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DETERMINATION OF THE VISCOSITY AND DENSITY OF VETERINARY VACCINES

DOROTA M. KRASUCKA[1], KATARZYNA KOS[1]*, ANETA D. WOŹNIAK[1] and WOJCIECH A. CYBULSKI[2]

[1]Department of Veterinary Pharmacy, [2]Department of Pharmacology and Toxicology, National Veterinary Research Institute, Al. Partyzantów 57, 24-100 Puławy, Poland

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The quality of veterinary vaccines is monitored by physicochemical, microbiological and biological tests. Two of the most important physicochemical parameters are viscosity and density, which are determined in the three different types of matrices: emulsions, suspensions and solvents.

Viscosity, known as internal friction or viscose medium, is a feature of fluids and plastic solids characterized by their internal resistance against flowing. Viscosity is one of the most important property of fluids (1). Newton's law states that the force of the impact (friction) between layers of flowing liquid depends on their ratio, and the viscosity are directly proportional to the difference in flowing velocity of layers, and inversely proportional to the distance between them. Shear rate (Fig. 1) is the quotient of the velocity of liquid layers and the distance between them (2). According to the European Pharmacopoeia (3), there are two types of viscosity: dynamic and kinematic. The unit of dynamic viscosity (which is described in this paper) is the pascal × second (Pa·s). The most commonly used submultiple is the millipascal × second (mPa·s = cP).

According to the European Pharmacopoeia (4), the relative density of a substance is the ratio of the mass of a certain volume of a substance at tempera-

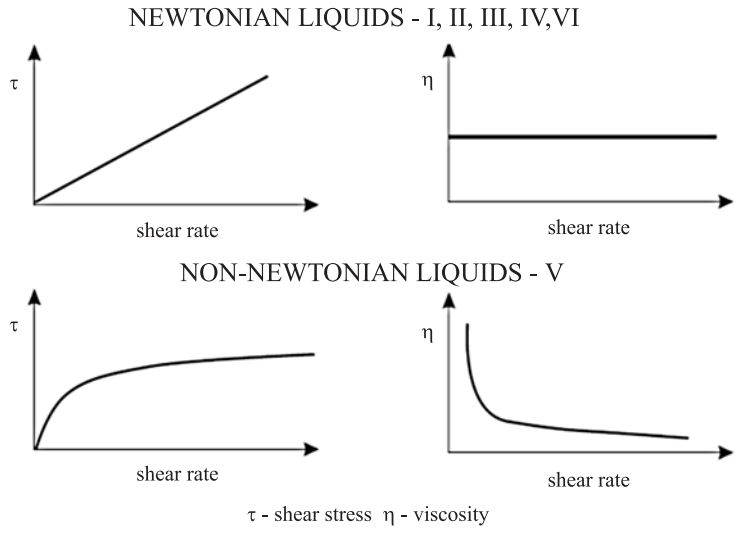

Figure 1. Shear stress. shear rate. and viscosity inter-relationship for vaccines as Newtonian and non-Newtonian liquids (explanation of vaccines symbols are in Table 1)

* Corresponding author: e-mail: katarzyna.pasik@piwet.pulawy.pl

ture t_1 to the mass of an equal volume of water at temperature t_2. Unless otherwise indicated, the relative density is used. Relative density is also commonly presented as density ρ20, defined as the mass of a unit volume of the substance at 20°C, expressed in kilograms per cubic meter or grams per cubic centimeter (1 kg/m^3 = 10^{-3} g/cm^3).

Viscosity and density are two of the most important parameters of vaccines' adjuvants like aluminium hydroxide or aluminium phosphate (5). The gels of this adjuvants are commonly used as adsorbents for antigens. All pre-formed gels are controlled by using internal standard methods, for example, determination of viscosity (6). Vaccine adjuvants improve immune responses to numerous antigens. Veterinary vaccines may contain a large number of adjuvants.

There are three types of emulsions used in veterinary medicine today. The first one are water-in-oil emulsions (W/O) that have a continuous oil phase. High viscosity makes these products difficult to inject. This emulsions induce a strong and long term immune response. The second type are oil-in-water emulsions (O/W). They have lower viscosity and thus are easier to inject. This emulsions are well tolerated and induce a short term immune response. The third type are the double emulsions: water-in-oil-in-water emulsions (W/O/W). These products are characterized by low viscosity but are less stable than the other two types. This multiphasic emulsions can induce short or long term immune responses depended on various antigens (7, 8). Burrel et al. studied the effect of sterilization on adjuvants without an antibody, which resulted in a decrease of the pH and a reduction of viscosity (9).

Beebe et. al. also monitored viscosity level in emulsion of vaccines. Measurements run at 0.1 intervals up to 3 months. A Brookfield RVDV-II Digital Viscometer with cone CP40 and sample cup CP-44Y equipped with a Neslab RTE-111 Refrigerated Bath Circulator were used to maintain a temperature of 25°C. The initial viscosity was 231 cP and remained stable over time, with a minimal decrease to 197 cP by 3 months and the results were expressed in centipoise units (cP). Analyses were carried out during the research on immunotherapy (10). Other findings proved that the viscosity of oily emulsions can reduce bacterial retention and filter capacity (11).

Yeu-Chun Kim et. al. reported that vaccine stability, as measured by an *in vitro* hemagglutination assay, was increased by decreased concentration of viscosity-enhancing compounds**.** They found that the influenza VLP vaccine could be coated onto microneedles and rapidly released into the skin or into solution. They also found that the inclusion of carboxymethylcellulose (CMC) used to increase viscosity of microneedles coating solution decreased vaccine activity (12).

In medicine, viscosity is an important parameter in the case of blood tests. Rajzer et al. described abnormal blood viscosity, accompanied by hypertension, atherosclerosis and stroke (2). There are scarce data found in the available literature devoted to stability of the veterinary vaccines in respect to methodological issues.

The aim of this study was to validate the method for determination of the viscosity and density of different types of veterinary vaccines.

MATERIALS AND METHODS

The linearity, repeatability, precision and uncertainty were comprised. Linearity was made at four levels of viscosity (within range 3.902–1110 mPa·s) and density (within range 0.81213–0.84262 g/cm^3) using Anton Paar company standards – SH L 122, M 116, H 120, C 120. Standards were used to check the instrument before each the day measurements. Six determinations of viscosity and density for each of six different vaccines were performed. All tested vaccines were of viral origin, except for the vaccine no. V, which was of bacterial origin.

Measurements of the viscosity and density were carried out by means of a rotational viscometer SVM 3000 with a density measurement, from Anton Paar (USA) with a fixed shear rate at 20°C. Rotating viscometers with simultaneous density measurement were used for measuring the viscosity of Newtonian (shear-independent viscosity, e.g., emulsions and solvents) or non-Newtonian liquids (shear dependent viscosity or apparent viscosity, e.g., suspensions) (13). The measurements were performed under the following conditions: ambient temperature of 18 to 25°C and relative humidity of 20 to 80% at the laboratory environment. The minimum quantity of sample dispensed into the measuring cell was optimized (2 mL).

The viscosity was measured in rotational viscometer with a cylinder geometry. It is based on a modified Couette principle with a rapidly rotating outer tube and an inner measuring bob which rotates more slowly. Rotational viscosity measurement is based on a torque and speed measurement. A rotating magnet in SVM 3000 produces an eddy current field with an exact speed dependent brake torque. The eddy current torque is measured with extremely high resolution. Combined with the integrated ther-

moelectric thermostating, this ensures unparalleled precision. The torque resolution is an unmatched 50 pico-Nm. That's why it only requires a very compact measuring cell. The very small measuring cell contains a tube which rotates at a constant speed. This tube is filled with the sample. A measuring rotor with a built-in magnet is floating in the sample. The low density of the rotor allows it to be centered by the centrifugal force. The freely swimming rotor requires no bearing – as it is not followed by a friction. This also makes the instrument insensitive to vibration. The small sample volume allows extremely quick temperature changes (Peltier) and very short equilibrium times. Shortly after the start of the measurement, the rotor reaches a stable speed. This is determined by the equilibrium between the effect of the eddy current brake and the shear forces at work in the sample. The dynamic viscosity is calculated from the rotor speed.

In order to calculate the kinematic viscosity from the dynamic viscosity, the density of the sample must be known. For this reason, SVM 3000 also has a density measuring cell that employs the well-known oscillating U-tube principle. Both cells are filled in one cycle. The measurements are carried out simultaneously (14).

All vaccines materials used in this studies are presented in Table 1. Before tests, the samples were stored at 2 to 8^{o}C. Tests were performed on six vaccines: (I) the vaccine against egg drop syndrome EDS'76, inactivated; (II) vaccine against feline rhinotracheitis birds, inactivated; (III) the vaccine against birds' reovirus infections, inactivated; (IV) rabies vaccine, calcivirose, viral rhinotracheitis and feline panleucopenia; (V) the vaccine against fungal infection of the skin of cattle, inactivated; (VI) the vaccine against myxomatosis and rabbit hemorrhagic disease of rabbits.

RESULTS AND DISCUSSION

Optimization of viscosity and density determination let to obtain very reproducible results. The maximum scatter of results (CV) for the viscosity was 3.87%, while the density was 0.261%. These results of measurements were for the suspension. It was the only bacterial vaccine in this study. For other types of vaccines, scattering measurements of viscosity did not exceed 1.7% and for a density did not exceed 0.09%. Based on the validation and subsequent monitoring studies, it was observed that the viscometer with a density measurement did not allow to obtain reliable results of viscosity and density determination of the suspensions (non-Newtonian liquids). The reason for this situation was the inability to change the settings of shear rate. Parameters of repeatability and precision of the viscosity and density determination are presented in Tables 2 and 3. The uncertainty of the viscosity were determined at 3.26% and density at 0.25% level. The method of dynamic viscosity was linear with r^2 = 1.000000. The method of relative density was linear with r^2 = 0.999995.

The main challenge in this research was to eliminate contamination of measuring apparatus after each analysis. Vaccines are an extremely diverse group of matrices. Each vaccine is an individual composition so that it was necessary to select of appropriate organic solvents for cleaning the instrument. Isopropanol, acetone and hexane were the most effective solvents. A sign of an effective apparatus purification were density less than 0.001

Table 1. Form of vaccines used in analysis.

Symbol	Form of vaccine	The commonly used name
I	emulsion for injection for chickens	the vaccine against egg drop syndrome EDS'76, inactivated
II	emulsion for injection for chickens	vaccine against feline rhinotracheitis birds, inactivated
III	emulsion for injection for chickens	the vaccine against birds' reovirus infections, inactivated
IV	lyophilizate and solvent for emulsion for injection for cats	rabies vaccine, calcivirose, viral rhinotracheitis and feline panleukopenia
V	suspension for injection	the vaccine against fungal infection of the skin of cattle, inactivated
VI	lyophilized, and suspension for injection	the vaccine against myxomatosis and rabbit haemorrhagic disease of rabbits

Table 2. Analytical and validation parameters of dynamic viscosity determination. Results of analysis for six different vaccines (explanation of vaccines symbols are in Table 1).

VALIDATION FEATURE		**VALIDATION RESULTS OF DYNAMIC VISCOSITY**					
LINEARITY	y = ax + b	y = 0.9980x – 0.4055 x – the level of plasma concentrations of analyte					
	r^2	1.000000					
	RANGE [mPa·s]	3.902 – 1110					
VETERINARY VACCINES		I	II	III	IV	V	VI
REPEATABILITY	$\bar{x}$	101.143	89.671	99.610	4.330	1.412	1.193
	s	0.728	0.774	0.969	0.070	0.055	0.013
	CV	0.720	0.863	0.972	1.611	3.870	1.130
PRECISION	$\bar{x}$	89.671	89.717	90.133	-	-	-
	S	0.774	0.205	0.400	-	-	-
	CV	0.863	0.228	0.444	-	-	-
UNCERTAINTY	$u_c(y)$ 1	1.63 %					
	k	2					
	U	3.26 %					

Table 3. Analytical and validation parameters of relative density determination. Results of analysis for six different vaccines (explanation of vaccines symbols are in Table 1).

VALIDATION FEATURE		**VALIDATION RESULTS OF RELATIVE DENSITY**					
LINEARITY	y = ax + b	y = 0.9765x + 0.0193 x – the level of plasma concentrations of analyte					
	r^2	0.999995					
	RANGE [mPa·s]	0.81213 – 0.84262					
VETERINARY VACCINES		I	II	III	IV	V	VI
REPEATABILITY	$\bar{x}$	0.918	0.916	0.914	0.964	1.006	1.007
	s	0.001	0.001	0.001	0.001	0.003	0.001
	CV	0.085	0.070	0.086	0.074	0.261	0.077
PRECISION	$\bar{x}$	0.916	0.917	0.917	-	-	-
	S	0.001	0.001	0.001	-	-	-
	CV	0.070	0.075	0.063	-	-	-
UNCERTAINTY	$u_c(y)$ 1	0.125 %					
	k	2					
	U	0.25 %					

g/cm^3 in dried measuring cell. Analysis of the literature data presented maximum values for the viscosity and density of veterinary vaccines – maximum 450 mPa·s and maximum of 1.05 g/cm^3.

Viscosity and density are two of the most important quality parameters of liquid vaccines that determine their stability. These parameters are officially controlled in Poland during the market monitoring research. The above-described method was set up, validated and used in this monitoring in years 2011–2013 and till now. Test specimens were randomly sampled from the market by inspection officers of pharmacovigilance from various pharmaceutical wholesalers across the country. Bioproducts

were intended to different animal species vaccination – poultry, cats, cattle and rabbits. Twenty seven vaccines were analyzed for density and six vaccines were analyzed for viscosity according to official EU actual guidelines.

Further investigation will be viscometry with a density measurement to explore dependence of viscosity and density on temperature.

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