Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Flat bulk-solvent model: obtaining optimal parameters

Andrei Fokine and Alexandre Urzhumtsev*

LCM3B, UPRESA 7036 CNRS, Faculté des Sciences, Université Henry Poincaré, Nancy I, 54506 Vandoeuvre-lés-Nancy, France

Correspondence e-mail: sacha@lcm3b.uhp-nancy.fr

Received 6 March 2002
Accepted 10 June 2002

A bulk-solvent correction is regularly used for macromolecular refinement. The flat model of the bulk solvent is considered to be the most reliable. It is shown that the standard procedure does not always result in the optimal values of the bulk-solvent correction parameters. A method to obtain the best values for parameters $k_{\rm sol}$ and $B_{\rm sol}$ of the flat-solvent model is discussed. The values of correctly determined parameters for crystallographic structures deposited in the Protein Data Bank are clustered around $k_{\rm sol} = 0.35$ e Å^{-3} and $B_{\rm sol} = 46$ Å^2, which have a reasonable physical meaning. Such a distribution allows the use of these mean values of solvent parameters for many practical applications when refined parameters cannot be obtained, especially when an atomic model in the unit cell is not yet known.

1. Introduction

The main goal of structural crystallography is to build a molecular model that has a reasonable physical interpretation and explains experimental structure-factor magnitudes. Macromolecular crystals contain a large part of disordered solvent whose contribution to low-resolution reflections is very important; an atomic macromolecular model without the contribution of the bulk solvent cannot correctly reproduce these diffraction data. Moreover, the low-resolution data are important for the map quality (Urzhumtsev, 1991), for the refinement (Kostrewa, 1997) and for the study of electrostatic potential (Lecomte, 1999). Therefore, bulk-solvent modelling is necessary to use correctly the whole amount of diffraction data.

Currently, the bulk-solvent correction is mostly used for refinement when an atomic model of the macromolecule in the crystal is already known. Several methods allowing the calculation of structure factors of the bulk solvent have been described (see Jiang & Brünger, 1994; Badger, 1997; Urzhumtsev, 2000), with only the exponential scaling model and the flat-solvent model being widely used.

The exponential scaling model (Moews & Kretsinger, 1975; Tronrud, 1997) is based on the assumption that the structure factors of the solvent are proportional to those of the protein and have the opposite direction,

$$\mathbf{F}_{\rm sol}(\mathbf{s};\, \chi_{\rm sol}, \beta_{\rm sol}) = -\chi_{\rm sol} \exp(-\beta_{\rm sol} s^2/4)\mathbf{F}_{\rm prot}(\mathbf{s}). \qquad (1)$$

[Here and in the following **s** represents a vector in reciprocal space defined by its Miller indices (*hkl*) and the scalar $s = |\mathbf{s}|$ is its modulus.] This approximation is correct only at very low resolution, lower than 15–20 Å (Podjarny & Urzhumtsev, 1997), and therefore the exponential scaling model is handi-

capped at higher resolution. In spite of its shortcoming, this model is used in several programs owing to its simplicity.

The flat bulk-solvent model (Phillips, 1980; Jiang & Brünger, 1994) is more reliable because it is based on the more reasonable assumption that the electron density in the solvent region has a uniform distribution. In this model the binary function M, the solvent mask, is introduced, which is equal to 1 inside the solvent region and equal to 0 outside. The structure factors of the bulk solvent are calculated as the scaled Fourier coefficients of this function,

$$\mathbf{F}_{\rm sol}(\mathbf{s}; k_{\rm sol}, B_{\rm sol}) = k_{\rm sol} \exp(-B_{\rm sol}s^2/4)\mathcal{F}(M). \quad (2)$$

The parameter $k_{\rm sol}$ describes the value of electron density in the solvent region (electron density of the crystallization solution) and the resolution-dependent multiplier is introduced to blur the sharp boundary between the macromolecular and the solvent regions.

If a flat macromolecular envelope complementary to the solvent mask is introduced, the formula (2) expressed in terms of its structure factors $\mathbf{F}_{\rm env}$ becomes very similar to (1),

$$\mathbf{F}_{\rm sol}(\mathbf{s}; k_{\rm sol}, B_{\rm sol}) = -k_{\rm sol} \exp(-B_{\rm sol}s^2/4)\mathbf{F}_{\rm env}(\mathbf{s}). \quad (3)$$

However, the principal difference between these formulae is that the parameters $k_{\rm sol}$ and $B_{\rm sol}$ in (2) and (3) have a physical meaning, while this is not the case for the parameters in (1).

When the bulk-solvent structure factors are calculated, the total structure factors of the crystal are calculated as their sum with the structure factors of the ordered atoms,

$$\mathbf{F}_{\rm total}(\mathbf{s}; k_{\rm sol}, B_{\rm sol}) = \mathbf{F}_{\rm atoms}(\mathbf{s}) + \mathbf{F}_{\rm sol}(\mathbf{s}; k_{\rm sol}, B_{\rm sol}). \quad (4)$$

The parameters $k_{\rm sol}$ and $B_{\rm sol}$ of the bulk solvent are usually chosen from the best fit of $\mathbf{F}_{\rm total}$ to diffraction data, for example

$$\sum_{\mathbf{s}} [|\mathbf{F}_{\rm obs}(\mathbf{s})| - |\mathbf{F}_{\rm total}(\mathbf{s}; k, B)|]^2 \rightarrow \min_{k,B}. \quad (5)$$

The bulk-solvent correction using the flat-solvent model improves the agreement between the experimental and calculated data. However, sometimes the standard procedure included in *CNS* (Brünger *et al.*, 1998) leaves a large discrepancy between the observed and calculated reflections at very low resolution (see, for example, Kostrewa, 1997). It should be noted also that this procedure requires the knowledge of the macromolecular atomic model already placed in the unit cell.

Analysis of the parameters $k_{\rm sol}$ and $B_{\rm sol}$ for refined structures deposited in the Protein Data Bank (PDB; Bernstein *et al.*, 1977) shows that the optimal parameters are distributed over quite a small region and that the outliers generally correspond to incorrectly determined values. We found that in most of these cases the origin of the problem is simply a wrong choice of the parameters $k_{\rm sol}$ and $B_{\rm sol}$. The correction of these parameters allows reduction of the discrepancy mentioned above. The mean values of the optimal parameters found from the statistical analysis of the PDB structures have a clear physical meaning and can be used to estimate bulk-solvent structure factors when the standard procedure cannot be applied; for example, when an atomic model is not yet known.

2. Determination of the bulk-solvent parameters

2.1. Bulk-solvent correction by the standard procedure

To study the bulk-solvent correction, two macromolecular crystals with a structure resolved at atomic resolution (1.8 and 1.1 Å, respectively) have been chosen for which the complete low-resolution data sets are available: ribonuclease Sa (Sevcik *et al.*, 1991) and protein G (Derrick & Wigley, 1994). Both proteins crystallize in the space group $P2_12_12_1$; the crystals of ribonuclease Sa have unit-cell parameters $a = 64.90$, $b = 78.32$, $c = 38.79$ Å and the crystals of protein G have unit-cell parameters $a = 34.90$, $b = 40.30$, $c = 42.20$ Å.

For both these proteins, the crystallographic R factor was calculated between experimental structure-factor magnitudes and those calculated from the corresponding refined atomic

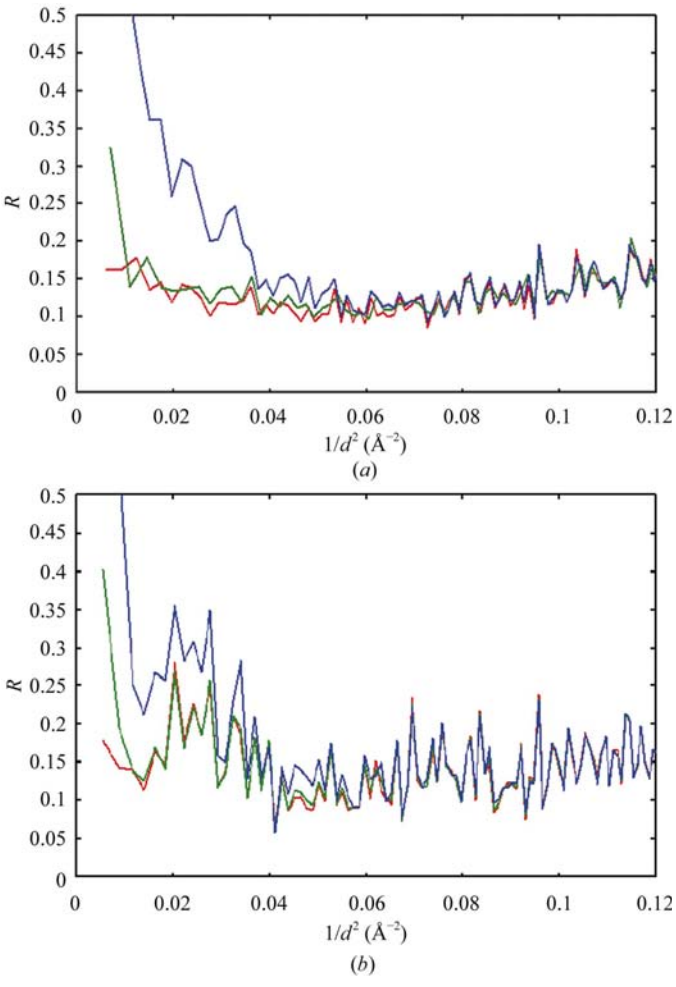

Figure 1
Dependence of the crystallographic R factor on $1/d^2$ (d is the resolution in Å) for ribonuclease Sa (*a*) and for protein G (*b*) without any bulk-solvent correction (blue curves), with bulk-solvent correction when the parameters $k_{\rm sol}$ and $B_{\rm sol}$ were determined by *CNS* (green curves) and with bulk-solvent correction when these parameters were determined by exhaustive search (red curves).

model. This criterion as a function of a resolution has a good value at high resolution, grows from the resolution of 5 Å and reaches very high values at the resolution of 10–12 Å or lower (Fig. 1), thus showing the necessity of the bulk-solvent correction. In the following, bulk-solvent correction using the flat-mask model (Jiang & Brünger, 1994) has been used since it has been shown to be of higher quality (Jiang & Brünger, 1994; Kostrewa, 1997). All calculations were performed with the program *CNS* (Brünger *et al.*, 1998) which searches for the optimal parameters by an iterative minimization procedure starting from the values $k_{sol} = 1.0$ e Å^{-3}, $B_{sol} = 0$ Å^2. Default *CNS* parameters for solvent-mask construction have been used.

The standard bulk-solvent correction (2) by *CNS* improved the agreement between the experimental and calculated data (Fig. 1) everywhere except in the very low resolution zone. Low-resolution reflections are not sensitive to errors in the atomic model and therefore the only possible explanation of this discrepancy is an imperfection of the bulk-solvent correction (we suppose that the data were measured correctly). At this low resolution, the hypothesis of the flat density distribution should be valid and the main reason for poor agreement must be incorrect choice of parameter values rather than the solvent model itself.

2.2. Physical meaning of the solvent parameters

A check of the solvent parameters k_{sol} and B_{sol} obtained from computations against their physically meaningful values was a first step in analysis of the problem. The flat-solvent model has a clear physical explanation, but this is much less true for the exponential model, where the initial hypothesis of the proportionality of structure factors from the macromolecular model and from solvent is weak.

The physical meaning of the parameter k_{sol} is very clear and has been discussed many times (see, for example, Kostrewa, 1997). This parameter corresponds to the mean value of the electron density in the solvent region and therefore depends on buffer composition. In neutron diffraction with contrast variation, this parameter can be higher than the mean density of the macromolecule. However, for X-ray analysis its value is below the mean density of a typical protein, which is 0.43 e Å^{-3}. As indicated by Kostrewa (1997), the electron density of pure water is 0.33 e Å^{-3} and the density of 4 *M* ammonium sulfate is 0.41 e Å^{-3}.

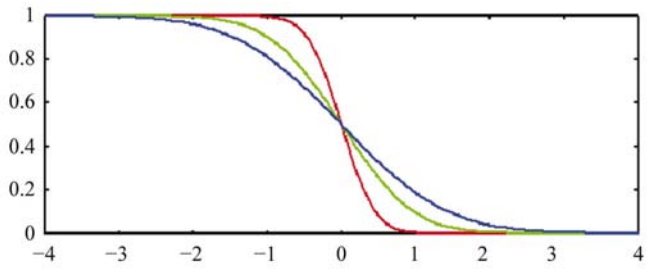

Figure 2
Convolution of the step function $f(\mathbf{r})$ with the Gaussian function $g(\mathbf{r}; B)$ (see text for details) for different B values: B = 10 (red), 46 (green), 100 Å^2 (blue).

Table 1
Correlation between solvent parameters.

The parameter k_{sol} was fixed and B_{sol} was calculated by minimization of the *CNS* target function (5) starting from $k_{sol} = 0.35$ e Å^{-3}, $B_{sol} = 50$ Å^2.

k_{sol} (e Å^{-3})	B_{sol} (Å^2)
0.10	21
0.15	26
0.20	32
0.25	38
0.30	50
0.35	78
0.40	80
0.45	127
0.50	300

The parameter B_{sol}, in contrast to k_{sol}, has been discussed much less and the range of possible values for this parameter is less clear. Several approaches have been used to understand its physical sense and therefore to estimate its limiting values.

First, the artificial situation of a flat solvent border was studied. The multiplication of the solvent-mask structure factors by a Gaussian function of the reciprocal resolution s is equivalent to convolution of this mask with the corresponding Gaussian function. Fig. 2 represents the convolution of the step function

$$f(x, y, z) = \begin{cases} 0 & \text{if } x > 0 \\ 1 & \text{otherwise} \end{cases} \quad (6)$$

with the Gaussian function

$$g(\mathbf{r}) = \exp(-|\mathbf{r}|^2/2\sigma^2)/(2\pi\sigma^2)^{1/2}, \quad (7)$$

with $\mathbf{r} = (x, y, z)$ and $B = 8\pi^2\sigma^2$. The larger B_{sol}, the deeper the electron density of the solvent penetrates into the macromolecular region; for values of B_{sol} of about 100 Å^2 and higher, the penetration distance is large in comparison with an atomic radius (Fig. 2).

At the same time, the lower limit for B_{sol} can be estimated from the mean value of the individual atomic temperature factors at the surface of the macromolecule. Analysis of several structures showed that this value is around 35–40 Å^2.

Secondly, it is clear that k_{sol} and B_{sol} are highly correlated. The analysis of this correlation has been performed using the data from ribonuclease Sa. Different values of k_{sol} have been tried and for each of them the optimal value of B_{sol} has been found from the minimization of the criterion in (5). From Table 1, it is clear that the increase in k_{sol} leads to the increase in B_{sol}. In other words, the overestimation of the solvent electron density leads to large values of B_{sol}. This is not surprising because the large B_{sol} produces a large expansion of the solvent region and thus decreases its mean density.

2.3. Analysis of the bulk-solvent parameters

A comparison of the parameters k_{sol} and B_{sol} determined for ribonuclease Sa and for protein G by the minimization procedure (Table 2) with physically reasonable estimations for these values showed a significant difference between them. In

Table 2
Bulk-solvent correction parameters for ribonuclease Sa and protein G determined by different methods.

	Ribonuclease Sa		Protein G	
Parameter	k_{sol} (e Å^{-3})	B_{sol} (Å^2)	k_{sol} (e Å^{-3})	B_{sol} (Å^2)
Standard *CNS* minimization procedure	0.45	133	0.41	116
Systematic search	0.31	55	0.30	65
CNS minimization starting from the mean values of the parameters	0.30	50	0.31	55

the case of ribonuclease Sa the value of k_{sol} is higher than that of protein and is equal to 0.45 e Å^{-3}. For protein G the corresponding value of k_{sol} is equal to 0.41 e Å^{-3}, which is also rather high. In both cases, the values for B_{sol} are also unreasonably large, being equal to 133 Å^2 for ribonuclease Sa and to 116 Å^2 for protein G.

This comparison confirmed the hypothesis that the solvent parameters should be checked first as a probable origin of a disagreement between calculated and observed structure factors at very low resolution.

3. Statistical analysis of the bulk-solvent parameters

3.1. Distribution of solvent parameters in the PDB

As the solvent parameters k_{sol} and B_{sol} can be estimated incorrectly using the standard *CNS* procedure, as demonstrated above, we analyzed the values of these parameters for the atomic models deposited in the Protein Data Bank (Bernstein *et al.*, 1977). The corresponding models have been selected using the software provided (3DB Browser; http://pdb-browsers.ebi.ac.uk/pdb-bin/pdbmain). From a total of 13 668 crystallographic structures deposited at the time of this study, 3120 contained search strings 'ksol' and 'bsol'; 1162 of these structures were refined using the flat model for the bulk solvent (corresponding PDB headers contained the string 'flat model' describing the method used for bulk-solvent modelling).

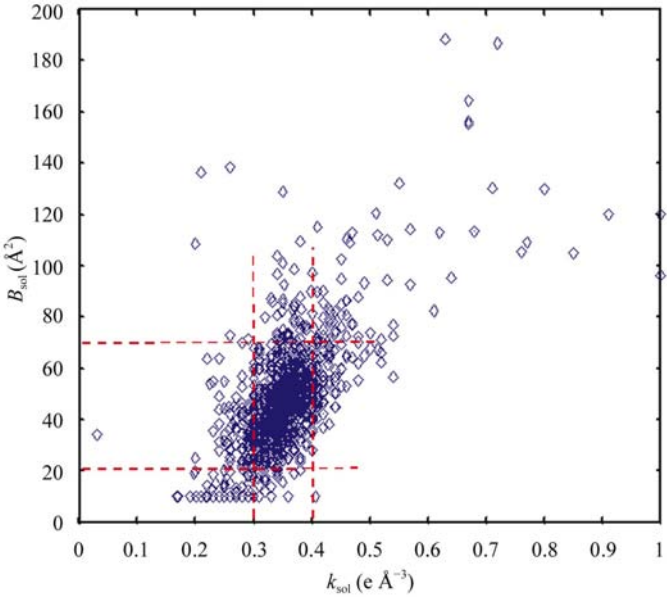

Figure 3
Distribution of values of parameters k_{sol} and B_{sol} of the flat model for the refined structures deposited in the PDB. Each rhomb corresponds to one structure.

Table 3
Some PDB structures with unreasonable solvent parameters.

The resolution range used previously to determine k_{sol} (PDB) and B_{sol} (PDB) is indicated. Columns k_{sol} (correct value) and B_{sol} (correct value) contain the correct values of parameters obtained using all data available in the PDB.

PDB ID	Resolution range, PDB (Å)	k_{sol} (PDB) (e Å^{-3})	B_{sol} (PDB) (Å^2)	k_{sol} (correct value) (e Å^{-3})	B_{sol} (correct value) (Å^2)
1b59	6.0–1.8	0.76	Not reported	0.38	67
1b6a	6.0–1.6	0.75	Not reported	0.38	56
1ev5	6.0–1.7	0.85	105	0.37	51
1ev8	6.0–2.6	1.11	137	0.36	43
1evf	6.0–1.7	0.91	120	0.39	56
1hw3	6.0–2.0	0.77	109	0.38	53
1hw4	6.0–2.1	0.76	106	0.39	55
4prg	10.0–2.9	0.69	300	0.34	87

For most of the structures (791 of 1162) the solvent parameters have reasonable physical values: parameter k_{sol} varies between 0.3 and 0.4 e Å^{-3} and B_{sol} varies in the interval 20–70 Å^2 (Fig. 3). The dispersion of the distribution of these parameters around their mean values, k^*_{sol} = 0.35 e Å^{-3} and B^*_{sol} = 46 Å^2, is rather small: 0.03 e Å^{-3} and 17 Å^2, respectively (the statistic was calculated for the models with $0 < k_{sol} < 0.6$ e Å^{-3} and $0 < B_{sol} < 100$ Å^2). This distribution is different from that obtained for the exponential scaling model (Glykos & Kokkinidis, 2000). In that case, the range of values of the adjustable parameters χ_{sol} and β_{sol} is very large and values of χ_{sol} do not correlate well with their assumed physical meaning (the ratio of the solvent electron density to the average density in the protein region). This is not surprising as the basic assumption of the exponential scaling model is not always held.

We analyzed some structures with completely unreasonable solvent parameters for which the diffraction data were available in the PDB (Table 3) and found that in all cases their solvent parameters were incorrectly determined. A typical error was that the authors excluded the low-resolution reflections during the calculation of the solvent parameters. When we repeated the search including these low-resolution data, we found much more realistic values for the bulk-solvent parameters (Table 3). For some other crystals such as ribonuclease Sa and protein G the standard *CNS* procedure simply failed to find the correct solution. In any case, all outliers for which we rechecked the solvent parameters were caused by errors.

It should also be noted that Fig. 3 shows clearly the correlation between the parameters k_{sol} and B_{sol}. Large values of k_{sol} usually appear with large values of B_{sol}.

Table 4
Dependence of the optimal solvent parameters on the low-resolution cutoff limit for ribonuclease Sa.

Reflections with resolution lower than limit indicated in the first column were excluded from the calculation of the solvent parameters. The parameters were calculated by the *CNS* minimization procedure starting from the mean values k_{sol} = 0.35 e Å^{-3} and B_{sol} = 50 Å^2. Compare with the data in Table 1.

Low-resolution limit (Å)	k_{sol} (e Å^{-3})	B_{sol} (Å^2)	No. of reflections
∞	0.30	49	17210
25.0	0.33	59	17200
20.0	0.33	59	17191
15.0	0.34	61	17164
12.0	0.35	65	17123
10.0	0.34	61	17073
9.0	0.34	61	17020
8.0	0.35	64	16953
7.5	0.36	65	16897
7.0	0.38	69	16828
6.5	0.39	70	16748
6.0	0.45	76	16626
5.5	0.49	79	16462
5.0	0.60	91	16233

3.2. Systematic search for the bulk-solvent parameters

As an alternative to the *CNS* minimization procedure, solvent parameters were found from the minimum of (5) by exhaustive search. In the cases of ribonuclease Sa and protein G, this search gave the optimal values of the parameters k_{sol} and B_{sol} with a much more reasonable physical meaning (Table 2). The bulk-solvent correction with parameters determined by exhaustive search is much better (Fig. 1). Moreover, the discrepancy between calculated and experimental data for very low resolution reflections decreased to the same level as for other resolution shells.

This study shows that sometimes the standard procedure of the search for the solvent parameters by iterative minimization of the criterion (4) can fail to find the correct values. The universal approach of the exhaustive search can find the optimal values in such cases.

3.3. Minimization starting from the mean values of the solvent parameters

Another reason why the *CNS* procedure can fail even with the complete data set, as was the case for ribonuclease Sa and protein G, may be that the starting search values are too far from the solution. As mentioned above, *CNS* searches for the solvent parameters by a local minimization which starts from the values k_{sol} = 1.0 e Å^{-3}, B_{sol} = 0 Å^2 (Brünger *et al.*, 1998). We supposed that the procedure can converge more rapidly and to the correct values if it starts from the mean values of the solvent parameters. Indeed, in all test cases (Table 2), including ribonuclease Sa and protein G, minimization starting from k_{sol} = 0.35 e Å^{-3} and B_{sol} = 50 Å^2 gives practically the same results as that obtained by the exhaustive search.

3.4. Solvent parameters and quality of data set

It has already been noted that the determination of the optimal solvent parameter requires low-resolution data because at high resolution the bulk-solvent contribution to diffraction data is quite weak and does not allow unambiguous definition of these parameters. However, if some reflections from the lowest resolution shells are absent, as usually happens in the X-ray diffraction experiment, this does not greatly influence the determined k_{sol} and B_{sol} values. For example, for ribonuclease Sa the values of parameters are still close to the correct values when all reflections lower than 10.0 Å are excluded (Table 4).

Table 5
Dependence of the optimal solvent parameters on the systematic absence of data.

The experimental data of ribonuclease Sa were used.

Reflections excluded	k_{sol} (e Å^{-3})	B_{sol} (Å^2)	No. of reflections	
			Excluded	Used
$h = 0$	0.31	54	651	16559
$h = 1$	0.30	50	709	16501
$h = 2$	0.30	50	712	16498
$k = 0$	0.30	50	542	16668
$l = 0$	0.31	54	1088	16122
$h = 0$ or $k = 0$ or $l = 0$	0.32	60	2237	14973
$l^2 < h^2 + k^2$	0.30	47	2072	15138
$k^2 < h^2 + l^2$	0.30	54	7728	9482
$h^2 < k^2 + l^2$	0.30	48	5636	11574

Table 6
Dependence of the solvent parameters on errors in the experimental modulus.

Errors were distributed randomly and uniformly in the interval $(-\delta F_{obs}, \delta F_{obs})$. The experimental data of ribonuclease Sa were used.

δ	k_{sol} (e Å^{-3})	B_{sol} (Å^2)
0.05	0.30	49
0.10	0.30	48
0.15	0.30	47
0.20	0.30	47
0.25	0.30	47
0.40	0.30	46
0.50	0.29	37
0.75	0.29	44
1.00	0.28	29

The systematic absence of data (reflections belonging to reciprocal-space planes or cones) also does not change the values of the solvent parameters (Table 5). The parameter values are not sensitive to random errors in the observed structure factors (Table 6) or to the step of the grid on which the solvent mask is calculated (Table 7).

4. Conclusions

The distribution of values of the bulk-solvent parameters k_{sol} and B_{sol} for crystallographic structures deposited in the PDB shows their tight clustering near reasonable physical values of the parameters k^*_{sol} = 0.35 e Å^{-3} and B^*_{sol} = 46 Å^2. Sometimes, the standard *CNS* procedure cannot find the optimal values for the bulk-solvent correction parameters k_{sol} and B_{sol} and leads to unreasonable physical values. A check of some

Table 7
Dependence of the optimal solvent parameters on the step of the grid used for the solvent-mask calculation.

The experimental data of ribonuclease Sa were used.

Grid step (Å)	k_{sol} (e Å^{-3})	B_{sol} (Å^2)
0.20	0.31	73
0.33	0.30	66
0.60	0.30	65
0.67	0.30	51
1.00	0.30	51
1.33	0.31	72
2.00	0.31	120
2.67	0.32	192
3.33	0.32	300
6.67	0.18	300

outliers confirmed that the reported parameters were incorrectly determined.

The procedure which searches for the solvent parameters through the local minimization can be improved if the default start values are replaced by the mean values indicated above. Otherwise, the optimal values can be found by exhaustive search. Data incompleteness, errors in observed structure factors and the grid step of the solvent mask do not greatly influence the result.

The existing procedures for bulk-solvent correction determine the optimal values of the solvent parameters of the flat-solvent model only when an atomic model is already placed in the unit cell. However, such a standard procedure cannot always be applied to obtain the optimal values of the parameters; for example, this happens when the atomic model is not yet known but only its envelope is placed in the unit cell or when the position of the model in the unit cell is unknown. For such situations, the clustering of the parameters around k^*_{sol} and B^*_{sol} suggests the use of these mean values as an approximation to the optimal values. This approach has already successfully been used for the bulk-solvent correction in the translation search in molecular replacement (Fokine & Urzhumtsev, 2002) and for improvement of electron-density maps by subtraction of bulk-solvent contribution (Fokine & Urzhumtsev, 2001).

The work was performed in the frame of the 'Pôle Intellegence Logiciels CPER-Lorraine' and in collaboration with CCH, Nancy and was partially supported by the grant from 'Region Lorraine' to AF. The authors thank C. Lecomte for his support of the project and L. Torlay for computing assistance. A referee's help allowed essential improvement of the manuscript.

References

Badger, J. (1997). *Methods Enzymol.* **277**, 344–352.
Bernstein, F. C., Koetzle, T. F., Williams, G. J., Meyer, E. F. Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535–542.
Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.
Derrick, J. P. & Wigley, D. B. (1994). *J. Mol. Biol.* **243**, 906–918.
Fokine, A. & Urzhumtsev, A. (2001). *CCP4 Newsl.* **39**, 71–78.
Fokine, A. & Urzhumtsev, A. (2002). *Acta Cryst.* A**58**, 72–74.
Glykos, N. M. & Kokkinidis, M. (2000). *Acta Cryst.* D**56**, 1070–1072.
Jiang, J.-S. & Brünger, A. T. (1994). *J. Mol. Biol.* **243**, 100–115.
Kostrewa, D. (1997). *CCP4 Newsl.* **34**, 9–22.
Lecomte, C. (1999). *Implications of Molecular and Materials Structure for New Technologies, NATO ASI and Euroconference, Serie E: Applied Science*, pp. 23–44. Dordrecht: Kluwer.
Moews, P. C. & Kretsinger, R. H. (1975). *J. Mol. Biol.* **91**, 201–228.
Phillips, S. E. V. (1980). *J. Mol. Biol.* **142**, 531–554.
Podjarny, A. D. & Urzhumtsev, A.G. (1997). *Methods Enzymol.* **276**, 641–658.
Sevcik, J., Dodson, E. J. & Dodson, G. G. (1991). *Acta Cryst.* B**47**, 240–253.
Tronrud, D. E. (1997). *Methods Enzymol.* **277**, 306–319.
Urzhumtsev, A. (1991). *Acta Cryst.* A**47**, 794–801.
Urzhumtsev, A. (2000). *CCP4 Newsl.* **38**, 38–49.