Summary of the Invention

[0005] The present invention provides improved methods for quantitation of analytes, especially biomolecules, in a sample, which require less time to perform as well as less sample volume than most methods known in the art and, further, do not require any special sample preparation steps, as do most methods known in the art. Further, the methods according to the present invention also obviate the need for a user to generate a calibration curve each time an analyte is quantitated and also do not require that the calibrant be the same molecule as the analyte being quantitated. The present invention also provides devices for use in the claimed methods.

[0006] In one aspect according to the present invention, an IR based method for quantitating one or more biomolecules in a sample is provided.

[0007] In ~~one method~~ a first aspect, the present invention provides a method for quantitation of one or more biomolecules in a sample ~~according to the present invention~~, the method comprises the steps of: (a) providing a sample holder comprising a porous membrane which comprises a hydrophilic region surrounded by a hydrophobic region for sample containment; (b) contacting the hydrophilic region of the membrane with a sample volume; (c) drying the sample volume on the membrane; (d) exposing the sample volume on the membrane to an infrared beam comprising a wavelength in the spectral range of 4000-400 cm^{-1}, or any portion of the spectral range, thereby to obtain an infrared ~~absorptio~~[n] absorption spectrum; where one or more absorption peak areas in the infrared absorption spectrum correlates with the quantity of the one or more biomolecules in the sample.

[0007a] The present invention also provides a sample holder card for use in the method of the first aspect of the invention, wherein the sample holder card comprises a porous membrane which comprises a hydrophilic region surrounded by a hydrophobic region, wherein the sample is contained within the boundaries of the hydrophilic region.

[0008] In ~~another method~~ a second aspect, the present invention provides a method for quantitation of one or more biomolecules in a sample ~~according to the present invention~~, the method comprises the steps of: (a) providing a sample holder comprising a porous membrane which comprises a hydrophilic region surrounded by a hydrophobic region for sample containment; (b) contacting the hydrophilic region of the membrane with a sample volume; (c) drying the sample volume on the membrane; (d) detecting the presence of water in the sample volume after step (c) by infrared absorbance and repeating step (c), if necessary, until presence of water is undetectable; and (e) exposing the dried sample volume on the membrane to an

infrared beam comprising a wavelength in the spectral range of 4000-400 cm^{-1} or any portion of the spectral range, thereby to obtain an infrared absorption spectrum; where one or more absorption peak areas in the infrared absorption spectrum correlates with the quantity of the one or more biomolecules in the sample.

absorption spectrum is obtained using the IR based methods described herein, the area under one or more peaks in the spectrum is calculated by drawing a baseline across the peak and measuring the area enclosed in the peak. The baseline is typically drawn based on points before and after the peak on the spectrum, ~~as depicted below~~ as in Figure 16.

[0054] In some embodiments according to the claimed methods, a peak area correlates with the concentration or quantity of an analyte in a sample.

[0055] In some embodiments, the concentration of protein in a sample is measured as follows. As a first step, a calibration curve is generated using a calibrant, which includes protein standards of known concentration, and the calibration curve is pre-loaded onto an IR spectrometer instrument. The same calibration curve can subsequently be used each time that the instrument is used for quantitating protein in a sample. Further, it is not required that the calibrant be present in the sample being analyzed, or in other words, it could be derived from a completely different source than the sample. For example, in a particular embodiment, solutions of a protein standard such as, for example, BSA, are prepared in a buffer at various known concentrations. The standard solutions are applied to the hydrophilic region of a membrane in a sample holder in the form of a card, the membrane is dried and the absorption spectrum ~~is measured~~ measurements using an IR instrument, e.g., the Bruker IR instrument. Without wishing to be bound by theory, it is contemplated that any suitable IR instrument may be used in the methods according to the present invention.

[0056] Most modem IR absorption instruments use Fourier-transform techniques with a Michelson interferometer. In some embodiments according to the claimed methods, in order to obtain an IR absorption spectrum, one mirror of the interferometer moves to generate interference in the radiation reaching the detector. Since all wavelengths are passing through the interferometer, the interferogram is a complex pattern. The absorption spectrum as a function of wavenumber (cm-') is obtained from the Fourier transform of the interferogram, which is a function of

Claims

1. A method for quantitation of one or more biomolecules in a sample, the method comprising the steps of:

(a) providing a sample holder comprising a porous membrane which comprises a hydrophilic region surrounded by a hydrophobic region for sample containment;

(b) contacting the hydrophilic region of the membrane with a sample volume;

(c) drying the sample volume on the membrane;

(d) exposing the sample volume on the membrane to an infrared beam comprising a wavelength in the spectral range of 4000-400 cm^{-1} or any portion of the spectral range of 4000-400 cm^{-1}, thereby to obtain an infrared absorption spectrum;

i) wherein one or more absorption peak areas in the infrared absorption spectrum correlates with the quantity of the one or more biomolecules in the sample.

2. The method of claim 1, wherein the one or more biomolecules is selected from the group consisting of nucleic acids, proteins, lipids, polysaccharides and lipopolysaccharides.

3. The method of claim 2, wherein the lipopolysaccharide is an endotoxin.

4. The method of any one of claims 1 to 3, wherein the method does not require a user to generate a calibration curve each time a sample is analyzed for quantitation of the one or more biomolecules.

5. The method of any one of claims 1 to 4, wherein sample volume ranges from 0.1-20 µl.

6. The method of claim 5, wherein sample volume is 1 µl or less.

7. The method of any one of claims 1 to 6, wherein the porous membrane is contained within a device.

8. The method of claim 7, wherein the device is a sample holder card.

9. The method of any one of claims 1 to 8, wherein the sample is a crude sample.

~~9.~~10. The method of any one of claims 1 to 9, wherein the sample comprises a biological fluid or comprises a cell or tissue lysate.

~~10.~~11. The method of claim ~~9~~10, wherein the biological fluid is selected from the group consisting of blood, plasma, serum and urine.

~~11. The method of claim 1, wherein the sample comprises cell or tissue lysate.~~

~~12. The method of claim 1, wherein the sample is a crude sample.~~

~~13.~~12. The method of claim 8, wherein the sample holder card comprises a porous membrane which comprises an area within which the sample is contained on the membrane.

~~14.~~13. The method of any one of claim 1 to 12, wherein the porous membrane is an ultrafiltration membrane or is a microporous membrane .

~~15. The method of claim 1, wherein the porous membrane is a microporous membrane.~~

~~16.~~14. The method of any one of claim 1 to 13, wherein the porous membrane comprises a polymeric material selected from the group consisting of PVDF (Polyvinylidene fluoride), polytetrofluoroethylene, hydrophilic polytetrofluoroethylene, polyethylene and polypropylene.

~~17.~~15. The method of claim ~~13~~12, wherein the area for sample containment on the membrane comprises a hydrophilic region surrounded by a hydrophobic region.

~~18.~~16. The method of claim ~~17~~15, wherein the hydrophobic region is created by plasma treatment of a hydrophilic porous membrane or is created by heat treatment of a hydrophilic porous membrane.

~~19.~~17. The method of claim ~~17~~15 or claim 16, wherein the sample is contained within the hydrophilic region.

~~20. The method of claim 17, wherein the hydrophobic region is created by heat treatment of a hydrophilic porous membrane.~~

~~21.~~18. A sample holder card for use in the method of any one of claims 1 to 17, wherein the sample holder card comprises a porous membrane which comprises a hydrophilic region surrounded by a hydrophobic region, wherein the sample is contained within the boundaries of the hydrophilic region.

~~22.~~19. The sample holder card of claim ~~21~~18, wherein the diameter of the hydrophilic region ranges from 2.0 mm through 10 mm or from 3.0mm through 6mm.

~~23. The sample holder card of claim 21, wherein the diameter of the hydrophilic region ranges from 3.0 mm through 6 mm.~~

~~24.~~20. A method for quantitation of one or more biomolecules in a sample, the method comprising the steps of:

(a) providing a sample holder card comprising a porous membrane which comprises a hydrophilic region surrounded by a hydrophobic region for sample containment;

(b) contacting the hydrophilic region of the membrane with a sample volume;

(c) drying the sample volume on the membrane;

(d) detecting the presence of water on the membrane using infrared absorbance and repeating step (c), if necessary, until no water is detected; and

(e) exposing the sample volume on the membrane to an infrared beam comprising a wavelength in the spectral range of 4000-400 cm-1 or any portion of the spectral range, thereby to obtain an infrared absorption spectrum;

i) wherein one or more absorption peak areas in the infrared absorption spectrum correlates with the quantity of the one or more biomolecules in the sample.