Correlation between Solvation of Peptide-Resins and Solvent Properties[1]

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The solvation properties of model resin and peptide-resins measured in *ca.* 30 solvent systems correlated better with the sum of solvent electron acceptor (AN) and electron donor (DN) numbers, in 1:1 proportion, than with other solvent polarity parameters. The high sensitivity of the (AN+DN) term to detect differentiated solvation behaviors of peptide-resins, taken as model of heterogeneous and complex solutes, seems to be in agreement with the previously proposed two-parameter model, where the sum of the Lewis acidity and Lewis basicity characters of solvent are proposed for scaling solvent effect. Besides these physicochemical aspects regarding solute−solvent interactions, important implications of this study for the solid phase peptide synthesis were also observed. Each class of peptide-resin displayed a specific solvation profile that was dependent on the amount and the nature of the resin-bound peptide sequence. Plots of resin swelling *versus* solvent (AN+DN) values allowed the visualization of a maximum solvation region characteristic for each class of resin. This strategy facilitates the selection of solvent systems for optimal solvation conditions of peptide chains in every step of the entire synthesis cycle. Moreover, only the AN and DN concepts allow the understanding of rules for solvation/shrinking of peptide-resins when in homogeneous or in heterogeneous mixed solvents.

Introduction

An optimized solvation of the whole polymeric matrix has been considered an essential prerequisite for the success of the solid phase peptide synthesis (SPPS) method.[2] A great variety of NMR,[3] CD,[4] and IR[5] approaches have been applied to investigate the influence of factors such as the solvent, resin, peptide sequence, etc., upon the complex solvation behavior of peptide-resin. Similarly, by using a novel paramagnetic amino acid derivative introduced by this laboratory,[6] we have alternatively initiated the application of the ESR method of labeled peptide-resins to investigate the influence of solvation parameters such as the swelling degree, viscosity of solvent, peptide chain mobility, intersite distances, etc., upon the yield of the synthesis.[7]

However, whatever the experimental protocol assayed, there is yet no well-established concept regarding the relationship between the solvation behavior of peptide-resin and the solvent properties of the medium. In this respect, only one recent paper[8] attempted to correlate solvation of peptide-resins with solvent polarity properties. In this report, differences in solvation behavior of peptide-resins was only detected when the Hildebrand solubility parameter (δ)[9] and its hydrogen bonding component (δ_h) of the solvent system were simultaneously considered in a contour solvation plot of resins.

Thus, the goal of the present report was to better investigate the correlation between solvation of peptide-resins, taken as a model of complex solute molecule and properties of the medium but stressing the following details in the approach: (1) a more accurate and sensitive method of microscopic determination of beads sizes (dry and swollen)[10] was employed for estimatives of the solvation degree of peptide-resins; (2) to magnify changes

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(1) (a) Preliminary accounts of some aspects of this work were described earlier as a communication in: Cilli, E. M., Oliveira, E., Marchetto, R., Paiva, A. C. M.; Nakaie, C. R. *Peptides 1992: Proceedings of the 22nd European Peptide Symposium;* Schneider, C. H., Eberle, A. N., Eds; Escom: Leiden, 1993; pp 425. (b) Abbreviations for amino acids and nomenclature of peptide structure follow the recommendations of the IUPAC-IUB (Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are as follow: Bz = benzyl; Boc = *tert*-butyloxycarbonyl; BOP = (benzotriazol-1-yloxy)-tris(dimethylamine)phosphonium hexafluorophosphate; DCM = dichloromethane; DIEA = diisopropylethylamine; DMF = *N*,*N*-dimethyl formamide; DMSO = dimethylsulfoxide; HOBt = 1-hydroxybenzotriazole; Fmoc = 9-fluorenylmethyloxycarbonyl, HPLC = high-performance liquid chromatography; NMP = *N*-methylpiperidinone; PIP = piperidine; TEA = triethylamine; TFA = trifuoroacetic acid; TFE = trifluoroethanol; THF = tetrahydrofuran.

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in the swelling degree of beads, very highly peptide-loaded resins (up to 75% of peptide content, weight/weight) assembled in highly substituted benzhydrylamine-resins (BHAR) were also investigated; (3) besides the variation in the peptide content and in the polarity of the sequence, the influence of chain aggregation on the solvation behavior of resin was investigated using a well-known resin-bound "difficult sequence"; (4) a large number of single and mixed solvents was selected to cover an as broad as possible range of polarity of the medium; (5) in contrast with other attempts where solvation studies are restricted to the critical coupling reaction, this solvation approach was also extended to other remaining steps of the peptide synthesis cycle.

Finally, (6) assuming that the solvation properties of peptide-resins can be affected by the total amount of the electrophilic (NH) and nucleophilic (C═O) groups mainly of its peptide backbones, we decided to emphasize in this correlation study, among other solvent parameters, the electron acceptor (AN) and donor (DN) numbers[11] of the solvent system. These two important solvent parameters were earlier proposed, implying that the solvent effect on a solute molecule can be considered in a generic sense, as an acid−base type interaction. The AN (acidic, electrophilic) and the DN (basic, nucleophilic) solvent properties have been employed as independent parameters to explain several chemical reactions and, to date, only the former has shown a linear relationship with other solvent polarity parameters.[12] In addition to AN and DN terms separately, the solvation behaviors of model peptide-resins were also correlated with the sum of these two solvent terms in different proportions. The Dimroth−Reichard's $E_T(30)$ parameter[13] and the dielectric constant ϵ were also assayed as representative of solvent properties. Besides the physicochemical aspect of the present work, implications of this study for the optimization of the solid phase peptide synthesis methodology were also evaluated.

Results

The repetitive tetrapeptide sequence (Asp-Ala-Asp-Pro)$_4$ bearing protecting benzyl groups at the Asp side chains and bound to a 1.4 mmol/g BHAR (resin **1**) and the well-known[14] aggregating sequence Ile-Asp-Gly [(72−74)-acyl carrier protein-fragment] bound to a 2.6 mmol/g BHAR (resin **2**) were synthesized and studied as to their swelling properties. These highly substituted BHARs, synthesized according to our forceful experimental protocol,[15] were deliberately used in order to magnify the swelling response of beads as a consequence of the solvation of a larger amount of attached peptide chains. The calculated peptide content (by amino acid analysis) of these resins reached 75% and 47%, respectively. For comparison, the same tripeptide sequence of resin **2** was alternatively assembled in a 0.2 mmol/g BHAR, giving a very low (6%) peptide content compound (resin **3**). The fourth resin studied was the 1.4 mmol/g BHAR (resin **4**), taken as a peptide-free resin model.

The percentage of the bead volume occupied by the solvent was chosen as the swelling parameter and is calculated according to the equation: [(swollen − dry volume of bead)/swollen volume of bead] × 100, where the swollen and dry bead volumes are calculated from measured average diameters of each resin in a microscope.[10]

Table 1 shows the swelling data of the four resins measured in 28 single and mixed solvents. These solvent systems were selected to cover a broad range of polarity, most of them having potential application as solvent for different steps of the peptide synthesis cycle. The measured percentage of bead volume occupied by the solvent ranged broadly from approximately 5% to approximately 90% in those swelling experiments.

To correlate the swelling of resins with solvent properties, Table 2 illustrates the values for the dielectric constant, ϵ, $E_T(30)$, AN, and DN found in the literature,[11a,12b,16] and also the additive (AN+DN) term, in 1:1 proportion, for 28 solvents. In the same manner as used for other empirical solvent properties,[8,9,17] values of solvent parameters for mixed solvents were calculated accordingly to the equation:

$$X_{1+2} = \phi_1 x_1 + \phi_2 x_2 \qquad (1)$$

where x_1 and x_2 are the solvent parameters under study for the two components of the mixture, and ϕ_1 and ϕ_2 are the corresponding volume fractions.

Figure 1 shows the solvation profiles for resin **1** when its swelling data are correlated comparatively with solvent ϵ, $E_T(30)$, AN and (AN+DN) values. In contrast to weak relationships observed with the first three solvent properties, the best fit in this correlation is observed with (AN+DN) (Figure 1D). A better contoured solvation curve is seen in this plot, with a characteristic maximum solvation region for this resin occurring with solvents having (AN+DN) values around 40.

Similarly, the best correlation found between swelling properties and solvent parameters for the other three resins is also with the (AN+DN) number (Figure 2). Characteristic maximum solvation regions are also observed and occur with solvents having (AN+DN) values around 50 for resin **2** and lower than 30 for resins **3** and **4.** All other figures which correlate swelling with ϵ, $E_T(30)$, and AN for these resins and the expected lack of correlation between swelling and DN number, irrespective of resin, are available as supporting information. In addition, the sum of AN and DN numbers was also tested in 1:2 and 2:1 proportions, but the correlations with swelling data of resins were weaker than those observed when the 1:1 proportion was employed (figures also in supporting information).

Interestingly, by analyzing the swelling *versus* solvent (AN+DN) number figures, only mixed solvents 21 and 22 (TFE/DMF and TFE/DMSO), which are designated by open circles, deviate significantly from the average sol-

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Table 1. Swelling Degree of Resins

		resins							
		1		2		3		4	
entry	solvent	diam swollen bead (μm)	solvent within bead (%)[a]	diam swollen bead (μm)	solvent within bead (%)[a]	diam swollen bead (μm)	solvent within bead (%)[a]	diam swollen bead (μm)	solvent within bead (%)[a]
1	toluene	100	50	82	11	93	81	132	92
2	DCM	117	69	89	31	88	77	99	85
3	chloroform	134	79	88	28	88	77	115	87
4	NMP	136	80	142	83	83	73	105	83
5	DMF	140	82	135	81	81	70	79	61
6	DMSO	142	83	136	81	70	54	66	46
7	TFE	135	80	154	87	58	17	73	51
8	EtOH	95	42	102	55	55	06	66	32
9	MeOH	98	47	113	66	58	19	68	38
10	formamide	91	33	95	44	56	09	69	31
11	50% TFE/toluene	146	84	130	78	90	78	121	90
12	20% TFE/DCM	136	80	124	74	90	79	125	90
13	50% TFE/DCM	140	82	143	83	73	59	89	73
14	80% TFE/DCM	147	84	147	85	60	27	80	62
15	20% DMSO/NMP	147	84	148	85	84	73	84	67
16	50% DMSO/THF	153	86	146	85	70	53	99	80
17	65% NMP/THF	159	88	130	78	90	78	109	85
18	50% DCM/DMF	138	81	131	78	80	69	96	78
19	50% DCM/DMSO	138	81	139	82	73	58	86	69
20	50% MeOH/DMSO	135	80	140	83	59	24	72	48
21	50% TFE/DMF	95	42	80	06	55	06	68	38
22	50% TFE/DMSO	106	58	89	30	55	07	70	42
23	10% TEA/DCM	127	75	100	52	85	74	101	79
24	10% TEA/DMF	150	85	134	80	78	67	89	69
25	10% TEA/DMSO	136	80	143	84	64	39	82	64
26	20% PIP/DCM	nd[b]	nd[b]	103	55	88	77	120	89
27	20% PIP/DMF	nd[b]	nd[b]	137	81	80	69	99	80
28	20% PIP/DMSO	nd[b]	nd[b]	136	81	66	46	101	81

[a] [(swollen volume − dry volume)/swollen volume] × 100 using the following values for measured diameters of dry beads: Resins: **1** = 79 μm, **2** = 79 μm, **3** = 54 μm, **4** = 58 μm. [b] Not determined.

Table 2. Solvent Parameters[11b,12b,16]

entry	solvent	ϵ	$E_T(30)$ [kcal/mol]	AN	DN	(AN+DN)
1	toluene	2.4	33.0	3.3[a]	0.1	3.4
2	dcm	8.9	40.7	20.4	1.0	21.4
3	chloroform	4.7	39.1	23.1	4.0	27.1
4	NMP	33.0	42.2	13.3	27.3	40.6
5	DMF	36.7	43.8	16.0	26.6	42.6
6	DMSO	46.7	45.1	19.3	29.8	49.1
7	TFE	26.7	54.1	53.5	0.0	53.5
8	EtOH	24.3	51.9	37.1	32.0	69.1
9	MeOH	32.6	55.4	41.3	30.0	71.3
10	formamide	109.5	55.8	39.8	24.0	63.8
11	50% TFE/toluene	14.6	43.6	28.4	0.1	28.5
12	20% TFE/DCM	12.5	43.4	27.0	0.5	27.5
13	50% TFE/DCM	17.8	47.4	37.0	0.5	37.5
14	80% TFE/DCM	23.1	51.4	46.9	0.5	47.4
15	20% DMSO/NMP	35.7	42.8	14.5	27.8	42.3
16	50% DMSO/THF	27.1	41.3	13.7	24.9	38.6
17	65% NMP/THF	24.1	40.5	11.4	24.7	36.1
18	50% DCM/DMF	22.8	42.3	18.2	13.8	32.0
19	50% DCM/DMSO	27.8	42.9	19.9	15.4	35.3
20	50% MeOH/DMSO	39.7	50.3	30.3	29.9	60.2
21	50% TFE/DMF	31.7	49.0	34.8	13.3	48.1
22	50% TFE/DMSO	36.7	49.6	36.4	14.9	51.3
23	10% TEA[b]/DCM	8.3	39.8	18.5	6.6	25.1
24	10% TEA[b]/DMF	33.3	42.6	14.5	30.0	44.5
25	10% TEA[b]/DMSO	42.3	43.8	17.5	32.9	50.4
26	20% PIP[b]/DCM	8.3	39.7	16.3	8.8	25.1
27	20% PIP[b]/DMF	30.5	42.1	12.8	29.3	42.1
28	20% PIP[b]/DMSO	38.5	43.2	15.4	31.8	47.2

[a] As AN_E number (ref 16b). [b] See Table 3 for values of TEA and PIP solvent parameters.

vation curve of resins. A much lower degree of swelling than that predicted by solvent parameters assayed in this study is observed in these two solvent systems, mainly with highly peptide-loaded resins **1** and **2** (Figures 1D and 2A, respectively). The explanation for these behaviors will be further discussed in light of the electron acceptor and donor properties of the two components of the two solvent mixtures.

Besides the coupling step, the present solvation report was also extended to both the α-amino group neutralization with TEA[18] and the deprotection/neutralization with piperidine (PIP) steps employed in the Boc- and Fmoc-peptide synthesis strategies, respectively. The PIP-containing mixed solutions 26 to 28 (Table 1) were not assayed for peptide-resin **1,** which contains a peptide sequence bearing benzyl group protection at the Asp residues and is only used in Boc/benzyl peptide synthesis strategy and, therefore, is not submitted to TEA treatment. The comparison of swelling degrees of four resins in the alkaline solvents 23 to 28 (Table 1) indicates that, as originally introduced for the use in Fmoc-strategy, DMF might be kept as the cosolvent for the PIP solution, regardless of the resin.

On the other hand, the much lower swelling behavior observed with resin **2** in 10% TEA/DCM, if compared with the corresponding TEA-solutions in DMF and DMSO (solvents 23, 24, and 25, respectively), suggests the replacement of the first cosolvent for the last two more polar solvents for the neutralization step in the Boc-strategy. However, this recommendation seems to be only valid for the special case of highly peptide-loaded resins containing aggregating and polar sequences (resin **2**).

Taking together, these findings demonstrate that the complex solvation behavior of peptide-resins correlates better with the (AN+DN) term than with the other solvent parameters. To further compare this additive term, its relationship with ϵ, $E_T(30)$, AN, and DN solvent properties was investigated in approximately 40 single solvents. In addition to 10 single solvents already

(18) DIEA showed similar solvation degree of resins if compared to TEA.

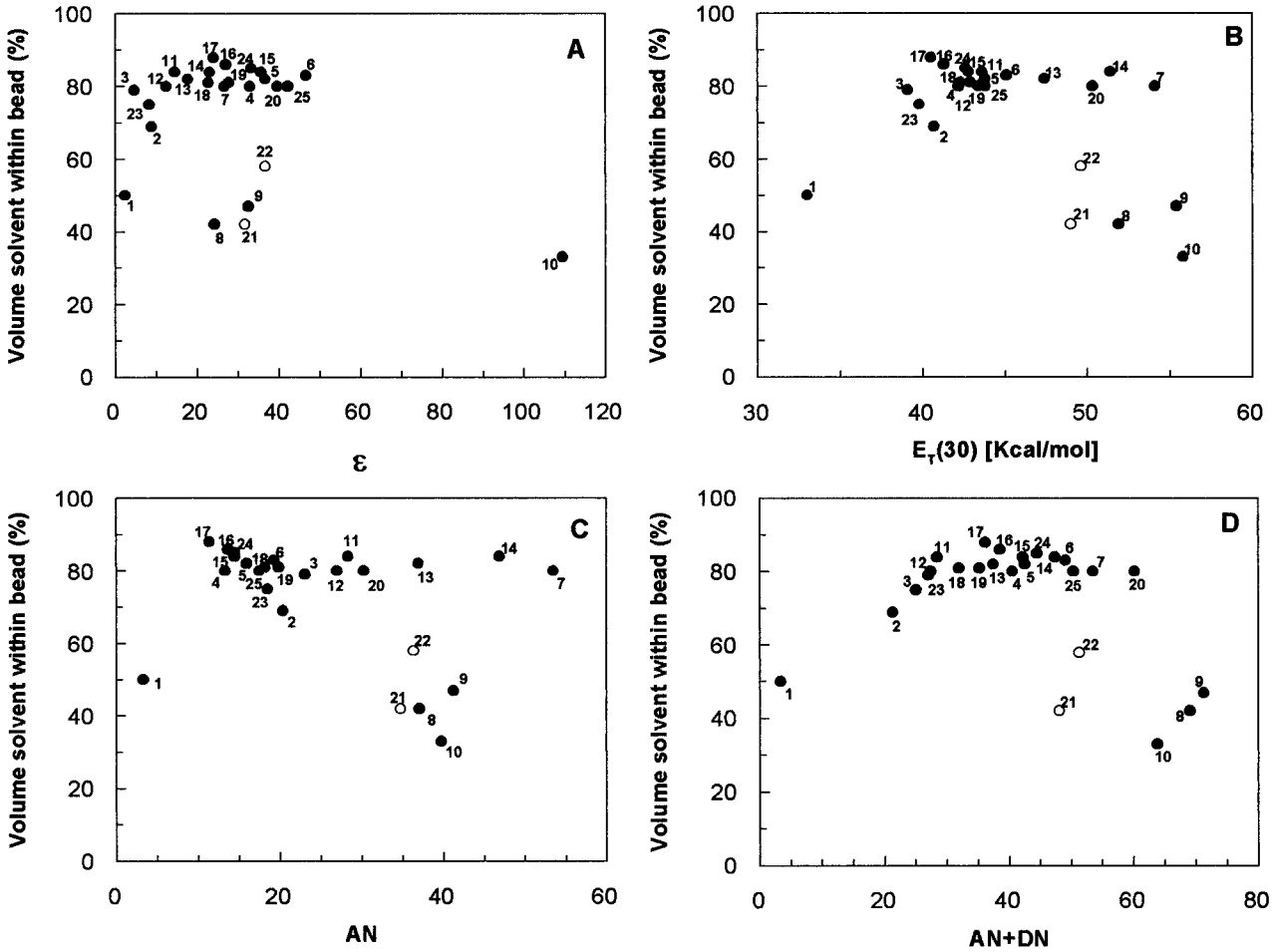

Figure 1. Swelling of resin **1** as a function of solvent ϵ [A], $E_T(30)$ [B], AN [C], and (AN+DN) [D] values.

employed for the solvation study (Tables 1 and 2), other solvents necessary to establish this correlation are shown in Table 3.

Table 4, summarizing the calculated and expected correlation values, shows that the DN term does not correlate with other solvent properties. On the other hand, the best binary correlation is found between E_T-(30) and AN with a regression coefficient (r) of 0.93. Finally, as predicted by the different swelling profiles of resins obtained with the (AN+DN) number, this term does not show a linear relationship, neither with ϵ ($r \cong 0.3$) nor with E_T (30) and AN parameters ($r \cong 0.7$).

Discussion

Resin Bead Swelling Measurements. We deem the use of the method of microscopic measurement of peptide-resins[10] essential to our work because of its accuracy and sensitivity. Usually, the swelling capacity of resins is determined by measurements of their swollen volume (mL/g or mL/mmol) in a glass-fritted burette. In addition to the low accuracy in the detection of small differences in swelling, this simple method presents limitations, depending on the approach to be carried out. Although valid to compare, for example, the solvation of a particular resin in different solvent systems, the measured solvated volume per gram is not always appropriate for comparing the swelling degrees of different resins. This is due to the fact that each resin has its own dry volume, which may contribute differently to the total swollen volume measured in a burette or in a column. This limitation also occurs even when swelling of a peptide-resin is measured in different positions during the peptide chain assembly. In this case, the increase in dry volume of beads will be observed as a consequence of progressive weight gain of a composite resin.

To overcome this shortcoming, we have employed the percentage of the volume of the swollen bead occupied by the solvent as the swelling parameter obtained through microscopic measurement of resins. This parameter allows a more reliable comparison between swelling capacities of resins with a sensitivity able to detect minimal swelling such as the 6% measured for resins **1** and **2** with solvent 21 (50% TFE/DMF, Table 1). On the other hand, values around 90% were obtained as the maximum solvated example (resin **4** in toluene, Table 1). To demonstrate the sensitivity of this swelling parameter, we have previously shown[7] that even small differences of its value (lower than 10%) between peptide-resins were sufficient to affect differently the yield of the coupling reaction obtained in comparative kinetic studies.

As an additional precaution in the experimental protocol, in order to assure reliable determination of resin swelling, a rigorous sizing procedure of beads was performed as detailed in the Experimental Section. This strategy allowed the lowering of the dispersion of resin bead population to approximately 4% in terms of geometric standard deviation.

The use of the simple weighed eq 1 for parameter calculation of mixed solvents is indeed rigorously valid in ideal solutions, when there is no preferential or selective solvation of the solute by one of the two

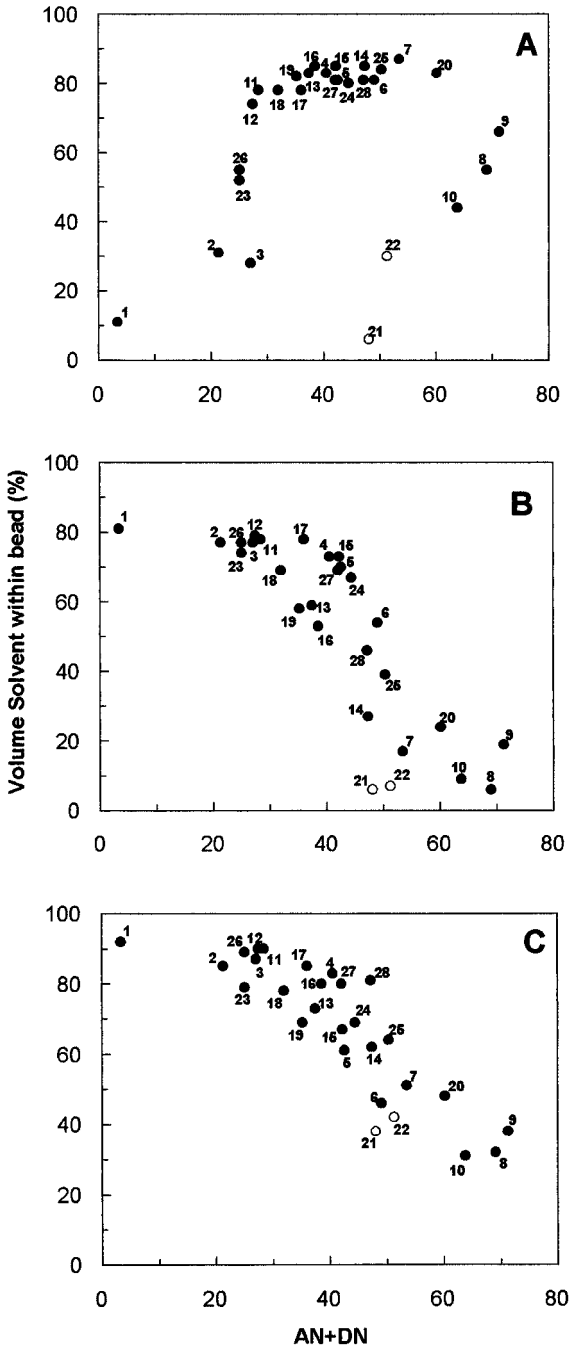

Figure 2. Swelling of resins **2** [A], **3** [B], and **4** [C] as a function of solvent (AN+DN) values.

components of the solvent mixture. Despite this conceptual shortcoming, successful applications of this equation for mixed solvents have been observed for solvent effect studies using, for example, Hildebrand's δ[8,9] and Snider's P'[17] parameters. Moreover, the removal of swelling data of mixed solvents in Figures 1 and 2, regardless of the resin, did not alter the solvation profile (not shown), thus evidencing the validity of the use of eq 1 in the present work.

Evaluation of the Solvent (AN+DN) Term. The search for the most appropriate scale which reflects the solvent effect has led initially to the proposition of the so-called empirical solvent parameters.[19] These are usually derived from a model reaction where experiments

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Table 3. Solvent Parameters[11b,12b,16]

solvent	ϵ	$E_T(30)$ [kcal/mol]	AN	DN	(AN+DN)
toluene	2.4	33.0	3.3[a]	0.1	3.4
DCM	8.9	40.7	20.4	1.0	21.4
chloroform	4.7	39.1	23.1	4.0	27.1
NMP	33.0	42.2	13.3	27.3	40.6
DMF	36.7	43.8	16.0	26.6	42.6
DMSO	46.7	45.1	19.3	29.8	49.1
TFE	26.7	54.1	53.5	0.0	53.5
EtOH	24.3	51.9	37.1	32.0	69.1
MeOH	32.6	55.4	41.3	30.0	71.3
formamide	109.5	55.8	39.8	24.0	63.8
2-propanol	18.3	48.4	33.5	36.0	69.5
1-butanol	17.5	49.7	36.8	29.0	65.8
tetrahydrofuran (THF)	7.5	37.4	8.0	20.0	28.0
acetone	20.7	42.2	12.5	17.0	29.5
piperidine (PIP)	5.8	35.5	0	40.0	40.0
triethylamine (TEA)	2.4	33.3	1.4	61.0	62.4
diethylamine	3.6	35.4	9.4	50.0	59.4
ethylamine	6.2	–	4.8	55.5	60.3
ethylenodiamine	12.9	–	20.9	55.0	75.9
pyridine	12.3	40.5	14.2	33.1	47.3
acetic acid	6.2	51.7	52.9	20.0	72.9
trifluoroacetic acid (TFA)	8.2	–	105	0	105.0
benzene	2.3	34.3	8.2	0.1	8.3
nitrobenzene	34.8	41.2	14.8	4.4	19.2
nitromethane	36.7	46.3	20.5	2.7	23.2
hexane	1.9	31.0	0	0	0
carbon tetrachloride	2.2	32.4	8.6	0	8.6
1,1-dichloroethane	10.0	39.4	16.2	0	16.2
1,2-dichloroethane	10.1	41.3	16.7	0	16.7
diethyl ether	4.2	34.5	3.9	19.2	23.1
dioxane	2.2	36.0	10.3	14.3	24.6
dimethoxyethane	7.2	38.2	10.2	20.0	30.2
acetonitrile	36.0	45.6	18.9	14.1	33.0
benzonitrile	25.2	41.5	15.5	11.9	27.4
hexamethylphosphoramide	29.6	40.9	10.6	38.8	49.4
diaminoethane	–	42.0	20.9	55.0	75.9
N-methylformamide	–	54.1	32.1	27.0	59.1
sulfolane	43.3	44.0	19.2	14.8	34.0
dimethylacetamide	37.8	42.9	13.6	27.8	41.4
diethylacetamide	–	41.4	13.6	32.2	45.8
methyl acetate	6.7	38.9	10.7	16.3	27.0
ethyl acetate	6.0	38.1	9.3	17.1	26.4
dichloroethylene carbonate	10.1	41.9	16.7	3.2	19.9
trimethyl phosphate	–	42.6	16.3	23.0	39.3
tributyl phosphate	–	39.6	9.9	23.7	33.6
water	78.4	62.8	54.8	18.0	72.8

[a] As AN_E number (ref 16b).

are designed to measure (spectrophotometrically, thermodynamically, etc.) a single or an average of several standard solute–solvent interactions. Besides $E_T(30)$, AN, and DN numbers, there are other parameters, such as Kosower's Z,[20] Kamlet–Taft's π^*,[21] Swain et al.'s acity and basity[22] values, etc. For example, $E_T(30)$, which was comparatively evaluated in the present report, is determined by measuring transition energies for the longest wavelength of the absorption band of a pyridinium *N*-phenoxide betaine, taken as the model probe for interaction with the solvent molecule.[13,16a] The shift in this transition energy increases with solvent polarity, and therefore the $E_T(30)$ values measured for various solvents have been applied to empirically establish the polarity scale. Successful application of this solvent parameter to investigate solute–solvent interaction has been demonstrated[23] and detailed in a recent review.[16a]

An alternative route to scale solvent effect was also developed by measuring the capacity of the solvent to act

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Table 4. Binary Correlations of Solvent Parameters[a]

Y/X	ϵ	$E_T(30)$	AN	DN	(AN+DN)
ϵ	1				
$E_T(30)$	$E_T(30) = 37.3 + 0.24\epsilon$ $r = 0.7233$; $n = 38$	1			
AN	AN = 14.8 + 0.29ϵ $r = 0.3177$; $n = 41$	AN = −58.6 + 1.82$E_T(30)$ $r = 0.9256$; $n = 43$	1		
DN	DN = 19.0 + 0.04ϵ $r = 0.0527$; $n = 41$	DN = 11.2 + 0.21$E_T(30)$ $r = 0.0957$; $n = 43$	DN = 24.3 − 0.15AN $r = -0.1653$; $n = 46$	1	
(AN+DN)	(AN+DN) = 33.8 + 0.33ϵ $r = 0.3015$; $n = 41$	(AN+DN) = −47.4 + 2.0$E_T(30)$ $r = 0.6944$, $n = 43$	(AN+DN) = 24.3 + 0.85AN $r = 0.6873$; $n = 46$	(AN+DN) = 24.2 + 0.82DN $r = 0.6028$; $n = 46$	1

[a] r = correlation coefficient of linear regression; n = number of solvent.

as an Lewis acid or Lewis base, a donor or acceptor of electron pairs, such as AN and DN numbers, respectively.[11] According to this theory, the solvent effect upon a solute molecule is generally considered as an acid−base type interaction. AN represents the electrophilic property of the solvent and is related to the relative chemical shift of ^{31}P in triethylphosphine in the particular solvent, with hexane as a reference solvent on the one hand, and triethylphosphine oxide−$SbCl_5$ in 1,2-dichoroethane on the other, to which the acceptor of 0 to 100 have been assigned. In contrast, the DN number is representative of the basic (nucleophilic) property of the solvent molecule and is defined as the molar enthalpy for the reaction of the donor with $SbCl_5$ as reference acceptor, also in 1,2-dichloroethane.[11]

Despite these conceptual differences which originate the $E_T(30)$ and AN parameters, they show a good linear relationship ($r = 0.93$, Table 4). This finding is in close agreement with previous reports[12] and explains the similarity of the swelling profiles of all resins assayed with these two parameters. However, the weak correlation observed between both parameters and swelling degree of resins suggests that they are not, at least for peptide-resin-type solutes, the most appropriate for scaling its solvent effect.

Similarly to what was observed with the $E_T(30)$ and AN parameters, the dielectric constant ϵ also showed a weak correlation with the resin-swelling properties. This macroscopic solvent parameter only takes into account the electrostatic solvent−solute interactions, but one must also consider whether the effective alignments of the solvent dipole for the maximum interaction with the solute molecule. This is conceptually the most serious limitation of the ϵ parameter[24] and probably explains its weak relationship with the solvation behavior of peptide-resins.

Comparatively, the DN number shows the worst correlation with resin solvation degree. This lack of correlation is also extended to binary relationships with other parameters and is summarized in Table 4. This result agrees with the previous concept that the DN number does not represent a true polarity scale,[12a] and its applicability seems to be more restricted to solvent-dependent processes which are primarily influenced by nucleophilic properties of solvents.

The presupposition that the solvation behavior of peptide-resins may be strongly affected by acidic N−H and basic C=O groups of the peptide backbone was the starting point to suggest an amphoteric solvent parameter which would be sensitive to the solvation of these groups throughout the resin matrix. The (AN+DN) number seems to match this character, since the degrees of resin solvation correlate slightly better with this term than with other properties, including the largely employed $E_T(30)$ parameter. Indeed, this strategy in considering simultaneously more than one solvent parameters (for example, AN and DN) to better investigate solvent effect phenomena as applied in the present report, has been proposed for other solute−solvent interactions. In this case, a two-parameter equation model,[25] where a physicochemical property (Q) of the solute (swelling degree of resin, in the case of the present work) measured in its interaction with the solvent may be represented by:

$$Q = Q_0 + aA + bB \qquad (2)$$

where A and B are Lewis acidity and Lewis basicity solvent parameters, respectively, and a and b are coefficients describing the sensitivity of Q to these two acidic and basic solvent properties. Q_0 is the Q value in a solvent with zero acidity and basicity on the A and B scale. *B*esides the Et(30) and DN number, originally tested[25] as the A and B terms, respectively, other solvent parameters such as Kamlet−Taft's α and β[21] and Swain's acity and basity[22] values are also accepted as measures of solvent acidity and basicity.

Following this empirical model, our first attempt to correlate solvation of resin with the sum of AN and DN numbers was reported in a preliminary communication.[1] At the same time, a more detailed solvation study of alkali metal and halide ions in protic and aprotic solvents also proposed the addition of Gutmann's parameters in the same equation.[26] The 1:1 proportion found as the most appropriate between AN and DN terms for scaling solvation of peptide-resins in the present report suggests unity values for the a and b constants in eq 2. This indicates that electrophilic and nucleophilic groups of the solute under study contribute equally to the interaction with solvent molecules.

However, attention should be paid in the application of the (AN+DN) parameters to correlate with the solvation process of peptide-resins. The empirical solvent effect model represented by the two-parameter eq 2 postulates that whatever the measured physicochemical property of the solute, it correlates linearly with the sum of all solvent parameters included in eq 2. If this is true for a great variety of kinetic, thermodynamic, or spectroscopic data of solute−solvent interactions found in the literature,[22,25] it does not occur with solvation degree of polymeric matrices. Instead, a curve is observed in Figures 1D and 2 with a characteristic maximum solvation region for each resin, more clearly observed in heavily peptide-loaded resins **1** and **2**, located in the

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middle of the (AN+DN) scale**.** A trend for a linear correlation seems to occur only at both sides of these maximum solvation region. Concerning low-loaded or peptide-free resins **3** and **4**, as their enhanced solvation regions seem to be shifted and hidden to the left side of Figures 2C and 2D, trends for linearity in this correlation are observed in decreasing swelling profile displayed in these figures.

The particularity of the solvation process of a polymeric matrix, if compared to any other solute−solvent interaction, is due to the fact that each resin has a characteristic degree of polarity and its matrix solvation is maximum when the solvent presents the same polarity.[24] For example, cross-linked polystyrene has a solvent Hildebrand's δ value of 9.1 and its maximum solvation occurred in solvents having δ values similar to those of the polymer.[27] To the best of our knowledge, only a recent paper[28] suggested a linear relationship between the swelling degree of a polyurethaneimide-type resin with the solvent $E_T(30)$ polarity parameter. But in this case, a maximum solvation region was not observed probably due to the restriction of the approach only to aliphatic alcohols and linear ethers solvents which do not encompass entirely the polarity scale as done in the present work.

In conclusion, in the special case of solvation of resins, the solvent (AN+DN) term evaluated here seems to be more appropriate as an optional solvent polarity parameter rather than a simple sum of parameters that correlates linearly with any solvent-dependent physicochemical properties of the solute. The idea in considering the sum of *A* and *B* parameters of eq 2 as a new measure of solvent polarity, as proposed here with the (AN+DN) number, was also previously postulated with the acity and basity terms.[16a,22] By taking into account that *a* and *b* coefficients are nearly 1 in the case of solvation of resins, one can disregard the problem of difference in the scale between AN and DN, and therefore eq 2 becomes: $Q = Q_0 + (AN+DN)$. Moreover, if for the sake of simplicity, one neglects the Q_0 term and the dimensional difference between AN and DN numbers, the simple (AN+DN) term may be used alternatively for scaling solvent effect, at least for polymeric compounds. According to the data shown in Table 3, the range of this polarity scale varies from 0 (hexane) to 105 (trifluoroacetic acid), and the best correlation of the (AN+DN) term with other solvent parameters assayed here is approximately 0.7 for correlation coefficients with AN and $E_T(30)$ properties (Table 4).

The search for a combined solvent term more appropriate than (AN+DN) or for another empirical equation applicable for the solvation study of polymeric material may probably involve a more complex theoretical approach including other solvent properties. The so-called multiparameter theory,[21,29] successfully applied for solvent effect studies, is being currently applied, including additional swelling values recently obtained with complementary sets of model peptide-resins.

Implications for the SPPS Methodology. Each class of peptide-resin displayed a characteristic swelling profile in the correlation with solvent (AN+DN) numbers, as shown in Figures 1 and 2. Solvent systems characterized by (AN+DN) values lower than 30 induced higher solvation of resins with low peptide-contents (resins **3** and **4)**. Apolar solvents such as toluene and DCM (Table 1) were more appropriate for solvation of these resins which are strongly influenced by the hydrophobic polystyrene chains of their polymeric matrices. These results also suggest that when in a very low peptide-loading condition, improved solvation of a peptide-resin, even containing an aggregating sequence, still occurs in apolar solvents (resin **3**).

Peptide-polystyrene resins are expected to have physicochemical properties that differ considerably from the initial polystyrene-resin, due mainly to the influence of the peptide backbone which introduces a polar component to the resin. This effect is clearly seen by the shift to higher (AN+DN) values (to approximately 40 and 50) of solvents which induced enhanced solvation of highly peptide-loaded resins **1** and **2**, respectively. However, the significant difference in solvent (AN+DN) values between these two resins suggest that other factors besides peptide-loading affect the swelling capacity of resins. In this comparison, the lower peptide-loaded resin **2** (peptide content of 47% against 75% of resin **1**) showed maximum solvation with solvents having higher (AN+DN) values (around 50) than resin **1** (around 40), and this is not in agreement with the postulated higher polarity character of peptide-resins containing a larger amount of peptide backbone. This apparent contradiction is probably due to the additional influence of amino acid side-chain protecting groups on the polarity of the peptide-resin, as already demonstrated in similar solvation studies of peptide-resins.[8,30] The maximum solvation of resin **1,** which occurs in lower (AN+DN) values, might be due to its higher overall hydrophobicity given by a total of eight apolar protecting benzyl groups bound to its $(Asp\text{-}Ala\text{-}Asp\text{-}Pro)_4$ sequence. On the other hand, the higher polarity of resin **2** may be due not only to the Ile-Asn-Gly sequence itself but also to the large amount of interchain hydrogen bonding induced by its strong aggregating tendency.

Besides facilitating the choice of more appropriate solvents for improved solvation of these two highly peptide-loaded resins, the correlation study involving solvent (AN+DN) values also allowed the detection of an additional difference between these two resins in the low (AN+DN) region (Figures 1D and 2A). By comparing, for instance, the swelling data measured in the apolar solvents toluene, DCM, and chloroform (Table 1), the resin **2** shows a much lower solvation with these solvents than the resin **1** (11%, 31%, and 28%, against 50%, 69%, 79%, respectively). This stronger shrinking of resin **2** beads in apolar solvents is in agreement with the previously reported aggravation of peptide chain interactions which occur specially in aggregating sequence-containing resins.[31]

Apart from the importance of applying the simple (AN+DN) term to investigate resin solvation, the electron acceptor−donor numbers theory, advantageously to most

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other solvent parameters, also allowed the prediction of the swelling/shrinking behavior of resins toward mixed solvents. The first example of this application is related to solvation properties of solvents 21 and 22 which do not swell the resins as expected from their solvent parameter values (Figures 1 and 2). A clear deviation in the swelling curve is observed with these solvents, and the explanation for this behavior resides in the fact that these two mixtures consist of strong electron acceptor (TFE) and strong electron donor (DMF or DMSO) components. In these solutions, the two components with high AN and high DN values tend to self-associate rather than to solvate peptide chains inside the bead, leading to lower swelling than predicted by solvent parameters. Obviously, this self-neutralizing effect of solvent components is maximal when the resin contains a large amount of strongly aggregated peptide chains to be disrupted. Accordingly, the most significant deviation of solvents 21 and 22 from the swelling curve of resins is mainly observed in the aggregating sequence-containing resin **2**.

This self-neutralizing effect (heterogeneous solution) is not observed with any of the other mixed solvents in Table 1, as they are homogeneous and consist either of a mixture of electron acceptor or of electron donor solvents. The heterogeneous mixtures seem to occur only when the solution is composed of strong electron acceptor and strong electron donor solvents. If the mixed solvents are, for instance, composed of medium electron acceptor (DCM and MeOH) and strong electron donor solvents (DMF, DMSO), these solutions behave as homogeneous systems, inducing the expected swelling of resins (see mixed solvents 18 to 20, Table 1).

In a series of correlated publications, Narita and coworkers have already successfully applied the electron acceptor−donor concept to explain peptide chain disrupting potentials of mixed solvents through IR spectroscopic studies of model peptide fragments.[32] The present findings are in agreement with these reports, thus emphasizing again the sensitivity and validity of this swelling approach to investigate the complex solvation phenomenon of peptide-resins.

For the first time in the peptide synthesis field, the swelling studies with TEA- or PIP-containing solutions (solvents 23−28, Table 1) were also designed to complete the solvation study of every step of the synthetic cycle, in both Boc and Fmoc strategies. Only in resin **2**, the swelling property of TEA solution in DCM is significantly lower (52%) than in DMF or DMSO (80% and 84%, respectively). This suggests the need for replacement of the former for the latter two more polar cosolvents during the neutralization step in Boc-chemistry. However, this alteration in the standardized synthesis protocol seems to be necessary only for very highly polar peptide-loaded resins.

The swelling data obtained with these mixed-alkaline solutions may again be interpreted in light of AN/DN theory. Accordingly, no strong shrinking of beads occurred in these TEA- or PIP-solutions as the two components of the mixtures always consist of either strong electron donor solvents (TEA, PIP, DMF, and DMSO) or strong electron donor and medium electron acceptor solvent (DCM).

To complete this solvation approach to the overall peptide synthesis cycle, there are the deprotection in TFA and corresponding washing steps, both routinely done in DCM as cosolvent during the Boc-chemistry synthesis strategy. Regarding the washing step, we have already demonstrated[33] that appropriate solvation of resin containing protonated amino groups (trifluoroacetate form) is strongly dependent on the peptide-content of the resin. In general, DCM is the solvent indicated for the washing step of low and medium peptide-loaded resins. However, its replacement by DMF seems to be necessary for heavily peptide-loaded resins as these resins containing protonated amino groups show a complete lack of swelling in apolar conditions.

Concerning the remaining step of the synthesis cycle (amino group deprotection in TFA), no swelling parameters can be obtained using the microscope due to the impossibility of measuring bead sizes in this acidic solution. However, personal observations in this[33] and other laboratories[34] have indicated that DCM is more swellable as cosolvent for TFA solution, irrespective of the peptide-resin employed. This finding is again explained by the electron acceptor/donor concept where the good solvation property of the TFA/DCM solution is due to its homogeneous character composed of the strongest electron acceptor solvent known so far (TFA, AN = 105 and DN = 0, Table 3) and a medium electron acceptor solvent (DCM). In this context, much weaker solvation properties might be expected from TFA/DMF or TFA/DMSO solutions, as they are heterogeneous and consist of strong electron acceptor and strong electron donor solvents.

The discussion of the present report was deliberately focused on the importance of the solvent systems for improving the solvation property of peptide-resins and, therefore, for increasing the overall yield of the synthesis. However, apart from affecting solvation of resins, one has to be always aware of additional influences of the solvent on other chemical processes involved in the synthesis cycle. A direct influence of the solvent on the mechanism of the coupling reaction is possible and occurs, for instance, in the carbodiimide-mediated coupling reaction.[2a−c] In this strategy, a much higher amount of the corresponding *N*-acyl urea formation as side product is observed when coupling is carried out in polar solvents. Also, the use of alcohols as single or mixed solvents for coupling requires, before the addition of the alcohol for solvation of the resin, the preformation of a stable activating species, preferably in apolar solvents.[35] This already employed strategy[36] is necessary since most alcohols consume the activator during the *in situ* acylation. And finally, the viscosity of the solvent may affect, as shown for other polymer-supported reactions,[37] the diffusion of reactants during the coupling and, therefore, the kinetics of the reaction. This influence was already demonstrated in a comparative swelling-kinetics of coupling approach,[7b] where in solvents which equally swell

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a resin, the faster rate of the coupling reaction was measured in the less viscous one.

Additional representative peptide-resins, which include very hydrophilic and very hydrophobic sequences with different lengths and, also, varying the peptide-loading of resins, are currently been synthesized. We think that a definitive and more comprehensive rule for solvation of peptide-resins may be obtained by investigating the correct set of model peptide-resins. Special attention will be also given to solvation studies on very long, hydrophobic, and aggregating sequences deliberately assembled in highly substituted resins. This special example of a difficult synthesis represents one of the last challenges to be overcome in the SPPS methodology, since its introduction more than three decades ago.[38]

Conclusions

We have investigated the complex solvation of peptide-resins in the light of solvent effect empirical models. In agreement with the proposed two-parameters theory, the sum of the Lewis acidity and Lewis basicity solvent properties, represented by Gutmann's electron acceptor (AN) and electron donor (DN) numbers, respectively, in 1:1 proportion, was more efficient to differentiate solvation behavior of peptide-resins than other solvent polarity parameters. However, differing from most kinetic, thermodynamic, or spectrophotometric solvent-dependent physicochemical properties of the solute, the solvation degree of peptide-resins did not correlate linearly with the sum of solvent properties of the two-parameter model. Instead, plots of swelling *versus* (AN+DN) display curves with maximum solvation regions characteristic for each class of resin which, in turn, depend on the nature and amount of resin-bound peptide sequence. Important for solid phase peptide synthesis methodology, the occurrence of the maximum solvation region in this plot facilitates the choice of the more appropriate solvent system for every step of the peptide synthesis cycle. Moreover, the present report demonstrates that only the solvent electron acceptor−donor concept allows a better understanding of the solvation/shrinking processes of peptide-resin induced by mixed solvents, irrespective of their compositions.

Experimental Section

Materials. All amino acids except Gly, are of the L-configuration. N^{α}-*tert*-butyloxycarbonyl (Boc)-Asn, -Ile, -Pro and β-benzyl (Bz) ester of Asp were purchased from Bachem, Torrance, CA. BHAR were synthesized as published[15] to obtain higher substituted resin batches. Solvents and reagents were purchased from Aldrich or Sigma Co. TEA (over ninhydrin), diisopropylethylamine (over CaH_2 and ninhydrin), and DMF (over P_2O_5 and ninhydrin under reduced pressure) were distilled before use. All solvents used for swelling studies were HPLC grade, and all chemicals met ACS standards.

Peptide Synthesis. The peptides were synthesized manually accordingly to the standard Merrifield Boc/Bz strategy.[2a−c] Briefly, the α-amino group deprotection and neutralization steps were performed in TFA, 30% (v/v) in DCM, and TEA, 10% (v/v) in DCM/DMF, respectively. The scale of synthesis was 0.2 mmol and all Boc-amino acids were coupled in DMF with BOP in presence of HOBt and DIEA (with a 4- and 5-fold excess over the amino component in the resin, respectively).[39] Boc-Asn was coupled in DCM/DMF (1:1) using diisopropylcarbodiimide and HOBt as acylating reagents (4-fold excess). To facilitate the quantitative incorporation of amino acids, the double coupling strategy with a 2-h reaction time each was used, and the qualitative ninhydrin test was performed to estimate the completeness of the reaction. Cleavage reactions were carried out with the low-high HF procedure,[40] the resin was rinsed with ethyl acetate, and the peptide was extracted in 10% (v/v) aqueous acetic acid solution and lyophilized. In addition to the expected yield for the scale employed, the purity of the crude peptides was characterized by high-voltage paper electrophoresis (pH 2.2, 4.9, and 9.9) and by HPLC. The analytical HPLC conditions were: 0.1 M NaH_2PO_4, pH 7.0 as solvent A, acetonitrile:H_2O (9:1) as solvent B; linear gradient from 5 to 50% of B in 45 min, flow rate of 3 mL/min, UV detection at 220 nm, Beckman Ultrasphere (5 μm) C-18 column, 10 × 250 mm. All crude peptides cleaved from resins were *ca.* 90% pure by analytical HPLC and mass spectra, and amino acid analyses were consistent with their theoretical peptide sequences.

Swelling Measurement of Beads. Before the use in the synthesis of peptide resins and microscopic measurement of bead sizes, the amino-protonated BHARs batches (Cl^- form) were exhaustively sized by the suspension in DCM and EtOH and sifted in pore metal sieves to lower the standard deviations of resin diameters to about 4%.[15] Swelling studies of these narrowly sized populations of beads were performed as published elsewhere,[10] with alteration only in the calculated swelling parameter. Briefly, 200 to 250 dry and swollen beads of each resin, allowed to solvate overnight, were spread over a microscope slide and measured directly at low magnification. Since the sizes in a sample of beads are not normally but log-normally distributed,[41] the central value and the distribution of the particle diameters were estimated by the more accurate geometric mean values and geometric standard deviations. All resins were measured with the amino groups in unprotonated form obtained by 3 × 5 min TEA/DCM/DMF (1:4.5:4.5) washings followed by 5 × 2 min DCM/DMF (1:1) and 5 × 2 min DCM washings. Resins were dried *in vacuo* using an Abderhalden-type apparatus and reflux in MeOH.

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Supporting Information Available: Figures showing swelling of peptide-resins as a function of solvent (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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