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(54) Title: DETECTION OF PHARMACEUTICAL PRODUCT FREEZING HISTORY USING WATER PROTON NMR

(57) Abstract: A method of using the relaxation rate (R_1 and/or R_2) of solvent NMR signal to invasively or noninvasively assess whether vaccines and other aqueous-based pharmaceutical products have been frozen during transport and/or storage.

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DETECTION OF PHARMACEUTICAL PRODUCT FREEZING HISTORY USING WATER PROTON NMR

STATEMENT OF FEDERAL SUPPORT

[0001] This invention was made with government support under Grant No. 01FD005946 awarded by the Food and Drug Administration. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. Provisional Patent Application No. 62/816,436, filed 11 March 2019 and entitled “Noninvasive and Quantitative Detection of Pharmaceutical Product Freezing History Using Water Proton NMR” in the name of Yihua (Bruce) YU et al., which is incorporated herein by reference in its entirety.

FIELD

[0003] The present invention relates to methods for quality control of pharmaceutical products, including drugs and vaccines, using solvent nuclear magnetic resonance (NMR). The methods can be used to determine whether individual pharmaceutical products have been damaged by freezing and should be removed from the distribution stream.

DESCRIPTION OF THE RELATED ART

[0004] Vaccines are critical in combating the spread of infectious diseases worldwide. Vaccines confer resistance to a particular disease by awakening the body's innate immune system through exposure to an antigen, or multiple antigens in the case of combination vaccines, specific to that disease, which in turn causes an adaptive immune system response that is protective in nature (1). In addition to antigens, vaccines often contain adjuvants, or substances that improve the immune response. Adjuvants are beneficial because they often allow the vaccine dose to be lowered. The most commonly used adjuvants are aluminum salts (1), although new adjuvants are currently being developed and licensed, such as squalene-based emulsions and virosomes. The mechanism by which antigens adsorbed onto aluminum salts enhance the immune response is still unclear, but it is proposed that they increase inflammation at the site of injection (1–3). The U.S. Food and Drug Administration (FDA) regulations allow for a maximum of 0.85 mg of Al(III) in the form of aluminum salt per dose to prevent serious irritation at the injection site, but vaccines containing aluminum-adjuvants, first introduced in 1926, have a long history of safety (4).

[0005] Despite their widespread use and critical importance, vaccines, in particular aluminum-adjuvanted vaccines, are thermally unstable outside their recommended storage temperature, either when they are exposed to lower or higher temperatures, or both (5, 6). Their instability is due to their susceptibility to degradation, agglomeration/aggregation, or other structural molecular changes (7). These molecular changes can be observed by several of the same techniques that are used in characterizing the vaccine components during development (e.g., scanning electron microscopy, flow cytometry, dynamic light scattering, microflow imaging, classical spectroscopic methods); however, the vials that are tested are compromised and therefore are no longer usable as vaccines (7–10). Freeze-induced damage to aluminum-adjuvanted vaccines leads to a loss of vaccine potency through a weakened immune response (6, 11). Even a slight potency loss could render a vaccine ineffective in its protective capacity against an infectious disease. For this reason, the temperature of a vaccine during its transportation and shipment, as well as storage prior to use, is carefully monitored and maintained. The World Health Organization presently recommends that all vaccines are stored at 2°C to 8°C (12).

[0006] The supply chain of vaccines and other pharmaceutical products that need to be kept refrigerated is also known as the “cold-chain” or “temperature controlled.” In the last decade, the cold-chain has improved through the use of new technology, such as temperature-sensitive vial container monitors, and new equipment, such as high performance carriers, solar direct-drive refrigerators, and long term cold-boxes (13, 14). When temperature monitoring devices or regular recording protocols observe that a temperature excursion has occurred, procedures are in place for trained personnel to take appropriate action. Despite this, the WHO has reported over 50% vaccine wastage worldwide because of frozen or suspected to be frozen vaccines, and in particular cold-chain lapses, being one of the major reasons for wastage (15, 16).

[0007] The first line of freezing detection is visual observation of ice. In actual cold chain management, if ice is visually observed in vials, then those vials should not be used. There is no ambiguity in such a case. Ambiguity arises when the temperature monitoring device records/reports exposure to subzero temperature, but no ice is seen in any vial. The Center for Disease Control (CDC) issued guidance advising that a batch of vaccines that may have experienced lower than recommended temperatures at any point during shipping and distribution should be separated from other vaccines and labeled “do NOT use.” Personnel are advised to wait for further directions from the distributor or manufacturer (17). Outside the U.S., the World Health Organization (WHO) recommends that the batch in question be tested using “the Shake Test,” a qualitative test whereby a few vials of the batch suspected to have been frozen are pulled and checked to infer whether the whole batch has been frozen. A trained person removes the vaccine labels from the pulled vials and visually compares an intentionally frozen (and then thawed) vial with a pulled vial (i.e., a vial suspected to have been unintentionally frozen). Upon shaking both vials, the frozen vaccine will have characteristic flakes that settle quicker than non-frozen vaccines, which appear more cloudy (18, 19). The benefit of “the Shake Test” is that it does not require any technology and can be performed “in the field” because it is based on freezing-induced

physical changes (7, 20, 21). Disadvantageously, the “Shake Test” requires a trained human observer, the removal of the vaccine label, requires that non-compromised vials have to be frozen, and takes approximately 5-45 minutes (22). It has been said that “[t]he key to deciding whether a shake test has passed or failed is the patience of the observer” (23).

[0008] There is a need for a fast and reliable technique which can be used for quality control in vaccine transport and storage to determine if the sealed vaccines in the distribution chain have been frozen. Towards that end, the present invention relates to a method of using the transverse relaxation rate of the solvent NMR signal, e.g., $R_2(^1\text{H}_2\text{O})$, to determine whether the contents of a filled and sealed pharmaceutical product container have been frozen. Advantageously, the method described herein is easy to use, can be used noninvasively, provides nearly immediate results, and is highly-sensitive.

SUMMARY

[0009] The present invention generally relates to a method of using NMR relaxation rates, specifically the transverse relaxation rate constant R_2 of water to determine if a pharmaceutical product such as a vaccine or a drug has experienced freeze-induced damage during transportation and storage.

[0010] In one aspect, a method of determining if a vaccine or aqueous-based pharmaceutical product has experienced freeze-induced damage is described, said method comprising: measuring the transverse relaxation rate of solvent $R_{2,m}$ in the vaccine or aqueous-based pharmaceutical product; and determining if the vaccine or aqueous-based pharmaceutical product has been frozen by comparing the measured $R_{2,m}$ to a reference transverse relaxation rate of solvent $R_{2,r}$, wherein the reference $R_{2,r}$ represents an acceptable range for non-frozen vaccine or non-frozen aqueous-based pharmaceutical product, wherein when the measured $R_{2,m}$ is inside the reference $R_{2,r}$ range, the vaccine or aqueous-based pharmaceutical product has not experienced freeze-induced damage.

[0011] In another aspect, a method of determining if a vaccine or aqueous-based pharmaceutical product has experienced freeze-induced damage is described, said method comprising: measuring the longitudinal relaxation rate of solvent $R_{1,m}$ in the vaccine or aqueous-based pharmaceutical product; and determining if the vaccine or aqueous-based pharmaceutical product has been frozen by comparing the measured $R_{1,m}$ to a reference longitudinal relaxation rate of solvent $R_{1,r}$, wherein the reference $R_{1,r}$ represents an acceptable range for non-frozen vaccine or non-frozen aqueous-based pharmaceutical product, wherein when the measured $R_{1,m}$ is inside the reference $R_{1,r}$ range, the vaccine or aqueous-based pharmaceutical product has not experienced freeze-induced damage.

[0012] Other aspects, features and advantages of the invention will be more fully apparent from the ensuing disclosure and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 is a photograph of a sealed unopened vial of the VAQTA vaccine drug product with label in two chilled (4°C) NMR tubes as it is lowered the bore of the benchtop NMR.

[0014] Figure 2A is a photo of vials 6-10 of DAPTACEL after three exposures to -18°C, i.e., freezing temperatures, each for a duration of 17-22 hours, tilted on their sides to distinguish which vials froze because the labels obstruct the side view.

[0015] Figure 2B is a photo of vials 6-10 of ENGERIX-B after three exposures to -18°C, i.e., freezing temperatures, each for a duration of 17-22 hours, tilted on their sides to distinguish which vials froze because the labels obstruct the side view.

[0016] Figure 2C is a photo of vials 6-10 of VAQTA after three exposures to -18°C, i.e., freezing temperatures, each for a duration of 17-22 hours, tilted on their sides to distinguish which vials froze because the labels obstruct the side view.

[0017] Figure 2D shows the front label of vial 1 (unexposed to -18°C) and the back label of vial 6 (exposed to -18°C) of each vaccine, illustrating the difficulty distinguishing frozen and unfrozen visually without removing the label. Three vaccines (shown left to right) are: DAPTACEL (Sanofi-Pasteur, 0.5 mL), ENGERIX-B (GlaxoSmithKline, 1.0 mL), and VAQTA (Merck, 1.0 mL).

[0018] Figure 3A illustrates the $R_2(^1\text{H}_2\text{O})$ values for shaken and suspended vaccine vials 1-5 (solid squares) in contrast to $R_2(^1\text{H}_2\text{O})$ values of fully settled/sedimented vaccine vials 1-5 (hollow square) for DAPTACEL. These vials were never exposed to freezing temperatures.

[0019] Figure 3B illustrates the $R_2(^1\text{H}_2\text{O})$ values for shaken and suspended vaccine vials 1-5 (solid squares) in contrast to $R_2(^1\text{H}_2\text{O})$ values of fully settled/sedimented vaccine vials 1-5 (hollow square) for ENGERIX-B. These vials were never exposed to freezing temperatures.

[0020] Figure 3C illustrates the $R_2(^1\text{H}_2\text{O})$ values for shaken and suspended vaccine vials 1-5 (solid squares) in contrast to $R_2(^1\text{H}_2\text{O})$ values of fully settled/sedimented vaccine vials 1-5 (hollow squares) for VAQTA. These vials were never exposed to freezing temperatures.

[0021] Figure 4A illustrates the $R_2(^1\text{H}_2\text{O})$ values of DAPTACEL vaccine suspensions vials 6-10 measured after the third exposure to freezing temperatures (-18°C) overnight (17-22 hours) (black triangles) relative to unstressed vials 1-5 (black squares).

[0022] Figure 4B illustrates the $R_2(^1\text{H}_2\text{O})$ values of ENGERIX-B vaccine suspensions vials 6-10 measured after the third exposure to freezing temperatures (-18°C) overnight (17-22 hours) (black triangles) relative to unstressed vials 1-5 (black squares).

[0023] Figure 4C illustrates the $R_2(^1\text{H}_2\text{O})$ values of VAQTA vaccine suspensions vials 6-10 measured after the third exposure to freezing temperatures (-18°C) overnight (17-22 hours) (black triangles) relative to unstressed vials 1-5 (black squares).

[0024] Figure 5A illustrates the $R_2(^1\text{H}_2\text{O})$ values of DAPTACEL vaccine vials 6 and 9 measured after the first exposure (light grey), second exposure (medium grey), and third exposure (black) to freezing temperatures (-18°C). The upper solid line for DAPTACEL is the average $R_2(^1\text{H}_2\text{O})$ of control vials 1-

5 (unexposed to freezing temperatures) measured 3 times ($2.566 \pm 0.020 \text{ s}^{-1}$). The lower dashed line is the 10-vial average of two settled $R_2(^1\text{H}_2\text{O})$ measurements for each vaccine.

[0025] Figure 5B illustrates the $R_2(^1\text{H}_2\text{O})$ values of ENGERIX-B vaccine vials 9 and 10 measured after the first exposure (light grey), second exposure (medium grey), and third exposure (black) to freezing temperatures (-18°C). The upper solid line for ENGERIX-B is the average $R_2(^1\text{H}_2\text{O})$ of control vials 1-5 (unexposed to freezing temperatures) measured 3 times ($1.245 \pm 0.006 \text{ s}^{-1}$). The lower dashed line is the 10-vial average of two settled $R_2(^1\text{H}_2\text{O})$ measurements for each vaccine.

[0026] Figure 6 illustrates the $R_2(^1\text{H}_2\text{O})$ of frozen/thawed vials of ultrapure water is no different than control vials.

[0027] Figure 7A illustrates $R_2(^1\text{H}_2\text{O})$ of ADJUPHOS unstressed vials 1-3 and freeze/thaw vials 4-6 measured at 25°C after the first (light triangle), second (medium triangle), and third (dark triangle) freeze-thaw cycle in the thawed liquid suspension state. Initial $R_2(^1\text{H}_2\text{O})$ measurements of vials 1-6 (pre-freeze stress) are shown as averages in the suspended adjuvant (solid line) and settled adjuvant (dashed line) states.

[0028] 7B illustrates $R_2(^1\text{H}_2\text{O})$ of ALHYDROGEL unstressed vials 1-3 and freeze/thaw vials 4-6 measured at 25°C after the first (light triangle), second (medium triangle), and third (dark triangle) freeze-thaw cycle in the thawed liquid suspension state. Initial $R_2(^1\text{H}_2\text{O})$ measurements of vials 1-6 (pre-freeze stress) are shown as averages in the suspended adjuvant (solid line) and settled adjuvant (dashed line) states.

[0029] Figure 8A is an image of the visual appearance of vials with 0 freeze-thaw (F/T) (left) relative to vials containing the same emulsion drug with 1 F/T (right).

[0030] Figure 8B illustrates the $R_2(^1\text{H}_2\text{O})$ for both DIPRIVAN and PROPOFOL with 0 F/T and 1 F/T.

[0031] Figure 9A is a photo of an unopened vial of DIPRIVAN.

[0032] Figure 9B illustrates $R_2(^1\text{H}_2\text{O})$ of unopened DIPRIVAN, opened DIPRIVAN, and opened PROPOFOL after 0 F/T and 1 F/T.

DETAILED DESCRIPTION, AND PREFERRED EMBODIMENTS THEREOF

[0033] The present invention generally relates to a method of using NMR relaxation times or rates (longitudinal and transverse relaxation rate constants, R_1 and R_2 , respectively) of solvent molecules, e.g., water, to determine if a pharmaceutical product such as a vaccine or a drug has experienced freeze-induced damage during transportation and storage. If damage is determined, the pharmaceutical product can be removed from the distribution chain to ensure it isn't administered to a patient.

[0034] As defined herein, a "pharmaceutical product" can include vaccines and drugs, including biologics and small molecules (i.e., non-biologics), in an aqueous medium. The pharmaceutical products can further comprise at least one adjuvant, at least one surfactant, at least one water-soluble organic solvent, at least one dispersant, at least one biocide, at least one buffering agent, at least one pH

adjusting agent (e.g., acids and/or bases), at least one peptide, at least one polypeptide, at least one protein, at least one antibody or fragment thereof, at least one nucleic acid, at least one oil, or any combination thereof, as readily determined by the person skilled in the art. In one embodiment, the pharmaceutical product comprises an emulsion. Notably, the methods described herein are preferentially used with pharmaceutical products that include in the instructions that the product should not be frozen prior to administration to a patient. It should be appreciated that a patient includes any human or other mammal, reptile, bird, fish, or amphibian.

[0035] For the purposes of the present application, reference will be made to vaccines comprising aluminum adjuvants, and emulsion drugs, however the method is not limited to vaccines and emulsion drugs and is intended to be extended to any pharmaceutical product as defined herein.

[0036] The prior art methods of determining if a vaccine has been exposed to freezing conditions, e.g., visual inspection and/or the Shake Test, are difficult and often impractical. To visually observe coloration, density, or sedimentation variations in a small vial containing volumes of 0.5 mL (e.g., most children's vaccines) and 1 mL (e.g., most adult vaccines) is not objectively reasonable, even when the label has been removed.

[0037] Further, vaccines are known to be homogenous, turbid, white suspensions when shaken before use. Package inserts for vaccines typically include instructions to visually inspect the vials containing the vaccines for particulate matter and discoloration prior to administration, whenever solution and container permit. It is possible to look from the bottom of the vial, but in the absence of an obviously large particle or discoloration, this visual observation at the point of administration would be very difficult to detect anything more than enormous changes or irregularities.

[0038] The method described herein is a reliable and simple method to assess whether vaccines and other pharmaceutical products were frozen during transportation and/or storage of same (i.e., while in the cold-chain). The method enables the assessment of the vaccines and other pharmaceutical products, formulated as aqueous suspensions, without the requirement of opening the vial or product container, without peeling off the label on the vial, and without waiting for a sedimentation time course. The method is quantitative and comprises measuring the nuclear spin relaxation rate constant, R_1 and/or R_2 , of solvent, e.g., water, as a quality control parameter. It is known that freeze-induced damage to vaccines leads to a loss of vaccine potency and even a slight potency loss can render a vaccine ineffective in its protective capacity against an infectious disease. In the present disclosure, freeze-induced damage to the vaccines and other pharmaceutical products is evidenced by a quantitative variation (either an increase or a decrease) of the nuclear spin relaxation rate constant, R_1 and/or R_2 , of solvent, e.g., water. The R_1 and/or R_2 constant of the non-frozen vaccine, or preferentially an acceptable range of R_1 and/or R_2 constants of said non-frozen vaccine, can be determined by the manufacturer. The R_1 and/or R_2 constant (or range of R_1 and/or R_2 constants) can be provided in the package insert, on the vial label, or both. Thereafter, the vaccine is released for sale and purchase and enters the "cold-chain," whereby it is transported and/or stored, and may be exposed to freezing temperatures. The R_1 and/or

R_2 constant of water can be measured by the transporter or distributor or the purchaser/user before use to confirm that the vaccine has not been frozen. If the measured R_1 and/or R_2 constant (or range of R_1 and/or R_2 constants) of the vaccine is outside of the reference range provided by the manufacturer, meaning that the contents may have been frozen, the specific vial can be rejected.

[0039] Recent breakthrough developments in the instrumentation for nuclear magnetic resonance (NMR) spectroscopy and imaging have opened up opportunities to design novel analytical techniques for the nanoparticle industry. Of special importance was the introduction of commercially available, relatively inexpensive benchtop and handheld NMR and magnetic resonance imaging (MRI) instruments and relaxometers (24). Benchtop NMR instruments enable highly accurate measurements of nuclear spin relaxation times T_1 and T_2 . Moreover, most of these instruments have a permanent or electronically cooled magnet with the variable bore from 10 mm to 45 mm and even larger which provides a great flexibility in the measurements of vials of various sizes.

[0040] Water proton NMR (w NMR) monitors water, which acts as a reporter for analytes dissolved in it. As a reporter, water has two tremendous advantages. First, its concentration far surpasses that of any analyte dissolved in it, by $10^3 - 10^6$ fold in most cases. This makes the $^1\text{H}_2\text{O}$ signal easily detectable by benchtop and handheld NMR instruments. Further, the solute association can be detected through the solvent NMR signal. In addition, water is “endogenous” to all biomanufacturing processes and all pharmaceutical products, including vaccines and drugs. This sets it apart from “exogenous” reporters such as fluorescent dyes or radiotracers. The high concentration of “endogenous” water make it possible for w NMR to be contact-free *in situ*.

[0041] The essence of w NMR is a consistency check, which makes it useful for the pharmaceutical industry, where consistency is both critical and expected. For example, as described herein, w NMR can be used to determine if the contents of the pharmaceutical, e.g., vaccine, vials have been frozen during transportation and/or storage. With the knowledge provided by the present invention, accidentally frozen pharmaceutical products can be identified with certainty, thus protecting patients from ineffective or harmful products. Additionally, since the present invention can be used non-invasively, the unnecessary waste of non-frozen pharmaceutical products in a batch exposed to freezing temperatures can be prevented.

[0042] As defined herein, the “aluminum-containing product” includes a product with nano- and micron-sized alum particles suspended in a solvent or a mixture of solvents. The aluminum-containing product can further comprise at least one surfactant, at least one water-soluble organic solvent, at least one dispersant, at least one biocide, at least one buffering agent, at least one pH adjusting agent (e.g., acids and/or bases), at least one oil, with or without antigens, or any combination thereof, as readily determined by the person skilled in the art. As is readily understood by the person skilled in the art, an aluminum-containing product includes many vaccines wherein the aluminum is present as an adjuvant.

[0043] As defined herein, a “vial” corresponds to a container, vessel, bottle, syringe, injection pen, or ampoule used to store the vaccine or pharmaceutical product, wherein the vial comprises glass, plastic,

ceramic, rubber, elastomeric material, and/or anything non-magnetic metal. The vial can have a screw top, a top that is closed using a cork or plastic stopper, a crimp vial (closed with a rubber stopper and a metal cap), a flip-top or snap cap. The vial can be tubular, or have a bottle-like shape with a neck. Other types and shapes of vials used to store particles as well as caps are readily understood by the person skilled in the art. The vials can be optically transparent or not optically transparent. There is no need to peel off any label on the vial, whether the label is transparent or not.

[0044] As defined herein, a “non-destructive” measurement is defined as a measurement performed without opening the vial or otherwise accessing, harming, or altering the contents of the vial (for example by withdrawing a portion through a rubber gasket). Moreover, a non-destructive measurement means that no additives or probes or the like are added to the vial prior to the measurement of the transverse relaxation rate of water R_2 in the vaccine or other pharmaceutical product. Non-destructive also means that there is no need to make the vials optically transparent and no need to peel off any labels on the vials.

[0045] The pharmaceutical products will have a freezing point that varies depending on the constitution of the specific product. That said, as will be discussed, two vials in the same batch exposed to the same temperature can yield different results whereby one may freeze and one may remain unfrozen at the same temperature. It is understood by the person skilled in the art that to “freeze” means to convert a liquid into a solid as a result of exposure to temperatures below a freezing point. For the purposes of this disclosure, to have been previously “frozen” corresponds to a product having a $T_2(^1\text{H}_2\text{O})$, $T_1(^1\text{H}_2\text{O})$, $R_2(^1\text{H}_2\text{O})$, or $R_1(^1\text{H}_2\text{O})$ value that is statistically different than the non-frozen control. For the purposes of this disclosure, “experienced freeze-induced damage” is intended to mean that the product has been frozen, intentionally or accidentally. A frozen product should be discarded immediately. Unexpectedly, just because the temperature of the vaccine or pharmaceutical product was for some period of time excursed below the freezing point for the specific product does not mean that the product has been frozen.

[0046] As understood by the person skilled in the art, refrigeration temperatures are in a range from about 2°C to about 8°C.

[0047] As defined herein, the aluminum in an aluminum-containing product comprises one or more of aluminum hydroxide, aluminum phosphate, aluminum sulfoxylphosphate, aluminum hydroxyphosphate sulfate, alum ($\text{KAl}(\text{SO}_4) \cdot 12\text{H}_2\text{O}$), as well as other known or proprietary aluminum salts that can be used as aluminum adjuvants in vaccines or in pharmaceutical products comprising aluminum.

[0048] The present inventors have surprisingly discovered that solvent NMR can be used to detect if vaccines and other pharmaceutical products in filled and sealed vials have been frozen upon exposure to freezing temperatures. The manufacturer as well as transporters, distributors, commercial end users, and researchers can use solvent NMR to inspect the vaccines and pharmaceutical products for freeze-induced damage. Advantages of low field solvent NMR includes low cost instrumentation (e.g., a benchtop, desktop, or hand-held NMR), simple and rapid data acquisition and analysis, and minimal

technical expertise requirement whereby the results are readily available within < 1 min. In a preferred embodiment, the method of using solvent NMR to inspect vaccines and pharmaceutical products for freeze-induced damage is non-invasive. It should be appreciated that the measurements can occur destructively as well, whereby the vial is opened, if needed. Further, the method described herein can utilize high field NMR, if needed and can be used in continuous-flow manufacturing processes. In addition, the methods described herein can be used with an originator pharmaceutical product or reference listed drug (RLD) or a follow-on version thereof.

[0049] It was discovered that once a vaccine has been frozen at least once, after thawing, the $R_2(^1\text{H}_2\text{O})$ value can be significantly different, as much as $\sim 50\%$ lower, relative to a non-frozen control. Interestingly though, the method described herein also revealed that exposing a vial of vaccine to freezing temperatures (e.g., -18°C , 15+ hrs) does not necessarily lead to a frozen product. Some vaccines can be exposed to freezing temperatures and not be frozen per se, and thus there is negligible or no change in the $R_2(^1\text{H}_2\text{O})$ value relative to a non-frozen control.

[0050] In practice, the manufacturer can provide the acceptable $R_2(^1\text{H}_2\text{O})$ or $R_1(^1\text{H}_2\text{O})$ range in sec^{-1} , e.g., a control or reference range, for the non-frozen vaccine or pharmaceutical product at refrigeration temperatures (e.g., about 2°C to about 8°C) and specific magnetic field strength (s) (e.g., 0.5 T). The user will then measure the $R_2(^1\text{H}_2\text{O})$ or $R_1(^1\text{H}_2\text{O})$ of the vaccine or pharmaceutical product at the same temperature and magnetic field strength and compare the measured $R_2(^1\text{H}_2\text{O})$ or $R_1(^1\text{H}_2\text{O})$ value with the manufacturer-specified acceptable range of $R_2(^1\text{H}_2\text{O})$ or $R_1(^1\text{H}_2\text{O})$, i.e., reference, as understood by the person skilled in the art, to determine if the vaccine or pharmaceutical product experienced freeze-induced damage.

[0051] Accordingly, in a first aspect, a method of determining if a vaccine or other aqueous-based pharmaceutical product has experienced freeze-induced damage is described, said method comprising: measuring the transverse relaxation rate of solvent $R_{2,m}$ in the vaccine or aqueous-based pharmaceutical product; and determining if the vaccine or aqueous-based pharmaceutical product has been frozen by comparing the measured $R_{2,m}$ to a reference transverse relaxation rate of solvent $R_{2,r}$, wherein the reference $R_{2,r}$ represents an acceptable range for non-frozen vaccine or aqueous-based pharmaceutical product, wherein when the measured $R_{2,m}$ is inside the reference $R_{2,r}$ range, the vaccine or aqueous-based pharmaceutical product has not experienced freeze-induced damage. In one embodiment, the pharmaceutical product comprises biologics or small molecules. In another embodiment, the vaccines or pharmaceutical products comprise at least one component selected from the group consisting of at least one adjuvant, at least one surfactant, at least one water-soluble organic solvent, at least one dispersant, at least one biocide, at least one buffering agent, at least one pH adjusting agent, at least one peptide, at least one polypeptide, at least one protein, at least one antibody or fragment thereof, at least one nucleic acid, at least one oil, and any combination thereof. The transverse relaxation rate of solvent R_2 can be determined using solvent NMR, preferably low field solvent NMR. Preferably, the measuring

of the transverse relaxation rate of solvent R_2 in the vaccine or pharmaceutical product can be done non-invasively in a vial, but it should be appreciated that the measurement can be done invasively as well, as readily understood by the person skilled in the art. The reference $R_{2,r}$ range, at a specified temperature and magnetic field strength, can be measured by the manufacturer and the result listed in the package insert and/or the vial of the vaccine or pharmaceutical product. Preferably $R_{2,m}$ is measured at substantially the same temperature and magnetic field strength as $R_{2,r}$. The distributor or purchaser can then use NMR, e.g., benchtop or handheld, to measure $R_{2,m}$ at the specified temperature and magnetic field strength and compare it with the reference $R_{2,r}$ range listed in the package insert or vial before distribution or usage. If the measured $R_{2,m}$ is inside the reference $R_{2,r}$ range, the vaccine or aqueous-based pharmaceutical product has not experienced freeze-induced damage, and as such can be distributed or used.

[0052] It should be appreciated that the method of the first aspect can be based on the water proton transverse relaxation time T_2 , instead of the rate R_2 , as readily determined by the person skilled in the art. In other words, when water is the solvent, the manufacturer provides $T_2(^1\text{H}_2\text{O})$ reference values for the acceptable range for non-frozen vaccine or aqueous-based pharmaceutical product and the measured $T_2(^1\text{H}_2\text{O})$ of the vaccine or aqueous-based pharmaceutical product is compared to the $T_2(^1\text{H}_2\text{O})$ reference values. The transverse relaxation time $T_2 (= 1/R_2)$ value can be extracted by fitting experimental data to Formula (1):

$$I(t) = I_0 \times \exp(-t/T_2) \quad (1)$$

where $I(t)$ is the $^1\text{H}_2\text{O}$ signal intensity at time t , I_0 is the initial $^1\text{H}_2\text{O}$ signal intensity when $t = 0$, and t is the T_2 delay time.

[0053] In a second aspect, a method of determining if a vaccine or other aqueous-based pharmaceutical product has experienced freeze-induced damage is described, said method comprising: measuring the longitudinal relaxation rate of solvent $R_{1,m}$ in the vaccine or aqueous-based pharmaceutical product; and determining if the vaccine or aqueous-based pharmaceutical product has been frozen by comparing the measured $R_{1,m}$ to a reference longitudinal relaxation rate of solvent $R_{1,r}$, wherein the reference $R_{1,r}$ represents an acceptable range for non-frozen vaccine or aqueous-based pharmaceutical product, wherein when the measured $R_{1,m}$ is inside the reference $R_{1,r}$ range, the vaccine or aqueous-based pharmaceutical product has not experienced freeze-induced damage. In one embodiment, the pharmaceutical product comprises biologics or small molecules. In another embodiment, the vaccines or pharmaceutical products comprise at least one component selected from the group consisting of at least one adjuvant, at least one surfactant, at least one water-soluble organic solvent, at least one dispersant, at least one biocide, at least one buffering agent, at least one pH adjusting agent, at least one peptide, at least one polypeptide, at least one protein, at least one antibody or fragment thereof, at least one nucleic acid, at least one oil, and any combination thereof. The longitudinal relaxation rate of solvent R_1 can be determined using solvent NMR, preferably low-field solvent NMR. Preferably, the measuring of the longitudinal relaxation rate of solvent R_1 in the vaccine or pharmaceutical product can

be done non-invasively in a vial, but it should be appreciated that the measurement can be done invasively as well, as readily understood by the person skilled in the art. The reference $R_{l,r}$ range, at a specified temperature and magnetic field strength, can be measured by the manufacturer and the result listed in the package insert and/or the vial of the vaccine or pharmaceutical product. Preferably $R_{l,m}$ is measured at substantially the same temperature and magnetic field strength as $R_{l,r}$. The distributor or purchaser can then use NMR, e.g., benchtop or handheld, to measure $R_{l,m}$ at the specified temperature and magnetic field strength and compare it with the reference $R_{l,r}$ range listed in the package insert or vial before distribution or usage. If the measured $R_{l,m}$ is inside the reference $R_{l,r}$ range, the vaccine or aqueous-based pharmaceutical product has not experienced freeze-induced damage, and as such can be distributed or used.

[0054] It should be appreciated that the method of the second aspect can be based on the water proton longitudinal relaxation time T_1 , instead of the rate R_1 , as readily determined by the person skilled in the art. In other words, when water is the solvent, the manufacturer provides $T_1(^1\text{H}_2\text{O})$ reference values for the acceptable range for non-frozen vaccine or aqueous-based pharmaceutical product and the measured $T_1(^1\text{H}_2\text{O})$ of the vaccine or aqueous-based pharmaceutical product is compared to the $T_1(^1\text{H}_2\text{O})$ reference values. The longitudinal relaxation time $T_1 (= 1/R_1)$ can be extracted by fitting experimental data to Formula (2) if the inversion recovery pulse sequence was used:

$$I(t) = I_0 \times [1 - 2 \cdot \exp(-t/T_1)] \quad (2)$$

or by fitting experimental data to Formula (3) if the saturation recovery pulse sequence was used:

$$I(t) = I_0 \times [1 - \exp(-t/T_1)] \quad (3)$$

where $I(t)$ is the $^1\text{H}_2\text{O}$ signal intensity at time t , I_0 is the $^1\text{H}_2\text{O}$ signal intensity when the signal is fully recovered, and t is the T_1 recovery time. The person skilled in the art will readily determine the situations where one of the above listed pulse sequences could be beneficially used to extract reliable values of T_1 .

[0055] The present inventors have thus disclosed a quality control technology using solvent NMR to determine if vaccines and other pharmaceutical products have been frozen and hence should be removed from the distribution stream because they may have a reduced potency and/or may be dangerous to a patient. The method described herein allows for the manufacturer and/or distributor and/or end user to monitor for freeze-induced damage during transport and/or storage. This can be done without opening the vial, i.e., non-invasively, or peeling off the label. Further advantages include the applicability of the method for both anti-bacterial and anti-viral vaccines, and any aqueous-based pharmaceutical products as defined herein.

[0056] As introduced herein, vaccines can be rendered thermally unstable when they are exposed to higher temperatures as well. Accordingly, in a third aspect, a method of determining if a vaccine or other aqueous-based pharmaceutical product has experienced heat-induced damage is described, said method comprising: measuring the relaxation rate of water R_m in the vaccine or aqueous-based

pharmaceutical product; and determining if the vaccine or aqueous-based pharmaceutical product has been heated by comparing the measured R_m to a reference relaxation rate of water R_r , wherein the reference R_r represents an acceptable range for non-heated vaccine or aqueous-based pharmaceutical product, wherein when the measured R_m is inside the reference R_r range, the vaccine or aqueous-based pharmaceutical product has not experienced heat-induced damage. In one embodiment, the pharmaceutical product comprises biologics or small molecules. In another embodiment, the vaccines or pharmaceutical products comprise at least one component selected from the group consisting of at least one adjuvant, at least one surfactant, at least one water-soluble organic solvent, at least one dispersant, at least one biocide, at least one buffering agent, at least one pH adjusting agent, at least one peptide, at least one polypeptide, at least one protein, at least one antibody or fragment thereof, at least one nucleic acid, at least one oil, and any combination thereof. The relaxation rate of water R_1 or R_2 can be determined using solvent NMR, preferably low field solvent NMR. Preferably, the measuring of the relaxation rate of water R_1 or R_2 in the vaccine or pharmaceutical product can be done non-invasively in a vial, but it should be appreciated that the measurement can be done invasively as well, as readily understood by the person skilled in the art. The reference R_r range, at a specified temperature and magnetic field strength, can be measured by the manufacturer and the result listed in the package insert and/or the vial of the vaccine or pharmaceutical product. Preferably R_m is measured at substantially the same temperature and magnetic field strength as R_r . The distributor or purchaser can then use NMR, e.g., benchtop or handheld, to measure R_m at the specified temperature and magnetic field strength and compare it with the reference R_r range listed in the package insert or vial before distribution or usage. If the measured R_m is inside the reference R_r range, the vaccine or aqueous-based pharmaceutical product has not experienced heat-induced damage, and as such can be distributed or used.

[0057] It should be appreciated that the method of the third aspect can be based on the water proton transverse relaxation time T_2 , instead of the rate R_2 , as readily determined by the person skilled in the art. In other words, when water is the solvent, the manufacturer provides $T_2(^1\text{H}_2\text{O})$ reference values for the acceptable range for non-heat-induced damaged vaccine or aqueous-based pharmaceutical product and the measured $T_2(^1\text{H}_2\text{O})$ of the vaccine or aqueous-based pharmaceutical product is compared to the $T_2(^1\text{H}_2\text{O})$ reference values. Further, the method of the third aspect can be based on the water proton longitudinal relaxation time T_1 , instead of the rate R_1 , as readily determined by the person skilled in the art. In other words, when water is the solvent, the manufacturer provides $T_1(^1\text{H}_2\text{O})$ reference values for the acceptable range for non-heat-induced damaged vaccine or aqueous-based pharmaceutical product and the measured $T_1(^1\text{H}_2\text{O})$ of the vaccine or aqueous-based pharmaceutical product is compared to the $T_1(^1\text{H}_2\text{O})$ reference values.

[0058] For the purposes of this disclosure, to have been rendered “thermally unstable” corresponds to a product having a $R_2(^1\text{H}_2\text{O})$ or $R_1(^1\text{H}_2\text{O})$ (or $T_2(^1\text{H}_2\text{O})$ or $T_1(^1\text{H}_2\text{O})$) value that is statistically different than the control that has not been rendered thermally unstable. For the purposes of this disclosure,

“experienced heat-induced damage” is intended to mean that the storage temperature of the product has been increased, intentionally or accidentally, above a storage temperature designated as suitable for storage of the product. For example, for vaccines that are intended to be stored at refrigeration temperatures of about 2°C to about 8°C, an increase in storage temperature above 8°C may render the product thermally unstable. Similar to the freezing results discussed herein, it should be appreciated that just because the temperature of the vaccine or pharmaceutical product was for some period of time excused above the refrigeration temperature for the specific product does not mean that the product has necessarily been rendered thermally unstable.

[0059] The features and advantages of the invention are more fully shown by the illustrative examples discussed below.

Example 1

[0060] The transverse relaxation rate of the water proton NMR signal, $R_2(^1\text{H}_2\text{O})$, of aluminum-containing vaccines exposed to a freeze-thaw cycle has been studied.

VACCINES TESTED

[0061] Three different vaccines were tested. On each of these labels, it is specifically stated, “do not freeze. If product has been frozen, discard and do not use.” Each vaccine has a different aluminum adjuvant, 0.5 mg Al(III) per dose/vial (0.5 mg/mL) for ENGERIX®-B (GlaxoSmithKline) as aluminum hydroxide, 0.33 mg Al(III) per dose/vial (0.66 mg/mL) for DAPTACEL® (Sanofi-Pasteur) as aluminum phosphate, and 0.45 mg Al(III) per dose/vial (0.45 mg/mL) for VAQTA® (Merck) as amorphous aluminum hydroxyphosphate sulfate. Each carton contained 10 vaccine vials and were labeled 1-10.

STORAGE AND MEASUREMENT PROCEDURES

[0062] Vaccines were stored according to their package insert instructions. All three vaccines required storage at 2-8°C. All vaccine cartons were stored in a 4°C miniature refrigerator, located in the 18°C room directly next to the benchtop NMR. The first four days of measurement, slight variations in the data were noticed, which were thought to be due to temperature variations due to the refrigerator cycling on and off as well as the refrigerator door opening and closing over the course of the measurements. Thereafter, the storage of the vials was adapted such that the cartons of vaccines were kept in temperature pre-equilibrated Styrofoam insulation boxes with lids, which were kept in the refrigerator. This helped to maintain a steady temperature of the vaccines throughout the course of measuring the 30 vials one at a time.

[0063] The $R_2(^1\text{H}_2\text{O})$ in sec^{-1} of each vaccine vial was measured at 0.56 T (23.8 MHz ^1H resonance frequency, Oxford Instruments MQC+ equipped with a PRO 1193 probe) at approximately 4°C. To measure the transverse relaxation rate constant R_2 , Carr-Purcell-Meiboom-Gill (CPMG) experiments

were used. It should be appreciated by the person skilled in the art that there are other methods to determine R_2 and the use of CPMG in the examples described herein are not intended to limit the determination of R_2 . Measurements of $R_2(^1\text{H}_2\text{O})$ in sec^{-1} were performed noninvasively, without opening the vial, since the wide bore (26 mm ID) of the probe of the low-field NMR spectrometer permitted the accommodation of the vial without drawing a portion of the sample and transferring it into standard NMR tube (Figure 1).

[0064] The order in which the vials were measured within a box was rotated with every day of measurement to account for temperature differences throughout the course of the experiment as well as operator flow. Measurements were collected for three vials in a row followed by a six minute wait so that the glass NMR tubes could be re-cooled to 4°C .

[0065] It should be noted that the precautions disclosed herein were taken to ensure quality of measurement to show the efficacy of the method disclosed herein. It should be appreciated by the person skilled in the art that the precautions are merely illustrative and are not intended to limit the method of determining if a vaccine or aqueous-based pharmaceutical product has experienced freeze-induced damage in any way.

FREEZING PROCEDURE

[0066] Five of the ten vials of each vaccine product brand were frozen overnight for 17-22 hours (vials 6-10), while the other five (vials 1-5) were kept in the refrigerator at 4°C as a control. The five vials that were frozen were exposed to freezing temperatures -17°C to -19°C followed by thawing at 4°C for a total of three times. After each thawing, the $R_2(^1\text{H}_2\text{O})$ was determined using a benchtop NMR for all ten vials, wherein vials 1-5 are the suspended, non-frozen control and vials 6-10 are the frozen vials. For the first freeze, vials 6-10 of the three vaccines (15 vials total) were set on the freezer floor, spaced at least two inches apart from each other and from anything else in the freezer. The three vaccines were then thawed in the refrigerator at 4°C . It was unexpected to have some vials of the same drug product not freeze while others froze. For the second freeze, the procedure was repeated except that this time all the vials to be frozen were shaken just as they were being placed in the freezer one by one. Additionally, to prevent any possible uneven freezing by touching the freezer floor, where there might be freeze coils beneath the floor, the vials were set on top of Styrofoam box lids. All the vials were spaced similarly to the first freezing. After each exposure to freezing temperatures and prior to thawing, visual observations of the physical state of the vaccine (solid frozen or liquid non-frozen) were written down. For the third freezing, all of the vials were unshaken/settled and had been undisturbed for 72 hours days prior to exposure to freezing temperatures.

[0067] Photos of the three vaccines after three exposures to -18°C , i.e., freezing temperatures, are shown in Figure 2, wherein Figure 2A is DAPTACEL, Figure 2B is ENERGIX-B, and Figure 2C is VAQTA. All vials numbered 6-10 of each vaccine were exposed to -18°C temperatures three separate times standing upright, each for a duration of 17-22 hours. They were tilted on their sides for the photos

to distinguish which vials froze because the labels obstruct the side view. After the third exposure to freezing temperatures, it appeared that DAPTACEL #9 did not freeze (Figure 2A), all of the ENGERIX-B vials froze (Figure 2B), and none of the VAQTA vials froze (Figure 2C). Vials were subsequently thawed at 4°C for at least 1.5 hours prior to $R_2(^1\text{H}_2\text{O})$ measurement at 4°C. Vial numbers are marked with a circle if they were frozen after the third exposure to -18°C. The circle is dashed if the vial did not freeze at either the first and/or second exposure to -18°C. Vials that did not freeze have no circle. Figure 2D shows the front label of vial 1 (unexposed to -18°C) and the back label of vial 6 (exposed to -18°C), illustrating the difficulty distinguishing frozen and unfrozen visually without removing the label. Out of necessity, the WHO Shake Test often requires labels to be removed, for example because a children's single dose vaccine is a low volume, only 0.5 mL, which would make it difficult to observe visual differences otherwise.

SETTLED PRODUCT MEASUREMENTS

[0068] All 30 vials were undisturbed for 1-2 weeks in a styrofoam 15 mL tube holder tray at 4°C in order to let the suspension particles settle. For these NMR measurements, the same NMR procedure as the vaccine monitoring and freeze/thaw experiments were followed with a few changes to minimize any disturbances to the contents of the vials. For each experiment, the two chilled glass NMR tubes were placed one inside the other and lowered into the NMR bore. Means for clasping the vial necks were attached to each vial (e.g., a string tied into a lasso) and the vial was carefully placed at the mouth of the glass NMR tubes inside the instrument and carefully lowered into the tubes inside the benchtop NMR instrument. The $R_2(^1\text{H}_2\text{O})$ was determined and the vials were then returned to the 4°C environment.

MEASUREMENTS OF VIALS EXPOSED TO FREEZING CONDITIONS

[0069] Figures 3A-3C illustrate the $R_2(^1\text{H}_2\text{O})$ of shaken and suspended vaccine vials 1-5 (solid squares) shown in contrast to $R_2(^1\text{H}_2\text{O})$ of fully settled/sedimented vaccine vials 1-5 (hollow square) for DAPTACEL (Figure 3A), ENGERIX-B (Figure 3B), and VAQTA (Figure 3C). These vials were never exposed to freezing temperatures. The average $R_2(^1\text{H}_2\text{O})$ for the control vials 1-5 (solid squares) from this third measurement, with standard deviation of 5 vials, was $2.541 \pm 0.016 \text{ s}^{-1}$ for DAPTACEL, $1.239 \pm 0.002 \text{ s}^{-1}$ for ENGERIX-B, and $3.238 \pm 0.011 \text{ s}^{-1}$ for VAQTA. The average $R_2(^1\text{H}_2\text{O})$ of the settled vaccine vials 1-5 (hollow squares) with standard deviation of 3 measurements for each of the 5 vials, was $0.653 \pm 0.023 \text{ s}^{-1}$ for DAPTACEL, $0.687 \pm 0.026 \text{ s}^{-1}$ for ENGERIX-B, and $0.690 \pm 0.035 \text{ s}^{-1}$ for VAQTA.

[0070] $R_2(^1\text{H}_2\text{O})$ of vaccine suspensions measured after the third exposure to freezing temperatures (-18°C) are shown in Figure 4A (DAPTACEL), Figure 4B (ENGERIX-B), and Figure 4C (VAQTA). Vials 6-10 (black triangles) were stressed, meaning they were exposed to -18°C overnight (17 - 22 hours), subsequently brought to 4°C, and then the $R_2(^1\text{H}_2\text{O})$ measured. This exposure to freezing

temperatures and $R_2(^1\text{H}_2\text{O})$ measurement cycle occurred three times. The data shown is from the third $R_2(^1\text{H}_2\text{O})$ measurement. The data for the control vials 1-5 (non-frozen, solid line) from this third measurement, with standard deviation of 6 measurements, as well as the average $R_2(^1\text{H}_2\text{O})$ for the settled vials (dashed lines) with standard deviation of 3 measurements for each of the 5 vials, are derived from the data presented in Figures 3A-3C and given in the previous paragraph.

[0071] Referring to Figure 4A, it can be seen that the $R_2(^1\text{H}_2\text{O})$ results verify that DAPTACEL vial 9 has approximately the same $R_2(^1\text{H}_2\text{O})$ value as the non-frozen suspended vaccine, suggesting that DAPTACEL vial 9 did not experience any freeze-induced damage (compare to Figure 2A). Referring to Figure 4B, it can be inferred that every one of vials 6-10 of ENGERIX-B suffered freeze-induced damage since it is clear that the $R_2(^1\text{H}_2\text{O})$ values are well below the average of the non-frozen suspended vaccine vials 1-5 (compare to Figure 2B). Lastly, referring to Figure 4C, it can be inferred that none of vials 6-10 of VAQTA suffered freeze-induced damage (compare to Figure 2C).

[0072] Interestingly, DAPTACEL vials 6 and 9, and ENGERIX-B vial 9 did not freeze initially upon first exposure to freezing temperatures (-18°C) overnight (17-22 hours). Referring to Figures 5A and 5B, this physical observation after the first freezing (light grey) exposure was detectable noninvasively by $R_2(^1\text{H}_2\text{O})$. The $R_2(^1\text{H}_2\text{O})$ was also measured after the second exposure to freezing temperatures (medium grey) and third exposure (black). ENGERIX-B vial 9 did not freeze after second exposure, while DAPTACEL vial 6 froze. Finally, after the third exposure to freezing temperatures, both DAPTACEL vial 6 and ENGERIX-B Vial 9 were frozen. In contrast, DAPTACEL vial 9 did not freeze after the second or third exposure to freezing temperatures. ENGERIX-B vial 10 froze all three times, but exhibited a slightly different trend over the course of the three exposures to freezing temperatures. All $R_2(^1\text{H}_2\text{O})$ measurements were of fully thawed, suspended vials at 4°C . Figures 5A and 5B highlight the non-uniform physical consequence of vaccine exposure to freezing temperatures (i.e., some vials froze, while others did not). The results of the vaccine suspensions exposed to freezing temperatures are easily compared to the average non-frozen suspended (solid line) and non-frozen settled (dashed line) results (corresponding to those shown in Figures 3A-3C).

ADVANTAGES OF THE DISCLOSED METHOD

[0073] Unexpectedly, some vials within the same carton experienced freeze-induced damage while others didn't (see, e.g., DAPTACEL vial 9 relative to vials 6-8 and 10). Further, some vaccines took multiple freeze-thaw cycles before freeze-induced damage was detected. Advantageously, the method described herein permits the user to easily determine, without having to destroy, alter or perturb the contents of the vial, whether each individual vial in a batch has experienced freeze-induced damage. This is a substantial improvement over the prior art, where one vial in a batch would be tested, for example using the WHO Shake Test, and if the vial showed evidence of freeze-induced damage, the entire batch was disposed of even if the rest of the batch had not experienced freeze-induced damage. At the same time, if the tested vial did not show evidence of freeze-induced damage, the entire batch

would be distributed for use, which could be detrimental to the public if some of the vials in the batch had experienced freeze-induced damage. The results obtained for vials 6-10 DAPTACEL are an excellent example of why each vial is preferentially tested to determine if freeze-induced damage occurred, thus minimizing waste and maximizing potency. Using the method disclosed herein, the viability and potency of an entire batch of vaccines is no longer dependent on whether just one vial in the batch was determined to be frozen or non-frozen, e.g., using the “Shake Test” or knowing that the vaccines may have been exposed to freezing temperatures, but instead can be quickly determined by measuring the $R_2(^1\text{H}_2\text{O})$ for each vial and comparing it to an acceptable $R_2(^1\text{H}_2\text{O})$ value for the suspended batch.

[0074] Further, it is evident from the results in Figures 4B and 4C that some vaccines experience freeze-induced damage more easily than others. Both ENGERIX-B and VAQTA were exposed to the same freezing temperatures but only the ENGERIX-B experienced freeze-induced damage. The method described herein allows for the rapid and nondestructive determination of which of multiple different vaccines, exposed to the same temperatures, remain potent.

[0075] Overall, it can be concluded that the method described herein ($w\text{NMR}$) is a substantial improvement over the current state of the art, i.e., the Shake test or instrument-based sedimentation tests. $w\text{NMR}$ is quantitative, and can be automated, removing the subjectivity of a highly trained human. $w\text{NMR}$ can nondestructively test the vaccines and other pharmaceutical products without removing any labels, and regardless of the volume of product in the vial. The Shake test cannot be accurately performed on low volume vials, e.g., 0.5 mL and 1 mL. Advantageously, the acceptance decision regarding vaccines suspected of freezing based on $w\text{NMR}$ is much more accurate. The use of $w\text{NMR}$ can substantially ensure all injected vials have not been frozen and that all rejected vials have been frozen. This is because $w\text{NMR}$ can be used to test every vial in a batch, unlike the Shake test which relies on the result from one vial in a batch to decide whether the entire batch should be injected or rejected. In other words, the results of the Shake test can potentially lead to situations where patients are injected with frozen vaccines or alternatively, where non-frozen vaccines have been disposed of. In addition, the $w\text{NMR}$ method assesses the fully suspended product, without the necessity of a time course experiment, making the $w\text{NMR}$ method very rapid, typically about 10-30 seconds. The Shake test and instrument-based sedimentation tests involve the monitoring of a time course as the product particles go from fully suspended to fully settled, which is very time consuming.

MEASUREMENT CONTROLS

[0076] Vials of either ultrapure pure water or aluminum adjuvants served as negative and positive controls, respectively. Two commercially available aluminum adjuvants, ADJUPHOS (the adjuvant in DAPTACEL) and ALHYDROGEL (the adjuvant in ENGERIX-B) were included as positive controls. The adjuvant in VAQTA is not commercially available and hence is not included. The freezing of pure

water and two adjuvant suspensions served two purposes. One, they helped to determine whether the observed freezing variability was due to flawed freezing procedures or a faulty freezer, i.e., whether pure water or adjuvant suspensions display variable freezing behavior (some vials freeze, some do not). Second, they help to determine whether the observed $R_2(^1\text{H}_2\text{O})$ decrease, in response to freeze/thaw, is a property of water alone, adjuvant in water, or the antigen-adjuvant complex in water.

[0077] Six vials of pure water were prepared and the $R_2(^1\text{H}_2\text{O})$ was measured prior to any freeze/thaw cycles to establish its baseline value. Vials 1-3 were kept at room temperature (23°C). Vials 4-6 were exposed to subzero temperatures (-18°C) overnight three times following the same freeze/thaw procedure as with the vaccine vials. The exposed vials 4-6 were visually observed to have frozen. However, unlike vaccine vials, for pure water, the $R_2(^1\text{H}_2\text{O})$ of frozen/thawed vials is no different than control vials (Figure 6). This result shows that the observed $R_2(^1\text{H}_2\text{O})$ decrease of the vaccines in response to a freeze/thaw event is not due to the freezing of water.

[0078] The next level assessed the aluminum adjuvant in water, without any antigens. Two suspensions were assessed, aluminum phosphate (ADJUPHOS, the adjuvant in DAPTACEL) and aluminum hydroxide (ALHYDROGEL, the adjuvant in ENGERIX-B), using the same freeze/thaw cycles as the vaccines. The aluminum concentration in the vials of ADJUPHOS was 0.66 mg/mL and in the vials of ALHYDROGEL was 0.50 mg/mL, matching the Al(III) in DAPTACEL and ENGERIX-B, respectively. Six vials of ADJUPHOS and six of ALHYDROGEL, each labeled 1-6, were measured initially to establish the high-quality adjuvant baseline in both suspended and settled states (Figure 7). Vials 4-6 were then exposed to -18°C for 19 hours. For both adjuvants, all the exposed vials were visually observed to have frozen. The frozen vials were then thawed at room temperature (23°C) and the $R_2(^1\text{H}_2\text{O})$ was measured. This process was repeated three times for both adjuvants.

[0079] Uniformity of freezing behavior was observed in the vials of water (Figure 6) or aluminum adjuvant suspensions (Figure 7). This uniformity in the freezing behavior of water and adjuvant vials demonstrates that the freezing procedure operation is not flawed, and the freezer is not faulty. The freeze/thaw of pure water led to no detectable changes in $R_2(^1\text{H}_2\text{O})$, while the freeze/thaw of aluminum adjuvant led to a significantly lower $R_2(^1\text{H}_2\text{O})$. Further, ADJUPHOS displayed a larger decrease in $R_2(^1\text{H}_2\text{O})$ than ALHYDROGEL (0.86 vs 0.40 s⁻¹), consistent with DAPTACEL displaying a larger $R_2(^1\text{H}_2\text{O})$ decrease than ENGERIX-B (0.61 vs 0.17 s⁻¹). This suggests that the observed $R_2(^1\text{H}_2\text{O})$ decrease in response to freeze/thaw events emerges at the adjuvant in water level. In vaccines, the magnitude of this decrease may be modulated by antigens and/or excipients.

Example 2

[0080] The transverse relaxation rate of the water proton NMR signal, $R_2(^1\text{H}_2\text{O})$, of PROPOFOL and DIPRIVAN exposed to a freeze-thaw cycle has been studied. PROPOFOL is the generic product of the brand name DIPRIVAN. They are injectable emulsions indicated for general anesthesia and sedation

that are administered intravenously. The package inserts of both products state "Do not freeze," rendering them freeze-sensitive products.

[0081] One vial of each product (DIPRIVAN and PROPOFOL) was opened and the contents (20 mL whitish emulsion) were aliquoted into six glass vials (3 mL/vial). The reason for opening the vial is because the 20-mL vial (o.d. 30 mm) cannot fit into the benchtop NMR that was available (limit: 26 mm). It should be appreciated by the person skilled in the art that had the NMR magnet bore been greater than the outside diameter of the vial, the vial could have been inserted directly into the NMR without opening the vial (i.e., a non-invasive measurement). Just before aliquoting, the product was inverted several times for uniform dispersion of emulsion. The six glass vials of each product were labelled 1-6 and were measured using w NMR. Following initial measurements, vials 1-3 of each product were stored at room temperature (i.e., unstressed), while vials 4-6 were subjected to overnight freezing at -30°C in a pre-equilibrated BioCool instrument for approximately 17 hours and then thawed at room temperature (i.e., stressed). Again, vials 1-6 for each product were measured using w NMR.

[0082] DIPRIVAN and PROPOFOL do not require cold storage and hence the NMR measurement was made at 25°C , which is the temperature of the benchtop NMR magnet. Three consecutive measurements were collected of each vial.

[0083] As seen from Figure 8A, visual detection of the freezing history of the emulsion drugs is not possible. Unstressed and stressed (previously frozen) vials look very similar if not identical. Figure 8B illustrates the average of vials 1-3 and vials 4-6, where 0 F/T refers to vials 1-3 with no freeze thaw, and 1 F/T refers to vials 4-6, which were frozen at -30°C and subsequently thawed prior to the measurement. It can be seen in Figure 8B that the $R_2(^1\text{H}_2\text{O})$ for both products increased by approximately 0.065 sec^{-1} after one freeze/thaw cycle. Accordingly, water proton relaxation rate $R_2(^1\text{H}_2\text{O})$ readily detects the difference between stressed and unstressed products and allows the user to easily identify a pharmaceutical product with a freezing history.

Example 3

[0084] An unopened vial of DIPRIVAN (10 mL) was stored at room temperature ($\sim 25^{\circ}\text{C}$), according to storage requirements detailed in the package insert. The $T_2(^1\text{H}_2\text{O})$ of this vial was measured 12 times, as described herein, once on 12 different non-consecutive days. $R_2(^1\text{H}_2\text{O})$ was calculated from the $T_2(^1\text{H}_2\text{O})$, as understood by the person skilled in the art. Referring to Figure 9B, the average $R_2(^1\text{H}_2\text{O})$ for unopened DIPRIVAN having zero freeze-thaws ("0 F/T") is $0.509 \pm 0.010\text{ s}^{-1}$, with an error bar showing the standard deviation of the 12 measurements (i.e. the measurement variability). The vial was subsequently exposed to -30°C overnight (16+ hours) and frozen, then thawed and equilibrated to 25°C . The $T_2(^1\text{H}_2\text{O})$ was measured, and the $R_2(^1\text{H}_2\text{O})$ was determined to be 0.564 s^{-1} (labeled "1 F/T" in Figure 9B, for one freeze-thaw). The magnitude of $R_2(^1\text{H}_2\text{O})$ change is 0.055 s^{-1} , well above the measurement

error of 0.010 s^{-1} . This result shows that $R_2(^1\text{H}_2\text{O})$ can readily detect the one F/T cycle experienced by unopened DIPRIVAN.

[0085] In comparison, when the vial is opened, $R_2(^1\text{H}_2\text{O})$ of DIPRIVAN increased from $0.549 \pm 0.0003 \text{ s}^{-1}$ (from an average of 3 vials, each measured 3 times) before freezing to $0.612 \pm 0.0003 \text{ s}^{-1}$ (from an average of 3 vials, each measured 3 times) after one F/T cycle. The magnitude of $R_2(^1\text{H}_2\text{O})$ change is 0.063 s^{-1} . The percentage of change (calculated by the magnitude of change in $R_2(^1\text{H}_2\text{O})$ before and after freezing divided by the $R_2(^1\text{H}_2\text{O})$ before freezing) for unopened DIPRIVAN and opened DIPRIVAN are both 11%.

[0086] Although the invention has been variously disclosed herein with reference to illustrative embodiments and features, it will be appreciated that the embodiments and features described hereinabove are not intended to limit the invention, and that other variations, modifications and other embodiments will suggest themselves to those of ordinary skill in the art, based on the disclosure herein. The invention therefore is to be broadly construed, as encompassing all such variations, modifications and alternative embodiments within the spirit and scope of the claims hereafter set forth.

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THE CLAIMS

What is claimed is:

1. A method of determining if a vaccine or aqueous-based pharmaceutical product has experienced freeze-induced damage, said method comprising:

measuring the transverse relaxation rate of solvent $R_{2,m}$ in the vaccine or aqueous-based pharmaceutical product; and

determining if the vaccine or aqueous-based pharmaceutical product has been frozen by comparing the measured $R_{2,m}$ to a reference transverse relaxation rate of solvent $R_{2,r}$, wherein the reference $R_{2,r}$ represents an acceptable range for non-frozen vaccine or non-frozen aqueous-based pharmaceutical product,

wherein when the measured $R_{2,m}$ is inside the reference $R_{2,r}$ range, the vaccine or aqueous-based pharmaceutical product has not experienced freeze-induced damage.

2. The method of claim 1, wherein the pharmaceutical product comprises biologics or small molecules.

3. The method of claim 1, wherein the vaccines or pharmaceutical products comprise at least one component selected from the group consisting of at least one adjuvant, at least one surfactant, at least one water-soluble organic solvent, at least one dispersant, at least one biocide, at least one buffering agent, at least one pH adjusting agent, at least one peptide, at least one polypeptide, at least one protein, at least one antibody or fragment thereof, at least one nucleic acid, at least one oil, and any combination thereof.

4. The method of claims 1 or 3, wherein the vaccine comprises an adjuvant comprising an aluminum-containing salts selected from the group consisting of aluminum hydroxide, aluminum phosphate, aluminum sulfoxylphosphate, aluminum hydroxyphosphate sulfate, alum ($\text{KAl}(\text{SO}_4) \cdot 12\text{H}_2\text{O}$), and any combination thereof.

5. The method of any of the preceding claims, wherein the $R_{2,m}$ is measured using nuclear magnetic resonance (NMR).

6. The method of any of the preceding claims, wherein the $R_{2,m}$ can be measured without opening a vial containing the vaccine or aqueous-based pharmaceutical product or otherwise accessing the contents of the vial containing the vaccine or aqueous-based pharmaceutical product.
7. The method of any of the preceding claims, wherein the solvent is water.
8. The method of any of the preceding claims, wherein a vaccine or aqueous-based pharmaceutical product that has not experienced freeze-induced damage can be used or distributed as intended.
9. The method of any of the preceding claims, wherein $R_{2,r}$ is a range.
10. The method of any of the preceding claims, wherein $R_{2,m}$ and $R_{2,r}$ are measured at substantially the same temperature.
11. The method of claim 10, wherein the pharmaceutical product is a vaccine and wherein the temperature is in a range from about 2°C to about 8°C.
12. The method of any of the preceding claims, wherein $R_{2,m}$ and $R_{2,r}$ are measured at substantially the magnetic field strength.
13. The method of any of claims 5 to 12, wherein possible heating from the radiofrequency pulses of the NMR is substantially prevented.
14. The method of any of the preceding claims, wherein the aqueous-based pharmaceutical product comprises an emulsion.

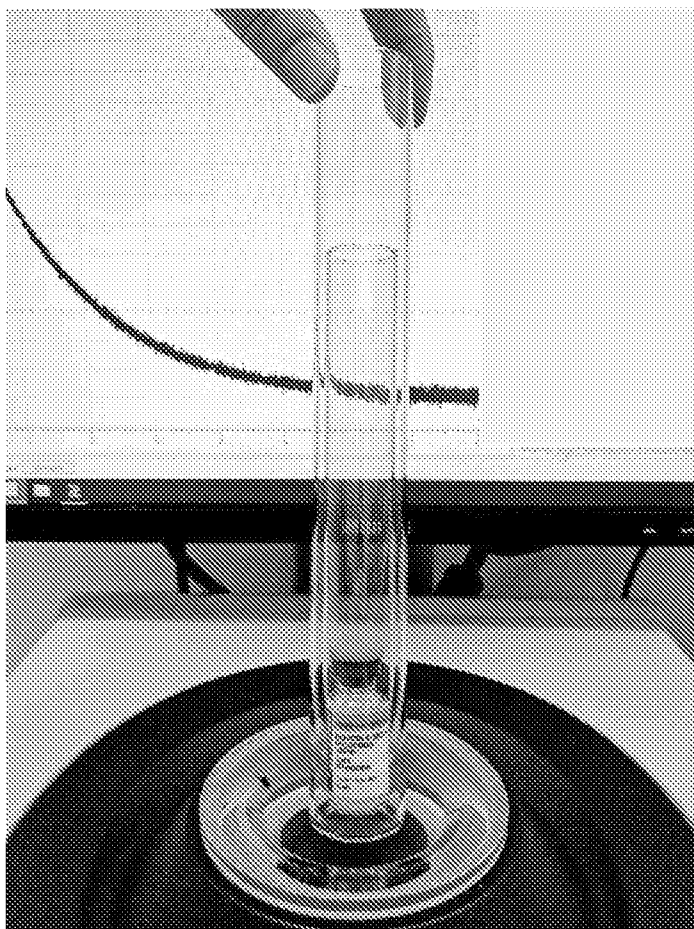


FIGURE 1

FIGURE 2A

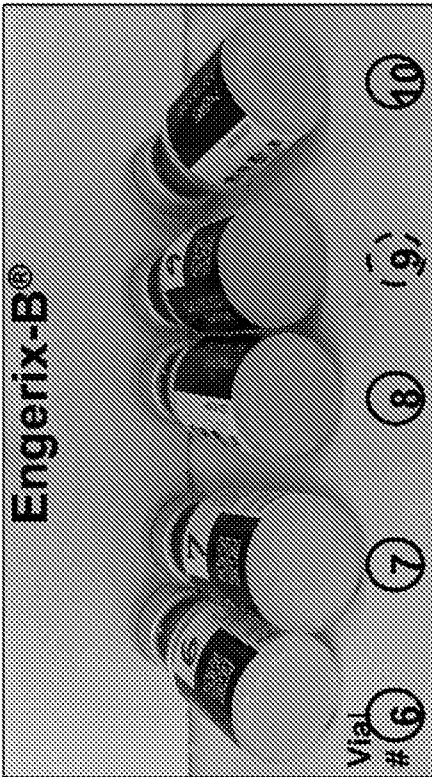
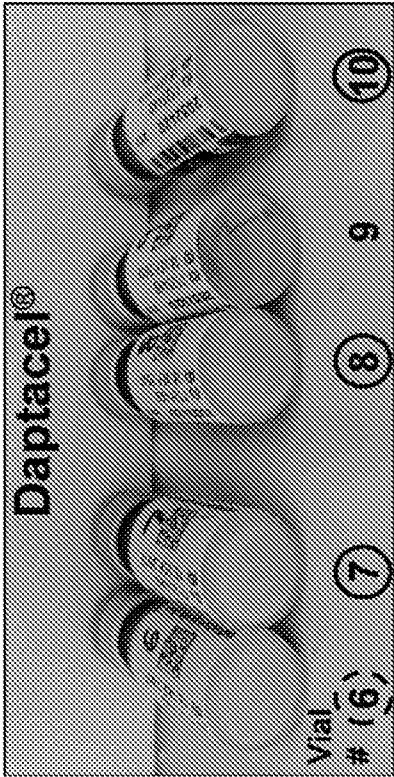


FIGURE 2C

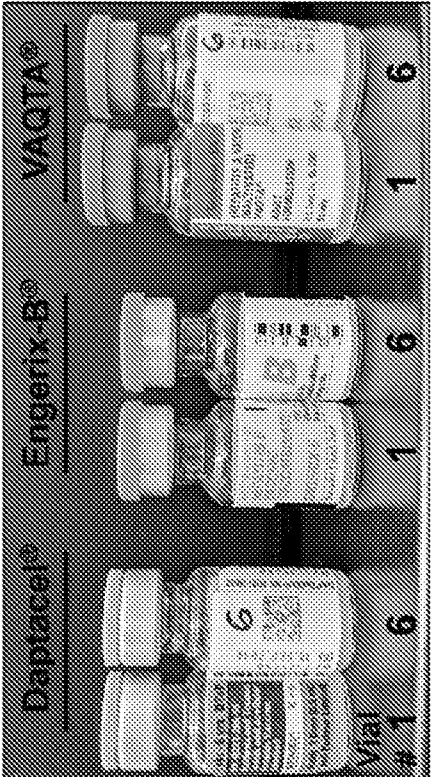
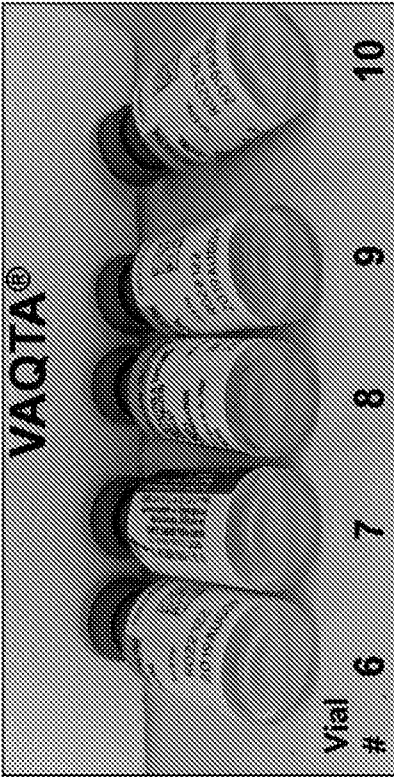


FIGURE 2B

FIGURE 2D

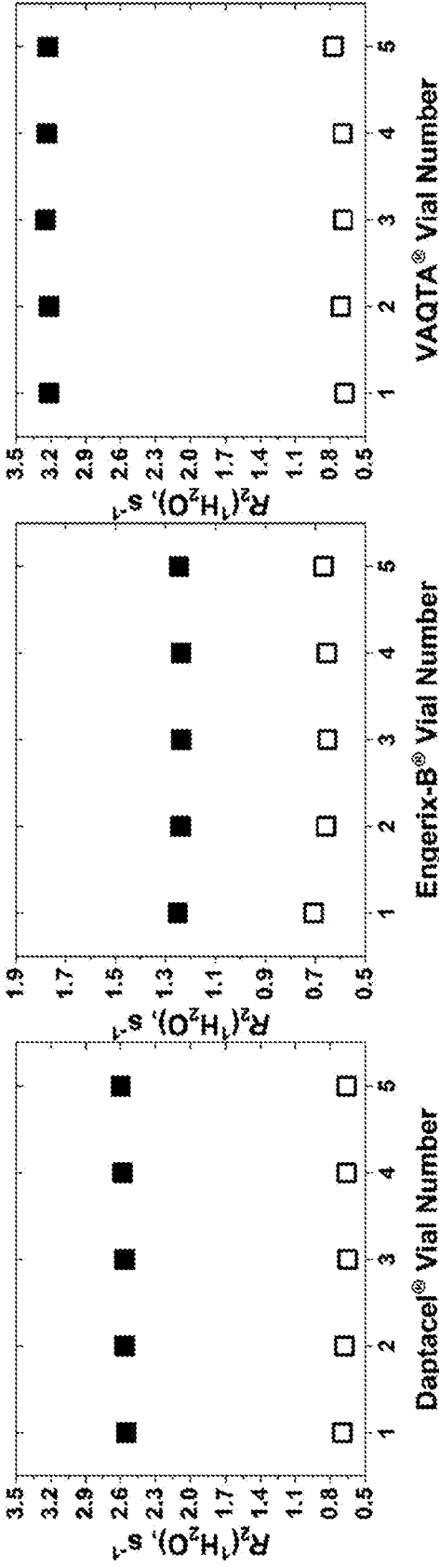


FIGURE 3A

FIGURE 3B

FIGURE 3C

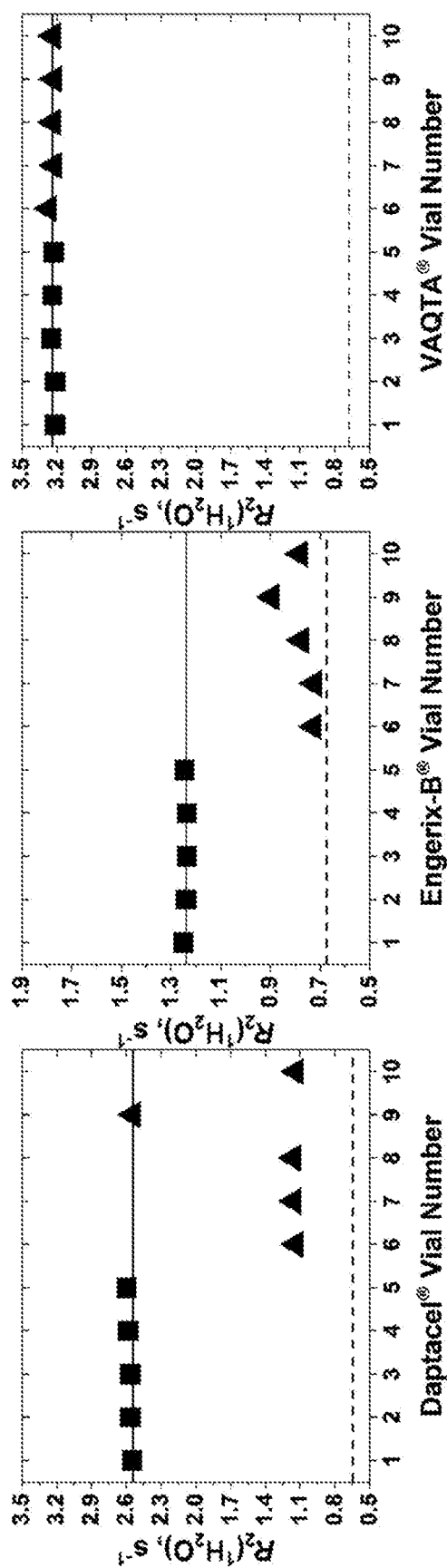


FIGURE 4A

FIGURE 4B

FIGURE 4C

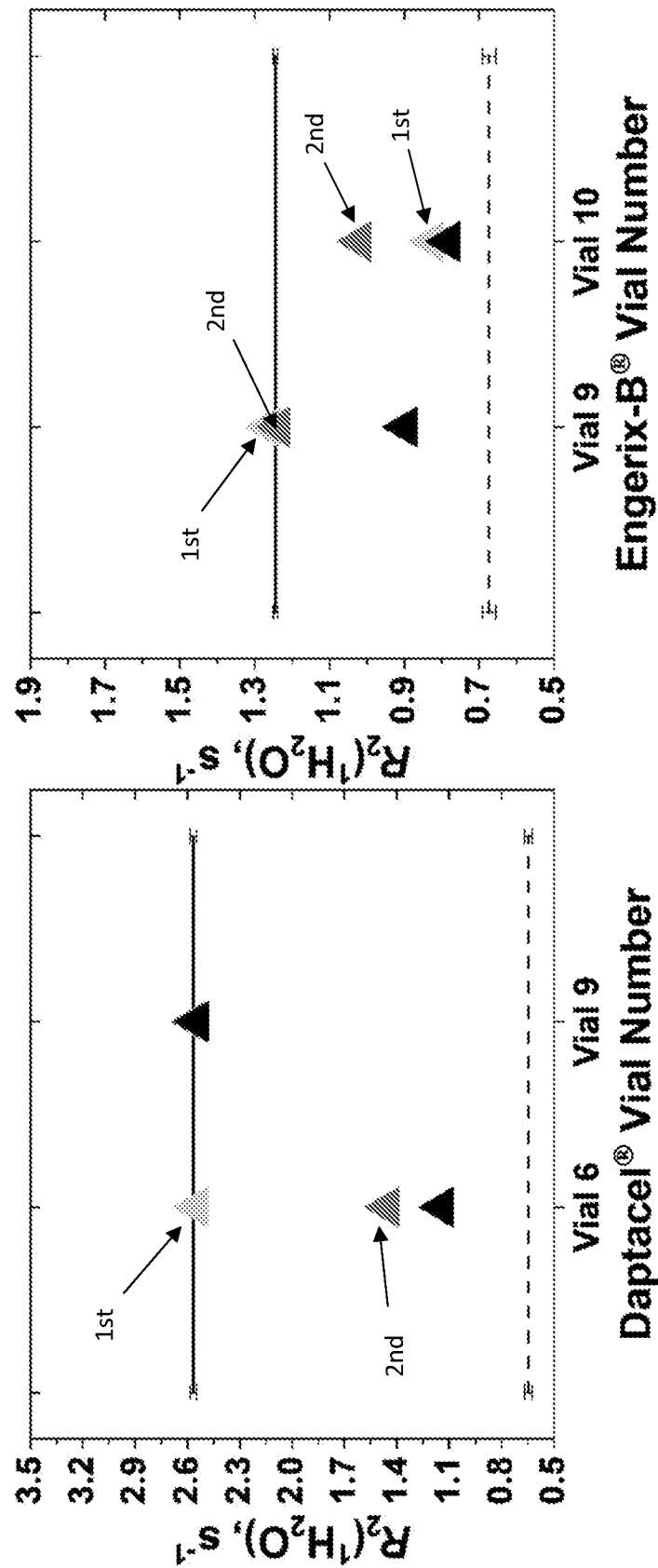


FIGURE 5A

FIGURE 5B

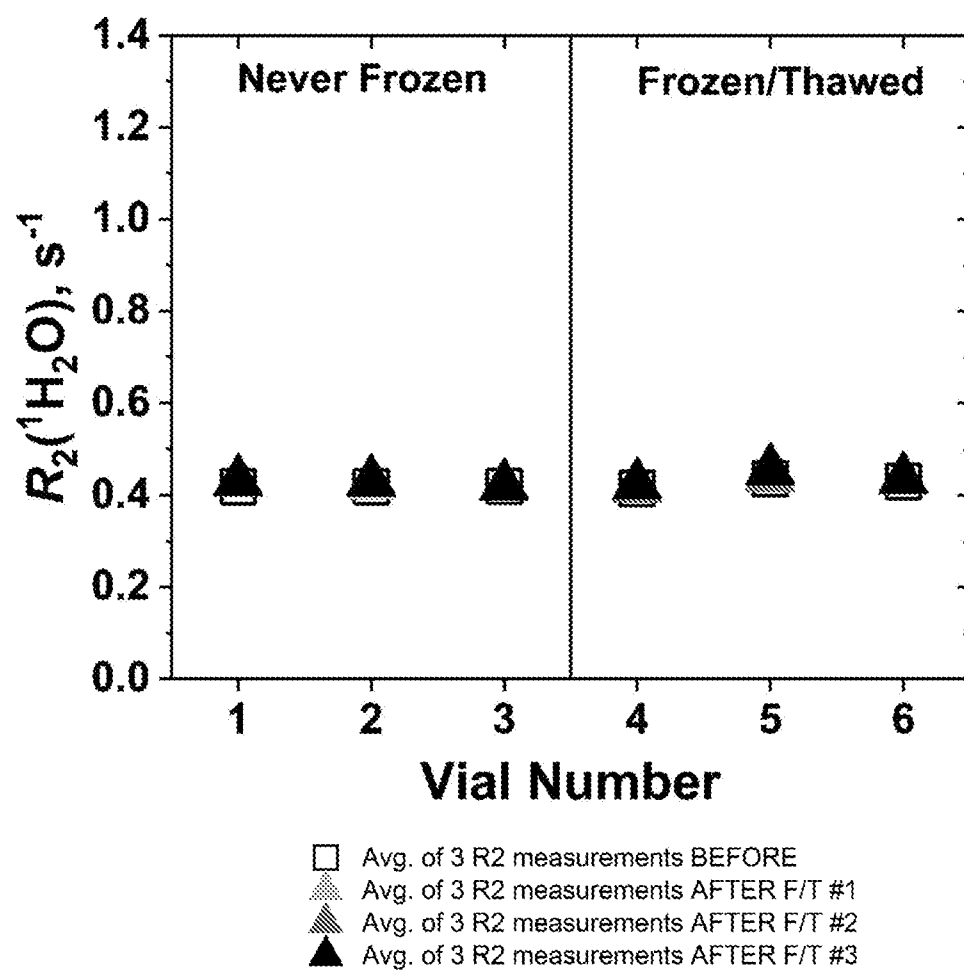


FIGURE 6

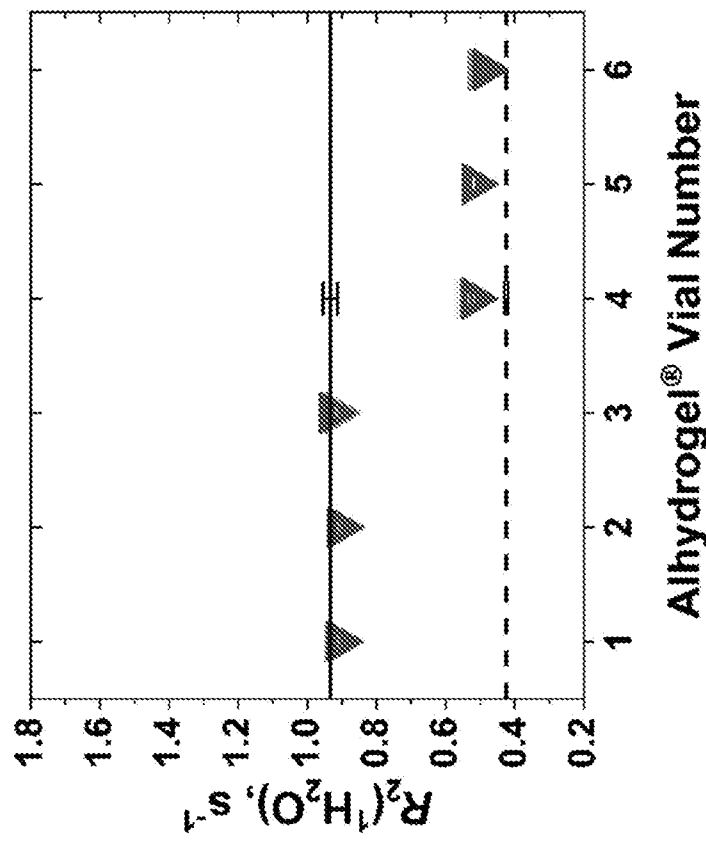


FIGURE 7B

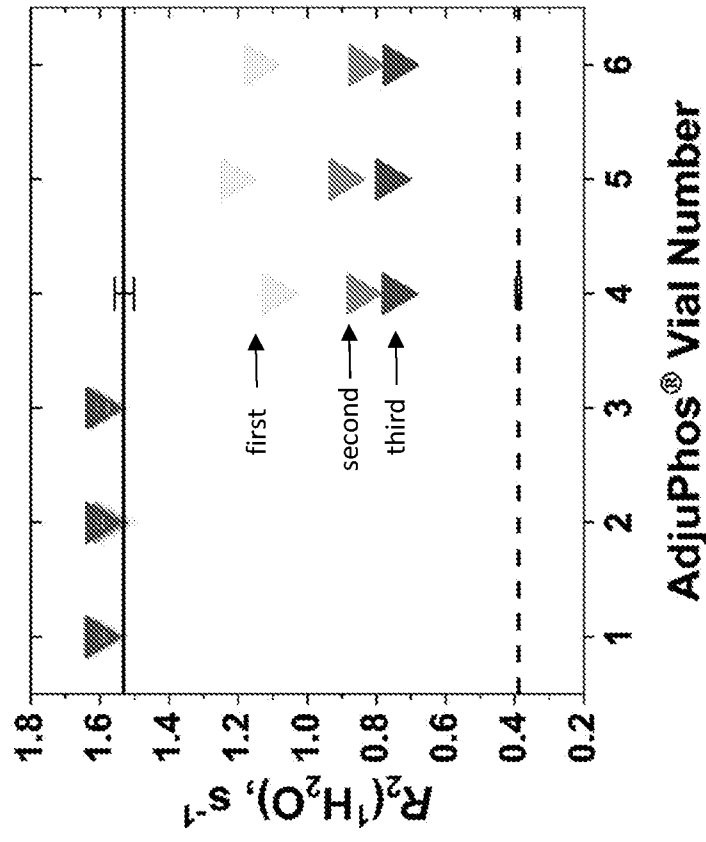


FIGURE 7A

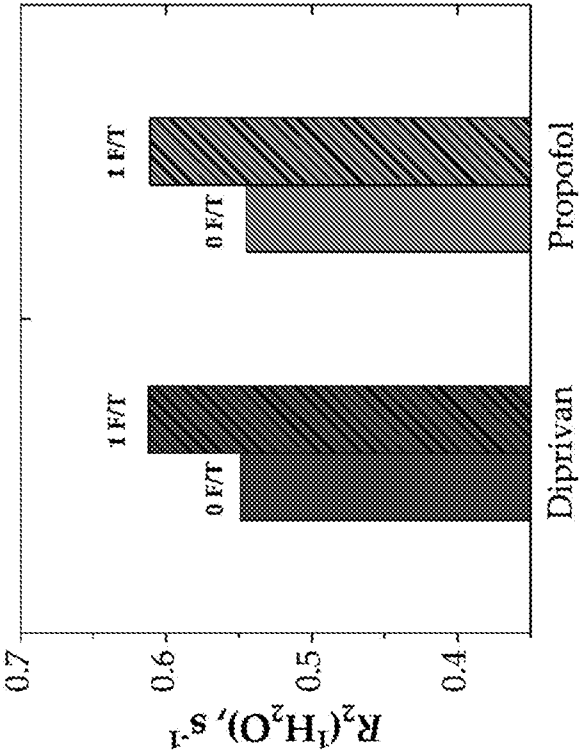


FIGURE 8B

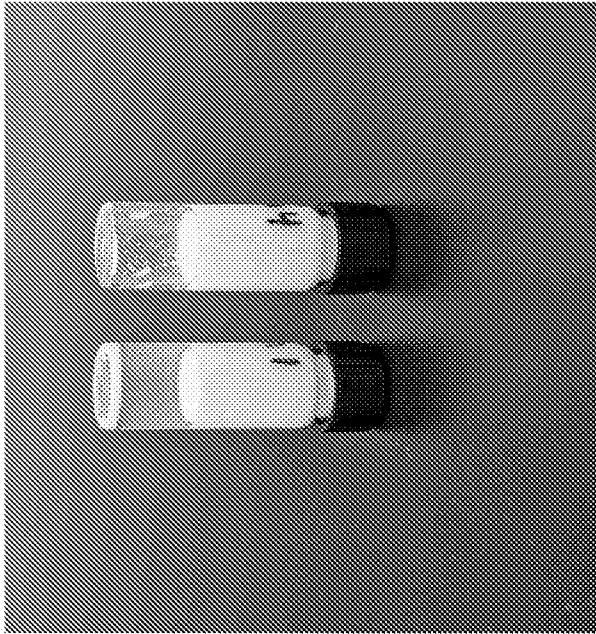


FIGURE 8A



FIGURE 9A

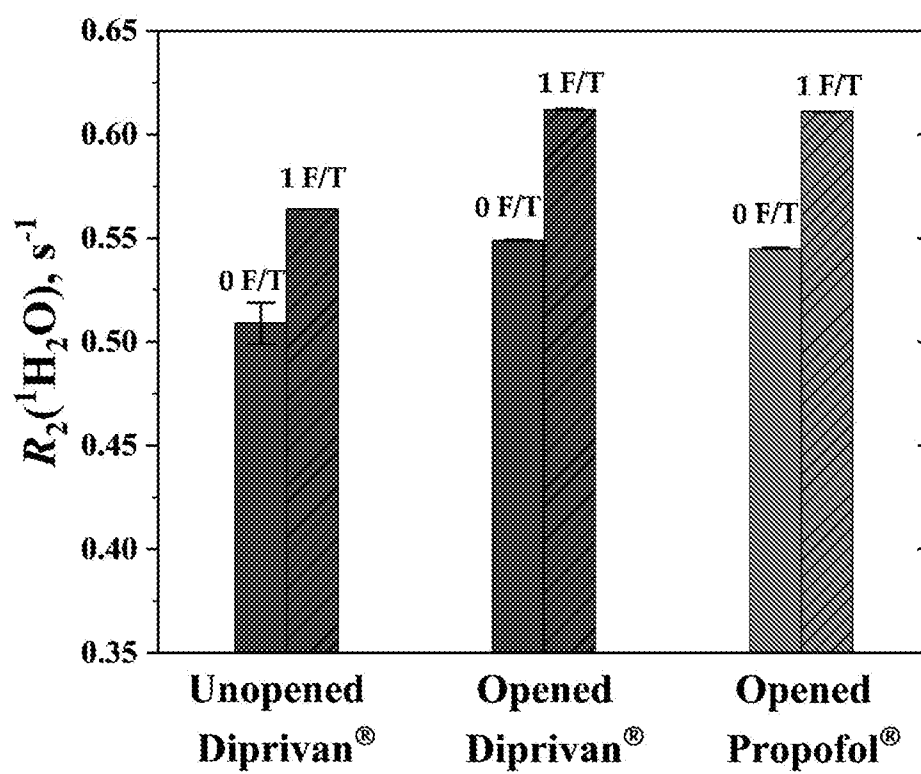


FIGURE 9B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/021983

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00; G01N 24/08 (2020.01)

CPC - A61K 39/00; G01N 24/088; G01R 33/448 (2020.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 324/309; 436/173 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/102681 A1 (UNIVERSITY OF MARYLAND, BALTIMORE et al) 07 June 2018	1-3
-	(07.06.2018) entire document	-----
Y		4
Y	US 2006/0228369 A1 (CHEN) 12 October 2006 (12.10.2006) entire document	4
A	US 8,314,618 B2 (BIERI et al) 20 November 2012 (20.11.2012) entire document	1-4
A	CLAPP et al. "Vaccines with Aluminum-Containing Adjuvants: Optimizing Vaccine Efficacy and Thermal Stability," Journal of Pharmaceutical Sciences, 25 August 2010 (25.08.2010), Vol. 100, No. 2, Pgs. 388-401. entire document	1-4
A	WO 2017/210226 A1 (Q BIO, INC.) 07 December 2017 (07.12.2017) entire document	1-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 April 2020

Date of mailing of the international search report

10 JUN 2020

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/021983

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-14
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.