structures on the surface of particles can be described by the following kinetic scheme (Figure 3).

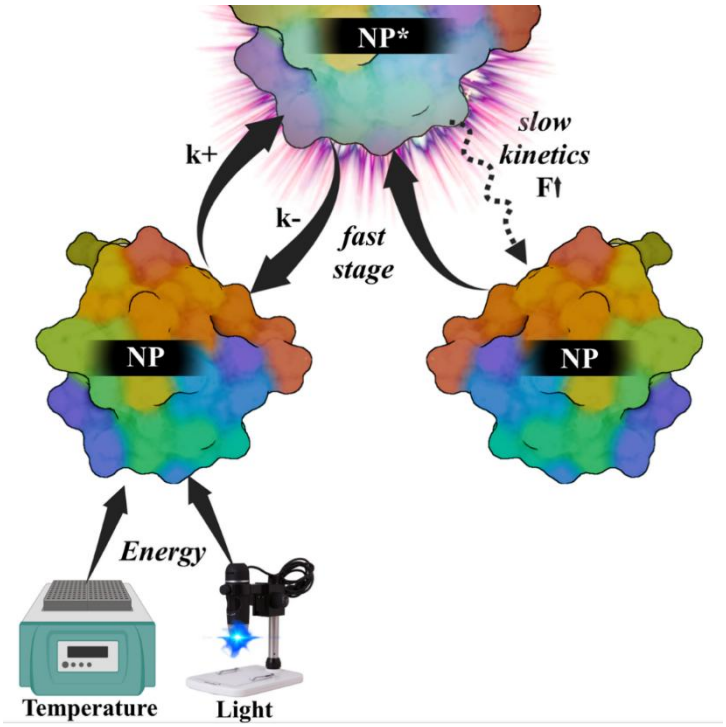

Figure 3: Kinetic scheme of formation of plasma-like regions on the surface of nanoprotein (NP), an insulin monomer, where NP of the insulin monomer are unexcited molecules, NP* is a particle with plasma–like regions, the relaxation time of the equilibrium transition takes less than 1 ms (fast stage), the rate constant of the slow transition is more than 1 s (slow kinetics), and F is the flux density of radiothermal emission.

Methodology for Enhancing the Flux Density of Intrinsic Radiothermal Emission for an Insulin Samples

In view of the aforementioned factors, it was imperative to ascertain the most efficacious methodology for enhancing the intensity of the flux density of the radiothermal emission of the insulin glulisine samples, or, in other words, the technique of "activating" the pharmaceutical agent. Earlier studies have demonstrated various approaches to the activation of radiothermal emission of interferon drugs and VLP vaccines [21], as well as therapeutic and preventive nutrition based on humic-fulvic acids [23]. As outlined in the aforementioned articles, both activation techniques – namely, the heating of the sample to 37°C and the irradiation of samples with light at a wavelength of 412 nm – were discussed. In order to ascertain the most suitable technique, a series of studies were conducted in order to examine the effect of two activation factors on the emission activity of the pharmaceutical compound insulin glulisine (Figure 4).

Increased values of the flux density were recorded for samples activated by temperature **(37°C)**, with a result of 9.5 ± 0.5 μW/m². In contrast, the flux density of non-activated samples was determined to be 5.3 ± 0.4 μW/m². Activation by irradiating the samples with light in the visible region **(*hv*)** did not lead to a significant increase in the intensity of its intrinsic radiothermal emission compared to non-activated samples.

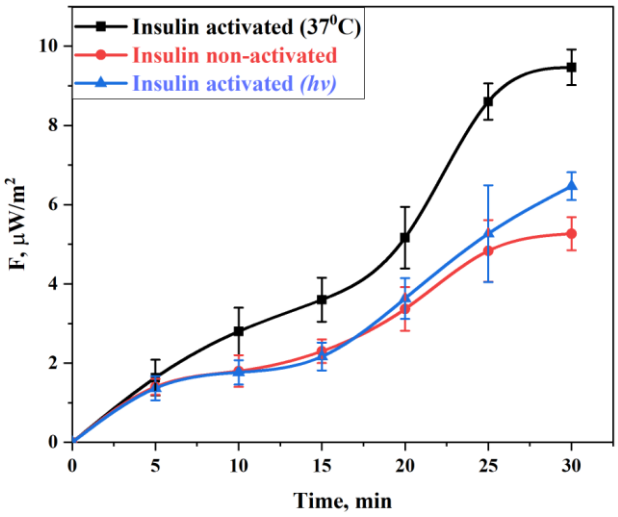

Figure 4: The time curve of the intensity of intrinsic radiothermal emission applying two methods of activating the insulin glulisine pharmaceuticals, where the black color shows the curve of the development of the flux density over time with temperature activation, blue – when irradiated with light in the visible region (λ = 412 nm), red – an insulin preparation of proper quality that was not influenced by external physical factors (n=7).

A more detailed study of the behaviour of the emission activity of samples and their ability to relax in the absence of an external energy source is demonstrated for both methods of increasing the intensity of the radiothermal emission flux density. This demonstration is illustrated in Figure 5, which shows the transition from the excited state to the initial state.

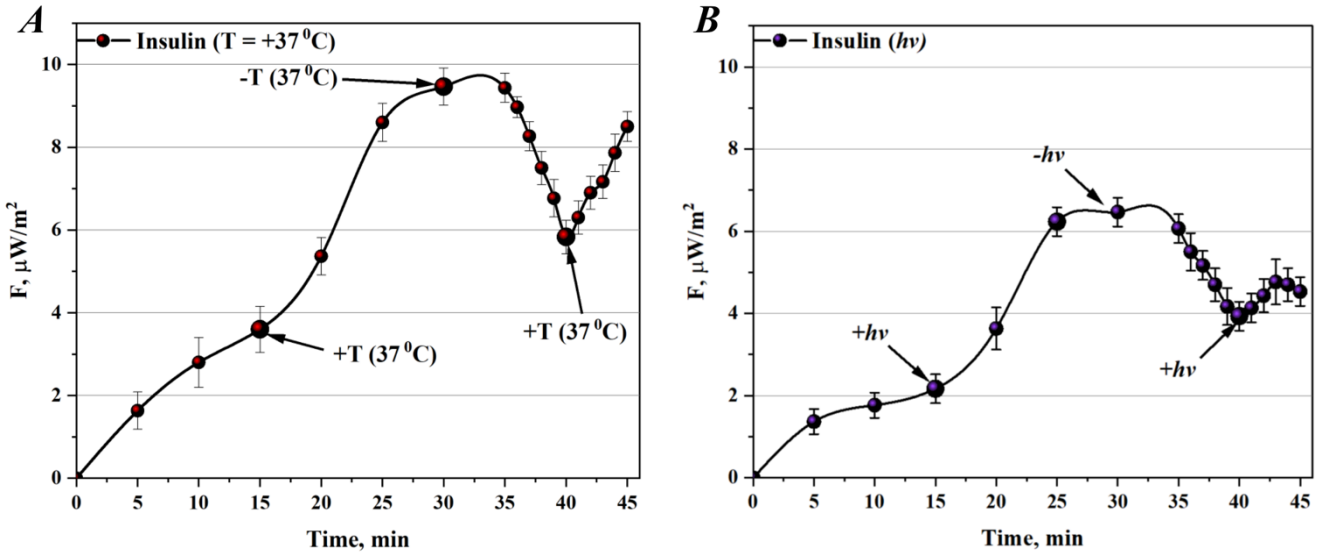

Figure 5: Curve of increase and relaxation of the flux density of intrinsic radiothermal emission of the insulin glulisine pharmaceuticals, where A is temperature activation, B is activation by irradiation with light. The arrows indicate the switching on and off the external physical factors. The measurement was carried out without opening the primary packaging (n=7).

The results obtained from the kinetics of increasing and relaxing the intensity of radiothermal emission demonstrate the dependence of the detected flux density on the presence of an activation factor for both cases. The increase in the average maximum value of the radiothermal emission occurs within a time frame of no more than 5 minutes, and the relaxation to the initial state following the deactivation of external excitation factors takes no more than 10 minutes for insulin glulisine samples. A series of measurements were conducted from 0 to 35 minutes, with a 5-minute interval established as the standard protocol. Following the deactivation of the activation source, the results were detected at 1-minute intervals for a more effective assessment of the relaxation characteristics of drugs. This approach utilized both techniques to ensure a comprehensive evaluation. Upon re-activation of the factors, an enhancement in the emission activity of the drug is observed. The kinetic results obtained in this study provide robust evidence to support the hypothesis that thermal activation is indeed an effective process, as illustrated in Figure 4. Subsequent measurements of the intrinsic radiothermal emission of insulin preparations were conducted applying this technique.

Investigation of the Dimensional Characteristics and Values of Intrinsic Radiothermal Emission of Insulin Glulisine Pharmaceuticals with Various Qualitative Characteristics

The method of detecting insulin glulisine's intrinsic radiothermal emission enables the determination of its quality characteristics without the opening of the primary packaging. Two samples with different qualitative characteristics were measured: the proper shelf life and the expired one (Table 2).

Table 2: The results of intrinsic radiothermal emission of insulin samples with different qualitative characteristics at different detection stages (0, 15, 30 minutes) (n=7).

Detection Stages	Proper Quality Insulin Glulisine	Expired Insulin Glulisine	Control (High resistance water)
	Flux Density F, μW/m² (Mean ± SD)		
The Beginning of the Measurements (0 min)	1.7 ± 0.2	1.2 ± 0.2	1.0 ± 0.2
The Beginning of the Activation of the Samples (15 min)	4.1 ± 0.5	1.7 ± 0.2	0.8 ± 0.3
The Last Point of the Measurements (30 min)	9.4 ± 0.5	2.8 ± 0.3	1.0 ± 0.1

Based on the results obtained, by the beginning of sample activation (15 minutes), increased emission activity was observed for a drug with an appropriate shelf life, while for a drug with an expired shelf life, the change in flux density indicators did not show a significant increase compared with the indicators obtained at the beginning of the measurement (0 minutes). At the 30-minute mark, the flux density of the drug from the proper shelf life was observed to be 9 times higher than the control values and more than 3 times higher than the values of the emission activity for the expired drug. The reduced emission activity can be interpreted as follows: following the expiration date of the pharmaceutical compound, the molecules of the active substance may undergo various changes as a consequence of the influence of various physicochemical factors. In the context of protein structures, the processes of denaturation or coagulation may occur. In order to confirm possible physicochemical changes, dimensional spectra were obtained for drugs with different qualitative characteristics (Figure 6).

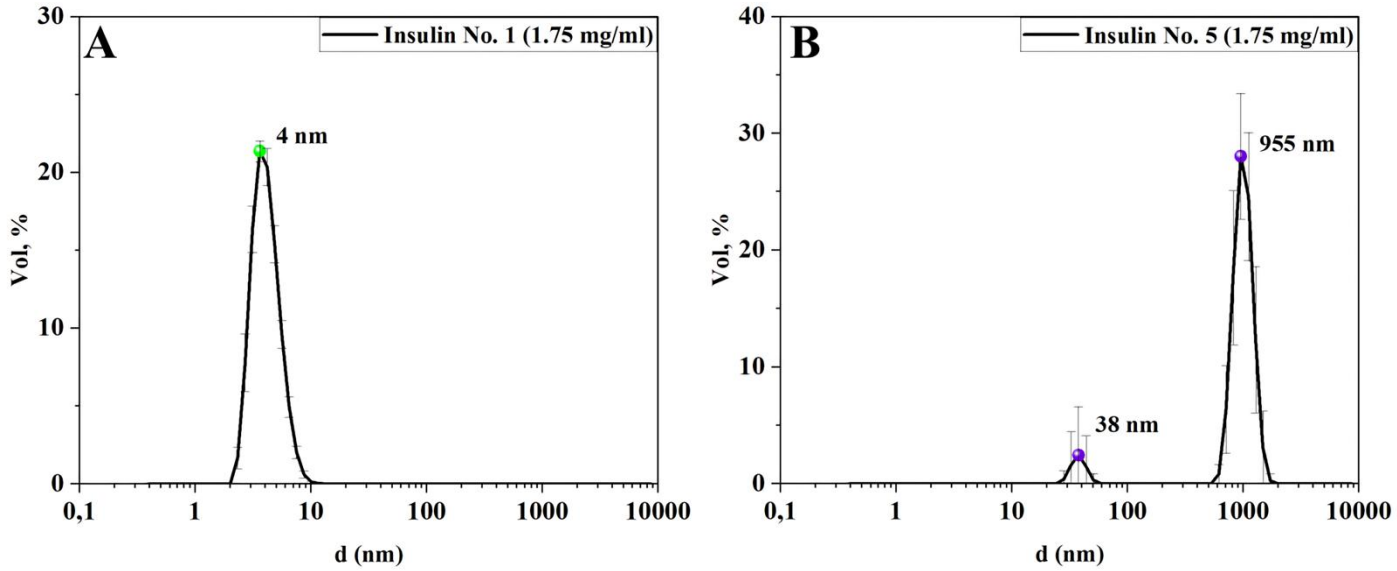

Figure 6: Comparison of the size spectra of insulin glulisine of proper No. 1 (A) and expired No. 5 (B) quality.

Consequently, for a sample with a proper shelf life (1.75 mg/ml), a monomodal particle volume distribution is observed (Figure 6 A) with a hydrodynamic radius of 4 nm. The data obtained provide indication of the size distribution characteristic of insulin glulisine. For example, the authors of the following articles demonstrate size ranges from 3 to 5 nm [26,27]. For the expired drug (1.75 mg/ml), a bimodal particle volume distribution was obtained, exhibiting two characteristic peaks of 38 and 955 nm (Figure 6 B). The presence of two peaks in the spectrum may indicate the occurrence of both coagulation and denaturation of the protein structure [31], thereby confirming the results of the detection of intrinsic radiothermal emission previously mentioned.

Effect of Stress Factors on the Size and Emission Characteristics of the Insulin Glulisine Pharmaceuticals

Protein substances characteristically exhibit thermally unstable structural properties, a factor which necessitates the maintenance of specific conditions during their storage. For all formulations of insulin, the recommended storage temperature range is from +2 to +8°C. It is imperative to note that any alteration in the storage temperature of these pharmaceuticals has the potential to result in a concomitant change in their pharmaceutical properties, thereby rendering their application impractical. For example, when insulin is stored at temperatures in excess of the optimal range, irreversible aggregation of the drug into fibrils occurs, reducing its biological activity [32]. However, it is permissible to store a number of drugs at temperatures ranging from +25 to +30°C following the opening of the primary packaging. Nevertheless, the shelf life of these pharmaceuticals is constrained to a period of several weeks [31,33]. Low storage temperatures, which result in the freezing of the preparation, also affect its activity, leading to the aggregation of insulin and heterogeneity of the system [35]. Furthermore, direct UV rays have been demonstrated to exert a deleterious effect on the pharmacological activity of the drug, leading to the degradation of insulin molecules [31,35]. In the context of the aforementioned facts, it was critical to investigate the influence of these physical factors on the emission activity and dimensional characteristics of the preparations.

Four dilutions of insulin glulisine samples of proper quality with a concentration of 1.75 mg/ml were subjected to critical stress factors, namely: heating at a temperature of 100°C for 1 hour; freezing at a temperature of -18°C for 1 day; direct irradiation with UV light for 1 hour (Table 3).

Table 3: Results of the intrinsic radiothermal emission of insulin glulisine pharmaceuticals exposed to stress factors (n=7).

Samples	Proper Shelf Life	Heated (+100 ^{0}C)	Frozen (-18 ^{0}C)	Irradiated by UV light	Background Value
	Flux Density F, μW/m^2 (Mean ± SD)				
Insulin Glulisine	9.5 ± 0.3	1.2 ± 0.1	1.7 ± 0.2	3.2 ± 0.7	1.0 ± 0.1

The results obtained demonstrated the almost complete absence of emission activity in the samples undergoing heating and freezing. In the case of the sample exposed to UV light, there was a 3-fold decrease in emission activity compared to the native sample. Furthermore, the dimensional spectra of these samples were obtained and are presented in Figure 7.

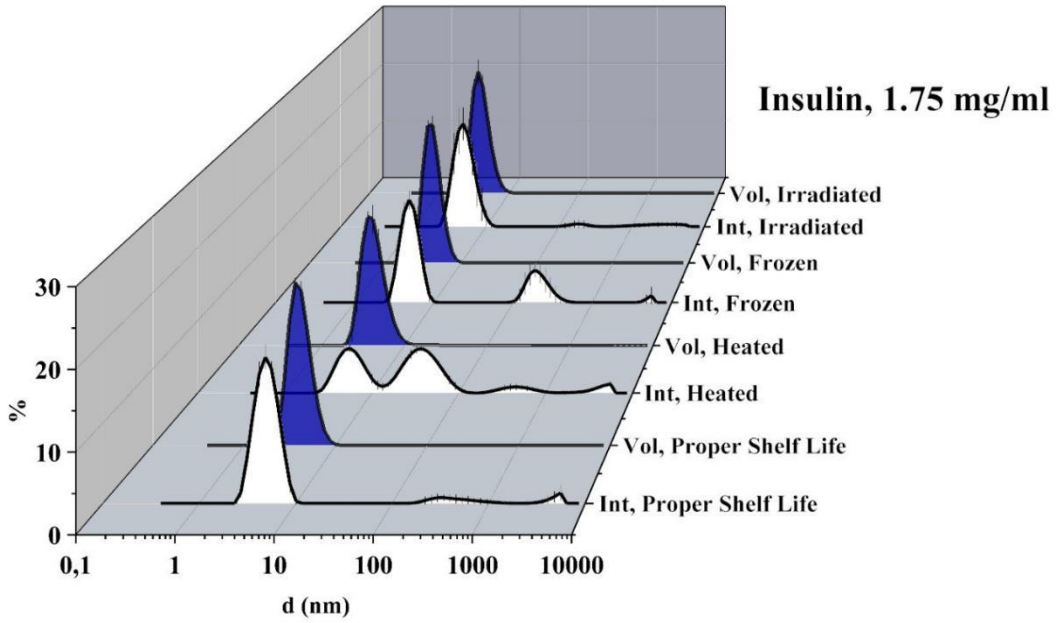

Figure 7: Comparison of the size spectra of proper shelf-life insulin glulisine with samples exposed to stress factors, where Int – Intensity distribution, Vol – Volume distribution.

Upon consideration of the volume distribution of the dimensional spectra, it was determined that for all four samples, the distribution was monomodal. However, when the intensity distribution spectra are considered, a decrease in the light scattering intensity is observed in the region of up to 5 nm in the heated sample, accompanied by the emergence of an additional peak at 38 nm relative to the native one. This phenomenon is analogous to the results obtained for the expired sample (see Figure 6 B). This may be indicative of physicochemical changes in the structure of insulin. In the case of the spectrum of the frozen sample, an additional peak emerges at 190 nm, which may signify the initiation of aggregation of insulin molecules. No alterations in the size spectrum were observed for the sample irradiated with UV light.

Sensitivity of the Method for Detecting Intrinsic Radiothermal Emission

It is logical to hypothesize that the phenomenon of intrinsic radiothermal emission will also depend on the number of emitting insulin nanostructures, where the flux density will increase linearly depending on their volume or quantity. Previous studies have demonstrated the sensitivity of this method, depending on the volume of active emitting structures and their concentration, using the example of interferon α-2b and virus-like vaccines [21]. In light of the aforementioned data, samples with a volume ranging from 100 to 1000 μl were prepared to evaluate the feasibility of determining the quantity of active substance "blindly" by employing the value of the flux density (Figure 8).

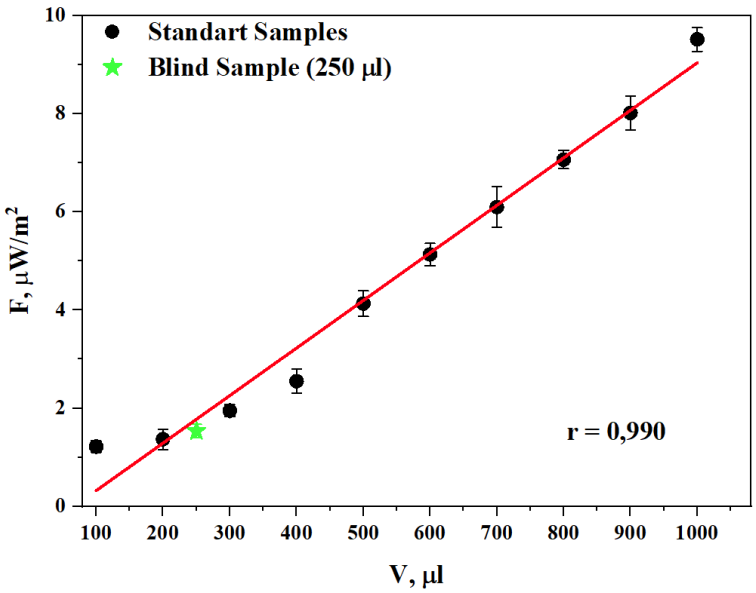

Figure 8: Linear dependence of the radiothermal emission flux density on the volume of insulin glulisine samples (1.75 mg/ml).

As demonstrated in Figure 8, there is a strong linear correlation between radiothermal emission and sample volume, as evidenced by a Pearson coefficient of 0.990. The volume of the blind sample was determined by the value of the flux density of its intrinsic radiothermal emission, which was found to be 1.5 ± 0.1 µW/m^2. The results obtained demonstrate the feasibility of employing this method to ascertain the quantity of active substance in a sample based on its volume.

Contemporary Approaches to Solve a Global Problem

Despite contemporary approaches to the treatment of diabetes, this problem remains a serious challenge for both scientists and the pharmaceutical industry. The necessity to develop contemporary pharmaceuticals to combat hyperglycemia remains a pressing concern in the present day. The present pharmaceutical market proffers a wide range of insulin-based pharmaceuticals, each exhibiting a distinct duration of action. From a pharmacological perspective, there are five main types of insulin preparations: rapid-acting (Lispro, Aspart, Glulisine), short-acting (Regular), intermediate (NPH Insulin), long (Detemir, Glargine) and ultralong (Degludec) [36]. However, despite the wide range of therapeutic insulins available on the market, factors such as the inconvenience of drug administration and side effects such as hypoglycemia and weight gain should not be excluded [37–39]. The scientific community is developing approaches to the alternative administration of insulin into the body by creating various nanocarriers. These nanocarriers have the potential to allow patients to use drugs orally in the future [40–42].

The primary objective of pharmaceutical chemistry is indisputably the quality control of pharmaceutical products, with the aim of ensuring that essential criteria for medicines are adhered to, namely their effectiveness and safety. The main analytical methods employed for the quality control of insulin-based preparations are as follows: the protein content of the final product is determined; high-performance liquid chromatography is used to assess the uniformity and concentration of the active substance; and mass spectrometry is employed to accurately determine the molecular weight of protein molecules and to detect the presence of posttranslational changes (oxidation, phosphorylation, glycosylation, etc.) [43]. The application of these methods is important for the quality control of the final production of insulin products. However, it is important to acknowledge the labour-intensive nature of these methods and the necessity for sample preparation, as well as the lack of non-invasive quality control [44–46]. The development of such an approach would facilitate the monitoring of the qualitative and quantitative characteristics of protein preparations without the need to open the primary packaging. This would enable the determination of the suitability of drugs at any stage of their life cycle. Guided by the possibilities described above, this study was proposed a contemporary approach that addresses the principal aspects necessary to achieve this objective.

CONCLUSION

In light of the results obtained in this study, it can be concluded that the method of detecting intrinsic radiothermal emission can be applied to control the qualitative and quantitative characteristics of protein preparations based on insulin, including the monomeric form of insulin glulisine. In order to achieve this objective, it was first necessary to determine the most appropriate methodology for activating the intrinsic radiothermal emissions of the insulin pharmaceuticals. The flux density of radiothermal emission from the samples was found to be 9.5 ± 0.5 µW/m^2 when heated to 37^{0}C, which is almost double the level observed with light activation (see Figure 4). The obtained data were confirmed by the relaxation characteristics of samples activated by two different techniques, which also made it possible to study the time-dependent behaviour of

insulin preparations in the presence or absence of activating factors (see Figure 5). The results of the study demonstrated the efficacy of the method for use in quality control without the need to open the primary packaging. The radiothermal emission exhibited by the drug in proper quality were found to be more than 3-fold higher than those observed in the expired drug (see Table 2 for details). The results obtained correlate with the size spectra, indicating possible denaturation or coagulation of the expired drug (bimodal distribution with peaks of 38 nm and 955 nm, versus 4 nm for the drug with the appropriate shelf life) (see Figure 6). Furthermore, the investigation encompassed critically important aspects affecting the shelf life of protein preparations, namely increased and decreased storage temperatures and the effects of direct UV rays. In the first two studies, a decrease in emission activity of the samples to background values was observed. In the case of direct UV rays, a 3-fold decrease in emission activity (3.2 ± 0.7 μW/m^2) was detected in comparison with the sample not affected by stress factors (9.5 ± 0.5 μW/m^2) (see Table 3). The obtained results were supported by the dimensional spectra of the samples described above (see Figure 7). The potential for quantitative analysis was illustrated by the sensitivity of the method (see Figure 8). The conducted studies of the emission activity of the "blind" sample made it possible to accurately determine the volume of the aliquot, which indicates the possibility of quantifying protein preparations using the method of detecting its intrinsic radiothermal emission.

As demonstrated by the results mentioned above, the method of detecting intrinsic radiothermal emission facilitates non-invasive observation of the qualitative and quantitative characteristics of insulin-based protein pharmaceuticals at any stage of their life cycle. In the future, the potential of the phenomenon of intrinsic radiothermal emission, as outlined in the article, and the method developed on its basis, can be employed in conjunction with existing approaches to the quality control of insulin products.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Contributors: All authors have read and approved the final manuscript.

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