[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~~plasmid DNA (pDNA) of interest from a mixture, the method comprising:
Contacting the mixture with an anion exchange material in the presence of a solution comprising a kosmotropic salt at a concentration that allows selective binding of the ~~biological molecule~~pDNA of interest to the anion exchange material;
wherein the kosmotropic salt is selected from the group consisting of: ammonium sulfate, ammonium citrate, ammonium phosphate, potassium phosphate, sodium citrate, sodium phosphate, and mixtures thereof;
~~optionally~~ wherein the ~~salt at a~~ concentration of the kosmotropic salt is greater than 0.5M ~~that allows selective binding of the biological molecule to the anion exchange material is a kosmotropic salt~~; and
eluting the ~~biological molecule~~pDNA of interest with an eluent comprising a chaotropic salt at a concentration that provides an eluant containing the ~~purified biological molecule~~pDNA;
~~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~~pDNA in the eluant is/are collected and/or
(ii) said ~~biological molecule~~pDNA 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, and/or 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.

Claims

1. A method for isolating or purifying a ~~biological molecule~~plasmid DNA (pDNA) of interest from a mixture, the method comprising:

contacting the mixture with an anion exchange material in the presence of a solution comprising a kosmotropic salt at a concentration that allows selective binding of the ~~biological molecule~~pDNA of interest to the anion exchange material;
wherein the kosmotropic salt is selected from the group consisting of: ammonium sulfate, ammonium citrate, ammonium phosphate, potassium phosphate, sodium citrate, sodium phosphate, and mixtures thereof;

~~optionally~~ wherein the ~~salt at a~~ concentration of the kosmotropic salt is greater than 0.5 M ~~that allows selective binding of the biological molecule to the anion exchange material is a kosmotropic salt~~; and

eluting the ~~biological molecule~~pDNA of interest with an eluent comprising a chaotropic salt at a concentration that provides an eluant containing the purified ~~biological molecule~~pDNA;
~~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~~ pDNA in the eluant is/are collected and/or
(ii) said ~~biological molecule~~ pDNA 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, and/or polyvinylpyrrolidone.

2. The method of claim 1, ~~wherein the biological molecule to be purified is a nucleic acid; optionally wherein the nucleic acid is plasmid DNA (pDNA),~~
~~further optionally~~ wherein
(i) the plasmid DNA is super-coiled plasmid DNA (sc pDNA); and/or
(ii) the plasmid DNA comprises mammalian DNA, bacterial DNA, non-coding DNA, or viral DNA; optionally wherein the plasmid DNA comprises DNA capable of expressing a polypeptide of interest; and/or
(iii) the mixture comprises super-coiled plasmid DNA, open-circular plasmid DNA, linear plasmid DNA, genomic DNA, RNA, lipopolysaccharides, endotoxins, proteins, or any combination thereof.

3. The method of claim 1 or claim 2, wherein the purity of the purified and/or isolated plasmid DNA is at least 90%, or at least 95%, or at least 98%.

4. The method of any one of claims 1 to 3, wherein

(i) the concentration of the kosmotropic salt ~~that allows selective binding of the biological molecule of interest to the anion exchange material is greater than about 0.5 M, optionally wherein the concentration of the salt that allows selective binding of the biological molecule to the anion exchange material~~ is ~~about~~ 0.8 M to ~~about~~ 4.0 M; and/or

(ii) the concentration of the chaotropic salt that provides an eluant containing the purified ~~biological molecule~~pDNA is ~~about~~ 0.25 M to ~~about~~ 4.0 M; and/or

(iii) the elution step comprises a gradient elution by varying the concentration of the chaotropic salt in the eluent; optionally wherein the concentration of the chaotropic salt in the eluent is increased.

5. The method of any one of claims 2 to 4, ~~wherein the biological molecule is plasmid DNA and~~ wherein the elution step includes fractionation of super-coiled plasmid DNA from other plasmid DNA forms such as linear plasmid DNA and open-circular plasmid DNA, and, optionally, other nucleic acid molecules.

6. The method of any one of claims 1 to 5, wherein

(i) the kosmotropic salt is ~~selected from the group consisting of:~~ ammonium sulfate~~, ammonium citrate, ammonium phosphate, potassium phosphate, sodium citrate, sodium phosphate, and mixtures thereof~~; optionally wherein the ~~kosmotropic salt is ammonium sulfate; further optionally wherein the~~ concentration of ammonium sulfate is ~~about~~ 0.5 M to ~~about~~ 2.0M; and/or

(ii) wherein the chaotropic salt is selected from the group consisting of: ammonium chloride, potassium chloride, sodium chloride, magnesium sulfate, magnesium chloride, magnesium nitrate, guanidinium hydrochloride, and mixtures thereof, optionally wherein the chaotropic salt is magnesium sulfate; further optionally wherein the concentration of magnesium sulfate is ~~about~~ 0.5M to ~~about~~ 1.0M; and/or

(iii) the solution comprising the kosmotropic salt has a pH in the range of ~~about~~ 5.0 to ~~about~~ 10.0, or of ~~about~~ 5.0 to ~~about~~ 8.0, or ~~about~~ 6.0 to ~~about~~ 8.0, or ~~about~~ pH 7.

7. The method of any one of claims 1 to 6, wherein the anion exchange material is selected from an anion exchange membrane, an ion exchange resin, a three-dimensional microporous hydrogel structure, a packed bed of superporous beads, macroporous beads, a monolith, agarose beads, cross-linked agarose, silica beads, large pore gels, methacrylate-based beads, polystyrene-based beads, cellulose-based beads, dextran-based beads, bisacrylamide-based beads, polyvinylether-based beads, ceramic-based beads, or polymer-based beads;

optionally wherein the anion exchange material is

(i) an anion exchange membrane; optionally wherein the anion exchange membrane has an average pore size of ~~about~~ 3.0μm to ~~about~~ 5.0μm; preferably wherein the average pore size is ~~about~~ 3.0μm; or

(ii) an ion exchange resin, optionally wherein the ion exchange resin has an average particle diameter from ~~about~~ 30 μm to ~~about~~ 300μm.

8. The method of any one of claims 1 to 7, wherein the method further comprises separating solid components from the mixture comprising the ~~biological molecule~~pDNA of interest prior to contacting the mixture with an anion exchange material in the presence of the solution comprising the salt at a concentration that allows selective binding of the ~~biological molecule~~pDNA to the anion exchange material,
optionally wherein said removal of solid components is achieved by filtration or phase separation.

9. The method of claim 8, wherein the removal of solid components is achieved via two-phase separation,
optionally wherein

(i) a buffer is added to increase the density of the mixture to be greater than ~~about~~ 1.1 kg/L, optionally wherein the buffer comprises a kosmotropic salt, preferably wherein the kosmotropic salt is ammonium sulfate; and/or

(ii) wherein the lower phase of the two-phase mixture having a higher density than the top phase is subjected to a depth filtration.

10. The method of any one of claims 1 to 9, wherein the method further comprises the step of

(i) precipitating the ~~biological molecule~~pDNA from the eluant; and/or

(ii) filtering the eluant by tangential-flow filtration to isolate the pDNA~~biological molecule~~; optionally wherein the average pore size of the filtration membrane used in the tangential-flow filtration step is ≤ 0.2 μm; and/or

(iii) lyophilization of the purified pDNA~~biological molecule~~ of interest, optionally wherein the lyophilization is carried out in the presence of a carbohydrate; and/or

(iv) collecting a flow-through following the step of contacting the mixture with an anion exchange material in the presence of the solution comprising the salt at a concentration that allows selective binding of the ~~biological molecule~~ pDNA to the anion exchange material; optionally wherein the flow-through comprises RNA, genomic DNA, proteins, cellular fractions, or combinations thereof, preferably wherein the flow-through comprises RNA; and/or

(v) washing the anion exchange material with a washing buffer solution prior to the elution of the pDNA ~~biological molecule~~ of interest; optionally wherein the washing buffer solution comprises a chaotropic salt at a concentration lower than the

concentration required for the elution of the pDNA ~~biological molecule~~ of interest, wherein the chaotropic salt is preferably selected from the group consisting of: ammonium chloride, potassium chloride, sodium chloride, magnesium sulfate, magnesium chloride, magnesium nitrate, and mixtures thereof.

11. The method of any one of claims 1 to 10, wherein the method further comprises a further purification step following the step of eluting and optionally isolating the pDNA~~biological molecule~~;
optionally wherein the second purification step comprises use of a thiophilic aromatic adsorption chromatography medium having a selectivity that allows super-coiled plasmid DNA to be separated from open circular and/or linear DNA.

12. The method of any one of claims 1 to 11, wherein the method further comprises the step of
(i) using the pDNA~~nucleic acid~~ to express a polypeptide of interest~~; preferably wherein the nucleic acid is plasmid DNA~~; or
(ii) using the pDNA ~~nucleic acid~~ to produce and harvest a polypeptide of interest~~; preferably wherein the nucleic acid is plasmid DNA~~; optionally wherein the method further comprises the step of formulating the polypeptide of interest into a pharmaceutical composition.

13. The method of any one of claims 2 to 11, wherein the method further comprises the step of using the purified plasmid DNA for the production of RNA.

~~13.~~14. The method of any one of claims 1 to 13~~12~~, wherein the method includes the steps of cell harvesting and washing, cell lysis, neutralization, and flocculate removal prior to contacting the resulting mixture comprising the pDNA ~~biological molecule~~ of interest with the anion exchange material.

~~14.~~15. The method of any one of claims 1 to ~~13~~14, wherein the method does not comprise a $CaCl_2$ precipitation step to remove RNA.

~~15. Use of a tulip-shaped vessel for the method of claim 8 or claim 9.~~