QSAR Study on the Biological Activity of Nonyl-phenyl-ethylene-oxide Polymers

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Abstract. Principal component analysis and factor analysis were applied to study the effect of different physico-chemical parameters (mass transfer coefficient, lipophilicity, adsorptivity, permeation growth of dipalmitoyl phosphatidylcholine vesicles) of nonyl-phenyl-ethylene-oxide polymers on their biological activity (growth inhibition of *Coronilla Rhizobium* and *Bacillus subtilis* strains). Both mathematical aproaches gave similar results, the first factor explained about 75%, the first two factors 92% of the total variance. All physicochemical parameters influence the biological activities, the compounds can be divided into three clusters containing the bulk and the two extreme ends of the linear ethylene-oxide chain.

Key words: Nonionic tenzides — Principal component analysis — Microbial effect of nonionic tenzides

Introduction

The nonionic tenzides present in the majority of pesticide formulations may influence the biological activity of active ingredients by modifying its adsorption on the surface of the target organism (Florence and Gillan 1975), their penetration through the different membranes (Turner 1972; Brian and Bland 1972; Sharma 1976), their movement inside the target organism (Brian 1972; Juniper 1972; Bland and Brian 1975) and in the soil (Mercer and Hill 1977; Shone and Wood 1977).

The tenzides alone may have curative effect (Clifford and Hislop 1975; Steiner and Watson 1965), modify the phytotoxicity (Parochetti 1975; Horowitz 1977), show synergistic or antagonistic effects (Sierra and Doll 1974; Moreira 1972; Peng et al. 1974; Polston and Robertson 1976; Bauer et al. 1972; Kramer and Manning 1971) influence the selectivity (Bayer 1972; Müller and Burth 1980), lipophilicity (Valkenburg and Yapel 1971) and the uptake (Sweet et al. 1979) of active ingredients.

The effect of tenzides depends considerably on their chemical structure (Wilson and Hines 1979; Bundick and Mitchell 1979), they increase the bioavaila-

bility (Marrs and Seaman 1978) and the sugar efflux from cells (Towne et al. 1978). The tenzides can modify membrane permeability too (Beckman 1978).

In spite ot the great practical and theoretical importance of the tenzide research the number of studies correlating the biological activities and physico-chemical parameters of tenzides is surprisingly low (Smith et al. 1966; Freed and Montgomery 1958; Cserháti and János 1979; Cserháti and Szőgyi 1980).

Our aim was to determine the microbiological activities and physico-chemical properties of some nonionic tenzides and to correlate them by up-to-date mathematical methods.

Material and Methods

The general structure of nonyl-phenyl-ethylene-oxide polymers belonging to the nonionic tenzides is shown in Fig. 1. The tenzides were produced by Hoechst AG (GFR) and pufiried by thick layer chromatography (TLC) on aluminiumoxide. The growth inhibitory effect of tenzides was determined on *Bacillus subtilis var. niger* and *Coronilla Rhizobium* strains. The strains (shaken liquid culture) were incubated at 26—28°C temperature in a nutrient broth (Oxoid CM 1) containing 50 *(Bac. subtilis)* and 300 ppm *(Coronilla Rhizobium)* tenzides. These concentrations were chosen for their very frequent

Fig. 1. Chemical structure of nonyl-phenyl-ethylene-oxide polymers (x = average number of ethylene-oxide groups per molecule: 4, 5, 6, 8, 9, 10, 11, 13, 15, 23 and 30).

occurrence in the agricultural practice. Untreated samples were used as controls. The starting microbe number was set to 10^6 microbes/cm^3. Growth was checked by measuring the turbidity of the culture at 600 nm wavelength. The experiments were carried out with 5 parallels in 2 repetitions.

The R_M values of tenzides characterizing the molecular lipophilicity were determined by reversed phase thin layer chromatography (Cserháti and János 1980). The data obtained in water: acetone 1:3 were used in the calculations. The R_f values of tenzides characterizing the adsorptivity on highly hydrophilic surfaces were measured on silicagel TLC plates in an eluent system of water: methanol 1:1 (for other conditions see Cserháti and János 1980).

The diffusion constant of tenzides was determined by the method developed by Tamás and Szőgyi 1973. The initial concentration of tenzides was set to 4 mg/cm^3 2% agarose, the tenzide concentration was determined spectrophotometrically at 224 nm wavelength (molar absorption coefficients: $1.55 - 2.61 \times 10^4$ l mol^{-1} cm^{-1}). We do not consider the diffusion constant calculated as a real diffusion constant because in our system (2% agarose matrix) some uncertainty exists about the exact solubility and about the exact critical micelle concentration of tenzides. Therefore we prefer to define the constant calculated as mass transfer coefficient determined at the concentration mentioned above.

In order to measure the permeability constant, liposomes were formed from dipalmitoyl phosphatidylcholine (DPPC) in 0.16 mol/l KCl solution containing tracer amounts of ^{42}KCl by sonication. (DPPC was produced by Sigma Ch.C., its purity was checked by TLC). After overnight equilibration the lipid dispersion was passed down a column of Sephadex G-50 (1.5×30 cm) to remove the excess tracer not trapped within the liposomes. The liposomes were eluted from the column with O.16 mol/l KCl solution

(flow rate 0.5 cm^3/min.); 3 cm^3 portions of eluted liposomes were dialysed against 10 cm^3 of 0.16 mol/l KCl solution. The efflux rate has been measured for consecutive 15 min periods. At the end the ^{42}K content of liposomes was measured by gamma scintillation counter. The tenzides investigated were added to the liposomes in molar ratio 1:99. The permeability constants were calculated according to the Johnson-Bangham model (Johnson and Bangham 1969). To correlate all the parameters measured principal component analysis (PCA) and factor analysis (FA) were applied to the data.

The initial data set contained the variables as follows:

1. Growth of *Coronilla Rhizobium* strain (in percent of the untreated control).
2. Growth of *Bacillus subtilis var. niger* strain (in percent of the untreated control).
3. The mass transfer coefficient ($D \times 10^{11}$ m^2/s).
4. The R_M value.
5. The R_f value.
6. The growth of the permeability constant $(P = P_{treated} - P_{untreated}) . 10^{12}$ m/s.

The data are summarized in Table 1.

Table 1. Biological and physico-chemical parameters of nonyl phenyl ethylene-oxide-polymers

Number of ethylene-oxide groups per molecule	Growth % Cor. rhiz.	Growth % Bac. subt.	$D.10^{11}$ m2/s	R_M	R_f	$P.10^{12}$ m/s
4	53.1	83.1	3.60	−0.11	0.79	0.1
5	57.4	22.8	4.59	−0.10	0.79	1.7
6	57.4	16.1	4.75	−0.09	0.77	3.8
8	49.7	12.0	6.95	−0.09	0.76	5.3
9	48.4	14.0	5.42	−0.08	0.76	6.8
10	47.7	46.4	7.20	−0.08	0.76	3.9
11	48.4	45.2	6.91	−0.07	0.76	3.3
13	48.1	78.0	6.85	−0.07	0.72	3.0
15	55.6	72.8	6.76	−0.06	0.70	2.7
23	90.2	92.5	10.35	0.02	0.53	0.9
30	98.4	98.3	10.2	0.07	0.41	0.8

Results and Discussion

The correlation matrix is shown in Table 2.

The effect of tenzides on the growth of the two strains investigated does not show correlation, they are differently affected by the tenzides, which can be explained by different membrane structure of the strains. The growth inhibition of *Coronilla Rhizobium* strain correlates well to the lipophilicity and adsorptivity of the tenzides and to a lesser extent to their mass transfer coefficient. The growth inhibition of *Bac. subtilis* strain correlates not so well to the lipophilicity and adsorptivity of the tenzides, rather to the permeability change. The discrepancies in the behaviour of the strains stress again that the determining factor in the biological activity of a homologous group of compounds can be different from strain to strain.

A fairly high grade of intercorrelation was found between the physico-chemi-

Table 2. Linear correlation matrix of the data ($r_{95\%} = 0.6319$; $r_{99\%} = 0.7646$; $r_{99.9\%} = 0.8721$)

	Variables					
Variables	1	2	3	4	5	6
1	1.0000	0.6033	0.7378	0.9239	−0.9431	−0.5819
2		1.0000	0.5417	0.6558	−0.6779	−0.7688
3			1.0000	0.8925	−0.8600	−0.2092
4				1.0000	−0.9945	−0.4183
5					1.0000	0.4588

cal parameters, the mass transfer coefficient correlates well to the lipophilicity and adsorptivity of tenzides. Because of the extremely high correlation between the lipophilicity and adsorptivity of tenzides the relative importance of these two parameters can not be established. It is interesting that the permeability growth does not correlate linearly to the other physico-chemical parameters.

It can be seen from the results of PCA (Table 3) that the first and the second factor explain practically all the variances (taking into consideration the experi-

Table 3. Eigenvalues of principal component analysis

Factor number	Eigenvalues	Explained variance %
1	4.4962	74.94
2	1.0498	92.43
3	0.3284	97.91
4	0.1068	99.69

mental errors in the data matrix), the first one explaining 75% of the total variance.

Due to the high eigenvalue of the first factor the loadings of all variables are high in the first factor that is the physico-chemical parameters and biological activities can be ascribed to one background factor. In the second factor only the growth inhibition of the *Bac. subtilis* strain, the mass transfer coefficient and the permeability growth show considerable loadings indicating some correlation between these three variables too (Table 4.).

Table 4. Loadings of the principal components

	Factors			
Variables	1	2	3	4
1	0.937	0.062	−0.332	0.012
2	0.799	−0.456	0.369	0.123
3	0.844	0.424	0.236	−0.227
4	0.964	0.241	−0.030	0.070
5	−0.972	−0.191	0.066	−0.112
6	−0.625	0.751	0.145	0.151

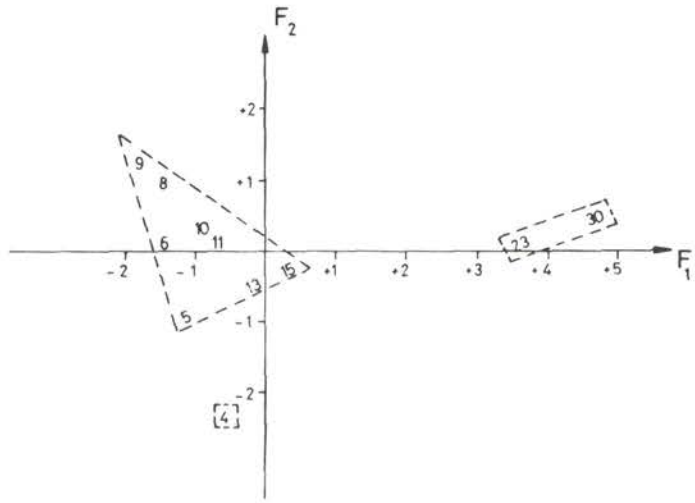

Fig. 2. Two-dimensional plot of principal component variables. The numbers indicate the number of ethylene-oxide groups per molecule.

From the linearly (equidistantly) changing structure of nonyl-phenyl-ethylene-oxide polymers a linearly or monotonously changing principal component plot could be expected. Instead of that three clusters were observed on the plot (Fig. 2.) containing the bulk and the two extremes of the linear ethylene-oxide chain. It means that the quantitative increase of ethylene-oxide groups per molecule causes a qualitative change in the behaviour of compounds concerning the variables investigated.

The results of the factor analysis were practically identical to those of the PCA pointing in our case to the eventual similarity and interchangibility of the two methods.

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