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INTERACTION OF PURPLE MEMBRANE WITH SOLVENTS

I. APPLICABILITY OF SOLUBILITY PARAMETER MAPPING

MICHAEL EISENBACH [a], S. ROY CAPLAN [a] and GERALD TANNY [b]

[a] *Department of Membrane Research and* [b] *Department of Plastics Research, The Weizmann Institute of Science, Rehovot (Israel)*

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Summary

We carried out spectral studies on the interaction between purple membrane fragments (isolated from *Halobacterium halobium*) and a series of different solvents, classified quantitatively according to their solubility parameters δ_d, δ_p, δ_h. These represent the contribution of dispersion forces, polar forces, and hydrogen bonding, respectively, to the cohesive energy density of the solvent. Purple membrane fragments, kept in the dark, were suspended in each of the solvents as well as in binary mixtures of solvents, and the spectrum of the resulting suspension was recorded in the wavelength region 250—700 nm. The interaction of each solvent with the membrane fragments can be represented by a point on either a ternary diagram, where each of the three axes represents one of the solubility parameters, or a binary diagram, where one of the two axes is a combination of two of the solubility parameters ($\delta_v = \sqrt{\delta_d^2 + \delta_p^2}$ or $\delta_a = \sqrt{\delta_p^2 + \delta_h^2}$). In the former type of solvent map the contribution of each of the parameters is distinct but only their relative contributions are expressed. In the latter the absolute values of δ_i are considered. In each of these modes of presentation an inner closed region is observed. The solvents inside its borders interact with bacteriorhodopsin with a resultant spectral change. Mixtures of solvents fit the maps according to their calculated δ values. Thus, a mixture of an apolar solvent with a highly polar solvent interacts with bacteriorhodopsin, even though each of these solvents alone does not.

Introduction

Due to their chemical complexity, the use of biological systems with various synthetic organic molecules has always been problematic in two major respects:

(a) 'inert' non-aqueous suspension media have been difficult to find, and (b) the functional changes in the system due to its operation in non-aqueous or mixed solvent media are not easily resolved into a rational pattern of behavior. However, both these problems are simply the result of a lack of a systematic definition of the properties of the solvents themselves. Qualitative terminology regarding the 'polarity' or hydrophobic character of solvents is insufficient to search rationally for suitable media or explain complex effects resulting from the interaction of organic molecules with the different components of the biological system.

The purpose of the present contribution is to remedy this situation by acquainting the reader with the concept of the 'solubility parameter', which has been utilized with great success in the physical chemistry of polymers and polymer solutions (for reviews, see Refs. 1—3). Furthermore, we shall demonstrate the application of this concept to the behavior of purple membrane fragments of *Halobacterium halobium* in mixed solvent media and to the definition of organic media in which these fragments are 'inert'. We shall demonstrate the applicability of the solubility parameter even to predict chemical interaction (rather than solubility) between purple membrane and organic solvents. Purple membrane fragments are a convenient preparation for this study due to their absorbance changes accompanying every chemical change. Since part of our work was concerned with dissolving these fragments in synthetic polymer media [4,5], the problem of 'inert' versus 'active' solvents (which were also solvents for the polymer) was of considerable importance.

Theory

Since the theory of solubility parameters is extensively described [6—11] and reviewed [1—3], the following is meant only to provide an outline of the fundamental ideas required for an understanding of their use.

Let us consider the change in internal energy, ΔU, of any substance in a condensed state brought about by removing all the intermolecular forces (e.g. by evaporating the liquid into the gaseous state). We can identify this value of ΔU with E_{coh}, the cohesive energy of the liquid (cal/mol). If we divide this energy by the molar volume, $\overline{V}$, we obtain the cohesive energy density, e_{coh}:

$$e_{coh} = E_{coh}/\overline{V} = (\Delta H_{vap} - RT)/\overline{V} \ (\mathrm{cal/cm^3}) \tag{1}$$

where ΔH_{vap} is the molar heat of evaporation. It is convenient in solution theory to define the square root of the cohesive energy density, the so-called solubility parameter, δ, as follows:

$$\delta = e_{coh}^{1/2} = (E_{coh}/\overline{V})^{1/2} \ (\mathrm{cal^{1/2}/cm^{3/2}}) \tag{2}$$

The reason for this choice of parameter rests on the following thermodynamic criterion of solubility. Two substances are mutually soluble when the free energy of mixing, ΔG_M, is negative, i.e.:

$$\Delta G_M = \Delta H_M - T\Delta S_M < 0 \tag{3}$$

where ΔH_M and ΔS_M are the enthalpy and entropy of mixing, respectively. Note that ΔS_M (with the exception of certain aqueous systems) usually favours

mixing, so that ΔH_M is most often the governing factor in determining the mutual miscibility of the substances. Hildebrand and Scott [6] have shown that:

$$\Delta h_M = \Delta H_M / \overline{V} = \phi_1 \phi_2 (\delta_1 - \delta_2)^2 \quad (4)$$

where Δh_M is the enthalpy of mixing/unit volume, ϕ_1 and ϕ_2 are the volume fractions of components 1 and 2 ($\phi_1 + \phi_2 = 1$), and δ_1 and δ_2 are their solubility parameters. Eqn. 4 predicts that the closer the values of δ_1 and δ_2, the more miscible the substances will be. It is the relation between δ_1 and δ_2 in Eqn. 4 which makes it convenient to deal with the square root of e_{coh}.

The cohesive energy density can be subdivided into component parts as follows:

$$e_{coh} = e_d + e_p + e_h \quad (5)$$

where e_d, e_p, and e_h are the contributions of dispersion (London) forces, polar forces, and hydrogen bonding, respectively, to the total cohesive energy density. Correspondingly,

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (6)$$

The values of δ_d and δ_p may be estimated from the index of refraction and the dipole moment of the substance, respectively, and consequently δ_h may be obtained from the value of δ (calculated from the heat of evaporation according to Eqns. 1 and 2) [11]. Values of δ_p, δ_d, and δ_h may be assigned to every liquid, and have been published in a tabular form for many common liquids [2,9—11]. In addition, for molecules whose values have not been experimentally determined, chemical group factor contributions may be used as a means of estimation [3]. Furthermore, the solubility parameters of a mixture of solvents can be also calculated [1]. Thus, for a parameter δ_i of a mixture of n components:

$$\delta_i = \sum_{j=1}^{n} \delta_{ij} \phi_j \quad (7)$$

Having obtained the relevant solubility parameters, the most common way to use them involves some form of parameter mapping. For the purpose of drawing two-dimensional maps, additional solubility parameters are defined, which are combinations of two of the previously defined parameters:

$$\delta_a = \delta \text{ of the association interactions} = \sqrt{\delta_h^2 + \delta_p^2} \quad (8)$$

$$\delta_v = \delta \text{ of the Van der Waals interactions} = \sqrt{\delta_d^2 + \delta_p^2} \quad (9)$$

Thus, δ_a represents all solvent-solvent interactions except for dispersion forces (i.e. it represents polar forces and hydrogen bonding), and δ_v represents all attraction forces which are inversely proportional to the 6th power of the intermolecular distance [3,7,11].

Materials and Methods

Purple membrane fragments were prepared from *H. halobium* M-1 strain according to Oesterhelt and Stoeckenius [12]. The fragments were freeze-dried, and 3 mg of the dried samples were suspended in the dark (using a Teflon

TABLE I

DETAILS OF SOLVENT USED

Abbreviations for purity grades: A, analar; C.P., chemically pure; D, distilled. The code numbers are according to Hansen and Beerbower [2].

Code No.	Solvent	Product of	Purity grade
3	*i*-pentane	Fluka	C.P.
4	*n*-hexane	Fluka	A
13	cyclohexane	Fluka	A
15	benzene	Frutarom	A
16	toluene	Frutarom	A
19.1	1-methylnaphthalene	Merck	C.P.
24	dichloromethane	BDH	C.P.
28	1,2-dichloroethane	BDH	C.P.
29	chloroform	Fluka	A
29.1	1,1-dichloroethane	Frutarom	A
36	carbon tetrachloride	Frutarom	A
37	1,1,1-trichloroethane	BDH	C.P.
39	chlorobenzene	Frutarom	C.P.
39.2	1,1,2,2-tetrachloroethane	E.K.	C.P.
40	bromobenzene	BDH	C.P.
41	*o*-dichlorobenzene	Fluka	C.P.
46	1-bromonaphthalene	E.K.	C.P.
51	furan	Fluka	C.P.
51.2	tetrahydrofuran	Frutarom	C.P.
51.3	1,4-dioxane	Fluka	A
52	diethyl ether	Fluka	D
53.1	anisole	Fluka	C.P.
55	acetone *	Frutarom	A
56	ethyl methyl ketone	Fluka	A
57	cyclohexane	Frutarom	C.P.
58	diethyl ketone	Fluka	C.P.
59	acetophenone	Fluka	C.P.
60	methyl *i*-butyl ketone	F.S.C.	A
65	benzaldehyde	Frutarom	C.P.
66.2	methyl acetate	Fluka	C.P.
67.1	propylene carbonate	Fluka	C.P.
69	ethyl acetate *	Frutarom	A
69.1	trimethyl phosphate	BDH	C.P.
72	*n*-butyl acetate	E.K.	C.P.
72.2	2-ethoxyethyl acetate	Carbide and Carbone	C.P.
75.1	triethyl phosphate	BDH	C.P.
89	ethanolamine	BDH	C.P.
89.3	2-pyrrolidone	Fluka	C.P.
90	pyridine	BDH	A
93	aniline	Fluka	A
95.1	diethylene triamine	E.K.	C.P.
96	cyclohexylamine	Fluka	C.P.
96.1	quinoline	BDH	C.P.
98	formamide	Fluka	A
99	dimethyl formamide	Fluka	A
100	carbon disulfide	Merck	A
101	dimethyl sulfoxide	Fluka	C.P.
120	methanol	Frutarom	A
121	ethanol	Fluka	A
121.1	ethylene cyanohydrine	E.K.	C.P.
122	1-propanol	F.S.C.	A
123	2-propanol	Merck	A
123.1	3-chloropropanol	BDH	C.P.
125	1-butanol	Frutarom	A
126	2-butanol	Frutarom	A
126.2	benzylalcohol	Fluka	A

TABLE I (continued)

Code No.	Solvent	Product of	Purity grade
127	cyclohexanol	E.K.	C.P.
128	1-pentanol	Fluka	C.P.
129.1	diacetone alcohol	Carbide and Carbone	C.P.
130	ethyl lactate	E.K.	C.P.
132.2	diethylene glycol monoethyl ether	E.K.	C.P.
134	1-octanol	Fluka	C.P.
140	formic acid	BDH	A
141	acetic acid	Frutarom	A
145	*m*-cresol	Fluka	A
148	water		
149	ethylene glycol	Frutarom	C.P.
150	glycerol	F.S.C.	A
150.1	propylene glycol	Fluka	C.P.
150.2	1,3-butanediol	BDH	C.P.
151	diethylene glycol	Fluka	C.P.
152	triethylene glycol	Fluka	C.P.
153	hexylene glycol	E.K.	C.P.

* Calculated values of δ according to van Krevelen and Hoftyzer [3].

homogenizer) in 6 ml of the solvent tested. The suspension was divided in the dark into two equal portions and treated as follows:

Portion I: after 24 h in the dark this was rehomogenized and spectrally measured (all processes carried out in the dark).

Portion II: after 24 h in the dark this was centrifuged for 10 min in a clinical centrifuge, and a spectrum of the supernatant taken (all processes carried out in the dark). The spectral measurements were carried out with a Cary 15 spectrophotometer in the wavelength range 250—700 nm.

The solvents used in this study are listed in Table I. They were used without further drying or purification. We are conscious of the fact that ideally all solvents should have been redistilled. However, in view of impurities present in any purple membrane preparation, this step seemed superfluous. The values of the solubility parameters and their code numbers are taken from Hansen and Beerbower [2].

Results and Discussion

Interaction of a solvent with the purple membrane was determined by its effect on the bacteriorhodopsin in the following way. An aqueous suspension of purple membrane fragments was frozen in liquid nitrogen, dried and resuspended in the test solvent. The spectra of the resulting suspension and of the supernatant after spinning down the purple membrane were measured as described under Materials and Methods. Those solvents in which purple membrane exhibited a different spectrum than that observed in water (i.e. the absorbance peak at 560 nm, typical of the dark-adapted form of bacteriorhodopsin in water [16,17], was either lost or blue shifted), were regarded as solvents which interacted with purple membrane. Some of the solvents exhibited new absorbance peaks, but nevertheless retained some of their

560 nm absorbance; these were considered as partial-interacting solvents. Those solvents which exhibited the same spectrum of purple membrane as in water, were considered as non-interacting. With ethylene glycol (No. 149) and glycerol (No. 150) a blue shift of 10—30 nm was observed, which was probably due to partial solvation of the membrane by these solvents accompanied by bacteriorhodopsin-trimer dissociation, somewhat similar to the action of detergents from the Triton X series [18—21]. Since in this case the interaction is probably not directly involved with the retinal (the purple color is retained), these solvents were also considered as non-interacting.

Since the thermodynamic properties of a solvent are defined by a set of three solubility parameters, it is impossible to represent a solubility system fully on a two-dimensional map. This is exactly the reason for the variety of modes of representation published, each having its advantages and disadvantages. Crowley et al. used three-dimensional solid models, obtaining irregular geometrical shapes in space [13,14]. Hansen, who also used a three-dimensional model, tried to obtain a regular spatial form by making the unit distance along the δ_d axis twice as long as that of the other axes (δ_p and δ_h); essentially spherical interaction regions were thus obtained [8,10]. However, because of the inconvenience involved in building solid models, graphic representation modes were examined. Crowley et al. presented the solubility characteristics of polymers on contour maps, the obvious disadvantage of this technique being that several contour maps were required to describe the solubility of one polymer [14]. Teas has shown that ternary diagrams on a triangular chart may also yield closed interaction areas [15]. In this mode of presentation only the relative contributions of the solubility parameters are considered. If the absolute values are preferred, one either has to neglect one of the three solubility parameters or to plot either of the combinations in Eqns. 8 and 9 vs. the remaining solubility parameter [3,10]. In so far as the chemical interaction between purple membrane and organic solvents is concerned, since we could not know a priori which, if any, of the graphical presentations was the most suitable, we examined four modes of representation.

Fig. 1 shows a binary diagram of δ_v vs. δ_h, where each solvent is represented by a point according to its tabulated δ_v and δ_h values. Though this 'solubility map' represents a chemical interaction of solvents with purple membrane rather than a solubility phenomenon, the interacting solvents are located in a region which can be described as a circle, exactly as was found by van Krevelen and Hoftyzer [3] for the solubility of polymers in organic solvents. Thus, 95% of the solvents inside the circle interact with purple membrane with a resultant spectral change. These include 14% which partially interact. On the other hand, 94% of the solvents excluded by this circle do not interact with purple membrane, and an additional 3% only partially interact.

A major requirement for mutual solubility is that the solubility parameters of a macromolecular phase (in this case purple membrane) and those of the solvent should be as close as possible [3]. On a solubility map the points representing the solvents should cluster about a 'focal' point representing the purple membrane, giving rise to an approximate circular area whose radius is not too well-defined. (One source of deviations is that the values attributed to the solvents are approximations.) This suggests that the center of each of the reac-

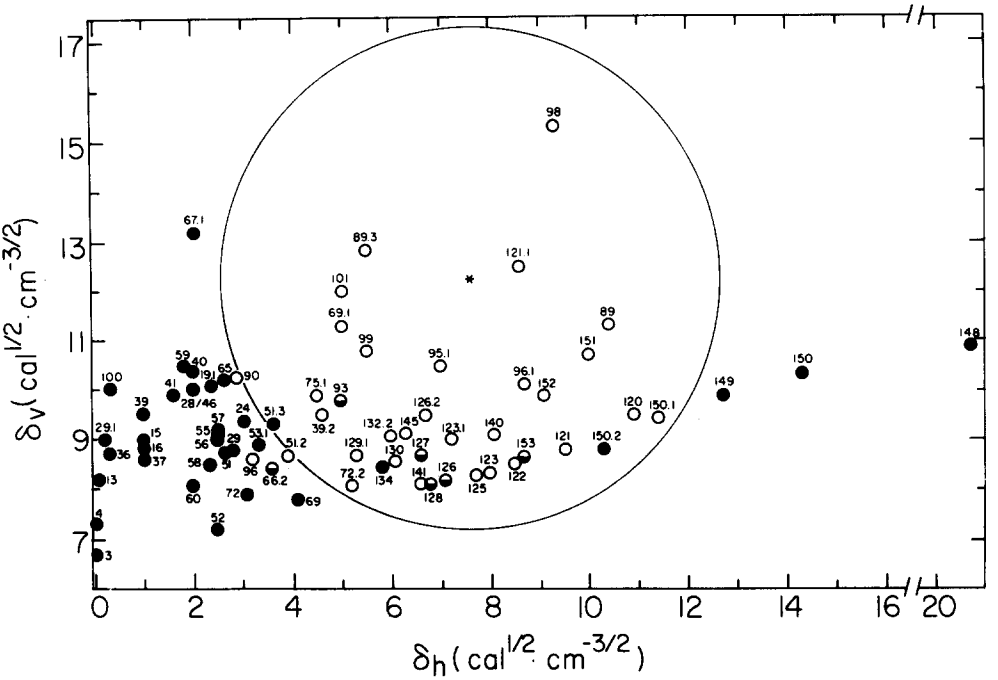

Fig. 1. Solubility parameter mapping of the interaction of purple membrane with solvents: binary presentation of δ_v vs. δ_h. The numbers are those appearing in Table I. *, the center of the circle; ○, solvent exhibiting interaction with purple membrane (total loss of 560 nm absorbance); ●, solvent exhibiting no interaction with purple membrane (560 nm absorbance retained); ◍, solvent exhibiting partial interaction with purple membrane (spectra of bacteriorhodopsin products present with some absorbance left at 560 nm).

tion circles represents a reasonable estimate of the solubility parameters (or, more correctly, the interaction parameters) of purple membrane. Thus, according to Fig. 1, these parameters are $\delta_v = 12.1$, $\delta_h = 7.6$, and any solvent within the circle centered at this point with a radius of 5.0 units (i.e. any solvent satisfying the rule $|[(\delta_v - 12.1)^2 + (\delta_h - 7.6)^2]^{1/2}| < 5.0$) will most probably interact with the purple membrane. This closely resembles the results and conclusions drawn for the solubility of polystyrene in solvents [3].

By using the δ_v/δ_h solubility map, the contributions of δ_d and δ_p to δ_v are not made explicit. We therefore examined the other possible binary representation, i.e. δ_d vs. δ_a, which emphasizes the role of hydrophobicity in the interaction of solvents with purple membrane. The results are shown in Fig. 2. Here the solvents are differently distributed, but nevertheless an interaction region is also observed. Note that in Fig. 2, in contrast to the other modes of representation, the unit distance along the δ_d axis is twice as long as that along the δ_a axis. This choice of scale was made following Hansen [8,10], with the hope of obtaining a circular interaction region. Though a circle could, in principle, be drawn, we resisted this temptation and did not draw any border. The reason for this is that the border is not unequivocally defined, and other choices of scales for the axes will also yield a definite interaction area.

In the above presentations the absolute values of the solubility parameters

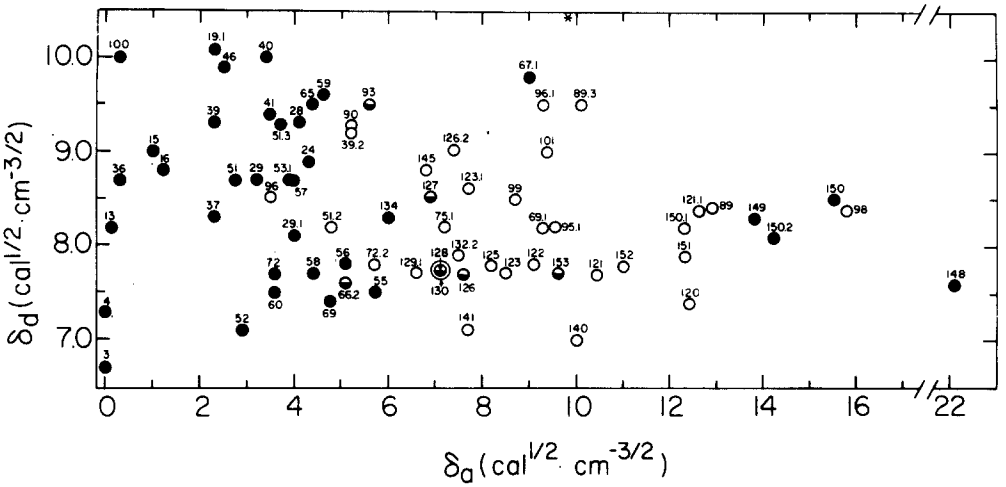

Fig. 2. Solubility parameter mapping of the interaction of purple membrane with solvents: binary presentation of δ_d vs. δ_a. *, the location of the solubility parameters of purple membrane as calculated from the centers of the circles in Figs. 1 and 3. The meanings of the numbers and symbols are as in Fig. 1.

were considered, but the separate contributions of each of the three independent parameters, δ_d, δ_p, and δ_h, could only be partially discerned in them. However, the very fact that both binary diagrams, δ_v vs. δ_h and δ_d vs. δ_a, exhibit a closed region of interaction in spite of the different relative locations of the solvents (with respect to each other), may indicate that the relative contributions of the solubility parameters are predominant in determining interaction versus non-interaction with the purple membrane, and their absolute values play only a minor role. The relative contribution of each of the solubility parameters can be observed on a ternary diagram, where the total solubility parameter of each solvent is normalized to 1. Such a representation is shown in Fig. 3, in which the relative solubility parameter δ'_d is defined by

$$\delta'_d = \delta_d/(\delta_d + \delta_p + \delta_h) \tag{10}$$

and so on. Here, too, a circle may readily be drawn to distinguish between interacting and non-interacting solvents. Again, 95% of the solvents interacting with purple membrane are mapped within a circle. Among these, however, 15% interact only partially. Outside of the circle 97% of the solvents do not interact with the purple membrane. It should be emphasized, however, that we do not know of any theoretical justification for a circle in this mode of presentation. Teas [15], for example, found for the solubility of resins in organic solvents irregular geometrical forms in this type of solubility map. In principle, a description of the interaction area by a triangle with its three sides parallel to the axes of the map would also do. However, in such a description, or any other one besides a circle, the number of exceptional points is larger. For this reason and because it is the simplest geometrical form, we felt that drawing a circle in this map is justified. If this is indeed the case, and if the circle center indeed represents the relative solubility parameters of purple membrane, it is possible to conclude that the relative solubility parameters of purple membrane

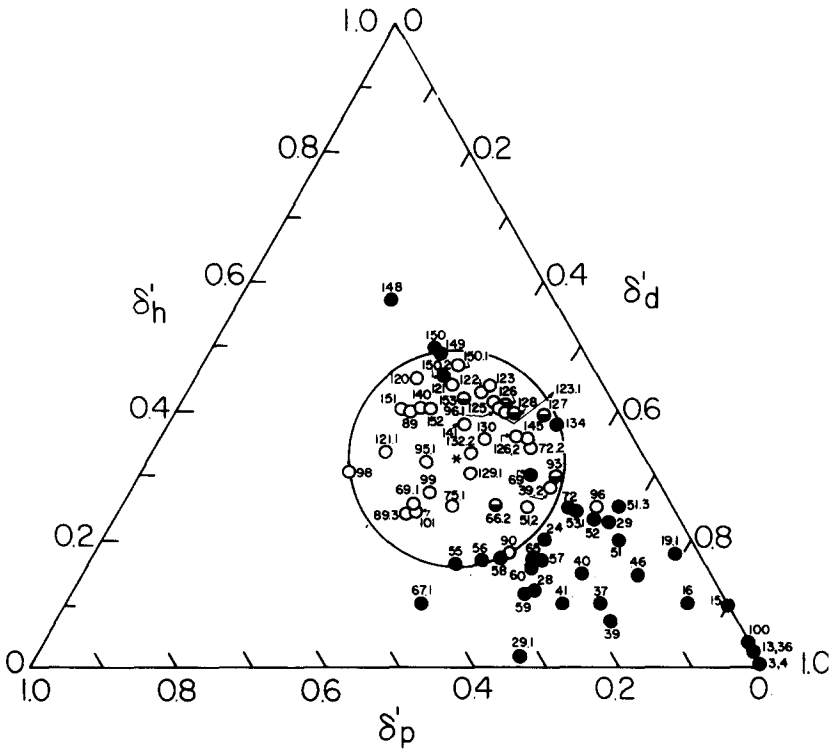

Fig. 3. Solubility parameter mapping of the interaction of purple membrane with solvents according to the relative contribution of each of the solubility parameters to the total solubility parameter. Each of the solubility parameters was normalized according to $\delta'_i = \delta_i/\Sigma\delta_i$. The significance of the symbols and numbers is as in Fig. 1.

are $\delta'_d = 0.43$, $\delta'_p = 0.25$, $\delta'_h = 0.32$. From these relative parameters and the absolute values of δ_v and δ_h (Fig. 1) it is possible to calculate the absolute values of δ_d and δ_p for purple membrane, yielding approximately 10.4 and 6.2, respectively. A remarkable feature of these results is the high hydrophobicity of purple membrane, as reflected in the high value of δ_d. This is especially prominent in Fig. 2, where the point representing the solubility parameters of purple membrane is asterisked. Extremely high hydrophobicity of purple membrane was indicated also from other approaches, e.g. in the works of Bridgen and Walker [23] and Ovchinnikov et al. [22].

Fig. 4 shows a triangular solubility map of the squares of the solubility parameters, i.e. of the normalized values of the cohesive energy densities (Eqn. 2). According to Eqn. 6, this might be expected to be a more informative mode of presentation. Here, too, it is possible to draw a circle for the interaction area; however, it is not justified in this case for the following reasons: (A) The center of this circle (not shown in the figure) does not fit the values of the centers in the other maps. (B) Mathematically, it is impossible to obtain a circle both in δ'_i representation (Fig. 3) and in e'_i representation (Fig. 4). (C) In fact, the number of exceptions is bigger if a circle is drawn instead of the geometrical shape shown in Fig. 4. We therefore drew an irregular, geometrical, closed curve, so that the same points which were included in the interaction

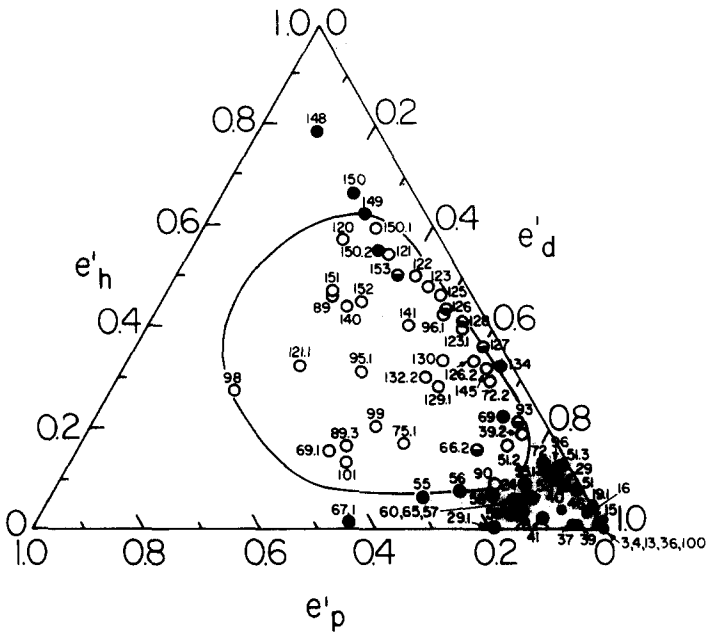

Fig. 4. Solubility parameter mapping of the interaction of purple membrane with solvents according to the relative values of the cohesive energy densities. Each of the values of the cohesive energy densities for a single solvent was normalized according to $e_i' = e_i/\Sigma e_i$. The significance of the symbols and numbers is as in Fig. 1.

area in Fig. 3 are also included here. We would like to emphasize that Fig. 4 is not just a slightly different presentation of Fig. 3. The information obtained from each of these maps is different, and it is impossible to know from the location of a point in one of the maps what its location in the other map would be, unless the absolute values are known as well. Thus, as shown in Fig. 5, methanol (No. 120) and a 1 : 1 mixture of acetone (No. 55) and water (No. 148) have exactly the same δ_i' values (and therefore both are represented by the same point in δ_i' map), but different e_i' values (and therefore distinct points in e_i' map). Whichever is the 'better' presentation, the previously discussed indication that the relative contributions of the dispersion forces, polar forces, and hydrogen bonding to the cohesive energy have higher weight in determining the interaction/non-interaction with purple membrane, seems to be correct.

The success of the solubility parameter mapping in defining the requirements for chemical interactions with purple membrane, can be demonstrated in our predictive capability even for solvent mixtures. Fig. 5 shows the four modes of presentation described above, in which the interaction/non-interaction of binary solvent mixtures and their individual constituents is drawn. The solubility parameters of the mixtures were calculated according to Eqn. 7. The borders were accurately redrawn from Figs. 1, 3, and 4, and, in fact, mixtures are inside or outside of the interaction areas, depending on whether they interact or do not interact with purple membrane, respectively, independent of interaction modes of their components taken separately. For example, a 1 : 1 mixture of

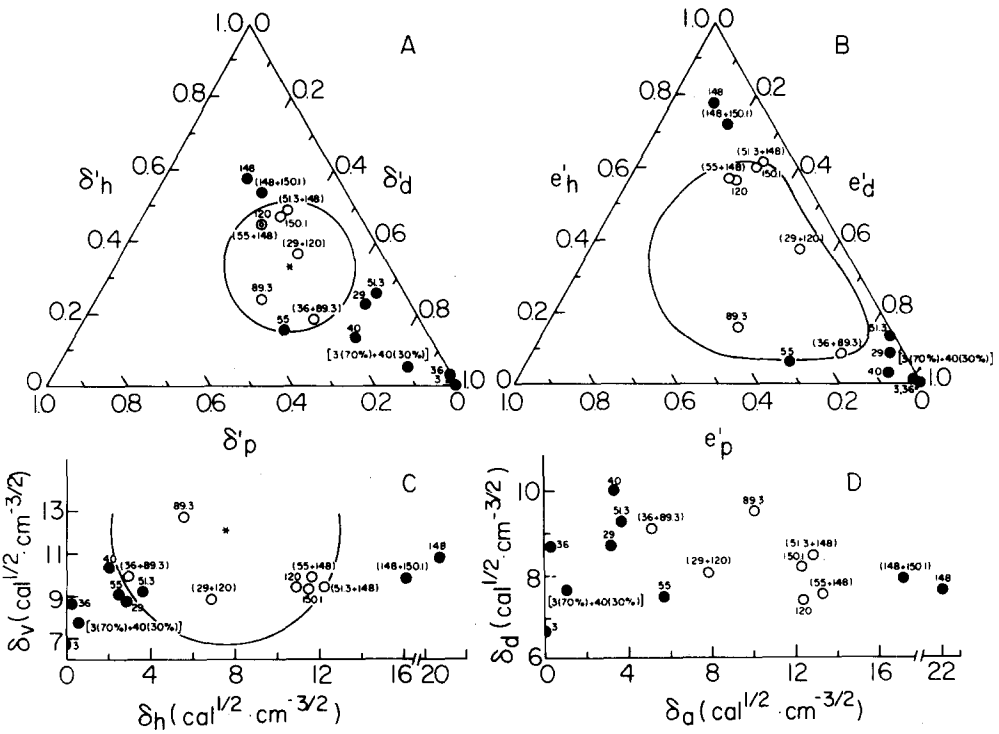

Fig. 5. Solubility maps of solvent mixtures and their individual components. All the mixtures were 1 : 1 by volume, unless otherwise shown. The boundaries and the asterisks are accurately depicted according to the appropriate figures: parts A, B, C, D relate to Figs. 3, 4, 1, 2, respectively. The significance of the symbols and numbers is as in Fig. 1.

acetone and water (located in the interaction area) interacts with purple membrane though neither acetone (No. 55) alone nor water (No. 148) alone (located outside the interaction area) interacts with purple membrane. A contrasting example is a 1 : 1 mixture of propylene glycol (No. 150.1) and water (No. 148). Propylene glycol alone interacts with purple membrane, but addition of water prevents this interaction by shifting the mixture out of the interaction area. We may therefore conclude that most combinations of solvents which together possess the appropriate hydrophobicity, hydrogen bonding capacity, and polar nature as defined by the solubility parameters will interact with purple membrane in exactly the same manner as a single solvent possessing the same parameters.

The significance of this study has both practical and theoretical aspects. Practically, any of the maps described above simplifies the choice of a single solvent or a solvent mixture, eliminating the need for experimentation. This is true for both the construction of a synthetic polymer containing purple membrane (see Introduction), or for any study which requires an addition of an organic solvent to the suspension. Theoretically, this study has implications for our understanding of the location of retinal in purple membrane, as will be detailed in the following report [24]. Of general importance is the demonstration that the chemical interactions of biological systems with organic solvents, and their mixtures, may be given a rational framework by means of solubility

parameter mapping. Such interactions are intimately connected with solubility and transport phenomena. Thus, the use of solubility parameter mapping, together with the group chemical contributions, should open the way for synthetic 'tailor-made' molecules possessing specific biological or physiological activity.

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