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Research Article

Non-destructive Remote Determination of Total Native Protein Concentration in Virus-Like Particle Vaccine Preparations

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Abstract

Introduction. Virus-Like Particles (VLPs) are nanoscale entities characterized by surface shape heterogeneity and enhanced emission activity in the millimeter wavelength range (30-300 GHz), which is influenced by external physical factors such as heating or electromagnetic radiation. This phenomenon presents opportunities for the noninvasive determination of accurate concentrations of the protein component in commercially prepared VLP vaccines.

Objective. To propose a modern and rapid approach for the quantitative determination of native proteins in VLP-containing vaccines, which enables research to be conducted without opening the primary packaging.

Materials and methods. Vaccines imitating rotavirus (Gam-VLP-Rota) and SARS-CoV2 virus (Gam-VLP-multivac) were studied. The density of the radiothermal emission flux at extremely high frequency (EHF) wavelengths was measured using a TES-92 device. The reference values for protein concentration in vaccines, prior to the addition of the adjuvant, were measured using the commercial "Micro BCA™ Protein Assay Kit". The dimensional characteristics of the VLP vaccine were analyzed using dynamic laser light scattering (Zetasizer Nano ZSP).

Results. A strong linear correlation (r = 0.99) was established between the flux density of radiothermal emission and the protein content in vaccines, allowing for analysis without opening the packaging. The validation procedure for this technique assessed linearity, accuracy, and intra-laboratory precision for Gam-VLP-multivac.

Conclusion. The values of the intrinsic radiothermal emission flux density enabled the determination of the native concentration of complex-shaped virus-like nanoparticles in vaccines without the need to open the primary packaging, irrespective of the presence of an adjuvant.

Keywords: *VLP, Virus-Like Particles, quality control, protein concentration determination, radiothermal emission.*

INTRODUCTION

Virus-Like Particle (VLP) vaccines are nanosized viral protein complexes capable of self-assembly. These vaccines mimic the virion and morphological characteristics of viruses while lacking fragments of matrix ribonucleic acid (mRNA), which prevents replication within the body, thereby establishing VLP vaccines as both effective and safe [1-3]. VLP-based vaccines exhibit significant immunogenicity, which facilitates the activation of antigen presentation mechanisms involving molecules of the major histocompatibility complex classes I and II (MHC-I and MHC-II). This process ensures the specific presentation of antigen epitopes to T-lymphocytes within the adaptive immune response. The structural characteristics of VLPs, including highly repetitive domains of surface proteins, create conformationally organized epitopes and simultaneously stimulate the activation of dendritic cells. This interaction enhances their engagement with components of innate humoral immunity, resulting in a more complex and effective immune response [4, 5].

The technology for producing virus-like particle (VLP) vaccines enables rapid modification of the antigen composition in response to the current epidemiological situation through site-directed mutagenesis. Consequently, contemporary methods for developing

vaccines based on VLPs facilitate the creation of a wide variety of designs and significantly reduce the time required to produce a vaccine *de novo* [6].

To ensure the native structure of virus-like particle preparations, a squalene-based adjuvant is incorporated into the final dosage forms [7]. The resulting preparation is a cloudy white emulsion. Classical colorimetric methods for determining protein concentration [8, 9] are not applicable in turbid and opaque media. Additionally, squalene interferes with color development when using both the Folin-Ciocalteu reagent and the Coomassie reagent, affecting the analytical results. Furthermore, the standard procedure for quality control of immunobiological preparations necessitates the determination of protein concentration in the final product, which consists of a mixture of VLP and adjuvant.

To address this issue, this article proposes utilizing the capabilities of virus-like particles, which are complex-shaped nanoparticles, to self-radiate thermal emission in the extremely high frequency (EHF) range of electromagnetic waves [10]. This study presents a novel rapid approach for assessing the concentration of native proteins in immunobiological drugs, specifically vaccines based on virus-like particles.

MATERIALS AND METHODS

The vaccine is a registered medicinal product composed of virus-like particles that mimic SARS-CoV-2 (Gam-VLP-multivac)[10] and rotavirus A (Gam-VLP-Rota)[11], produced as previously described. Key stages in the production process include the synthesis of virus-like particles using a baculovirus expression system and their purification through differential ultracentrifugation. Recombinant baculoviruses contain the genes for four proteins: S, E, M, and N of SARS-CoV-2, with the S protein genes derived from relevant clades [10].

The total protein concentration in VLP samples without adjuvant was determined using the "Micro BCA Protein Assay Kit" (Thermo Fisher Scientific, USA).

The emission activity of vaccines in the extremely high frequency (EHF) wavelength range was determined using a TES-92 apparatus (TES Electrical Electronic Corp., Taipei, Taiwan), based on the methodology described in reference [12]. Samples and sensors were placed in a Faraday chamber configured in an anechoic mode. Additionally, a reference signal from the microwave range of electromagnetic radiation, with a frequency of 3.5 GHz, was employed. The background radiation in the Faraday chamber, with the reference signal activated, did not exceed 0.8 $\mu W/m^2$. All measurements were conducted seven times, and the standard deviation is presented in the figures. A key aspect of the study was the ability to monitor the radiothermal emission of VLP vaccines without opening the primary packaging. Figure 1 illustrates a comparison of the emission activity of VLPs in experiments conducted with and without opening the primary packaging during the activation of samples using blue light (λ = 407 nm, power density – 50 mW/cm^2, with a spectral line width of 4 nm). The electromagnetic flux density was recorded every five minutes until the values stabilized at a plateau.

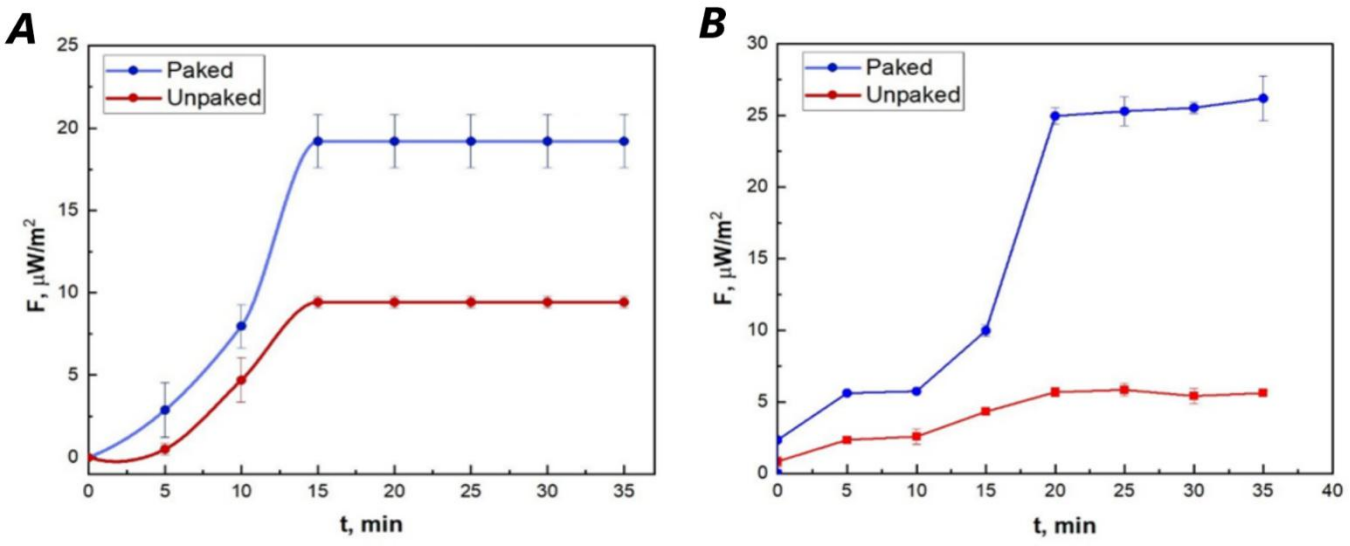

Figure 1: The time curve of flux density of intrinsic radiothermal emission upon blue light irradiation (λ = 407 nm) of the vaccines: A – Gam-VLP-multivac, B – Gam-VLP-Rota (n=7).

The particle sizes of the dispersed phase of VLP vaccines were monitored using dynamic light scattering (DLS) with a Zetasizer Nano ZSP (Malvern, UK) in the nanometer range. The refractive index of the background buffer solution was determined through refractometry (n = 1.334), while the refractive index of the VLP vaccine solutions was assumed to be equivalent to that of the proteins, as per the Malvern protocol (n = 1.4500). Each measurement was repeated 7 times and replicated three times.

RESULTS

The effectiveness of immunobiological preparations depends directly on strict adherence to the cold chain. For most vaccines, the recommended storage temperature ranges from +2 to +8 °C. However, violations of this storage protocol frequently occur during both the transportation and distribution of these products [13]. Consequently, the effects of two temperatures, +4 °C and +25 °C, on the intrinsic radiothermal emission of VLP vaccines were primarily investigated (Table 1). Vaccine stability measurements were conducted over a three-hour period, with a constant temperature of +4 °C maintained using cooling elements.

Table 1: Effect of different temperatures on the intrinsic radiothermal emission of the Gam-VLP-multivac vaccines (n=7).

Temperature, °C	Flux density F, μW/m²		Protein content, μg/mL
	Packed	Unpacked	
VLP with adjuvant			
+4 °C	10±2	18±1.2	40
+25 °C	1 1±1.5	21±2	
VLP without adjuvant			
+4 °C	6±1	9±1	80
+25 °C	7±1.5	12±1.5	

Adjuvants play a crucial role in vaccine manufacturing by enhancing the presentation of antigens and boosting the immunogenicity of the formulations. It is important to note the variation in intrinsic radiothermal emission levels between vaccines containing adjuvants and those without. The observed increase in emission activity of nanoscale vaccine formulations, which is about ten times greater than the background level (1 μW/m^2), could result from direct physical and chemical interactions between VLPs and adjuvants.

The effect of critical temperatures during artificial aging on the emission capacity of VLP vaccines was investigated. A vaccine without an adjuvant was selected as the test sample, allowing for the determination of the radiothermal emission flux density from the native sample and the exclusion of results related to the impact of high temperature on the adjuvant (Table 2). The vaccines were heated at 100°C for 30 and 60 minutes. All experiments were conducted without opening the primary packaging, followed by confirmation of coagulation using dynamic light scattering. The control measurement was the intrinsic radiothermal radiation of the sample at +4°C.

Table 2: The effect of critical temperatures on the emission activity of the Gam-VLP-multivac vaccines (80 μg/mL) (n=7).

Heating time, min	F, μW/m²	Particle size, nm
Control / Control	10.0±1.5	190 ±15
30	4.0±1.2	255 ±18
60	2.0±0.8	55 ±8

As shown, long-term exposure to elevated temperatures caused the denaturation of the virus-like particles. The peak size in the control sample's volume distribution, initially at 190 nm, shifted to 55 nm under artificial aging conditions. Additionally, the emission activity of the preparations decreased fivefold, nearly reaching background levels.

As previously described, the addition of an adjuvant to immunobiological preparations is a crucial step in their production, as it enhances their immunogenicity [14]. At the same time, incorporating an adjuvant into vaccine formulations for intramuscular administration facilitates the formation of emulsions. Traditional methods for monitoring protein content are ineffective in turbid and opaque media containing adjuvants. However, the flux density of radiothermal emission in the microwave wavelength range is independent of the medium's characteristics and depends solely on the qualitative and quantitative properties of the protein components. Calibration curves demonstrating the direct relationship between intrinsic radiothermal emission and protein content were established for the Gam-VLP-multivac (Figure 2, A) and Gam-VLP-Rota (Figure 2, B) vaccine preparations, with Pearson correlation coefficients of r = 0.99 and r = 0.98, respectively.

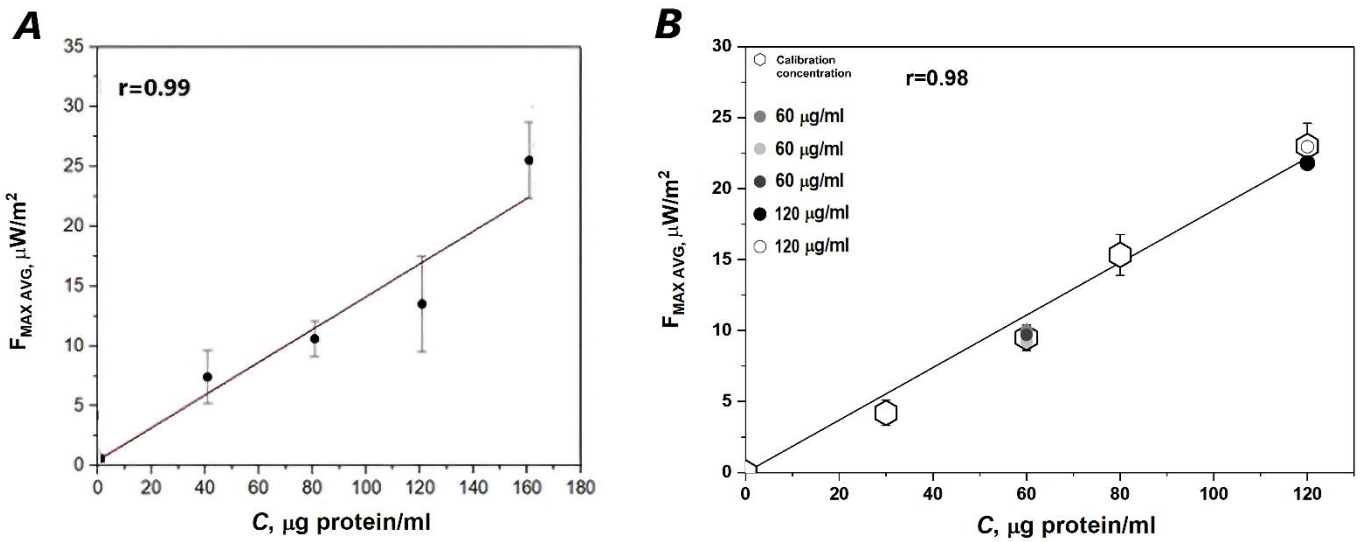

Figure 2: The dependence of intrinsic radiothermal emission on protein concentration in vaccines without opening the primary packaging. A – Gam-VLP-multivac; B – Gam-VLP-Rota (n=7).

During the experiment, five Gam-VLP-Rota vaccines in sealed vials, with protein concentrations of 60 and 120 μg/ml, were studied. The protein content, determined by intrinsic radiothermal emission measurements without opening the primary packaging, corresponded to the reference values (Figure 2, B).

In addition to validating the method for linearity and accuracy parameters, an assessment of intralaboratory precision was conducted using Gam-VLP-multivac vaccines without opening the package. Table 3 presents the results of seven consecutive measurements of the intrinsic radiothermal emission values for Gam-VLP-multivac vaccines.

Table 3: The radiothermal emission flux density of the Gam-VLP-multivac vaccine.

No.	Flow density of radiothermal emission (μW/m2)	
	Background values	Gam-VLP-multivac vaccines
1	0.5	25.8
2	0.7	23.3
3	0.5	23.3
4	0.6	25.8
5	0.6	21.0
6	0.8	25.8
7	0.7	23.3
Average Mean	0.6	24.0
Standard Deviation	0,1	1.8

DISCUSSION

The general chapters of United States Pharmacopeia [8] and European Pharmacopoeia [9] provide several methods for the quantitative determination of protein in immunobiological medicinal products. The *spectrophotometric method* relies on the ability of aromatic amino acids—tyrosine, tryptophan, and phenylalanine—to absorb ultraviolet radiation at a wavelength of 280 nm. The analytical range of this technique is 200–2000 μg/mL. The *colorimetric Lowry method* quantifies the same aromatic amino acids in proteins after their interaction with copper (II) salts and reduction with Folin–Ciocalteu's phenol reagent at λ = 750 nm, enhancing sensitivity to 10–200 μg/mL. *The Bradford method* involves colorimetric measurement of optical density at the absorption maximum (λ = 595 nm) following the interaction of arginine and lysine residues with Coomassie reagent; the protein concentration range is 100–1000 μg/mL. The *bicinchoninic acid (BCA) assay*, which detects colored protein complexes at λ = 562 nm, is based on the reducing properties of cysteine, cystine,

tryptophan, and tyrosine residues, with an analytical range of 10–1000 µg/mL. The *Biuret assay* monitors protein content by measuring optical density at λ = 545 nm, based on the interaction of peptide bonds with the biuret reagent, covering a range of 500–10,000 µg/mL. *Fluorescence* intensity measurements between 440 and 455 nm (excitation at λ = 340 nm) of protein reaction products with ortho-phthalaldehyde enable protein quantification at levels of 10–200 µg/mL. Finally, protein determination by *nitrogen content* involves a mathematical conversion using a coefficient relating protein content to nitrogen amount, established after sample mineralization.

All the pharmacopoeial methods listed rely on measuring the total aromatic amino acid residues through fluorescence intensity or absorption during color development with specific reagents. These methods are highly sensitive to the composition of the medium and are clearly unsuitable for turbid or opaque media. In this article, we propose a novel method for determining protein concentration by measuring the flux density of intrinsic radiothermal radiation emitted from oligomeric protein nanoparticles, based on our previously developed approaches and theoretical assumptions [10]. As shown in Figure 2, the range of protein concentrations measurable by this new method is comparable to that of the Lowry method.

It should be emphasized that radiothermal emission in the extremely high frequency range is detected only from the native protein. As shown in Table 2, VLP coagulation is accompanied by a decrease in radiothermal radiation. This finding aligns well with data on the artificial aging of other immunobiological drugs [14].

The relative “transparency” of the glass vials routinely used to store finished vaccine preparations for EHF radiation enabled the developed method to control the concentration of native protein without opening the primary packaging. This approach will allow future users not only to determine protein concentration but also to repeatedly assess the vaccine’s suitability for use during storage.

In this study, the radio emission of virus-like particles (VLPs), which are complex nanoparticles, was utilized for quality control by assessing native protein concentration. It is important to note that the phenomenon of self-emission by viruses, acting as unique nanoantennas [10], is an integral part of the Deryagin interaction mechanism between virus and receptor surfaces. This phenomenon can be employed in novel ways to investigate virus interactions with cells and the organism as a whole [15, 16].

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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