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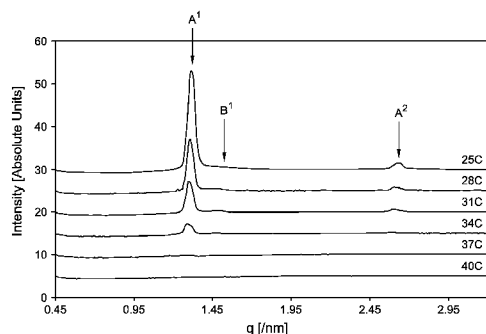
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ML, MR, NE, SN, TD, TG).

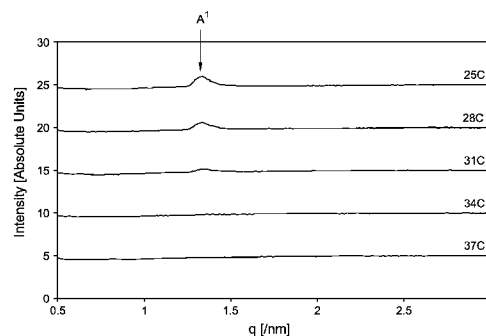
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(54) Title: METHOD FOR DIAGNOSING DRY EYE AND MEIBOMIAN GLAND DISEASE USING MEIBOMIAN SECRE-
TIONS



A



B

(57) Abstract: The present invention relates to methods for diagnosing dry eye and/or meibomian gland disease using measurements of meibum. In one embodiment, polarized light microscopy is used to observe the melting behavior of meibum. In another embodiment, dry eye and/or meibomian gland disease can be diagnosed if a meibum sample has a single melting point.

METHOD FOR DIAGNOSING DRY EYE AND MEIBOMIAN GLAND DISEASE USING MEIBOMIAN SECRETIONS

CROSS-REFERENCE TO RELATED APPLICATION

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This application claims priority under 35 U.S.C. §119 to U.S. Provisional Patent Application No. 61/642866, filed May 4, 2012, the entire contents of which are incorporated herein by reference.

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TECHNICAL FIELD OF THE INVENTION

The present invention generally relates to methods for diagnosing dry eye and meibomian gland disease using measurements of meibomian gland secretions. The present invention specifically relates to methods for diagnosing dry eye and meibomian gland disease using small angle x-ray scattering and polarized light microscopy evaluation of meibomian gland secretions. The present invention also relates to treatments for dry eye and meibomian gland disease using information relative to meibomian gland secretions.

20

BACKGROUND OF THE INVENTION

Dry eye, also referred to as keratoconjunctivitis sicca, is a common ophthalmological disorder affecting millions of persons each year. The condition is particularly widespread among post-menopausal women due to hormonal changes following the cessation of fertility. Dry eye may afflict an individual with varying severity. In mild cases, a patient may experience burning, a feeling of dryness, and persistent irritation such as is often caused by small bodies lodging between the eye lid and the eye surface. In severe cases, vision may be substantially impaired.

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Approximately 20-75% of the adult population is afflicted with dry eye disease and meibomian gland disease, making these conditions the most common source of patient complaints in common ophthalmology practice. Both diseases are associated with irritating and sometimes painful symptoms as well as varying degrees of damage to the ocular surface epithelium, that if left untreated can lead to serious ocular sequelae. Despite the prevalence of these diseases, treatment strategies have yet to be optimized and sensitive clinical diagnostics remain elusive. Without the ability to accurately diagnosis and differentiate dry eye from meibomian gland disease and/or other ocular surface diseases, the selection of appropriate treatment modalities is difficult at best.

35

Meibum is a lipid rich secretion that is a critical component of the tear film. The meibum originates from the meibomian glands located in the upper and lower eyelids where it is slowly released and spread over the tear film through the blinking
5 action. Meibomian gland disease (MGD) is one of the leading causes of dry eye disease that is marked by changes to the quality and quantity of meibum. Changes to either the amount or the quality of meibum being secreted by the glands can lead to instability of the tear film and ultimately contribute to inflammation of the ocular surface. Currently MGD is evaluated using a clinical grading scale for the appearance
10 of both the meibomian glands and the secreted meibum, monitoring changes in the number of glands, the color, thickness, and amount of secreted meibum, and the presence of inflammation along the lid.

BRIEF SUMMARY OF THE INVENTION

Understanding and possibly being able to quantify specific physical characteristics of meibum may provide insight into how the meibum functions as a critical component of the tear film and further evaluate severity of the disease and efficacy of treatments.

Polarized light microscopy is a technique used to evaluate the unique crystalline structures of a variety of materials. By polarizing the light to a single wave, materials which have an ordered, crystalline structure (such as lipids) will present a specific birefringent pattern. Addition of temperature cycling can provide further insight into these crystalline patterns by establishing phase transition temperatures that may correlate to differences in the lipid composition of different samples. The present inventors have found that the use of PLM and other techniques such as small angle x-ray scattering to evaluate meibomian gland secretions (meibum) can be used as a diagnostic tool to determine the presence of dry eye disease.

The present invention relates to methods of diagnosing dry eye in a patient using measurements of meibum. In certain aspects, a method of the invention comprises: a) obtaining a meibum sample from a patient; b) heating the meibum sample and observing the melting behavior of the sample; and c) determining that the patient has dry eye and/or meibomian gland disease if the meibum sample has a single melting point.

The invention also provides methods of determining the efficacy of an agent for treating dry eye, the methods comprising: a) obtaining a first meibum sample from a patient; b) administering the agent to the patient; c) obtaining a second meibum sample from the patient; d) determining the melting behavior of the first and second patient meibum samples; e) comparing the melting behavior of the meibum samples to a normal meibum sample; and f) determining that the agent is effective for treating dry eye if the second meibum sample exhibits melting behavior consistent with a normal meibum sample.

The invention also provides kits for diagnosing dry eye and meibomian gland disease in a patient.

The foregoing brief summary broadly describes the features and technical advantages of certain embodiments of the present invention. Additional features and

technical advantages will be described in the detailed description of the invention that follows. Novel features which are believed to be characteristic of the invention will be better understood from the detailed description of the invention when considered in connection with any accompanying figures. However, figures provided herein are
5 intended to help illustrate the invention or assist with developing an understanding of the invention, and are not intended to be definitions of the invention's scope.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete understanding of the present invention and the advantages thereof may be acquired by referring to the following description, taken in
5 conjunction with the accompanying drawings and wherein:

FIGURE 1A shows small angle x-ray scattering (SAXS) profiles of samples obtained from patients with meibomian gland disease; and

10 FIGURE 1B shows small angle x-ray scattering (SAXS) profiles of samples obtained from normal patients.

DETAILED DESCRIPTION OF THE INVENTION

The tear film is a complex biological fluid composed of a large number of lipids, mucins, and proteins mixed in an electrolyte rich aqueous medium. Alterations
5 in this complex fluid can manifest into various disease states; the two most common of which are dry eye (DE) and meibomian gland disease (MGD). DE is defined as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and
10 inflammation of the ocular surface. The definition for MGD or posterior blepharitis is not as clear, but is known to involve an abnormality of the lipid layer of the tear film as a result of aberrant or reduced secretions of meibum from the meibomian glands. The deficient lipid layer of the tear film associated with MGD manifests as many common signs and symptoms associated with DE. Despite similar clinical
15 symptomology, treatment modalities for the two diseases are different owing to the issue of how best to replace or supplement the specific tear film deficiency. As the primary distinction between DE and MGD is at the level of meibomian gland secretions, current diagnostic criteria are aimed at quantifying parameters such as color and consistence of the meibum secretion. These criteria however are subjective.
20 A significant improvement in the ability to differentiate DE from MGD (and other ocular surface diseases such as allergy) would be accomplished through direct physical characterization of the meibum secretion.

The present inventors have found that meibum samples have an organized
25 crystalline structure which is sensitive to temperature and can be monitored using techniques such as polarized light microscopy (PLM) and small angle x-ray scattering (SAXS). The molecular order associated with meibum can be used to understand lipid composition changes and provide valuable information relative to the diagnosis of dry eye disorders and meibomian gland disease.

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The present invention relates in one embodiment to methods of diagnosing dry eye in a patient using measurements of meibum. In certain aspects, a method of the invention comprises: a) obtaining a meibum sample from a patient; b) heating the meibum sample and observing the melting behavior of the sample; and c) determining
35 that the patient has meibomian gland disease if the meibum sample has a single melting point.

The invention also provides methods of determining the efficacy of an agent for treating dry eye, the methods comprising: a) obtaining a first meibum sample from a patient; b) administering the agent to the patient; c) obtaining a second meibum sample from the patient; d) determining the melting behavior of the first and
5 second patient meibum samples; e) comparing the melting behavior of the meibum samples to a normal meibum sample; and f) determining that the agent is effective for treating dry eye if the second meibum sample exhibits melting behavior consistent with a normal meibum sample. As used herein, the term “agent” refers to a chemical compound, a mixture of chemical compounds, a biological molecule, a mixture of
10 biological molecules, an extract made from biological materials, or any other such composition.

A normal meibum sample can be, for example, a statistical aggregate of multiple samples obtained from patients not having dry eye symptoms, dry eye
15 disease, and/or meibomian gland disease, or a single sample obtained from a patient not experiencing such symptoms or pathologies. One of skill is cognizant of accepted protocols for generating a “control” population which would be appropriate as a normal meibum sample in the present invention.

20 An agent of the present invention may be administered to a subject via aerosol, buccal, dermal, intradermal, inhaling, intramuscular, intranasal, intraocular, intrapulmonary, intravenous, intraperitoneal, nasal, ocular, oral, otic, parenteral, patch, subcutaneous, sublingual, topical, or transdermal administration, for example.

25 Meibum samples can be obtained from a patient or subject using any method known in the art. In one technique, samples of meibum are collected from the lower lids of individuals using a Mastrota paddle and digital pressure.

In some embodiments, the present invention provides kits for the detection of
30 dry eye and/or meibomium gland disease in a patient. In certain embodiments, the kits contain reagents and/or apparatus for conducting an assay to evaluate meibum. In other embodiments, the kits contain a means for collecting a meibum sample from a patient.

35 As noted above, small angle x-ray scattering is a methodology that can be employed to characterize meibum. The lamellar spacing of meibum samples can indicate the presence of defining secondary structures. Also, SAXS can be used to

calculate meibum melting temperature-associated changes in peak intensity, width and center point of temperature sweeps.

The following examples are presented to further illustrate selected
5 embodiments of the present invention.

EXAMPLE 1

Pin-head sized samples of meibum were collected from the lower lids of
10 individuals using a Mastrotta paddle and digital pressure. Samples collected from the left and right lids were pooled and stored neat at room temperature in sealed amber vials. Samples were prepared for analysis by transferring a small portion of the sample to a microscope slide using a tungsten needle then gently pressing the sample with a 5mm square coverslip. Microscopic analysis was performed using an Olympus
15 polarized light microscope with a temperature-controlled chamber and digital imaging system. Characterization of inherent crystal structure and melt behavior was accomplished by temperature cycling between 21°C and 60°C at a rate of 2°C per minute while capturing live video. Individual images were taken from the live video to represent changes to the crystalline structure over the temperature cycling. Visual
20 observations of the recorded images were used to estimate melting point(s) as well as to make observations as to the size and shape of the crystalline structures.

Initial observation under crossed polarization demonstrated distinct
birefringent areas of human meibum. Crystals within the meibum ranged from 10-30
25 uM and exhibited low order (white/gray) retardation colors. Because of the low order and intensity of the birefringent crystals, use of a 450nm filter was employed to increase contrast, resulting in increased visibility and observation of bright blue/yellow retardation colors. After initial observation of samples at room temperature, heating of the samples demonstrates greater fluidity and loss of
30 birefringent intensity at ~32°C with completion of the first melt phase between 39°C-40°C. Normal samples retain areas of birefringence beyond this initial melt phase. The birefringence of the normal samples remains constant until ~45°C where they begin to diminish and undergo a second melt transition with completion between 48°C-55°C. Upon cooling, all samples appear to undergo a restructuring, where
35 smaller (1-2um), more uniform crystals begin to form at ~35°C, and complete recrystallization occurs between 30°C-32°C.

In contrast to normal meibum samples, after initial observation at room temperature, heating of samples obtained from patients suspected of having MGD demonstrated greater fluidity and loss of birefringent intensity starting at $\sim 32^{\circ}\text{C}$ with complete loss of birefringence (melting) occurring by $\sim 40^{\circ}\text{C}$. The single, lower temperature transition temperature seen in meibum obtained from MGD subjects suggests that the secondary, higher temperature transition is unique to normal subjects.

EXAMPLE 2

Small angle x-ray scattering (SAXS) was used to evaluate 32 samples. 16 of the samples were obtained from patients with MGD and 16 of the samples were from normal patients (control). Data from the study is presented in FIGURE 1A (control) and FIGURE 1B (MGD samples).

The data indicates differences between the MGD and normal groups. The MGD group has only one lamellar first order phase with a melt temperature of 33°C . The control group contains two or more lamellar phases with melting temperatures around 37°C . The intensity of the single peak in the MGD group is lower than that of the control group, and the peak width is greater, indicating that the lamellar phase in the MGD group is less ordered. The melt temperature of phase A in FIGURE 1A (control) is 36.92°C . The melt temperature of phase A in FIGURE 1B (MGD samples) is 32.96°C .

The present invention and its embodiments have been described in detail. However, the scope of the present invention is not intended to be limited to the particular embodiments of any process, manufacture, composition of matter, compounds, means, methods, and/or steps described in the specification. Various modifications, substitutions, and variations can be made to the disclosed material without departing from the spirit and/or essential characteristics of the present invention. Accordingly, one of ordinary skill in the art will readily appreciate from the disclosure that later modifications, substitutions, and/or variations performing substantially the same function or achieving substantially the same result as embodiments described herein may be utilized according to such related embodiments of the present invention. Thus, the following claims are intended to encompass within their scope modifications, substitutions, and variations to processes, manufactures, compositions of matter, compounds, means, methods, and/or steps disclosed herein.

CLAIMS

What is claimed is:

- 5 1. A method of diagnosing dry eye or meibomian gland disease in a patient, the method comprising:
- a) obtaining a meibum sample from a patient;
- b) heating the meibum sample and observing the melting behavior of the sample; and
- 10 c) determining that the patient has dry eye and/or meibomian gland disease if the meibum sample has a single melting point.
2. The method according to claim 1, wherein said melting point is less than 40°C.
- 15 3. The method according to claim 1, wherein polarized light microscopy is used to observe the melting behavior of the sample.
4. The method according to claim 1, wherein small angle x-ray scattering is used
- 20 to observe the melting behavior of the sample.

5. A method of diagnosing dry eye or meibomian gland disease in a patient, the method comprising:

- a) obtaining a first meibum sample from a patient;
- b) administering the agent to the patient;
- 5 c) obtaining a second meibum sample from the patient;
- d) determining the melting behavior of the first and second patient meibum samples;
- e) comparing the melting behavior of the meibum samples to a normal meibum sample; and
- 10 f) determining that the agent is effective for treating dry eye if the second meibum sample exhibits melting behavior consistent with a normal meibum sample.

6. The method according to claim 5, wherein said melting point is less
15 than 40°C.

7. The method according to claim 5, wherein polarized light microscopy is used to observe the melting behavior of the sample.

20 8. The method according to claim 5, wherein small angle x-ray scattering is used to observe the melting behavior of the sample.

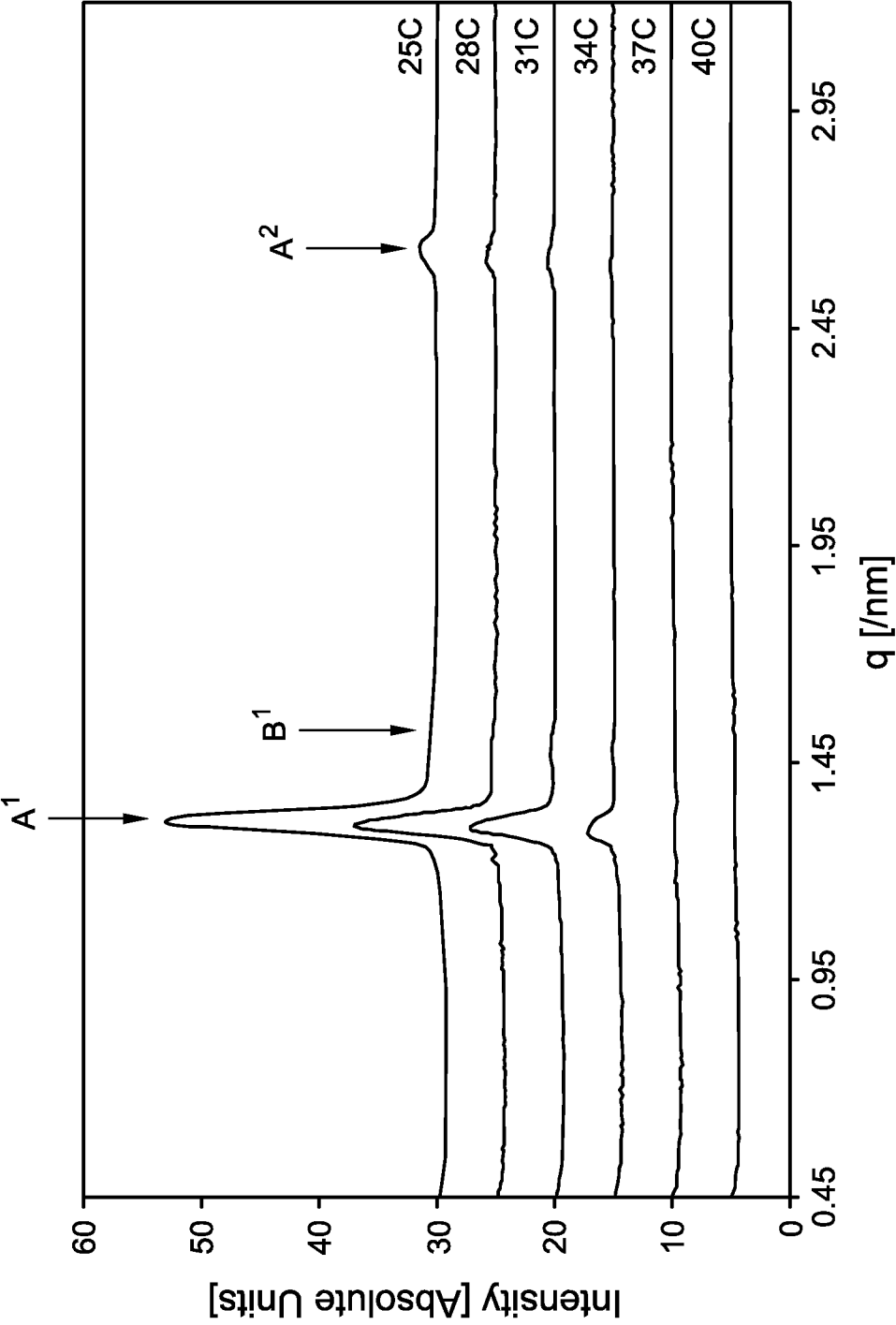


FIG. 1A

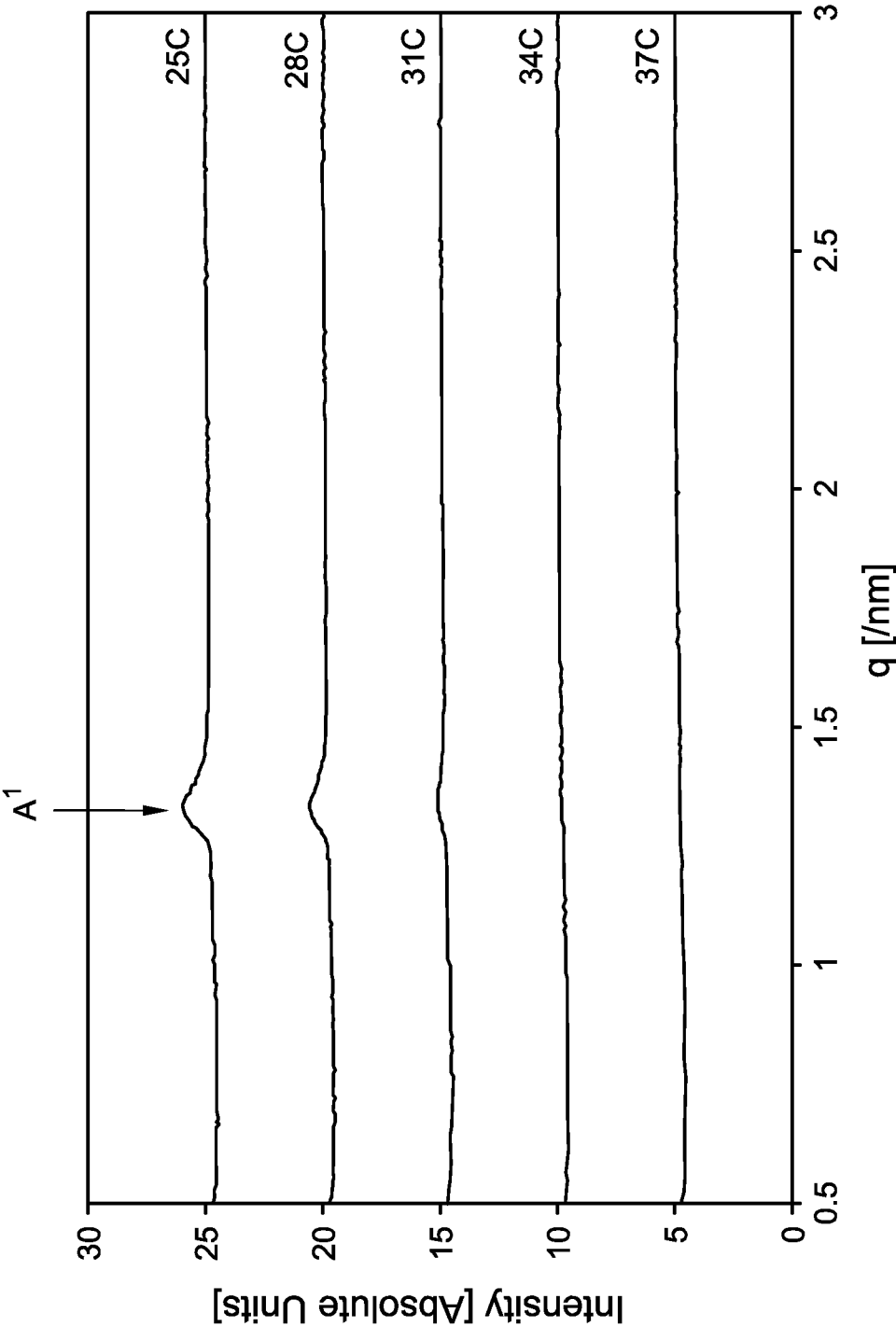


FIG. 1B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/039399

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61B3/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	D. BORCHMAN ET AL: "Human Meibum Lipid Conformation and Thermodynamic Changes with Meibomian-Gland Dysfunction", INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, vol. 52, no. 6, 1 June 2011 (2011-06-01), pages 3805-3817, XP055069343, ISSN: 0146-0404, DOI: 10.1167/iov.10-6514 page 3805, left-hand column, paragraph 1 - page 3809, right-hand column, paragraph 1 page 3813, left-hand column, paragraph Discussion - page 3815, left-hand column, paragraph 2 figures 1-3 ----- -/--	1-3



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

3 July 2013

Date of mailing of the international search report

17/07/2013

Name and mailing address of the ISA/

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Artikis, T

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/039399

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DANIELLE LEISKE ET AL: "Temperature-Induced Transitions in the Structure and Interfacial Rheology of Human Meibum", BIOPHYSICAL JOURNAL, BIOPHYSICAL SOCIETY, US, vol. 102, no. 2, 7 December 2011 (2011-12-07), pages 369-376, XP028444957, ISSN: 0006-3495, DOI: 10.1016/J.BPJ.2011.12.017 [retrieved on 2011-12-17] the whole document -----	1-4
A	ONG AND J R LARKE B I: "Meibomian gland dysfunction: some clinical, biochemical and physical observations", OPHTHALMIC AND PHYSIOLOGICAL OPTICS, PERGAMON PRESS, OXFORD, GB, vol. 10, no. 2, 1 April 1990 (1990-04-01), pages 144-148, XP009170776, ISSN: 0275-5408, DOI: 10.1111/J.1475-1313.1990.TB00968.X [retrieved on 2007-12-19] abstract page 144, left-hand column, paragraph 1 - page 145, right-hand column, paragraph 4 figure 3 -----	1,2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/039399

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 5-8
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.