

WHAT IS CLAIMED:

1. A method of conducting direct detection ^1H solid state NMR ("ssNMR") on a macromolecule-containing solid state formulation, the method comprising:
 - (a) equilibrating a solid state formulation comprising a macromolecule at a first temperature;
 - (b) conducting a ^1H spin-lattice relaxation time (" T_1 ") experiment on the solid state formulation at the first temperature using direct detection ^1H ssNMR to generate at least three free induction decay ("FID") plots at the first temperature, wherein the T_1 experiment comprises a saturation recovery sequence having at least three variable delay times from which each FID plot is generated;
 - (c) equilibrating the solid state formulation at a second temperature, and repeating step (b) at the second temperature to generate at least three FID plots at the second temperature;
 - (d) equilibrating the solid state formulation at a third temperature, and repeating step (b) at the third temperature to generate at least three FID plots at the third temperature;
 - (e) generating a saturation recovery curve at each temperature; and
 - (f) fitting each saturation recovery curve to a nonlinear regression equation to generate a T_1 value at each temperature.
2. The method of claim 1, further comprising:
 - (g) converting each T_1 value to ^1H spin-lattice relaxation rate (" R_1 "), and
 - (h) plotting R_1 versus temperature to generate a relaxation rate curve for the solid state formulation.
3. The method of claim 2, further comprising analyzing the relaxation rate curve to determine the molecular mobility of the macromolecule in the solid state formulation, the degree of aggregation in the solid state formulation, and/or the stability of the solid state formulation.
4. The method of any one of claims 1-3, further comprising repeating step (d) at 5 or more additional temperatures.
5. The method of claim 4, wherein step (d) is repeated at 10 or more additional temperatures.

6. The method of claim 5, wherein step (d) is repeated at 25 or more additional temperatures.
7. The method of any one of claims 1-6, wherein each temperature is in a range from about -200 °C to about 150 °C.
8. The method of claim 7, wherein each temperature is in a range from about -100 °C to about 150 °C.
9. The method of claim 8, wherein each temperature is in a range from about -50 °C to about 150 °C.
10. The method of any one of claims 1-9, wherein the highest and lowest temperatures have a difference of at least about 25 °C.
11. The method of any one of claims 1-10, wherein the lowest temperature is in a range from about -200 °C to about 25 °C.
12. The method of claim 11, wherein the lowest temperature is in a range from about -100 °C to about 0 °C.
13. The method of claim 12 wherein the lowest temperature is in a range from about -50 °C to about -30 °C.
14. The method of any one of claims 1-13, wherein the T_1 experiment comprises baseline suppression.
15. The method of any one of claim 1-14, wherein in each equilibrating step the solid state formulation is held at the temperature for a duration in the range of about one minute to about one hour before conducting the T_1 experiment.
16. The method of claim 15, wherein in each equilibrating step the solid state formulation is held at the temperature for a duration in the range of about one minute to about 10 minutes before conducting the T_1 experiment.
17. The method of claim 16, wherein the solid state formulation is held at the temperature for about five minutes before conducting the T_1 experiment.
18. The method of any one of claims 1-17, wherein said method excludes one or both of retuning and recalibrating the ssNMR probe after equilibrating at the first temperature.

19. The method of any one of claims 1-18, wherein each T_1 experiment comprises at least five variable delay times at each temperature to generate at least five FID plots at each temperature.

20. The method of claim 19, wherein each T_1 experiment comprises nine variable delay times at each temperature to generate nine FID plots at each temperature.

21. The method of any one of claims claim 1-20, wherein the saturation recovery curve is generated by:

(a) plotting the signal intensity of each of the at least three FID plots versus delay time;

or

(b) Fourier transforming each of the at least three FID plots to generate a plot of intensity versus frequency; and

(i) plotting peak height versus delay time; or

(ii) plotting integral peak intensity versus delay time.

22. The method of claim 21, wherein the saturated recovery curve is generated by plotting the signal intensity of the FID plot versus delay time.

23. The method of any one of claims 1-22, wherein the T_1 experiment comprises a magnetic field having a frequency in a range from about 200 MHz to about 1.2 GHz.

24. The method of claim 23 wherein the frequency range is from about 300 MHz to about 1 GHz.

25. The method of claim 24, wherein the frequency is about 500 MHz.

26. The method of any one of claims 1-25, wherein the T_1 experiment comprises magic angle spinning.

27. The method of any one of claims 1-26, wherein all of the T_1 experiments are conducted within a time period of up to 48 hours.

28. The method of claim 27, wherein all of the T_1 experiments are conducted within a time period of up to 24 hours.

29. The method of claim 28, wherein all of the T_1 experiments are conducted within a time period of up to 12 hours.

30. The method of claim 29, wherein all of the T_1 experiments are conducted within a time period of up to 6 hours.

31. The method of any one of claims 1-30, wherein macromolecule is a biologic molecule.

32. The method of claim 31, wherein the biologic molecule is a protein.

33. The method of claim 32, wherein the protein is an antibody or a bispecific antibody construct.

34. The method of any one of claims 1-33, wherein the solid state formulation is a lyophilized formulation.

35. The method of claim 34, wherein the fitting of step (f) is monoexponential.

36. The method of claim 34 or 35, wherein the highest temperature is in a range from about 50 °C to about 150 °C.

37. The method of claim 36, wherein the highest temperature is in a range from about 100 °C to about 150 °C.

38. The method of any one of claims 34-37, wherein the highest and lowest temperatures have a difference of at least about 50 °C.

39. The method of any one of claims 34-38, wherein the T_1 experiment comprises a variable delay period in a range from about 0.01 seconds to about 60 seconds.

40. The method of any one of claims 1-33, wherein the solid state formulation is a frozen formulation.

41. The method of claim 40, wherein the fitting of step (f) is biexponential.

42. The method of claim 40 or 41, wherein the highest temperature is from about -15 °C to about 0 °C.

43. The method of claim 42, wherein the highest temperature is from about -15 °C to about -10 °C.

44. The method of any one of claims 40-43, wherein the highest and lowest temperatures have a difference of at least about 40 °C.

45. The method of any one of claims 40-44, wherein each T_1 experiment comprises nine variable delay times at each temperature to generate nine FID plots at each temperature.

46. The method of any one of claims 40-45, wherein the T_1 experiment comprises a variable delay period in a range from about 0.1 seconds to about 240 seconds.

47. The method of claim 1, wherein the solid state formulation is a lyophilized formulation and:

the saturation recovery curve is generated by plotting the signal intensity of each of the at least three FID plots versus delay time;

the T_1 experiment comprises baseline suppression and magic angle spinning;

steps (d)-(f) are repeated at 15 or more temperatures;

each temperature is in a range from about -50 °C to about 150 °C;

the highest and lowest temperatures have a difference from about 75 °C to about 100 °C;

in each equilibrating step the solid state formulation is held at the temperature for a duration in the range of about one minute to about ten minutes before conducting the T_1 experiment;

the method excludes retuning and recalibrating the ssNMR probe after equilibrating at the first temperature;

the variable delay period is in a range from about 0.1 seconds to about 60 seconds; and

each T_1 experiment comprises six variable delay times at each temperature to generate six FID plots at each temperature.

48. The method of claim 1, wherein the solid state formulation is a frozen formulation and:

the saturation recovery curve is generated by plotting the signal intensity of each of the at least three FID plots versus delay time;

the T_1 experiment comprises baseline suppression and magic angle spinning;

steps (d)-(f) are repeated at 25 or more temperatures;

each temperature is in a range from about -50 °C to about 0 °C;

the highest and lowest temperatures have a difference from about 25 °C to about 40 °C;
in each equilibrating step the solid state formulation is held at the temperature for a duration in the range of about one minute to about ten minutes before conducting the T_1 experiment;

the method excludes retuning and recalibrating the ssNMR probe after equilibrating at the first temperature;

the variable delay period is in a range from about 0.01 seconds to about 240 seconds;
and

each T_1 experiment comprises nine variable delay times at each temperature to generate nine FID plots at each temperature.

49. The method of claim 47 or 48 further comprising:

(g) converting each T_1 value to ^1H spin-lattice relaxation rate (" R_1 "), and

(h) plotting R_1 versus temperature to generate a relaxation rate curve for the solid state formulation.

50. The method of claim 49, further comprising analyzing the relaxation rate curve to determine the molecular mobility of the macromolecule in the solid state formulation, the degree of aggregation in the solid state formulation, and/or the stability of the solid state formulation.

51. The method of any one of claims 47-50, wherein the macromolecule is a biologic molecule.

52. The method of claim 51, wherein the biologic molecule is a protein.

53. A method of selecting a macromolecule-containing solid state formulation among a group of test macromolecule-containing solid state formulations, the method comprising:

(I) generating a relaxation rate curve for each macromolecule-containing solid state formulation in the group of test macromolecule-containing solid state formulations; wherein the relaxation rate curve for each macromolecule-containing solid state formulation is generated by:

(a) equilibrating the solid state formulation at a first temperature;

(b) conducting a ^1H spin-lattice relaxation time (" T_1 ") experiment on the solid state formulation at the first temperature using direct detection ^1H ssNMR to generate at least three free induction decay ("FID") plots at the first

temperature, wherein the T_1 experiment comprises a saturation recovery sequence having at least three variable delay times from which each FID plot is generated;

- (c) equilibrating the solid state formulation at a second temperature, and repeating step (b) at the second temperature to generate at least three FID plots at the second temperature;
- (d) equilibrating the solid state formulation at a third temperature and repeating step (b) at the third temperature to generate at least three FID plots at the third temperature;
- (e) generating a saturation recovery curve at each temperature;
- (f) fitting each saturation recovery curve to a nonlinear regression equation to generate a T_1 value at each temperature;
- (g) converting each T_1 value to ^1H spin-lattice relaxation rate (" R_1 "), and
- (h) plotting R_1 versus temperature to generate a relaxation rate curve for the solid state formulation;

(II) comparing the maximum R_1 peak value of each curve, the temperature of the maximum R_1 peak of each curve, the width of the maximum R_1 peak of each curve, or a combination thereof; and

(III) selecting the solid state formulation which has the lowest maximum R_1 peak value, the highest temperature of the maximum R_1 peak, or the narrowest R_1 peak width.

54. A method of selecting a formulation excipient for use in a macromolecule-containing solid state formulation, the method comprising:

- (I) generating a relaxation rate curve for each macromolecule-containing solid state formulation in a group of test macromolecule-containing solid state formulations, each formulation having a different composition of excipients, a different amount of one or more excipients, or both; wherein the relaxation rate curve for each macromolecule-containing solid state formulation is generated by:
 - (a) equilibrating the solid state formulation at a first temperature;
 - (b) conducting a ^1H spin-lattice relaxation time (" T_1 ") experiment on the solid state formulation at the first temperature using direct detection ^1H ssNMR

- to generate at least three free induction decay ("FID") plots at the first temperature, wherein the T_1 experiment comprises a saturation recovery sequence having at least three variable delay times from which each FID plot is generated;
- (c) equilibrating the solid state formulation at a second temperature, and repeating step (b) at the second temperature to generate at least three FID plots at the second temperature;
 - (d) equilibrating the solid state formulation at a third temperature, and repeating step (b) at the third temperature to generate at least three FID plots at the third temperature;
 - (e) generating a saturation recovery curve at each temperature;
 - (f) fitting each saturation recovery curve to a nonlinear regression equation to generate a T_1 value at each temperature;
 - (g) converting each T_1 value to ^1H spin-lattice relaxation rate (" R_1 "), and
 - (h) plotting R_1 versus temperature to generate a relaxation rate curve for the solid state formulation;
- (II) comparing the maximum R_1 peak value of each curve, the temperature of the maximum R_1 peak of each curve, the width of the maximum R_1 peak of each curve, or a combination thereof; and
- (III) selecting an excipient that is present in the solid state formulation that has the lowest maximum R_1 peak value, the highest temperature of the maximum R_1 peak, or the narrowest R_1 peak width.