

DISINFECTANT SOLUTIONS

The present invention relates to disinfectant solutions effective against a wide range of viruses and micro organisms.

5 It is known that a disinfectant solution containing lithium dodecyl sulphate and non-ionic detergent dissolved in a water/aliphatic alcohol mixture and comprising not less than 50% by volume of an aliphatic alcohol is effective against viruses, extra cellular and intra-cellular micro-organisms and further inhibits the intra-cellular replication
10 of certain viruses.

We have now found that combinations of two components selected from an aqueous solutions of a non-ionic detergent, an aqueous solution of an alkali metal or
15 ammonium salt of an alkyl sulphuric acid containing 8-20 carbon atoms and an aliphatic alcohol are effective in inactivating a wide range of viruses and other micro-organisms. The selective combinations were unexpectedly found to exhibit a dramatic level of synergism between the
20 components, the activity being in some cases increased many thousandfold compared with the activity of the compounds tested independently.

The non-ionic detergent may be an ethylene oxide condensate, for example a non-idet sold by Shell Chemicals
25 U.K. Limited under the designation NONIDET P40 (octylphenol-ethyleneoxide condensate).

The most preferred alkyl sulphuric acid salt is lithium or sodium dodecyl sulphate.

5 The aliphatic alcohol preferably has up to three carbon atoms in the chain, and is most preferably ethyl alcohol (EtOH).

The disinfectant of the present invention may be used as an aqueous solution, in aerosol sprays, and, with suitable carriers, as ointments or creams.

10 The following examples illustrate the present invention but are not limited thereto.

The following aqueous solutions and mixtures with water were prepared:

0.02% (w/v) lithium dodecyl sulphate (LDS)

0.02% (w/v) sodium dodecyl sulphate (SDS)

15 0.02% (v/v) non-idet (P40)

50% alcohol/water (v/v)

Final concentrations of the compounds in the presence of the organism dilutions are noted in the table below.

20 Inactivation of Viruses

Example 1

Herpes simplex virus type 1 (strain Troisbel) and type 2 infected cell extracts were diluted 1/100 to 10^7 pfu/ml (plaque forming units per ml.) in sterile water and mixed
25 with equal volumes of the prepared solutions. The virus

dilutions were incubated with solutions for 10 minutes at room temperature (20°C). An aliquot of reaction mixture was diluted 1/100 in Eagles medium and tested for virus infectivity following the method of Russell (Nature 195 1962 pages 1028-1029). Virus inactivation was measured as the reduction ($\log_{10}$) in virus titre when the virus was exposed to a component or a mixture of components of the disinfectant compared with the virus titre in a control medium. Results are given in Table 1 for type 1 HSV (Examples 1 and 2) and type 2 HSV Example 3).

TABLE 1

Experiment No	Components	1		2		3	
		Expd.	Obs.	Expd.	Obs.	Expd.	Obs.
15	EtOH (25% v/v)	--	0.19	--	0.18	--	0.0
	P40 (0.01% v/v)	--	0.29	--	0.16	--	0.37
	LDS (0.01% w/v)	--	0.16	--	0.34	--	0.13
20	EtOH + P40	0.48	0.50	0.34	0.75	0.37	0.42
	EtOH + LDS	0.35	0.89	0.52	0.16	0.13	0.74
	P40 + LDS	0.45	>5.3	0.50	4.42	0.50	>5.1

The effective synergism is shown as the difference between the observed virus inactivation minus the expected

virus inactivation (the sum of the virus inactivations for the individual components). The results show a very marked synergism in respect of the combination of the non-ionic detergent and lithium dodecyl sulphate.

5

Example 2

Example 1 was repeated except that sodium dodecyl sulphate (SDS replaced the lithium salt). The results are given in Table 2 below.

TABLE 2

10

Experiment No. 4

	Expected Virus Inactivation	Observed Virus Inactivation
<u>Components</u>		
15 EtOH (25% v/v)	-	0.13
P40 (0.01% v/v)	-	0.04
SDS (0.01% w/v)	-	0.10
20 EtOH + P40	0.17	0.39
EtOH + SDS	0.23	0.55
P40 + SDS	0.14	3.63
EtOH + P40 + SDS	0.27	1.11

25 The results show a very significant synergistic effect for the non-ionic detergent and sodium dodecyl sulphate combination and also for the combination of all three

components, namely alchohol non-ionic detergent and sodium dodecyl sulphate.

Example 3

Tests were carried out using a series of viruses under the same conditions as for Example 1 but using different concentrations of disinfectant components. The viruses tested were:-

Pseudorabies virus - experiment number 5

Bovine mamillitis - experiment number 6

Equine abortion virus - experiment number 7

The results are given in Table 3 and the concentration of components in Table 4 below.

TABLE 3

Virus Inactivation

Experiment No.	5		6		7	
	Exptd.	Obs.	Exptd.	Obs.	Exptd.	Obs.
EtOH	--	0.39	--	0.00	--	0.00
P40	--	0.00	--	0.00	--	0.01
LDS	--	0.16	--	0.16	--	2.04
EtOH + P40	0.39	1.03	0.00	0.00	0.01	0.00
EtOH + LDS	0.55	1.12	0.16	>4.36	2.04	>5.90
LDS + P40	0.16	2.56	0.16	>4.36	2.05	5.42

TABLE 4

Experiment No.	5	6	7	8
<u>Component</u>				
P40 v/v	0.010%	0.010%	0.01%	
5 LDS w/v	0.001%	0.005%	0.01%	
EtOH	25%	10.00%	10%	

In each case there was a marked synergistic effect in the combination of lithium dodecyl sulphate and the nonionic detergent and also in the combination of lithium dodecyl sulphate and alcohol.

Example 4

The effect of varying the concentration of components in respect of the synergistic activity of the combination of lithium dodecyl sulphate and the nonionic detergent was tested by measuring the inactivation of herpes simplex virus type 1 under the same conditions as in Example 1. The results are given in Table 5 below.

TABLE 5
Experiment No. 9
LDS (W/V %)

	0.02	0.01	0.005	0.002	H ₂ O
5 P40 (V/V%)					
0.01	>5.00 (3.13)	>5.00 (1.03)	2.75 (0.80)	NS	0.76
0.005	>5.00 (2.47)	2.89 (0.37)	1.37 (0.05)	NS	0.10
10 0.002	NS	NS	NS	NS	0.00
H ₂ O	2.37	0.27	0.04	0.00	0.00

NS = Not significant

The numbers represent virus inactivation ($\log_{10}$) when the virus is exposed to the combined components at the given concentrations; the numbers in brackets represent the expected level of virus inactivation calculated by taking the sum of the inactivation when the two components are used separately at these concentrations.

20 In order to achieve a significant synergistic effect both components should be at a concentration exceeding 0.002% and preferably at least 0.005%.

Example 5

25 The activity of the components was tested in respect of the inactivation of influenza A virus (NWS strain) under the same conditions as for Example 1. The results are given in Table 6 below.

TABLE 6
Influenza A virus (NWS strain)

	<u>Component</u>	<u>Virus inactivation</u>					
		<u>Experiment 10</u>		<u>Experiment 11</u>		<u>Experiment 12</u>	
5		Exptd.	Obs.	Exptd.	Obs.	Exptd.	Obs.
	EtOH		0.06	--	0.03	--	0.02
	P40	--	0.12	--	0.67	--	0.43
	LDS	--	0.00	--	0.04	--	0.00
	EtOH + P40	0.18	0.52	0.70	0.43	0.45	0.31
10	EtOH + LDS	0.06	1.26	0.07	1.94	0.02	1.92
	P40 + LDS	0.12	0.01	0.71	0.14	0.43	0.00

Concentration of components	P40	0.025%)	
	LDS	0.025%)	Exp 10
	EtOH	25.000%)	
15	P40	0.05%)	
	LDS	0.05%)	Exps 11+12
	EtOH	25.00%)	

A marked synergistic effect is noted between alcohol and lithium dodecyl sulphate but, in contrast to the activity against the herpes virus strains, the combination of lithium dodecyl sulphate and non-ionic detergent produced a marked inhibitory effect.

Inactivation of Bacteria

Example 6

Bacterial cultures were subjected to combinations of components. The components, individually or in combination, were added to a bacterial culture of 10^8 organisms/ml, in a ratio of 9:1 for 5 minutes at 25°C. Viable organisms were then titrated on appropriate media. The bacteria tested and the concentrations of the components used were as follows:-

10 *Staphylococcus aureus* - Experiment 13; P40 -

0.5%; LDS - 0.2%; EtOH 25%;

Streptococcus viridians - Experiment 14; P40 - 0.5%; LDS - 0.05%; EtOH - 25%;

15 *Streptococcus pneumoniae* "mucoid" - Experiment 15; P40 - 0.1%; LDS - 0.005%; EtOH - 20%;

Escherichia coli - Experiment 16; P40 - 0.5%; LDS - 0.2%; EtOH - 25%;

Klebsiella aerogenes - Experiment 17; P40 - 1.0%; LDS - 0.4%; EtOH - 35%;

20 *Pseudomonas aeruginosa* - Experiment 18 P40 0.5%; LDS - 0.05%; EtOH 25%;

Clostridium perfringens - Experiment 19; P40 0.5%; LDS - 0.005%; EtOH - 25%;

The results are given in Table 7.

TABLE 7
Bacterial Inactivation

	Expt No	Component	EtOH	P40	LDS	EtOH+ P40	EtOH+ LDS	P40+ LDS
5	13	Expected	--	--	--	0.00	4.28	4.28
		Observed	0.00	0.00	4.28	3.82	6.18	0.00
	14	Expected	--	--	--	0.01	0.14	0.15
		Observed	0.00	0.01	0.14	2.19	6.04	0.08
10	15	Expected	--	--	--	0.19	0.15	0.04
		Observed	0.15	0.04	0.00	3.04	>1.31	0.00
	16	Expected	--	--	--	1.98	4.26	2.68
		Observed	1.78	0.20	<2.48	5.53	>6.84	0.00
	17	Expected	--	--	--	3.34	3.32	0.38
		Observed	3.14	0.20	0.18	>6.11	6.11	0.15
15	18	Expected	--	--	--	0.01	0.02	0.03
		Observed	0.00	0.01	0.02	1.63	1.38	0.00
	18	Expected	--	--	--	0.68	0.04	0.64
		Observed	0.04	0.64	0.00	>6.26	1.20	0.43

A marked synergistic effect is shown by the combinations of alcohol with either lithium dodecyl sulphate or the non-ionic detergent but, as with the influenza virus A strain NWS there was marked inhibition of activity between lithium dodecyl sulphate and the non-ionic detergent in aqueous solutions.

Example 7

Example 5 was repeated using *Streptococcus pneumoniae* "classic" (Experiments 20 - 23) and *Branhamella catarrhalis* (Experiment 24). The concentrations of components used

were:-

	P40	LDS	EtOH
Experiments 20-24	0.05%	0.02%	20%
Experiment 25	0.01%	0.02%	25%

The results are given in Table 8 below.

TABLE 8

Bacterial Inactivation

Expt No	Components	EtOH	P40	LDS	EtOH+ P40	EtOH+ LDS	P40+ LDS
15	20	Expected	--	--	--	0.47	0.32
		Observed	0.32	0.15	0.00	>4.58	2.20
	21	Expected	--	--	--	0.38	0.32
		Observed	0.16	0.22	0.16	0.70	1.56
20	22	Expected	--	--	--	0.02	0.02
		Observed	0.02	0.00	0.00	>6.15	2.13
	23	Expected	--	--	--	0.22	0.53
		Observed	0.00	0.22	0.53	3.33	5.06
	24	Expected	--	--	--	0.00	0.02
		Observed	0.00	0.00	0.02	1.21	>5.93

Experiments 20 - 22 used the same organism, Experiment 23

used a different isolate of the same morphology.

A synergistic effect was noted for each combination, the most dramatic being that between alcohol and the non-ionic detergent.

Example 8

The interaction of the components against *Mycoplasma arginini* was tested as described in Example 5 except mycoplasma were titrated on PPLO medium and colonies allowed to develop for 5 days prior to enumeration. The results are shown in Table 9.

TABLE 9

Component	<u>Bacterial Inactivation</u>			
	<u>Exp 25</u>		<u>Exp 26</u>	
	Expected	Observed	Expected	Observed
10 EtOH 20%		0.00		0.00
P40 0.01%	--	0.21	--	0.00
15 LDS 0.005%	--	0.00	--	0.00
EtOH + P40	0.21	0.00	0.00	0.00
EtOH + LDS	0.00	0.25	0.00	0.00
P40 + LDS	0.21	>3.59	0.00	2.62

20 Marked synergistic reaction was observed between lithium dodecyl sulphate and the non-ionic detergent.

Yeast Activation

Example 9

Interactions against *Candida albicans* were investigated as described in respect of Example 5 except that the estimation of surviving yeast cells was carried out on Sabouraud's medium. The results are given in Table 10.

TABLE 10

<u>Component</u>		<u>Yeast Inactivation</u>			
		<u>Exp 27</u>		<u>Exp 28</u>	
		<u>Expected</u>	<u>Observed</u>	<u>Expected</u>	<u>Observed</u>
10					
	EtOH 25%	--	0.12	--	0.00
	P40 0.5%	--	0.02	--	0.00
15	LDS 0.2%	--	0.80	--	0.33
	EtOH + P40	0.14	2.26	0.00	0.57
	EtOH + LDS	0.92	2.14	0.33	0.59
	P40 + LDS	0.82	0.32	0.33	0.00

20 A synergistic effect is noted between the combinations of alcohol and the non-ionic detergent and between alcohol and lithium dodecyl sulphate.

Virus Infected Cells

Example 10

BHK - 21 cells were infected with herpes simplex virus type 1 (HSV 1) (Experiment 29) or influenza A virus (Experiment 30) at a multiplicity of infection to ensure that every cell was infected by the virus. The cells were gently harvested (to avoid cell disruption) and washed with phosphate buffered saline. In these two experiments cells were diluted prior to ultrasonic disruption and these solutions disrupted prior to virus titration. In this way inactivation of extra cellular virus by components of disinfectant was minimised. The virus titration was carried out according to the method described in Example 1.

Results are given in Table 11.

TABLE 11

Component	Virus Inactivation			
	Experiment No. 29		Experiment No. 30	
	Expected	Observed	Expected	Observed
EtOH (25% v/v)	--	1.49	--	0.97
P40 (0.01% Expt. 29 (0.05% Expt. 30)	--	0.47	--	0.04
LDS (0.01% Expt. 29 (0.05% Expt. 30)	--	0.53	--	1.72
EtOH + P40	1.96	3.77	1.01	0.72
EtOH + LDS	2.02	5.39	2.69	3.02
P40 + LDS	1.00	0.56	1.76	0.00

Synergism was noted between alcohol and the non-ionic detergent and between alcohol and lithium dodecyl sulphate in respect of cells infected with HSV and between alcohol and lithium dodecyl sulphate in respect of cells 5 infected with influenza virus.



CLAIMS

1. A disinfectant solution effective against viruses and micro organisms consisting of a combination of two components selected from an aqueous solution of a non-ionic detergent, an aqueous solution of an alkali metal or ammonium salt of an alkyl sulphuric acid containing 8-20 carbon atoms and an aliphatic alcohol.
2. A disinfectant solution according to claim 1 wherein the non-ionic detergent is an alkyl phenol ethoxylate.
3. A disinfectant solution according to claim 2 wherein the non-ionic detergent is an octyl phenol-ethylene oxide condensate.
4. A disinfectant solution according to claim 1 wherein the non-ionic detergent is an alcohol ethoxylate.
5. A disinfectant solution according to any one of the preceding claims wherein the alkali metal is sodium or lithium.
6. A disinfectant solution according to any one of the preceding claims wherein the alkyl sulphuric acid salt is sodium or lithium dodecyl sulphate.
7. A disinfectant solution according to any one of the preceding claims wherein the aliphatic alcohol is an alkyl alcohol having a linear chain length of up to 3 carbon atoms.



8. A disinfectant solution according to claim 9 wherein the alcohol is ethyl alcohol.

9. A disinfectant solution according to claim 1 comprising an aqueous solution of lithium dodecyl sulphate and a non-ionic detergent.

10. A disinfectant solution according to claim 9 wherein the non-ionic detergent is an octyl phenol-ethylene oxide condensate.

11. A disinfectant solution according to claim 9 or 10 wherein the concentration of lithium dodecyl sulphate is at least 0.002% w/v and the concentration of the non-ionic detergent is at least 0.002% v/v; preferably at least 0.005% w/v and v/v respectively.

12. A disinfectant solution according to claim 1 comprising an aqueous solution containing ethyl alcohol and a non-ionic detergent.

13. A disinfectant solution according to claim 12 wherein the non-ionic detergent is an octyl phenol-ethylene oxide condensate.

14. A disinfectant solution according to claim 12 or 13 wherein the concentration of the alcohol is at least 20% v/v and the concentration of the non-ionic detergent is at least 0.01% v/v.

15. A disinfectant solution according to claim 1 comprising an aqueous solution containing ethyl alcohol and lithium dodecyl sulphate.



16. A disinfectant solution according to claim 15 wherein the concentration of the ethyl alcohol is at least 20% v/v and the concentration of lithium dodecyl sulphate is at least 0.001% w/v, preferably at least 0.01% w/v.
- 5 17. A disinfectant solution according to claim 1 comprising an aqueous solution of sodium dodecyl sulphate and a non-ionic detergent.
18. A disinfectant solution according to claim 17 wherein the non-ionic detergent is an octyl phenol-ethylene oxide
10 condensate.
19. A disinfectant solution according to claim 18 when the concentration of the sodium dodecyl sulphate is at least 0.01% w/v and the concentration of the non-ionic detergent is at least 0.01% v/v.
- 15 20. A disinfectant solution according to any one of claims 17 - 19 containing not less than 25% v/v ethyl alcohol.
21. A disinfectant solution according to claim 1 substantially as herein described with reference to any one
20 of the examples.
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