Supplementary Material

A flow-through strategy using supported ionic liquids for L-asparaginase purification

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The axial points are encoded at a distance α from the central point calculated according to Eq. (S1).

$$\alpha = (2^k)^{\frac{1}{4}} \text{ (S1)}$$

To analyze the operating conditions and identify the most significant parameter in ASNase purification, response surface methodology (RSM) was applied. In a 2^k RSM, there are k factors that contribute to a distinct response and data is treated according to the second-order polynomial Eq. (S2).

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j \text{ (S2)}$$

where y is the response variable, β_0, β_i, β_{ii} and β_{ij} are the adjusted coefficients for the intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j are independent variables.

Table S1 - 2^2 factorial planning.

Experiment	X_1	X_2
1	-1	-1
2	1	-1
3	-1	1
4	1	1
5	-1.41	0
6	1.41	0
7	0	-1.41
8	0	1.41
9	0	0
10	0	0
11	0	0

Table S2 - Independent variables coded levels used in factorial planning for ASNase purification using [Si][N_{3114}]Cl.

Independent variables	Axial point −1.41	Factorial point −1	Coded levels Central point 0	Factorial point 1	Axial point 1.41
pH	2.9	3.5	5.0	6.5	7.1
S/L R	5.05	6.50	10.00	13.50	14.95

§ Abbreviations: solid/liquid ratio (S/L R).

Table S3 - Experimental data and response surface predicted values of the factorial planning for ASNase purification regarding purification factor using [Si][N_{3114}]Cl.

	pH	S/L R	Exp.	Pred.	RE (%)
1	3.5	6.5	3.28	3.03	8.2
2	6.5	6.5	1.51	1.44	4.5
3	3.5	13.5	3.54	3.22	9.9
4	6.5	13.5	2.27	2.13	6.4
5	2.9	10.0	3.26	3.59	-9.1
6	7.1	10.0	1.65	1.71	-3.8
7	5.0	5.0	1.80	1.94	-7.1
8	5.0	15.0	2.33	2.57	-9.2
9	5.0	10.0	2.33	2.22	5.1
10	5.0	10.0	2.22	2.22	0.0
11	5.0	10.0	2.11	2.22	-5.0

§ Abbreviations: solid/liquid ratio (S/L R), experimental data (Exp.), response surface predicted values (Pred.), relative error (RE).

Table S4 - Regression coefficients of the predicted second-order polynomial model for purification factor of ASNase from factorial planning using [Si][N_{3114}]Cl. R^2: 0.915; Adj: 0.830.

	Reg. Coef.	St. Dev.	t-student (5)	p-value
Interception	7.574	2.226	3.403	0.019
pH	-1.654	0.603	-2.744	0.041
pH^2	0.097	0.054	1.813	0.130
S/L R	-0.084	0.235	-0.356	0.736
S/L R^2	0.001	0.010	0.148	0.888
pH x S/L R	0.024	0.027	0.881	0.418

§ Abbreviations: solid/liquid ratio (S/L R), regression coefficients (Reg. Coef), standard deviation (St. Dev.).

Table S5 - ANOVA results for purification factor of ASNase from factorial planning using [Si][N_{3114}]Cl.

	Sum of squares	Degrees of freedom	Mean square	F-value	p-value
Regression	4.272	5	0.854	10.770	0.010
Residuals	0.397	5	0.079		
Total	4.669				

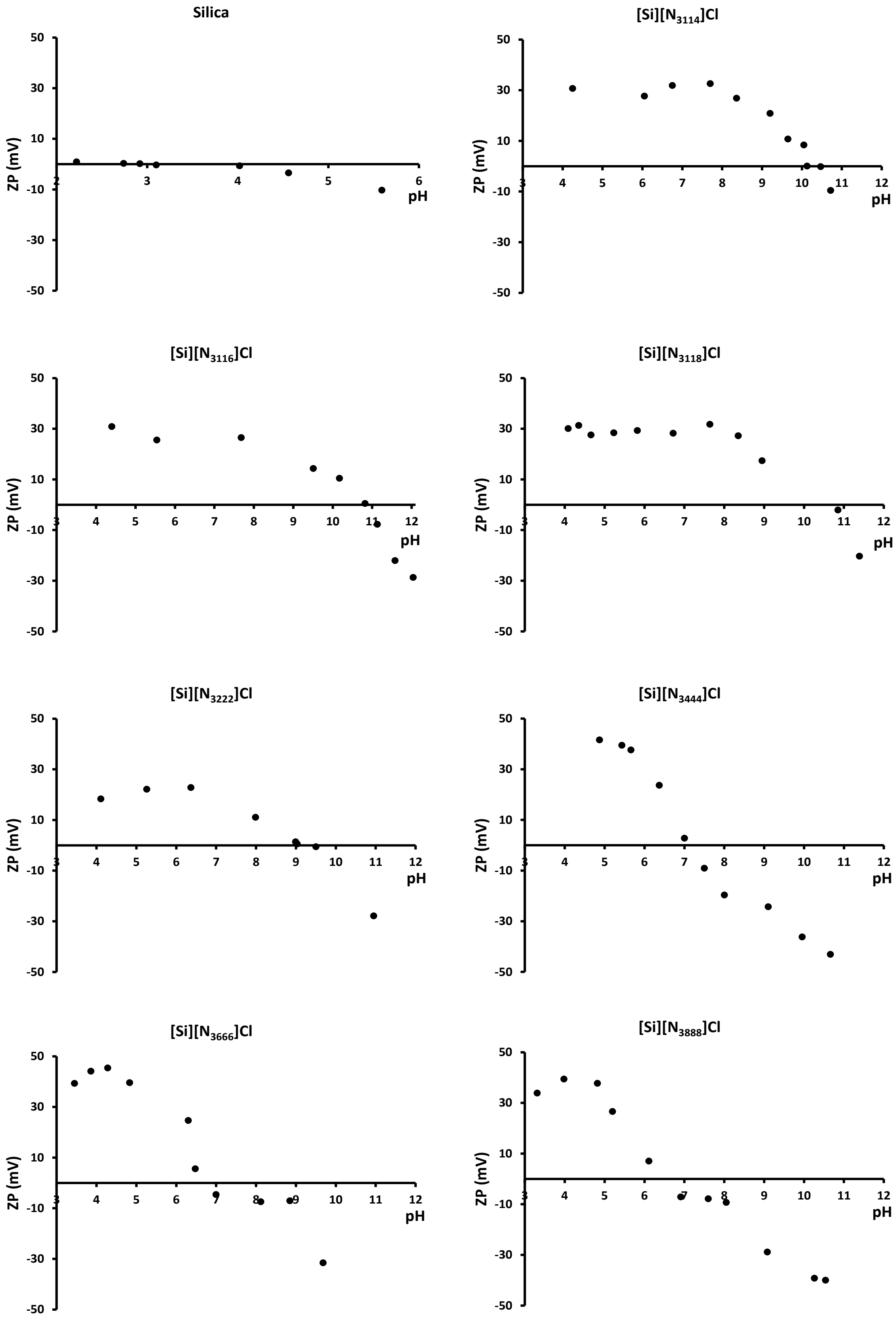

Fig. S1. Zeta potential (ZP) of activated silica and all SSILLP materials ([Si][N_{3114}]Cl, [Si][N_{3116}]Cl, [Si][N_{3118}]Cl, [Si][N_{3222}]Cl, [Si][N_{3444}]Cl, [Si][N_{3666}]Cl, and [Si][N_{3888}]Cl) as a function of pH.

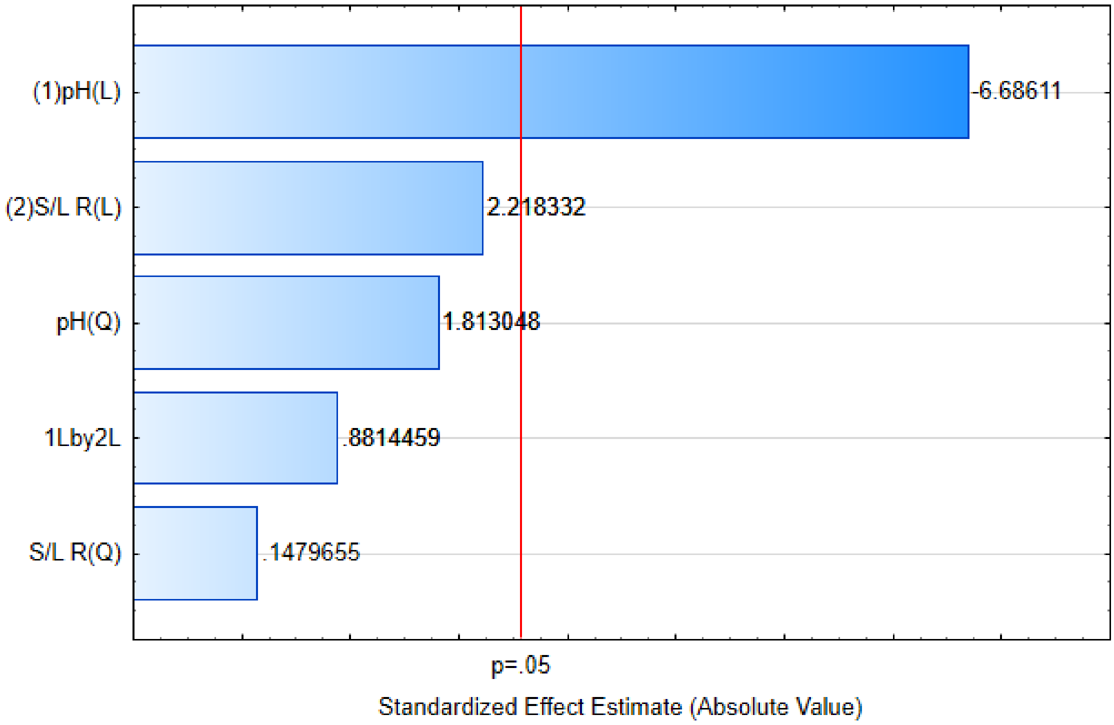

Fig. S2. Pareto charts for the standardized main effects in the factorial planning using [Si][N_{3114}]Cl for ASNase purification regarding purification factor. Vertical line indicates the statistical significance of the effects.

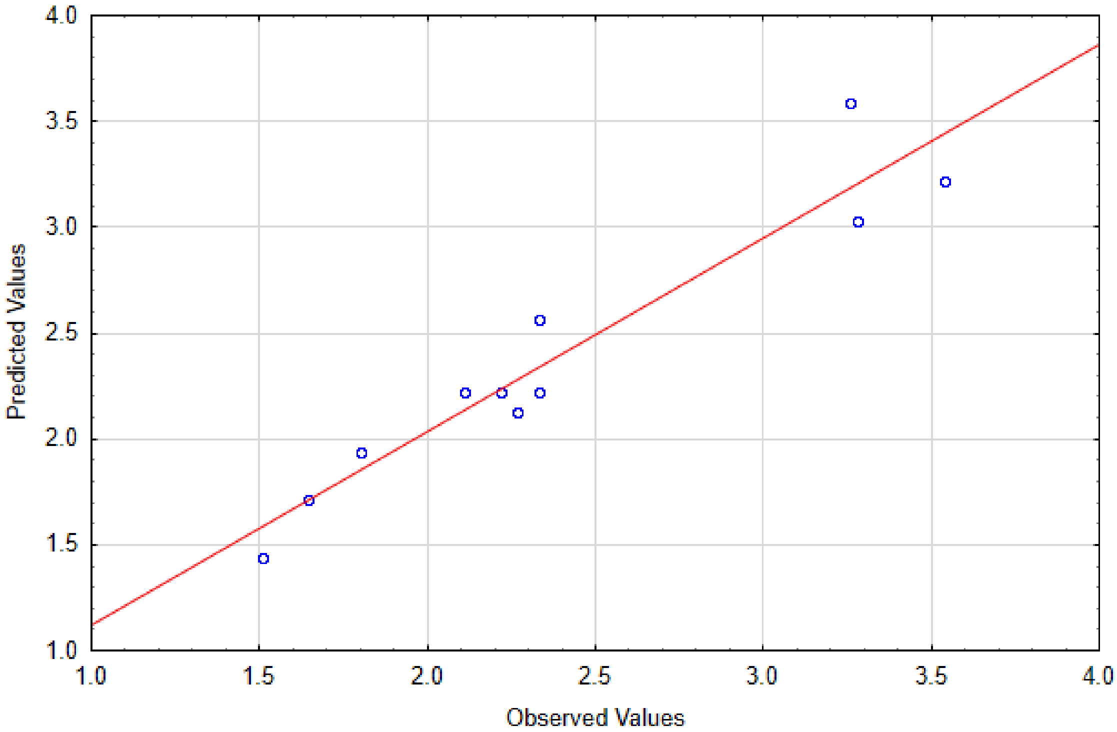

Fig. S3. Observed values vs Predicted values in the factorial planning using [Si][N_{3114}]Cl for ASNase purification regarding purification factor.

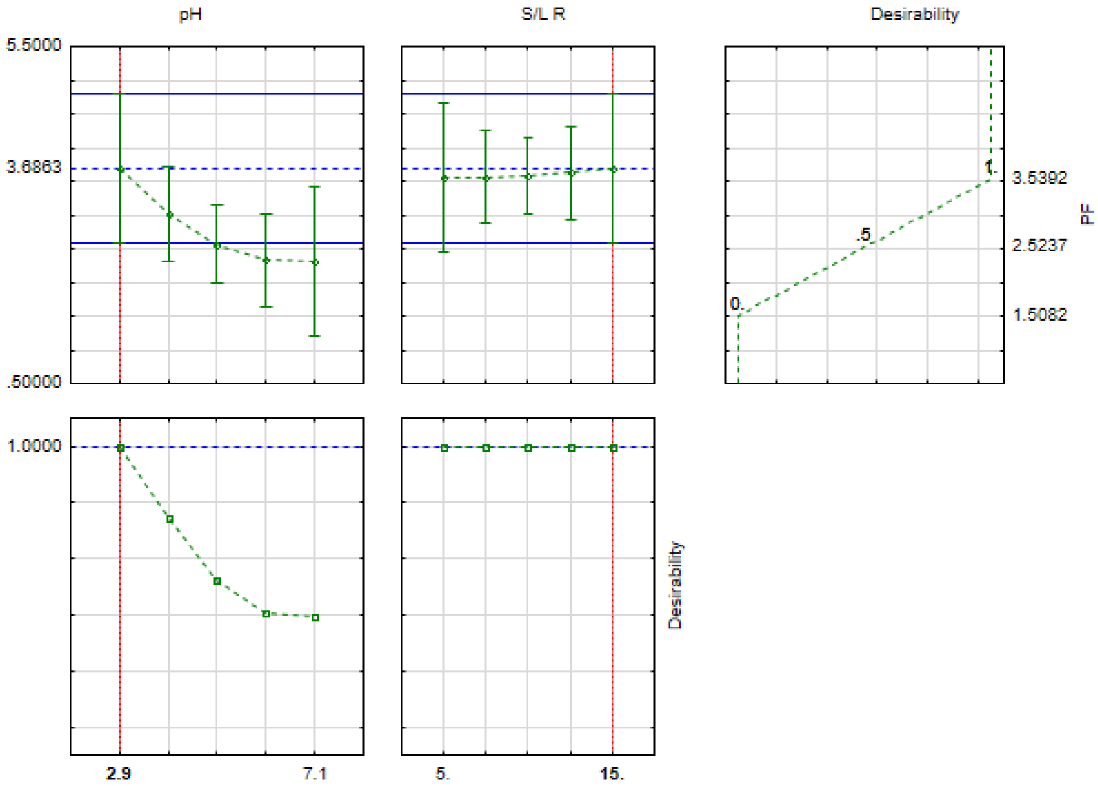

Fig. S4. Profile of prediction of the optimal operating conditions to obtain the maximum ASNase purification using [Si][N_{3114}]Cl regarding purification factor.

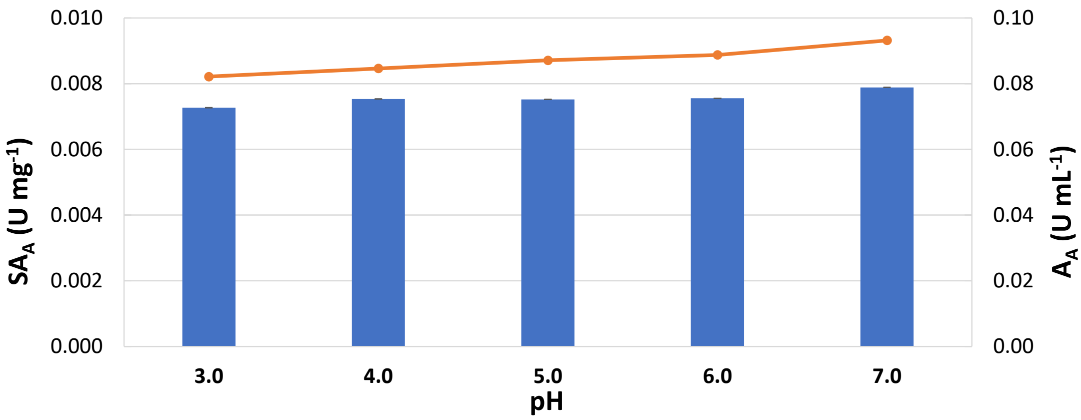

Fig. S5. ASNase activity (A_A, orange line) and specific activity of ASNase (SA_A, blue bars) of the initial enzymatic extract at different pH values (pH 3.0, 4.0, 5.0, 6.0, and 7.0).