Virkon™ S: stability and microbiological Performance at sub-zero temperatures

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STUDY SUMMARY

Additions of glycol based chemical agents to use dilutions of Virkon™ S can be employed in order to help protect the solutions from freezing when employed under cold climate conditions. This process may be extended to include commercially available automotive antifreeze preparations, based upon diethylene glycol (DEG).

Addition of suitable glycol additives to Virkon™ S 1:100, 1:200 dilutions can substantially reduce the freezing point of the mixed solution. Two commercial glycol antifreezes (noted as DEG1, DEG2) were evaluated in this study, alongside monopropylene glycol (MPG).

Dilution	Ratio of water: glycol	Stable liquid dilution at
1/100	80:20	-10°C
1/200	75:25	-10°C

Microbiological performance was demonstrated by a series of bactericidal studies on Virkon™ S show no loss of biocidal activity, when the diluted solutions including the presence of the MPG or a DEG based solvent were challenged. Bactericidal studies were performed on solutions at the stress points (end of shelf life as determined by the chemical stability assessment).

Dilution	Glycol	%	Storage Temp (°C)	Solution Age (hours)	Efficacy vs. Pseudomonas aeruginosa reduction in initial count, 30 minutes
1:100	MPG	20	20	48	> 5 log
1:100	DEG 1				> 5 log
1:100	DEG 2				> 5 log
1:200	MPG	25			> 5 log
1:200	DEG 1				> 5 log
1:200	DEG 2				> 5 log
1:100	MPG	20	-10	168	> 5 log
1:200	MPG	25			> 5 log

Addition of glycols may reduce the viable lifetime of the diluted solution, compared to the same dilutions prepared in the water alone, however this validity is very much dependent on the temperature of storage.

Temperature (°C)	Dilution	Shelf life	Glycols
-10	1:100	7 days	All tested
	1:200	7 days	All tested
20	1:100	48 hours	All tested
	1:200	48 hours	All tested

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Virkon™ S stability and microbiology performance at sub-zero temperatures

Introduction

Dilutions of Virkon™ S (1/100 and 1/200) were made up in municipal hard water (average hardness- typically 380 ppm as $CaCO_3$) with inclusions of glycol based anti-freezes to prevent the solution freezing at sub-zero temperatures. A 1% Virkon™ S solution in water will begin to freeze if held at -4^{0}C.

The ratios of water to glycol required to prevent freezing at -10^{0}C are shown in the table below.

Dilution	Ratio of water: glycol	Stable liquid dilution at
1/100	80:20	-10^{0}C
1/200	75:25	-10^{0}C

This data will benefit colder climates, where temperatures often reach below zero degrees Celsius where a standard Virkon™ S dilution would freeze.

The chemical stability and microbiology performance of variations of these solutions were tested to observe the negative effect the glycol has, if any.

3 glycols were chosen for this trial. A reference sample of commercial grade monopropylene glycol (100%) was used as well as two commercial automotive ethylene glycol based anti-freezes which are referred to here as DEG1 (80-90% ethylene glycol) and DEG2 (30-60% ethylene glycol). It should be noted that these samples were specifically chosen for their absence of silicates (which would likely have a highly destabilising effect on Virkon™ S in solution) and other grades of anti-freeze would likely perform differently to the ones chosen here and should be tested independently before use in this application. Contact Antec for advice.

During the stability trials, two temperatures were trialled (20^{0}C and -10^{0}C) to see how this would affect the speed of degradation of the sample. These were seen as the upper and lower temperature limits of storage of this type of sample. The microbiological performance was tested at each of these extremes at a designated end of shelf life period determined by

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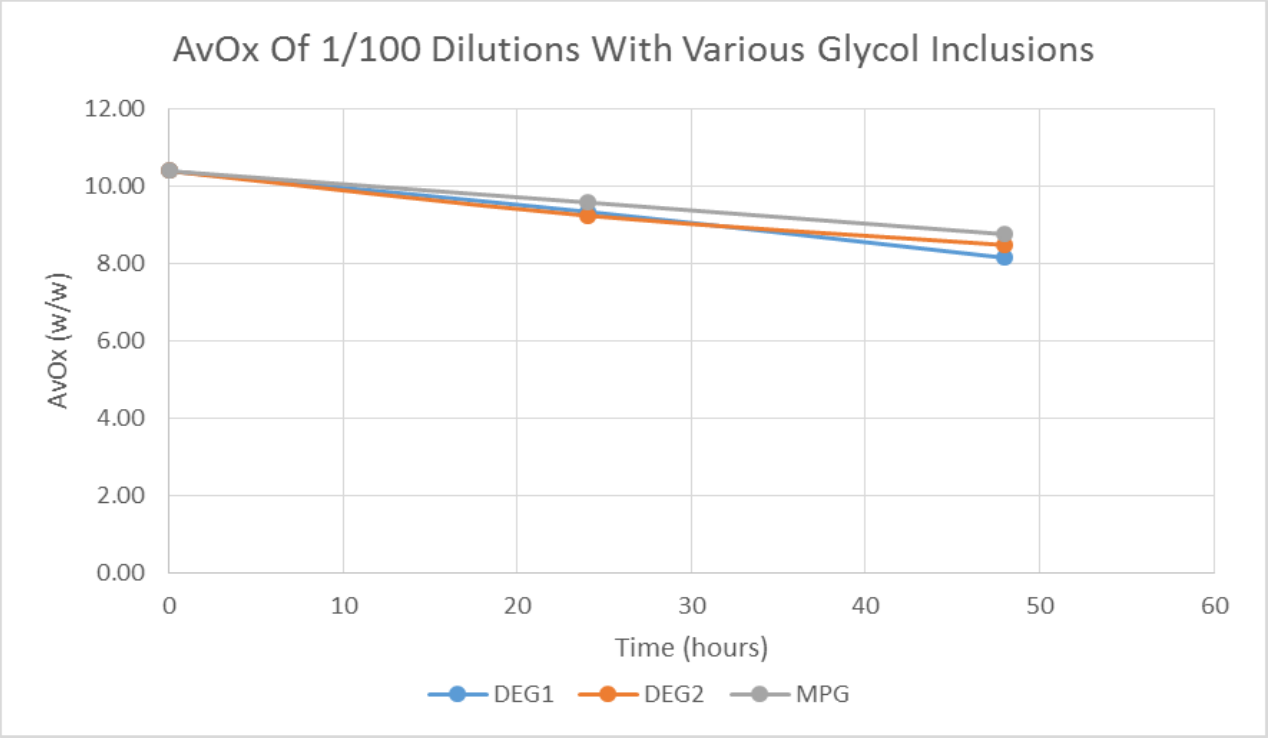
the available oxygen level, so that efficacy at this point could be proven. Samples of this type stored outside of these limits or beyond the shelf life do not have guaranteed efficacy.

A 1% Virkon™ S dilution in local tap water, held at room temperature (20^{0}C) is expected to have a shelf life of 5-7 days (effective biocidal duration). To monitor stability, a titration was carried out which determines the available oxygen (AvOx) in the dilution. The method employed for this titration can be found in Appendix 1 of this report. The available oxygen values have been related back to the powder that was originally added to create the dilution. This way, each of the dilutions can be easily compared and the stability of the sample is clearer.

Chemistry Results

Comprehensive data from this section can be found in Appendix 2. The graphs show the variance in AvOx with time (AvOx values are quoted in relation to the powder inclusion for the dilution)

Stability with different glycol inclusions at 20°C:



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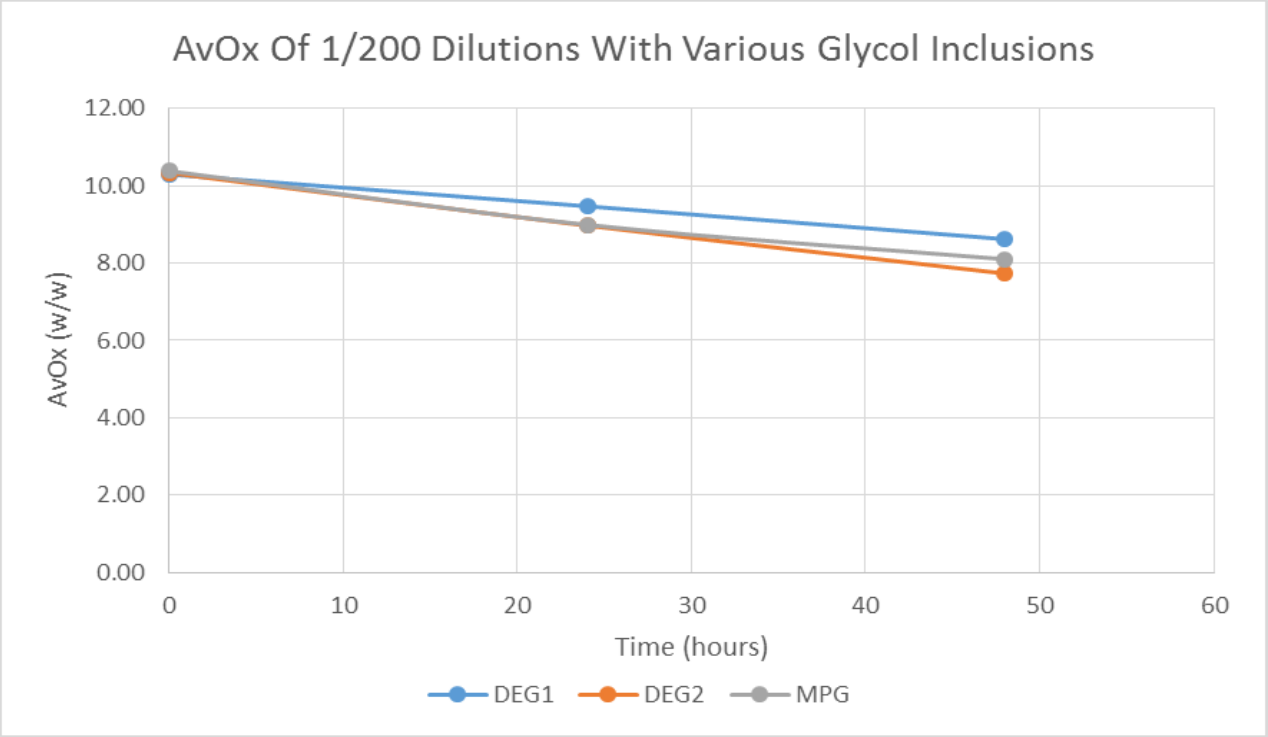
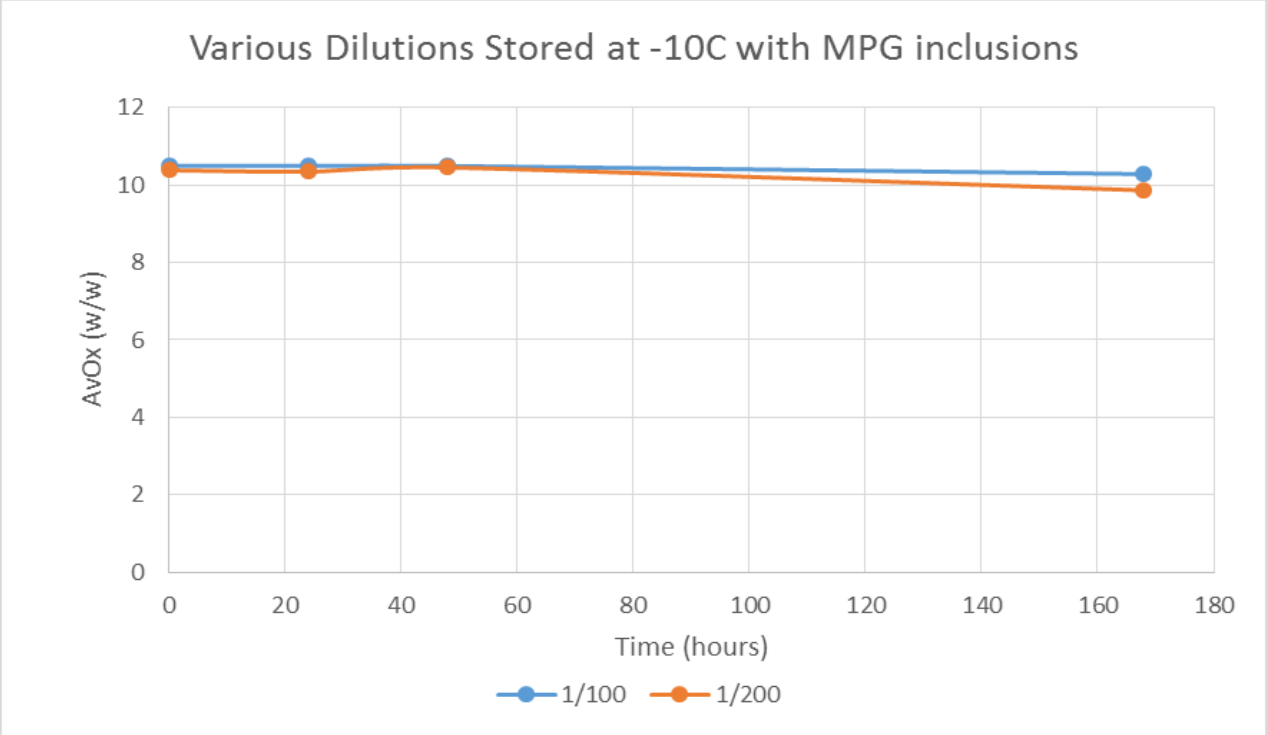


Stability with monopropylene glycol inclusions at -10°C:



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Micro Results

A summary confirming the efficacy of the sample dilutions with glycol inclusions at the pre-determined end of shelf life is below

Dilution	Glycol	%	Storage Temp ^{0}C	Solution Age (hours)	Efficacy vs. Pseudomonas aeruginosa reduction in initial count, 30 minutes
1:100	MPG	20	20	48	> 5 log
1:100	DEG 1				> 5 log
1:100	DEG 2				> 5 log
1:200	MPG	25			> 5 log
1:200	DEG 1				> 5 log
1:200	DEG 2				> 5 log
1:100	MPG	20	-10	168	> 5 log
1:200	MPG	25			> 5 log

Detailed results of the microbiological assessments can be found in Appendix 3

Discussion

From the data collected at the various temperatures, the following retention times have been recommended for a solution of Virkon™ S under the specified conditions. If a solution is kept for longer than the recommended time, it cannot be guaranteed that the product will be efficacious.

Temperature, storage	Dilution	Glycol additive, type	Glycol additive, %	Shelf life proposed (hours / days)
-10C	1:100	MPG	20%	7
		DEG1		7
		DEG2		7
	1:200	MPG	25%	7
		DEG1		7
		DEG2		7
20C	1:100	MPG	20%	48 hour
		DEG1		48 hour
		DEG2		48 hour
	1:200	MPG	25%	48 hour
		DEG1		48 hour
		DEG2		48 hour

The points at which the microbiological performance have been tested are based on the stability results which can be found in chemistry section. We have tested each of the solutions against pseudomonas.

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Appendix 1

Accurately pipette 50.0 ml of Virkon S solution 250 ml conical flask. Add 10 ml of 10% acetic acid and approximately 1 g of potassium iodide. Titrate with 0.1 M sodium thiosulphate solution until the test solution returns to its original colour; record the titre, T ml. Calculate the Available Oxygen using the following formula:

$$\text{AvOx\%w/w as Chlorine} = \frac{T \times 0.1}{1000} \times \frac{35.45}{W} \times \frac{5000}{50} \times 100 = \frac{T \times 35.45}{W}$$

NB-Recorded weight of powder: W g

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Appendix 2

AvOx values of 1/100 Virkon™ S with 20% Glycol*				
Time (h)	20^{0}C			-10^{0}C
	DEG1	DEG2	MPG	MPG
0	10.38	10.38	10.38	10.49
1	-	-	-	-
2	-	-	-	-
4	-	-	-	-
6	-	-	-	-
24	9.33	9.23	9.57	10.49
48	8.14	8.47	8.75	10.49
72	-	-	-	-
96	-	-	-	-
120	-	-	-	-
144	-	-	-	-
168	-	-	-	10.28

AvOx values of 1/200 Virkon™ S with 25% Glycol*				
Time (h)	20^{0}C			-10^{0}C
	DEG1	DEG2	MPG	MPG
0	10.29	10.33	10.38	10.38
1	-	-	-	-
2	-	-	-	-
4	-	-	-	-
6	-	-	-	-
24	9.47	8.97	8.99	10.34
48	8.62	7.73	8.10	10.45
72	-	-	-	-
96	-	-	-	-
120	-	-	-	-
144	-	-	-	-
168	-	-	-	9.86

*All AvOx values are quoted in reference to the powder inclusion for the dilution.

Note: "-" indicates that the sample wasn't tested at this point

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Appendix 3

Microbiological studies

A series of laboratory based microbiological studies have been conducted to support the mixture of a 1:100 (1%) use-solution of Virkon™ S in water, with variable added levels of glycol . Mixtures are prepared immediately prior to the commencement of the testing (typically within 1 hour of preparation).The general microbiological suspension test methodology employed in this study is outlined in *Appendix 3.*

1. Virkon™ S 1:100 & 1:200 dilutions (20% -25% glycol), ambient (20C) temperature , bactericidal suspension test

Virkon™ S						
Study reference	**Test solutions**	**Solvent**	**Conditions**	**Method**	**Initial count (N) log_{10}**	**Reduction in count (R) log_{10}**
Bac129-1016	Virkon™ S 1:100	80% water: 20% MPG	Temperature: 20C Organic soil: 5% horse serum Contact time: 30 minutes Hard water (>300ppm) Organism : Ps aeruginosa NCIMB 10421	Bactericidal suspension test	8.5	>5.5
		80% water: 20% DEG 1				>5.5
		80% water: 20% DEG2				>5.5

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Virkon™ S						
Study reference	**Test solutions**	**Solvent**	**Conditions**	**Method**	**Initial count (N) log_{10}**	**Reduction in count (R) log_{10}**
Bac129-1016	Virkon™ S 1:200	75% water: 25% MPG	Temperature: 20C Organic soil: 5% horse serum Contact time: 30 minutes Hard water (>300ppm) Organism : Ps aeruginosa NCIMB 10421	Bactericidal suspension test	8.5	>5.5
		75% water: 25% DEG 1				>5.5
		75% water: 25% DEG2				>5.5

2. Virkon™ S 1:100 & 1:200 dilutions (20% -25% glycol), ambient (-10C) temperature , bactericidal suspension test

Virkon™ S						
Study reference	**Test solutions**	**Solvent**	**Conditions**	**Method**	**Initial count (N) log_{10}**	**Reduction in count (R) log_{10}**
Bac134-1016	Virkon™ S 1:100	80% water: 20% MPG	Temperature: -10C Organic soil: 5% horse serum Contact time: 30 minutes Hard water (>300ppm) Organism : Ps aeruginosa NCIMB 10421	Bactericidal suspension test	8.3	>5.3
		80% water: 20% DEG 1				>5.3
		80% water: 20% DEG2				>5.3

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Virkon™ S						
Study reference	**Test solutions**	**Solvent**	**Conditions**	**Method**	**Initial count (N) log_{10}**	**Reduction in count (R) log_{10}**
Bac134-1016	Virkon™ S 1:200	75% water: 25% MPG	Temperature: -10C Organic soil: 5% horse serum Contact time: 30 minutes Hard water (>300ppm) Organism : Ps aeruginosa NCIMB 10421	Bactericidal suspension test	8.3	>5.3
		75% water: 25% DEG 1				>5.3
		75% water: 25% DEG2				>5.3

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Summary & conclusion:

Virkon™ S is an effective biocidal product when employed under conditions of low temperatures.

Microbiological studies of solutions stored at both 20C & and at -10C have shown that the product remains effective at the end of the storage period, for 1:100 & 1:200 dilutions of the product.

Glycol additives can reduce the shelf life of the stored solutions, & this storage period is very much temperature & dilution dependent.

Bacteriological test method (outline):

Bacteriological suspension tests are conducted broadly in line with the requirement set out in EU CEN type methodology (method is a quantitative suspension test typically used for the evaluation of bactericidal activity of chemical disinfectants).

The technique that we employ in this study is based on a suspension test, using a bacterial strain of Pseudomonas aeruginosa.

Modifications to the test conditions may be made by varying the temperature, using different soil levels, & also by altering the contact times.

Test Conditions:

Organisms:

Ps. aeruginosa strain NCIMB 10421

Organic soil(s): 5% horse serum

Test temperature(s):

a. -10C (for study 1)
b. 20C (for study 2)

Diluent: Hard water, > 300ppm (as $CaCO_3$)

Neutraliser: Universal polyvalent neutraliser *

Agar: Tryptone soya agar (TSA), for organism enumeration, & organism recovery.

Initial inoculum: initial count (N) between a log value range of 8.2 to 8.7

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Pass criteria:

In line with many international test standards for assessing efficacy, a reduction in the initial bacterial count of >5 log is accepted as a suitable demonstration of activity of the disinfectant, under the conditions of the test.

*Universal Neutraliser formulation:

Universal Disinfectant Neutraliser	
Distilled water	960ml
Sodium thiosulphate	5g
0.25N phosphate buffer (34g KH_2PO_4 dissolved in 1 litre distilled water)	10ml
Tween 80	30ml
L-Histidine	1g
Lecithin	7g

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