[0008] As will be appreciated by those of skill in the art, yet another drawback to known processes for the purification of biomolecules (such as pDNA) is the difficulty to apply such methods in large-scale production, particularly large-scale production of pharmaceutical-grade pDNA. There are many existing constraints on large-scale production, for example personal safety issues and hazardous waste considerations. Further, the application of bench-scale purification processes of pDNA on a larger scale almost always results in decreased yield of the purified pDNA. Given these limitations, it would be desirable to provide for a method of purification or isolation of pDNA that not only provides pDNA with improved purity and in high yield, but that may also be used for large-scale production.

SUMMARY

[0008a] In an aspect, there is provided a method for isolating or purifying a biological molecule of interest from a mixture, the method comprising:
contacting the mixture with an anion exchange material in the presence of a solution comprising a salt at a concentration that allows selective binding of the biological molecule of interest to the anion exchange material;
optionally wherein the salt at a concentration that allows selective binding of the biological molecule to the anion exchange material is a kosmotropic salt; and
eluting the biological molecule of interest with an eluent comprising a salt at a concentration that provides an eluant containing the purified biological molecule; optionally wherein the salt at a concentration that provides an eluant containing the purified biological molecule is a chaotropic salt;
further optionally wherein
(i) the fraction(s) comprising said biological molecule in the eluant is/are collected and/or
(ii) said biological molecule of interest is isolated from any one or all of the collected fractions; and/or
(iii) the method is conducted in the absence of organic solvents, detergents, glycols, hexamine cobalt, spermidine, polyvinylpyrrolidone.

[0009] An example of the present disclosure relates to a method for isolating or purifying a biological molecule of interest from a mixture, the method comprising contacting the mixture comprising the biological molecule of interest with an anion exchange (AEX) material in the presence of a solution comprising a salt at a concentration that allows selective binding of the biological molecule of interest to the anion exchange material; and eluting the biological molecule of interest with an eluent comprising a salt at a concentration that provides an eluant containing the purified biological molecule. In some examples, the fraction or fractions comprising said biological molecule of interest in the eluant is/are optionally collected. The purified biological molecule of interest

may in some instances be subsequently isolated from any one or all of the collected fractions.

[0010] In some examples, the salt used at a concentration that allows selective binding of the biomolecule of interest to the AEX material is a kosmotropic salt, for example ammonium citrate, ammonium sulfate, ammonium phosphate, potassium phosphate, sodium citrate, sodium phosphate, or mixtures of said salts.

[0011] In some examples, the salt that is present in the eluent at a concentration that provides an eluant containing the purified biological molecule is a chaotropic salt, such as ammonium chloride, potassium chloride, sodium chloride, magnesium sulfate, magnesium chloride, magnesium nitrate, guanidinium hydrochloride, or mixtures thereof.

[0012] The biological molecule to be purified by the methods of the disclosure is typically a highly polar / charged biomolecule such as a nucleic acid. It was found that the novel method described herein is particularly suitable for a fast and highly effective purification of plasmid DNA, in particular pharmaceutical-grade super-coiled plasmid DNA (sc pDNA), which must be free from bacterial genomic DNA, RNA, protein and endotoxins.

[0013] In another example, the present disclosure provides an advantageous sample preparation step that can be advantageously used before contacting the mixture containing the biomolecule of interest with the AEX material.

[0014] In this example, solid components (such as cell debris or other materials insoluble in the buffer system) of the mixture comprising the biological molecule of interest are conveniently separated by phase separation and/or filtration. In some examples, the removal of the solid components is achieved via a simple two-phase separation, wherein a buffer is added to increase the density of the mixture to be greater than about 1.1 kg/L. The increase in the density of the mixture causes the solid components to float on top, and the liquid part of the mixture (comprising the biological molecule of interest) can then be conveniently drained off, without requiring a filtration step (or at least offering a higher filtration capacity due to the significantly reduced solid material content, thereby allowing scalability of the process, i.e., making it useful for large-scale production). This sample preparation step can in some examples be advantageously combined with the novel purification method described herein.

[0015] Another, related example of the present disclosure relates to the use of a tulip-shaped vessel or tank for the above phase separation step. The tulip-shaped vessel was found to be particularly advantageous for the convenient and effective separation of the solid components from the liquid part of the mixture comprising the biological molecule of interest (such as plasmid DNA).

[0015a] In another aspect, there is provided a use of a tulip-shaped vessel for the method as defined above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **Figure 1** depicts a schematic view of plasmid vector pUC19 used as a sample biomolecule to be purified in the working examples.

[0017] **Figure 2A - D** shows agarose gel electrophoresis (AGE) data on the results of a binding buffer screening using Sartobind Q as AEX material.

[0018] **Figure 3A - D** shows agarose gel electrophoresis (AGE) data on the results of an elution buffer screening using Sartobind Q as AEX material.

[0019] **Figure 4** depicts a chromatography profile of the AEX chromatography unit (Sartobind Q) zoomed in on the elution gradient.

[0020] **Figure 5** shows agarose gel electrophoresis data of fractions from the AEX chromatography step (Sartobind Q).

[0021] **Figure 6** shows AEX-HPLC traces from the Sartobind Q elution pool 1 as depicted in Figure 3 (dashed line) and the standard (solid line).