and complex sample illumination, limiting that capabilities of iSCAT for the robust and accurate detection, imaging and characterisation of particles as small as single molecules. However, improvements to the instrument have been developed, such that this technology has evolved into a powerful means for looking at single objects. An exemplary design of an iSCAT instrument is described in Cole *et al* ACS Photonics, 2017, 4 (2), pp 211–216 and Arroyo et al. Nat Protocols 2016, 617-633, both herein incorporated by reference. Further details about the instrument are provided in an earlier application by the present applicant, WO2018/011591, herein incorporated by reference.

iSCAT has been described previously for detection of purified single proteins (Cole *et al* (ACS Photonics, 2017, 4(2), pp 211-216), but the detection and quantification of lipoproteins has not previously been explored, since detection of particles in a complex solution was not thought to be feasible.

Diagnostic assays for cardiovascular disease may include detection of lipoproteins (also described as lipoprotein particles). Various methods for detection of lipoproteins are reviewed in Circulation. 2009 May 5; 119(17): 2396–2404; Curr Opin Lipidol. 2017 Jun;28(3):261-266. These methods have disadvantages of being time-consuming, expensive, providing incomplete information on lipoprotein particles and/or having limited application to clinical settings.

SUMMARY

The present inventors have surprisingly identified that is possible to use single particle light scattering, preferably single particle interferometric scattering microscopy to determine the concentration of a particle in solution. Additionally, the present inventors have unexpectedly illustrated that it is possible to determine the concentration of particles by single particle light scattering (such as by using iSCAT) directly from a biological sample (such as blood or blood plasma) without purification of the particles away from other components, despite the background signal provided by these other components. This has proven to be a particularly useful technique for lipoproteins in biological samples, for example.

In one aspect of the present disclosure, there is provided a method of measuring the concentration of a particle in solution, comprising contacting the solution with a surface and detecting the binding of said particle to the surface using light scattering.

In another aspect of the present disclosure, there is provided a method for detection of lipoprotein particles in a sample, comprising detecting said particles by light scattering, optionally interferometric scattering microscopy.

In a further aspect of the present disclosure, there is provided a method for diagnosing a disease or condition associated with size and/or number of lipoprotein particles in an individual, or for determining the risk that the individual will develop said disease or condition, comprising detecting the size and/or number of lipoprotein particles in a sample obtained from said individual by light scattering, optionally by interferometric scattering microscopy.

In another aspect of the present disclosure, there is provided a method of selecting an individual to whom a substance or composition is to be administered, or to whom a regimen is to be prescribed, wherein said substance or composition or regimen is suitable for treating or preventing a disease or condition associated with size and/or number of lipoprotein particles, comprising detecting the size and/or number of lipoprotein particles in a sample from said individual by a method disclosed herein, and selecting said patient for said administration or said regimen if the size and/or number of lipoprotein particles detected is indicative of the presence of said disease or condition, or of a risk thereof.

In a further aspect of the present disclosure, there is provided a substance or composition for use in treating or preventing a disease or condition associated with size and/or number of lipoprotein particles in an individual, wherein said individual is diagnosed or determined at risk or selected by a method disclosed herein.

In another aspect of the present disclosure, there is provided use of a substance or composition in the manufacture of a medicament for treatment or prevention of a disease or condition associated with size and/or number of lipoprotein particles in an individual, wherein said individual is diagnosed or determined at risk or selected by a method disclosed herein.

The present disclosure provides a method of measuring the concentration of a particle in solution, comprising contacting the solution with a surface and detecting the binding of said particle to the surface using light scattering.

The light scattering method for detection of the particle binding is preferably iSCAT. The method may preferably be performed using a suitable microscope. The concentration is preferably absolute concentration. The binding of the particle to said surface may be detected or visualised using single particle light scattering.

The present disclosure provides a method for determining the concentration of a particle in solution comprising contacting said solution with a measuring solution and a surface, and detecting the binding of the particle to said surface using light scattering.

This feature of the present disclosure is particularly useful for situations in which the solution is thought to be concentrated. A volume of the solution containing the particle may be included with a volume of a measuring solution in a sample holder prior to detection of the binding of the particle to a surface. The advantage of this example is to ensure that the concentration measured represents the state of the particle in the original, more concentrated solution.

The present disclosure provides a method for determining the concentration of a particle in solution comprising contacting the solution with a surface in the presence of a calibrant and detecting the binding of the particle to the surface using light scattering.

Therefore, the present disclosure enables the concentration measurement of a particle in solution by means of detecting the binding rate of said particle to a surface.

Further, the present disclosure enables absolute concentration measurement of a particle in solution by analysis of the changes or decay in binding rate of the particle to a surface as detected by light scattering over time.

Absolute concentration in solution may be determined by measuring binding events between particles in the sample and a surface, and be calibrated against those observed with samples of particles of known concentrations. The surface may be part of a sample holder. , In a sample holder with a high surface to volume ratio the binding of a particle to the surface decreases over time as a function of the remaining number of particles in solution and accessible sites for binding. Initial binding rates for a series of concentrations may be extrapolated from the binding data and used to generate a standard curve allowing

for conversion of a measured initial binding rate in the sample to the respective absolute concentration of the particles in solution.

The binding of the particle to the surface may be detected or visualised using light scattering, preferably iSCAT.

Alternatively, concentration in solution may be determined by use of a passivated or activated surface, such that the binding rate of particles can be controlled by controlling the binding activity between the surface and the particle. In this setting, surface activation or passivation can either increase binding affinity and thus the measurement range to lower concentrations, or lower the binding affinity and therefore be able to measure at higher concentrations.

The surface preferably forms part of a sample holder for said solution. Said sample holder may be an element of a light scattering microscope. The sample holder may be a high surface-to-volume chamber.

In order to calculate the concentration, it may be necessary to include correction for diffusion rates. Diffusion rates can be readily calculated based on known properties of the particle, such as mass and shape.

Therefore, the present disclosure provides a method for measuring the concentration of particle a solution, the method comprising:

i) contacting the solution with a surface;

ii) detecting the particle binding to the surface as visualised by light scattering;

iii) repeating the detection step and calculating changes in binding rate and the initial binding rate over time of the particle to the surface;

iv) providing a calibration curve of initial binding rate against concentration based on data from solutions of known particle concentration;

v) using the calibration curve in step (iv) to convert the initial binding rate recorded for the sample in step (iii) to the concentration of the particle in solution.

Preferably, the concentration is absolute concentration.

In some variations, the solution may be pre-treated before or simultaneously with contact to the surface, for example with a measuring solution or calibrant.

The particle in solution may be contacted with a measuring solution and a surface.

The advantage of this example is to ensure that the concentration measured represents the state of the particle in the original, more concentrated solution.

The measuring solution may be a buffering solution. Generally, the volume of the measuring solution is known in order to enable calculation of dilution effects.

Various techniques may be required to prepare the solution for concentration determination. For example, a volume of the solution may be placed into a sample holder, preferably with a known geometry. This enables the introduction of a volume of a measuring solution (or introduction of the particle solution into the measuring solution), and using dilution behaviour, this allows the detected binding rate of the particle to the surface to be converted to concentration of the particle.

The detection of the binding of the particle to the surface may be taken at one or more different time intervals following the introduction of the measuring solution. If the detection step is repeated after introduction of measuring solution at different time intervals, this will allow for correction for dilution effects, such as dissociation. If the measurements are taken at different time point after addition (and thus dilution) a change in, for example, species distribution if the particle dissociates or clusters at lower concentrations, can be visualised. This approach, therefore, allows a more detailed view of the particle and enables the verification of the concentration measurement.

Alternatively or additionally, the volume of one or both of the solutions (particle or measuring) can be varied, and/or the geometry of the sample holder can be varied. Both allow for correction of dilution effects. If the detection step is repeated in one or more different solution volumes and/or in sample holders of one or more different geometries this can also correct for dilution effects such as dissociation.

Therefore, the present disclosure provides a method for determining the concentration of a particle in solution, the method comprising:

i) contacting the solution with a surface and a volume of measuring solution in a sample holder with a geometry;

ii) detecting the particle binding to the surface as visualised by light scattering;

iii) repeating the detection step and calculating changes in binding rate and the initial binding rate over time of the particle to the surface;

iv) providing a calibration curve of initial binding rate against concentration based on data from solutions of known particle concentration;

v) using the calibration curve in step (iv) to convert the initial binding rate recorded for the sample in step (iii) to the absolute concentration of the particle in solution.

The method may further involve repeating steps (i) to (ii) using one or more of a sample holder of different geometry or using a different volume of measuring solution in order to verify the determined concentration.

Alternatively, particle in solution may be contacted with a surface in the presence of a calibrant.

The calibrant in solution has a pre-determined concentration and mass. Once it is introduced into the particle solution, the detection of the binding rate of the calibrant and the particle to said surface occurs separately but simultaneously. Repeated measurements of both the particle and the calibrant binding to the surface may be taken, allowing for the determination of the initial binding rate for the particle and the initial binding rate for the calibrant. The initial binding rate of the known concentration of calibrant permits the conversion of the initial binding rate of the particle to concentration of said particle. In one embodiment, the calibrant is selected to resemble the particle with respect to its characteristics in binding to the surface. For example, it is desirable to select a calibrant with similar surface properties (e.g. charge, hydrophobicity, hydrophilicity). Such selection allows more precise measurements over a variety of particles. Effectively, the calibrant and the particle should have the same affinity for the surface, and therefore the determination of the binding rate of the known concentration of calibrant permits the conversion of the binding rate of the particle into a concentration.

Therefore, the present disclosure provides a method for measuring the concentration of particle a solution, the method comprising:

i) contacting the solution with a surface in the presence of a calibrant;

ii) detecting the particle binding to the surface as visualised by light scattering;

iii) simultaneously detecting the calibrant binding to the surface as visualised by light scattering;

iv) repeating the detection steps (ii) and (iii) and calculating changes in binding rate and the initial binding rate over time of the particle and the calibrant to the surface;

v) using the initial binding rates calculated in step (iv) for the calibrant to convert the initial binding rate of the particle calculated in step (iv) to concentration of the particle in solution.

It is preferred that the calibrant is of known concentration, and has similar characteristics to the particle, particularly in respect of binding to the surface.

Furthermore, the present inventors have surprisingly identified that is possible to detect lipoprotein particles, which represent heterogeneous mixtures of different biomolecules including various non-protein components, by light scattering, in particular using interferometric scattering microscopy. Single lipoprotein particles are able to be visualised optically. This allows for numbers and size of lipoprotein particles (and of different classes of lipoprotein particles) to be determined directly in a sample. The relative proportions of different lipoprotein particles in a sample can also be determined in a single measurement. The method of detection of the present disclosure thus provides a rapid and simple means of detecting lipoprotein particles and provides extensive information on the nature and distribution of lipoprotein particles in the sample. The optical detection carried out is also relatively inexpensive and advantageously suited to clinical settings, and avoids complex processing protocols associated with previous methods for detection of lipoproteins.

Additionally, the present inventors have unexpectedly illustrated that it is possible to detect lipoprotein particles by light scattering (such as by using iSCAT) directly from a biological sample (such as blood or blood plasma) without purification of the particles away from other components, despite the background signal provided by these other components.

The method of detection of the present disclosure is advantageously employed in clinical settings for detection of lipoprotein particles from patient samples, to assist diagnosis and treatment of diseases and conditions associated with size and/or number of lipoprotein particles.

The present disclosure therefore provides a method for detection of lipoprotein particles in a sample, comprising detecting said particles by light scattering, preferably by interferometric scattering microscopy. The present disclosure further provides a method for diagnosing a disease or condition associated with size and/or number of lipoprotein

particles in an individual, or for determining the risk that the individual will develop said disease or condition, comprising detecting the size and/or number of lipoprotein particles in a sample from said individual by interferometric scattering microscopy.

The present disclosure additionally provides a method for selecting an individual to whom a substance or composition is to be administered, or to whom a regimen is to be prescribed, wherein said substance or composition or regimen is suitable for treating or preventing a disease or condition associated with size and/or number of lipoprotein particles, comprising detecting the size and/or number of lipoprotein particles in a sample from sapid individual by a method for detection of the present disclosure, and selecting said patient for said administration or said regimen if the size and/or number of lipoprotein particles detected is indicative of the presence of said disease or condition, or of a risk thereof.

The present disclosure also provides a method of treating or preventing a disease or condition associated with size and/or number of lipoprotein particles in an individual, the method comprising diagnosing or determining risk of said disease or condition in said individual or selecting said individual by a method of the present disclosure, and administering a substance or composition to the individual, or carrying out a regimen thereon, which is effective to treat or prevent said disease or condition in the individual.

The present disclosure further provides a substance or composition for use in a method of treating or preventing a disease or condition associated with size and/or number of lipoprotein particles in an individual, wherein said individual is diagnosed or determined at risk or selected by a method of the present disclosure.

The present disclosure also provides use of a substance or composition in the manufacture of a medicament for treatment of prevention of a disease or condition associated with size and/or number of lipoprotein particles in an individual, wherein said individual is diagnosed or determined at risk or selected by a method of the present disclosure.

BRIEF DESCRIPTION OF FIGURES

Figure 1 provides a chart depicting different types of lipoprotein particles present in human blood and their respective diameters (nm) and density (g/ml). The table also provides information on the relative proportions of proteins, cholesterol, phospholipids and triglycerides in each type of particle.

CLAIMS

1. A method of measuring the concentration of a particle in solution, comprising contacting the solution with a surface and detecting the binding of said particle to the surface using light scattering.

2. A method as claimed in claim 1, wherein said surface is passivated, activated, coated, treated or derivatised.

3. A method as claimed in claim 1 or claim 2, wherein the detection binding of the particle to the surface is repeated after one or more time intervals.

4. A method as claimed in claim 3, wherein the repeated measurements permit calculation of the initial rate of binding of the particle to the surface.

5. A method as claimed in claim 4, wherein the initial binding rate of the particle is compared to the initial binding rate for a known concentration of the particle.

6. A method as claimed in any one of claims 1 to 3 wherein the measurements permit calculation of the constant binding rate of the particle, and optionally this is compared to the constant binding rate for a known concentration of the particle.

7. A method as claimed in claim 1, for measuring the concentration of particle a solution, said method comprising:

i) contacting the solution with a surface;

ii) detecting the particle binding to the surface as visualised by light scattering;

iii) repeating the detection step and calculating the changes in binding rate and/or initial binding rate over time of the particle to the surface;

iv) providing a calibration curve of initial binding rate against concentration based on data from solutions of known particle concentration;

v) using the calibration curve in step (iv) to convert the initial binding rate recorded for the sample in step (iii) to the concentration of the particle in solution.

8. The method as claimed in any preceding claim, wherein the concentration is absolute concentration.

9. The method as claimed in any preceding claim, wherein said particle is also contacted with a measuring solution.

10. The method as claimed in any one of claims 1 to 4, wherein said solution is contacted with said surface in the presence of a calibrant and the binding of said calibrant to said surface is detected using light scattering.

11. A method for detection of lipoprotein particles in a sample, comprising detecting said particles by light scattering, optionally interferometric scattering microscopy.

12. The method of claim 11, wherein said detecting comprises determining the size and/or number of said particles.

13. The method of claim 11 or 12, comprising detecting the size and/or number of more than one different type of lipoprotein particle in said sample, and optionally detecting the ratio of different types of lipoprotein particle in said sample.

14. The method of any one of claims 11 to 13, comprising detecting high density lipoprotein (HDL) particles and/or low density lipoprotein (LDL) particles.

15. The method of any one of claims 11 to 14, comprising determining the ratio of HDL to LDL particles in said sample.

16. A method for diagnosing a disease or condition associated with size and/or number of lipoprotein particles in an individual, or for determining the risk that the individual will develop said disease or condition, comprising detecting the size and/or number of lipoprotein particles in a sample obtained from said individual by light scattering, optionally by interferometric scattering microscopy.

17. The method of claim 16, comprising detecting the size and/or number of more than one different type of lipoprotein particle, or the ratio of different types of lipoprotein particle, in said sample.

18. The method of claim 16 or claim 17, comprising detecting high density lipoprotein (HDL) particles and/or low density lipoprotein (LDL) particles, optionally comprising determining the ratio of HDL to LDL particles in said sample.

19. The method of claim 17 or claim 18, wherein a decreased number of HDL particles as compared with a control sample or reference sample/level, is indicative that the individual has said disease or condition, or has an increased risk of developing said disease or condition.

20. The method of claim 17 or claim 18, wherein an increased number and/or a decreased size of LDL particles as compared with a control sample or reference sample/level, is indicative that the individual has said disease or condition, or has an increased risk of developing said disease or condition.

21. The method of any one of claims 16 to 19, wherein said disease is cardiovascular disease.

22. The method of any one of claims 1 to 21, wherein said solution or sample is a biological sample, optionally obtained from a human individual.

23. The method of claim 22, wherein said biological sample is blood, plasma or serum, optionally obtained from a human individual.

24. A method of selecting an individual to whom a substance or composition is to be administered, or to whom a regimen is to be prescribed, wherein said substance or composition or regimen is suitable for treating or preventing a disease or condition associated with size and/or number of lipoprotein particles, comprising detecting the size and/or number of lipoprotein particles in a sample from said individual by a method according to any one of claims 11 to 15, and selecting said patient for said administration or said regimen if the size and/or number of lipoprotein particles detected is indicative of the presence of said disease or condition, or of a risk thereof.

25. A substance or composition for use in treating or preventing a disease or condition associated with size and/or number of lipoprotein particles in an individual, wherein said individual is diagnosed or determined at risk or selected by a method according to any one of claims 16 to 23.

26. Use of a substance or composition in the manufacture of a medicament for treatment or prevention of a disease or condition associated with size and/or number of lipoprotein particles in an individual, wherein said individual is diagnosed or determined at risk or selected by a method according to any one of claims 16 to 23.