Two-dimensional system optimizer

A software for optimization of two-dimensional HPLC systems using a Pareto-optimization strategy

Version: 1.2

Developer: Dr. Gabriel Vivó-Truyols
Tecnometrix
Amsterdam, NL

CONTENTS

1. Installation Instructions

To install the software, double-click on the TwoDimensionalSO_pkg.exe. It will automatically install the MCR component and extract the AroHPLC.exe. To start up the software, double click on TwoDimensionalSO.exe. Once the MCR component has been installed in a certain computer, it doesn't need to be installed again. This means that more advanced versions of this software can be installed just updating the TwoDimensionalSO.exe file.

2. Aim of the Software

Comprehensive two-dimensional chromatography consists of the separation of a sample according to two retention mechanisms in order to obtain higher peak capacities. In on-line, column mode, the sample is separated in the two dimensions in the following way: fractions of the sample eluted from the first-dimension column are injected in the second dimension. In order to preserve the separation achieved in the first dimension, the second dimension separation should be extremely fast, compared to the elution speed of the first dimension. In a recent publication[1], it was demonstrated that the existence of a two-dimensional system hampers the peak capacity in both separation dimensions. On one hand, the first-dimension peak capacity gets worsened due to the low sampling frequency that the second-dimension separation provides. On the other, the second-dimension separation gets worsened due to the (relatively) high injection band-broadening effects. As a result, the selection of the optimal parameters (column lengths, particle sizes, flow rates modulation times, etc) is particularly challenging, as lots of effects should be taken into account. This software is intended to help the user to optimize the chromatographic parameters in this type of chromatography. The user is referred to the reference below ([1]) for details about the mathematical treatment involved.

3. Description Of The Main Panel

Once a file is loaded (see section 4 for more information on how to load files), the main panel of the software looks like the following:

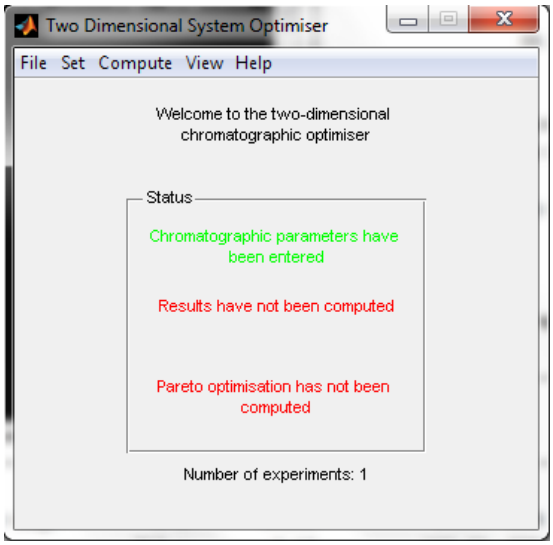


FIGURE 1: Main panel of the Aro-HPLC software

[1] G. Vivó-Truyols, S. van der Wal, P.J. Schoenmakers, Anal. Chem. 82 (2010) 8525–8536

The status panel informs about the operations that have been performed and the data that is present or absent.

4. File Management

The data produced by the current software can be saved (and later loaded) for revision and/or re-computing. All the information (including parameters, equations, results, and Pareto-optimal experiments) contained is stored in a single file (*.cso extension). To save this type of file, click on "File" >> "Save". To load this type of file, click on "File" >> "Load".

All the results from the analysis can be exported to Excel. The Excel table created contains information about the chromatographic parameters (column lengths, particle sizes, flow rates, etc.). Each row in the Excel table correspond to a chromatographic case. To export the data, click on "File" >> "Export to Excel". Two options are available: "Export all experiments" or "Export only Pareto experiments". For the first option, the chromatographic parameters for all experiments are exported to Excel. For the second option, only the Pareto-optimal experiments are exported. This second option is beneficial when lots of combinations of parameters are calculated (it can arrive to two million), as exporting all experiments would create a too large file.

5. Setting the Chromatographic Parameters

To define the chromatographic parameters, click on "Set" >> "Chromatographic Parameters". The following table appears:

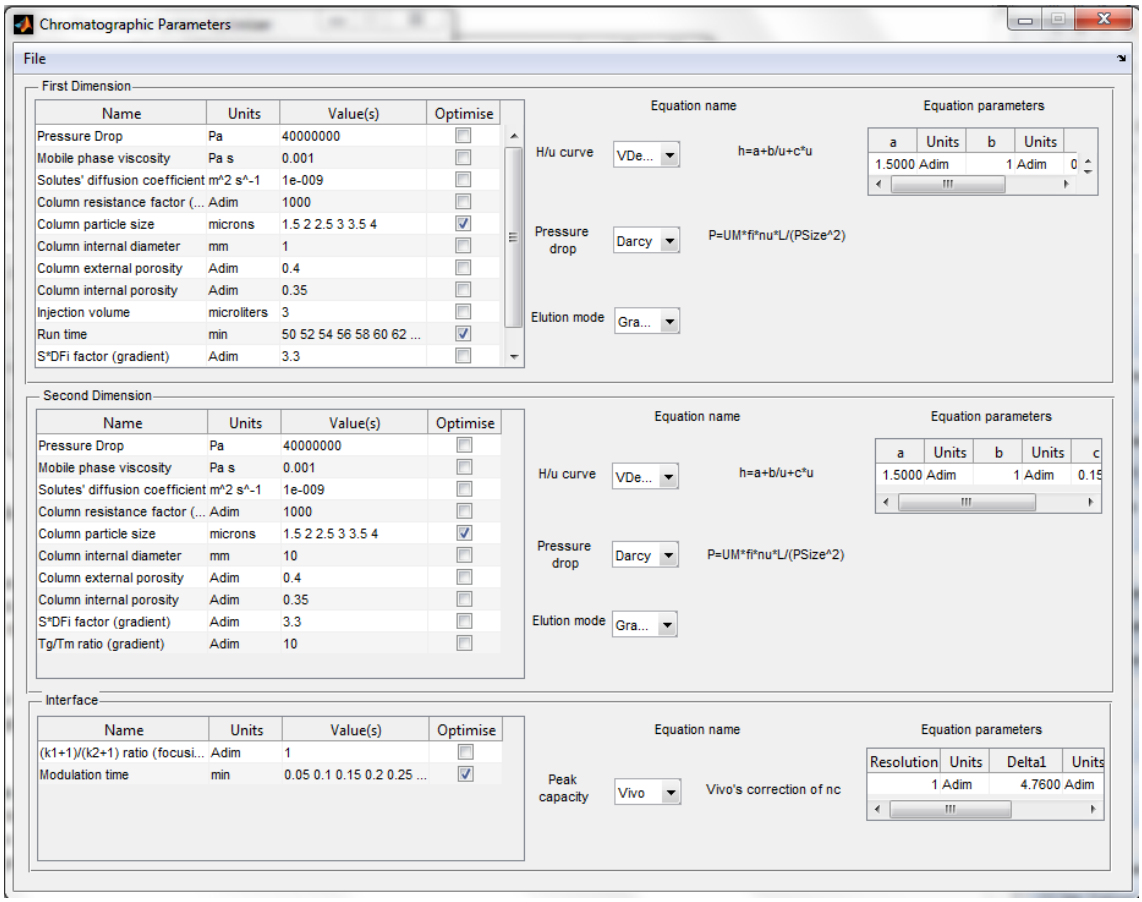


FIGURE 2: Chromatographic parameters

The top panels contain information about the first-dimension parameters –left- and equations –right-. The middle panels contain information about the second-dimension parameters –left- and equations –right-. The bottom panel contains information about the interface between the two dimensions (left: focusing factors and modulation times), as well as the equations used to calculate peak capacity (right). To consider a factor for the optimization, write several values in a parameter in the left tables. For example, if the user wants to optimize the particle size in the first dimension, (s)he should write the collection of values that are considered available (e.g., "2, 3, 4, 5 microns"). Write the different values separated by a space. If the user wants to consider a large set of equally-spaced parameters (e.g., run times from 50 to 180 min, stepped by 5 min), it is possible to write the numbers in the following way: "50:5:180". In this example, the software automatically will create the following set of data: 50, 55, 60, 65, …, 180. Note that, when a parameter contains several values (and therefore it is considered for optimization), the check-box "Optimise" is selected.

Select the different equations (and equation parameters) on the right side. For each dimension, the user should select the plate-height equation ("Van Deemter" or "Knox"), the pressure-drop equation (only Darcy equation is available in this software) and the elution mode ("Isocratic" or "Gradient"). Note that some parameters on the left panels may change depending on the elution mode.

Three options are available for the equations used in peak capacity: "Giddings", "Tanaka" and "Vivo". Giddings equation refers to the theoretical equation of peak capacity, without considering any worsening of the first or the second dimension. "Tanaka" equation refers to the equation published elsewhere[2], where the worsening in the peak capacity in the first-dimension is accounted due to the low sampling frequency. "Vivo" equation refers to the equation published[1], in which both the worsening in the first dimension (due to low sampling frequency) and in the second (due to injection band broadening) are taken into account.

Once the chromatographic parameters are decided, click on "File" >> "Ok & return" to return to the main panel.

6. Setting the Objectives

Click on "Set" >> "Pareto optimization parameters" to select which objectives does the user want to optimize. Two or three objectives can be selected. Typical objectives are total peak capacity (maximized), total time (minimized) and total dilution (minimized).

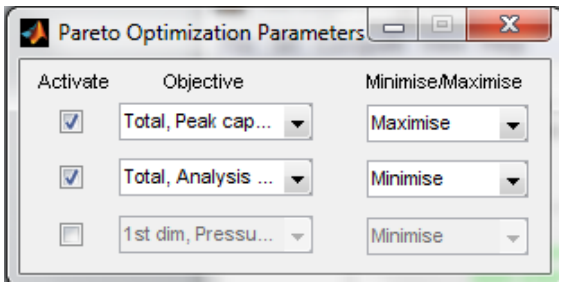


FIGURE 3: Pareto Optimization Parameters window

[2] K. Horie, H. Kimura, T. Ikegami, A. Iwatsuka, N. Saad, O. Fiehm, N. Tanaka, Anal. Chem. 79 (2007), 3764–3770.

7. Computations

Once the parameters and the objectives have been set, click on “Compute” >> “Chromatographic values” to compute all the chromatographic values (e.g., column lengths, flow rates, dilution factors, peak capacities, etc.) for all combination of parameters that have been defined in section 5.

8. Calculating the Pareto-optimal experiments

The user should note that the optimization problem that we are facing is a case of multi-objective optimization. Indeed, we aim to several objectives (e.g., maximum peak capacity and minimum time). One way to optimize a system with more than one objective is the Pareto-optimal approach. With this approach, the Pareto-optimal configurations are distinguished from the rest of the configurations and create the so-called “Pareto front”. A configuration (or experiment) is Pareto-optimal if it is impossible to improve one of the objectives without worsening the other(s). In our example, suppose that we want to maximize the total peak capacity and minimize the analysis time. A certain chromatographic combination of column lengths, particle sizes etc. would be Pareto-optimal if it is impossible to find another configuration that increases the peak capacity without decreasing the analysis time. In other words, the Pareto-optimal experiments represent the limit of our possibilities.

Once all the chromatographic values are computed, click on “Compute” >> “Pareto…” >> “Pareto optimization” to flag out which of all these chromatographic conditions are pareto-optimal according to the objectives selected in section 6. See section 10 for the computation of the Smart Pareto.

9. Visualizing the results

Once the Pareto-optimal cases have been calculated, click on “View” >> “Multiple Experiments (Pareto)” to inspect the Pareto-optimal cases.

The Pareto front is plotted in the upper panel. The color code in which the line of the Pareto front is depicted corresponds to the optimal values of the parameter selected in the drop-down list in the upper-right corner, following the color code depicted in the top of the plot. For example, in Figure 4 the “First-dimension column particle size” has been selected. In this case, the color of the Pareto front is blue at low peak capacities, indicating that the optimal particle size for the experiments at low peak capacity is around 2.5 microns (see the color scale at the top). However, the color of the Pareto front is red at high peak capacities, indicating that the optimal particle sizes are around 3.5 microns for these experiments.

The panel at the bottom describes the same information as the color code of the Pareto front. It depicts the values of the optimal parameters along the Pareto front. In the example depicted in the figure, it indicates that the optimal particle sizes in the first dimension are 2.5 for lower peak capacities (and faster analysis times), and around 3.5 for higher peak capacities. If the user wants to inspect the variation of another parameter along the Pareto front, this should be selected in the drop-down list of the upper-right corner.

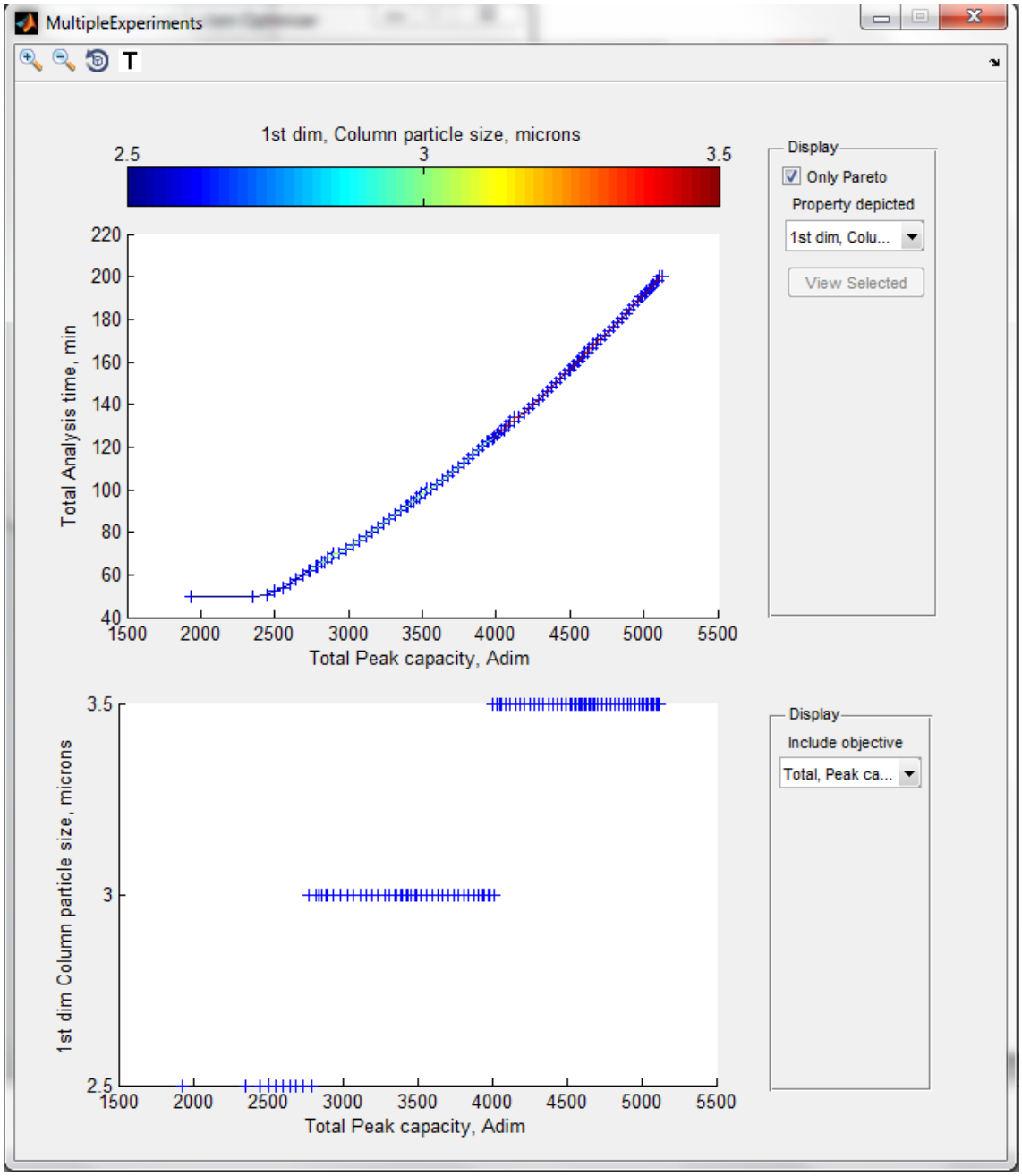
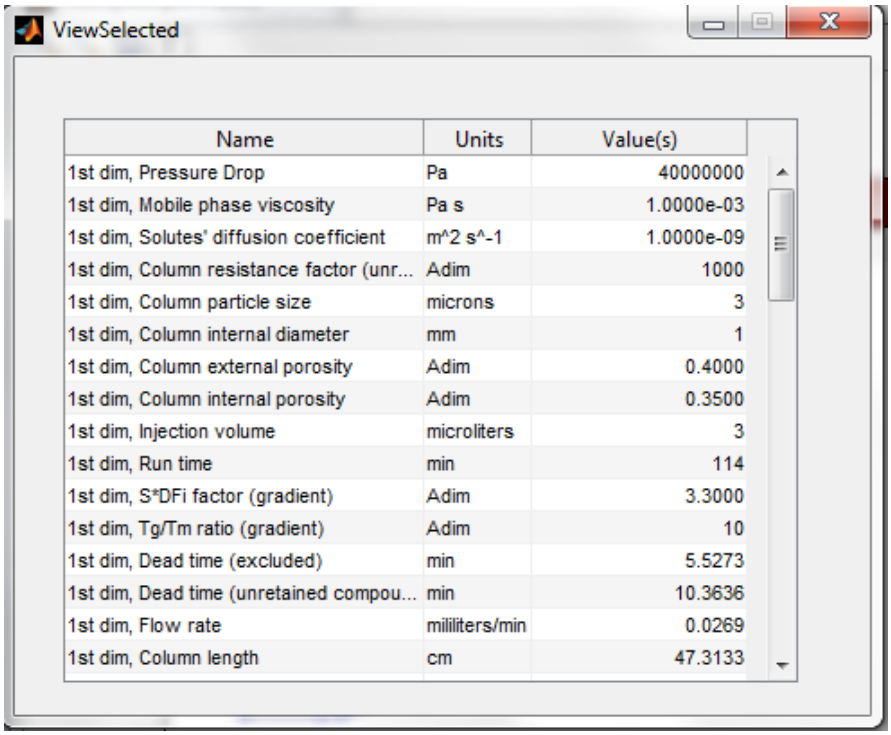


FIGURE 4: View panel.

In order to view all the experiments (and not only the Pareto-optimal experiments), de-select the check-box “Only Pareto” in the “View” upper-right pane.

Select the “T” tool (upper left corner) to inspect all the parameters defining a certain experiment. With the “T” pressed, click with the mouse to any experiment of the top panel (the selected experiment changes its color). Then click on the button “View Selected”. A table with all the chromatographic parameters appears:



Name	Units	Value(s)
1st dim, Pressure Drop	Pa	40000000
1st dim, Mobile phase viscosity	Pa s	1.0000e-03
1st dim, Solutes' diffusion coefficient	m^2 s^-1	1.0000e-09
1st dim, Column resistance factor (unr...	Adim	1000
1st dim, Column particle size	microns	3
1st dim, Column internal diameter	mm	1
1st dim, Column external porosity	Adim	0.4000
1st dim, Column internal porosity	Adim	0.3500
1st dim, Injection volume	microliters	3
1st dim, Run time	min	114
1st dim, S*DFi factor (gradient)	Adim	3.3000
1st dim, Tg/Tm ratio (gradient)	Adim	10
1st dim, Dead time (excluded)	min	5.5273
1st dim, Dead time (unretained compou...	min	10.3636
1st dim, Flow rate	milliliters/min	0.0269
1st dim, Column length	cm	47.3133

FIGURE 5: Chromatographic parameters of the selected experiments.

10.The “Smart-Pareto” Algorithm

In some cases, the computations could be too large and time consuming. Also, in certain cases, the number of possible combinations is too large to be handled by the RAM memory of the computer. In order to avoid these problems, massive optimization can be performed setting only the limits (maximum and minimum) of the parameters that should be optimized. Clicking on “Compute” >> “Pareto…” >> “Smart Pareto” (selecting a reasonable number of pareto experiments, usually 1000 is sufficient) to follow this option. In this case, a less coarse Pareto front is obtained (compare Figure 4 with Figure 6), and the optimal values for each parameters can be inspected in a nearly-continuous way.

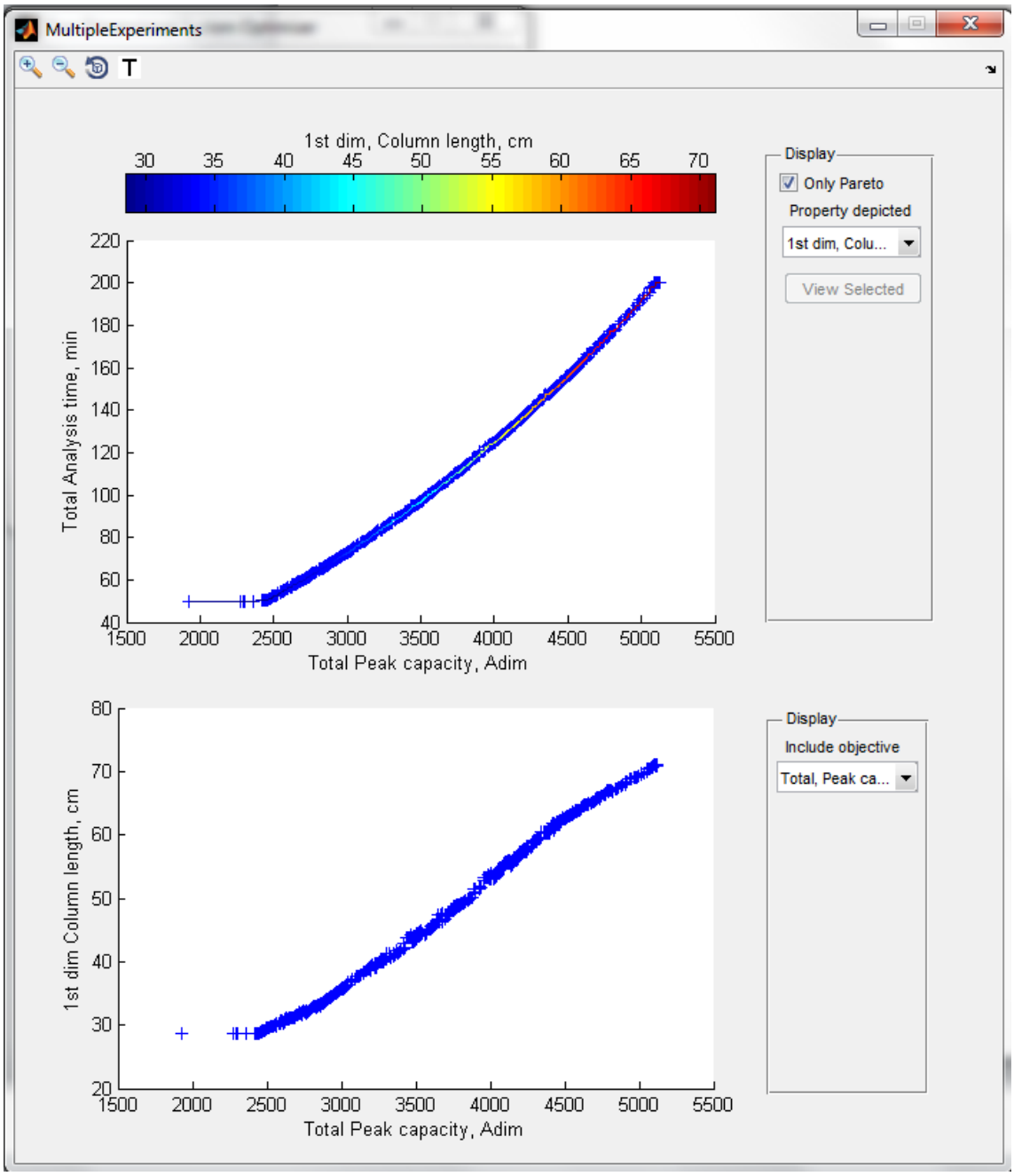


FIGURE 6: View panel after performing a "Smart Pareto" calculation. Note that the variation in the parameter depicted at the bottom is almost continuous.

11.Further Notes

1. When opening the software for the first time (after any restart of the system), the software can take a significant amount of time to start (~30 s). This is because the Matlab Common Runtime has to be started previously.

2. Some RAM memory problems may appear if the user selects more than 1.5 milion combination of parameters. If this happens, use the Smart-Pareto approach. A

RAM-memory problem could induce a crash of the software. Just re-start the software again if this happens.

12. Support

Contact Gabriel Vivó Truyols (g.vivotruyols@tecnometrix.com) for help when using this software. These instructions are also embedded in the software (click on "Help" >> "Help"). Please, report any bugs or suggestions to this e-mail address.

13. Disclaimer

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