co-mix

Application Note
AN-001

Vitl
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Mixing High Viscosity Solutions with the Vitl Co-Mix Laboratory Mixer

Abstract

The viscosity of a solution has a significant effect on the mixing efficiency of an orbital mixer. Viscous liquids such as glycerol and polyethylene-glycol (PEG) are widely used as a non-aqueous mixing solvent for drugs, vaccines and dyes, or as a stabilizing agent in protein solutions.

This work was carried out to examine the performance of the Vitl Co-Mix when used to mix solutions with a high concentration of glycerol in different microplates and tubes.

The results presented in this paper show that the Co-Mix is able to efficiently mix glycerol solutions of up to 90%wt (percentage by weight) in most vessel types.

Introduction

The Vitl Co-Mix combines a microplate orbital mixer and tube vortexer in a single unit, with independent speed controls for each module. It was developed to allow variable speed mixing of all types of multiple-well plates that are readily available and widely used in laboratories. The Co-Mix also comes with adapters for mixing an array of 0.5, 1.5 and 2ml microtubes.

Previous tests have shown that aqueous solutions of viscosity close to that of water are easily mixed within 1 minute (mostly less than 10 seconds) independent of the well geometry. However, it was decided to further examine the mixing capability of the Co-Mix when mixing highly viscous solutions, such as glycerol, which is widely used in the pharmaceutical and food industry.

The viscosity of a solution varies significantly with substance, concentration and temperature. The viscosity of glycerol reduces with increasing temperature and concentration. This is demonstrated in the References section at the end of this paper.

Materials and Method

The mixing performance of the Co-Mix was tested for different plate and tube types (vessels) with a range of fill volumes (from 10% to 100% of the maximum recommended working fill volume - WV) and at a tightly controlled ambient temperature of 20°C to 23°C.

The sample used was a solution of Glycerol (SIGMA-7893) in water at a concentration of 90%wt giving a viscosity of 219 cP at 20°C. The colourant was Ponceau 4R Red Dye at a ratio of 1 part in 30 by volume.

All tests were performed with the same viscosity solution - unless the solution failed to mix completely within one minute, in which case a lower viscosity solution (reduced concentration) was used and noted in the test results. The glycerol solution was dispensed into the sample vessel and the red dye pipetted to overlay it. Each combination was mixed on the Co-Mix for 60 seconds at the maximum permissible speed and the mixing efficiency was determined by visual inspection.

Results and Conclusion

The tests were designed to examine the mixing performance of the Co-Mix with highly viscous solutions.

Table 1 below shows the tested vessel types, their physical properties and the achievable mixing viscosity for increasing fill volumes. As indicated by the test results, the vessel type and fill volume did not have a significant effect on the mixing efficiency other than for the 1.5ml micro-tubes.

The experiments demonstrated that the instrument is capable of mixing a 90%Wt glycerol solution, in most cases, independent of sample vessel geometry and fill volumes.

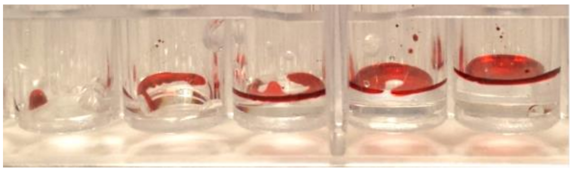
Table 1. vessel types, physical properties and achievable mixing viscosity for increasing fill volumes

Vessel Type	96 Well MTP	96 Well MTP	96 Well PCR	96 Well DWP	384 Well MTP	1.5ml Micro Tube	2ml Micro Tube
Vessel Shape	Circular	Circular	Circular	Circular	Square	Circular	Circular
Vessel Bottom	Flat	U-shaped	Conical	U-shaped	V-shaped	Conical	Round
Mixing Speed	2000rpm	2000rpm	3000rpm	3000rpm	3000rpm	2000rpm	2000rpm
Vessel Volume	350µl	350µl	150µl	1200µl	250µl	1500µl	2000µl
Working Volume (WV)	100µl 28%	100µl 28%	100µl 66%	500µl 42%	100µl 40%	1500µl 100%	2000µl 100%
Fill Volume 10% of WV	90%wt	90%wt	90%wt	90%wt	90%wt	85%wt	90%wt
Fill Volume 25% of WV	90%wt	90%wt	90%wt	90%wt	90%wt	85%wt	90%wt
Fill Volume 50% of WV	90%wt	90%wt	90%wt	90%wt	90%wt	80%wt	90%wt
Fill Volume 75% of WV	90%wt	90%wt	90%wt	90%wt	90%wt	80%wt	80%wt
Fill Volume 100% of WV	90%wt	90%wt	90%wt	90%wt	85%wt	Working Volume same as Maximum Volume	

Reference Viscosity at 20°C	90%wt 219cP	85%wt 109cP	80%wt 60cP

Pictures 1 and 2 below show the mixing of the 96 well flat-bottomed microtiter plate (MTP) at the five tested fill volumes of the glycerol and 1:30 dye solution:

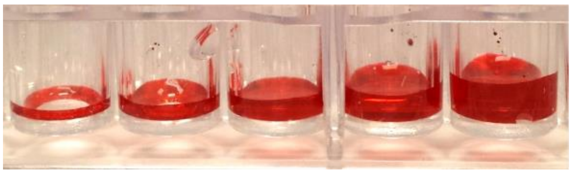
Picture 1. 96-well MTP before mixing



10µl 25µl 50µl 75µl 100 µl

2000
RPM
⇨
60 Secs

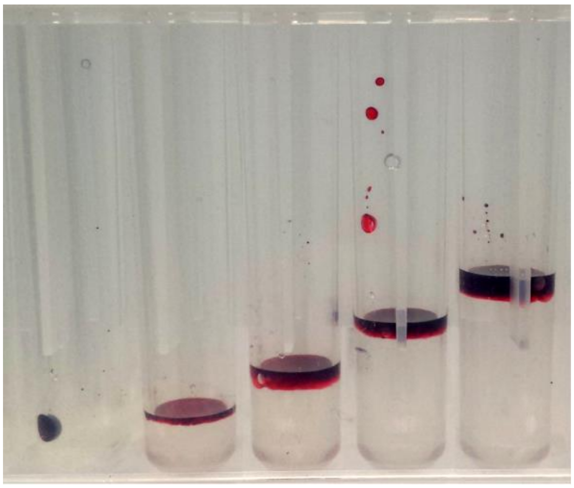
Picture 2. 96-well MTP after mixing



10µl 25µl 50µl 75µl 100 µl

Similarly, pictures 3 and 4 below show the mixing of the U-shaped 96 well deep well plate (DWP) at the five fill volumes:

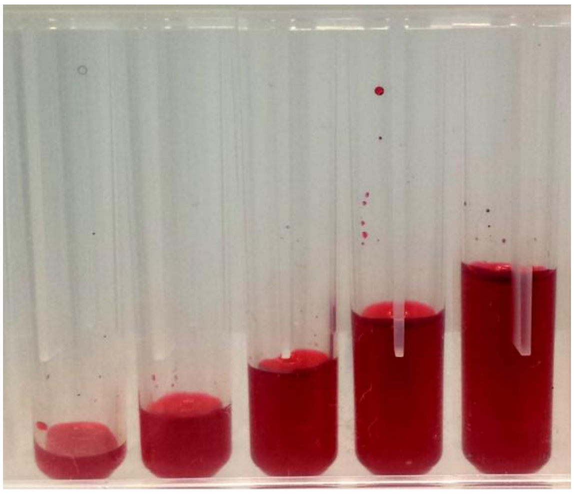
Picture 3. U-shaped 96-well plate before mixing



50µl 125µl 250µl 375µl 500 µl

3000
RPM
⇨
60 Secs

Picture 4. U-Shaped 96-well plate after mixing



50µl 125µl 250µl 375µl 500 µl

It can be clearly seen from the images above that both sets of tests demonstrate excellent mixing within a short space of time without excessive vertical vortexing of the samples.

Reference Data

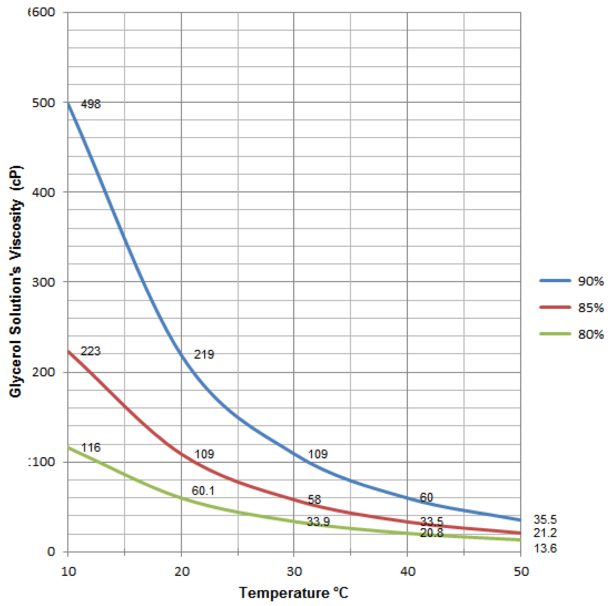
The viscosity of glycerol at different concentrations (%wt percentage weight dilution in water) and temperatures is given in the table below. The viscosity data has been plotted for the three concentrations used in this experiment in the range 10°C to 50°C to allow the reader to interpolate from the quoted 20°C values.

Table 2. Viscosity of Aqueous Glycerine Solutions in Centipoises/mPa s

Glycerine percent weight	Temperature (°C)										
	0	10	20	30	40	50	60	70	80	90	100
0 [1]	1.792	1.308	1.005	0.8007	0.6560	0.5494	0.4688	0.4061	0.3565	0.3165	0.2838
10	2.44	1.74	1.31	1.03	0.826	0.680	0.575	0.500	–	–	–
20	3.44	2.41	1.76	1.35	1.07	0.879	0.731	0.635	–	–	–
30	5.14	3.49	2.50	1.87	1.46	1.16	0.956	0.816	0.690	–	–
40	8.25	5.37	3.72	2.72	2.07	1.62	1.30	1.09	0.918	0.763	0.668
50	14.6	9.01	6.00	4.21	3.10	2.37	1.86	1.53	1.25	1.05	0.910
60	29.9	17.4	10.8	7.19	5.08	3.76	2.85	2.29	1.84	1.52	1.28
65	45.7	25.3	15.2	9.85	6.80	4.89	3.66	2.91	2.28	1.86	1.55
67	55.5	29.9	17.7	11.3	7.73	5.50	4.09	3.23	2.50	2.03	1.68
70	76	38.8	22.5	14.1	9.40	6.61	4.86	3.78	2.90	2.34	1.93
75	132	65.2	35.5	21.2	13.6	9.25	6.61	5.01	3.80	3.00	2.43
80	255	116	60.1	33.9	20.8	13.6	9.42	6.94	5.13	4.03	3.18
85	540	223	109	58	33.5	21.2	14.2	10.0	7.28	5.52	4.24
90	1310	498	219	109	60.0	35.5	22.5	15.5	11.0	7.93	6.00
91	1590	592	259	127	68.1	39.8	25.1	17.1	11.9	8.62	6.40
92	1950	729	310	147	78.3	44.8	28.0	19.0	13.1	9.46	6.82
93	2400	860	367	172	89	51.5	31.6	21.2	14.4	10.3	7.54
94	2930	1040	437	202	105	58.4	35.4	23.6	15.8	11.2	8.19
95	3690	1270	523	237	121	67.0	39.9	26.4	17.5	12.4	9.08
96	4600	1580	624	281	142	77.8	45.4	29.7	19.6	13.6	10.1
97	5770	1950	765	340	166	88.9	51.9	33.6	21.9	15.1	10.9
98	7370	2460	939	409	196	104	59.8	38.5	24.8	17.0	12.2
99	9420	3090	1150	500	235	122	69.1	43.6	27.8	19.0	13.3
100	12070	3900	1410	612	284	142	81.3	50.6	31.9	21.3	14.8

[1] Viscosity of water taken from "Properties of Ordinary Water-Substance." N.E. Dorsey, p. 184. New York (1940)

Graph 1. Glycerol Viscosity /°C



Viscosity is specified in either the SI unit pascal-second (Pa.s) or in the CGS system (centimetre-gram-second) poise (p), or more commonly centipoise (cP). The relationship between these two units of measurement is:

1 Pa.c = 1000 cP (or as per the table above) 1cp = 1mPa.s

Vitl
LIFE SCIENCE SOLUTIONS

www.vitlproducts.com
sales@vitlproducts.com
USA: T: +1 804-381-0905
UK: T: +44 (0)1233 638383
China: T: +86 (021) 585 854 63