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APPLICATION NOTE No. 159

Mixing of Highly Viscous Liquids Using Eppendorf MixMate®

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Abstract

Viscous liquids such as glycerol or polyethylene glycol (PEG) are often used as a nonaqueous solvent for drugs, vaccines or dyes or serve as antifreeze and stabilizing agents in protein solutions. Because of their high viscosity these solvents are hard to mix and place specific demands on the performance of laboratory mixers.
The aim of this work was to analyze the performance of Eppendorf benchtop mixer MixMate in mixing highly viscous solutions. Therefore, we determined experimentally the maximum glycerol concentration being efficiently mixed by MixMate in different plate and tube formats. Results presented herein show that MixMate is able to efficiently mix glycerol solutions up to 90 % at 20 °C which corresponds to a viscosity of 219 Centipoise (cP), i.e., approx. 219-fold the viscosity of water. The best mixing performance was found for vessels with a wide diameter and flat bottom. In general, the mixing performance of MixMate allows fast and efficient mixing of aqueous solutions as well as highly viscous samples.

Introduction

MixMate is a high speed mixer that was especially developed for fast and controlled mixing of small reaction volumes in all types of plates, including 384-well plates.
As proven in previous publications, MixMate mixes all kinds of molecular biological samples within 1 minute, independent of sample properties or plate geometry [1] . While most samples and reaction buffers used in molecular biology are based on aqueous solutions having a viscosity comparable to water we also wanted to test the capability of MixMate in mixing highly viscous solutions. These highly viscous solutions such as glycerol, propylene glycol or polyethylene glycol (PEG) are often used in pharmaceutical or food industry as nonaqueous solvents for drugs, vaccines or dyes. In addition, with molecular biology enzyme solutions, these substances serve as antifreeze and stabilizing agents.

Viscosity, commonly perceived as "thickness", is a measure that describes the resistance of a fluid to deform under shear stress. The viscosity varies with the substance, its concentration and with temperature. The higher the temperature, the lower the viscosity.

There are different physical units in use to express the dynamic viscosity of a solution: The International System of Units (SI unit) expresses viscosity in pascal-second (Pa·s) whereas the centimeter-gram-second system (CGS) uses poise (P), or more commonly centipoise (cP). Centipoise is commonly used because water has a viscosity of 1.0020 cP (at 20 °C) [2,3] . The relation between both units is:
1 Pa.s = 10 P
1 mPa.s = 1 cP

For example, the viscosity of some undiluted substances are:
> Glycerol: 1410 mPa.s at 20 °C
> PEG-200 (MW 190 – 210): 50 mPa.s at 25 °C

> PEG-300 (MW 280 – 320): 70 mPa.s at 25 °C
> PEG-400 (MW 380 – 420): 90 mPa.s at 25 °C
> PEG-600 (MW 570 – 630): 135 mPa.s at 25 °C
> Propylene glycol: 40.4 mPa.s at 25 °C
> Triton® X-100: 240 mPa.s at 25 °C

In this work we analyzed the performance of MixMate in mixing highly viscous solutions. Therefore, we determined experimentally the maximum glycerol concentration being efficiently mixed by Eppendorf MixMate in different kind of tubes and 96-well plates and at different filling volumes. All experiments were performed at 20 °C. The tested glycerol concentrations were afterwards correlated to their corresponding viscosity in mPa.s as given within the literature [4].

Materials and Methods

The mixing performance of MixMate was tested with different vessel types (micro test tubes and 96-well DWP, MTP and PCR plates; please refer to Table 1 for details) and different filling volumes (12.5, 25, 50 and 75 % of the recommended working volume).

For the assay, glycerol (Rotipuran® ≥ 99.5 %, p.a. water-free, Carl Roth GmbH, Karlsruhe, Germany) solutions of different concentrations (50, 60, 70, 75, 80, 85, 90, 95 % (v/v) in dH2O) were pipetted to the bottom of the vessels and were carefully overlayed with 1/30 volume of Ponceau-4R colored dH2O (Figure 1). After 1 min mixing with Eppendorf MixMate at maximum speed (3,000 rpm for MTP and PCR plates; 2,000 rpm for tubes and DWP) the vessels were visually checked for completely mixed glycerol solutions. A solution was rated as completely mixed if the solution was stained homogeneously red without any cords (Figure1).
All experiments were performed at 20 °C room temperature. Solutions, plates, tubes, MixMate, pipettes and tips were equilibrated to room temperature.

Results and Discussion

Previous publications have shown that the unique 2DMix-Control technology of MixMate enables fast mixing of small reaction volumes independent of sample properties or vessel geometry [1]. Even difficult mixing applications such as resuspension of bacterial cell pellets can be achieved with MixMate in less than 1 minute [6]. Furthermore, the 2Dmixing stroke ensures a controlled mixing motion which reduces the risk for lid-wetting or cross-contamination [7].

Figure 1: Experimental setup before (left) and after (right) mixing with MixMate. Left: Glycerol solutions overlayed with 1/30 volume of red colored water. Right: A completely mixed solution as indicated by the homogeneously red staining without any cords.

In this Application Note we analyzed the mixing performance of MixMate by mixing glycerol solutions in differentkind of tubes and 96-well plates. The experiments showed that MixMate can efficiently mix glycerol solution up to 90 % in less than 1 minute (Table 1). According to data provided in the literature a 90 % glycerol concentration corresponds to a viscosity of 219 mPa.s, 219-fold the viscosity of water [4,5].

As can be seen by the differences between mixing results in 1.5 ml and 2.0 ml tubes and micro test plates with round and flat bottom, the shape of the vessel bottom largely influences the mixing performance. Flat bottom vessels were easier to mix than those with round bottom or conical shape.

Looking at the relationship between sample viscosity and total sample volume, the data show that the best performance at high viscosities was achieved with Eppendorf Plates Deepwell 96 2 ml (DWP 96/2000) as up to 500 µl of 90 % glycerol solution (viscosity = 219 mPa.s) were efficiently mixed by MixMate. These data prove that MixMate, besides its superior performance in mixing aqueous solutions, also enables efficient mixing of highly viscous samples.

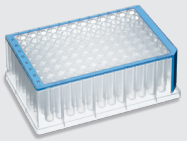
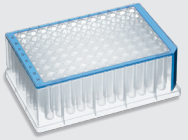
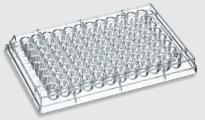
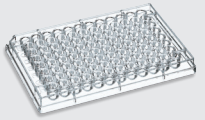
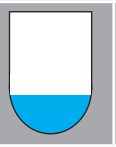
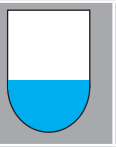
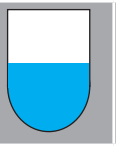
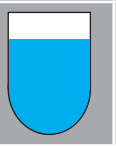
	Tubes		Plates (96-well)				
	Micro test tubes (max. 2,000 rpm)		Deepwell plate (DWP) (max. 2,000 rpm)		Micro test plate (MTP) (max. 3,000 rpm)		PCR plate (max. 3,000 rpm)
							
	Eppendorf Tube Safe-Lock 2.0 ml	Eppendorf Tube Safe-Lock 1.5 ml	Eppendorf Plate Deepwell 96/2000 µl	Eppendorf Plate Deepwell 96/1000 µl	Nunc F96 MicroWell™ Plates (# 260836)	Nunc 96 MicroWell™ Plates (# 249570)	Eppendorf twin.tec® PCR Plate 96, skirted
Total volume:	2,000 µl	1,500 µl	2,400 µl	1,200 µl	400 µl	300 µl	150 µl
Working volume:	2,000 µl	1,500 µl	2,000 µl	1,000 µl	200 µl	150 µl	150 µl
Bottom shape:	round	conical	RecoverMax conical	RecoverMax conical	flat	U-bottom	conical
12.5 % filling volume 	85 % (109 mPa s)	80 % (60 mPa s)	90 % (219 mPa s)	85 % (109 mPa s)	90 % (219 mPa s)	90 % (219 mPa s)	80 % (60 mPa s)
25 % filling volume 	85 % (109 mPa s)	75 % (35 mPa s)	90 % (219 mPa s)	80 % (60 mPa s)	90 % (219 mPa s)	90 % (219 mPa s)	80 % (60 mPa s)
50 % filling volume 	80 % (60 mPa s)	75 % (35 mPa s)	80 % (60 mPa s)	70 % (22 mPa s)	90 % (219 mPa s)	85 % (109 mPa s)	80 % (60 mPa s)
75 % filling volume 	75 % (35 mPa s)	50 % (6 mPa s)	70 % (22 mPa s)	50 % (6 mPa s)	90 % (219 mPa s)	85 % (109 mPa s)	50 % (6 mPa s)

Table 1: Mixing of highly viscous solutions in tubes and 96-well plates using Eppendorf MixMate. Vessels were filled with glycerol solution up to 12.5, 25, 50 and 75 % of the recommended working volume. The maximum glycerol concentration (in percent) that was completely mixed after 1 minute at either 2,000 rpm (tubes and DWP) or 3,000 rpm (MTP and PCR plate) is listed. In parentheses the viscosity of the aqueous glycerol solutions in millipascal-seconds (mPas) is given. All experiments were performed at a temperature of 20°C.

References

[1] Osterhoff C, Dauch D, Mueller P, Borrmann L. Comparison of mixing performance in 96- and 384-well plates of Eppendorf MixMate and competitor devices. Eppendorf Application Note 130, 2006.
[2] Swindells JF, Coe JR, Godfrey TB. Absolute viscosity of water at 20 °C. J. Res. Nat. Bur. Stand. 1952; 48(1):1-31.
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[4] Segur JB and Oberstar HE. Viscosity of Glycerol and Its Aqueous Solutions. Industrial and Engineering Chemistry. 1951; 43(9):2117 – 2120.
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[6] Osterhoff C, Mueller P, Borrmann L. Eppendorf MixMate – Resuspension of bacteria pellets in deepwell plates (96- and 384-well) and micro test tubes. Eppendorf Application Note 131, 2006.
[7] Osterhoff C, Mueller P, Borrmann L. Eppendorf MixMate – Experimental evidence of controlled mixing using a PCR-based chessboard assay. Eppendorf Application Note 129, 2006.

Ordering information

Description	Order no. International	Order no. North America
MixMate®, basic device without tube holders		
230 V, 50–60 Hz	5353 000 510	2231000804
110 V, 50–60 Hz	5353 000 529	
Eppendorf Safe-Lock Tubes 1.5 ml, Eppendorf quality, colorless	0030 120 086 (1,000 pcs)	022363204 (500 pcs)
Eppendorf Safe-Lock Tubes 2.0 ml, Eppendorf quality, colorless	0030 120 094 (1,000 pcs)	022363352 (500 pcs)
Deepwell Plate 96/1000 µL, PCR clean, Blue, 20 plates (5 bags of 4)	0030 501 241	951032689
Deepwell Plate 96/2000 µL, PCR clean, Blue, 20 plates (5 bags of 4)	0030 501 349	951033481
Eppendorf twin.tec® PCR Plate 96, skirted 150 µL, colorless, 25 pcs	0030 128 508	951020401

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