Claims

1. A method for purification of a hydrated target species of biological origin in a sample comprising the steps of contacting the sample with a hydrated surface of an undissolved material and contacting the sample with a constraining agent in an amount sufficient to cause at least a fraction of the target species to be retained at the hydrated surface of the undissolved material under conditions providing an absence of a significant chemical attraction between the target species and the surface of the undissolved material,

wherein the constraining agent is an aqueous soluble uncharged linear or branched non-ionic polymer

wherein the undissolved material is a convective chromatography material comprising a monolith, and

wherein the target species is a protein, antibody, clotting factor, cellular organelle, virus, virus-like particle, gene therapy vector or cell.

2. The method of claim 1 wherein more than 50% of the hydrated target species is retained at the hydrated surface of the undissolved material.

3. The method of claim 2 wherein substantially all of the hydrated target species is retained at the hydrated surface of the undissolved material.

4. The methods of claims 1-3 wherein at least one individual of the hydrated target species is retained at the hydrated surface exclusively by constrained co-hydration due to the presence of at least one constraining agent.

5. The methods of claims 1-4 wherein the sample is a cell culture harvest, a cell culture supernatant, a protein-containing solution derived from a cell culture, an antibody-containing solution derived from a cell culture, a virus-containing solution derived from cell culture, or a target species-containing solution from a previous stage of purification.

6. The method of claims 1-5 wherein the target species is a protein.

7. The method of claim 6 wherein the target species is a clotting factor.

8. The method of claims 1-5 wherein the target species is a polyclonal or monoclonal antibody of the class IgA, IgD, IgE, IgG, or IgM or a fragment or compound recombinant construct thereof.

9. The method of claims 1-5 wherein the target species is a prokaryotic cell, eukaryotic cell, stem cell.

10. The method of claims 1-5 wherein the target species is a virus, lipid

enveloped virus, protein capsid virus or virus like particle.

11. The method of claims 1-5 wherein the target species is an exosome, liposome, mitochondrion, chloroplast, lysosome, or other cellular organelle.

12. The method of claims 1-11 wherein the surface of the undissolved material has one or more polar chemical moieties.

13. The method of claim 12 wherein the polar chemical moiety is selected from the group of hydroxyl, amine, imine, ureide, carbohydrate, amino acid, peptide, a cationic charged group or an anionic charged group.

14. The method of claim 13 wherein the polar chemical moiety is a carbohydrate selected from the group consisting of glucose, mannose, galactose, lactose, a monosaccharaide, a disaccharide and a polysaccharide.

15. The method of claim 13 wherein the polar chemical moiety is a ureide selected from the group consisting of urea, uric acid, allantoin, hydantoin.

16. The method of claim 13 wherein the polar chemical moiety is histidine, glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, lysine, arginine, or selenocysteine.

17. The method of claims 1-16 wherein the constraining agent is selected from the group of polyethylene glycol, polypropylene glycol, polyvinylpyrrolidone, dextran, starch, cellulose.

18. The method of claim 17 wherein the constraining agent is polyethylene glycol.

19. The method of claim 18 wherein the polyethylene glycol has an average polymer weight between 100 and 10,000 D.

20. The method of claim 19 wherein the polyethylene glycol has an average polymer weight between 600 and 8,000 D.

21. The method of claims 18-20 wherein the polyethylene glycol is provided at a concentration between 2% and 50% (w/v).

22. The method of claim 21 wherein the polyethylene glycol is provided at a concentration between 2% and 25% (w/v) and the polyethylene glycol has an average polymer weight above 6,000 D.

23. The method of claim 21 wherein the polyethylene glycol is provided at a concentration between 15% and 50% (w/v) and the polyethylene glycol has an average polymer weight below 6,000 D.

24. The method of claim 21 wherein the polyethylene glycol is provided at a concentration between 2% and 25% (w/v) and the polyethylene glycol has an average polymer weight between 4,000 D and 8000D.

25. The method of claims 1-24 wherein the undissolved material constitutes organic nucleation centers.

26. The method of claim 25 wherein the organic nucleation center comprises a carbohydrate, ureide or a peptide.

27. The method of claim 26 wherein the ureide is allantoin.

28. The method of claim 26 wherein the carbohydrate is starch or cellulose.

29. The method of claim 27 wherein the organic nucleation center comprises a core to which an outer layer of the target species is bound by chemical or electrostatic forces of attraction or retained by constrained co-hydration where such outer layer of target species provides the hydrated surface of the undissolved material.

30. The method of claim 28 wherein the organic nucleation center retains multiple layers of the target species solely by constrained co-hydration.

31. The method of claims 26-30 wherein the sample is contacted with the organic nucleation center prior to the step of contacting the sample with the constraining agent.

32. The method of claim 31 comprising the additional steps of separating the supernatant containing contaminants from the organic nucleation centers having the target species retained at the hydrated surface of the organic nucleation centers.

33. The method of claim 32 comprising the additional step of recovering the target species from the organic nucleation center by exposing the organic nucleation centers to conditions which promote the dissociation of the target species from the organic nucleation center.

34. The method of claim 33 wherein the target species is dissociated from the organic nucleation center by substantially reducing the concentration of the constraining agent.

35. The method of claims 33-34 comprising the step of contacting the

organic nucleation center with a solution which does not contain the constraining agent.

36. The method of claims 33-35 wherein the salt concentration is increased sufficiently to reduce the effectiveness of the constraining agent to retain the target species at the hydrated surface of the organic nucleation center.

37. The method of claims 33-35 wherein the sugar concentration is increased sufficiently to reduce the ability of the constraining agent to constrain the target species at the hydrated surface of the organic nucleation center.

38. The method of claim 37 wherein the sugar is selected from the group consisting of sorbitol, xylitol, and sucrose and the constraining agent is polyethylene glycol.

39. The methods of claims 33-38 wherein method comprises one or more additional steps of washing the organic nucleation centers prior to dissociating the target species from the organic nucleation centers.

40. The methods of claims 33-39 wherein the solutions used in the steps of dissociating the target species from the organic nucleation centers are substantially the same as the solutions used for the recovery of the target species.

41. The method of claim 1 wherein the monolith is comprised of a material selected from the group consisting of polyacrylate, polymethacrylate, styrenedivinylbenzene and silica.

42. The method of claims 1-41 wherein the monolith comprises average channel size between 1 micron and 200 micron.

43. The method of claim 42 wherein the channel size is between 1 micron and 5 microns.

44. The method of claim 42 wherein the channel size is between 10 micron and 20 microns.

45. The method of claim 42 wherein the channel size is between 50 micron and 200 microns.

46. The method of claims 1-45 wherein the monolith is coated with a polymer.

47. The method of claims 1-45 wherein the monolith is chemically modified to increase its capacity for the hydration of its surface.

48. The method of claims 1-47 wherein the convective chromatography material surface is hydroxylated, glycosylated or polyhydroxylated.

49. The method of claims 1-48 wherein if the convective chromatography material surface includes charged moieties, then the pH, conductivity and salt concentration of the buffers delivered to the column are selected to discourage direct interaction between the target species and such charged moieties.

50. The method of claims 1-48 wherein if the convective chromatography material surface includes charged moieties, then the pH, conductivity and salt concentration of the buffers delivered to the column are selected to suspend direct interaction between the target species and such charged moieties during at least a portion of the step of contacting the sample with the constraining agent so that during that portion the target species is retained at the hydrated surface exclusively by constrained cohydration.

51. The method of claims 1-48 wherein the step of contacting the sample with the hydrated surface of the convective chromatography material is performed in the presence of a constraining agent, wherein the sample is mixed with a concentrated solution of the constraining agent immediately before the sample contacts the convective chromatography material.

52. The method of claims 1-48 wherein the step of contacting the sample with the hydrated surface of the convective chromatography material is performed by applying the sample through one inlet line, simultaneously applying concentrated constraining agent through another line, mixing the two flow streams at a mixer placed immediately before the convective chromatography material so that the pre-column residence time of the mixture is too short to permit formation of sufficient precipitates to clog the convective chromatography material or otherwise interfere with the method.

53. The method of claims 51-52 wherein the sample is combined with a buffer containing at least one constraining agent prior to delivery of the sample to the convective chromatography material and wherein such convective chromatography material is equilibrated with the constraining agent prior to such delivery of the target sample.

54. The method of claims 51-53 comprising the additional steps of washing the hydrated convective chromatography material with a solution comprising the constraining agent in an amount sufficient to remove contaminants not retained at the hydrated surface of the undissolved material, and subsequently dissociating the target species from the convective chromatography material.

55. The method of claim 54 wherein the step of dissociating the target species comprises washing the convective chromatography material with a solution where the solution either (i) does not contain the constraining agent, (ii) contains the constraining agent in an amount insufficient to retain the target species at the hydrated surface of the convective chromatography material, (iii) contains an agent which effects dissociation of the target species from the hydrated surface of the convective chromatography material, (iv) a combination of (i) and (iii), or (iv) a combination of (ii) and (iii).

56. The method of any of claims 1-55 wherein the method is performed prior to or after a method for fractionating the target species from other materials is performed and the method for fractionating is selected from the group consisting of high performance liquid chromatography, affinity chromatography, protein A chromatography, protein G chromatography, anion exchange chromatography, cation exchange chromatography, hydrophobic interaction chromatography, immobilized metal affinity chromatography, precipitation methods, polyethylene glycol precipitation, octanoic acid precipitation, centrifugation, and ultracentrifugation.

57. The method of claim 55 wherein the agent which effects dissociation of the target species from the hydrated surface of the convective chromatography material in the presence of a constraining agent is selected from the group consisting of a surfactant, a ureide, a sugar, a salt, a chelating agent, or a chaotrope.

58. The methods of claims 1-57 wherein the undissolved material possesses chemical or functional moieties which can bind with the target species under a first set of conditions and not bind with the target species under a second set of conditions and wherein the method comprises a step of retaining the target species at the hydrated surface of the undissolved material in the presence of the constraining agent under the second set of conditions.

59. The method of claim 58 wherein the first set of conditions and the second set of conditions differ only by the presence of the constraining agent under the second set of conditions.

60. The method of claims 58-59 wherein prior to the step of retaining the target species at the hydrated surface of the undissolved material under the second set of conditions, the method comprises an additional step of washing the sample under the first set of conditions while the target species is bound to the undissolved material.

61. The method of claim 60 wherein the first and second set of conditions are differentiated with respect to at least one of pH, conductivity and salt concentration.

62. A kit comprising organic nucleation centers, constraining agents configured for the convenient practice of any of the methods of claims 1 to 61.

Claims

1. A method for purification of a hydrated target species of biological origin in a sample comprising the steps of contacting the sample with a hydrated surface of an undissolved material and contacting the sample with a constraining agent in an amount sufficient to cause at least a fraction of the target species to be retained at the hydrated surface of the undissolved material under conditions providing an absence of a significant chemical attraction between the target specires and the surface of the undissolved material,

wherein the constraining agent is an aqueous soluble uncharged linear or branched non-ionic polymer.

2. The method of claim 1 wherein more than 50% of the hydrated target species is retained at the hydrated surface of the undissolved material.

3. The method of claim 2 wherein substantially all of the hydrated target species is retained at the hydrated surface of the undissolved material.

4. The methods of claims 1-3 wherein at least one individual of the hydrated target species is retained at the hydrated surface exclusively by constrained co-hydration due to the presence of at least one constraining agent.

5. The methods of claims 1-4 wherein the target species is a protein, antibody, clotting factor, cellular organelle, virus, virus-like particle, gene therapy vector or cell.

6. The methods of claims 1-5 wherein the sample is a cell culture harvest, a cell culture supernatant, a protein-containing solution derived from a cell culture, an antibody-containing solution derived from a cell culture, a virus-containing solution derived from cell culture, or a target species-containing solution from a previous stage of purification.

7. The method of claims 1-6 wherein the target species is a protein.

8. The method of claim 7 wherein the target species is a clotting factor.

9. The method of claims 1-6 wherein the target species is a polyclonal or monoclonal antibody of the class IgA, IgD, IgE, IgG, or IgM or a fragment or compound recombinant construct thereof.

10. The method of claims 1-6 wherein the target species is a prokaryotic cell, eukaryotic cell, stem cell.

11. The method of claims 1-6 wherein the target species is a virus, lipid

enveloped virus, protein capsid virus or virus like particle.

12. The method of claims 1-6 wherein the target species is an exosome, liposome, mitochondrion, chloroplast, lysosome, or other cellular organelle.

13. The method of claims 1-12 wherein the surface of the undissolved material has one or more polar chemical moieties.

14. The method of claim 13 wherein the polar chemical moiety is selected from the group of hydroxyl, amine, imine, ureide, carbohydrate, amino acid, peptide, a cationic charged group or an anionic charged group.

15. The method of claim 14 wherein the polar chemical moiety is a carbohydrate selected from the group consisting of glucose, mannose, galactose, lactose, a monosaccharaide, a disaccharide and a polysaccharide.

16. The method of claim 14 wherein the polar chemical moiety is a ureide selected from the group consisting of urea, uric acid, allantoin, hydantoin.

17. The method of claim 14 wherein the polar chemical moiety is histidine, glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, lysine, arginine, or selenocysteine.

18. The method of claims 1-17 wherein the constraining agent is selected from the group of polyethylene glycol, polypropylene glycol, polyvinylpyrrolidone, dextran, starch, cellulose.

19. The method of claim 18 wherein the constraining agent is polyethylene glycol.

20. The method of claim 19 wherein the polyethylene glycol has an average polymer weight between 100 and 10,000 D.

21. The method of claim 20 wherein the polyethylene glycol has an average polymer weight between 600 and 8,000 D.

22. The method of claims 19-21 wherein the polyethylene glycol is provided at a concentration between 2% and 50% (w/v).

23. The method of claim 22 wherein the polyethylene glycol is provided at a concentration between 2% and 25% (w/v) and the polyethylene glycol has an average polymer weight above 6,000 D.

24. The method of claim 22 wherein the polyethylene glycol is provided at a concentration between 15% and 50% (w/v) and the polyethylene glycol has an average polymer weight below 6,000 D.

25. The method of claim 22 wherein the polyethylene glycol is provided at a concentration between 2% and 25% (w/v) and the polyethylene glycol has an average polymer weight between 4,000 D and 8000D.

26. The method of claims 1-25 wherein the undissolved material constitutes organic nucleation centers.

27. The method of claim 26 wherein the organic nucleation center comprises a carbohydrate, ureide or a peptide.

28. The method of claim 27 wherein the ureide is allantoin.

29. The method of claim 27 wherein the carbohydrate is starch or cellulose.

30. The method of claim 28 wherein the organic nucleation center comprises a core to which an outer layer of the target species is bound by chemical or electrostatic forces of attraction or retained by constrained co-hydration where such outer layer of target species provides the hydrated surface of the undissolved material.

31. The method of claim 29 wherein the organic nucleation center retains multiple layers of the target species solely by constrained co-hydration.

32. The method of claims 27-31 wherein the sample is contacted with the organic nucleation center prior to the step of contacting the sample with the constraining agent.

33. The method of claim 32 comprising the additional steps of separating the supernatant containing contaminants from the organic nucleation centers having the target species retained at the hydrated surface of the organic nucleation centers.

34. The method of claim 33 comprising the additional step of recovering the target species from the organic nucleation center by exposing the organic nucleation centers to conditions which promote the dissociation of the target species from the organic nucleation center.

35. The method of claim 34 wherein the target species is dissociated from the organic nucleation center by substantially reducing the concentration of the constraining agent.

36. The method of claims 34-35 comprising the step of contacting the

organic nucleation center with a solution which does not contain the constraining agent.

37. The method of claims 34-36 wherein the salt concentration is increased sufficiently to reduce the effectiveness of the constraining agent to retain the target species at the hydrated surface of the organic nucleation center.

38. The method of claims 34-36 wherein the sugar concentration is increased sufficiently to reduce the ability of the constraining agent to constrain the target species at the hydrated surface of the organic nucleation center.

39. The method of claim 38 wherein the sugar is selected from the group consisting of sorbitol, xylitol, and sucrose and the constraining agent is polyethylene glycol.

40. The methods of claims 34-39 wherein method comprises one or more additional steps of washing the organic nucleation centers prior to dissociating the target species from the organic nucleation centers.

41. The methods of claims 34-40 wherein the solutions used in the steps of dissociating the target species from the organic nucleation centers are substantially the same as the solutions used for the recovery of the target species.

42. A kit comprising organic nucleation centers, constraining agents configured for the convenient practice of any of the methods of claims 1-41.

43. The method of claims 1-25 wherein the undissolved material comprises particles.

44. The method of claims 42-43 wherein the particles are nanoparticles or microparticles.

45. The method of claims 43-44 wherein the particles are magnetic, paramagnetic or high-density.

46. The method of claims 43-45 wherein the particles comprise a material selected from the group consisting of metal-core particles having a polymer coating, metal core particles having a cellulose coating, glass particles, polyacrylate, polymethacrylate, styrenedivinylbenzene, thiophilic magnetic particles, cellulose coated tungsten carbide particles, silica particles, agarose particles, cellulose particles, and composite particles.

47. The method of claims 43-46 wherein the particle size is between 100nm and 500μm.

48. The method of claim 47 wherein the particle size is between 100nm and 50μm.

49. The method of claim 47 wherein the particle size is between 100nm and 4μm.

50. The method of claim 47 wherein the particle size is between 100nm and 3μm.

51. The method of claim 47 wherein the particle size is between 100nm and 1μm.

52. The method of claim 47 wherein the particle size is between 200nm and 2μm.

53. The method of claim 47 wherein the particle size is between 200nm and 500nm.

54. The method of claim 47 wherein the particle size is between 500nm and 1μm.

55. The method of claim 47 wherein the particle size is between 5μm and 50μm.

56. The method of claim 43 wherein the step of contacting the target sample with the particles occurs prior to the step of containing the sample with the constraining agent.

57. The method of claims 43-56 comprising the additional steps of separating the particles with the target species associated with the hydrated surface of the particles from the liquid phase and dissociating the target species from the particles.

58. The method of claims 43-57 wherein if the surface of the particles includes charged moieties, then the pH, conductivity and salt concentration of the buffers delivered to the column are selected to discourage direct interaction between the target species and such charged moieties.

59. The method of claims 43-57 wherein if the convective chromatography material surface includes charged moieties, then the pH, conductivity and salt concentration of the buffers delivered to the column are selected to suspend direct interaction between the target species and such charged moieties during at least a portion of the step of contacting the sample with the constraining agent so that during

that portion the target species is retained at the hydrated surface exclusively by constrained cohydration.

60. The method of claim 57 wherein the step of dissociating the target species comprises washing the particles with a solution where the solution either (i) does not contain the constraining agent, (ii) contains the constraining agent in an amount insufficient to retain the target species at the hydrated surface of the particles, (iii) contains an agent which effects dissociation of the target species from the hydrated surface of the particles, (iv) a combination of (i) and (iii), or (iv) a combination of (ii) and (iii).

61. The method of claim 60 wherein the agent which effects dissociation of the target species from the hydrated surface of the particles in the presence of a constraining agent is selected from the group consisting of a surfactant, a ureide, a sugar, a salt, a chelating agent, or a chaotrope.

62. The method of claims 43-61 wherein the constraining agent is added to the sample and the particles over a period of time from one minute to five hours; between 2 min and 15 min; between 2 min and 30 min; between 10 min and 30 min; between 2 min and 2 hours; or between 2 min and 1 hour.

63. The method of claims 43-62 wherein the particles are separated from the liquid component of the mixture by centrifugation, sedimentation, decantation, subjecting the mixture to a magnetic field, or by filtration.

64. The method of claim 63 wherein the separated particles are washed with a solution comprising the constraining agent.

65. The methods of claims 57-64 wherein the step of dissociating is carried out in a single step of adding a dissociating buffer to the particles.

66. The methods of 57-64 wherein the step of dissociating the target species is carried out incrementally through the reduction of the concentration of the constraining agent.

67. The method of claims 43-66 wherein the method is performed prior to or after a method for fractionating the target species from other materials is performed and the method for fractionating is selected from the group consisting of high performance liquid chromatography, affinity chromatography, protein A chromatography, protein G chromatography, anion exchange chromatography, cation exchange chromatography, hydrophobic interaction chromatography, immobilized metal affinity chromatography, precipitation methods, polyethylene glycol precipitation, octanoic acid precipitation, centrifugation, and ultracentrifugation.

68. The method of claims 1-25 wherein the undissolved material is a convective chromatography material.

69. The method of claim 68 wherein the convective chromatography material is selected from the group consisting of a monolith, a membrane, and nonporous particles.

70. The method of claim 69 wherein the convective chromatography material comprises nonporous silica particles packed in a column.

71. The method of claim 69 wherein the convective chromatography material is a monolith.

72. The method of claim 71 wherein the monolith is comprised of a material selected from the group consisting of polyacrylate, polymethacrylate, styrenedivinylbenzene and silica.

73. The method of claims 71-72 wherein the monolith comprises average channel size between 1 micron and 200 micron.

74. The method of claim 73 wherein the channel size is between 1 micron and 5 microns.

75. The method of claim 73 wherein the channel size is between 10 micron and 20 microns.

76. The method of claim 73 wherein the channel size is between 50 micron and 200 microns.

77. The method of claims 71-76 wherein the monolith is coated with a polymer.

78. The method of claims 71-76 wherein the monolith is chemically modified to increase its capacity for the hydration of its surface.

79. The method of claims 68-78 wherein the convective chromatography material surface is hydroxylated, glycosylated or polyhydroxylated.

80. The method of claims 68-79 wherein if the convective chromatography material surface includes charged moieties, then the pH, conductivity and salt concentration of the buffers delivered to the column are selected to discourage direct interaction between the target species and such charged moieties.

81. The method of claims 68-79 wherein if the convective chromatography

material surface includes charged moieties, then the pH, conductivity and salt concentration of the buffers delivered to the column are selected to suspend direct interaction between the target species and such charged moieties during at least a portion of the step of contacting the sample with the constraining agent so that during that portion the target species is retained at the hydrated surface exclusively by constrained cohydration.

82. The method of claims 68-79 wherein the step of contacting the sample with the hydrated surface of the convective chromatography material is performed in the presence of a constraining agent.

83. The method of claims 68-79 wherein the step of contacting the sample with the hydrated surface of the convective chromatography material is performed by applying the sample through one inlet line, simultaneously applying concentrated constraining agent through another line, mixing the two flow streams at a mixer placed immediately before the convective chromatography material so that the pre-column residence time of the mixture is too short to permit formation of sufficient precipitates to clog the convective chromatography material or otherwise interfere with the method.

84. The method of claims 82-83 wherein the sample is combined with a buffer containing at least one constraining agent prior to delivery of the sample to the convective chromatography material and wherein such convective chromatography material is equilibrated with the constraining agent prior to such delivery of the target sample.

85. The method of claims 82-84 comprising the additional steps of washing the hydrated convective chromatography material with a solution comprising the constraining agent in an amount sufficient to remove contaminants not retained at the hydrated surface of the undissolved material, and subsequently dissociating the target species from the convective chromatography material.

86. The method of claim 85 wherein the step of dissociating the target species comprises washing the convective chromatography material with a solution where the solution either (i) does not contain the constraining agent, (ii) contains the constraining agent in an amount insufficient to retain the target species at the hydrated surface of the convective chromatography material, (iii) contains an agent which effects dissociation of the target species from the hydrated surface of the convective chromatography material, (iv) a combination of (i) and (iii), or (iv) a combination of (ii) and (iii).

87. The method of any of claims 68-86 wherein the method is performed prior to or after a method for fractionating the target species from other materials is performed and the method for fractionating is selected from the group consisting of

high performance liquid chromatography, affinity chromatography, protein A chromatography, protein G chromatography, anion exchange chromatography, cation exchange chromatography, hydrophobic interaction chromatography, immobilized metal affinity chromatography, precipitation methods, polyethylene glycol precipitation, octanoic acid precipitation, centrifugation, and ultracentrifugation.

88. The method of claim 86 wherein the agent which effects dissociation of the target species from the hydrated surface of the convective chromatography material in the presence of a constraining agent is selected from the group consisting of a surfactant, a ureide, a sugar, a salt, a chelating agent, or a chaotrope.

89. The methods of claims 1-88 wherein the undissolved material possesses chemical or functional moieties which can bind with the target species under a first set of conditions and not bind with the target species under a second set of conditions and wherein the method comprises a step of retaining the target species at the hydrated surface of the undissolved material in the presence of the constraining agent under the second set of conditions.

90. The method of claim 89 wherein the first set of conditions and the second set of conditions differ only by the presence of the constraining agent under the second set of conditions. .

91. The method of claims 89-90 wherein prior to the step of retaining the target species at the hydrated surface of the undissolved material under the second set of conditions, the method comprises an additional step of washing the sample under the first set of conditions while the target species is bound to the undissolved material.

92. The method of claim 91 wherein the first and second set of conditions are differentiated with respect to at least one of pH, conductivity and salt concentration.