absorbance measurement). In such instruments, the pathlength is defined by the distance between the tip of the light delivering Fibrette and the inside bottom of the sample vessel. The pathlength is dynamically controlled, through movement of the Fibrette up and down in the sample, by integrated hardware means. Variable pathlength instruments address the issues of concentration range of direct measurement. In fixed-pathlength instruments, the direct measurement range is narrow, therefore, in practice sample dilution is always required. With variable pathlength instruments, however, since such instruments can scan variable pathlengths, it can directly measure high protein concentration without need of any dilution. Such instruments, however, are typically expensive to acquire and maintain, and, fundamentally, are also prone to measurement error due to the mechanical movement and manipulation of the light delivering Fibrette. For example, lack of routine calibration or maintenance of the Fibrette, and/or slight variations in the mechanics of the variable light instruments, generally results in errors where the results of the measurement are offset by the degree of mechanical failure, mis-calibration, or mechanical variations. These errors generally lead to variance in sample measurement and prevent repeatable results and sufficient calibration within acceptable limits, including, for example, for product measurement and quality control purposes.

[0011] For the foregoing reasons, there is a need for UV based imaging systems and methods for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration.

BRIEF SUMMARY

[0011a] According to a first aspect of the present invention, there is provided an ultraviolet (UV) based imaging system configured to determine protein concentrations of unknown protein samples based on automated multi-wavelength calibration, the UV based imaging system comprising:

a light source configured to project a multi-wavelength light beam;

a monochromator configured to receive the multi-wavelength light beam and to project, based on the multi-wavelength light beam, a range of single-wavelength light beams of a UV spectra;

a sample holder operable for receiving a protein sample, the protein sample being either of (1) a standard protein sample selected from a set of standard protein samples, or (2) an unknown protein sample selected from a set of unknown protein samples, the unknown protein

sample having an unknown protein concentration, wherein the sample holder is positioned to allow the range of single-wavelength light beams to pass through at least a portion of the protein sample;

a detector operable to detect the range of single-wavelength light beams passing through the at least the portion of the protein sample;

a memory storing program instructions; and

a processor communicatively coupled to the memory, wherein the

the processor is configured to execute the program instructions to cause the processor to:

receive a standard set of wavelength data as recorded by the detector for each standard protein sample of the set of standard protein samples, wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from the range of the single-wavelength light beams, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value,

generate a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data,

receive an unknown set of wavelength data as recorded by the detector for each unknown protein sample of the set of unknown protein samples, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value, and

determine with the multi-wavelength calibration model, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

[0011b] According to a second aspect of the present invention, there is provided an ultraviolet (UV) based imaging method for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration, the UV based imaging method comprising:

receiving, at a processor, a standard set of wavelength data as recorded by a detector for each standard protein sample of a set of standard protein samples ,

wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from a range of a single-wavelength light beams of a UV spectra, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value;

generating, with the processor, a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data;

receiving, at the processor, an unknown set of wavelength data as recorded by the detector for each unknown protein sample of a set of unknown protein samples, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value; and

determining, with the multi-wavelength calibration model as implemented on the processor, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

[0011c] According to a third aspect of the present invention, there is provided a tangible, non-transitory computer-readable medium storing instructions for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration, that when executed by one or more processors of a computing device, cause the computing device to:

receive, at a processor, a standard set of wavelength data as recorded by a detector for each standard protein sample of a set of standard protein samples, wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from a range of a single-wavelength light beams of a UV spectra, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value;

generate, with the processor, a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data;

receive, at the processor, an unknown set of wavelength data as recorded by the detector for each unknown protein sample of a set of unknown protein samples, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value; and

determine, with the multi-wavelength calibration model as implemented on the processor, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

[0011d] According to a fourth aspect of the present invention, there is provided an imaging apparatus for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration, the imaging apparatus comprising:

a means for receiving a standard set of wavelength data for each standard protein sample of a set of standard protein samples, wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from a range of a single-wavelength light beams of a UV spectra, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value;

a means for generating a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data;

a means for receiving an unknown set of wavelength data for each unknown protein sample of a set of unknown protein samples, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value; and

a means for determining, with the multi-wavelength calibration model, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

[0012] The present disclosure generally relates to the automation and testing of therapeutic pharmaceuticals, including the development, processing, monitoring, and/or product quality attribute control related to such therapeutic pharmaceuticals. In various embodiments, UV based imaging systems and methods are disclosed for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration. The determined protein concentrations may be concentrations of unknown samples of therapeutic pharmaceuticals or products. The determination of such protein concentrations may be used process monitoring and product quality attribute control, for example, during manufacturing of therapeutics, or other protein-based products. The determination of such protein concentrations may also be used in

CLAIMS

1. An ultraviolet (UV) based imaging system configured to determine protein concentrations of unknown protein samples based on automated multi-wavelength calibration, the UV based imaging system comprising:

a light source ~~(102)~~ configured to project a multi-wavelength light beam ~~(104)~~;

a monochromator ~~(106)~~ configured to receive the multi-wavelength light beam and to project, based on the multi-wavelength light beam, a range of single-wavelength light beams of a UV spectra;

a sample holder ~~(110)~~ operable for receiving a protein sample, the protein sample being either of (1) a standard protein sample selected from a set of standard protein samples ~~(140s)~~, or (2) an unknown protein sample selected from a set of unknown protein samples ~~(140u)~~, the unknown protein sample having an unknown protein concentration, wherein the sample holder is positioned to allow the range of single-wavelength light beams to pass through at least a portion of the protein sample;

a detector ~~(114)~~ operable to detect the range of single-wavelength light beams passing through the at least the portion of the protein sample;

a memory storing program instructions; and

a processor communicatively coupled to the memory, wherein the

the processor is configured to execute the program instructions to cause the processor to:

receive a standard set of wavelength data as recorded by the detector for each standard protein sample of the set of standard protein samples, wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from the range of the single-wavelength light beams, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value,

generate a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data,

receive an unknown set of wavelength data as recorded by the detector for each unknown protein sample of the set of unknown protein samples, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the

range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value, and

determine with the multi-wavelength calibration model, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

2. The UV based imaging system of claim 1, wherein the light source, the monochromator, the sample holder, and the detector comprise a portion of a spectrophotometer device.

3. The UV based imaging system of either claim 1 or claim 2, wherein the processor is communicatively coupled to the detector.

4. The UV based imaging system of any one of claims 1-3, wherein the memory or the processor is remote to the detector.

5. The UV based imaging system of any one of claims 1-4, wherein the set of standard protein samples comprises a plurality of standard protein samples, and wherein the set of unknown protein samples comprises a single protein sample having the unknown protein concentration.

6. The UV based imaging system of any one of claims 1-5, wherein the set of standard protein samples are protein standard solutions in the range of 0.1 to 220 milligrams (mg) per milliliter (ml).

7. The UV based imaging system of any one of claims 1-6, wherein the range of single-wavelength light beams comprises single-wavelength light beams in the UV spectra from 200 nanometers (nm) to 400 nanometers (nm).

8. The UV based imaging system of claim 7, wherein each single-wavelength light beam in the UV spectra is separated by a one nanometer interval.

9. The UV based imaging system of any one of claims 1-8, wherein generating the multi-wavelength calibration model includes generating a single-wavelength calibration model for each of the first range of wavelengths selected from the range of the single-wavelength light beams, each single-wavelength calibration model comprising a slope value and a y-intercept value corresponding to UV absorbance of particular single wavelength.

10. The UV based imaging system of any one of claims 1-9, wherein the multi-wavelength calibration model is trained as a machine-learning model.

11. The UV based imaging system of any one of claims 1-10, further comprising a standard wavelength data filter, the processor further configured to execute the program instructions to cause the processor to implement the standard wavelength data filter against the range of the single-wavelength light beams to select the first range of wavelengths.

12. The UV based imaging system of claim 11, wherein the processor is further configured to execute the program instructions to cause the processor to implement the standard wavelength data filter to limit each first series of first absorbance-to-wavelength value pair based on UV absorbance values within a first filter range of UV absorbance values.

13. The UV based imaging system of any one of claims 1-12, further comprising an unknown wavelength data filter, the processor further configured to execute the program instructions to cause the processor to implement the unknown wavelength data filter against the range of the single-wavelength light beams to select the second range of wavelengths.

14. The UV based imaging system of claim 13, wherein the processor is configured to execute the program instructions to cause the processor to implement the unknown wavelength data filter to limit each second series of second absorbance-to-wavelength value pair based on UV absorbance values within a second filter range of UV absorbance values.

15. The UV based imaging system of any one of claims 1-14, wherein the processor is further configured to generate an enhanced prediction based on the plurality of protein concentration values.

16. The UV based imaging system of claim 15, wherein the generating of the enhanced prediction comprises averaging each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths.

17. The UV based imaging system of any one of claims 1-16, wherein the multi-wavelength calibration model is used to monitor or measure protein concentration of a protein-related product.

18. The UV based imaging system of claim 17, wherein the protein concentration is monitored or measured during manufacture of the protein-related product during manufacture of the protein-related product.

19. The UV based imaging system of claim 17, wherein the protein concentration of the protein-related product is compared against known specifications for the protein-related product.

20. The UV based imaging system of claim 17, wherein the protein-related product is a therapeutic product.

21. The UV based imaging system of any one of claims 1-20, wherein the multi-wavelength calibration model inputs the second series of second absorbance-to-wavelength value pairs to predict each plurality of protein concentration values.

22. An ultraviolet (UV) based imaging method for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration, the UV based imaging method comprising:

receiving, at a processor, a standard set of wavelength data as recorded by a detector ~~(114)~~ for each standard protein sample of a set of standard protein samples ~~(140s)~~, wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from a range of a single-wavelength light beams of a UV spectra, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value;

generating, with the processor, a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data;

receiving, at the processor, an unknown set of wavelength data as recorded by the detector for each unknown protein sample of a set of unknown protein samples ~~(140u)~~, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value; and

determining, with the multi-wavelength calibration model as implemented on the processor, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

23. The UV based imaging method of claim 22, wherein the detector comprises a portion of a spectrophotometer device.

24. The UV based imaging method of either claim 22 or claim 23, wherein the processor is communicatively coupled to the detector.

25. The UV based imaging method of any one of claims 22-24, wherein the processor is remote to the detector.

26. The UV based imaging method of any one of claims 22-25, wherein the set of standard protein samples comprises a plurality of standard protein samples, and wherein the set of unknown protein samples comprises a single protein sample having the unknown protein concentration.

27. The UV based imaging method of any one of claims 22-26, wherein the set of standard protein samples are protein standard solutions in the range of 0.1 to 220 milligrams (mg) per milliliter (ml).

28. The UV based imaging method of any one of claims 22-27, wherein the range of single-wavelength light beams comprises single-wavelength light beams in the UV spectra from 200 nanometers (nm) to 400 nanometers (nm).

29. The UV based imaging method of claim 28, wherein each single-wavelength light beam in the UV spectra is separated by a one nanometer interval.

30. The UV based imaging method of any one of claims 22-29, wherein generating the multi-wavelength calibration model includes generating a single-wavelength calibration model for each of the first range of wavelengths selected from the range of the single-wavelength light beams, each single-wavelength calibration model comprising a slope value and a y-intercept value corresponding to UV absorbance of particular single wavelength.

31. The UV based imaging method of any one of claims 22-30, wherein the multi-wavelength calibration model is trained as a machine-learning model.

32. The UV based imaging method of any one of claims 22-31, further comprising a standard wavelength data filter, the processor further configured to execute the program instructions to cause the processor to implement the standard wavelength data filter against the range of the single-wavelength light beams to select the first range of wavelengths.

33. The UV based imaging method of claim 32, wherein the processor is further configured to execute the program instructions to cause the processor to implement the standard wavelength data filter to limit each first series of first absorbance-to-wavelength value pair based on UV absorbance values within a first filter range of UV absorbance values.

34. The UV based imaging method of any one of claims 22-33, further comprising an unknown wavelength data filter, the processor further configured to execute the program instructions to cause the processor to implement the unknown wavelength data filter against the range of the single-wavelength light beams to select the second range of wavelengths.

35. The UV based imaging method of claim 34, wherein the processor is configured to execute the program instructions to cause the processor to implement the

unknown wavelength data filter to limit each second series of second absorbance-to-wavelength value pair based on UV absorbance values within a second filter range of UV absorbance values.

36. The UV based imaging method of any one of claims 22-35, wherein the processor is further configured to generate an enhanced prediction based on the plurality of protein concentration values.

37. The UV based imaging method of claim 36, wherein the generating of the enhanced prediction comprises averaging each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths.

38. The UV based imaging method of any one of claims 22-37, wherein the multi-wavelength calibration model is used to monitor or measure protein concentration of a protein-related product.

39. The UV based imaging method of claim 38, wherein the protein concentration is monitored or measured during manufacture of the protein-related product.

40. The UV based imaging method of claim 38, wherein the protein concentration of the protein-related product is compared against known specifications for the protein-related product.

41. The UV based imaging system of claim 38, wherein the protein-related product is a therapeutic product.

42. The UV based imaging method of any one of claims 22-41, wherein the multi-wavelength calibration model inputs the second series of second absorbance-to-wavelength value pairs to predict each plurality of protein concentration values.

43. A tangible, non-transitory computer-readable medium storing instructions for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration, that when executed by one or more processors of a computing device, cause the computing device to:

receive, at a processor, a standard set of wavelength data as recorded by a detector ~~(114)~~ for each standard protein sample of a set of standard protein samples ~~(140s)~~, wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from a range of a single-wavelength light beams of a UV spectra, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value;

generate, with the processor, a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data;

receive, at the processor, an unknown set of wavelength data as recorded by the detector for each unknown protein sample of a set of unknown protein samples ~~(140u)~~, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value; and

determine, with the multi-wavelength calibration model as implemented on the processor, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

44. An imaging apparatus for determining protein concentrations of unknown protein samples based on automated multi-wavelength calibration, the imaging apparatus comprising:

a means for receiving a standard set of wavelength data for each standard protein sample of a set of standard protein samples ~~(140s)~~, wherein the standard set of wavelength data includes, for each standard protein sample, a first series of first absorbance-to-wavelength value pairs across a first range of wavelengths selected from a range of a single-wavelength light beams of a UV spectra, each first absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value;

a means for generating a multi-wavelength calibration model based on each of the first series of first absorbance-to-wavelength value pairs of the standard set of wavelength data;

a means for receiving an unknown set of wavelength data for each unknown protein sample of a set of unknown protein samples ~~(140u)~~, wherein the unknown set of wavelength data includes, for each unknown protein sample, a second series of second absorbance-to-wavelength value pairs across a second range of wavelengths selected from the range of the single-wavelength light beams, each second absorbance-to-wavelength value pair comprising a UV absorbance value and a wavelength value; and

a means for determining, with the multi-wavelength calibration model, for each unknown protein sample of the set of unknown protein samples, a plurality of protein concentration values, each protein concentration value of the plurality of protein concentration values corresponding to the second range of wavelengths selected from the range of the single-wavelength light beams.

45. The UV based imaging method of claim 22, wherein at least one unknown protein sample of the set of unknown protein samples comprises a protein therapeutic.

46. The UV based imaging method of claim 45, wherein the protein therapeutic is selected from the group consisting of an antibody, an antigen-binding antibody fragment, an antibody protein product, a Bi-specific T cell engager (BiTE) molecule, a bispecific antibody, a trispecific antibody, an Fc fusion protein, a recombinant protein, and an active fragment of a recombinant protein.