SLOW QUASIKINETIC CHANGES IN WATER-LACTOSE COMPLEXES DURING STORAGE

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Introduction

For more than ten years among the antiviral drugs duly registered in the State Register of the Russian Federation, there have been several drugs of the so-called release-active forms of antibodies. They (ЛП-N (000035)-(РГ-RU), ЛП-N (000023)-(РГ-RU), ЛП-N (000031)-(РГ-RU)) are widely used in practice for the successful treatment of various infectious diseases not only in Russia but also in many foreign countries. The effectiveness has been proven in preclinical and clinical studies and noted in many publications.

Our previous works were devoted to the study of the influence of sample preparation conditions (grinding, saturation with a solvent, etc.) on the physicochemical characteristics of lactose monohydrate powder, which is used as a carrier of the active substance in drugs with release-active (RA) forms of antibodies (Abs) [1]. The presence of critical differences in the IR spectra, in the spectra within the terahertz region between the sample of intact lactose substance and the powder, which underwent the fluidization procedure in an aerosol chamber, was shown [2].

We substantiated the results obtained by the possibility of the formation of supramolecular structures (SMC) of water-lactose complexes in the amorphous and quasicrystalline state (fig. 1).

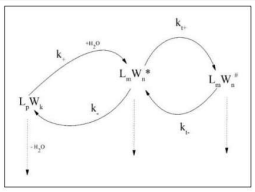

Fig. 1: The formation of a water-lactose complex (SMC) and reversible transitions between the conformational states of the amorphous RA forms of Abs system (L–lactose, W–H_2O, L_mW_n*–RA forms of Abs)

It is noteworthy that all of the above properties disappear upon drying of powdered preparations, which directly indicates the role of water in the formation of release-active SMCs.

In preliminary experiments, we showed that SMCs undergo slow conformational transformations with monthly kinetics. The observed kinetic transitions suggest the existence of a free energy barrier between the states of the supramolecular structure. The process can be compared with the kinetic trapping phenomenon, well studied, and described for protein molecules, associated with conformational differences between the long-lived transitional and stable final states [3, 4].

This paper presents the results of a study of the multi-month kinetics of the supramolecular structure oscillations, that were registered as characteristic changes in the FTIR spectra. Bearing in mind that the energy of saturated supramolecular complexes must undergo hysteresis and trigger conformational transitions (fig. 1), we also used trace elements as a kind of sensors for changes in the dielectric constant inside the SMCs. By analogy with the use of EPR lables in the study of conformational transitions of high molecular systems, we used the method of X-ray fluorescence spectroscopy to track changes in the fluorescence signal at certain wavelengths.

Methodology

The lactose powder we used presented the powder of lactose monohydrate $C_{12}H_{22}O_{11}{\cdot}H_2O$ (Super Tab 30GR, DFE Pharma, Germany), which was saturated with release-active forms of affinity-purified polyclonal rabbit antibodies to recombinant human interferon-gamma using the technology of applying substances in a fluidized bed [1]. Flat-cylindrical tablets were produced from this substance by direct compression after the addition of excipients (microcrystalline cellulose, magnesium stearate) and were not commercially available drugs. RA forms of Abs was produced by the technology of ultra-high dilutions as described in [1] and in the US patent 8 535 664 with the initial substance dilution factor (10^{24}). As a control, we used powdered and tableted placebo samples obtained by the same method, from the same series of lactose and excipients, but only saturated during fluidization with technologically processed (i.e. underwent the same technological dilution process as the antibodies) water (saturation control), technologically processed phosphate-buffered saline (control of antibody solvent) and ethanol (process control).

Table 1: Technological characteristics of powdered samples

Sample	Composition	Loss on drying, %	Flowability±SD*, s per 100 g
1p	Lactose monohydrate	0.20	17.1±0.7
2p	Lactose monohydrate saturated with RA forms of Abs	0.18	15.7±1.0
3p	Lactose monohydrate saturated with technologically processed phosphate-buffered saline	0.16	17.6±4.2
4p	Lactose monohydrate saturated with technologically processed water	0.20	16.5±3.6
5p	Lactose monohydrate saturated with ethanol	0.16	12.9±0.7

*n = 5, SD-standard deviation

Table 2: Technological characteristics of tableted samples

Sample	Composition	Mean mass±SD*, mg	Disintegration±SD**, s
1t	Lactose monohydrate	296.3±3.1	246.7±44.7
2t	Lactose monohydrate saturated with RA forms of Abs, microcrystalline cellulose, magnesium stearate	299.8±4.0	286.7±55.0
3t	Lactose monohydrate saturated with technologically processed phosphate-buffered saline, microcrystalline cellulose, magnesium stearate	302.9±3.2	366.2±68.5
4t	Lactose monohydrate saturated with technologically processed water, microcrystalline cellulose, magnesium stearate	301.5±1.6	339.5±62.6
5t	Lactose monohydrate saturated with ethanol, microcrystalline cellulose, magnesium stearate	301±2.2	270.5±60.3

*n = 10, SD-standard deviation, **n = 6, SD-standard deviation

Kinetic changes in SMC of release-active drugs were studied using an Agilent Cary 630 FTIR spectrophotometer with a diamond ATR accessory (Agilent Technologies, USA). The spectra of the samples were recorded weekly for 6 months. For samples containing only intact lactose, the average spectrum was calculated based on the results of measurements of 6 samples. The spectral data of all saturated lactose samples were further transformed as follows: the corresponding "background"–the spectrum of intact lactose averaged over the results of 6 measurements–was subtracted from the spectrum of each sample. The obtained results–difference spectra–for each sample were averaged over the number of replicates (n = 6) and the standard deviation was calculated. The results were presented graphically in the coordinates "the wavenumber, cm^{-1} – the ratio of the average difference spectrum to the standard deviation spectrum (signal to noise ratio–S/N)". To estimate the frequency of SMC oscillations in powders and tablets for the entire observation period, kinetic curves of the weekly change in the S/N value were plotted at points 1700 (oscillation frequency of the carbonyl group), 3250 and 3450 cm^{-1} (oscillation frequencies of the O-H bond).

An EDX-7000 Shimadzu energy dispersive X-ray fluorescence spectrometer was used in the study. The investigated wavelengths were K Kα 3.313 keV (3.7424 Å); Si Kα 1.740 keV (7.1255 Å). The measurement time was 50 seconds at each wavelength. The results obtained using the XRF method are presented in values of irradiation intensity expressed in cps/μA (cps is the line intensity in standard units (counting per second), μA is the current intensity). For each of 10 samples, 6 spectra were recorded weekly. The kinetic curves of changes in the signal intensity at a certain wavelength were plotted based on averaged values

Results

For 6 months, once a week, we recorded and processed the FTIR spectra of powdered and tableted lactose samples in the infrared range according to the method described above. The differential spectra of the samples presented graphically in the "wave number-S/N" coordinates did not coincide with each other from week to week, which confirms the assumption about the conformational variability of SMCs. Below are some of the obtained kinetic curves of changes in the S/N value for the samples under investigation at points 1700, 3250, and 3450 cm^{-1} – the results of the kinetic study of powders in fig. 4, tablets–in fig. 5.

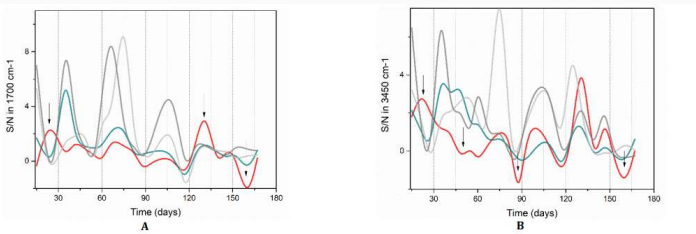

Fig. 4: Kinetics of S/N values for powders at 1700 cm^{-1} (A) and 3450 cm^{-1} (B), red–sample 2p, green–3p, dark gray–4p, gray–5p, n=6. The arrows indicate the time moments when the SMCs of RA forms of Abs exhibit oscillations in antiphase relative to the controls

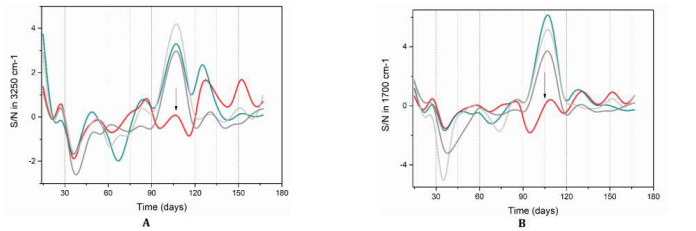

Fig. 5: Kinetics of S/N values for tablets at 1700 cm^{-1}(A) and 3250 cm^{-1} (B), red–sample 2t, green–3t, dark gray–4t, gray–5t, n=6. The arrows indicate the time moments when the SMCs of RA forms of Abs exhibit oscillations in the antiphase relative to the controls

The XRF was used as a marker of the transition of conformational states of the supramolecular complex of the water-lactose conglomerate. The elemental composition of the samples could not change qualitatively and quantitatively during the experiment. However, the kinetics of the fluorescence intensity at certain wavelengths indicates the possibility of a periodic cooperative trigger transition of the system, which was recorded during the study. Thus, according to the data presented in fig. 6, such reversible conformational transitions are observed for powders on the 30th and 130th days (Kα 3.313 keV). For tablets, no pronounced transitions were observed, but at Kα 3.313 keV and Kα 1.740 keV, small changes were visualized just on those days (100–110th day) when hysteresis phenomena were recorded in the IR spectra of these samples (fig. 5).

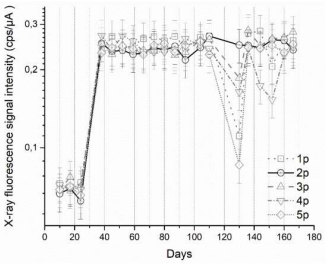

Fig. 6: Kinetics of changes in the intensity of X-ray fluorescence in the studied samples of powders at the wavelength of Kα 3.313 keV (3.7424 Å), n=6

Hysteresis phenomena in water-lactose SMCs illustrated by the results of two spectral methods of analysis are most likely associated with the influence of environmental factors such as variations in relative humidity, temperature, background variations in the level of natural radioactivity, including the flux of thermal neutrons, which can be a source of energy for conformational rearrangements.

Conclusion

It was shown that differential IR spectra of the saturated lactose substance and the finished dosage form revealed kinetic changes reflecting the energy transitions of the supramolecular system and appearing at two-week intervals. The XRF data, where the fluorescence signal was used as a tag of conformational mobility, correlates with the results obtained. At the same time, it was shown that in case of long-term (>10 weeks) observation of changes in the IR spectra, the median S/N values (at 1700, 3250, and 3450 cm^{-1}) of RA forms of Abs are always minimal in comparison with placebo samples for powders and vice versa are maximal for tablets. Thus, the features of kinetic changes confirm that lactose state is changed by saturation with the RA forms of Abs and it should be considered when the product is tested in vitro by different analytical tools.

References

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