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Physicochemical basis for the microbicidal action of disinfection solutions. I. Polyvinylpyrrolidinone-iodine

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Summary

A new potency coefficient, termed the disinfectant activity coefficient of solution (DACS), for aqueous polyvinylpyrrolidinone-iodine (PVP-iodine) solutions is proposed. The DACS index, which is the sum of four terms (fluidity, surface tension, redox potential and osmolality), results in good correlation with the germicidal activity at different dilutions of PVP-iodine. Factor statistical analysis was applied to all the terms; the first two factors account for over 96% of the total variation in the data set (factor 1, 71.7%; factor 2, 24.5%).

Introduction

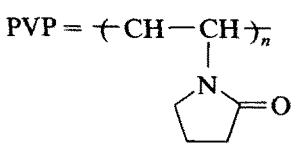
At first sight, it might appear that a substance such as PVP-iodine which has been known for almost 30 years and which has been used very successfully for over 20 years as a local broad-spectrum antiseptic requires no further discussion as concerns its analytical aspects. However, as a result of recent work in this field, it has become necessary to investigate the particular and possibly surprising properties of PVP-iodine (Gottardi, 1983; Rackur, 1985; Pollack and Iny, 1985, 1986). Moreover, the first part of the 3rd World Congress of Antisepsis, which was held in London in 1984, considered the role of free non-complex iodine in the effectiveness of PVP-iodine solutions.

In antiseptic aqueous solutions containing polymers with iodophor properties, the chemistry of iodine is complex, since macromolecules interact with iodine forms:

$$\text{PVP-iodine aggregated} \xrightarrow{\text{aq.}}$$

$$\text{PVP} + I_3^- \underset{\searrow \text{PVP}-I_2 \nearrow}{\overset{\nearrow \text{PVP}-I_3^- \searrow}{\longleftrightarrow}} \text{PVP} + I_2 + I^-$$

$$\text{PVP} = -(\text{CH}-\text{CH})_n- \qquad (1)$$



The iodine moiety of the PVP-iodine complex is present in an aqueous iodophor solution in the

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different thermodynamically stable anionic iodine species and as diatomic iodine. According to many authors (Gottardi, 1983; Pinter et al., 1984; Pollack and Iny, 1985, 1986), it is the equilibrium iodine alone that exerts the antiseptic action of the preparation at any given moment. Efforts were directed towards developing methods of measuring the quantitative extent of complexing between the iodine and the organic polymer in order to formulate iodophor antiseptic preparations with improved stability and reproducibility.

The aim of this paper was to develop a new empirical and statistically adequate equation to express the dependence of the various physicochemical properties of aqueous solution of PVP-iodine on bactericidal activity. We proposed a new potency coefficient, referred to as the disinfection activity coefficient of a solution (DACS). It is hoped that this general approach will find further applications in the design of disinfection solutions.

Materials and Methods

Materials

PVP-iodine solutions were provided by Mundipharma GmbH, Limburg. Solutions of different PVP-iodine concentrations (10–0.05%) were prepared by diluting a 10% PVP-iodine stock solution with sterile water.

Determination of bactericidal activity

The bactericidal activity was examined against *Staphylococcus aureus*, according to a modified in vitro method recommended in the guidelines for testing and assessing chemical methods of disinfection (Richtlinien manual, 1981). The exposure time was 30 s. The bactericidal activity of solutions was expressed by RF (reduction factor) values:

$$RF = \log_{10}(CFU_1) - \log_{10}(CFU_2) \quad (2)$$

where CFU_1 and CFU_2 represent, respectively, the number of colony forming units per ml without action and after action of the preparation.

Determination of the physicochemical parameter values

The physicochemical constants were determined at 25 °C. The viscosity of PVP-iodine solutions of various concentrations was measured on an Ostwald viscosimeter (Swindells and Ullmann, 1959). The surface tension of the solutions was determined using a stalagmometer (Harkinks, 1959). For measurements of the total osmolality, a KNAUER vapor-pressure osmometer with a universal thermistor was used (Wagner and Moore, 1959).

Results and Discussion

The results obtained on the bactericidal activity of PVP-iodine solutions of various dilutions are presented in Table 1 and Fig. 1.

Our data for the physicochemical parameter values of PVP-iodine stock solutions of various dilutions are listed in Table 2.

Microbicidal activity of aqueous PVP-iodine solutions

On consulting the literature dealing with correlations between the concentration of aqueous PVP-iodine solution and microbicidal activity, it is

TABLE 1

Bactericidal activity of PVP-iodine solutions of varying concentration on Staphylococcus aureus (exposure time 30 s)

	PVP-iodine solution (%)							
	10	7.5	5	2.5	1	0.5	0.1	0.05
$\log(CFU_1)$	6.89	6.89	6.89	6.89	6.89	6.89	6.89	6.89
$\log(CFU_2)$	4.92	4.26	4.08	3.25	2.02	2.78	3.87	5.09
RF	1.97	2.63	2.81	3.64	4.87	4.11	3.02	1.80

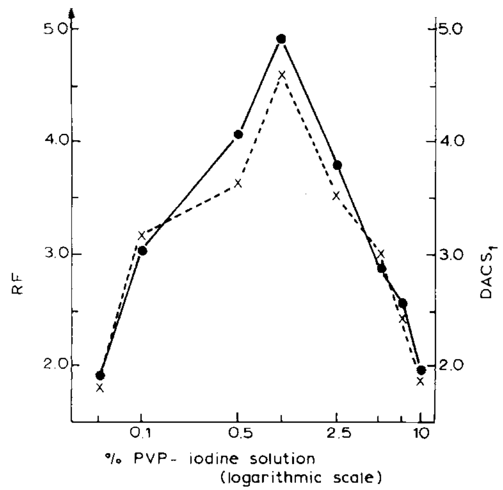

Fig. 1. Relationship of reduction factor (RF) for *S. aureus* (●——●) and disinfection activity coefficient of solution ($DACS_1$) for short action time (×----×) vs different concentrations of aqueous PVP-iodine solutions.

possible to draw the following controversial conclusions: Bactericidal activity increases with dilution of PVP-iodine solution (Berkelman et al., 1982). Starting with the 10% solution, the content of equilibrium iodine increases, reaching a maximum at about 0.1% solution (Gottardi, 1983; Pinter et al., 1984). There is no significant distinction between bactericidal activity of the 5 and 10% PVP-iodine solutions tested on *Escherichia coli* ($RF_{10\%} = 4.71$; $RF_{5\%} = 4.43$; the content of equilibrium iodine not determined) (Newsom and Matthews, 1985).

TABLE 2

Viscosity (η), surface tension (σ), redox potential (redox) and osmolality (o) of PVP-iodine solutions of various concentrations

No.	PVP-iodine (solution) (%)	η (mPa s)	σ (μN/cm)(×10)	Redox (V)	o (mosmol/l)
1	10	3.572	41.10	0.586	348
2	7.5	2.137	41.70	0.587	250
3	5	1.478	42.24	0.589	166
4	2.5	1.155	45.21	0.597	66
5	1	1.040	47.55	0.613	26
6	0.5	1.007	57.29	0.625	14
7	0.1	0.975	72.13	0.654	2
8	0.05	0.914	72.11	0.660	0.1

Our results on the microbicidal activity of aqueous PVP-iodine solutions show that, commencing with a commercially available 10% stock solution, it initially increases with increasing dilution, reaching maximum values at about 1% solution, and then falls on further dilution (see Table 1 and Fig. 1).

The question as to whether there is a correlation between the content of the so-called equilibrium iodine and the microbicidal activity of aqueous solutions of PVP-iodine remains to be resolved. From our experience, the concentrations of equilibrium iodine, as measured potentiometrically and spectrophotometrically, do not allow an answer to this question. In addition, the present pharmacopoeia specifications are inadequate for prediction of their microbicidal efficacy.

Disinfection activity coefficient of a solution (DACS)

As is well known, the mechanisms by which disinfectants kill or inhibit the growth of microorganisms are complex. Sequential or simultaneous changes in the properties of disinfection solutions often occur, which cause difficulties in differentiating primary from secondary effects. The disinfection activity of a solution may be expressed as a function of at least four characteristic properties in the general manner demonstrated by Eqn 3:

$$\text{disinfection activity} = A_{\phi} + A_{\sigma} + A_{ch} + A_{o} \qquad (3)$$

We have designated this parameter the disinfection activity coefficient of the solution (DACS).

The different symbols represent the separate contributions to the disinfection activity due to fluidity (A_{ϕ}), surface tension (A_{σ}), chemical properties (A_{ch}) and osmolality (A_{o}). Each of these will be discussed below.

For short periods of exposure to disinfection solution, the fluidity (A_{ϕ}) and surface tension (A_{σ}) terms are evidently important in the reaction with microorganisms. Both of these terms allow one to determine the passive transport of a substance in the solution, across a concentration, electrical and osmotic gradient. In the case of the active transport of substances in the solution, the terms denoting the chemical properties (A_{ch}) and osmolality (A_o) are applicable.

The aqueous solutions of PVP-iodine mentioned above show a remarkable extent of disinfection activity due to their properties as oxidizing agents, and consequently, the redox potential provides a measure of the chemical activity.

Fluidity term (A_ϕ)

Eqn 4 gives the common expression for A_ϕ

$$A_\phi = 1/\eta \quad (4)$$

where η is the viscosity of the solution.

Surface tension term (A_σ)

The contribution of the surface tension term to the microbicidal action of a solution was assumed to be defined as expressed by Eqn 5:

$$A_\sigma = 30/\sigma \quad (5)$$

where σ is the surface tension of the solution.

With rise in the surface tension of a solution the value of the surface tension term decreases. It should be noted that commercial disinfectant solutions usually contain substances that lower the surface tension.

Redox potential term (A_{redox})

We propose a simple expression for the electrochemical potential of the solution as given by Eqn 6:

$$A_{\mathrm{redox}} = (\mathrm{redox} - \mathrm{redox}_{\mathrm{sol.}}) \times 10 \quad (6)$$

where 'redox' designates the potentiometrically measured potential of a solution using a platinum electrode.

In accordance with Eqn 6, it was expected that the contribution of the redox potential term to the microbicidal action of pure water would be zero. Since the bacterial surface is normally negatively charged, the oxidizing agents are probably more effective because of the attraction of the oxidant molecule to the membrane surface.

Osmolality term (A_o)

This contribution may be quite considerable in the DACS of certain types of solutions. On the other hand, the expression for the osmolality term (A_o) must be capable of reflecting the considerable variability due to different kinds of bacteria, and even different bacterial strains.

For short exposure times to disinfectant solutions, such as when disinfecting one's hands, we propose the following empirical expression for the osmolality term

$$A_{o1} = \{\exp[-a \cdot \log(1+o)] - \exp[-b \cdot \log(1+o)]\} \cdot c \quad (7)$$

where a, b and c are constants with the values of 0.8, 1 and 17, respectively. Eqn 7 provides quantification of the osmolality contribution to the disinfection activity of PVP-iodine aqueous solution vs *S. aureus*. However, for broad-spectrum disinfectants, in the case of longer than 120 s contact time, the expression for A_o becomes

$$A_{o2} = 1/2 \log(1+o) \quad (8)$$

The values obtained for the terms representing the fluidity (A_ϕ), surface tension (A_σ), redox potential (A_{redox}) and osmolality (A_{o1} and A_{o2}) of PVP-iodine aqueous solutions of varying concentration are listed in Table 3.

Finally, for brief periods of exposure to disinfection solution, the DACS is estimated using Eqn 9 and for prolonged contact, Eqn 10:

$$\mathrm{DACS}_1 = nA_\phi + pA_\sigma + qA_{\mathrm{redox}} + rA_{o1} + s \quad (9)$$

$$\mathrm{DACS}_2 = nA_\phi + pA_\sigma + qA_{\mathrm{redox}} + rA_{o2} + s \quad (10)$$

where *n, p, q, r* and *s* are the respective regression coefficients. The observed disinfection activities were fitted to Eqn 9 by a least-squares regression method using the Statgraphics Computer Program (STATGRAPHICS manual, 1988). A summary of the statistics for the final expression (Eqn 11) is given in Table 4. The development of Eqns 9 and 10 was based mainly on theoretical rather than statistical grounds. The results for $DACS_1$ and

TABLE 3

Fluidity (A_ϕ), surface tension (A_σ), redox potential (A_{redox}), osmolality (A_{o1}, A_{o2}) terms and disinfection activity coefficient of solution ($DACS_1$, $DACS_2$) of PVP-iodine solution of varying concentration

No.	PVP-iodine solution (%)	A_ϕ (Eqn 4)	A_σ (Eqn 5)	A_{redox} (Eqn 6)	A_{o1} (Eqn 7)	A_{o2} (Eqn 8)	$DACS_1$ (Eqn 9)	$DACS_2$ (Eqn 10)
1	10	0.28	0.73	0.89	0.89	1.27	1.875	2.776
2	7.5	0.47	0.72	0.88	0.95	1.20	2.366	2.959
3	5	0.68	0.71	0.86	1.04	1.10	3.044	3.186
4	2.5	0.87	0.66	0.78	1.21	0.91	3.578	2.867
5	1	0.96	0.63	0.62	1.35	0.71	4.686	3.170
6	0.5	0.99	0.52	0.50	1.39	0.59	3.765	1.869
7	0.1	1.03	0.42	0.21	1.06	0.24	3.309	1.366
8	0.05	1.09	0.42	0.15	0.13	0.02	1.653	1.392

$DACS_2$ obtained by using these equations are listed in Table 3.

Our data on the $DACS_1$ values of aqueous PVP-iodine solutions in fact show that, starting from a commercially available 10% stock solution, the $DACS_1$ values initially increase with increasing dilution, reaching a maximum at about 1% solution, and subsequently fall on further dilution (see Table 3 and Fig. 1). On the other hand, the $DACS_2$ values (referring to the disinfection efficiency for more than 120 s contact time) demonstrate that the 5% PVP-iodine solution has the greatest microbicidal potency (Table 3).

TABLE 4

Parameters and statistics for the linear regression of $DACS_1$ on A_ϕ, A_σ, A_{redox} and A_{o1}

Variable	Regression coefficient	Standard error	T value significance at 5% level	Prob. ($>T$)
Intercept	−7.49	2.90	−2.58	0.82
A_ϕ	2.39	0.88	2.70	0.07
A_σ	17.24	7.81	2.21	0.12
A_{redox}	−6.74	3.66	−1.84	0.16
A_{o1}	2.37	0.47	5.03	0.02

$r^2 = 0.958$; standard error of estimate = 0.326; degree of freedom = 4.3; $F = 17$.

$$DACS_1 = 2.39 \cdot A_\phi + 17.24 \cdot A_\sigma - 6.74 \cdot A_{redox} + 2.37 \cdot A_{o1} - 7.49 \quad \text{(Eqn 11)}$$

It was pointed out earlier that certain bacteria were more readily killed by a dilute aqueous solution of PVP-iodine than with the traditional 10% stock solution (Gottardi, 1983; Pinter et al., 1984; Newsom and Matthews, 1985). The phenomenon of the maximum microbicidal effect of PVP-iodine solutions being exerted at specific concentrations can be seen particularly clearly with *S. aureus*. In addition, we find that the disinfection activity coefficient of solution ($DACS_1$) is correlated directly with the observed disinfection activity of *S. aureus* after 30 s of action (Fig. 2). The agreement between the experimentally determined bacteri-

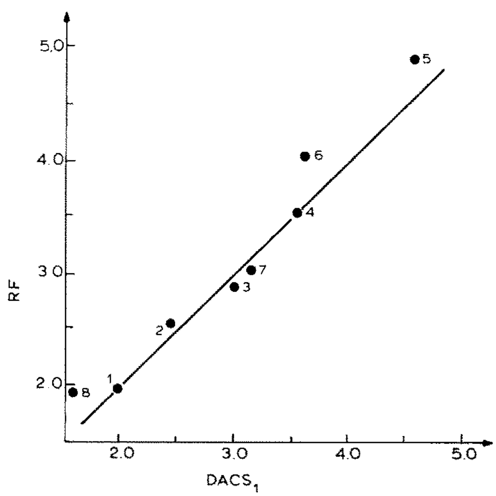

Fig. 2. Plot of the observed disinfection activity of *S. aureus* after 30 s of action vs the predicted activity of $DACS_1$.

cidal activity of solutions, RF, and the calculated value, $DACS_1$, is analyzed according to the linear least-square equation:

$$RF = 0.998(\pm 0.085) \cdot DACS_1 - 0.008(\pm 0.271) \quad (11)$$

$n = 8$; $r = 0.979$; $s = 0.231$; $F = 137$

From a physicochemical point of view, while the values of the surface tension and redox potential terms in the 1% PVP-iodine solution do not differ markedly from those of the 10% solution, the variation in the fluidity and osmolality terms provides a suitable opportunity for adjusting the maximum $DACS_1$ values (Table 3), which results in the desired information on the microbicidal kinetics of PVP-iodine solutions.

The relatively low standard errors of the predicted values (Eqn 11) suggest that the regression parameters are acceptable. According to the *T*-statistic, the relative importance of the various terms in the predicted disinfection activity ($DACS_1$) decreases in the order: $A_o > A_\phi > A_\sigma > A_{redox}$. On the other hand, the sensitivity of $DACS_1$ to changes in the various terms, based on the absolute values of the standardized regression coefficients, follows the order: $A_\sigma > A_{redox} > A_\phi > A_o$.

Factor statistical analysis based on the principal components method was performed (STATGRAPHICS manual, 1988) in order to estimate

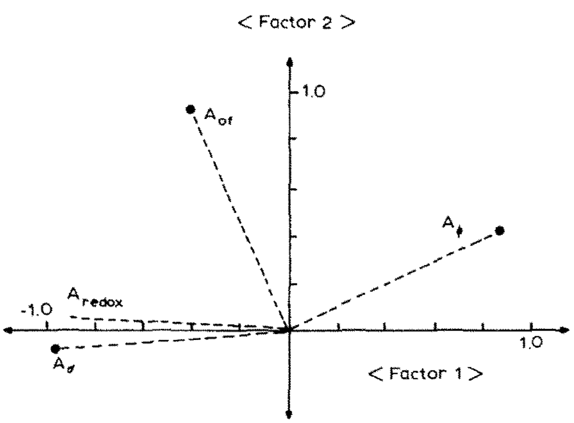

Fig. 3. R-mode factor analysis showing the interrelationship of the four terms in factor 1 and factor 2 space.

TABLE 5

R-mode relative contribution to factor loadings

	Factor 1	Factor 2	Factor 3	Factor 4
A_ϕ	0.871	0.397	0.288	0.003
A_σ	−0.983	−0.047	0.171	−0.056
A_{redox}	−0.989	0.024	0.134	0.061
A_{o1}	−0.408	0.905	−0.121	−0.006

the relative contributions of the individual terms to the $DACS_1$. Factor analysis is a multivariate statistical technique that creates new independent variables (i.e. factors) that are linear combinations of the original variables (i.e. four terms). The primary objective of statistical factor analysis is to reduce the dimensionality of the data to the most important components or factors that best explain the variation in the data. In R-mode factor analysis, both the variables and observations are scaled so that they can be plotted on the same set of factor axes and readily visualized (Jöreskog, 1977). Fig. 3 illustrates the results of factor analysis of the data in Table 3. Together, the first two factors account for over 96% of the total variation in the data set (factor 1, 71.7%; factor 2, 24.5%). Factors 3 and 4 describe 3.6 and 0.2% of the variations, respectively. Table 5 lists the relative contributions from each of the four terms to factors 1–4. A high relative factor loading for a particular variable (Table 5) is reflected in Fig. 3 as a vector parallel to the factor axis (e.g. variables A_σ and A_{redox} for factor 1), whereas those with a low relative factor loading tend toward being perpendicular. The surface tension (A_σ) and redox potential (A_{redox}) terms account for most of the loadings (60%) on factor 1, while the osmolality term (A_{o1}) comprises most (66%) of factor 2.

Care must be taken that the functions used in DACS calculations are empirical, being chosen because they are easy to handle numerically, and because of their ability to give a quantitative description of disinfection solutions. Therefore, the DACS shows potential for use in the designing of disinfection solutions. However, more work is needed in order to establish the range of its applicability.

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