EXPRESS METHOD FOR QUALITY CONTROL OF PRODUCTS AFTER THE FLUIDIZED BED AEROSOL CHAMBER BY DETECTING RADIO THERMAL EMISSION OF NANOPARTICLES

Petrov G.V. [1]*, Taranov V.V.[2] and Syroeshkin A.V.[3]
[1,3]*Department of Pharmaceutical and Toxicological Chemistry, Peoples Friendship University of Russia (RUDN University), 6, Miklukho- Maklaya st, Moscow, 117198, Russian Federation*
[2]*National Technical University "Kyiv Polytechnic Institute" named after Sikorsky, 37 Pobedy Street, Kyiv, Ukraine, 03056*
gleb98.2014@gmail.com

ABSTRACT

Objective: *The main objective was to study physical and chemical activity of biotechnological pharmaceutical drugs (BPD) produced in a fluidized bed chamber and to compare it with placebo in order to further develop an up-to-date quality control method.*

Methods: *Lactose monohydrate as a filler; affinity purified polyclonal rabbit antibodies (Ab) for recombinant human interferon gamma (INFγ) as an active pharmaceutical ingredient (API); intact lactose (IL) powder. Pilotlab fluidized bed chamber, which was used to saturate lactose powder with solutions of pharmaceutical substances prior to granulation; Built-in flux density meter TES-92 (TES Electrical Electronic Corp., Taipei, Taiwan), which was used to determine the flux density of radio thermal emission in the gigahertz range.*

Results: *In the experiment aimed at studying the intrinsic radio thermal emission of BPD containing antibodies for INFγ, it was found that, as compared with the placebo preparation and IL, BPD exhibits radio thermal emission with a flux density of 80 ± 10 μW/m^2 at 37^{0}C, which is more than an order of magnitude higher than background values obtained using IL. It is observed when all samples are transferred from the powder state to the solution. When transformed into 5% solutions, BPD emit with a flux density of 30 ± 4 μW/m^2, which is 15 times higher than similar values for 5% placebo solutions and IL.*

Conclusion: *The developed method based on broadband detection of intrinsic radio thermal emission can be used to control the efficiency when BPD is prepared in a fluidized bed chamber.*

Keywords*: antibodies for interferon γ, supramolecular complex, radio thermal emission, lactose, interferon*

DOI: 10.48047/ecb/2023.12.6.273

INTRODUCTION

Fluidized bed chambers are used for efficient mixing of solid and liquid aerosols. In particular, they are used to apply highly diluted Ab solutions on lactose media. Such drugs have proven themselves well for treatment of a wide range of diseases: infectious diseases, metabolic and psycho-emotional disorders, etc. Their efficacy and safety have been proven in many preclinical and clinical randomized trials (Castagne V., 2008; Chu X., 2008; Kheyfets I.A., 2012; Nicoll J., 2013; Bailbe D., 2013; Kardash E.V., 2018; Mkrtumyan A., 2018). Quality control of such products by traditional methods, namely the full range of biopharmaceutical analysis, from ELISA and PCR to biotesting, is a challenging task for the manufacturer since it takes more than 4 man-hours to analyze one sample by one method.

Earlier, we proposed approaches based not on the analysis of low Ab concentrations but on the analysis of the properties of supramolecular water-lactose complexes (Morozova M.A. 2021, Uspenskaya E.V. 2020, Syroeshkin A.V. 2016) formed in the fluidized bed aerosol chamber. In this

case, the excessive surface Gibbs energy allows the formation of sophisticated specific supramolecular complexes, the structure of which is induced by the Ab used in the technology of preparation of the above mentioned substances. The earlier used methods of terahertz spectroscopy (Morozova et al. 2021), differential FTIR spectroscopy (Morozova et al. 2021), biotesting (Uspenskaya et al. 2020), forward and backward scattering (Syroeshkin et al. 2016) allow us to state that the thermodynamically superheated supramolecular water-lactose complex undergoes slow conformational changes. These conformational changes are specific, that is, they are peculiar only to the drug preparation substance and are absent for the placebo substance, in the preparation of which specific antibodies are not used.

It is assumed that preparations produced in fluidized bed chambers and containing specific supramolecular complexes will emit in the gigahertz and sub-terahertz ranges, due to the mentioned conformational transitions. Such a broadband emission with appropriate activation is described in this article as a method of non-destructive express analysis.

MATERIALS AND METHODS

In this experiment, a powdery substance of BPD contaning antibodies for INFγ was used. The corresponding placebo preparation is a substance prepared using the same technical process as for the biotech preparation (containing antibodies for INFγ), except for the solvent used, which does not contain antibodies for INFγ. IL is lactose monohydrate powder.

The investigated drug preparation substance (as a component of registered medicinal product No. P (000023)-(RG-RU)-140422) was obtained from the manufacturer (Materia Medica Holding) in the form of a powder saturated with a water-alcohol solution of antibodies for INFγ in a fluidized bed aerosol chamber (Uspenskaya et al. 2020). The placebo preparations of antibodies for INFγ were also produces by the manufacturer for this purpose in the aerosol chamber. In the placebo preparations, the solutions of antibodies for INFγ were replaced with a phosphate buffer solution (Levitskaya et al. 2020, Syroeshkin et al. 2019). The medium was also investigated: intact powder of lactose monohydrate used for the manufacture of a pharmaceutical substance.

FLUX DENSITY OF THERMAL RADIO EMISSION

The flux density of thermal radio emission in the microwave range was determined using a TES92 instrument (TES Electrical Electronic Corp., Taipei, Taiwan) with the device set to anisotropic measurement along Z axis. The measurement results were recorded as maximum average value of the flux density over a time interval of 300 ms.

The powders or solutions were heated using a solid state thermostat with Peltier elements with control of the sample temperature using a remote laser infrared thermometer. All measurements were taken on the same installation to eliminate the influence of electromagnetic emission scattering due to change in the mutual arrangement of parts of the instrument. The instrument was placed in a room where the distribution of microwave background emission in space and time was controlled with the height, width, and length pitch of 50 cm. The background emission in the experimental room did not exceed 1 $\mu W/m^2$ at all monitoring points with hourly control.

The aqueous solutions were applied in drops of 100 µl to the bottom in the center of sterile 10 cm Petri dishes in the axial direction along Z axis at the distance of 10 mm from the measuring head of the device. The powders were poured in doses of 30 g into sterile Petri dishes 3 cm in diameter with uniform shrinkage.

Each measurement was taken at least 7 times. The standard deviation is shown in plots in the results section. Electric field meter TES92 was calibrated with a relative error of 1 dB and had a low temperature error (0.2 dB in the range from 0°C to 50°C).

RESULTS

During the experiment, BPD containing antibodies for INFγ was compared with the corresponding placebo prepared using the same technology. IL served as a control sample for measurements. In

turn, the measurement of powders was carried out in a strict sequence. First, the IL intrinsic radio thermal emission was measured to establish a reference value. The readings for the powdery IL did not exceed 1 ± 0.5 μW/m^2 in the temperature range from 23 to 37°C, which corresponds to the background values.

BPD with antibodies to κ INFγ shows its intrinsic radio thermal emission of 10±6 μW/m^2 at 23 °C. After the activation by heating to 37°C, the intrinsic radio thermal emission of the preparation reaches 80 ± 7 μW/m^2. We worked for months to determine the reproducibility of the results obtained. It turned out that the flux density of the intrinsic radio thermal emission of activated BPD preparations is always an order of magnitude higher than the corresponding values for placebo and IL (Fig. 1).

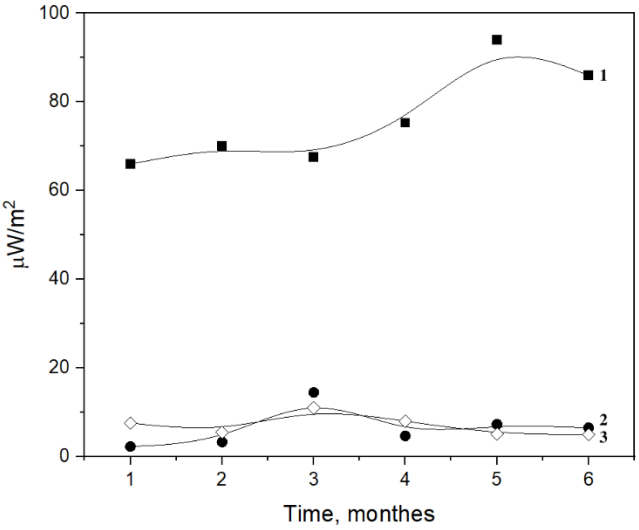

Fig.1 Half-year time curve of reproducibility of results obtained in measurements of intrinsic radio thermal emission of samples, where 1 – BPD, 2 – Placebo, 3 – IL.

We demonstrated the temperature-dependent activation of the intrinsic radio thermal emission of the BPD not only for the powder, but also for the solutions (Fig. 2). It should be emphasized that the thermal activation of BPD is determined by its specific properties. Neither IL nor placebo exhibited the same temperature dependence as the original drug preparation substance.

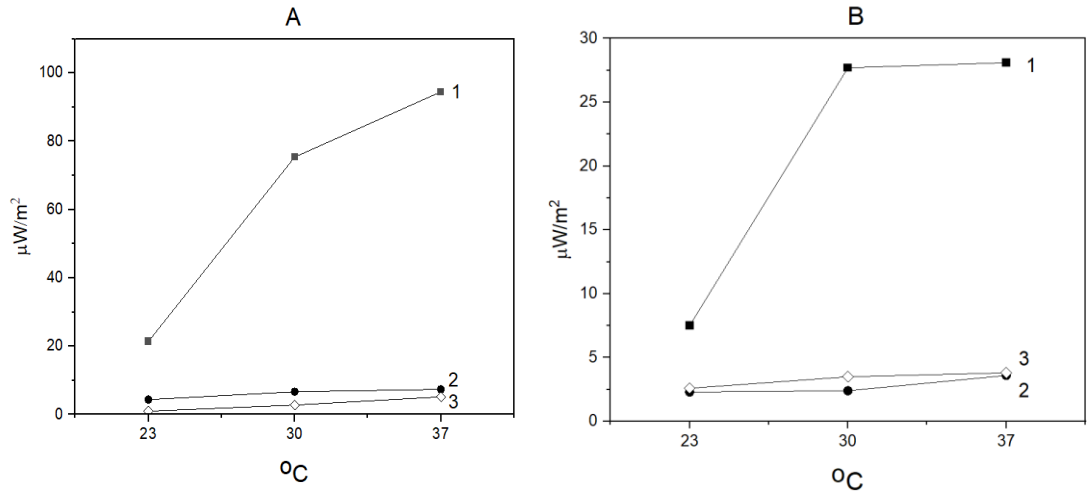

Fig. 2: Temperature dependence of the radio thermal emission of powdery samples (A) and 5% solutions (B), where 1 – BPD, 2 –Placebo, 3 – IL

The intrinsic radio thermal emission of BPD is probably associated with its slow conformational transitions earlier described by other methods (Morozova M.A. 2021, Uspenskaya E.V. 2020, Syroeshkin A.V. 2016, 2023). Indeed, the kinetics of a set of average maximum values develops in the 10-minute range for both powdery substances and aqueous solutions (Fig. 3 A, B). The existence of this effect for aqueous solutions (Fig. 3b) can only be explained

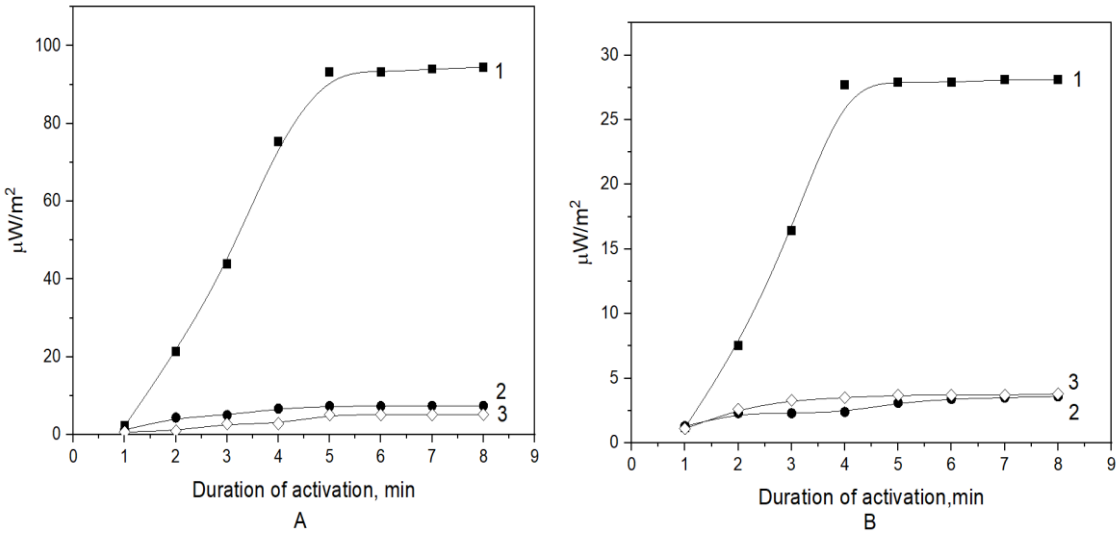

Fig. 3 (A,B) intrinsic radio thermal emission of powdery samples (A) and 5% solutions (B) within the time interval from 1 min to 35 min (1–8 along X axis), where 1 – BPD, 2 – Placebo, 3 – IL.

with taking into account the latest physical and chemical description of real aqueous solutions, for example, the existence of bubstons (Bunkin et al. 2021, Smirnov et al. 2005). In this case, the traditional Debye-Hückel model for diluted aqueous solutions becomes a particular case.

The weight of the sample under study affects the intrinsic radio thermal emission of the BPD (Fig. 4 A, B). Such a weight-volume dependence is associated with increased number of "emitters" in the detection field of the meter. In this measurement, important time points lie in the time interval between the 15thand the 30th minute of the study, corresponding to the detection of signal from the BPD substance heated to 37^{o}C (Fig. 4B).

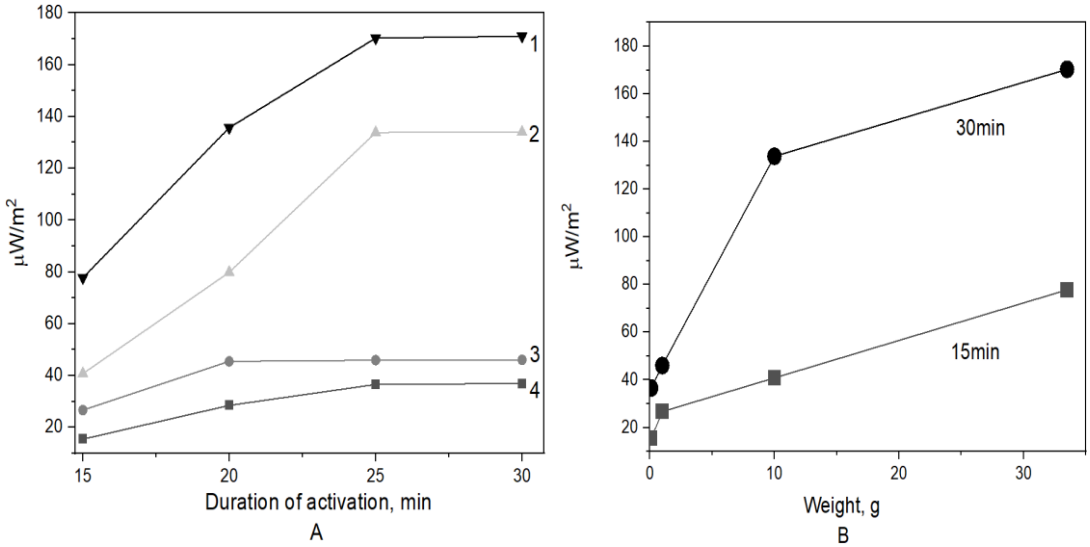

Fig. 4 A) Intrinsic radio thermal emission of the BPD powder sample with different weight as a function of time between 15th and 30th minute of measurement, where 1 – 33 g (full Petri dish, 90mm in diameter), 2 – 10 g, 3 – 1 g, 4 – 0.1 g; B) Comparison of intrinsic radio thermal emission

readings for different weights of the BPD powder sample for 15th minute and for 30th minute of measurement.

The difference in radio thermal emission readings, as expected, occurs with a change of the detected surface area (Fig. 5).

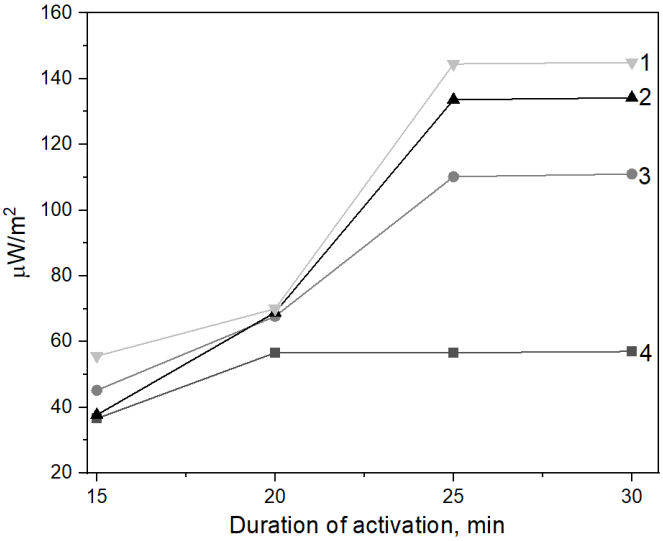

Fig. 5 Comparison of intrinsic radio thermal emission of the BPD powder sample in the time interval from 20 to 35 min, where 1 – 90 mm, completely filled with powder; 2 – 90 mm, half-filled with powder in volume and completely filled in height; 3 – 60 mm, completely filled with powder; 4 – 60 mm half-filled with powder in height.

DISCUSSION

Lactose-based drugs produced in aerosol chambers (fluidized bed chambers) with low levels of antibodies, are among the most sophisticated preparations in terms of authenticity identification and quality control. In such drugs, quasicrystalline supramolecular complexes of lactose with water are formed (Baranova et al. 2022). These supramolecular complexes can be detected by terahertz spectroscopy data, long-term observations in the mid-IR range, biological activity on test cultures (Uspenskaya et al. 2021, Morozova et al. 2021). We assumed that these antibody-induced supramolecular complexes would spontaneously emit in the broadband gigahertz and sub-terahertz ranges due to the formation of thermodynamically “overheated” structures in the aerosol chamber, when a certain part of free surface Gibbs energy is spent on the activation of supramolecular complexes during agglomeration of liquid and amorphous aerosols. In this paper, for the first time for preparations of this class, we propose a method for express quality control in terms of "authenticity". It should be noted that spectral characteristics of the studied drug preparation and the corresponding placebo purpose-made using aerosol chamber are very similar (Syroeshkin A.V. et al. 2018). Therefore, methods of pharmaceutical analysis are very labour-intensive. In this paper, we have demonstrated the elements of validation of the new method:

1) Specificity (differences from placebo and raw materials) – Fig. 1–3,

2) Stability of signal in case of long-term storage of the preparation – Fig. 2,

3) Dependence on the emitter area and weight – Fig. 4, 5.

It is worth mentioning that when the drug preparation is dissolved, the possibility of using the thermal radio emission method to control the specificity of supramolecular complexes in the aqueous solution is preserved (Fig. 2, 3). This is in good agreement with the work (Woods K.

2021) which shows how such aqueous solutions mimic the conformational response of INFγ receptors.

CONCLUSIONS

The developed express method for quality control of BPD based on the measurement of intrinsic radio thermal emission in gigahertz and sub-terahertz ranges can be used for industrial challenges in the field of quality control of products that have been activated in fluidized bed chamber.

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