In WO 2018/102681, a method of using the transverse relaxation rates of solvent NMR signal to noninvasively assess particle-containing products formulated as suspension or emulsion in solvent(s) is reported.

Hills et al. (Mol. Phys. 67 (1989) 903-918) reported the transverse water proton relaxation in solutions of native bovine serum albumin (BSA).

Feng et al. (Chem. Commun. 51 (2015) 6804) reported that the transverse relaxation rate of water protons can be used to quantify protein aggregation and surfactant micellization in water.

Using human insulin preparations, Taraban et al. (J. Pharm. Sci. 104 (2015) 4132-4141) demonstrate that the transverse relaxation rate of water protons can serve as a reliable and sensitive indicator to detect and quantify both visible and sub-visible protein aggregates.

Taraban et al. (Anal. Chem. 89 (2017) 5494-5502) reported differences in the sensitivity of water NMR and conventional techniques, such as size-exclusion chromatography, microflow imaging and dynamic light scattering, toward detection of the presence of monoclonal antibody aggregates generated by different stresses.

Taraban et al. reported that in-line measurement of water NMR is possible and dependent on flow system configuration, flow rate, protein concentration, and protein aggregation (7th Annual PANIC Conference, 2019, Poster presentation P35: "Water Flow-NMR - A Prospective Contact-Free In-Line Analytical Tool for Continuous Biomanufacturing").

Summary

Herein is reported a method for the determination of the loading status of viral particles in a sample.

The current disclosure is based, at least in part, on the finding that the transverse nuclear magnetic spin relaxation time T_2 and the transverse nuclear magnetic spin relaxation rate R_2, respectively, of protons of water molecules in an aqueous solution comprising viral particles depends on the loading status of the viral particle.

Thus, the current disclosure provides a method for determining the ratio/fraction/concentration of loaded viral particles in a sample,

the method comprising the following steps:

- determining a nuclear magnetic resonance (NMR) parameter related to the protons of the water molecules present in an aqueous solution comprising a mixture of loaded and empty viral particles by applying an NMR measurement to the solution, and

- determining the ratio/fraction/concentration of loaded viral particles with the NMR parameter determined in the previous step using/based on a calibration function.

In one aspect, the current disclosure provides a method for determining the ratio of DNA-containing AAV particles to empty AAV particles in a sample, comprising the following steps:

- determining a nuclear magnetic resonance (NMR) parameter of the protons of the water molecules in the sample, wherein the sample is an aqueous solution comprising a mixture of DNA-containing AAV particles and empty AAV particles, by applying an NMR measurement to the solution, and

- determining the ratio of DNA-containing AAV particles to empty AAV particles with the determined NMR parameter using a calibration function.

In one example, the NMR parameter is indicative of the transverse nuclear magnetic spin relaxation of water in the solution or fraction thereof, specifically of the protons of the water molecules in the aqueous solution.

In one example, the NMR parameter is the transverse nuclear magnetic spin relaxation time T_2 or the transverse nuclear magnetic spin relaxation rate R_2 of the protons of the water molecules in the aqueous solution.

In one example, the isolated, (monomeric) viral particle has a molecular weight in the range of 5,000 kDa to 6,000 kDa. In one preferred example, the isolated, (monomeric) viral particle has a molecular weight of 5,000 kDa to 5,250 kDa.

In one example, the viral particle is a virus, a virus-like particle (VLP), or a liposome. In a further example, the viral particle is a virus or a VLP. In another example, the viral particle is a non-enveloped virus or a VLP of a non-enveloped virus. In one preferred example, the viral particle is an adeno-associated virus particle (AAV particle).

In one example, the loaded viral particles are DNA-containing viral particles. In one preferred example, the loaded viral particles are DNA-containing AAV particles.

In one example, the total concentration of the viral particles in the aqueous solution is at least $1*10^{12}$ vp/mL (viral particles per mL), in one example at least $5*10^{12}$ vp/mL, in one preferred example at least $1*10^{13}$ vp/mL, in one example at least $2*10^{13}$ vp/mL.

In one example, the aqueous solution is a phosphate buffered saline solution. In one preferred example, the aqueous solution is a phosphate buffered saline solution comprising a detergent and optionally citrate.

In one example, the method is for determining the ratio/fraction/concentration of loaded viral particles in a sample, wherein the sample comprises up to 80 % of DNA-containing viral particles, in one preferred example up to 80 % of DNA-containing AAV particles, and the calibration function is a linear function.

In one example, the calibration function is a second order polynomial function.

In one example, the method is for determining the ratio/fraction/concentration of loaded viral particles in a sample, wherein the sample comprises up to 100 % of DNA-containing viral particles, in one preferred example up to 100 % of DNA-containing AAV particles, and the calibration function is a second order polynomial function.

In one example, the calibration function is based on the same NMR parameter determined for at least two (calibration) samples with different ratios of DNA-containing and empty AAV particles.

The method according to the current disclosure is in certain examples an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate, e.g., to producing an aqueous solution comprising (a mixture of) DNA-containing and empty AAV particles, and/or the steps indicated elsewhere herein. Moreover, one or more of said steps may be performed by automated equipment.

The present disclosure also relates to a use of the method according to the present disclosure, for a purpose selected from the group consisting of producing a recombinant AAV particle and release of recombinant AAV particle preparations for therapeutic application, especially real-time release.

Thus, the current disclosure provides the use of the transverse nuclear magnetic spin relaxation time T_2 for the determination of the loading status of viral particles, in one preferred example of AAV particles. In another aspect, the current disclosure provides use of the transverse nuclear magnetic spin relaxation time T_2 for the determination of the loading status of AAV particles.

In one example, the loading status is the ratio of DNA-containing viral particles to empty viral particles.

In one example, the loading status is the fraction of DNA-containing viral particles of the total viral particles.

In one example, the loading status is the concentration of DNA-containing viral particles.

In one example, the loading status is determined in a sample.

In one example, the determination is in an aqueous solution and the transverse nuclear magnetic spin relaxation time T_2 is the transverse nuclear magnetic spin relaxation time T_2 of the protons of the water molecules in the aqueous solution.

In one example, the use is an in vitro use.

In addition to the various examples depicted and claimed, the disclosed subject matter is also directed to other examples having other combinations of the features disclosed and claimed herein. As such, the particular features presented herein, especially presented as aspects or examples, can be combined with each other in other manners within the scope of the disclosed subject matter such that the disclosed subject matter includes any suitable combination of the features disclosed herein. The description of specific examples of the disclosed subject matter has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the disclosed subject matter to those examples disclosed.

Detailed Description of the Invention

Herein is reported a method for determining the ratio/fraction/concentration of DNA-containing AAV particles in a sample, wherein the method comprises the steps of determining the water proton transverse relaxation time (T2) or the water proton transverse relaxation rate (R2), of the protons of the water molecules in an aqueous solution comprising a mixture of DNA-containing and empty AAV particles, by applying an NMR measurement to the solution and thereafter determining the ratio/fraction/concentration of DNA-containing AAV particles with the determined nuclear magnetic spin relaxation based on a calibration function.