A microdevice assisted approach for the preparation, characterization and selection of continuous aqueous two-phase systems: from micro to bench-scale

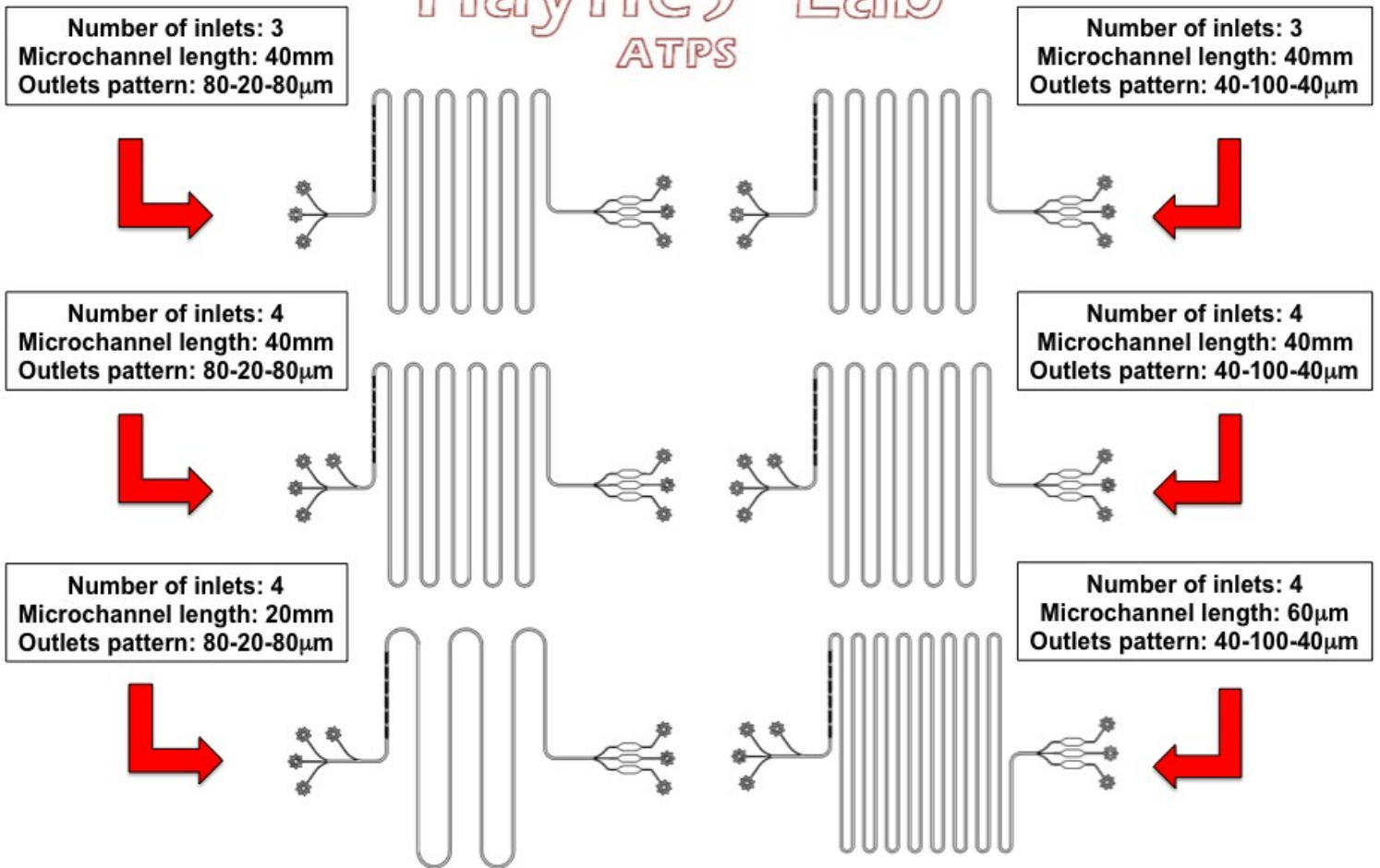By:

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Electronic Supplementary Information

Figure S1. Detailed information of the first microdevice design. All the designs as they are depicted are fitted in a 10 cm x 10 cm silicon wafer prepared using conventional photolithography. These first designs were arbitrarily employed to carry out the poof of concept and prepare the binodal diagrams for the different ATPSs employed in this work. As well, the design was employed for the selection of a system to further scale an application of recover invertase from spent brewery yeast. All design contains 6 staggered herringbone chaotic (SHC) micromixers sets included right after the stream inlets.
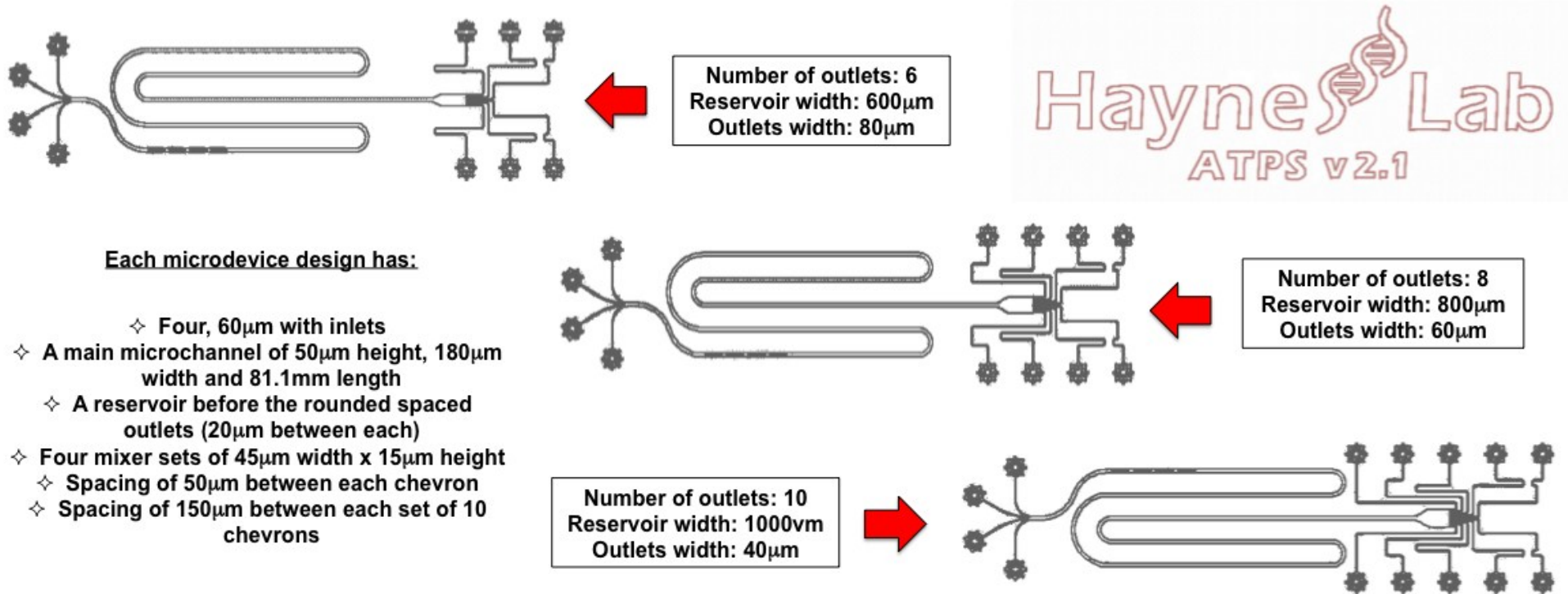
Number of outlets: 6
Reservoir width: 600μm
Outlets width: 80μm

Number of outlets: 8
Reservoir width: 800μm
Outlets width: 60μm

Number of outlets: 10
Reservoir width: 1000vm
Outlets width: 40μm

Each microdevice design has:

◇ Four, 60μm with inlets
◇ A main microchannel of 50μm height, 180μm width and 81.1mm length
◇ A reservoir before the rounded spaced outlets (20μm between each)
◇ Four mixer sets of 45μm width x 15μm height
◇ Spacing of 50μm between each chevron
◇ Spacing of 150μm between each set of 10 chevrons

Figure S2. Detailed information of the second microdevice design. All the designs are duplicated and fitted in a 10cmx 10cm silicon wafer using conventional photolithography (6 designs in total). This design was employed to poof the solute partitioning and recovery of samples at the end of the microdevice. The solutes employed were BSA and RBCs. The design with 6 outlets was employed, however, if needed, one can employ use until 10 outlets to recover more sample. All the designs have a main channel length of 81.1mm to allow phases to coalesce and 4 staggered herringbone chaotic (SHC) micromixers sets included right after the stream inlets.

Table S1. Estimated density and weight percent of the ATPS phase components dilutions.	
Dilution % (w/w)	**Density (g/cm^3)**
Ethanol 70%	**0.85**
Phosphate 45%	**1.30**
Phosphate 25%	**1.22**
Citrates 45%	**1.31**
PEG 1000 70%	**1.11**
PEG 1000 40%	**1.08**
PEG 1000 35%	**0.98**
PEG 8000 60%	**1.20**
PEG 8000 30%	**1.05**
PEG 8000 20%	**1.03**
DEX 500 10%	**0.87**

Note: All the stocks are diluted in bidestilated water. After the dilutions were prepared, 1 mL of them was weighted and compared to the same amount of water in order to estimate their density. The reported density is the average of measurements done by triplicate. Given that the standard deviation was negligible it is not included
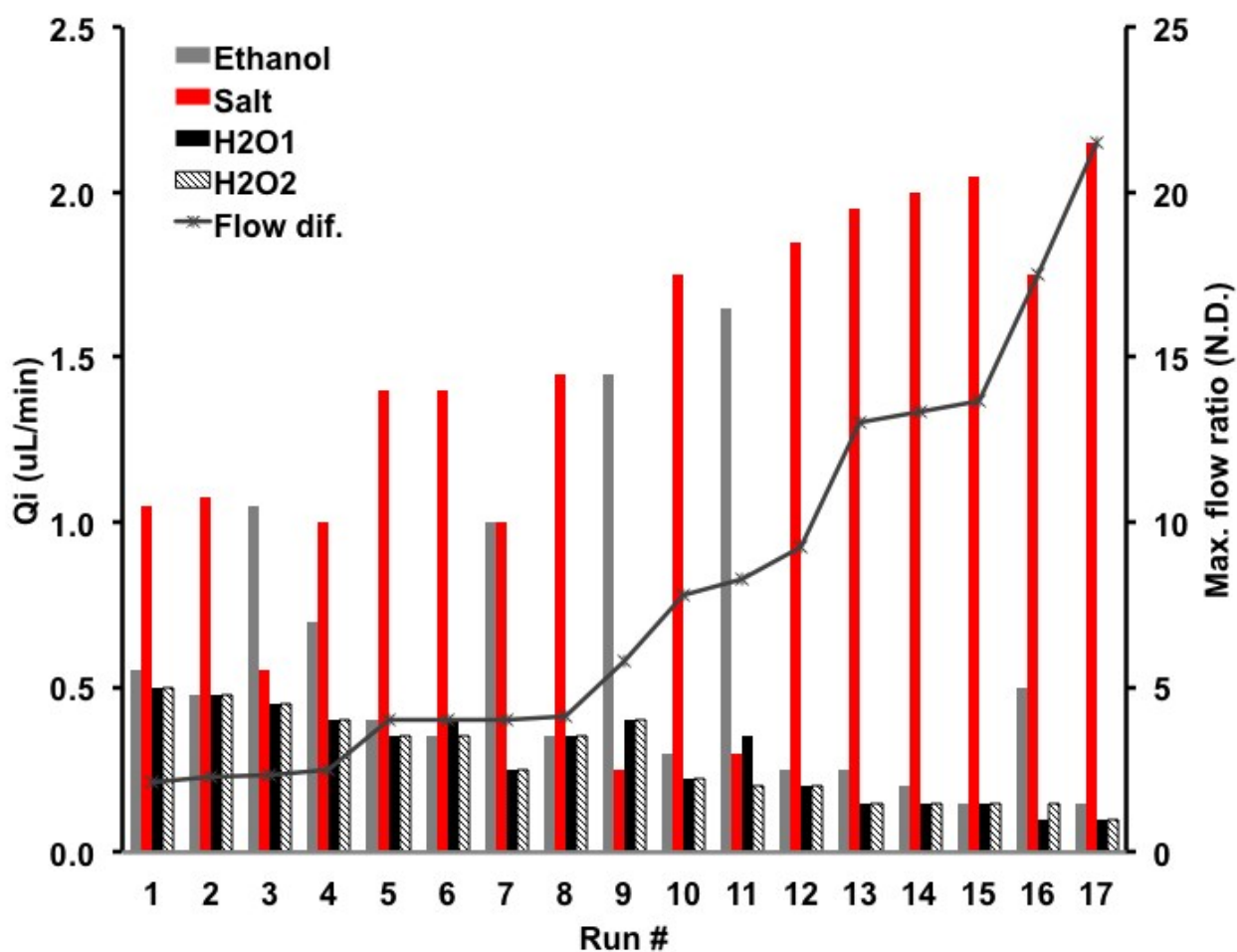
Figure S3. Record of the flows of the phase forming components employed to perform the experiments of binodal curve for the Ethanol-phosphate aqueous two-phase system (ATPS). The left axis represent the flow rate of the different components in μL/min, while the right axis depicts the maximum flow ratio calculated among the smallest and the highest of all the inlet streams. As observed, for an Ethanol-salt system, the ratio between the minimum flow rate and the maximum flowrate goes from 2.1 to 21.5 fold. Thus, the working limits of the device, so laminar flow is allowed for this system in particular, goes from 0.1 to 2.15 μL/min.
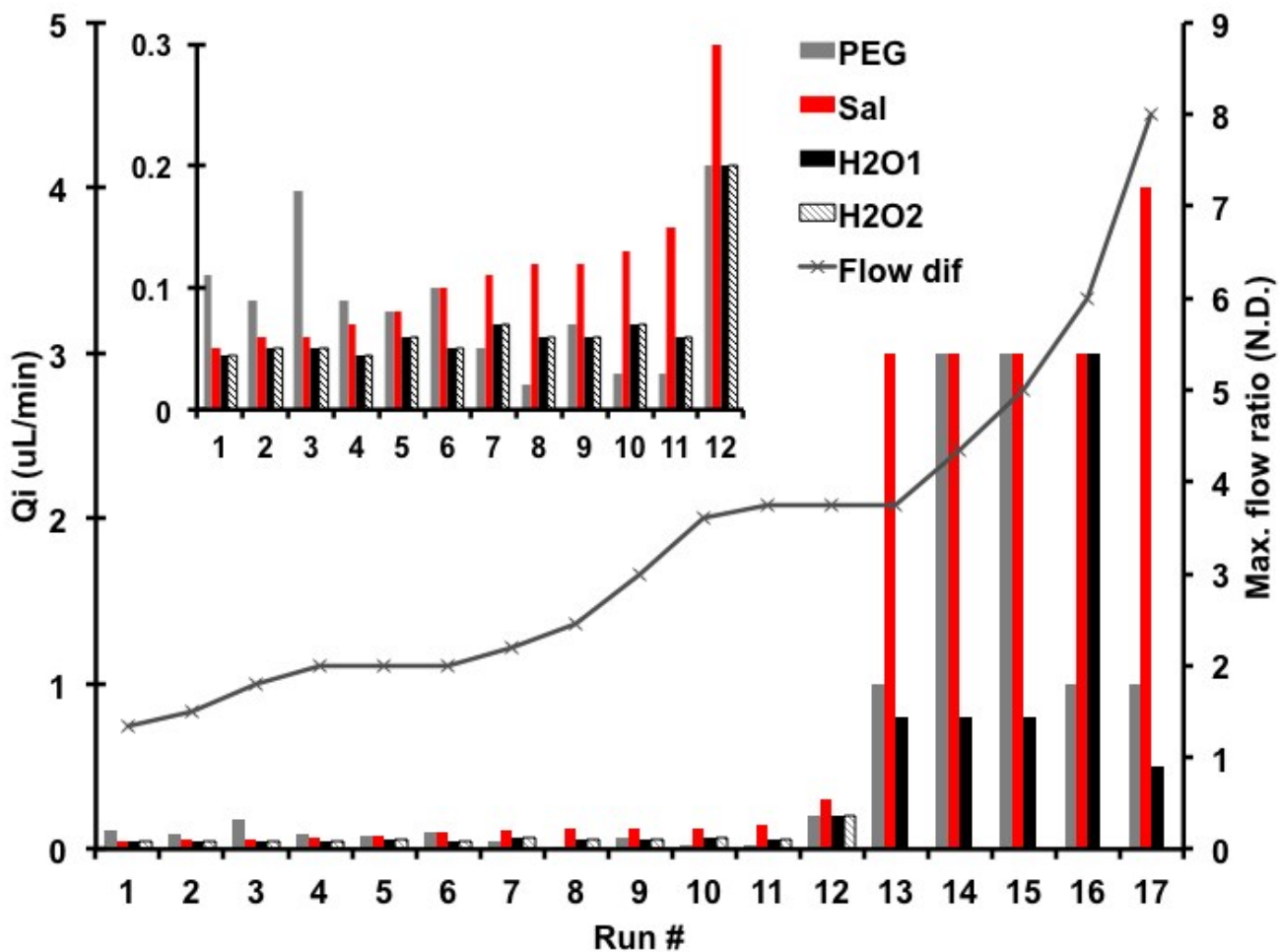
Figure S4. Densities and record of the flows of the phase forming components employed to perform the experiments of binodal curves for the PEG1000-phosphate aqueous two-phase system (ATPS). The left axis represent the flow rate of the different components in μl/min, while the right axis depicts the maximum flow ratio calculated among the smallest and the highest of all the inlet streams. For a PEG1000-Phosphate system, the ratio between the minimum flow rate and the maximum flowrate goes from 1.3 to 8.0 fold. Thus, the working limits of the device, so laminar flow is allowed for this system in particular, goes from 0.5 to 4.0 μl/min, and from 0.02 to 0.3 μl/min to obtain well defined of a discontinuous phase drops on a continuous phase flowing in the microchannel. For laminar flows, there is no need to use a fourth inlet of water to dilute the polymer/salt streams.
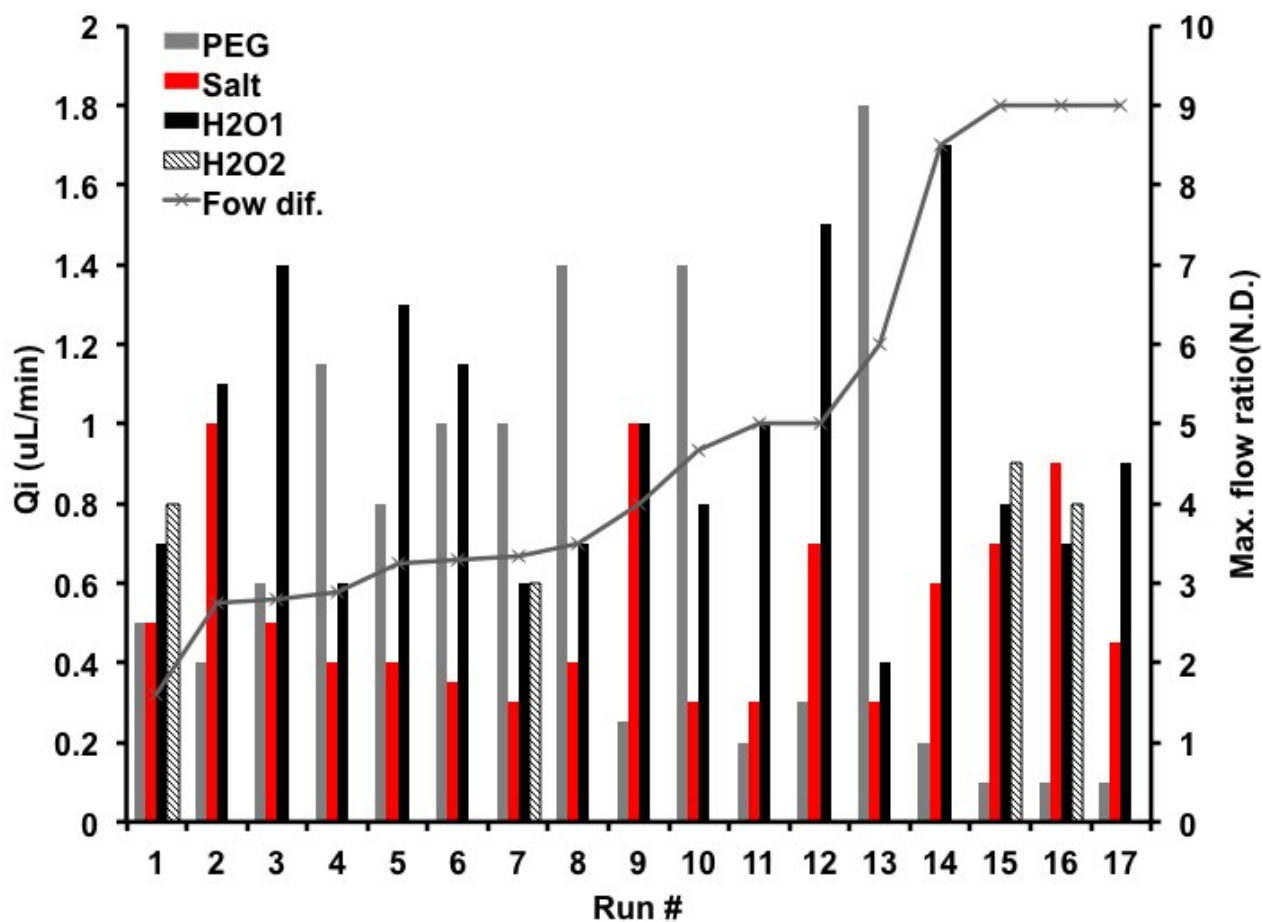
Figure S5. Densities and record of the flows of the phase forming components employed to perform the experiments of binodal curves for the PEG8000-phosphate aqueous two-phase system (ATPS). The left axis represent the flow rate of the different components in µl/min, while the right axis depicts the maximum flow ratio calculated among the smallest and the highest of all the inlet streams. For a PEG8000-Phosphate system, the ratio between the minimum flow rate and the maximum flowrate goes from 1.6 to 9.0 fold. Thus, the working limits of the device, so laminar flow is allowed for this system in particular, goes from 0.1 to 1.8 µl/min. As can be observed, the more viscous are the phases components the lower is the flow rates that generates laminar behaviour of the liquid streams.
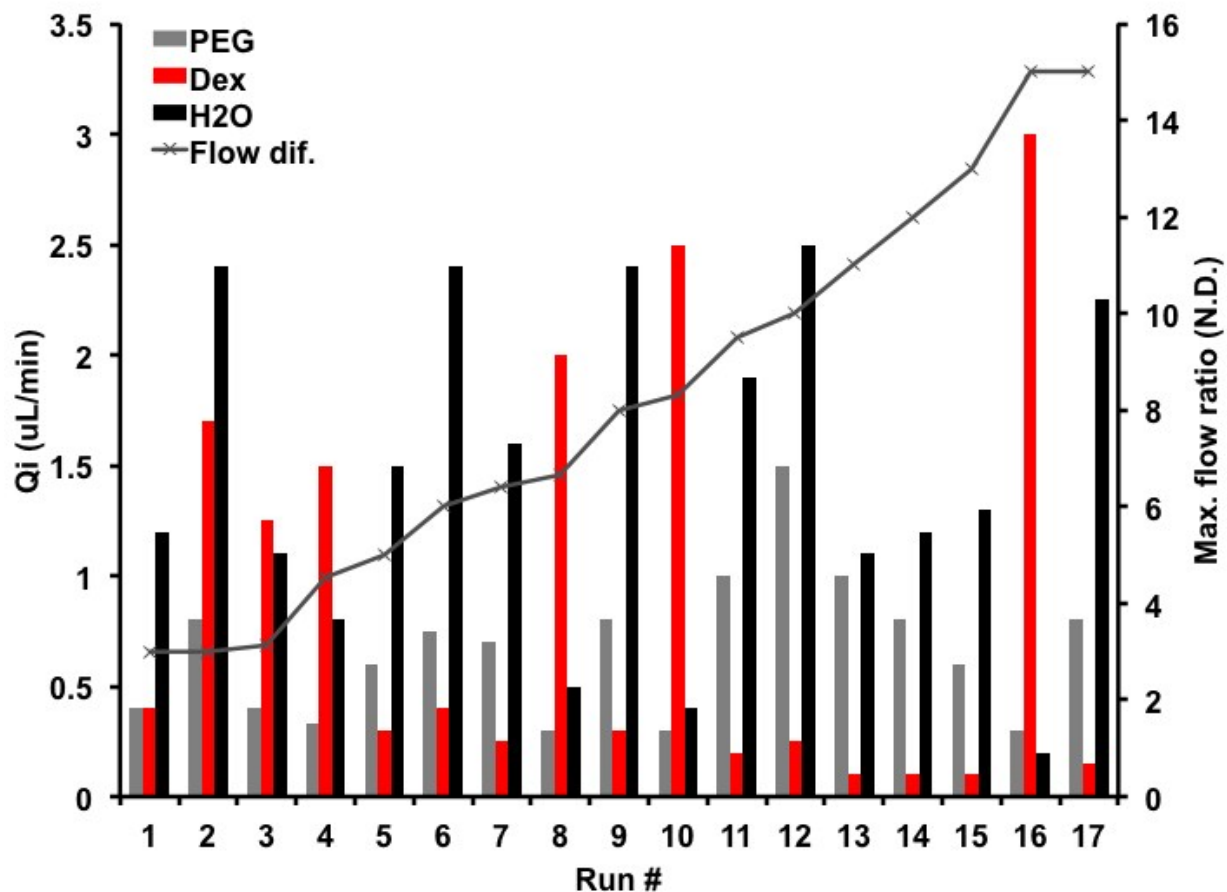
Figure S6. Densities and record of the flows of the phase forming components employed to perform the experiments of binodal curves for the PEG8000-DEX500 aqueous two-phase system (ATPS). The left axis represent the flow rate of the different components in μl/min, while the right axis depicts the maximum flow ratio calculated among the smallest and the highest of all the inlet streams. For a PEG8000-DEX500 system, the ratio between the minimum flow rate and the maximum flowrate goes from 3 to 15 fold. As can be observed, the more similar are the phases components streams (viscosity) the higher is the maximum flow ratio. The working limits of the device, so laminar flow is allowed for this system in particular, goes from 0.1 to 3 μl/min. For a polymer-polymer system such the one presented here, where small concentration of polymer can give a two-phase composition, there is no need of an additional water stream.
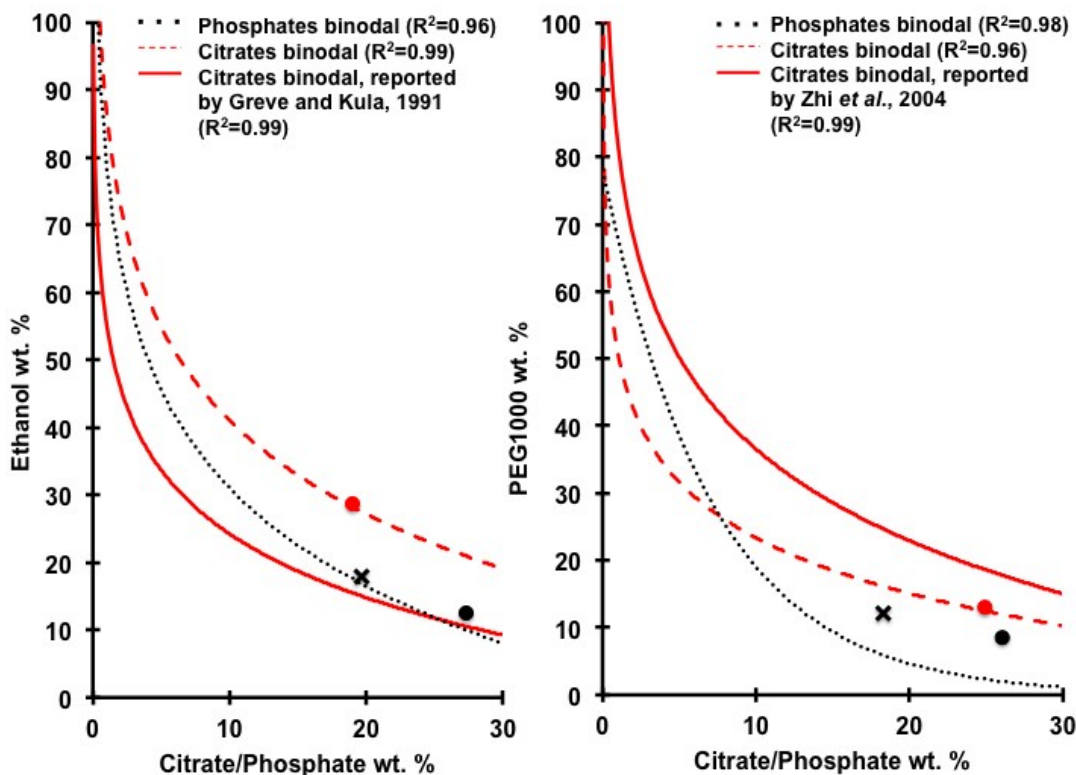
Figure S7. Binodal curves of the PEG-salt and Ethanol-salt ATPS prepared using the microdevice showing the working phase components concentrations used at micro and bench-scale to recover invertase from spent brewery yeast. Black circles represent the total composition of the phosphate systems used for the microdevice application (12% wt ethanol – 27% wt. phosphates and 12% wt. PEG1000 – 18% wt. phosphates); red circle represent the citrate systems for the microdevice experiments (29% wt. ethanol – 19% wt. citrates and 13% wt. PEG1000 25% wt. citrates); black X represents the compositions employed in the batch and bench-scale systems (18% wt. ethanol – 20% wt. phosphates and 12% wt. PEG1000 – 18% wt. phosphates). As observed, the compositions in bench scale are different than those used for the microscale. This was due the working limits of the microdevice are different than in the bench-scale device. However, all the compositions allow a salt-rich continuous phase (this is the phase which is present in higher amount inside the system). Also, there is a considerable difference among the predicted points of the prepared microscale binodal curve and the points of the reported binodal curve for the citrate systems. This may have been a cause for the differences in the invertase partition behaviour among scales. Reported data obtained from: Greve and M. R. Kula, *Fluid Phase Equilib.,* 1991, **62**, 52-63. And W. Zhi, J. Song, J. Bi and F. Ouyang, *Bioprocess Biosyst Eng.,* 2004, **27**, 3-7.
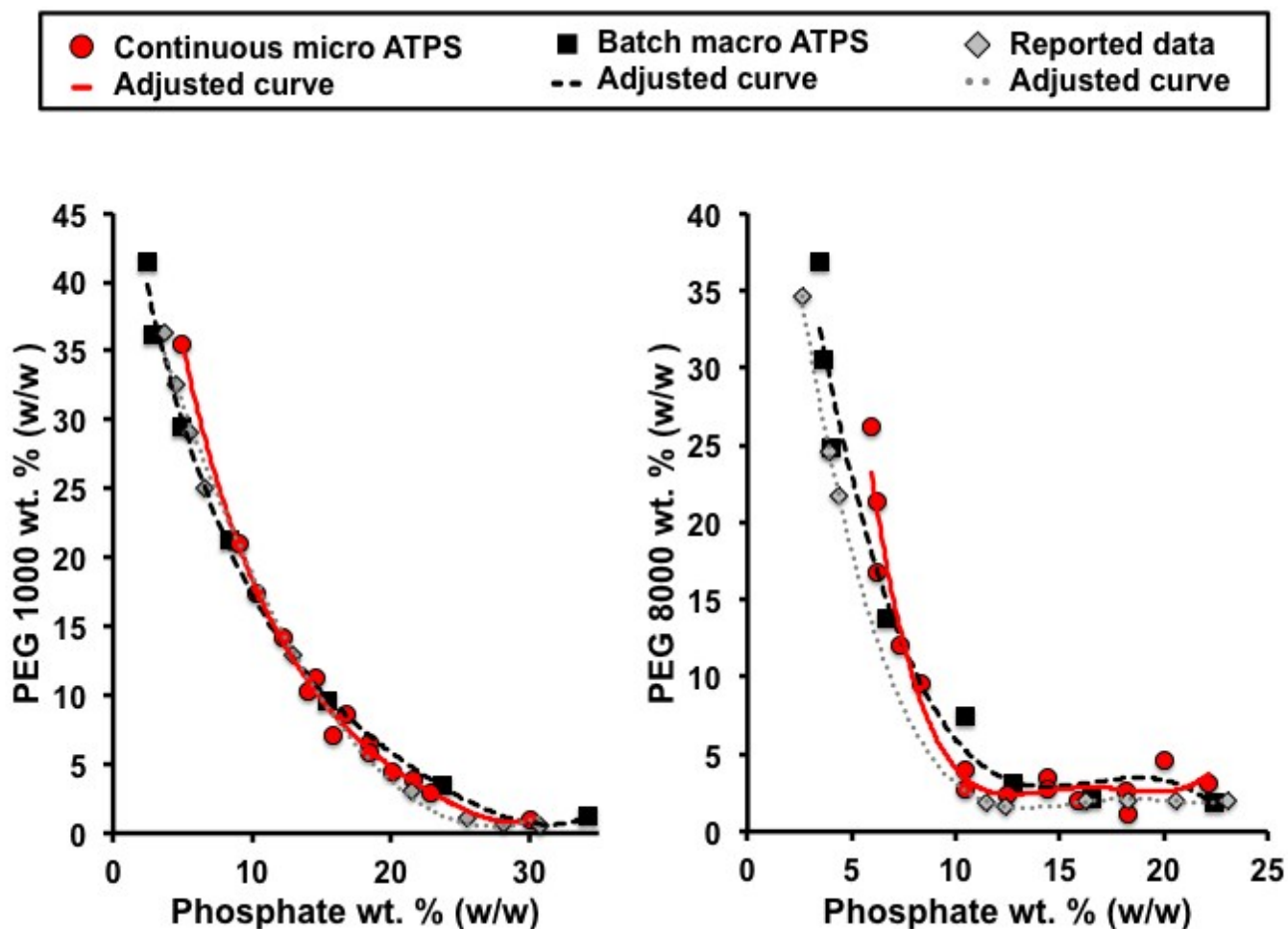
Figure S8. **Comparison of binodal curves obtained at microscale with prepared curves at batch macroscale for two selected ATPS.** To show whether or not the differences of the binodal prepared and reported data were arise from user error, differences in polymer/chemical batches, etc., a more rigorous validation of the method for binodal determination was made. As observed, the prepared binodal curves of the PEG1000-Phosphate and PEG-8000 Phosphate systems are better adjusted to experimental data obtained by the cloud-point method at 1-2.5 g batch macroscale. It is expected that all other binodal curves prepared in this work have similar adjustment behaviour. Reported data obtained from: S. M. Snyder, K. D. Cole and D. C. Sziag, *J. Chem. Eng. Data*, 1992, **37**, 266-274.