Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2990818 A1 2017/01/05

(21) 2 990 818

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2016/06/30
(87) Date publication PCT/PCT Publication Date: 2017/01/05
(85) Entrée phase nationale/National Entry: 2017/12/22
(86) N° demande PCT/PCT Application No.: US 2016/040563
(87) N° publication PCT/PCT Publication No.: 2017/004452
(30) Priorités/Priorities: 2015/06/30 (US62/187,090); 2016/01/15 (US62/279,262)

(51) Cl.Int./Int.Cl. *C09B 61/00* (2006.01), *A23L 5/43* (2016.01)

(71) Demandeurs/Applicants:
MARS, INCORPORATED, US;
THE OHIO STATE INNOVATION FOUNDATION, US

(72) Inventeurs/Inventors:
SIGURDSON, GREGORY, US;
COLLINS, THOMAS M., US;
GIUSTI, MARIA MONICA, US;
ROBBINS, REBECCA J., US

(74) Agent: CASSAN MACLEAN IP AGENCY INC.

(54) Titre : COMPOSITION DE COLORANT A BASE D'ANTHOCYANINE ACYLEE ET PROCEDES D'UTILISATION ASSOCIES

(54) Title: ACYLATED ANTHOCYANIN BASED COLOURANT COMPOSITION AND METHODS OF USE THEREOF

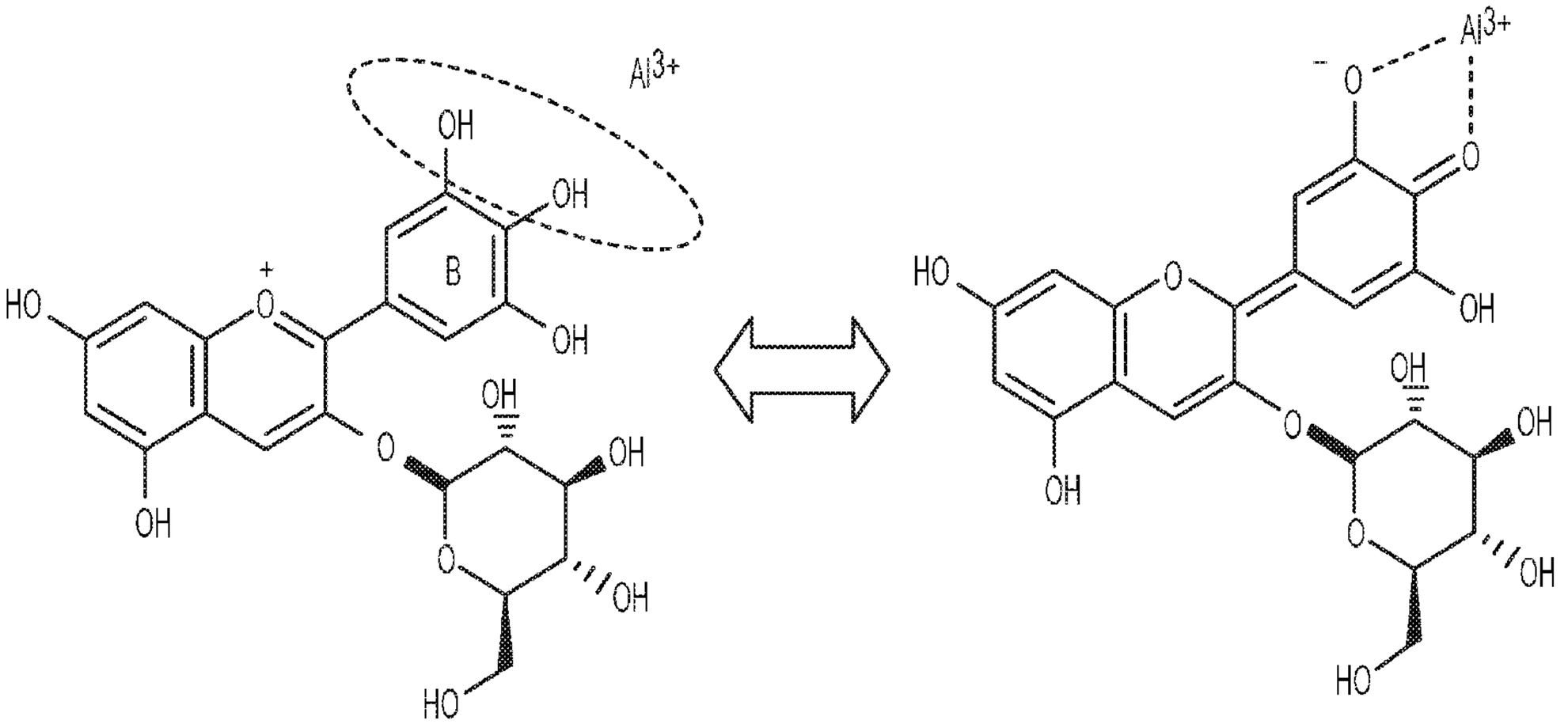


FIG. 1

(57) Abrégé/Abstract:
Natural blue colorant compositions and methods of use thereof are provided. The colorant compositions include at least one acylated anthocyanin, such as a diacylated anthocyanin. The colorant composition can further include a buffer solution having a pH from about 6.0 to about 8.0 and a metal ion or salt form thereof. The colorant compositions can be derived from a natural product and provide a stable, blue colorant that can be used in food products.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 January 2017 (05.01.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/004452 A1

(51) **International Patent Classification**:
C09B 61/00 (2006.01) ***A23L 5/43*** (2016.01)

(21) **International Application Number**:
PCT/US2016/040563

(22) **International Filing Date**:
30 June 2016 (30.06.2016)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
62/187,090 30 June 2015 (30.06.2015) US
62/279,262 15 January 2016 (15.01.2016) US

(71) **Applicants**: **MARS, INCORPORATED** [US/US]; 6885 Elm Street, Mclean, VA 22101-3883 (US). **THE OHIO STATE INNOVATION FOUNDATION** [US/US]; 1524 North High Street, Columbus, OH 43201 (US).

(72) **Inventors**: **SIGURDSON, Gregory**; 461 Elsmere St., Columbus, OH 43206 (US). **COLLINS, Thomas, M.**; 800 High Street, Hackettstown, NJ 07840 (US). **GIUSTI, Maria, Monica**; 7716 Cashel Ct., Dublin, OH 43017 (US). **ROBBINS, Rebecca, J.**; 800 High Street, Hackettstown, NJ 07840 (US).

(74) **Agent**: **LEE, Sandra, S.**; Baker Botts LLP, 30 Rockefeller Plaza, New York, NY 10112-4498 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) Title: ACYLATED ANTHOCYANIN BASED COLOURANT COMPOSITION AND METHODS OF USE THEREOF

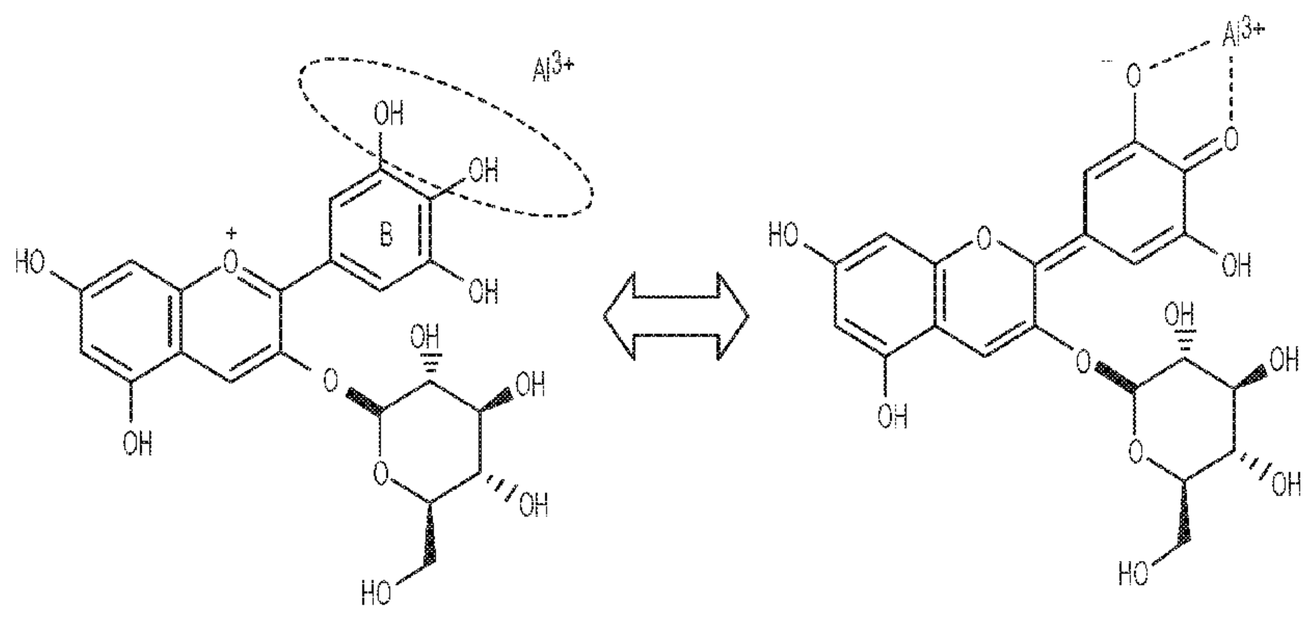


FIG. 1

(57) Abstract: Natural blue colorant compositions and methods of use thereof are provided. The colorant compositions include at least one acylated anthocyanin, such as a diacylated anthocyanin. The colorant composition can further include a buffer solution having a pH from about 6.0 to about 8.0 and a metal ion or salt form thereof. The colorant compositions can be derived from a natural product and provide a stable, blue colorant that can be used in food products.

WO 2017/004452 A1

ACYLATED ANTHOCYANIN BASED COLOURANT COMPOSITION AND METHODS OF USE THEREOF

This application claims priority to United States Provisional Application No. 62/187,090, filed June 30, 2015, and United States Provisional Application No 62/279,262, filed January 15, 2016, which are incorporated by reference herein in their entireties.

FIELD

The present disclosure relates to natural blue colorant compositions that comprise one or more acylated anthocyanin, and methods of use thereof. The colorant compositions of the present disclosure can comprise a diacylated anthocyanin and/or a pH buffer and/or a metal ion, and can be used to produce various edible products including hard coated confectionery products.

BACKGROUND

The use and demand of alternatives for synthetic food colorants has been increasing due to possible health concerns and consumer demand. However, natural food colorants have not been able to achieve the same color characteristics as their synthetic counterparts, for example, FD&C Blue No. 1. The lack of a suitable natural blue colorant has also made the development of a desirable natural green hue, from a combination of natural blue and yellow colorants, difficult.

Anthocyanins are known as natural food colorants responsible for reds and blues of fruits and vegetables. It is known in the art that anthocyanin-containing juices and extracts generally exhibit red hues at low pH and the hue shifts towards purple as pH increases. International Patent Publication No. WO 2014/152417 discloses the isolation of fractions of anthocyanin molecules from vegetable and fruit extracts, including red cabbage, at select pHs to provide different color characteristics than those provided by the source vegetable or fruit.

As a class, anthocyanins encompass a large number of structurally diverse compounds based on differences in primary structure, glycosylation and acylation patterns. Anthocyanins comprise an anthocyanidin esterified to one or more sugar molecules. Examples of sugar molecules found in anthocyanin structures are arabinose, galactose, glucose, rhamnose, rutinose, sambubiose, sophorose and xylose. An anthocyanin may be substituted with hydrogen, hydroxyl, and/or methoxyl groups

at various positions. Anthocyanins can also be acylated, where they may have one or more molecules esterified to the sugar molecules at the 2-, 3-, 4- and/or 6-position of a monosaccharide.

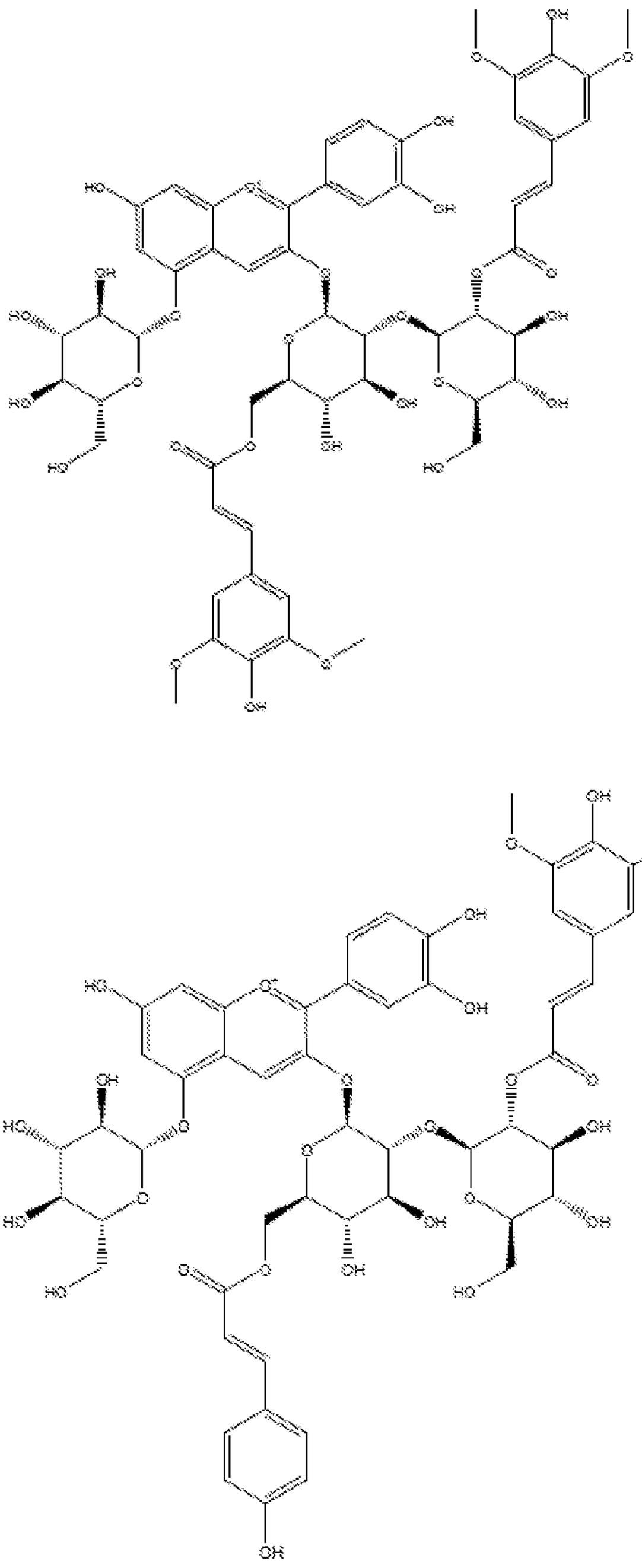
The currently available blue colorant compositions that are produced from natural sources have not been satisfactory for use in food products. For example, natural blue colorant compositions have been shown to be unstable and undergo color shifts when incorporated into a food product. Therefore, there remains a need for a natural blue anthocyanin colorant composition that provides the similar color characteristics and stability as a synthetic counterpart, such as FD&C Blue No. 1.

SUMMARY OF THE INVENTION

The present disclosure relates to colorant compositions, *e.g.*, blue colorant compositions, comprising acylated anthocyanins, and methods of generating and using such compositions. Specifically, the present disclosure is directed to natural colorant compositions *e.g.*, natural blue colorant compositions, comprising at least one acylated anthocyanin, *e.g.*, at least one diacylated anthocyanin. The present disclosure further provides methods of generating food products containing the natural colorant compositions.

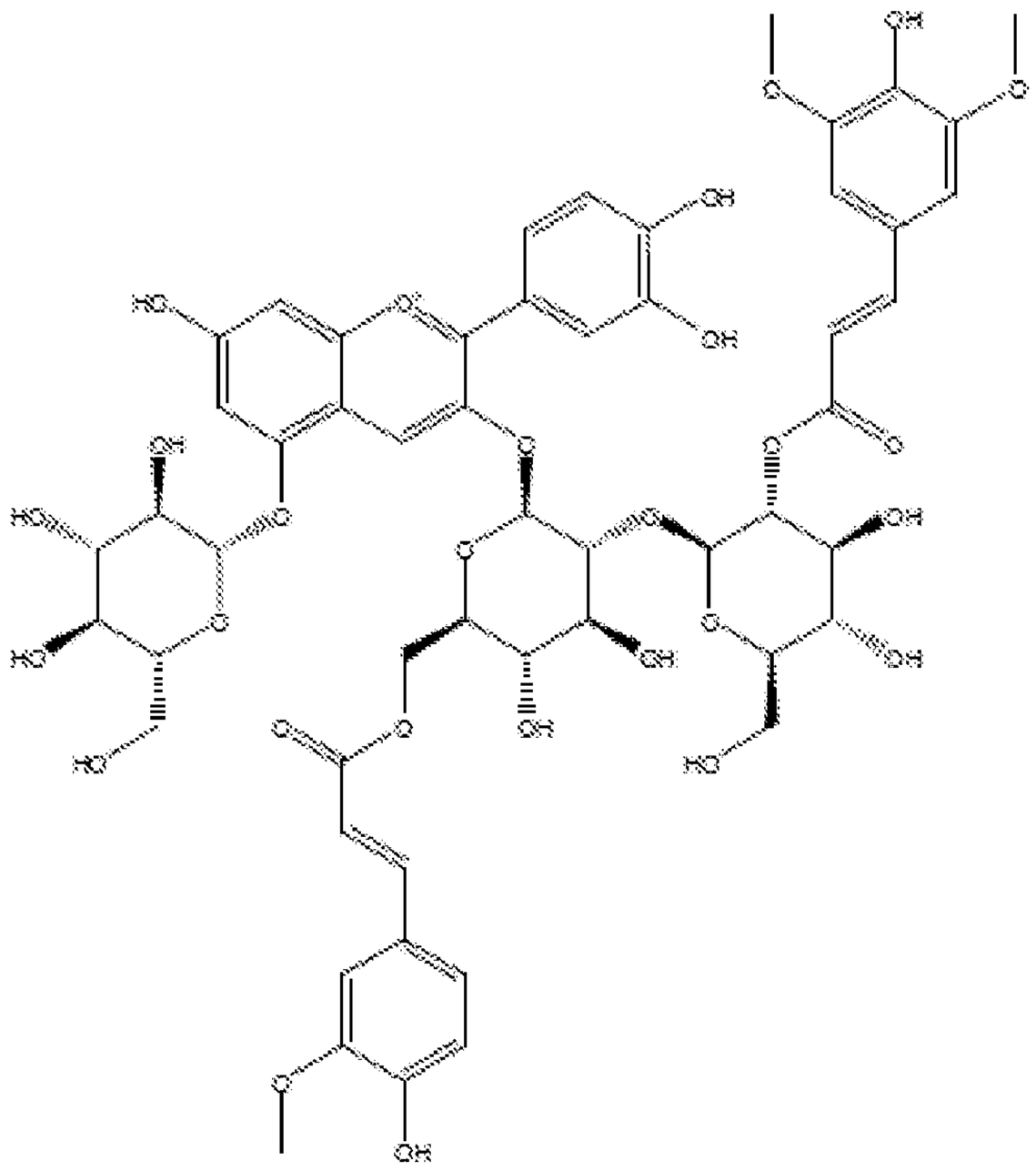
In certain embodiments, a natural blue colorant composition is provided, including a fraction of a natural juice or extract comprising at least one acylated anthocyanin, a buffer solution having a pH of from about 6.0 to about 8.0, and a metal ion or salt thereof.

In certain embodiments, the acylated anthocyanin can comprise at least one diacylated anthocyanin. In certain embodiments, the diacylated anthocyanin can be one or more of



,

, and



,

and salt forms thereof, and combinations thereof.

In certain embodiments, the metal ion can be a trivalent metal ion. The trivalent metal ion can be selected from the group consisting of a Fe^{3+}, Al^{3+}, Ga^{3+}, and combinations thereof. In certain embodiments, the trivalent metal ion can be Fe^{3+}, Al^{3+}, or a combination thereof. In certain embodiments, the buffer solution has a pH of from about 7.0 to about 8.0.

In certain embodiments, the concentration of the acylated anthocyanin can be from about 1 μM to about 200 μM. In certain embodiments, the metal ion or salt thereof can have a concentration of less than about 10 times, or from about 0.25 to about 0.5 times the concentration of the acylated anthocyanin. In certain embodiments, the metal ion or salt thereof can have a concentration that is less than or equal to the concentration of the acylated anthocyanin.

In certain embodiments, the buffer solution includes a buffer selected from the group consisting of potassium phosphate, tris(hydroxymethyl)-aminomethane, sodium hydroxide, and combinations thereof.

As embodied herein, the natural blue colorant composition can provide a color having a ΔE value of about 12 or less when compared to a color defined by L*a*b* values of 50 ppm and 100 ppm FD&C Blue No. 1 in an aqueous solution. In certain

embodiments, the ΔE value can be about 11 or less. The natural blue colorant composition can exhibit color stability for greater than about 14 days.

In certain embodiments, the fraction is obtained from a natural product, or an extract or juice thereof, selected from the group consisting of red cabbage, purple sweet potato, red potato, blue potato, red radish, black carrot, purple carrot, purple corn, red corn, red onion, purple broccoli, red broccoli, purple cauliflower, rhubarb, black bean, red leaf lettuce, black rice, eggplant, strawberry, raspberry, cranberry, lingonberry, red grape, apple, black currant, red currant, cherry, blueberry, elderberry, bilberry, crowberry, blackberry, chokeberry, gooseberry, acai, nectarine, peach, plum, blood orange, blue tomato, "Heavenly Blue" Morning Glory flower petal, "Better Times" Rose flower petal, and combinations thereof. In particular embodiments, the fraction is obtained from red cabbage.

The present disclosure further provides a green colorant composition. The green colorant composition can include a natural blue colorant composition and one or more yellow colorants. The one or more yellow colorants can be natural yellow colorants.

The present disclosure further provides a food product comprising a natural blue colorant composition. In certain embodiments, the food product can be a confectionary product. The confectionary product can further include a yellow colorant. In certain embodiments, the natural blue colorant composition can be present in a coating applied to a surface of the confectionary product.

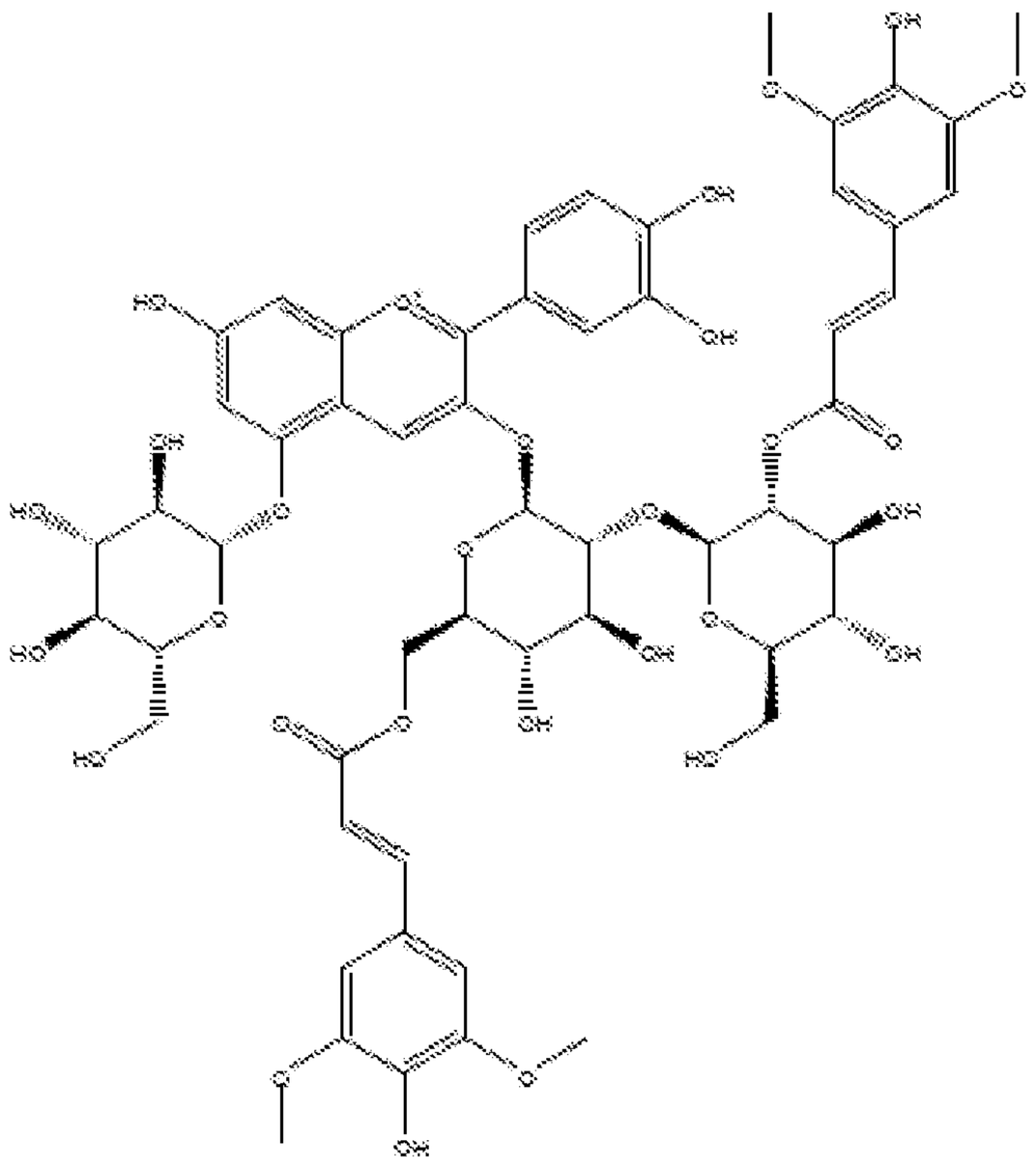
In certain embodiments, a natural blue colorant can include a fraction of a natural juice or extract including at least one diacylated anthocyanin selected from the group shown above, and salt forms thereof, and combinations thereof, where the diacylated anthocyanin has a concentration of from about 1 μM to about 200 μM. The natural blue colorant can further include a buffer solution, where the buffer solution has a pH of from about 7.0 to about 8.0, and a metal ion comprising Fe^{3+} or a salt form thereof, where the metal ion has a concentration of from about 0.25 to about 0.5 times the concentration of the diacylated anthocyanin.

In certain embodiments, a natural blue colorant can include a fraction of a natural juice or extract including at least one diacylated anthocyanin selected from the group shown above, and salt forms thereof, and combinations thereof, where the diacylated anthocyanin has a concentration of from about 1 μM to about 200 μM. The natural blue colorant can further include a buffer solution, where the buffer solution

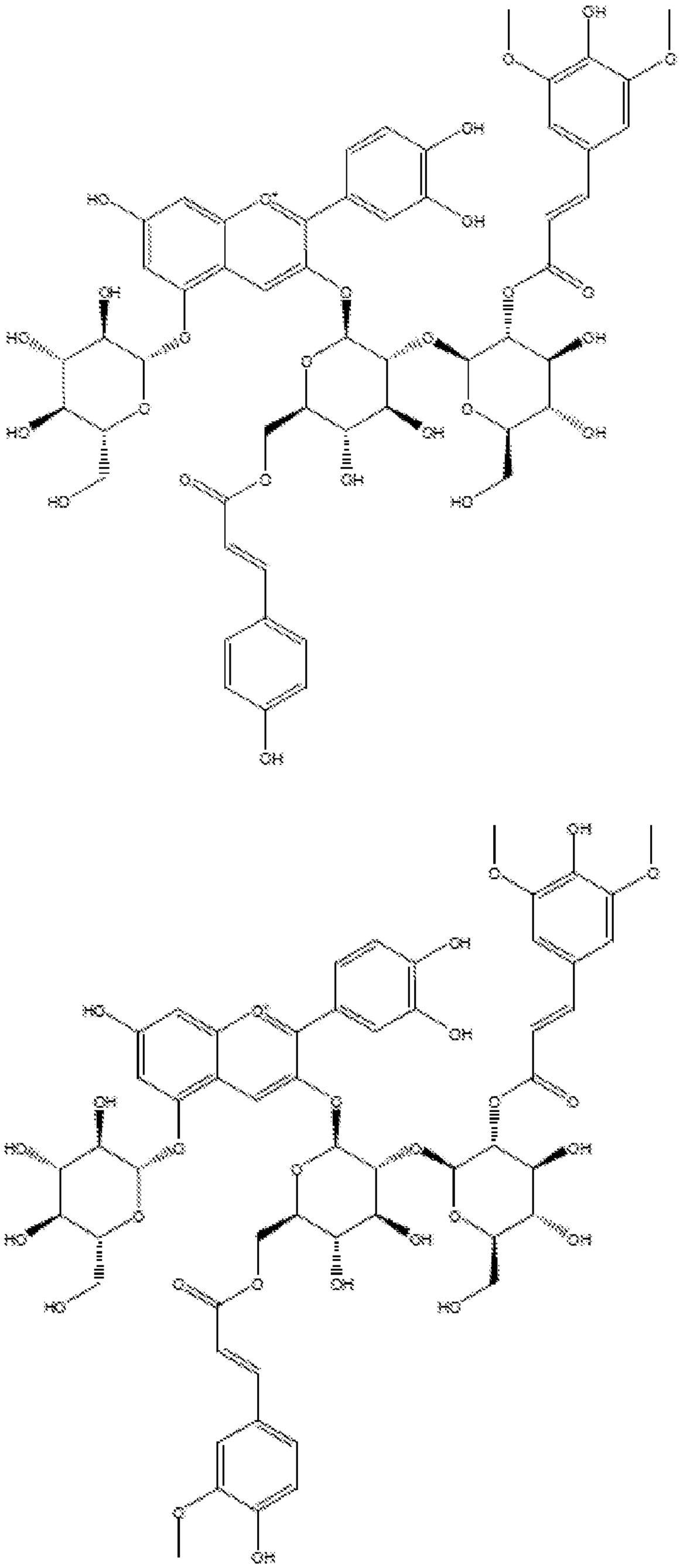
has a pH of from about 7.0 to about 8.0, and a metal ion comprising Al^{3+} or a salt form thereof, where the metal ion has a concentration of less than or equal to the concentration of the diacylated anthocyanin.

The presently disclosed subject matter further includes methods of preparing a natural blue colorant composition. For example, a method of preparing a natural blue colorant composition can include obtaining a fraction of a natural juice or extract comprising at least one acylated anthocyanin from a natural product, diluting the acylated anthocyanin in a buffer solution having a pH of from about 6.0 to about 8.0, and admixing a metal ion or salt thereof in the buffer solution.

In certain embodiments, the acylated anthocyanin can comprise at least one diacylated anthocyanin. In certain embodiments, the diacylated anthocyanin can be one or more of



,



, and

,

or salt forms thereof, or combinations thereof.

In certain embodiments, the natural product can be red cabbage. The buffer solution can have a pH of from about 7.0 to about 8.0. The metal ion or salt thereof can include Fe^{3+}, Al^{3+}, or a combination thereof.

In certain embodiments, the concentration of the acylated anthocyanin can be from about 1 μM to about 200 μM. In certain embodiments, the metal ion or salt thereof can have a concentration of less than about 10 times, or from about 0.25 to about 0.5 times the concentration of the acylated anthocyanin. In certain embodiments, the metal ion or salt thereof can have a concentration that is less than or equal to the concentration of the acylated anthocyanin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts Al^{3+} complexed with delphinidin-3-*O*-glucoside (Dpn-3-glu).

FIG. 2 shows the color of chokeberry and red cabbage anthocyanins at pH 6 in the presence of Al^{3+} at a concentration of zero to 5 times the concentration of the total amount of anthocyanins.

FIG. 3 shows the visible absorbance and λ_{max} of 50 μM cyanidin (Cy) with Al^{3+} at a pH of 6.

FIG. 4 shows monoacylated and diacylated red cabbage anthocyanins at a pH of 6, complexed with Al^{3+} at a concentration of zero to 10 times the concentration of anthocyanins. The anthocyanins complexed with the metal ion were stored for 28 days at about 23°C.

FIG. 5 shows the λ_{max} and absorbance of diacylated red cabbage anthocyanins, pH 6, in the presence of Al^{3+} 0-10 times the concentration of anthocyanins and stored for 28 days.

FIG. 6 shows the visible absorbance of 50 μM monoacylated red cabbage anthocyanins in buffers pH 5-8, complexed with Al^{3+} zero to 100 times the concentration of the total amount of anthocyanins.

FIG. 7 shows monoacylated red cabbage anthocyanins with Al^{3+} zero to 100 times the concentration of the total amount of anthocyanins.

FIG. 8 shows the λ_{max} and hue of monoacylated red cabbage anthocyanins with Al^{3+} 0-100 times the concentration of the total amount of anthocyanins.

FIG. 9 depicts the calculated ΔE of red cabbage extract in a solution having pH from 3 to 8 with various metal ions (Al^{3+}, Ga^{3+}, Cr^{3+}, and Fe^{3+}) at a concentration equimolar to the concentration of anthocyanins in the red cabbage extract.

FIG. 10 depicts the calculated ΔE of red cabbage extract in a solution having pH of 7 with various metal ions (Al^{3+}, Ga^{3+}, Cr^{3+}, and Fe^{3+}) at a concentration from zero to 500 times concentration of anthocyanins in the red cabbage extract.

FIGS. 11A-11C provide three-dimensional depictions of color characteristics based on pH and metal ion concentration for Fe^{3+} chelates, where FIG. 11A depicts ΔE, FIG. 11B depicts hue, and FIG. 11C depicts λ_{max}.

FIG. 12 depicts absorbance for Fe^{3+} chelated anthocyanins with metal ion concentrations of zero to one times the anthocyanin concentration at a pH of 7.

FIG. 13 depicts absorbance for Al^{3+} chelated anthocyanins with metal ion concentrations of zero to 100 times the anthocyanin concentration at a pH of 6.

FIGS. 14A-14C provide three-dimensional depictions of color characteristics based on pH and metal ion concentration for Al^{3+} chelates, where FIG. 14A depicts ΔE, FIG. 14B depicts hue, and FIG. 14C depicts λ_{max}.

FIGS. 15A-15C depict the absorbance of a fraction of anthocyanins after treatment with Al^{3+} at metal ion concentrations from zero to one times the anthocyanin concentration at various pHs. FIG. 15A depicts absorbance at a pH of 6; FIG. 15B depicts absorbance at a pH of 7; and FIG. 15C depicts absorbance at a pH of 8.

FIGS. 16A-16B provide the λ_{max} and absorbance, respectively, of Al^{3+} chelated diacylated anthocyanins at pHs of 6, 7, and 8.

FIGS. 17A-17C depict the absorbance of a fraction of anthocyanins after treatment with Fe^{3+} at metal ion concentrations from zero to one times the anthocyanin concentration at various pHs. FIG. 17A depicts absorbance at a pH of 6; FIG. 17B depicts absorbance at a pH of 7; and FIG. 17C depicts absorbance at a pH of 8.

FIGS. 18A-18B provide the λ_{max} and absorbance, respectively, of Fe^{3+} chelated diacylated anthocyanins at pHs of 6, 7, and 8.

FIGS. 19A-19B depict the absorbance of a fraction of anthocyanins after treatment with Al^{3+} at metal ion concentrations from zero to ten times the anthocyanin concentration and at a pH of 7. FIG. 19A shows the absorbance on day zero and FIG. 19B shows the absorbance on day 28.

FIGS. 20A-20B depict the λ_{max} and absorbance, respectively, of a fraction of anthocyanins after treatment with Al^{3+} at metal ion concentrations from zero to ten times the anthocyanin concentration and at a pH of 7 over 28 days of storage.

FIGS. 21A-21B depicts the absorbance (at the λ_{max} wavelength of day zero) of Al^{3+} chelates at pHs from 5 to 8 over 28 days of storage. FIG. 21A shows the absorbance of chelates having a metal ion concentration of 0.5 times the anthocyanin concentration and FIG. 21B shows the absorbance of chelates having a metal ion concentration of 10 times the anthocyanin concentration.

FIGS. 22A-22B depict the absorbance of a fraction of anthocyanins after treatment with Fe^{3+} at metal ion concentrations from zero to ten times the anthocyanin concentration and at a pH of 7. FIG. 22A shows the absorbance on day zero and FIG. 22B shows the absorbance on day 28.

FIGS. 23A-23B depict the λ_{max} and absorbance, respectively, of a fraction of anthocyanins after treatment with Fe^{3+} at metal ion concentrations from zero to ten times the anthocyanin concentration and at a pH of 7 over 28 days of storage.

FIGS. 24A-24B depicts the absorbance (at the λ_{max} wavelength of day zero) of Fe^{3+} chelates at pHs from 5 to 8 over 28 days of storage. FIG. 24A shows the absorbance of chelates having a metal ion concentration of 0.5 times the anthocyanin concentration and FIG. 24B shows the absorbance of chelates having a metal ion concentration of 10 times the anthocyanin concentration.

FIGS. 25A-25B depict the λ_{max} and absorbance, respectively, of red cabbage extract treated with Al^{3+} and/or Fe^{3+} at metal ion concentrations from zero to 10 times the anthocyanin concentration and a pH of 7.

FIG. 26 provides a chromatogram of red cabbage extract, showing Peaks 4-6 corresponding to diacylated anthocyanins.

FIGS. 27A-27B depict the hue angles of metallo-anthocyanins comprising Peaks 4-6 at metal ion concentrations from zero to 5 times the anthocyanin concentration and a pH of 7. FIG. 27A provides the hue angles of Al^{3+} chelates and FIG. 27B provides the hue angles of Fe^{3+} chelates.

FIGS. 28A-28B depict the hue angles of metallo-anthocyanins comprising Peaks 4-6 at metal ion concentrations from zero to 5 times the anthocyanin concentration and a pH of 8. FIG. 28A provides the hue angles of Al^{3+} chelates and FIG. 28B provides the hue angles of Fe^{3+} chelates.

FIGS. 29A-29B depict the chroma of metallo-anthocyanins comprising Peaks 4-6 at metal ion concentrations from zero to 5 times the anthocyanin concentration and a pH of 7. FIG. 29A provides the chroma of Al^{3+} chelates and FIG. 29B provides the chroma of Fe^{3+} chelates.

FIGS. 30A-30B depict the chroma of metallo-anthocyanins comprising Peaks 4-6 at metal ion concentrations from zero to 5 times the anthocyanin concentration and a pH of 8. FIG. 30A provides the chroma of Al^{3+} chelates and FIG. 30B provides the chroma of Fe^{3+} chelates.

FIGS. 31A-31B depict λ_{max} of Peak 4 at metal ion concentrations from zero to 5 times the anthocyanin concentration and at a pH of 6 over 48 hours of storage. FIG. 31A shows λ_{max} of Peak 4 after treatment with Al^{3+} and FIG. 31A shows λ_{max} of Peak 4 after treatment with Fe^{3+}.

FIGS. 32A-32F depict λ_{max} of Peaks 4, 5, and 6 at metal ion concentrations from zero to 5 times the anthocyanin concentration and at a pH of 7 over 48 hours of storage. FIGS. 32A-32C show λ_{max} of Peaks 4, 5, and 6, respectively, after treatment with Al^{3+}. FIGS. 32D-32F show λ_{max} of Peaks 4, 5, and 6, respectively, after treatment with Fe^{3+}.

FIGS. 33A-33F depict λ_{max} of Peaks 4, 5, and 6 at metal ion concentrations from zero to 5 times the anthocyanin concentration and at a pH of 8 over 48 hours of storage. FIGS. 33A-33C show λ_{max} of Peaks 4, 5, and 6, respectively, after treatment with Al^{3+}. FIGS. 33D-33F show λ_{max} of Peaks 4, 5, and 6, respectively, after treatment with Fe^{3+}.

DETAILED DESCRIPTION

The present disclosure relates to colorant compositions, *e.g.*, blue colorant compositions, that include at least one acylated anthocyanin. The colorant composition can further include a metal ion and/or a buffer having a particular pH. In certain embodiments, the colorant compositions can be used in wide variety of food products. For example, and not by way of limitation, a colorant composition of the present disclosure can be used in a coating of a hard panned confection.

1. Definitions

The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them.

As used herein, the use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." Still further, the terms "having," "including," "containing" and "comprising" are interchangeable and one of skill in the art is cognizant that these terms are open ended terms.

The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 3 or more than 3 standard deviations, per the practice in the art.

As used herein, the term "acylated anthocyanin" refers to an anthocyanin molecule having one or more molecules esterified to one or more sugar molecules at the 2-, 3-, 4-, and/or 6-position of a monosaccharide.

As used herein, the term "monoacylated anthocyanin" refers to an anthocyanin molecule that has one molecule esterified to a sugar molecule at a single position of the sugar molecule.

As used herein, the term "diacylated anthocyanin" refers to an anthocyanin molecule that has one molecule esterified to the monosaccharide at two positions of the monosaccharide.

As used herein, the term "monovalent metal ion" refers to a metal atom having one valence electron. Non-limiting examples of monovalent metal ions include Na^{+} and K^{+}.

As used herein, the term "multivalent metal ion" refers to a metal atom having more than one valence electron. Non-limiting examples of multivalent metal ions include divalent metal ions, trivalent metal ions, Cr^{+6}, Cr^{+4} and Mn^{+4}.

As used herein, the term "divalent metal ion" refers to a metal atom having two valence electrons. Non-limiting examples of divalent metal ions include Ca^{2+}, Mg^{2+}, Cu^{2+}, and Fe^{2+}.

As used herein, the term "trivalent metal ion" refers to a metal atom having three valence electrons. Non-limiting examples of trivalent metal ions include Al^{3+}, Fe^{3+}, and Ga^{3+}.

As used herein, the term "colorant" refers to any substance that imparts color by absorbing or scattering light at different wavelengths.

As used herein, the term "colorant composition" refers to any composition that imparts color by absorbing or scattering light at different wavelengths.

As used herein, the term "natural colorant" refers to any substance that exists in or is produced by nature or is obtained from a natural source. In certain embodiments, the term "natural colorant" refers to a colorant that comprises one of more anthocyanins obtained from a natural source, *e.g.*, a vegetable, a plant, or a flower (or a flower petal).

As used herein, the term "natural colorant composition" refers to any composition that comprises a colorant that exists in or is produced by nature or is obtained from a natural source. In certain embodiments, the term "natural colorant composition" refers to a colorant composition that comprises one of more anthocyanins obtained from a natural source, *e.g.*, a vegetable, a plant, or a flower (or a flower petal).

As used herein, the term "blue colorant" refers to a colorant that reflects light at wavelengths in the region of 450 to 495 nanometers and has a maximum UV/VIS wavelength absorbance ranging from 615 to 635 nanometers.

As used herein, the term "blue colorant composition" refers to a colorant composition that reflects light at wavelengths in the region of 450 to 495 nanometers and has a maximum UV/VIS wavelength absorbance ranging from 615 to 635 nanometers.

As used herein, the term "green colorant" refers to a colorant that reflects light at wavelengths in the region of 490 to 570 nanometers and has a maximum UV/VIS wavelength absorbance ranging from 380 to 420 nanometers or from 630 to 750 nanometers.

As used herein, the term "green colorant composition" refers to a colorant composition that reflects light at wavelengths in the region of 490 to 570 nanometers and has a maximum UV/VIS wavelength absorbance ranging from 380 to 420 nanometers or from 630 to 750 nanometers.

As used herein, the term "yellow colorant" refers to a colorant that reflects light at wavelengths in the region of 570 to 585 nanometers and has a maximum UV/VIS wavelength absorbance ranging from 420 to 430 nanometers.

As used herein, the term "yellow colorant composition" refers to a colorant composition that reflects light at wavelengths in the region of 570 to 585 nanometers

and has a maximum UV/VIS wavelength absorbance ranging from 420 to 430 nanometers.

As used herein, "maximum absorbance," "lambda max," or "λ_{max}," refers to the wavelength in nanometers at which the maximum fraction of light is absorbed by a substance, colorant and/or colorant composition.

As used herein, "FD&C Blue No. 1" includes the various names given to the identical synthetic blue colorant, Brilliant Blue FCF and European Commission E133. The lambda max of FD&C Blue No. 1 is 630 nm.

As used interchangeably herein, the terms "color" and "color characteristics" refer to the color properties such as hue, chroma, purity, saturation, intensity, vividness, value, lightness, brightness and darkness, and color model system parameters used to describe these properties, such as Commission Internationale de l'Eclairage CIE 1976 CIELAB color space L*a*b* values and CIELCH color space L*C*h° values. The CIELAB and CIELCH color models provide more perceptually uniform color spaces than earlier color models. In certain embodiments, the colorant compositions of the present disclosure can be analyzed with a spectrophotometer, and CIELAB L*a*b* and CIELCH L*C*h° values can be calculated from the spectral data, as described in greater detail below. The L*a*b* and L*C*h° values provide a means of representing color characteristics and assessing the magnitude of difference between two colors. Methods for determining the CIELAB and CIELCH values of a presently disclosed colorant composition are disclosed in International Patent Publication Nos. WO 2014/150230 and WO 2014/152417, the contents of which are hereby incorporated by reference in their entireties.

As used herein, "hue" or "hue angle" refers to the color property that gives a color its name, for example, red, blue and violet.

As used herein, "chroma" is a color property indicating the purity of a color. In certain embodiments, a higher chroma is associated with greater purity of hue and less dilution by white, gray or black.

As used herein, "value" is a color property indicating the lightness or darkness of a color wherein a higher "value" is associated with greater lightness.

As used herein "admixing," for example, "admixing a colorant composition of the present disclosure with a food product," refers to the method where a colorant composition of the present disclosure is mixed with or added to the completed product or mixed with some or all of the components of the product during product formation

or some combination of these steps. When used in the context of admixing the term "product" refers to the product or any of its components. Admixing can include a process that includes adding the colorant composition to the product, spraying the colorant composition on the product, coating the colorant composition on the product, painting the colorant composition on the product, pasting the colorant composition on the product, encapsulating the product with the colorant composition, mixing the colorant composition with the product or any combination thereof. The colorant compositions, *e.g.*, those that are admixed with the product, can be a liquid, dry powder, spray, paste, suspension or any combination thereof.

"Fractionation" is the process of selecting and separating a portion of compounds from the complex mixture of an anthocyanin-containing vegetable, fruit, or flower juice or extract. A "fraction" is the product of fractionation. Anthocyanin fractions are separated from the juice or extract at a select pH based on differences in charge and polarity of the different anthocyanin molecules present. An "fraction of a natural juice or extract" is the product of the fraction of a juice or extract from a natural source, *e.g.*, a vegetable, fruit, or flower. A fraction of a natural juice or extract contains a mixture of anthocyanins that is different from the mixture of anthocyanins in the anthocyanin-containing juice or extract from which the fraction was separated.

As used herein, "food grade," refers to any substance, metal ion and/or colorant composition that is of a grade acceptable for use in edible food products.

As used herein, "food product" refers to an ingestible product, such as, but not limited to, human food, animal foods and pharmaceutical compositions.

As used herein, "sugar syrup" refers to a liquid material comprising at least a sugar and water. In certain embodiments, a sugar syrup can include a syrup where a sugar is dissolved in the water in an amount of at least 60% sugar solids by weight of the syrup. In certain embodiments, other components can also be present within the sugar syrup. For example, and not by way of limitation, a colorant composition of the present disclosure can be present within a sugar syrup.

As used herein, "coating layer" refers to a layer obtained by one application of a coating material, *e.g.*, a sugar syrup, to a substrate, *e.g.*, a food product, being coated.

As used herein, "coating" refers to the total amount of coating material, *e.g.*, one or more sugar syrups, applied to a substrate, *e.g.*, a food product, upon completion

of a coating process. In certain embodiments, the coating process can include one or more steps of applying a coating material, *e.g.*, a sugar syrup containing one or more colorant compositions of the present disclosure, to the substrate.

As used herein, "beverage category" refers to beverages, beverage mixes and concentrates, including but not limited to, alcoholic and non-alcoholic ready to drink and dry powdered beverages. Additional non-limiting examples of beverages include carbonated and non-carbonated beverages, *e.g.*, sodas, fruit or vegetable juices.

As used herein, "frozen food category" refers to chilled or frozen food products. Non-limiting examples of food products of the frozen food category include ice cream, impulse ice cream, single portion dairy ice cream, single portion water ice cream, multi-pack dairy ice cream, multi-pack water ice cream, take-home ice cream, take-home dairy ice cream, ice cream desserts, bulk ice cream, take-home water ice cream, frozen yogurt, artisanal ice cream, frozen ready meals, frozen pizza, chilled pizza, frozen soup, frozen pasta, frozen processed red meat, frozen processed poultry, frozen processed fish/seafood, frozen vegetables, frozen processed vegetables, frozen meat substitutes, frozen potatoes, frozen bakery products and frozen desserts.

As used herein, "snack food category" refers to any food that can be a light informal meal including, but not limited to sweet and savory snacks and snack bars. Examples of snack foods include, but are not limited to, fruit snacks, chips/crisps, extruded snacks, tortilla/corn chips, popcorn, pretzels, nuts and other sweet and savory snacks. Examples of snack bars include, but are not limited to granola/muesli bars, breakfast bars, energy bars, fruit bars and other snack bars.

As used herein, "confectionery product" or "confection" refers to a sweet or candy food product. Non-limiting examples of confectionery products include cakes, cookies, pies, candies, chocolates, chewing gums, gelatins, ice creams, sorbets, puddings, jams, jellies, salad dressings, and other condiments, cereal, and other breakfast foods, canned fruits and fruit sauces.

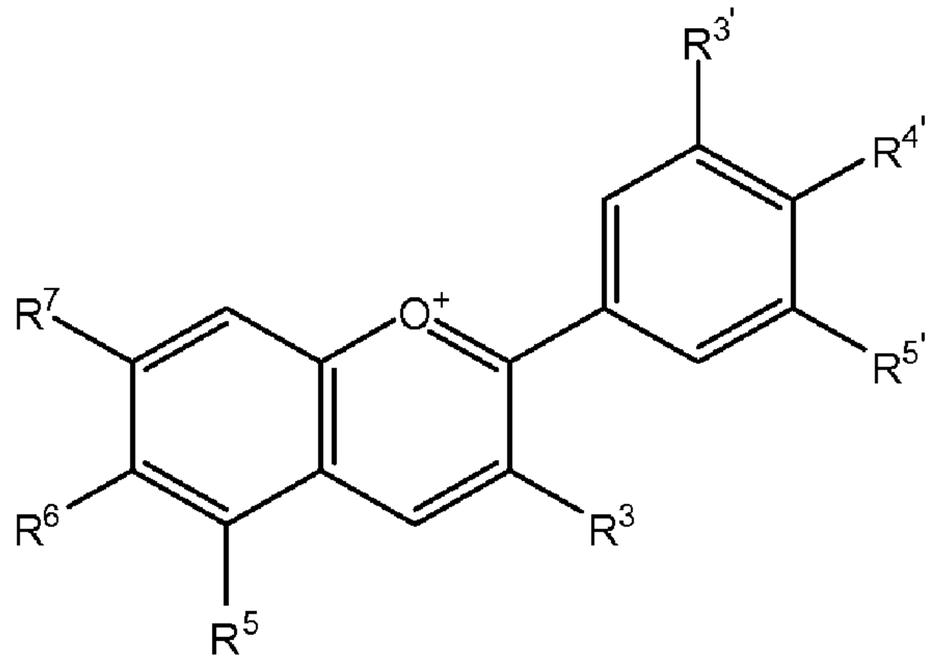
2. Acylated Anthocyanins

The present disclosure relates to colorant compositions that include at least one acylated anthocyanin, *e.g.*, a diacylated anthocyanin. In certain embodiments, the colorant composition is a natural colorant composition, in which the anthocyanin is naturally derived, *e.g.*, from a natural vegetable, plant, or flower (or flower petal)

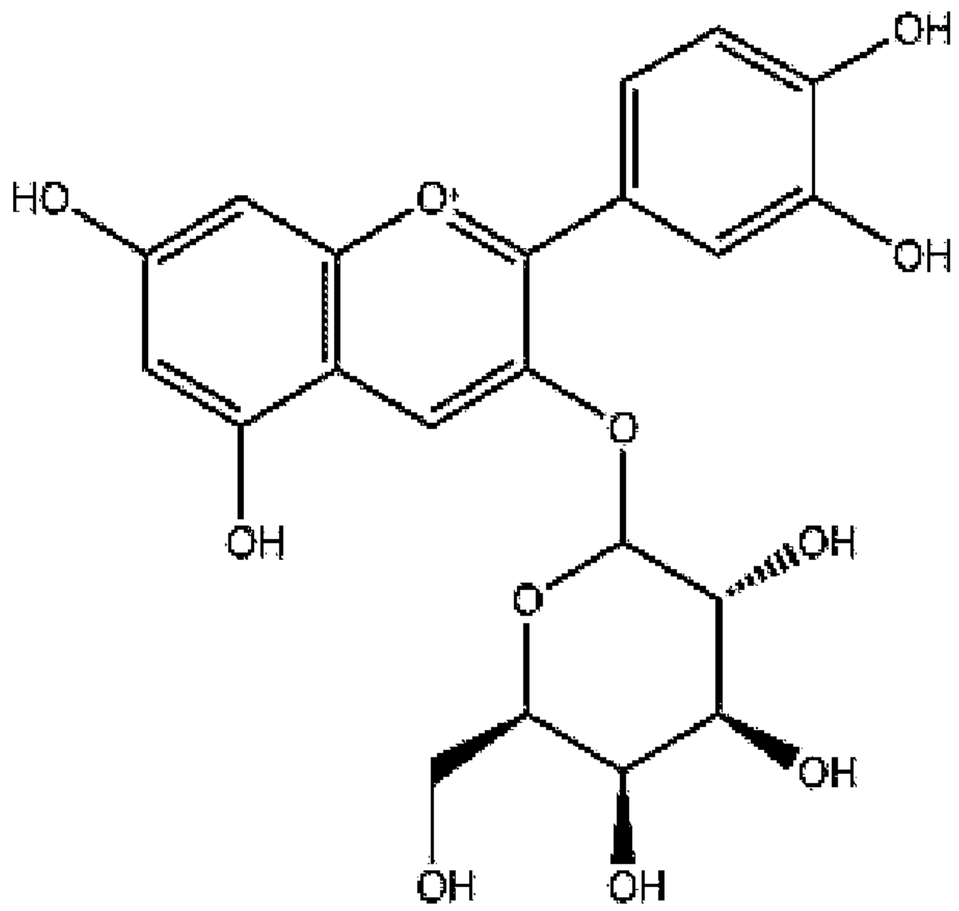
source. In certain embodiments, the colorant composition is a blue colorant composition, in which the anthocyanin produces a blue color. The color compositions disclosed herein can include one or more acylated anthocyanins, *e.g.*, diacylated anthocyanins, and/or one or more metal ions, or salt forms thereof, and/or one or more buffers.

The colorant compositions of the present disclosure can be added to food products, for example, to alter the color characteristics of the food product. For example, and not by way of limitation, the colorant composition can be used within a coating for confections to produce a blue colored coating. Alternatively or additionally, the blue colorant composition can be combined with another colorant, *e.g.*, a yellow colorant, to produce a colorant composition of a different color, *e.g.*, a green colorant composition.

In certain embodiments, an anthocyanin of the present disclosure, *e.g.*, an acylated anthocyanin, can be substituted with hydrogen, hydroxyl and/or methoxyl groups at various positions. An anthocyanin includes an anthocyanidin esterified with one or more sugar molecules to form a glycoside. Anthocyanidins can have the general structure:



For example, and not by way of the limitation, the anthocyanin can be an anthocyanin of Formula I (also referred to herein as cyanidin-3-*O*-glucoside (Cy-3-



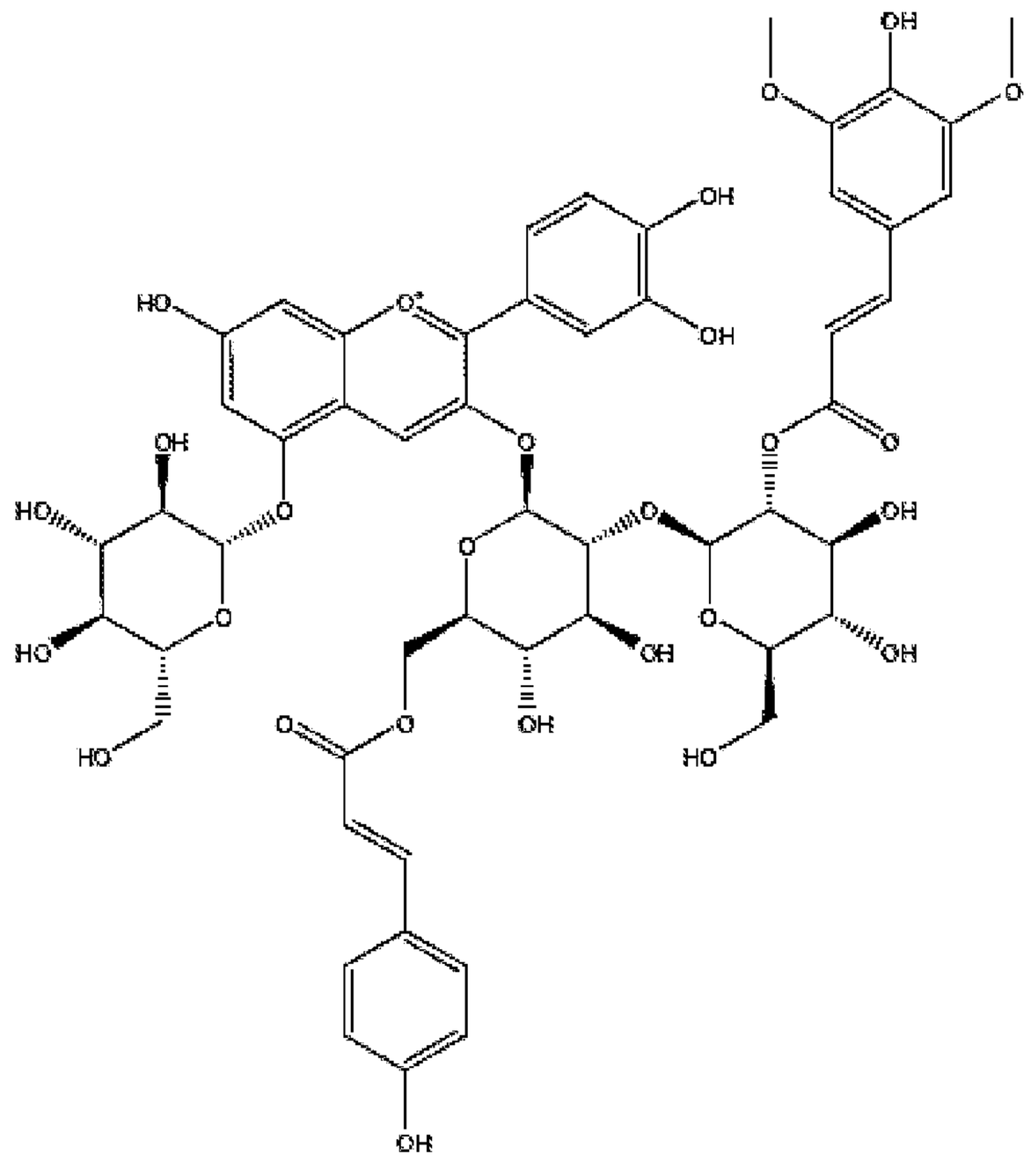
glu)):

In certain embodiments, the anthocyanin can be a derivative, *e.g.*, glycoside, of cyanidin, delphinidin, aurantinidin, europinidin, pelargonidin, malvidin, peonidin, petunidin, rosinidin or combinations thereof.

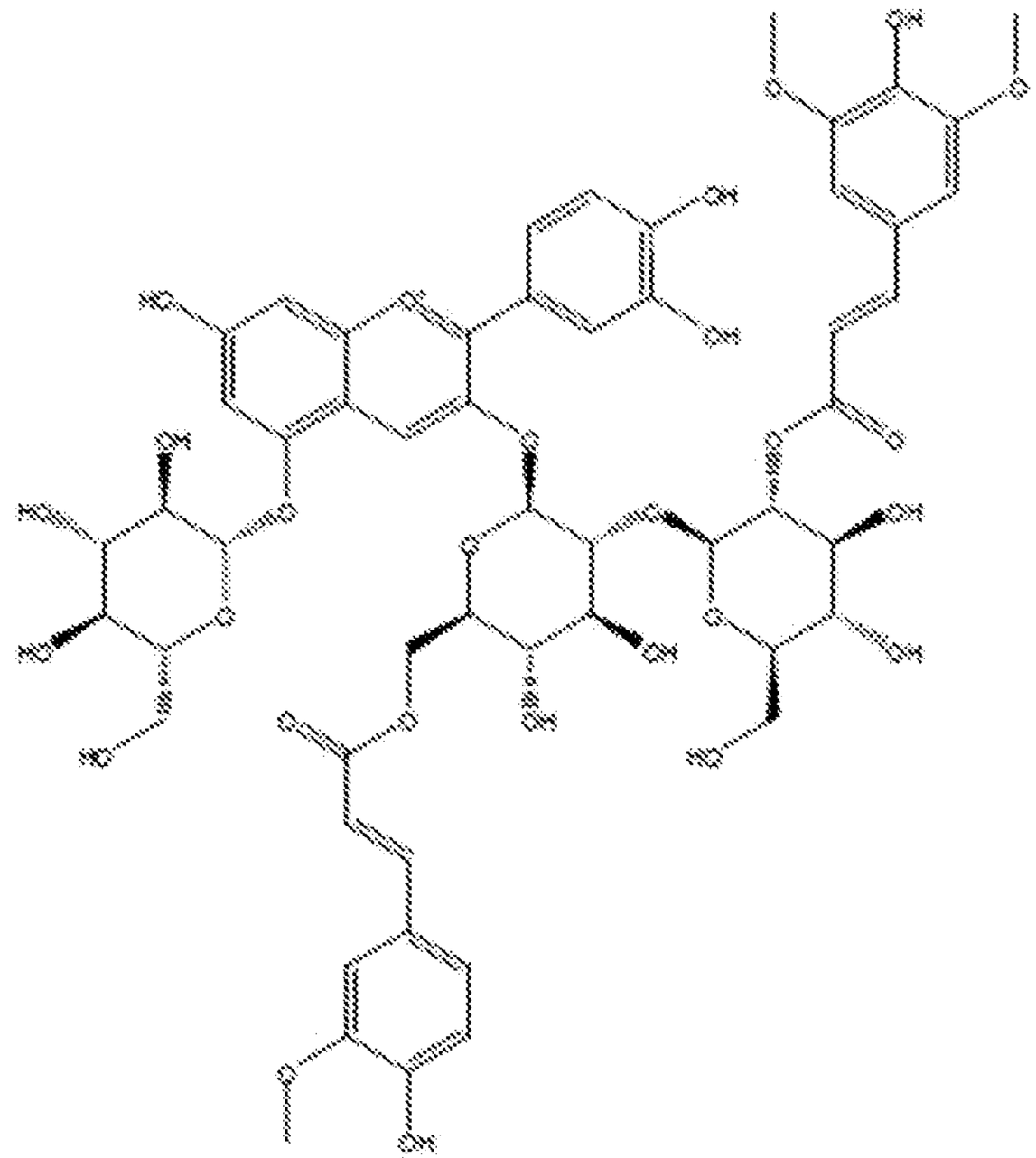
In certain embodiments, the anthocyanin of the present disclosure can be acylated. For example, and not by way of limitation, the anthocyanin can have one or more molecules esterified to the sugar molecules at the 2-, 3-, 4-, and/or 6-position of a monosaccharide. In certain embodiments, the anthocyanin of the present disclosure can include one or more acyl groups, *i.e.*, be acylated. In certain embodiments, the acyl group is derived from coumaric, ferulic, caffeic, sinapic, gallic, malonic, acetic, malic, succinic, vanillic, hydroxycinnamic, oxalic acids or combinations thereof.

In certain embodiments, the acylated anthocyanin is a monoacylated cyanidin glucoside, a diacylated cyanidin glucoside or a mixture thereof. In certain embodiments, the anthocyanin of the present disclosure can be an anthocyanin of Formula I that includes one or more acyl groups. In certain embodiments, the acylated anthocyanin can be monoacylated or diacylated forms of cyanidin, delphinidin, aurantinidin, europinidin, pelargonidin, malvidin, peonidin, petunidin or rosinidin derivatives, *e.g.*, glucosides and galactosides.

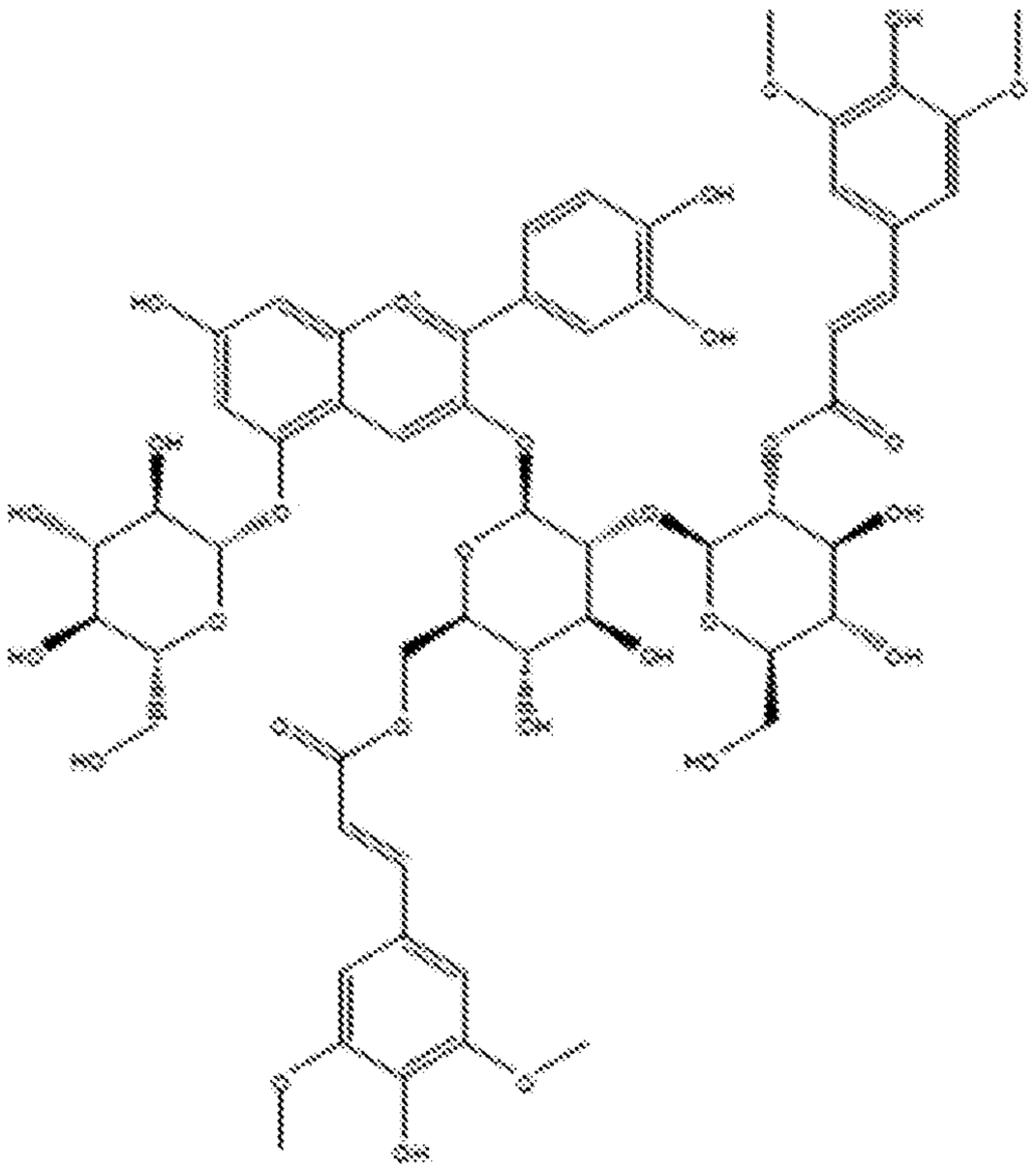
In certain embodiments, an anthocyanin of the present disclosure is a diacylated anthocyanin of Formula II (also referred to herein as Cy-3-diglu-*p*-coumaric-sinapic-5-glu):



In certain embodiments, an anthocyanin of the present disclosure is a diacylated anthocyanin of Formula III (also referred to herein as Cy-3-diglu-*p*-ferulic-sinapic-5-glu):



In certain embodiments, an anthocyanin of the present disclosure is a diacylated anthocyanin of Formula IV (also referred to herein as Cy-3-diglu-*p*-sinapic-sinapic-5-glu):



The anthocyanins of the present disclosure can be obtained from natural sources and/or juices or extracts thereof. For example, and not by way of limitation, the anthocyanins of the present disclosure can be obtained from vegetables (or extracts or juices thereof) such as red cabbage, purple sweet potato, red potato, blue potato, red radish, black carrot, purple carrot, purple corn, red corn, red onion, purple broccoli, red broccoli, purple cauliflower, rhubarb, black bean, red leaf lettuce, black rice, eggplant or combinations thereof. In certain embodiments, the anthocyanins can be obtained from fruits (or extracts or juices thereof) such as, but not limited to, strawberry, raspberry, cranberry, lingonberry, red grape, apple, black currant, red currant, cherry, blueberry, elderberry, bilberry, crowberry, blackberry, chokeberry, gooseberry, acai, nectarine, peach, plum, blood orange, blue tomato or combinations thereof. In certain embodiments, the anthocyanins of the present disclosure can be obtained from flower petals (or extracts or juices thereof) such as those of "Heavenly Blue" Morning Glory, "Better Times" Rose or combinations thereof.

In certain embodiments, a vegetable, fruit, and flower petal juice can be obtained by pressing liquid out of the fruit, vegetable, or flower. In certain

embodiments, a vegetable, fruit, and flower petal extract can be obtained by washing a macerated fruit, vegetable, or flower with a solvent (*e.g.*, water or alcohol). Juices and extract can contain anthocyanins as well as other naturally occurring compounds, including, for example, carbohydrates, acids, flavonoids, metal ions, phenolic acids, phenolic acid esters, and vitamins.

In certain embodiments, vegetable, fruit, and flower petal juices or extracts can include processed juices and extracts. Non-limiting examples of processed juices and extracts include reconstituted juices and extracts, deodorized juices and extracts, and juices and extracts subjected to other processes for removing specific or broad classes of compounds.

In certain embodiments, the anthocyanin is obtained from red cabbage or an extract or juice thereof.

3. Colorant Compositions

The present disclosure provides colorant compositions that comprise one or more acylated anthocyanins, *e.g.*, diacylated anthocyanins, disclosed above, and/or a metal ion (or salt form thereof) and/or a buffer of a particular pH. In certain embodiments, a color composition of the present disclosure can be a blue colorant composition, *e.g.*, the anthocyanins present with the color composition produce a blue color.

In certain embodiments, one or more of the colorant compositions of the present disclosure can be added to a food product, in an amount effective to increase, enhance and/or modify the color characteristics of a food product. For example, and not by way of limitation, a colorant composition of the present disclosure can enhance the blue color characteristics of the food product. In certain embodiments, colorant compositions of the present disclosure can be used to increase, enhance and/or modify the color characteristics of a food product, such as, but not limited to, a chocolate confection.

In certain embodiments, the colorant composition can comprise one or more acylated anthocyanins, *e.g.*, diacylated anthocyanins, at a concentration of about 1 μM to about 1 M. In certain embodiments, the colorant composition can comprise one or more acylated anthocyanins, *e.g.*, diacylated anthocyanins, at a concentration of about 1 μM to about 200 μM. For example, and not by way of limitation, the anthocyanins can be present in the colorant composition at a concentration from about 1 μM to

about 150 μM, from about 1 μM to about 125 μM, from about 1 μM to about 100 μM, from about 1 μM to about 75 μM, from about 1 μM to about 50 μM, from about 1 μM to about 25 μM, from about 1 μM to about 20 μM, from about 1 μM to about 15 μM, from about 1 μM to about 10 μM, from about 1 μM to about 5 μM, from about 5 μM to about 200 μM, from about 10 μM to about 200 μM, from about 15 μM to about 200 μM, from about 20 μM to about 200 μM, from about 25 μM to about 200 μM, from about 50 μM to about 200 μM, from about 75 μM to about 200 μM, from about 100 μM to about 200 μM, from about 125 μM to about 200 μM, from about 150 μM to about 200 μM or from about 175 μM to about 200 μM. In certain embodiments, the colorant composition can comprise one or more anthocyanins at a concentration of about 25 μM to about 75 μM. In certain embodiments, the colorant composition can comprise one or more acylated anthocyanins, *e.g.*, diacylated anthocyanins, at a concentration of about 50 μM.

In certain embodiments, the one or more anthocyanins comprise about 1% to about 100% by weight of the total colorant composition. In certain embodiments, the one or more anthocyanins comprise about 10% to about 90% by weight of the colorant composition. In certain embodiments, the one or more anthocyanins comprise about 20% to about 80% by weight of the colorant composition. In certain embodiments, the one or more anthocyanins comprise about 30% to about 70% by weight of the colorant composition. In certain embodiments, the one or more anthocyanins comprise about 40% to about 60% by weight of the colorant composition. In certain embodiments, the one or more anthocyanins comprise about 1% to about 20%, about 5% to about 15% or about 8% to about 12% by weight of the colorant composition. In certain embodiments, the one or more anthocyanins comprise about 10% by weight of the total colorant composition.

In certain embodiments, the colorant compositions of the present disclosure exhibit increased color stability, *e.g.*, increased blue color stability. As shown in the Examples below, the stability of the color composition can depend on the acylation of the anthocyanin, the concentration of the metal ion in relation to the concentration of the anthocyanins in the composition, and the pH of the buffer. For example, and not by way of limitation, a composition comprising a diacylated anthocyanin, a metal ion, and a buffer having a pH of about 6 exhibits greater color stability than a color composition comprising a monoacylated anthocyanin, a metal ion, and a buffer having a pH of about 6. In certain embodiments, a colorant composition, *e.g.*, a

colorant composition that comprises an diacylated anthocyanin, a metal ion, and a buffer that has a pH range of about 7, exhibits greater color stability than a composition that comprises a mono-acylated anthocyanin. In certain embodiments, a composition comprising a diacylated anthocyanin, a metal ion, and a buffer having a pH of about 7 exhibits greater color stability than a color composition comprising a diacylated anthocyanin, a metal ion, and a buffer having a pH of about 8. In certain embodiments, a composition that comprises an acylated anthocyanin, a metal ion, and a buffer that has a pH range of about 5 to about 8 exhibits greater color stability than a colorant composition that includes the acylated anthocyanin alone. In certain embodiments, a colorant composition, *e.g.*, a colorant composition that comprises an acylated anthocyanin, a metal ion, and a buffer that has a pH range of about 5 to about 8, exhibits greater color stability than a composition that comprises a non-acylated anthocyanin.

In certain embodiments, a colorant composition of the present disclosure in solution exhibits color stability, *e.g.*, blue color stability, for a time period greater than about 1 day, greater than about 2 days, greater than about 3 days, greater than about 4 days, greater than about 5 days, greater than about 6 days, greater than about 7 days, greater than about 8 days, greater than about 9 days, greater than about 10 days, greater than about 11 days, greater than about 12 days, greater than about 13 days, greater than about 14 days, greater than about 15 days, greater than about 16 days, greater than about 17 days, greater than about 18 days, greater than about 19 days, greater than about 20 days, greater than about 21 days, greater than about 22 days, greater than about 23 days, greater than about 24 days, greater than about 25 days, greater than about 26 days, greater than about 27 days, or greater than about 28 days.

In certain embodiments, the colorant composition of the present disclosure can further include one or more stabilizers to enhance stability.

3.1. Metal Ions

In certain embodiments of the present disclosure, the colorant composition comprises at least one acylated anthocyanin, *e.g.*, a diacylated anthocyanin, as disclosed above, and at least one metal ion or salt form thereof. In certain embodiments, the metal ion can bind to one or more hydroxyl groups present on the anthocyanin molecule (*see, e.g.,* FIG. 1), for example, to increase the color stability of the color composition and/or enhance the blue color of the color composition.

In certain embodiments, the metal ion can be a cation. In certain embodiments, the metal ion can be a multivalent metal ion such as, but not limited to, a divalent metal ion or a trivalent metal ion. Non-limiting examples of a divalent cation include Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Cr^{2+}, Fe^{2+}, Co^{2+}, or Ni^{2+}. In certain embodiments, the metal cation is a trivalent cation such as Al^{3+}, Au^{3+}, Cr^{3+}, Mn^{3+}, Ga^{3+}, Fe^{3+}, Co^{3+}, Ni^{3+}, Ce^{3+}, Sb^{3+}, or Bi^{3+}. In certain embodiments, the metal ion is Al^{3+}. In certain embodiments, the metal ion is Ga^{3+}. In certain embodiments, the metal ion is Fe^{3+}.

In certain embodiments, the metal ion can be present in the colorant composition as a metal salt. For example, and not by way of limitation, the metal salt can be $Al_2(SO_4)_3$, $FeCl_3$, $GaCl_3$, and/or $CrCl_3$.

In certain embodiments, thereof can be a food grade metal ion or salt form thereof. In certain embodiments, the metal ion or salt form thereof can be selected to be suitable for use in an edible product, *e.g.*, Al^{3+} or Fe^{3+}.

In certain embodiments, the concentration of the metal ion (or salt thereof) in the colorant composition can be from about 0.01 times to about 100 times the concentration of anthocyanins. For example, and not by way of limitation, the concentration of the metal ion, or salt form thereof, can be from about 0.01 times to about 10 times the anthocyanin concentration. In certain embodiments, the concentration of the metal ion, or salt form thereof, can be from about 0.01 times to about 10 times, from about 0.01 times to about 8 times, from about 0.01 times to about 5 times, from about 0.01 times to about 3 times, from about 0.01 times to about 2 times, from about 0.01 times to about 1 times, from about 0.01 times to about 0.75 times, from about 0.01 times to about 0.5 times, from about 0.1 times to about 5 times, from about 0.1 times to about 2 times, from about 0.1 times to about 1 times, from about 0.1 times to about 0.75 times, from about 0.1 times to about 0.5 times, from about 0.25 times to about 5 times, from about 0.25 times to about 2 times, from about 0.25 times to about 1 times, from about 0.25 times to about 0.75 times, from about 0.25 times to about 0.5 times the anthocyanin concentration.

In certain embodiments, the concentration of the metal ion, or salt thereof, in the colorant composition can be less than about 0.5 times, about 0.75 times, about 1.0 times, about 1.5 times, about 2.0 times, about 2.5 times, about 3.0 times, about 3.5 times, about 4.0 times, about 4.5 times, about 5.0 times, about 5.5 times, about 6.0 times, about 7.0 times, about 7.5 times, about 8.0 times, about 8.5 times, about 9.0

times, about 9.5 times or about 10.0 times the anthocyanin concentration. In certain embodiments, the concentration of the metal ion, or salt thereof, in the colorant composition can be from about 0.25 to about 0.75 times the anthocyanin concentration. In certain embodiments, the concentration of the metal ion, or salt thereof, in the colorant composition can be about 0.25 times, about 0.5 times, or about 0.75 times the anthocyanin concentration. In certain embodiments, the concentration of the metal ion, or salt form thereof, in the colorant composition is about 1.0 times the anthocyanin concentration. In certain embodiments, the concentration of the metal ion, or salt thereof, in the colorant composition can be equal to or less than about 1.0 times the anthocyanin concentration (*i.e.*, equal to or less than the anthocyanin concentration).

3.2. Buffers

In certain embodiments, a composition of the present disclosure comprises at least one acylated anthocyanin, *e.g.*, a diacylated anthocyanin, and a buffer of a particular pH.

In certain embodiments, a composition of the present disclosure comprises at least one acylated anthocyanin, *e.g.*, a diacylated anthocyanin, and/or a metal ion, or salt thereof, and/or a buffer solution of a particular pH.

Non-limiting examples of the buffer solution include potassium and sodium-based buffers. In certain embodiments, the buffer can include potassium phosphate, tris(hydroxymethyl)-aminomethane, sodium acetate, or combinations thereof.

In certain embodiments, the buffer has a pH of about 1.0 to about 14.0. For example, and not by way of limitation, the pH of the buffer can be from about 3.0 to about 11.0. In certain embodiments, for a blue colorant composition, the pH can be from about 5.0 to about 8.0, or from about 6.0 to about 8.0, or from about 7.0 to about 8.0. In certain non-limiting embodiments, the pH of the buffer present within a colorant composition, *e.g.*, a blue colorant composition, is about 6.0. In certain embodiments, the pH of the buffer present within a colorant composition, *e.g.*, a blue colorant composition, is about 6.5. In certain embodiments, the pH of the buffer present within a colorant composition, *e.g.*, a blue colorant composition, is about 7.0. In certain embodiments, the pH of the buffer present within a colorant composition, *e.g.*, a blue colorant composition, is about 7.5. In certain embodiments, the pH of the

buffer present within a colorant composition, *e.g.*, a blue colorant composition, is about 8.0.

In certain embodiments, the pH of the buffer depends on the concentration of the metal ion in the colorant composition. For example, and not by way of limitation, if the composition contains higher concentrations of the metal ion, *e.g.*, a metal ion concentration greater than or equal to the concentration of anthocyanins, the pH of the buffer can be lower than the naturally occurring pH, for example, a pH of about 6.0 and still achieve the desired color. Alternatively, at a higher pH, for example, a pH of about 8.0, lower concentrations of the metal ion, *e.g.*, a metal ion concentration less than or equal to the concentration of anthocyanins, can be used to achieve the desired color.

3.3 Color Characteristics

As embodied herein, color characteristics of the presently disclosed colorant compositions, *e.g.*, blue colorant compositions, can be determined. Such color characteristics can include hue, chroma, purity, saturation, intensity, vividness, value, lightness, brightness and darkness, and color model system parameters used to describe these properties, such as Commission Internationale de l'Eclairage CIE 1976 CIELAB color space L*a*b* values and CIELCH color space L*C*h° values. For example, L*a*b* values consist of a set of coordinate values defined in a three-dimensional Cartesian coordinate system. L* is the value, or lightness, coordinate. L* provides a scale of lightness from black (0 L* units) to white (100 L* units) on a vertical axis, a* and b* are coordinates related to both hue and chroma, a* provides a scale for greenness (- a* units) to redness (+ a* units), with neutral at the center point (0 a* units), on a horizontal axis; b* provides a scale for blueness (- b* units) to yellowness (+ b* units), with neutral at the center point (0 b* units), on a second horizontal axis perpendicular to the first horizontal axis. The three axes cross where L* has a value of 50 and a* and b* are both zero.

L*C*h° values consist of a set of coordinate values defined in a three-dimensional cylindrical coordinate system. L* is the value, or lightness, coordinate. L* provides a scale of lightness from black (0 L* units) to white (100 L* units) on a longitudinal axis. h° is the hue coordinate. h° is specified as an angle from 0° to 360° moving counterclockwise around the L* axis. Pure red has a hue angle of 0°, pure yellow has a hue angle of 90°, pure green has a hue angle of 180°, and pure blue has a

hue angle of 270°. The C* coordinate represents chroma and is specified as a radial distance from the L* axis. C* provides a scale from achromatic, i.e., neutral white, gray, or black, at the L* axis (0 C* units) to greater purity of hue as the coordinate moves away from the L* axis (up to 100 or more C* units). C* and h° can be calculated from a* and b* using Equations 1 and 2:

$$C^* = (a^{*2} + b^{*2})^{0.5} \quad (1)$$

$$h^\circ = \arctan(b^*/a^*) \quad (2)$$

"Delta E," "ΔE_{ab}^*," or "ΔE" is a measure of the magnitude of total color difference between two colors represented in CIELAB L*a*b* color space. It has been reported that an experienced color observer cannot distinguish any difference between two colors when the ΔE is about 2.3 or less. The ΔE of two different colors with L*a*b* values, $L^*_1a^*_1b^*_1$ and $L^*_2a^*_2b^*_2$, is calculated using Equation 3:

$$\Delta E_{ab}^* = \sqrt{(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2} \quad (3)$$

The CIELAB L*a*b* and CIELCH L*C*h° values of FD&C Blue No. 1 at seven different concentrations in aqueous solution are presented in Table 1.

Table 1

Concentration	L*	a*	b*	C*	h°
1000 ppm	10.49	15.82	-44.99	47.69	289.37
500 ppm	24.07	9.80	-58.18	59.00	279.56
100 ppm	52.43	-29.57	-57.38	64.55	242.74
50 ppm	63.64	-43.71	-48.31	65.14	227.86
10 ppm	84.25	-37.23	-23.42	43.99	212.17
5 ppm	90.65	-24.40	-14.28	28.27	210.33
1 ppm	97.69	-6.43	-3.57	7.36	209.02

These L*a*b* and L*C*h° values for FD&C Blue No. 1 can be used as target values for a natural blue colorant alternative to FD&C Blue No. 1. Natural blue colorants having L*a*b* values that fall within a ΔE of 2.3 or less from these target values would be expected to provide color characteristics sufficiently similar to those provided by FD&C Blue No. 1 that a human eye could not distinguish the difference in color provided by the natural colorant versus the synthetic. However, natural blue colorants having L*a*b* values that fall outside a ΔE of 2.3 can also be used as a natural substitute for FD&C Blue No. 1. The closer the L*a*b* values for a natural

blue colorant come to the synthetic target values (*i.e.*, yielding smaller values of ΔE), the better replacement the natural blue colorant will be for FD&C Blue No. 1 in an edible application.

Mathematical models can be generated to represent the color characteristics provided by FD&C Blue No. 1 at any concentration in the L*a*b* and L*C*h° color spaces. For example, the color characteristics may be represented by a segmented line model connecting the L*a*b* or L*C*h° data points of Table 1. A line (L) connecting two points (P_1 and P_2) representing two different concentrations of FD&C Blue No. 1 in L*a*b* space can be calculated with the following Equation 4:

$$L = \{P_1 + t * (P_2 - P_1\} \quad (4)$$

wherein P_1 is (L^*_1, a^*_1, b^*_1); P_2 is (L^*_2, a^*_2, b^*_2); and t is any real number.

Consequently, a segmented line model for FD&C Blue No. 1 in L*a*b* color space can be interpolated based on the L*a*b* values for the seven different concentration points using Equation 4 as follows.

For concentrations between 500 and 1000 ppm, $0 < t < 1$:

L* = 10.49 + 13.58 * t

a* = 15.82 + -6.02 * t

b* = -44.99 + -13.19 * t

For concentrations between 100 and 500 ppm, $0 < t < 1$:

L* = 24.07 + 28.36 * t

a* = 9.80 + -39.37 * t

b* = -58.18 + 0.80 * t

For concentrations between 50 and 100 ppm, $0 < t < 1$:

L* = 52.43 + 11.21 * t

a* = -29.57 + -14.14 * t

b* = -57.38 + 9.07 * t

For concentrations between 10 and 50 ppm, $0 < t < 1$:

L* = 63.64 + 20.61 * t

a* = -43.71 + 6.48 * t

b* = -48.31 + 24.89 * t

For concentrations between 5 and 10 ppm, $0 < t < 1$:

$L^* = 84.25 + 6.40 * t$

$a^* = -37.23 + 12.83 * t$

$b^* = -23.42 + 9.14 * t$

For concentrations between 1 and 5 ppm, $0 < t < 1$:

$L^* = 90.65 + 7.04 * t$

$a^* = -24.40 + 17.97 * t$

$b^* = -14.28 + 10.71 * t$

In addition, colors having L*a*b* values falling within a specific ΔE range of the FD&C Blue No. 1 model can be mathematically modeled in L*a*b* color space. Selecting a specific ΔE value, *e.g.*, 15, with respect to FD&C Blue No. 1 and plotting that ΔE in L*a*b* color space results in a tube-like structure around the FD&C Blue No. 1 segmented line model.

To determine whether a point (X_0) in L*a*b* color space falls within a specific ΔE value from the FD&C Blue No. 1 model, the minimum distance, d_{min}, between the point and the model (represented by line segment X_1 to X_2) must be calculated. Equation 5 can be used to calculate d_{min}:

$$d_{min} = \frac{\left|(x_0 - x_1) \times (x_0 - x_2)\right|}{\left|x_2 - x_1\right|} \qquad (5)$$

wherein x denotes the cross product of two vectors and vertical bars denote the magnitude of a vector expression.

If the value of d_{min} is less than or equal to the chosen ΔE value, then the point in L*a*b* color space falls within that specific ΔE value from the FD&C Blue No. 1 model.

The colorant compositions disclosed herein include natural blue colorant compositions comprising a fraction of diacylated anthocyanins sourced from a natural product, wherein the colorant composition can provide color characteristics having a ΔE value of 12 or less compared to the color characteristics defined by the segmented line defined by the L*a*b* values of 50 ppm and 100 ppm FD&C Blue No. 1 in

aqueous solution. In other embodiments the ΔE value may be less than 11, 10, 9, 8, 7, 6, 5, 4, or 3. The colorant composition may also be measured against a plurality of segmented lines defined by different concentrations of FD&C Blue No. 1 in aqueous solution, *e.g.*, 1 ppm and 5 ppm, 5 ppm and 10 ppm, 10 ppm and 50 ppm, 100 ppm and 500 ppm, 500 ppm and 1000 ppm, or any combination selected therefrom. However, if ΔE value is used to describe the colorant composition, only one segmented line is required to define the colorant composition.

4. Food Products

The colorant composition of the present disclosure can be used in a wide variety of food products. Non-limiting examples of suitable food products include chocolates, chewing gum compositions, hard and soft confectionery products, dairy products, food products of the beverage category including juice products and soft drinks, food products of the frozen food category including frozen dairy products, pharmaceuticals and food categories described herein.

4.1. Confectionery Products

In certain embodiments, a colorant composition of the presently disclosed subject matter can be incorporated into a confectionery product. Non-limiting examples of confectionery products include cakes, cookies, pies, candies, chocolates, chewing gums, gelatins, ice creams, sorbets, puddings, jams, jellies, salad dressings, and other condiments, cereal, and other breakfast foods, canned fruits and fruit sauces.

In certain embodiments, a colorant composition of the present disclosure can be incorporated into the confections by admixing the colorant composition into a confectionery product, *e.g.*, a hard or soft confectionery product. For example, and not by way of limitation, the present disclosure provides methods for enhancing or modulating the blue color of an edible product that comprises (a) providing at least one food product, or a precursor thereof, and (b) combining the food product or precursor thereof with one or more blue colorant compositions, disclosed herein, so as to form a modified edible food product. Additionally, the methods can be used for enhancing or modulating the green color of an edible product by (a) providing at least one food product, or a precursor thereof, and (b) combining the food product or precursor thereof with one or more green colorant compositions, *e.g.*, a blue colorant

composition and a yellow colorant composition, so as to form a modified edible food product.

In certain embodiments, certain amounts of a colorant composition of the present disclosure can be incorporated into a confectionery product. The amount of the colorant composition that is used can depend on a number of factors including, but not limited to, the type of bulking agent or carrier employed, method of application, use rate, the type of colorant employed and the intensity of color desired. In certain embodiments of the present disclosure, the colorant composition is admixed with a confection, where the colorant composition is present in an amount of from about 0.0001 to about 10% weight/weight (w/w), or from about 0.001 to about 8% w/w, or from about 0.01 to about 6% w/w, or from about 0.025 to about 4% w/w, or from about 0.05 to about 2% w/w, or from about 0.075 to about 1% w/w, or from about 0.1 to about 0.5% w/w, and values in between.

In certain embodiments, the colorant composition of the present disclosure can be incorporated in a confectionery product of the dragée type, which can include a core and a layer of granulated sugar. Non-limiting examples of the type of cores in a dragée type confectionery product can include a natural center (*e.g.*, almond, hazelnut or groundnut) or a "confectionery" center (*e.g.*, caramel, fondant or chocolate). The cores can then be coated with chocolate, with successive layers of sugars or other substances such as polyols, gums and natural polymers, that can further include one or more color compositions of the present disclosure. In certain embodiments, the present disclosure provides for confectionery products that are coated with a blue color composition disclosed herein.

In certain embodiments, the methods for manufacturing compositions of the dragée type can comprise the deposition of a plurality of sublayers, for example between about 5 and about 30, by a succession of phases of application and drying carried out, for example, in a pan. In a hard panning process, multiple applications of a highly concentrated sugar syrup can be used to build up the uncolored portion of a sugar coating on an edible product center. This can be followed by multiple applications of a concentrated sugar syrup containing a colorant composition of the present disclosure. In certain embodiments, the hard panning process comprises the repetitive application of thin layers of a coating solution or composition onto an intermixed mass of centers, and the drying of each layer of coating solution or composition during which the sugar in the coating crystallizes between the

applications of layers. Additional non-limiting examples of methods for producing hard panned confectionaries are provided in International Patent Publication Nos. WO 2014/150438 and WO 2014/152417, the disclosures of which are incorporated herein by reference.

If the coating is to be colored, a colorant composition of the present disclosure can be added to the coating solution in the later stages of the coating process. For example, and not by way of limitation, the colorant composition can be a natural blue colorant composition comprising an acylated anthocyanin, a metal ion, or salt form thereof, and a buffer having a pH of about 6 to about 8. For a hard panned confectionery, following the application of a number of layers of the uncolored sugar syrup to build up the sugar coating, a number of applications of a sugar syrup comprising a colorant composition, disclosed herein, are applied to provide the color coat. In certain embodiments, the color coat can require 30 or more applications of a colored coating solution to achieve the desired color.

In certain embodiments, when a colorant composition is included in one or more sugar syrups used for hard panned coating, the hard panned coating has a visible color provided by the colorant composition. A hard panned confection comprising an edible product center coated with this same hard panned coating also has a visible color provided by the colorant composition. In certain embodiments, the hard panned coating has a blue color, and a hard panned confection coated with this same hard panned coating has a blue color. The blue color can be provided, at least in part, by a blue colorant composition, disclosed herein, incorporated in the coating.

In certain embodiments, the hard panned coating has a green color, and a hard panned confection coated with this same hard panned coating has a green color. In certain embodiments, the green color can be provided by the combination of a blue colorant composition, disclosed herein, and a natural yellow colorant incorporated in the coating. Examples of natural yellow colorants can include, but are not limited to, curcuminoids (*e.g.*, from turmeric), carotenoids (*e.g.*, from saffron and gac), annatto (*e.g.*, from achiote) and combinations thereof. In certain embodiments, the natural yellow colorant is derived from turmeric.

In certain embodiments, the present disclosure provides edible products in the form of a hard coated confectionery product comprising a center core and at least one coating layer that comprises the colorant composition disclosed herein and crystallized sugar. For example, and not by way of limitation, the present disclosure

provides a hard panned confection that comprises (a) an edible core and (b) a hard panned coating that comprises a plurality of coating layers, wherein at least one of the coating layers comprises a color composition of the present disclosure. For example, and not by way of limitation, the edible core can comprise chocolate.

5. Methods of Preparation

5.1. Fractionation of Anthocyanins

In certain embodiments, the anthocyanins of the present disclosure can be obtained from natural sources such as, but not limited to, vegetables, fruits, and flowers as disclosed above.

In certain embodiments, the anthocyanins can be present in a fraction extracted from a natural source, such as a vegetable, fruit, or flower, or juice or extract thereof. For example, the anthocyanins can be present in a fraction of a juice or extract from the natural source. The fraction can include one or more diacylated anthocyanins.

In certain embodiments, the fraction can be derived from the natural source using any suitable method known in the art. For example, and not by way of limitation, a fraction, *e.g.*, containing a diacylated anthocyanin, can be purified from a natural source using solid phase extraction. In certain embodiments, a fraction can be purified from a natural source using semi-preparative high performance liquid chromatography (HPLC). In certain embodiments, a fraction of the present disclosure can be purified by a pH differential method. Additional methods of purification can be found in International Patent Publication No. WO 2014/152417, the disclosure of which is hereby incorporated by reference in its entirety.

In certain embodiments, the natural source can be fractionated based on the degree of acylation of the anthocyanins. For example, one or more fractions can correspond to the degree of acylation of the anthocyanins within the natural source. For example, the fractionation can result in a first fraction comprising anthocyanins having one degree of acylation, *e.g.*, monoacylated anthocyanins, and a second fraction comprising anthocyanins having another degree of acylation, *e.g.*, diacylated anthocyanins.

5.2. Isolation of Anthocyanins

In certain embodiments, a fraction of anthocyanins can be further purified such that one or more species of anthocyanins are isolated from other anthocyanins. For example, a fraction can include two or more, or three or more different anthocyanins, *e.g.*, diacylated anthocyanins, that can be further isolated from each other.

In certain embodiments, an acylated anthocyanin, *e.g.*, a diacylated anthocyanin, can be further isolated using semi-preparative high performance liquid chromatography (HPLC). Additionally or alternatively, an anthocyanin, *e.g.*, a diacylated anthocyanin, can be further isolated using solid phase extraction. In certain embodiments, the purity of an acylated anthocyanin, *e.g.*, a diacylated anthocyanin, of the present disclosure can be evaluated by analytic high performance liquid chromatography.

5.3. Preparation of the Colorant Compositions

In certain embodiments, the colorant compositions of the present disclosure can be prepared from one or more anthocyanins purified from a natural source, as disclosed above.

In certain embodiments, the colorant compositions, *e.g.*, blue colorant compositions, of the present disclosure can be prepared by diluting fraction comprising at least one acylated anthocyanin, *e.g.*, at least one diacylated anthocyanins, in a buffer solution and admixing a metal salt. For example, and not by way of limitation, an acylated anthocyanin, *e.g.*, a diacylated anthocyanin, can be diluted to a concentration of about 50 μM in a buffer solution to generate a diluted anthocyanin composition. In certain embodiments, the buffer solution can have a pH from about 6.0 to about 8.0. In certain embodiments, a metal salt solution, as a source of metal ions, can be added to the diluted anthocyanin composition. In certain embodiments, the metal salt can be added to the diluted anthocyanin composition in concentrations from about 0.01 times to about 100 times the anthocyanin concentration to prepare the colorant composition.

The color compositions of the present disclosure can be prepared and used in any form. For example, and not by way of limitation, a color composition of the present disclosure can be liquid, crystal, paste, suspension or powder.

EXAMPLES

The presently disclosed subject matter will be better understood by reference to the following Examples, which are provided as exemplary of the invention and should not be construed as limiting the scope of the invention in any way.

EXAMPLE 1 - METALLO-ANTHOCYANIN COMPLEXES

The use and demand of alternatives for synthetic food colorants has been increasing due to possible health concerns and consumer demand. Anthocyanins are known as natural food colors responsible for reds and blues of fruits and vegetables. In acidic conditions common to foods, anthocyanins (ACNs) appear red-purple; however, self-association, co-pigmentation, and metal chelation can expand this color range to blue-purple. Metal ions (M+) can displace hydrogen ions from B-ring hydroxyl groups, transforming red cations to quinoidal anions (*see*, *e.g.*, FIG. 1).

This Example shows the effect pH and the formation of metal ion complexes has on the color produced by acylated anthocyanin compounds.

Materials and Methods

Cyanidin (Cy) derivatives were purified from chokeberry and red cabbage using solid-phase extraction with a C18 resin, ethyl acetate and methanol as previously described (Rodriguez et al., 2001). Monoacylated and diacylated rich anthocyanin (ACN) fractions were isolated from red cabbage by semi-preparative high performance liquid chromatography (HPLC). The monomeric ACN were quantitated by the pH differential method and diluted to 50 μM concentrations in buffers ranging from pH 5-8. To each sample, Cr^{3+}, Al^{3+}, Ga^{3+} or Fe^{3+} salts were added in 1 times to 100 times the ACN concentration. Each sample was evaluated by UV-Visible light spectrophotometry for transmittance, and the CIE-Lab color values were calculated.

Results

Effect of Acylation on Al^{3+}-Anthocyanin Complexes. As shown in FIG. 2, acylation of anthocyanins was determined to be important for the development of attractive blue colors similar to those of synthetic blue dyes in foods. Diacylated cyanidin (Cy) from red cabbage produced a more attractive blue color than non-acylated cyanidin from chokeberry (FIG. 2). Bathochromic shifts were greatest for Cy > Di-Acyl Cy > Mono-Acyl Cy, but λ_{max} was still greater for acylated Cy (FIG. 3).

With increasing acylation, λ_{max} was greatest and the observed color was increasingly blue. In ACN:Al^{3+} ratios of 1:1, both bathochromic and hyperchromic shifts ranged depending on pH (5-8), and were greatest for Cy (8-54 nm; 0.14-0.83, respectively) followed by diacylated Cy (12-30 nm; 0.04-0.58) followed by monoacylated Cy (2-31 nm; -0.04-0.36) (FIG. 4). The λ_{max} increased with pH and ranged from 567-624 nm for diacylated Cy, which is greater than 552-604 nm for monoacylated Cy and 548-568 nm for Cy.

With ACN:M^{3+} ratios of 1:1, the largest bathochromic and hyperchromic shifts occurred with non-acylated cyanidin (-1-62nm) > diacylated cyanidin (-3-42nm) > monoacylated cyanidin (11-47nm). The λ_{max} of these samples was highest for diacylated cyanidin > monoacylated cyanidin > cyanidin and increased with increasing pH, developing more blue colors. Largest bathochromic shifts were induced by Fe^{3+} (-1-62nm) ≈ Ga^{3+} (-3-61nm) > Al^{3+} (-2-54nm) but greatest change in absorbance was caused by Al^{3+} or Ga^{3+}.

Effect of pH on Al^{3+}-Anthocyanin Complexes. Increasing the pH of the samples led to larger λ_{max} (hues became more blue) but decreased bathochromic shift induced by the metal ion (M^{3+}). By increasing the pH of the samples, lower concentrations of M^{3+} ([M^{3+}]) were needed for bluing effect (FIGS. 6-8). Hypsochromic effects occurred with large [M^{3+}] with increasing pH and absorbance was increased with [M^{3+}] chelation. Precipitation occurred with increasing pH and [M^{3+}].

Effect of pH on other [M^{+3}]-Anthocyanin Complexes. The λ_{max} of the samples was also dependent on M^{3+} structure. Increased M^{3+} electron density promoted larger bathochromic shifts and λ_{max}. The largest bathochromic shifts were induced by Fe^{3+} (-1-62nm) ≈ Ga^{3+} (-3-61nm) > Al^{3+} (-2-54nm) with λ_{max} greatest for ACN-Fe^{3+} > Ga^{3+} > Al^{3+}. The greatest change in absorbance was caused by Al^{3+} or Ga^{3+}.

Stability. With increasing degree of acylation, the pigments were found to exhibit more intense color for longer periods of time. Metal chelation by anthocyanins not only allowed for development of blue colors by anthocyanins at lower pH than naturally occurring but also provided beneficial effects on the color stability of these pigments. Although λ_{max} and absorbance decreased over time, anthocyanin color stability was increased in the presence of M^{3+} (FIG. 4). As shown in FIG. 4, at a pH of 6.0, anthocyanin color stability was shown to increase with

increasing [M^{3+}]. At a pH of 6.0, highest color intensity was maintained with [M^{3+}] ≥ 5× [ACN] (FIG. 5). Interestingly, the anthocyanin-M^{3+} precipitates showed higher stability than the pigments in solution.

Conclusion

The pH environment, metal ion and anthocyanin structure affected the color of anthocyanin-M^{3+} chelates. In the presence of the M^{3+}, anthocyanin samples underwent bathochromic and hyperchromic shifts in absorbance, becoming bluer and more intensely colored. Acylation of anthocyanins was found to be important for the development of blue colors similar to synthetic counterparts. Although the largest bathochromic and hyperchromic shifts occurred with non-acylated cyanidin > diacylated cyanidin > monoacylated cyanidin, diacylated cyanidins consistently created bluer colors than either non-acylated or monoacylated cyanidins. Increasing pH from 5-8 led to the development of more blue colors as λ_{max} increased with pH, reducing the amount of M^{3+} necessary to achieve blue colors. λ_{max} of ACN-M^{+3} was greatest for diacylated cyanidin > monoacylated cyanidin > cyanidin. Increasing degree of acylation and the presence of M^{3+} improved the longevity of the pigment in terms of intensity and desirable color formation. The anthocyanin-M^{3+} chelates based pigments developed in this Example showed desirable hues with increased stability that could act as alternatives to synthetic blue colorants.

EXAMPLE 2 - EFFECTS OF DEGREE AND STRUCTURE OF ACYLATION

Anthocyanins (ACN) are a versatile class of naturally occurring pigments that produce red-purple-blue hues in nature. The pH conditions of many food products result in ACN typically appearing red-purple. However, through metal ion chelation and molecular co-pigmentation, ACN can express blue-purple colors.

This Example shows the effect of the degree and structure of acylation, as intramolecular co-pigments, on the spectral responses and stability of metallo-anthocyanin complexes.

Materials and Methods

Non-, mono-, and di- acylated cyanidin (Cy) derivatives were isolated from food sources by semi-preparative high performance liquid chromatography (HPLC). The monomeric ACN were diluted to 50 μM concentrations in buffers ranging of pH

6 and 7. To each sample, Al^{3+} or Fe^{3+} salts were added in 1 times to 5 times the ACN concentration. Each sample was evaluated by UV-Visible light spectrophotometry for transmittance (380-700 nm), and the CIE-Lab color values were calculated during dark, ambient storage (48 hours).

Results

pH. At a pH of 6 with equimolar Al^{3+} ratios, the λ_{max} (nm) of the tested samples were as follows: malonic acid monoacylated Cy (563 nm) < triglycosylated Cy (576 nm) < monoacylated with hydroxycinnamic acids (sinapic (579 nm) < coumaric (580 nm) < ferulic (583 nm)) < diacylated Cy (sinapic-sinapic (596 nm) > ferulic-sinapic (609 nm) > *p*-coumaric-sinapic (610 nm)). Similar patterns were observed at pH 7 regarding λ_{max} and also with the bathochromic shifts induced by metal chelation.

$[M^{+3}]$-Anthocyanin Complexes. With metals (Al^{3+} or Fe^{3+}), all ACN became increasingly purple and blue. Diacylated Cy showed the most blue colors. At pH of 7, all ACN showed blue hues (negative a* and b* values), with the exception of Cy bearing aliphatic acylation which expressed a* = 1.0 and b* = -16.4.

Stability. Degradation of ACN-metal chelates was found to follow 1st order kinetics. Pigment stability was improved with increasing proportion of metal ions and degree of acylation. At pH of 7, the half-lives (hr) of Al^{3+} chelates (5× [ACN]) were as follows: diacylated Cy (*p*-coumaric-sinapic (149.1 hr) > ferulic-sinapic (40.2 hr) ≈ sinapic-sinapic (41.0 hr)) > monoacylated (malonic (62.4 hr) > ferulic (52.9 hr) > sinapic (31.9 hr) > *p*-coumaric (22.5 hr)).

Conclusion

Metal chelation induced color evolution of ACN towards more purple and blue hues. Stability of these naturally occurring pigments was also increased, fulfilling current demands for alternatives to synthetic colorants.

References

Yoshida K, Mihoko M, Kondo T. 2009. Blue flower color development by anthocyanins: from chemical structure to cell physiology. Nat Prod Rep 26(7):857-964.

Takeda K, Yamashita T, Takahashi A, Timberlake CF. 1990. Stable blue complexes of anthocyanin-aluminum-3-p-coumaroyl- or 3-caffeoyl-quinic acid involved in the blueing of Hydrangea flower. Phytochemistry 29(4):1089-91.

Schreiber HD, Swink AM, Godsey TD. 2010. The chemical mechanism for Al3+ complexing with delphinidin: A model for the bluing of hydrangea sepals. J Inorg Biochem 104: 732–9.

Bayer E, Egeter H, Fink A, Nether K, Wegman K. 1966. Complex Formation and Flower Color. Angew Chem Interna Edit 5(9):797-8.

Buchweitz M, Carle R, Kammerer DR. 2012. Bathochromic and stabilizing effects of sugar beet pectin and an isolated pectic fraction on anthocyanins exhibiting pyrogallol and catechol moieties. J Food Chem 135:3010-9.

Rodriguez-Saona LE, Wrolstad RE. 2001. Extraction, Isolation, and Purification of Anthocyanins. In Handbook Food Anal Chem. Ed RE Wrolstad, SJ Schwartz. NY, USA: John Wiley and Sons, Inc. 7-18.

Giusti, MM; Wrolstad, RE. 2005. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In Handbook Food Anal Chem. Ed RE Wrolstad, SJ Schwartz. NY, USA: John Wiley and Sons, Inc. 19-31.

EXAMPLE 3 - METALLO-ANTHOCYANIN COMPLEXES WITH RED CABBAGE EXTRACT

Anthocyanins are known to be present within red cabbage extract, which can be used to produce red to blue-purple natural colorants. As described above, metal ions (M+) can displace hydrogen ions from B-ring hydroxyl groups in anthocyanins, transforming red cations to quinoidal anions (*see*, *e.g.*, FIG. 1).

This Example shows the effect pH and the formation of metal ion complexes has on the color produced by anthocyanin compounds as compared to FD&C Blue No. 1.

Materials and Methods

Red cabbage extract was diluted to an anthocyanin concentration of 50 μM in a buffer solution to a pH ranging from 3 to 8. For solutions having a pH from 3 to 6, a sodium acetate buffer solution was used. For solutions having a pH from 7 to 8, a Tris(hydroxymethyl)-aminomethane buffer solution was used.

Four different metal salts were added to the solutions at various concentrations: $Al_2(SO_4)_3$, $FeCl_3$, $GaCl_3$, and $CrCl_3$. The ratio between the anthocyanin concentration and the metal ion (*i.e.*, Al^{3+}, Fe^{3+}, Ga^{3+}, or Cr^{3+}) concentration ranged from 1:0 to 1:500.

The CIELAB L*a*b* and CIELCH L*C*h° values for the solutions were calculated from spectral data obtained from a Konica Minolta Spectrophotometer CM-3500d operated in transmittance mode, with CIE Standard Illuminant D65 and 10° observer angle. The ΔE values described in this Example are as compared to FD&C Blue No. 1 in an aqueous solution at a concentration of from 5 ppm to 10 ppm, and calculated using the methods described above.

Results

pH. In general, increasing the pH from 3 to 8 was found to decrease the ΔE across all metal ions. A pH from about 6 to about 8 yielded the lowest ΔE, with a pH of 7 or 8 resulting in particularly low ΔE values. At a pH of 7 and an equimolar ratio between the anthocyanin concentration and the metal ion concentration, the ΔE of the tested samples were as follows: Ga^{3+} (10.25) < Al^{3+} (10.82) < Fe^{3+} (11.08) < Cr^{3+} (13.53). At a pH of 8 and an equimolar ratio, the ΔE of the tested samples were as follows: Al^{3+} (9.16) < Ga^{3+} (9.18) < Fe^{3+} (9.71) < Cr^{3+} (9.99). These data are shown in FIG. 9, along with ΔE values for solutions having a pH from 3 to 6 and an equimolar ratio.

[M^{3+}]. A lower concentration of metal ions was generally found to decrease ΔE. As shown in FIG. 10, ΔE was decreased when the concentration of metal ions ([M^{3+}]) was lower relative to the concentration of anthocyanins ([ACN]), particularly for Ga^{3+}, Fe^{3+} , and Al^{3+}. Moreover, at these lower concentrations, Ga^{3+}, Fe^{3+} , and Al^{3+} had improved ΔE values as compared to Cr^{3+}.

Conclusion

The color characteristics of anthocyanins can be induced towards more purple and blue hues by selecting a particular metal ion, increasing the pH, or decreasing metal ion concentration. Trivalent metal ions, such as Ga^{3+}, Fe^{3+} , and Al^{3+} can be used in a metallo-anthocyanin complex, for example, at a pH from about 6 to about 8, to achieve color characteristics similar to FD&C Blue No. 1.

EXAMPLE 4 - METALLO-ANTHOCYANIN COMPLEXES WITH FRACTIONATION OF RED CABBAGE EXTRACT

As described above in Example 2, anthocyanins having a diacylated structure were found to produce a desirable blue color characteristics.

This Example shows the effect pH and the formation of metal ion complexes has on the color produced by such diacylated anthocyanin compounds as compared to FD&C Blue No. 1.

Materials and Methods

A concentrated red cabbage extract (with an anthocyanin concentration of ≥150 mg cy-3-glu/L) was fractionated based on anthocyanin structure. A Shimadzu LC (Shimadzu Corporation, Tokyo, Japan) system consisting of a SIL-20A prominence auto sampler and a SPD-M20A photo diode array detector (PDA) was used. The column oven and auto sampler were set at room temperature. A Sun Fire Prep C18 OBD 5 μm column (19x100 mm, Waters Corp., MA, USA) was used. The chromatographic conditions were set as follows: flow rate of 12 mL/min; mobile phase A was 4.5% formic acid in LCMS grade water; B was LCMS acetonitrile; gradient of 0-15 min, 9-25% B; 15-20 min, 25-100% B; 20-25 min, 100% B. Injection volume was 2000 μL.

Two fractions (a first with λ_{max} of about 520nm and a second with λ_{max} of about 530nm), each containing three peaks, were collected one at a time. The procedure was performed manually. The fractions containing the 520nm and 530nm peaks were collected between 11.50-12.50 min and 12.82-13.86 min, respectively. Spectral data and peaks elusions were monitored at 520nm using a LC solution software.

The fractions were diluted to an anthocyanin concentration of 50 μM in a buffer solution to a pH ranging from 6 to 8. For a pH of 6, a sodium acetate buffer was used. For a pH of 7 or 8, a potassium phosphate buffer was used. The fractions were chelated with Fe^{3+} at concentrations from zero ("control") to one times ("equimolar" or "1x") the anthocyanin concentration. The fractions were also chelated with Al^{3+} at concentrations from zero ("control") to 100 times ("100x") the anthocyanin concentration. The CIELAB L*a*b* and CIELCH L*C*h° values for the solutions were calculated as described in connection with Example 3. The ΔE values described in this Example are as compared to FD&C Blue No. 1 in an aqueous

solution at a concentration of from 50 ppm to 100 ppm, and calculated using the methods described above.

Results

Fe^{3+} metal ions. Tables 2-4 below show the effect ΔE, Hue (h°), and λ_{max}, respectively, for metallo-anthocyanin complexes with Fe^{3+} and diacylated anthocyanins at various pH values and concentrations. These data are also presented in three-dimensions in FIGS. 11A-11C. A pure blue has a hue angle of 270, and FD&C Blue No. 1 has a hue angle from about 210 to about 230 when in solution. Additionally, FD&C Blue No. 1 has a λ_{max} of 630 nm, and ideally a natural blue colorant composition will have a similar λ_{max}.

Table 2. ΔE for Fe^{3+} chelated diacylated anthocyanins.

	Concentration ([Fe^{3+}] : [ACN])				
pH	**Control**	**0.25x**	**0.5x**	**0.75x**	**1x**
6	45.3	31.2	30.9	33.4	34.5
6.5	40.6	29.1	27.2	28.5	29.5
7	35.9	27.0	23.6	23.6	24.5
7.5	25.6	19.2	17.8	18.4	19.7
8	15.3	11.4	11.9	13.3	14.9

Table 3. Hue for Fe^{3+} chelated diacylated anthocyanins.

	Concentration ([Fe^{3+}] : [ACN])				
pH	**Control**	**0.25x**	**0.5x**	**0.75x**	**1x**
6	292	262	246	239	239
6.5	287	259	243	236	236
7	281	256	239	233	234
7.5	267	244	236	234	234
8	253	233	233	235	235

Table 4. λ_{max} for Fe^{3+} chelated diacylated anthocyanins.

	Concentration ([Fe^{3+}] : [ACN])				
pH	**Control**	**0.25x**	**0.5x**	**0.75x**	**1x**
6	583	605	626	629	628
6.5	583	608	624	629	629
7	583	610	621	629	630
7.5	598	618	627	633	634
8	613	625	632	636	637

In general, increasing the pH from 6 to 8 was found to decrease the ΔE for Fe^{3+} chelated anthocyanins The solutions having a pH of 8 had the lowest ΔE across all concentrations.

A lower concentration of metal ions was generally found to decrease ΔE, depending on the pH. For example, for Fe^{3+} at a pH of 8, the relative ΔE of the tested samples were as follows: 0.25x < 0.5x < 0.75x < 1x < Control.

Moreover, the additional of Fe^{3+} metal ions at various concentrations was found to create bathochromic and hyperchromic shifts in absorbance, resulting in bluer and more intense colors relative to the control (FIG. 12).

Al^{3+} metal ions. Tables 5-7 below show the effect ΔE, Hue (h°), and λ_{max}, respectively, for metallo-anthocyanin complexes with Al^{3+} and diacylated anthocyanins at various pH values and concentrations. These data are also presented in three-dimensions in FIGS. 14A-14C.

Table 5. ΔE for Al^{3+} chelated diacylated anthocyanins.

	Concentration ([Al^{3+}] : [ACN])						
pH	**Control**	**0.5x**	**1x**	**5x**	**10x**	**50x**	**100x**
6	50.8	28.6	27.9	28.6	29.2	30.3	30.8
6.5	39.2	20.0	19.8	21.2	21.5	25.4	26.6
7	27.6	11.4	11.7	13.8	13.8	20.4	22.4
7.5	21.5	11.4	11.8	13.5	14.3	17.6	26.6
8	15.3	11.4	11.9	13.3	14.9	14.9	30.8

Table 6. Hue for Al^{3+} chelated diacylated anthocyanins.

	Concentration ([Al^{3+}] : [ACN])						
pH	**Control**	**0.5x**	**1x**	**5x**	**10x**	**50x**	**100x**
6	294	267	271	275	276	279	281
6.5	288	254	257	261	261	268	270
7	282.5	242	243	247	247	257	259
7.5	268	237	238	241	241	246	249
8	253	233	233	235	235	235	238

Table 7. λ_{max} for Al^{3+} chelated diacylated anthocyanins.

	Concentration ([Al^{3+}] : [ACN])						
pH	**Control**	**0.5x**	**1x**	**5x**	**10x**	**50x**	**100x**
6	578	605	605	601	599	594	591
6.5	589	614	616	607	604	599	597
7	599	622	626	613	608	603	602
7.5	606	624	626	618	615	607	605
8	612	626	625	622	621	610	607

In general, increasing the pH from 6 to 8 was found to decrease the ΔE for Al^{3+} chelated anthocyanins. The solutions having a pH of 8 had the lowest ΔE for both metal ions and across all concentrations.

A lower concentration of metal ions was generally found to decrease ΔE, depending on the pH. For example, for Al^{3+} at a pH of 8, the ΔE of the tested samples were as follows: $0.5x < 1x < 5x < 10x \approx 50x <$ Control.

Moreover, the additional of Al^{3+} metal ions at various concentrations was found to create bathochromic and hyperchromic shifts in absorbance, resulting in bluer and more intense colors relative to the control (FIG. 13).

Conclusion

By adjusting the pH and metal ion concentration depending on the metal ion, the color characteristics of a diacylated anthocyanin fraction can be developed into a blue or blue-purple color and achieve color characteristics similar to FD&C Blue No. 1. For example, increasing the pH to up to 8 was found to decrease ΔE for both Fe^{3+} and Al^{3+} chelates. Moreover, ΔE was found to improve at lower metal ion concentrations, *e.g.*, less than 1x for Fe^{3+} chelates and less than 10x for Al^{3+} chelates. Indeed, the best blue colors were developed by increasing pH while maintaining a low metal ion concentration, *e.g.*, 0.5x or less.

EXAMPLE 5 - COLOR OF METALLO-ANTHOCYANIN COMPLEXES WITH LOW METAL ION CONCENTRATION

As shown above, lower metal ion concentrations in metallo-anthocyanin complexes resulted in bluer color characteristics (*see, e.g.,* Example 4).

This Example further observes the effect of pH and metal ion concentration on the color produced by anthocyanin compounds.

Materials and Methods

A concentrated red cabbage extract was fractionated based on anthocyanin structure using the methods described in Example 4.

A fraction comprising diacylated anthocyanins had a composition as shown in Table 8 below:

Table 8

ACN	MW	% Area of HPLC	Absorbance	[ACN] (mg/L)
Cy-3-diG-5-G+p-coumaric&Ferulic	1160	14.1	0.034	94.14
Cy-3-diG-5-G+Sinapic&Ferulic	1185	21.1	0.05	160.47
Cy-3-diG-5-G+Sinapic&Sinapic	1220	53.4	0.13	496.42
Other anthocyanins (except Cy-3-diG-5-G)	809	11.4	0.03	35.73
Total		**100%**	**0.24**	**786.76**

The fraction was diluted to an anthocyanin concentration of 50 μM in a buffer solution to a pH ranging from 6 to 8. For a pH of 6, a 0.5 M sodium acetate buffer was used. For a pH of 7 or 8, a 1 M Tris(hydroxymethyl)-aminomethane buffer was used. The fraction was chelated with Fe^{3+} (*i.e.*, $FeCl_3$ salt) or Al^{3+} (*i.e.*, $Al_2(SO_4)_3$ salt) at concentrations from 0.25 times ("1:0.25x") to one times ("1:1x") the anthocyanin concentration. Visible spectrometry was used to determine the absorbance and λ_{max} of each sample.

Results

Al^{3+}. Increasing hyperchromic and bathochromic shift was observed with increasing Al^{3+} concentration at an acidic pH of 6 (FIG. 15A). However,

hyperchromic and bathochromic effects were about equal between the various Al^{3+} concentrations at pH of 7 and 8 (FIGS. 15B- 15C). Within each pH, λ_{max} and absorbance were not found to vary significantly between the various low Al^{3+} concentrations (FIGS. 16A-16B). All samples were blue in appearance.

Fe^{3+}. Increased absorbance was observed at increased pH (FIG. 17C). Hyperchromic shift was observed with increasing Fe^{3+} concentration, particularly at a pH of 8 (FIGS. 17A-17C). Within each pH, λ_{max} increased until a Fe^{3+} concentration of 0.75 times the anthocyanin concentration (FIG. 18A). For a pH of 6 and 7, absorbance increased until a Fe^{3+} concentration of 0.75 times the anthocyanin concentration, but showed little change at a pH of 8 (FIG. 18B). All samples were blue in appearance. Generally, the Fe^{3+} chelates had larger bathochromic shifts than the Al^{3+} chelates, having a λ_{max} of about 630-636 nm, as compared to 623-625 nm. Absorbance was similar for both metal ions within the respective pH.

Conclusion

The metal ion and concentration thereof can affect the color characteristics of a diacylated anthocyanin fraction, and can including a metal ion at a low concentration can result in various shifts in the spectral data.

EXAMPLE 6 - STABILITY OF METALLO-ANTHOCYANIN COMPLEXES WITH LOW METAL ION CONCENTRATION

As shown above, lower metal ion concentrations in metallo-anthocyanin complexes resulted in bluer color characteristics (*see, e.g.,* Examples 4 and 5). This Example provides the stability of such metallo-anthocyanin complexes.

Materials and Methods

A concentrated red cabbage extract was fractionated based on anthocyanin structure using the methods described in Example 4. The fractions were diluted to an anthocyanin concentration of 50 μM in a buffer solution to a pH ranging from 5 to 8. For a pH of 5 or 6, a 0.5 M sodium acetate buffer was used. For a pH of 7 or 8, a 1 M Tris(hydroxymethyl)-aminomethane buffer was used. The fractions were chelated with Fe^{3+} (*i.e.*, $FeCl_3$ salt) or Al^{3+} (*i.e.*, $Al_2(SO_4)_3$ salt) at concentrations from zero times ("1:0" or "Control") to ten times ("1:10") the anthocyanin concentration. 2mL samples were stored in a darkened chamber at a temperature of 23.4 ℃ +/- 2.1 for a

total of 28 days. Visible spectrometry was used to determine the absorbance and λ_{max} of each sample at various time points.

Results

Al^{3+}. At a pH of 7, absorbance was substantially reduced over the 28 day period for all concentrations of metal ions (FIGS. 19A-19B). However, an increasing concentration of Al^{3+} was found to increase stability, and the presence of Al^{3+} improved stability as compared to the control (FIGS. 20A-20B). The highest absorbance was maintained at Al^{3+} concentrations of five times the anthocyanin concentration and above (FIG. 20B), although precipitates were also observed in these samples during the 28 day period. Over time, λ_{max} also decreased in all samples (FIG. 20A). The pH was also found to affect stability in Al^{3+} chelates, with a pH of 7 providing the greatest stability (*i.e.*, least change in absorbance), followed by a pH of 6 (FIGS. 21A-21B).

Fe^{3+}. For Fe^{3+} chelates at a pH of 7, absorbance was substantially reduced over the 28 day period for all concentrations of metal ions (FIGS. 22A-22B). An increasing concentration of Fe^{3+} was found to increase stability, and the presence of Fe^{3+} improved stability as compared to the control (FIGS. 23A-23B). Over time, λ_{max} generally decreased in all samples (FIG. 23A) although it decreases less as higher metal ion concentrations. The shades of blue were visibly less attractive over time. The highest absorbance was maintained at Fe^{3+} concentrations of ten times the anthocyanin concentration and above (FIG. 23B), although precipitates were also observed in samples having a Fe^{3+} concentration of five times or above during the 28 day period. The pH was similarly found to affect stability in Fe^{3+} chelates, with a pH of 6 or 7 providing the greatest stability (*i.e.*, least change in absorbance) (FIGS. 24A-24B).

Conclusion

The Al^{3+} chelates generally have better color stability than the Fe^{3+} chelates over time. Regardless, a metal ion can improve the stability of a diacylated anthocyanin fraction over time, with greater concentrations of metal ions improving color stability.

EXAMPLE 7 - AFFINITY OF DIACYLATED ANTHOCYANINS FOR Al^{3+} AND Fe^{3+} METAL IONS

As shown above, the Al^{3+} and Fe^{3+} metal ions can improve the color characteristics and color stability of anthocyanin fractions (*see, e.g.*, Examples 4-6). This Example provides the affinity of diacylated anthocyanins for such metal ions.

Materials and Methods

Red cabbage extract diluted to an anthocyanin concentration of 50 μM in a buffer solution to a pH of 7 using a 1 M Tris(hydroxymethyl)-aminomethane buffer. The extract was chelated with Fe^{3+} or Al^{3+} at concentrations from zero times ("1:0" or "Control") to five times ("1:5") the anthocyanin concentration. For some samples, Al^{3+} was added first, then equilibrated for about 45 minutes. Subsequently, Fe^{3+} was added at the same concentration and equilibrated for about 45 minutes. For other samples, this procedure was reverse (*i.e.*, Fe^{3+} was added first). Additionally, in some samples, Al^{3+} and Fe^{3+} were added simultaneously at the same concentration.

Visible spectrometry was used to determine the absorbance and λ_{max} of each sample. The spectral data was collected at 380 to 700 nm.

Results

The anthocyanins in the red cabbage extract generally had stronger affinity for Al^{3+} as compared to Fe^{3+}. For example, there was no significant change in λ_{max} in a sample containing Al^{3+} before ("Al") and after ("Al(1) + Fe(2)") Fe^{3+} addition (FIG. 25A). However, there was a decrease in λ_{max} in a sample containing Fe^{3+} before ("Fe") and after ("Fe(1) + Al(2)") Al^{3+} addition (FIG. 25A). Adding Al^{3+} and Fe^{3+} simultaneously ("Al & Fe") did not significantly affect λ_{max}. Absorbance was reduced by the addition of a second metal ion, regardless of which was added first (FIG. 25B).

Conclusion

Metallo-anthocyanin complexes can be formed between diacylated anthocyanins and Al^{3+} and/or Fe^{3+} to improve color characteristics and color stability, and the anthocyanins present in red cabbage extract generally have greater affinity for Al^{3+} as compared to Fe^{3+}.

EXAMPLE 8 - ISOLATED DIACYLATED ANTHOCYANINS CHELATED WITH Al^{3+} AND Fe^{3+} METAL IONS

As shown above, the Al^{3+} and Fe^{3+} metal ions can improve the color characteristics and color stability of anthocyanin fractions (*see, e.g.*, Examples 4-6). Moreover, diacylated anthocyanins were found to have improved color characteristics and stability as compared to other anthocyanins (*see, e.g.*, Example 2). This Example provides the color characteristics and color stability of isolated diacylated anthocyanin derived from red cabbage extract when chelated with Al^{3+} and Fe^{3+} metal ions.

Materials and Methods

A concentrated red cabbage extract was fractionated based on anthocyanin structure using the methods described in Example 4. Subsequently, each diacylated anthocyanin ("peak") within the fraction was isolated using semi-preparative HPLC. As shown in FIG. 26, each fraction includes three peaks, each of which corresponds to a different acylated anthocyanin. The individual peaks corresponding to diacylated anthocyanins (Peaks 4-6 in FIG. 26) were obtained using reverse phase HPLC with a Phenomenex Luna PFP column with a 5 μm particle size, 100 Å pore size, and 250 × 21.2 mm column size. The flow rate was 10.0 mL/min over a run time of 50 min. The solvents were: Phase A: 4.5% formic acid in water; Phase B: acetonitrile. The binary gradient for the solvent was as follows: B: 0 min: 15% B, 0-30 min for 15-30% B. The composition of the fraction containing the three diacylated anthocyanin peaks is shown in Table 9 below. As indicated in Table 9, the fraction primarily included the diacylated anthocyanins Cy-3-diG-5-G+Coumaric&Sinapic ("Peak 4"), Cy-3-diG-5-G+Sinapic&Ferulic ("Peak 5"), and Cy-3-diG-5-G+Sinapic&Sinapic ("Peak 6"), but further included a small amount of other anthocyanins.

Table 9

ACN	MW	% Area of HPLC	Absorbance	[ACN] (mg/L)
Cy-3-diG-5-G+Coumaric&Sinapic	1160	18.5	0.11	300.3
Cy-3-diG-5-G+Sinapic&Ferulic	1185	25.4	0.15	469.9
Cy-3-diG-5-G+Sinapic&Sinapic	1220	48.8	0.28	1103.9
Other anthocyanins (except Cy-3-diG-5-G)	809	7.4	0.04	56.3
Total		**100%**	**0.58**	**1930.3**

The samples were diluted to an anthocyanin concentration of 50 μM in a buffer solution to a pH ranging from 6 to 8. For a pH of 6, a 0.5 M sodium acetate buffer was used. For a pH of 7 or 8, a 1 M Tris(hydroxymethyl)-aminomethane buffer was used. The fractions were chelated with Fe^{3+} or Al^{3+} at concentrations from zero times ("1:0" or "Control") to ten times ("1:10") the anthocyanin concentration. 2mL samples were stored in a darkened chamber at a temperature of about 23 ºC for 48 hours.

Visible spectrometry was used to determine the absorbance and λ_{max} of each sample. The spectral data was collected at 250 to 700 nm with anthocyanin elution monitored at 520 nm. The CIELAB L*a*b* and CIELCH L*C*h° values for the solutions were calculated as described in connection with Example 3.

Results

Hue. Each of the isolated diacylated anthocyanins exhibited hue angles similar to FD&C Blue No. 1 (*i.e.*, from about 210 to about 230) when chelated with either Al^{3+} or Fe^{3+}. At a pH of 7 and with Al^{3+}, Peak 6 had a greater hue angle than Peak 5, which had a greater hue angle than Peak 4 (FIG. 27A). Depending on the metal ion concentration, this pattern was also observed for Fe^{3+} (FIG. 27B). At a pH of 8 and with either Al^{3+} or Fe^{3+}, Peak 5 had a greater hue angle than Peak 6, which had a greater hue angle than Peak 4 (FIGS. 28A-28B).

Chroma. At a pH of 7 and with Fe^{3+}, Peak 4 had a greater chroma than Peak 5, which had a greater hue angle than Peak 6 (FIG. 29B). With Al^{3+}, the chroma of Peaks 5 and 6 were substantially similar (FIG. 29A). At a pH of 8 and with either

Al^{3+} or Fe^{3+}, Peak 4 had a greater chroma than Peak 6, which had a greater chroma than Peak 5 (FIGS. 30A-30B).

Bathochromic Response. Tables 10 and 11 below provide the λ_{max} of Peaks 4-6 at various pH levels, as well as the bathchromic shifts, when chelated with Al^{3+} and Fe^{3+}, respectively. λ_{max} (control) is the λ_{max} of Peaks 4-6 at these pH levels when they have not been chelated with a metal ion. At a pH of 7, the bathochromic shift was greatest for Peak 5. At a pH of 8 and with Al^{3+}, the bathochromic shift was greatest for Peak 4. Moreover, samples with Fe^{3+} generally had a greater bathochromic shift that those with Al^{3+}.

Table 10 (Al^{3+} chelates)

H	Peak	λ_{max} (control)	λ_{max} (treated)	Bathochromic Shift (nm)	[ACN] : [Al^{3+}]
	4	555.3	617.0	61.7	1:0.25
	4	625.0	625.0	0.0	1:0.25
	5	623.0	624.0	1.0	1:5
	6	618.7	619.0	0.3	1:1
	4	612.7	627.0	14.3	1:0.5
	5	623.0	625.0	2.0	1:5
	6	620.7	622.3	1.6	1:5

Table 11 (Fe^{3+} chelates)

H	Peak	λ_{max} (control)	λ_{max} (treated)	Bathochromic Shift (nm)	[ACN] : [Fe^{3+}]
	4	555.3	640.3	85.0	1:0.5
	4	625.0	629.3	4.3	1:0.5
	5	623.0	635.7	12.7	1:0.5
	6	618.7	629.0	10.3	1:1
	4	612.7	627.0	14.3	1:0.5
	5	623.0	640.7	17.7	1:1
	6	620.7	636.3	15.6	1:1

Hyperchromic Response. Tables 12 and 13 below show the hyperchromic shift, as measured by the percentage increase in absorption, at a pH of 7 and 8, respectively. Peak 4 generally had a greater hyperchromic shift than Peaks 5 or 6. Moreover, samples with Fe^{3+} generally had a greater hyperchromic shift that those with Al^{3+}.

Table 12 (pH of 7)

	Al^{3+} Chelates			Fe^{3+} Chelates		
ACN:M+	**Peak 4**	**Peak 5**	**Peak 6**	**Peak 4**	**Peak 5**	**Peak 6**
1:0.25	8.9	-3.2	2.8	2.2	-7.2	-6.1
1:0.5	8.9	-2.3	3.3	0.9	-7.0	-5.5
1:1	6.9	-2.8	4.2	0.1	-15.4	-7.1
1:5	5.7	-1.9	3.0	-6.7	-9.6	-6.8

Table 13 (pH of 8)

	Al^{3+} Chelates			Fe^{3+} Chelates		
ACN:M+	**Peak 4**	**Peak 5**	**Peak 6**	**Peak 4**	**Peak 5**	**Peak 6**
1:0.25	4.6	-0.3	-0.2	-3.8	-3.6	-9.2
1:0.5	5.0	-1.4	0.6	-1.1	-7.2	-12.7
1:1	3.0	-2.9	-0.2	-3.7	-9.2	-14.7
1:5	-3.0	-3.6	-2.1	-8.5	-13.0	-19.8

Stability. The color stability of the samples was observed over 48 hours. For Peak 4 at a pH of 6, λ_{max} decreased over the time period for both Al^{3+} and Fe^{3+} chelates (FIGS. 31A-31B). Additionally, stability was observed for Peaks 4, 5, and 6 at a pH of 7 for both Al^{3+} and Fe^{3+} chelates (FIGS. 32A-32F). Peak 4 generally had the best stability (*i.e.*, least decrease in λ_{max}), followed by Peak 6 and Peak 5 with both metal ions. Similarly, at a pH of 8, Peak 4 also generally had the best stability (FIGS. 33A-33F).

Conclusion

Metallo-anthocyanin complexes with isolated diacylated anthocyanins can provide natural blue colorant compositions having desirable color characteristics and color stability.

* * *

Although the presently disclosed subject matter and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in

the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the presently disclosed subject matter, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the presently disclosed subject matter. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods or steps.

Patents, patent applications publications product descriptions, and protocols are cited throughout, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

WHAT IS CLAIMED IS:

1. A natural blue colorant composition, comprising:

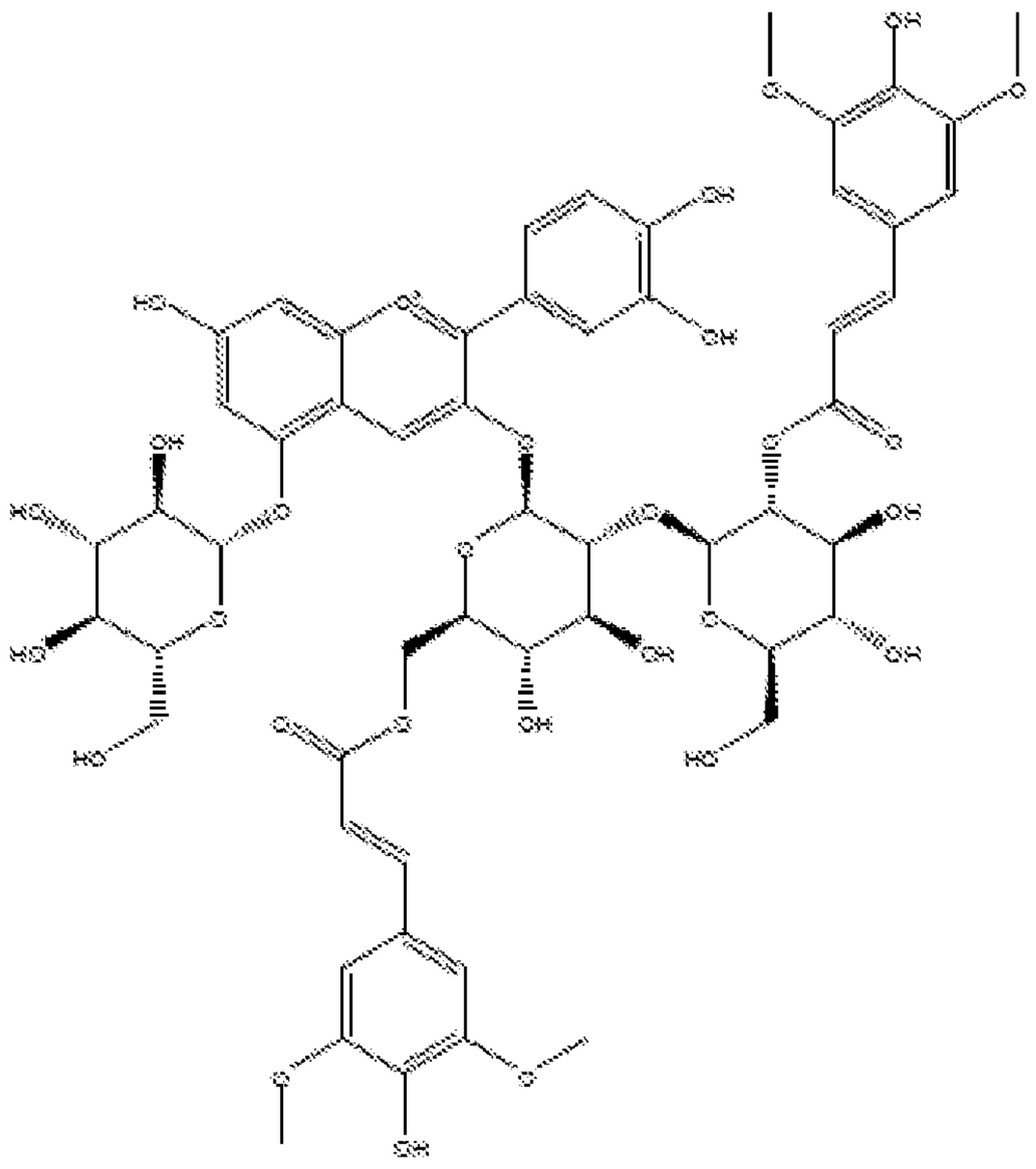
a) a fraction of a natural juice or extract comprising at least one acylated anthocyanin;

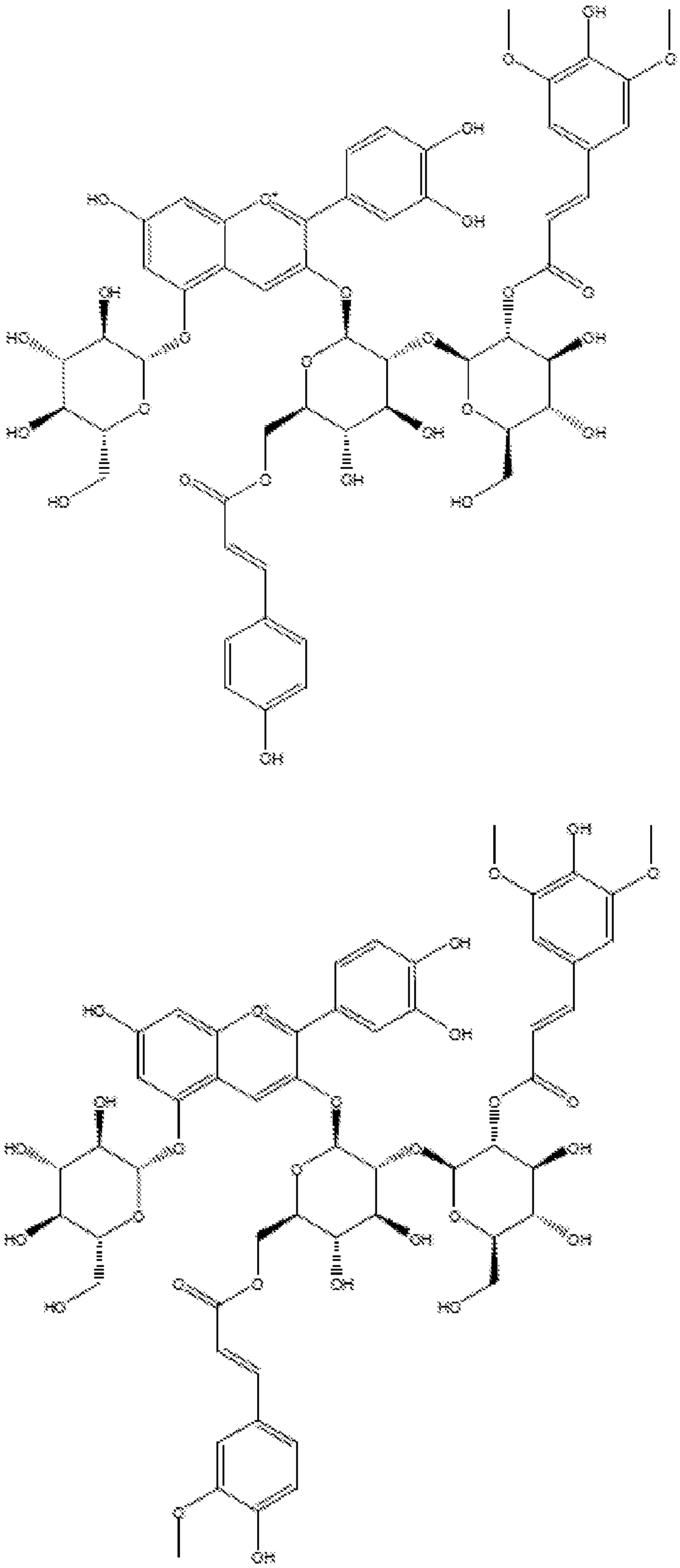
b) a buffer solution, wherein the buffer solution has a pH of from about 6.0 to about 8.0; and

c) a metal ion or salt thereof.

2. The natural blue colorant composition of claim 1, wherein the acylated anthocyanin comprises at least one diacylated anthocyanin.

3. The natural blue colorant composition of claim 2, wherein the diacylated anthocyanin comprises a compound selected from the group consisting of





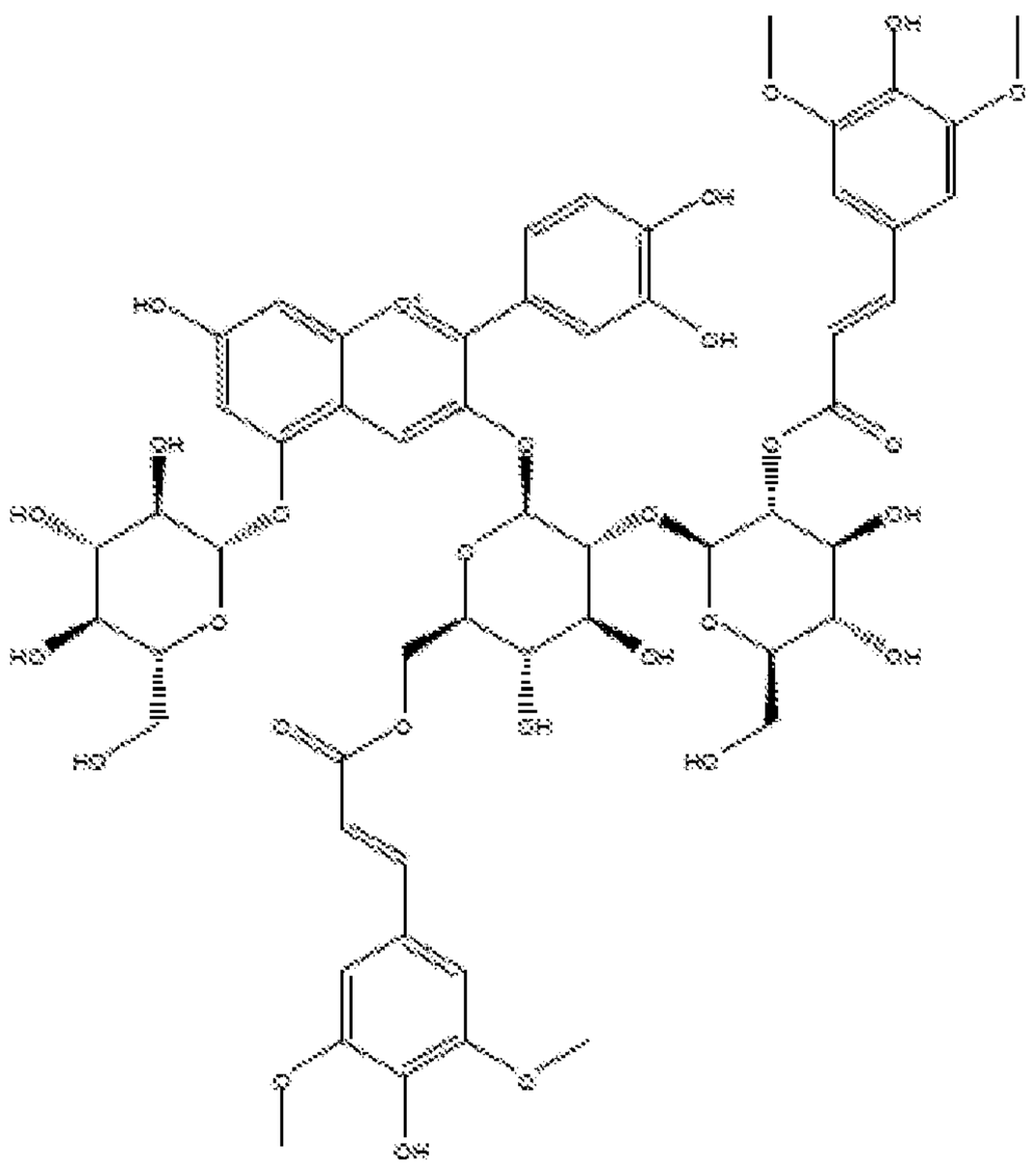
,

and salt forms thereof, and combinations thereof.

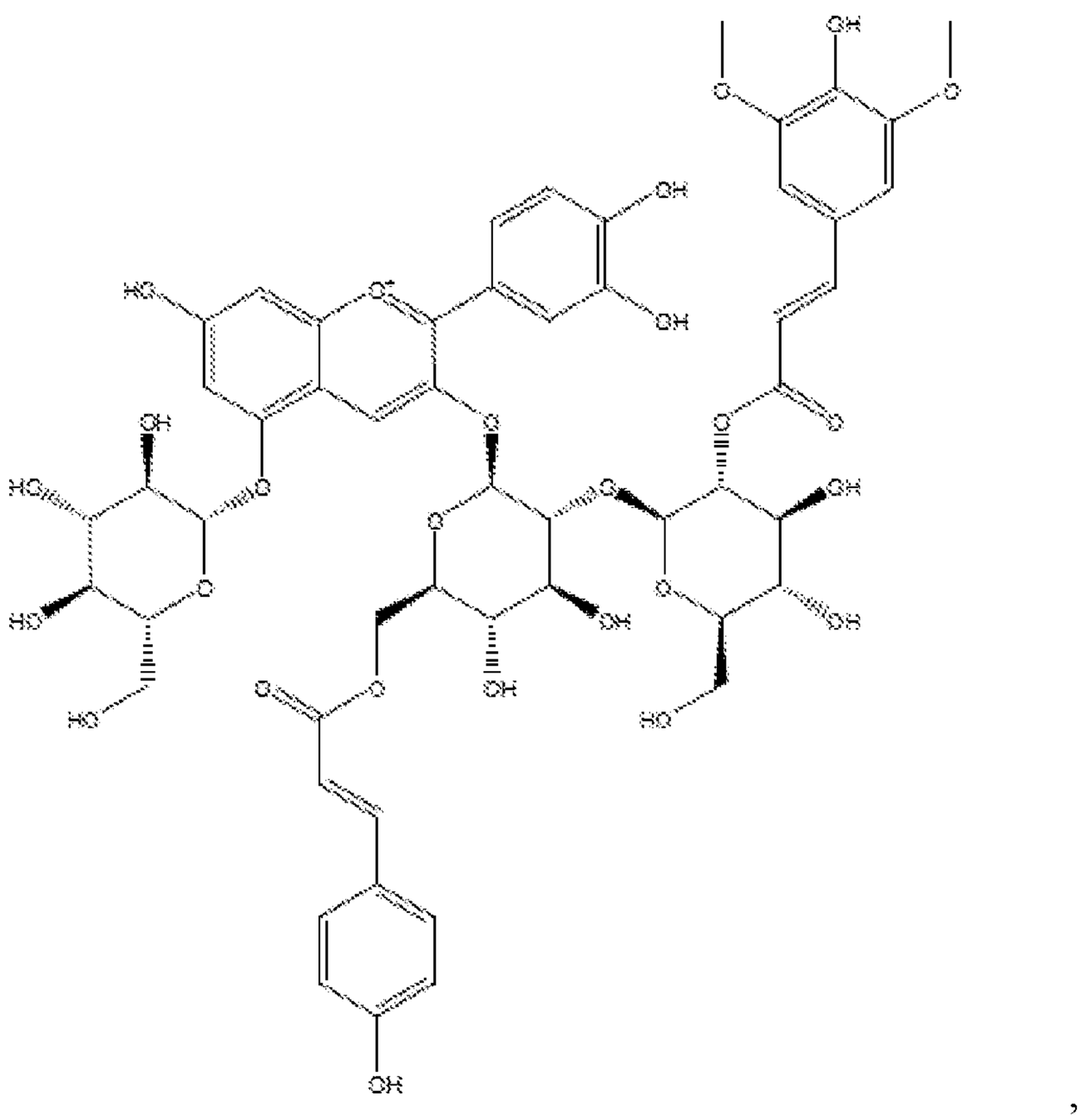
4. The natural blue colorant composition of any one of the preceding claims, wherein the metal ion is a trivalent metal ion.
5. The natural blue colorant composition of any one of the preceding claims, wherein the trivalent metal ion is selected from the group consisting of a Fe^{3+}, Al^{3+}, Ga^{3+}, and combinations thereof.
6. The natural blue colorant composition of claim 5, wherein the trivalent metal ion is Fe^{3+}, Al^{3+}, or a combination thereof.
7. The natural blue colorant composition of any one of the preceding claims, wherein the pH is from about 7.0 to about 8.0.
8. The natural blue colorant composition of any one of the preceding claims, wherein the acylated anthocyanin has a concentration of from about 1 μM to about 200 μM.
9. The natural blue colorant composition of any one of the preceding claims, wherein the metal ion or salt thereof has a concentration of less than about 10 times the concentration of the acylated anthocyanin.
10. The natural blue colorant composition of claim 9, wherein the metal ion or salt thereof has a concentration of less than or equal to the concentration of the acylated anthocyanin.
11. The natural blue colorant composition of any one of claims 9-10, wherein the metal ion or salt thereof has a concentration of from about 0.25 to about 0.5 times the concentration of the acylated anthocyanin.
12. The natural blue colorant composition of any one of the preceding claims, wherein the buffer solution comprises a buffer selected from the group consisting of potassium phosphate, tris(hydroxymethyl)-aminomethane, sodium hydroxide, and combinations thereof.
13. The natural blue colorant composition of any one of the preceding claims, wherein the natural blue colorant composition provides a color having a ΔE value of about 12 or less when compared to a color defined by L*a*b* values of 50 ppm and 100 ppm FD&C Blue No. 1 in an aqueous solution.
14. The natural blue colorant composition of claim 13, wherein the ΔE value is about 11 or less.
15. The natural blue colorant composition of any one of the preceding claims, wherein the natural blue colorant composition exhibits color stability for greater than about 14 days.

16. The natural blue colorant composition of any one of the preceding claims, wherein the fraction is obtained from a natural product, or an extract or juice thereof, selected from the group consisting of red cabbage, purple sweet potato, red potato, blue potato, red radish, black carrot, purple carrot, purple corn, red corn, red onion, purple broccoli, red broccoli, purple cauliflower, rhubarb, black bean, red leaf lettuce, black rice, eggplant, strawberry, raspberry, cranberry, lingonberry, red grape, apple, black currant, red currant, cherry, blueberry, elderberry, bilberry, crowberry, blackberry, chokeberry, gooseberry, acai, nectarine, peach, plum, blood orange, blue tomato, "Heavenly Blue" Morning Glory flower petal, "Better Times" Rose flower petal, and combinations thereof.
17. The natural blue colorant composition of claim 16, wherein the fraction is obtained from red cabbage.
18. A green colorant composition, comprising the natural blue colorant composition of any one of the preceding claims and one or more yellow colorants.
19. The green colorant composition of claim 18, wherein the yellow colorant is a natural yellow colorant.
20. A food product, comprising the natural blue colorant composition of any one of the preceding claims.
21. The food product of claim 20, wherein the food product is a confectionary product.
22. The confectionary product of claim 21, further comprising a yellow colorant.
23. The confectionary product of any one of claims 21-22, wherein the natural blue colorant composition is present in a coating applied to a surface of the confectionary product.
24. A natural blue colorant composition, comprising:

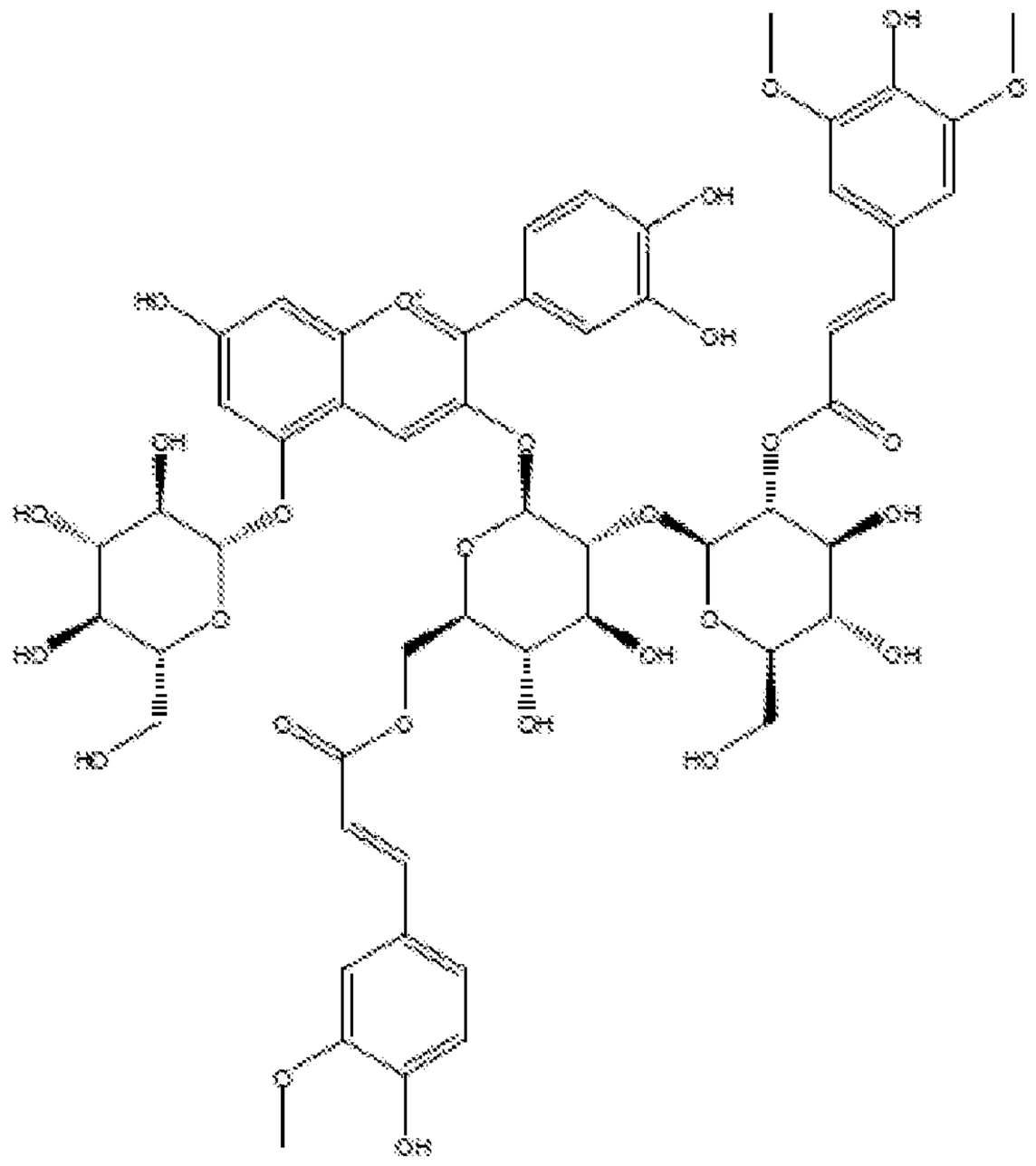
a) a fraction of a natural juice or extract comprising at least one diacylated anthocyanin selected from the group consisting of



,



,



,

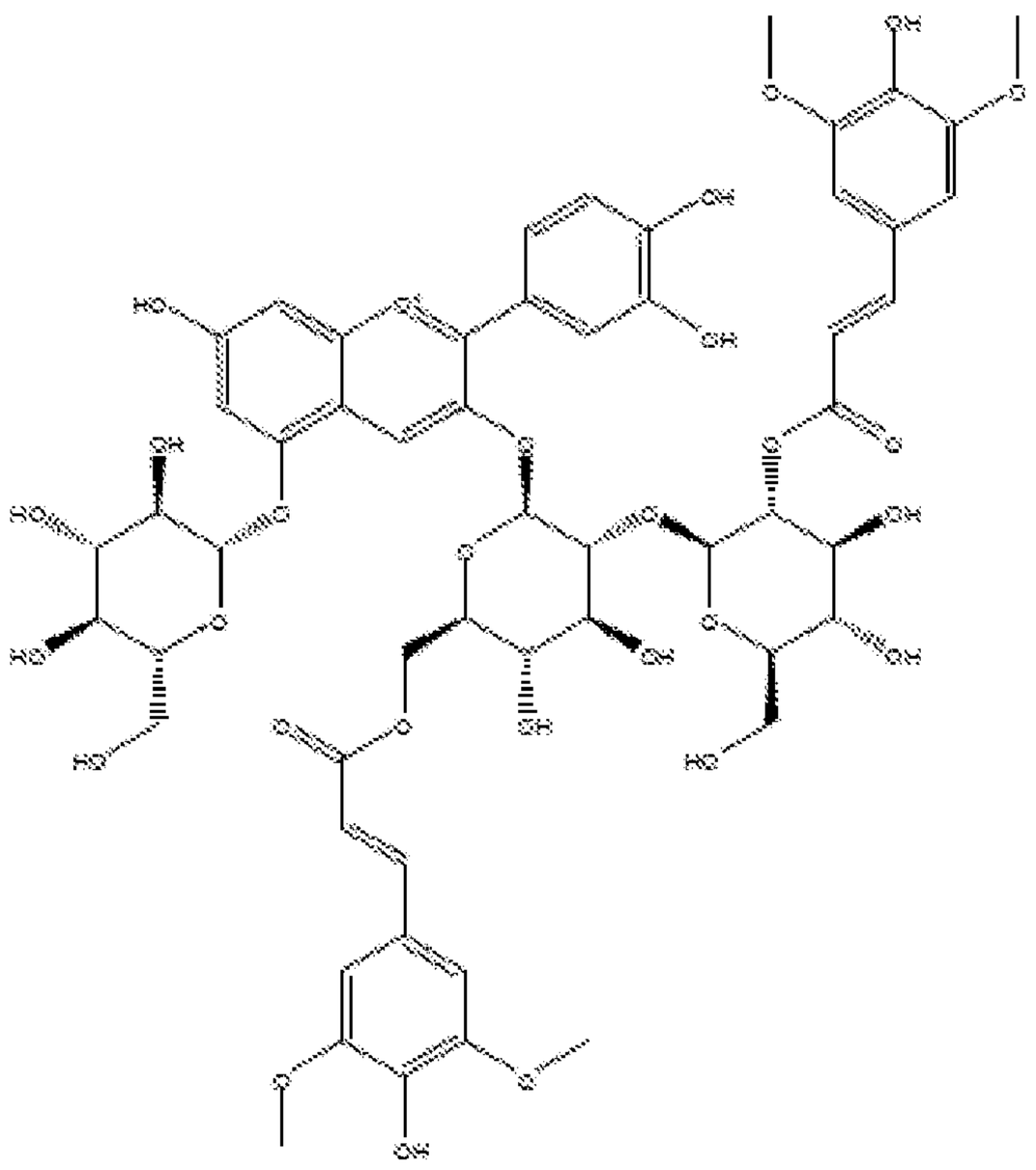
and salt forms thereof, and combinations thereof, wherein the diacylated anthocyanin has a concentration of from about 1 μM to about 200 μM;

b) a buffer solution, wherein the buffer solution has a pH of from about 7.0 to about 8.0; and

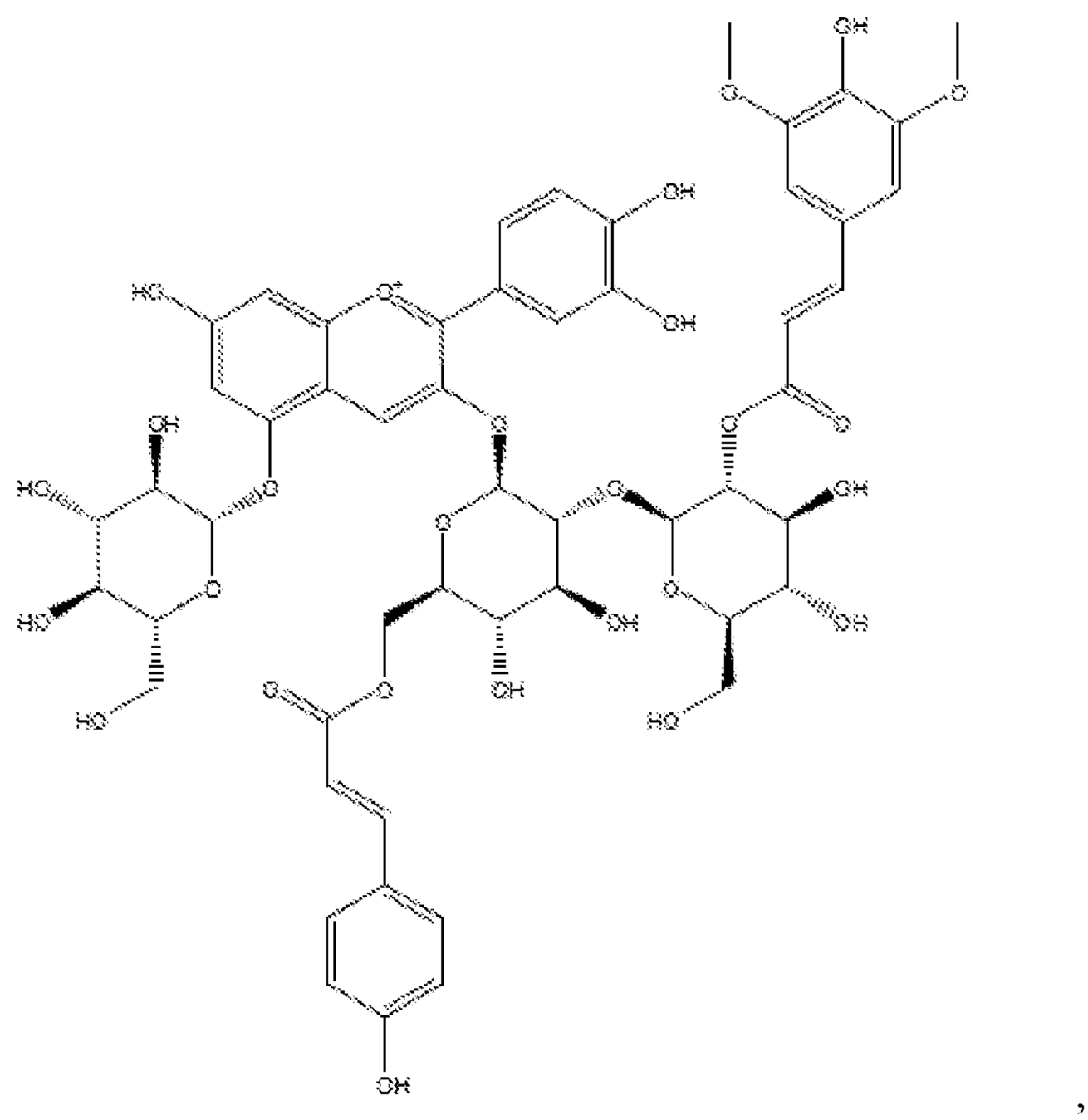
c) a metal ion comprising Fe^{3+} or a salt form thereof, wherein the metal ion has a concentration of from about 0.25 to about 0.5 times the concentration of the diacylated anthocyanin.

25. A natural blue colorant composition, comprising:

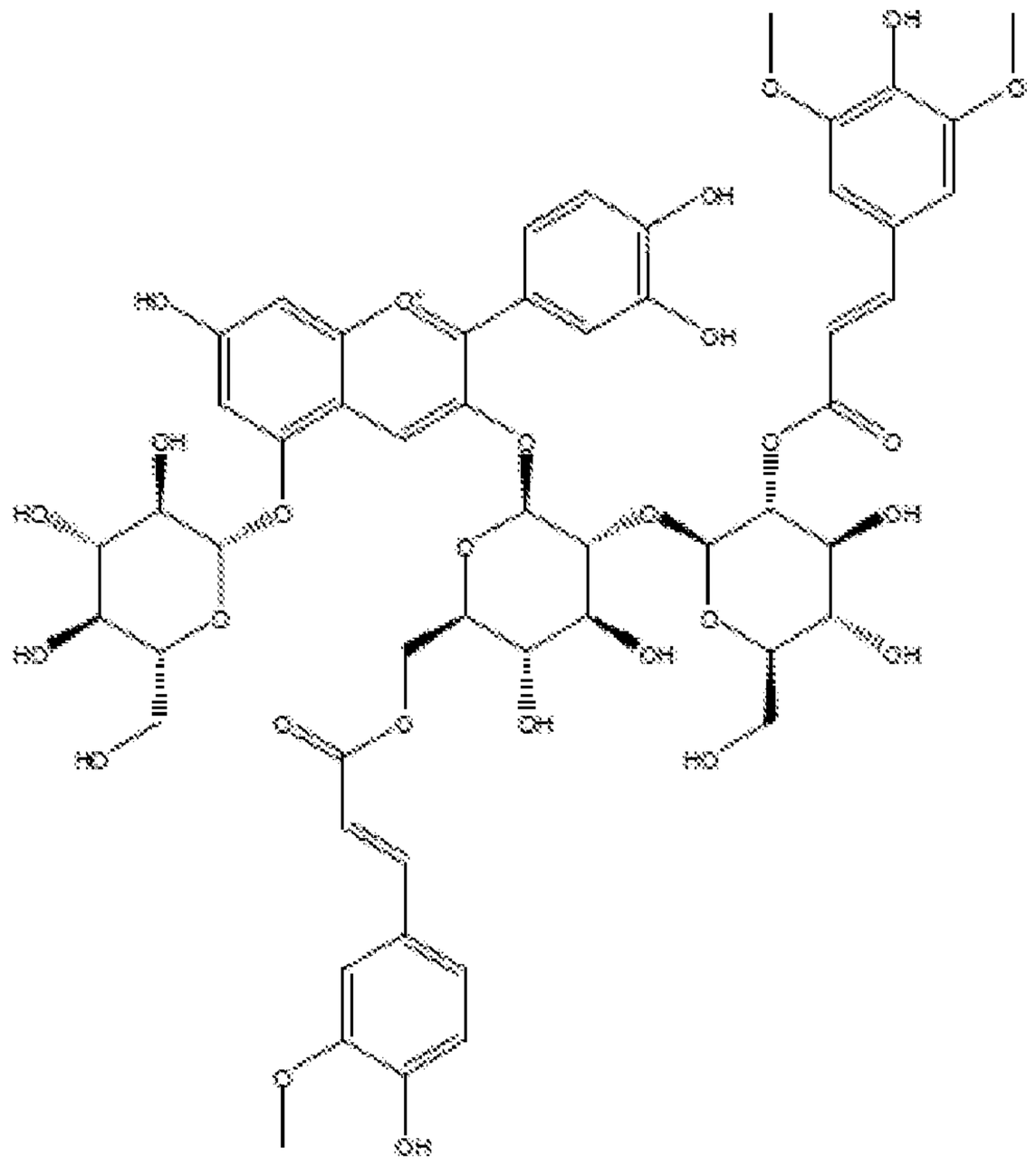
a) a fraction of a natural juice or extract comprising at least one diacylated anthocyanin selected from the group consisting of



,



,



,

and salt forms thereof, and combinations thereof, wherein the diacylated anthocyanin has a concentration of from about 1 μM to about 200 μM;

b) a buffer solution, wherein the buffer solution has a pH of from about 7.0 to about 8.0; and

c) a metal ion comprising Al^{3+} or a salt form thereof, wherein the metal ion has a concentration of less than or equal to the concentration of the diacylated anthocyanin.

26. A method of preparing a natural blue colorant composition, comprising:

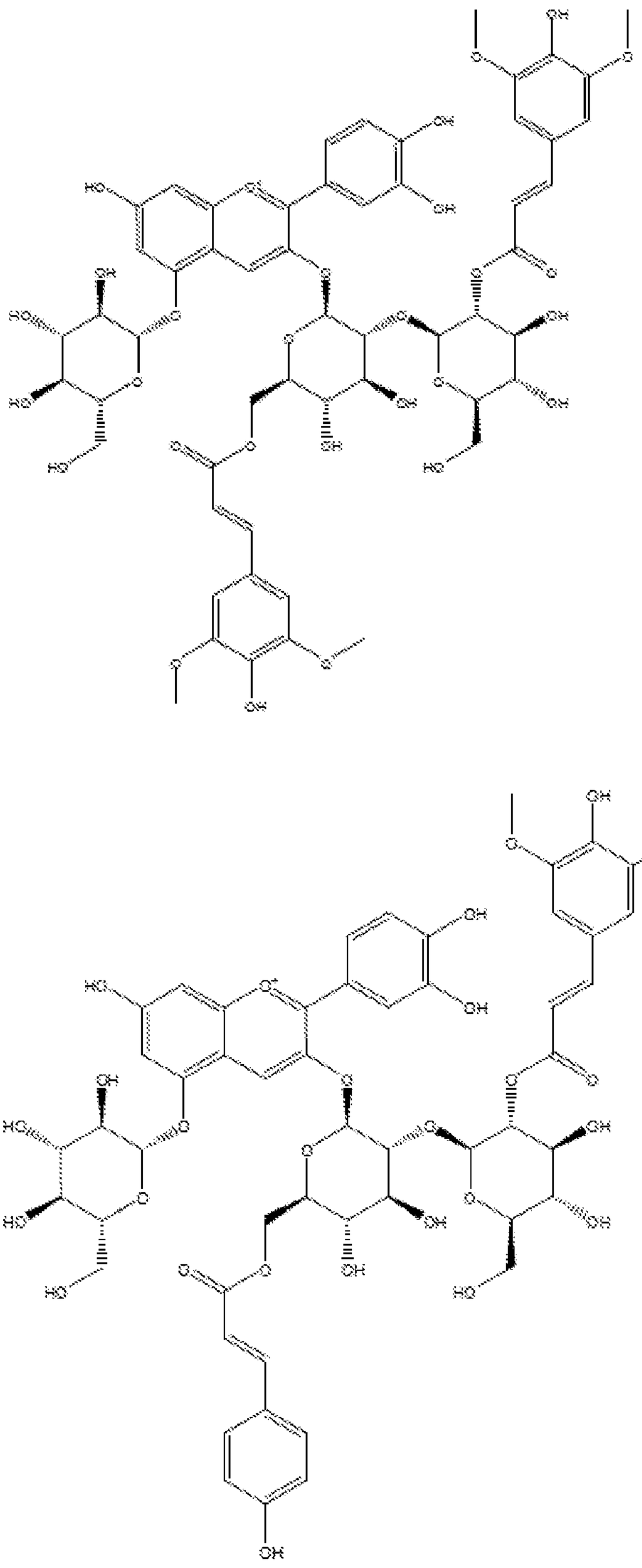
a) obtaining a fraction of a natural juice or extract comprising at least one acylated anthocyanin from a natural product;

b) diluting the acylated anthocyanin in a buffer solution having a pH of from about 6.0 to about 8.0; and

c) admixing a metal ion or salt thereof in the buffer solution.

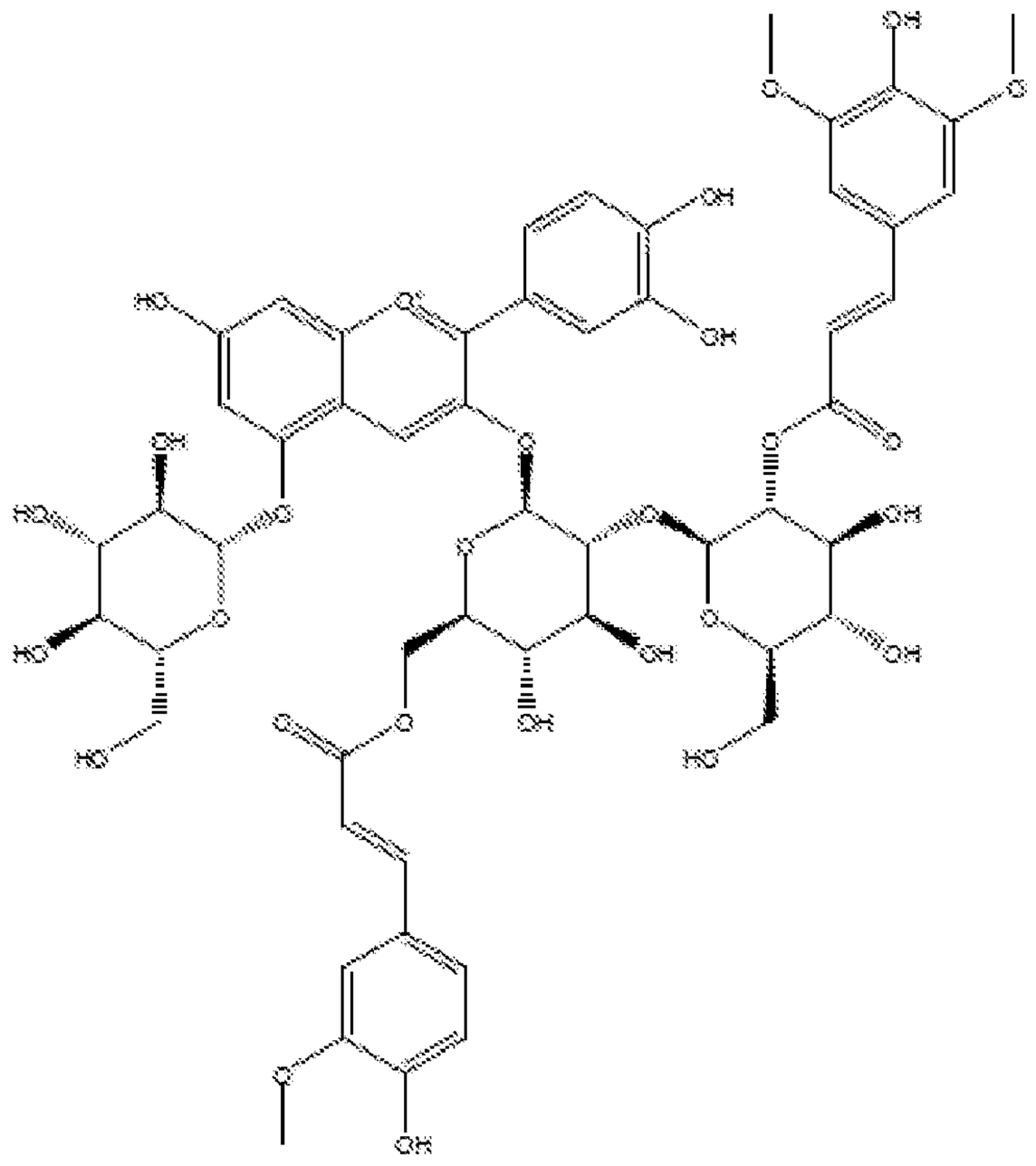
27. The method of claim 26, wherein the acylated anthocyanin comprises at least one diacylated anthocyanin.

28. The method of any one of claims 26-27, wherein the diacylated anthocyanin comprises a compound selected from the group consisting of



,

,



,

and combinations thereof.

29. The method of any one of claims 26-28, wherein the natural product comprises red cabbage.

30. The method of any one of claims 26-29, wherein the buffer solution has a pH of from about 7.0 to about 8.0.

31. The method of any one of claims 26-30, wherein the metal ion or salt thereof comprises Fe^{3+}, Al^{3+}, or a combination thereof.

32. The method of any one of claims 26-31, wherein the acylated anthocyanin has a concentration of from about 1 μM to about 200 μM.

33. The method of claim 32, wherein the metal ion or salt thereof has a concentration of less than about 10 times the concentration of the acylated anthocyanin.

34. The method of any one of claims 32-33, wherein the metal ion or salt thereof has a concentration of less than or equal to the concentration of the acylated anthocyanin.

35. The method of any one of claims 32-34, wherein the metal ion or salt thereof has a concentration of from about 0.25 to about 0.5 times the concentration of the acylated anthocyanin.

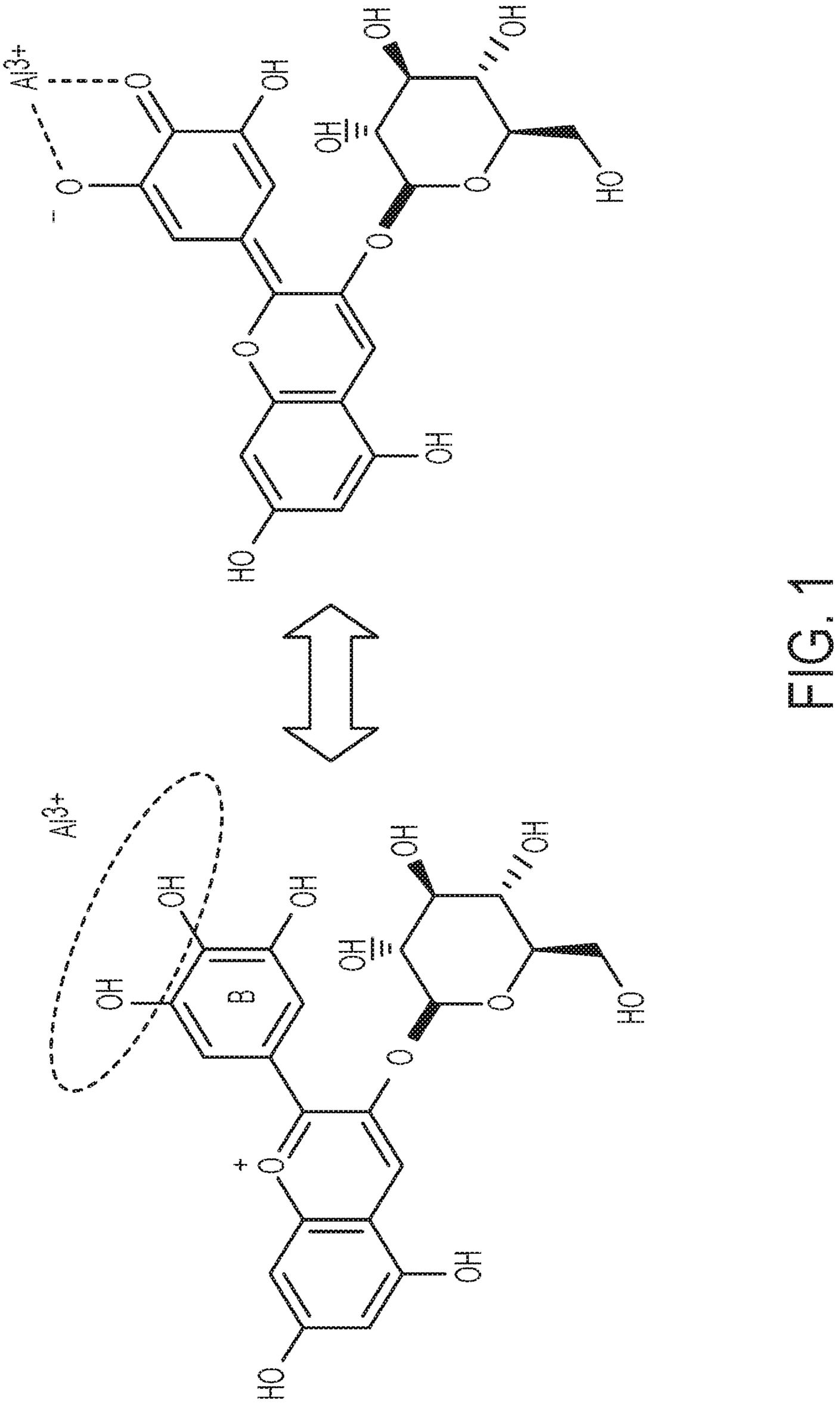


FIG.

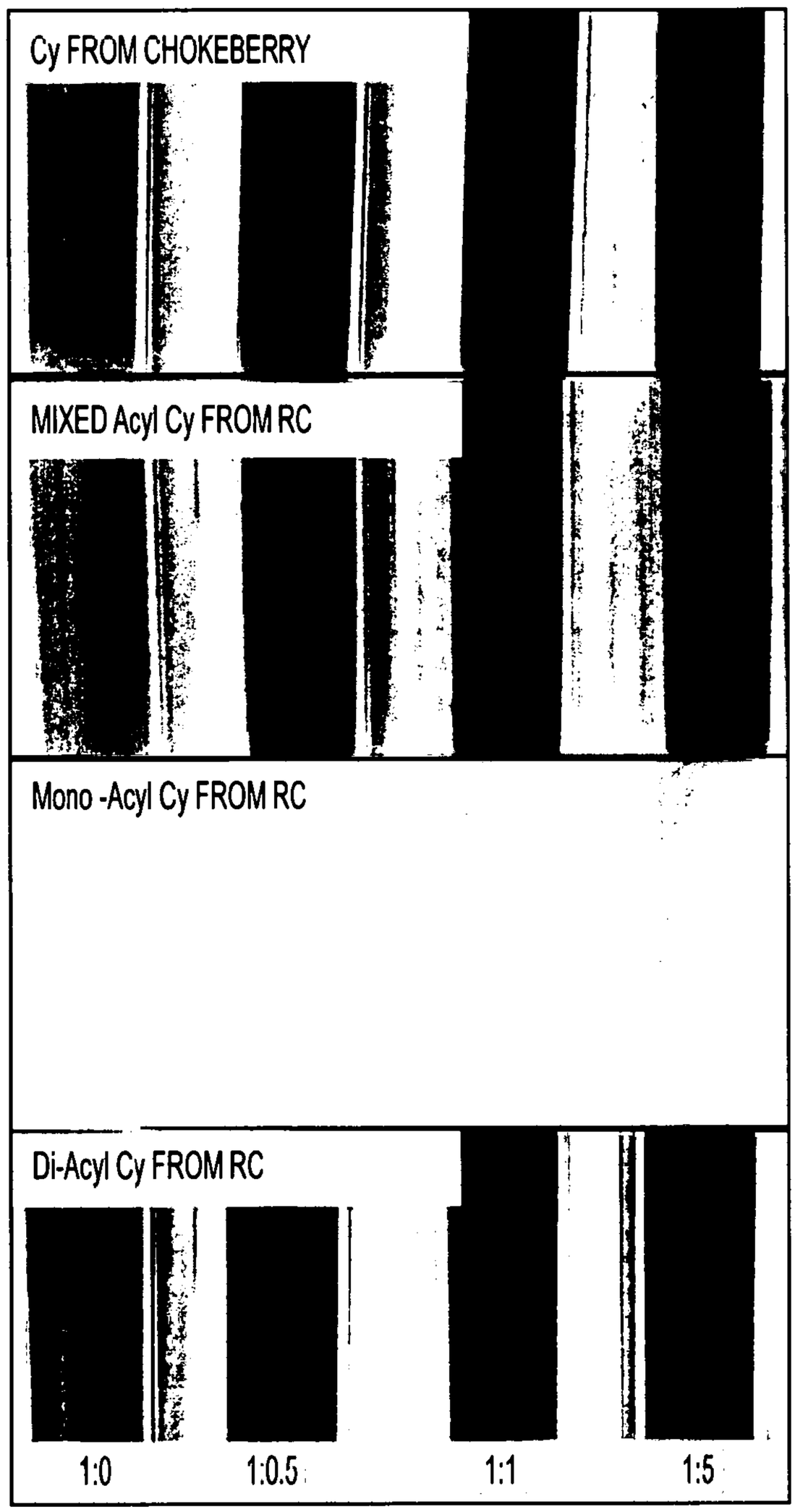

Cy FROM CHOKEBERRY
MIXED Acyl Cy FROM RC
Mono -Acyl Cy FROM RC
Di-Acyl Cy FROM RC
1:0
1:0.5
1:1
1:5

FIG. 2

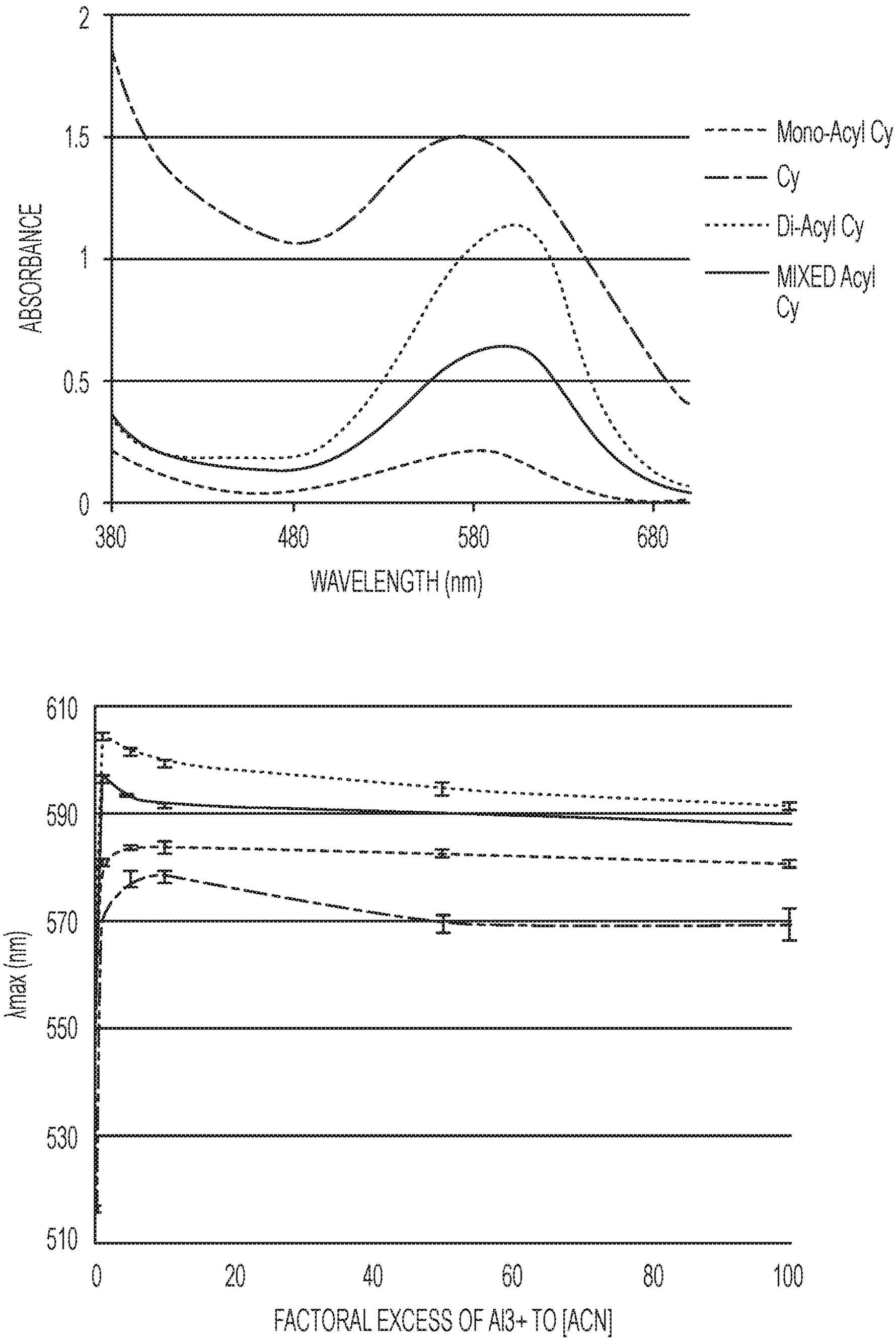

2
1.5
1
0.5
0
ABSORBANCE
380
480
580
680
WAVELENGTH (nm)
Mono-Acyl Cy
Cy
Di-Acyl Cy
MIXED Acyl Cy
610
590
570
550
530
510
λmax (um)
0
20
40
60
80
100
FACTORAL EXCESS OF Al3+ TO [ACN]

FIG. 3

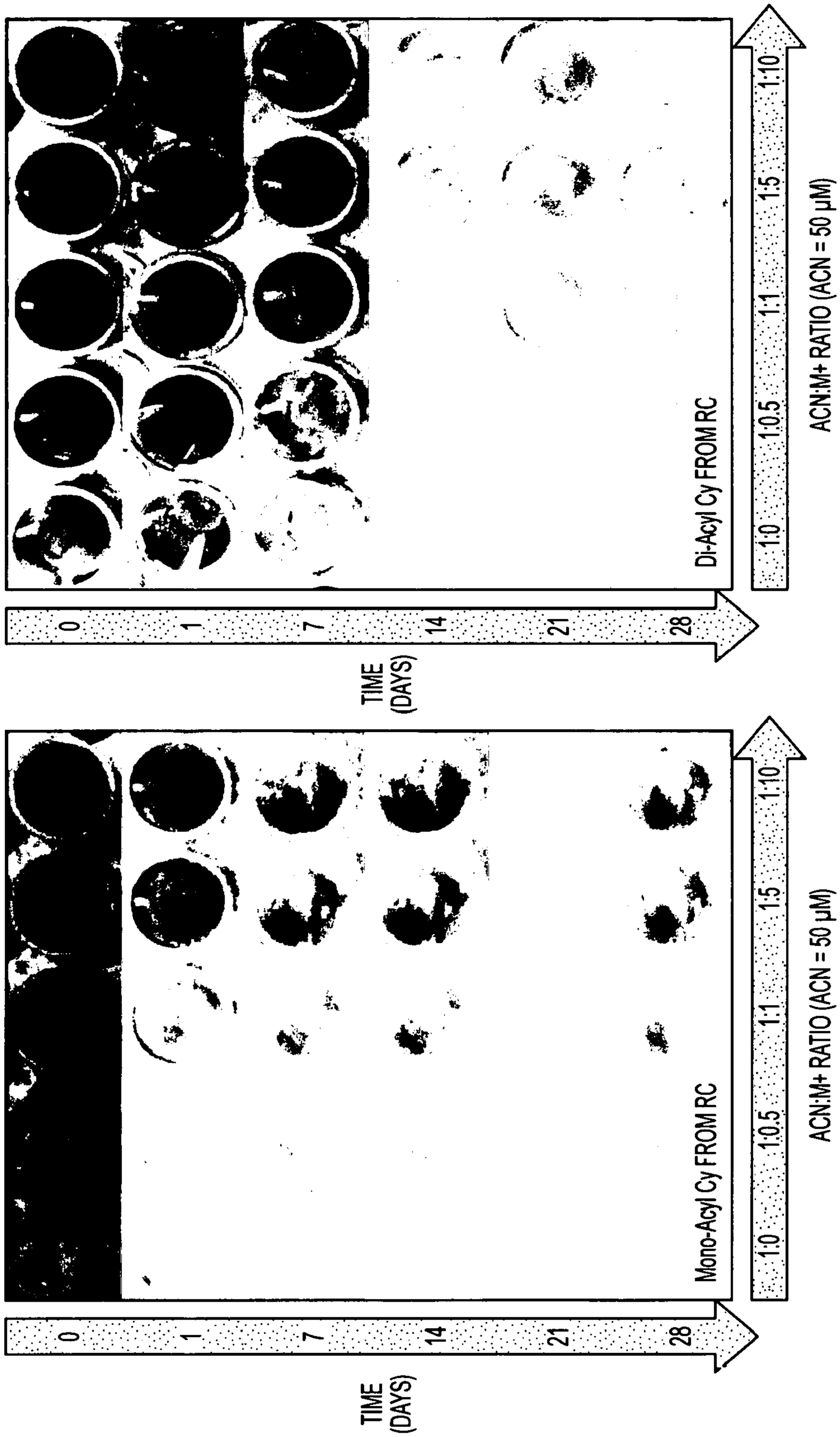
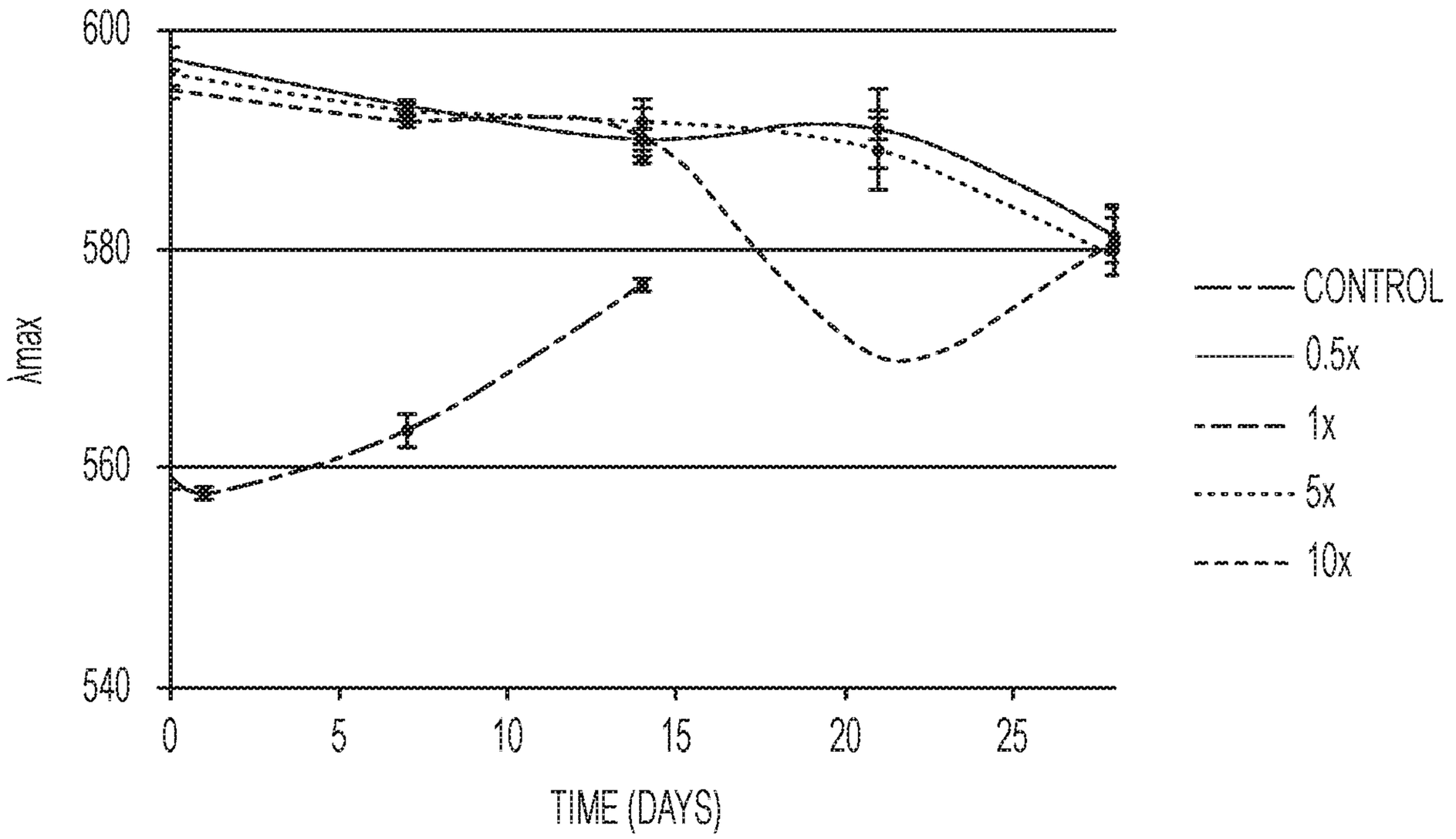

Di-Acyl Cy FROM RC
Mono-Acyl Cy FROM RC
1:0
1:0.5
1:1
1:5
1:10
ACN:M+ RATIO (ACN = 50 µM)
0
1
7
14
21
28
TIME (DAYS)

FIG. 4


600
580
560
540
λmax
0
5
10
15
20
25
TIME (DAYS)
CONTROL
0.5x
1x
5x
10x

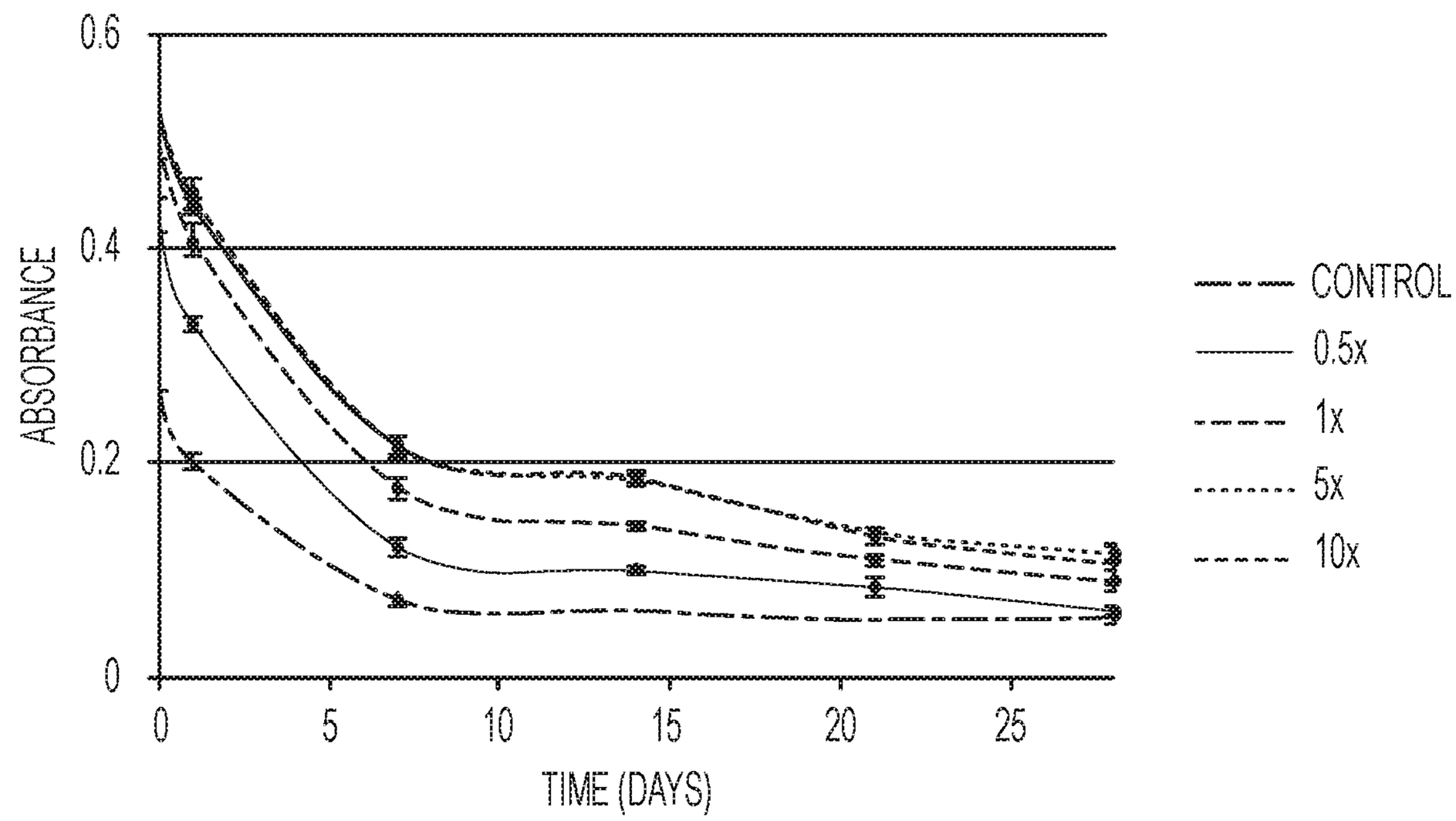

0.6
0.4
0.2
0
ABSORBANCE
0
5
10
15
20
25
TIME (DAYS)
CONTROL
0.5x
1x
5x
10x

FIG. 5

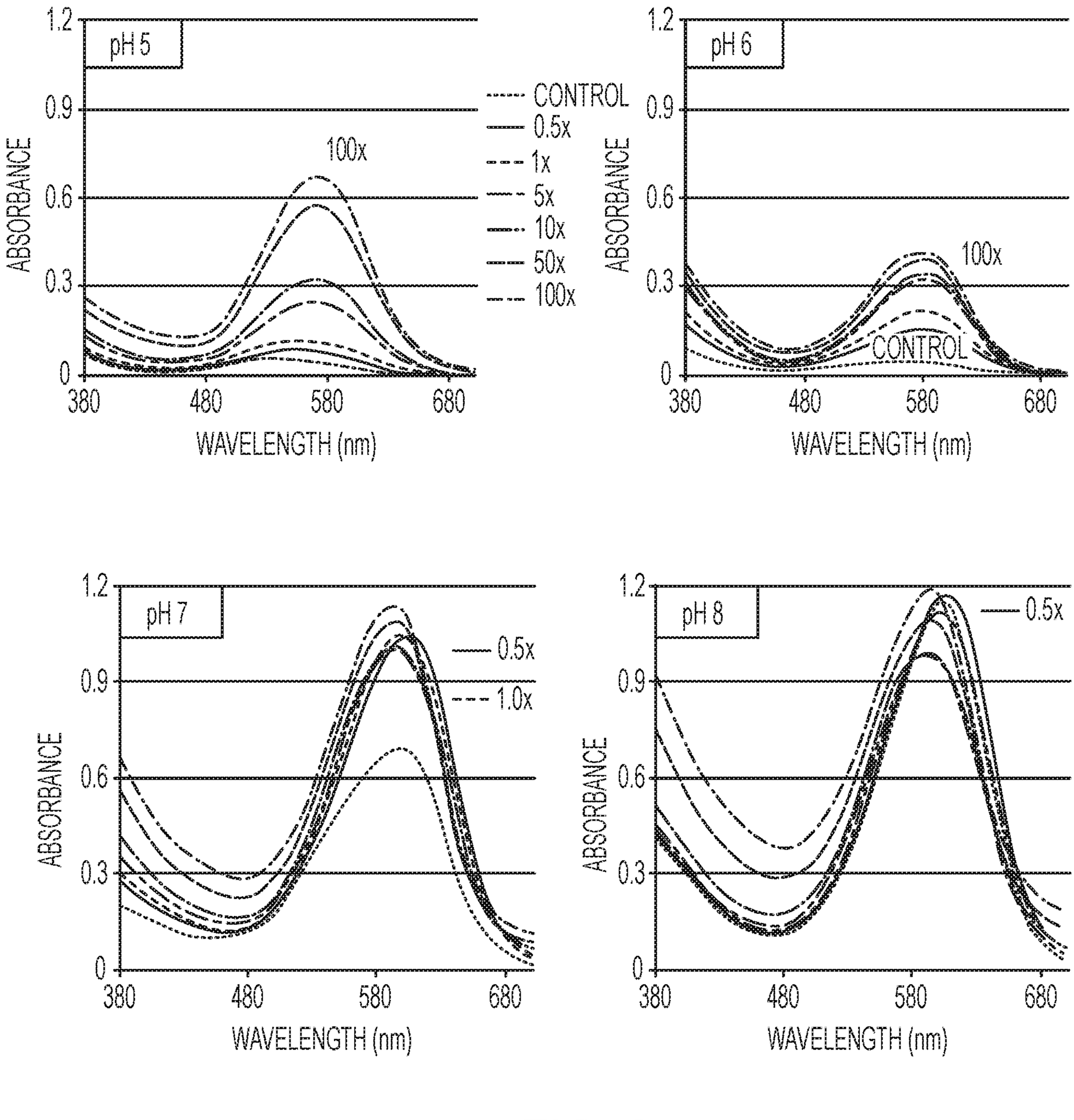

pH 5
pH 6
pH 7
pH 8
CONTROL
0.5x
1x
5x
10x
50x
100x
1.0x
ABSORBANCE
WAVELENGTH (nm)
0
0.3
0.6
0.9
1.2
380
480
580
680

FIG. 6

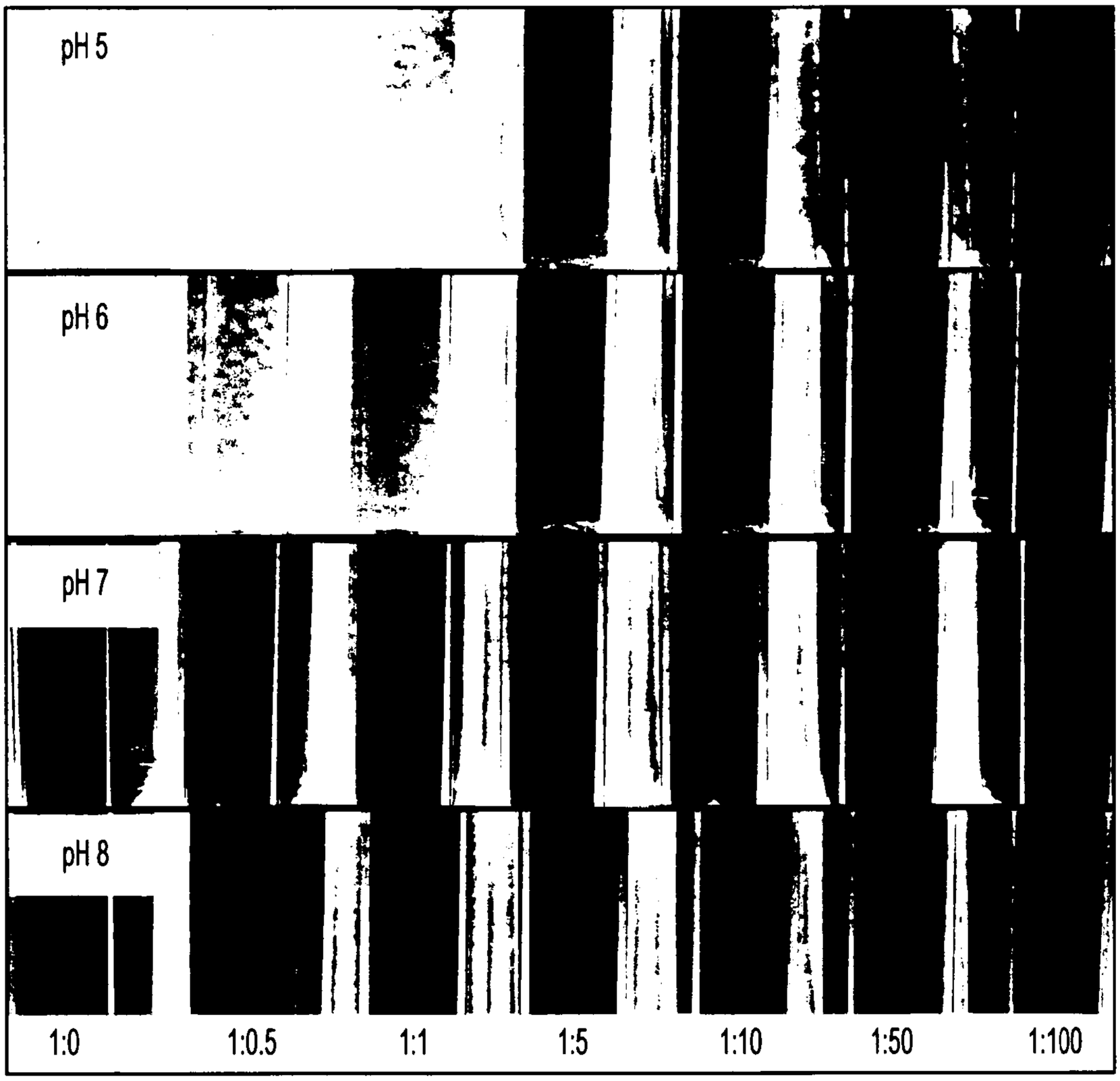
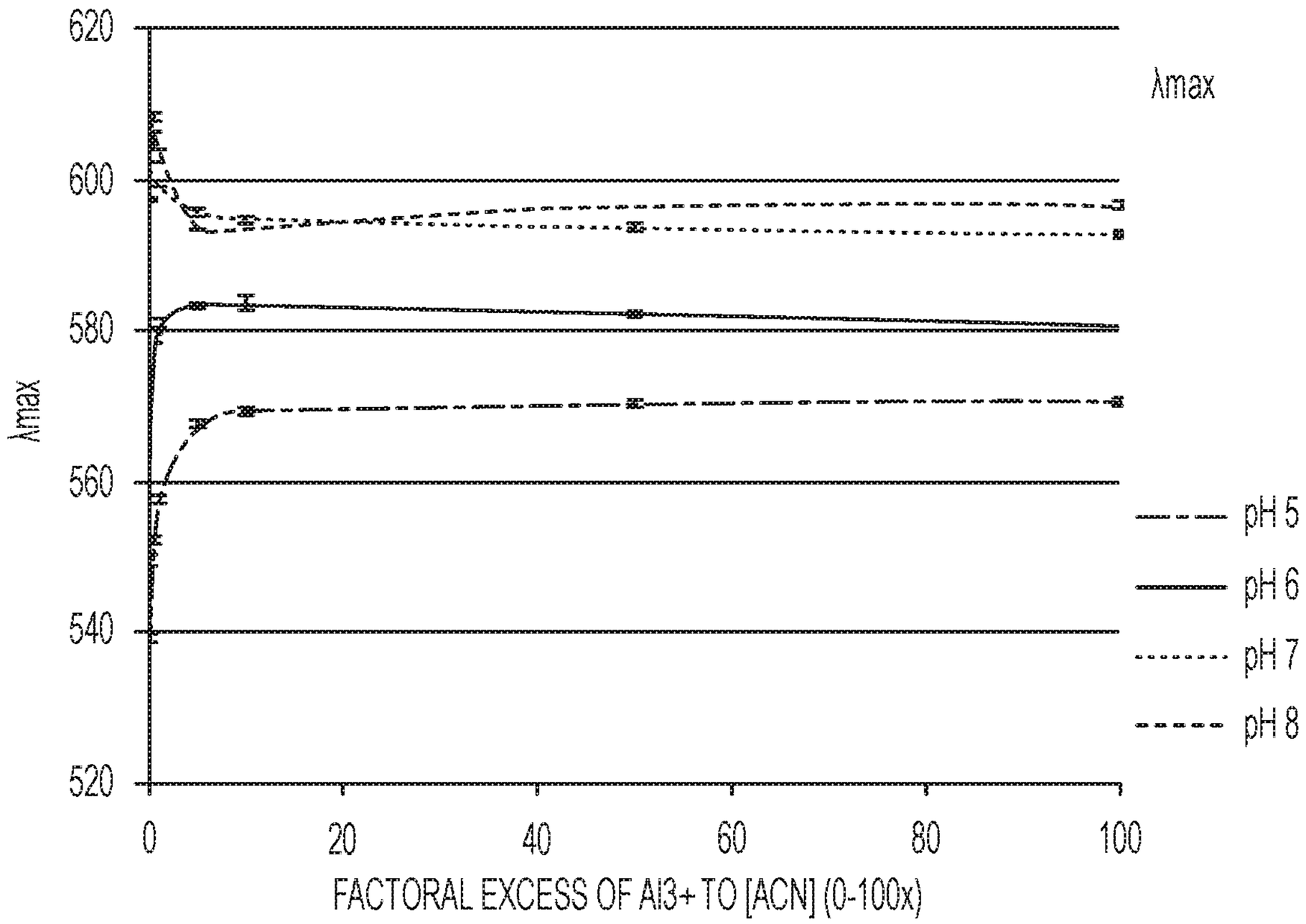


FIG. 7


620
600
580
560
540
520
λmax
λmax
pH 5
pH 6
pH 7
pH 8
0
20
40
60
80
100
FACTORAL EXCESS OF Al3+ TO [ACN] (0-100x)

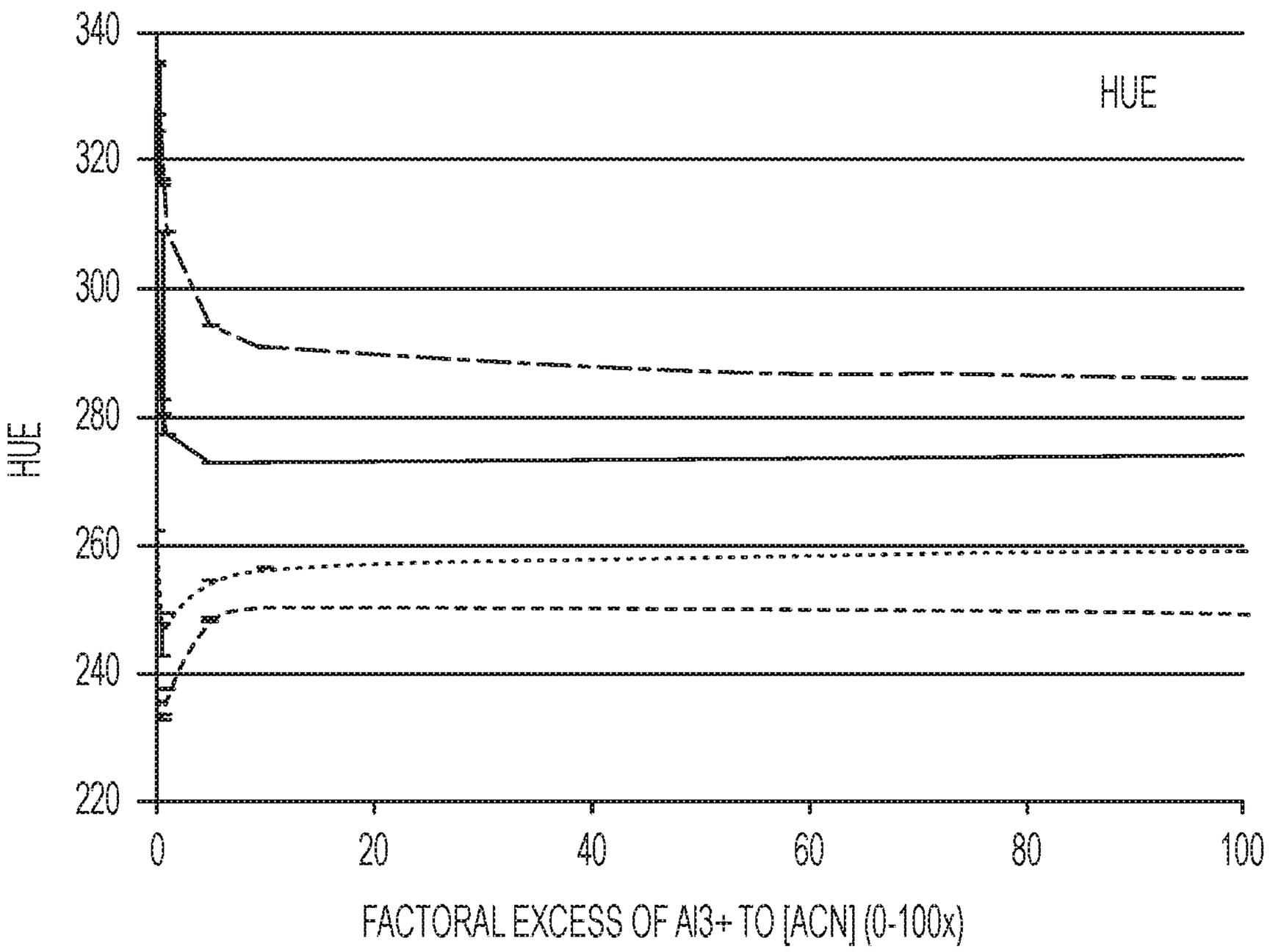

340
320
300
280
260
240
220
HUE
HUE
0
20
40
60
80
100
FACTORAL EXCESS OF Al3+ TO [ACN] (0-100x)

FIG. 8

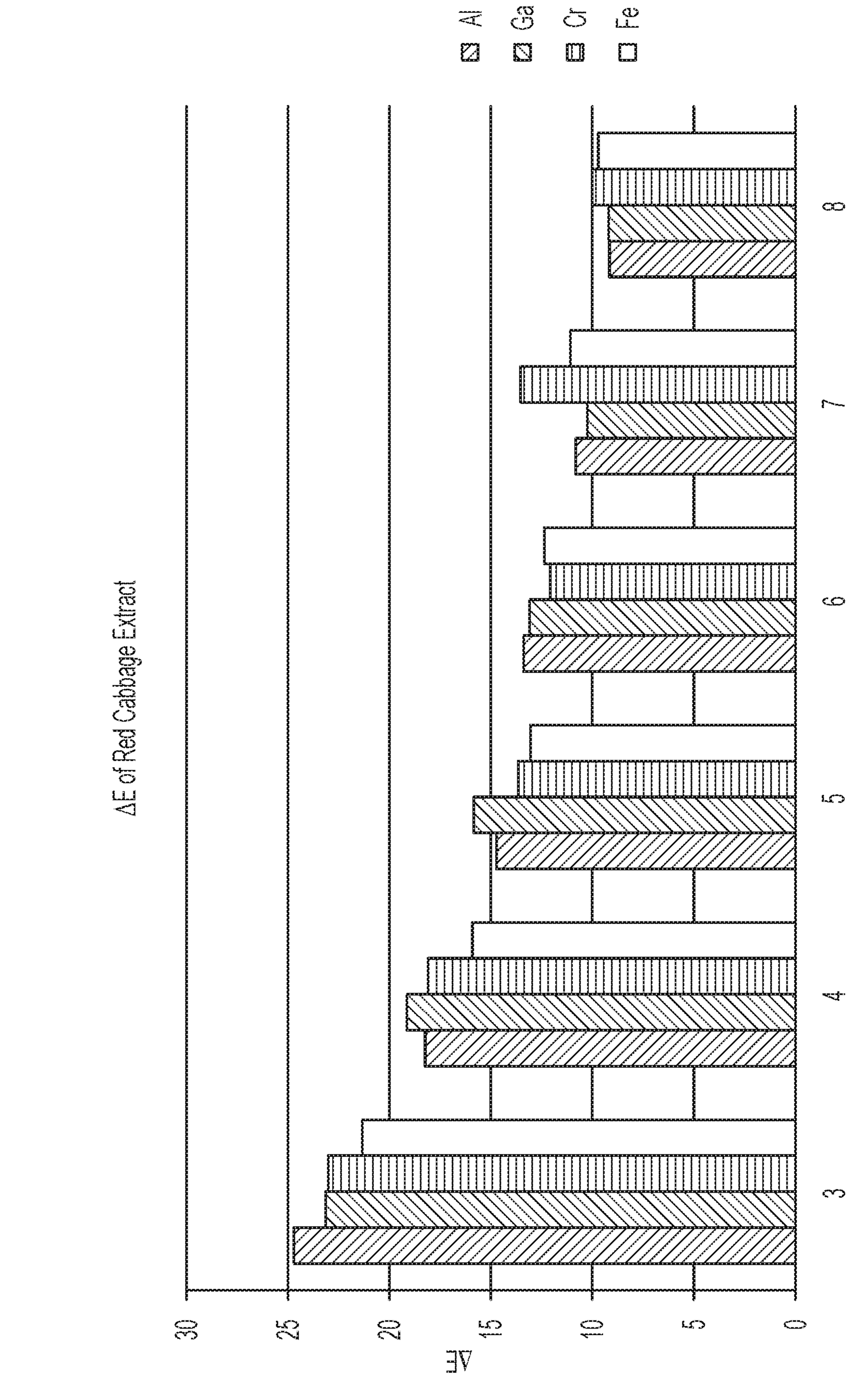

Al
Ga
Cr
Fe
ΔE of Red Cabbage Extract
pH
ΔE
30
25
20
15
10
5
0
3
4
5
6
7
8

FIG. 9

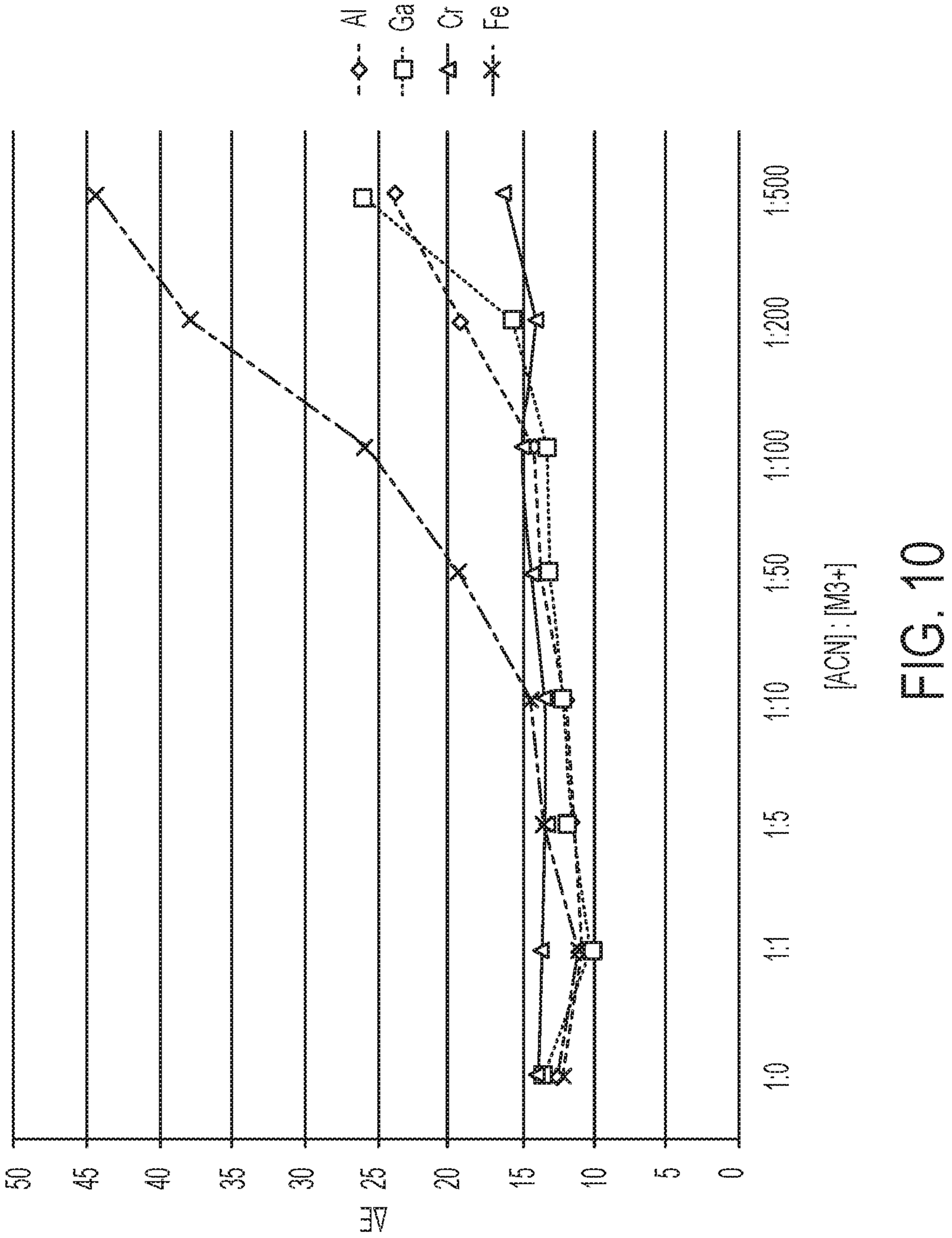

Al
Ga
Cr
Fe
ΔE of Red Cabbage Extract at pH7
50
45
40
35
30
25
20
15
10
5
0
ΔE
1:0
1:1
1:5
1:10
1:50
1:100
1:200
1:500
[ACN] : [M3+]

FIG. 10

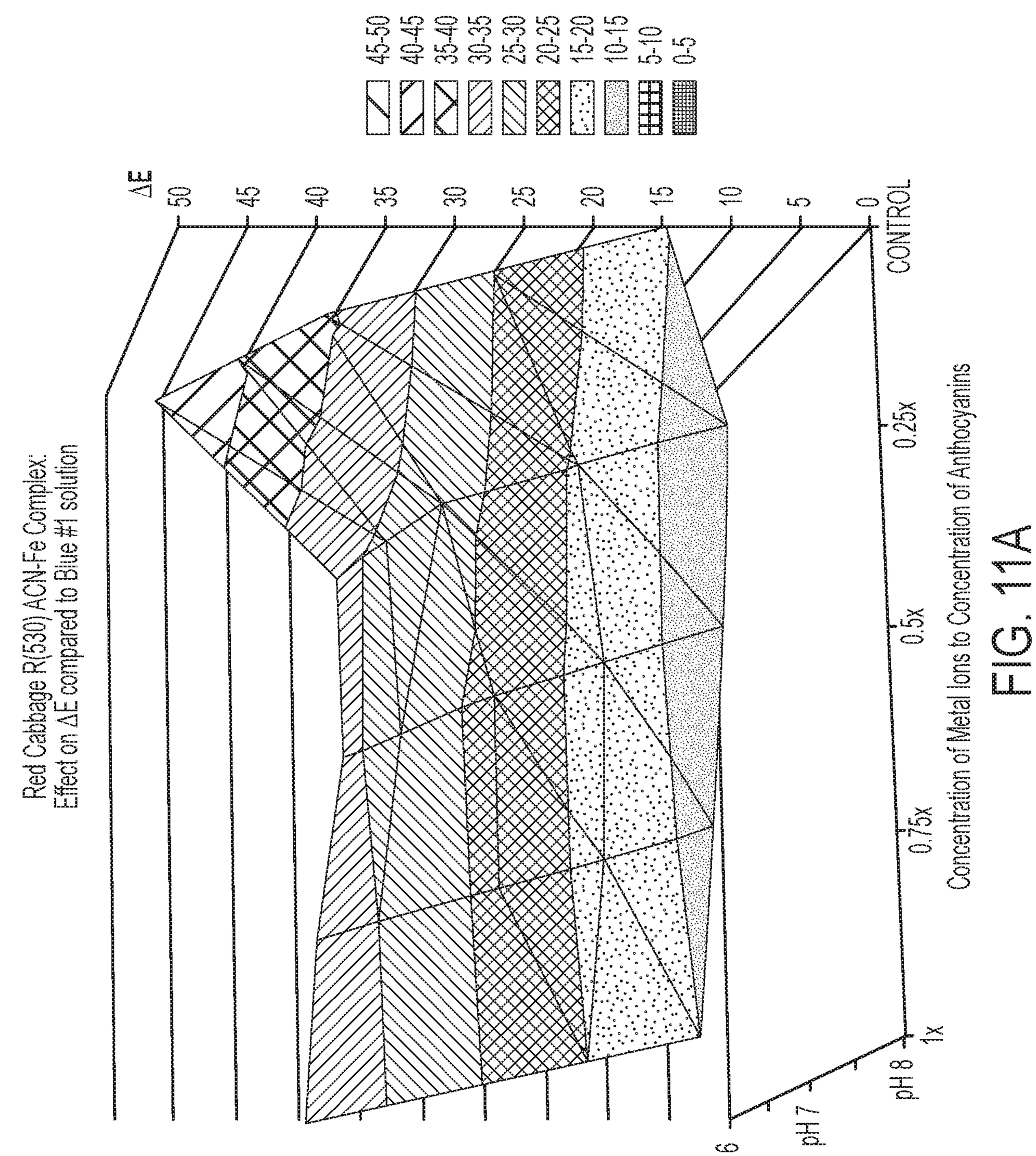

45-50
40-45
35-40
30-35
25-30
20-25
15-20
10-15
5-10
0-5
ΔE
50
45
40
35
30
25
20
15
10
5
0
CONTROL
0.25x
0.5x
0.75x
1x
pH 8
pH 7
pH 9
Concentration of Metal Ions to Concentration of Anthocyanins
Red Cabbage R(530) ACN-Fe Complex:
Effect on ΔE compared to Blue #1 solution

FIG. 11A

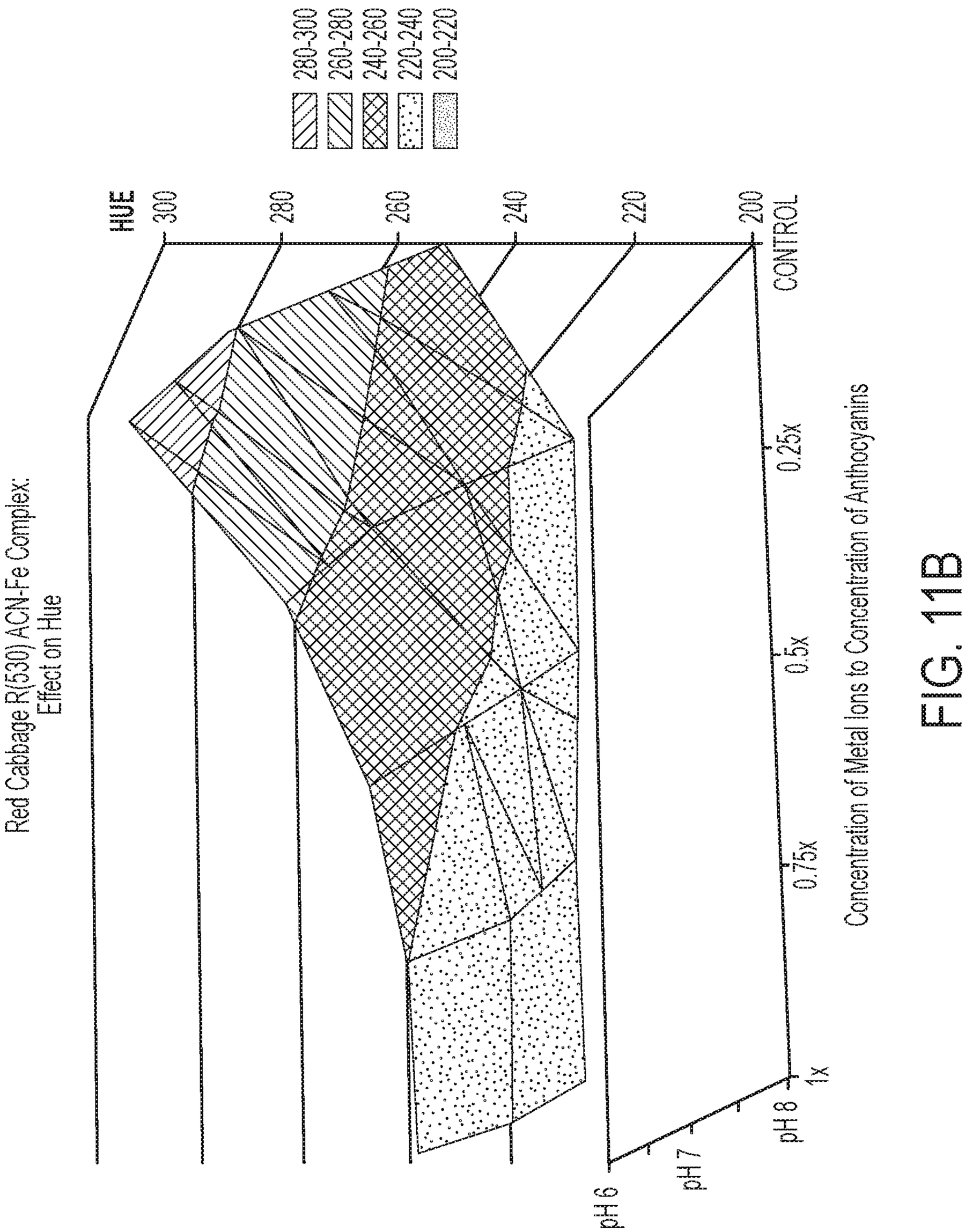

280-300
260-280
240-260
220-240
200-220
HUE
300
280
260
240
220
200
CONTROL
0.25x
0.5x
0.75x
1x
pH 8
pH 7
pH 6
Concentration of Metal Ions to Concentration of Anthocyanins
Red Cabbage R(530) ACN-Fe Complex:
Effect on Hue

FIG. 11B

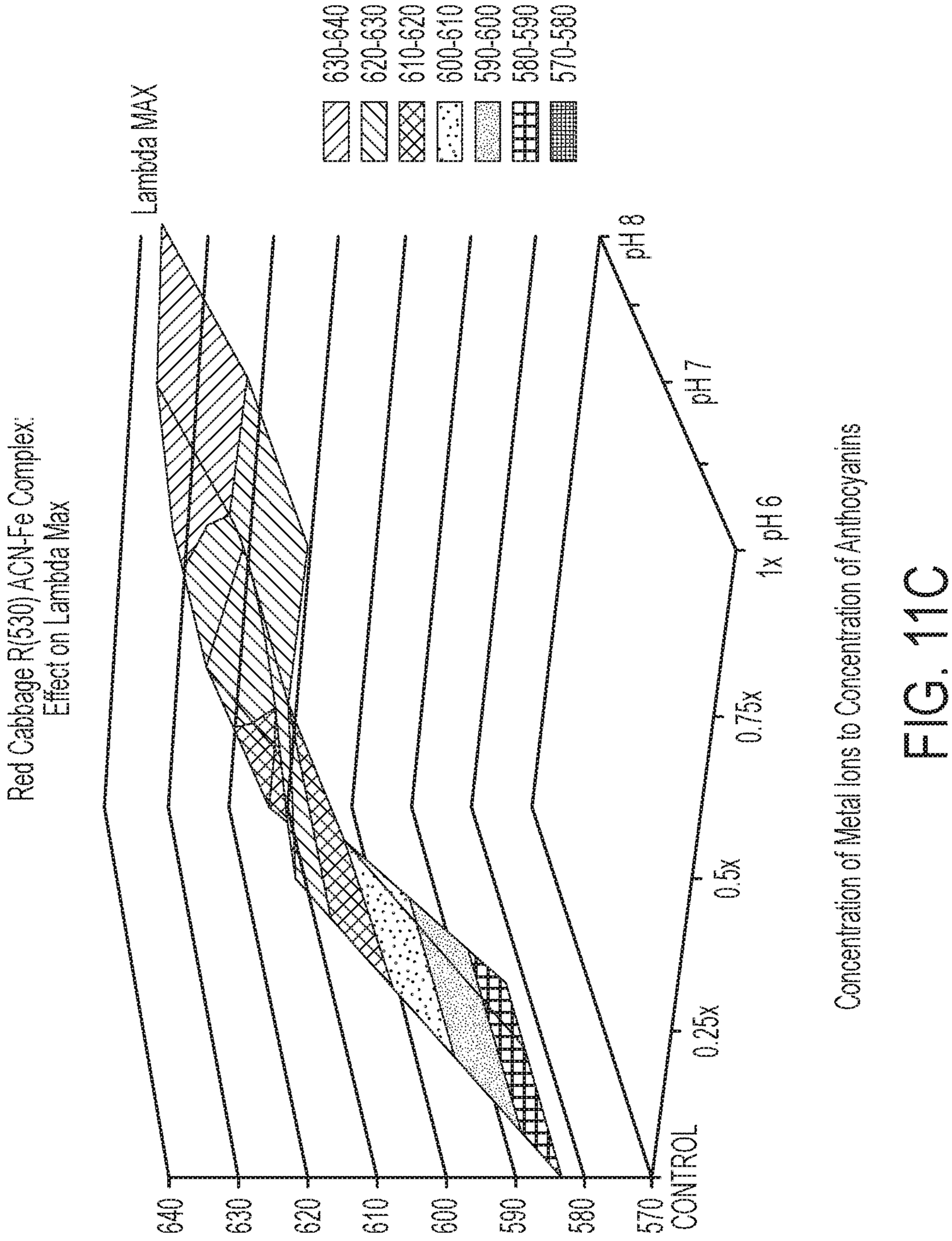

Red Cabbage R(530) ACN-Fe Complex:
Effect on Lambda Max
Lambda MAX
640
630
620
610
600
590
580
570
pH 8
pH 7
1x pH 6
CONTROL
0.25x
0.5x
0.75x
Concentration of Metal Ions to Concentration of Anthocyanins

FIG. 11C

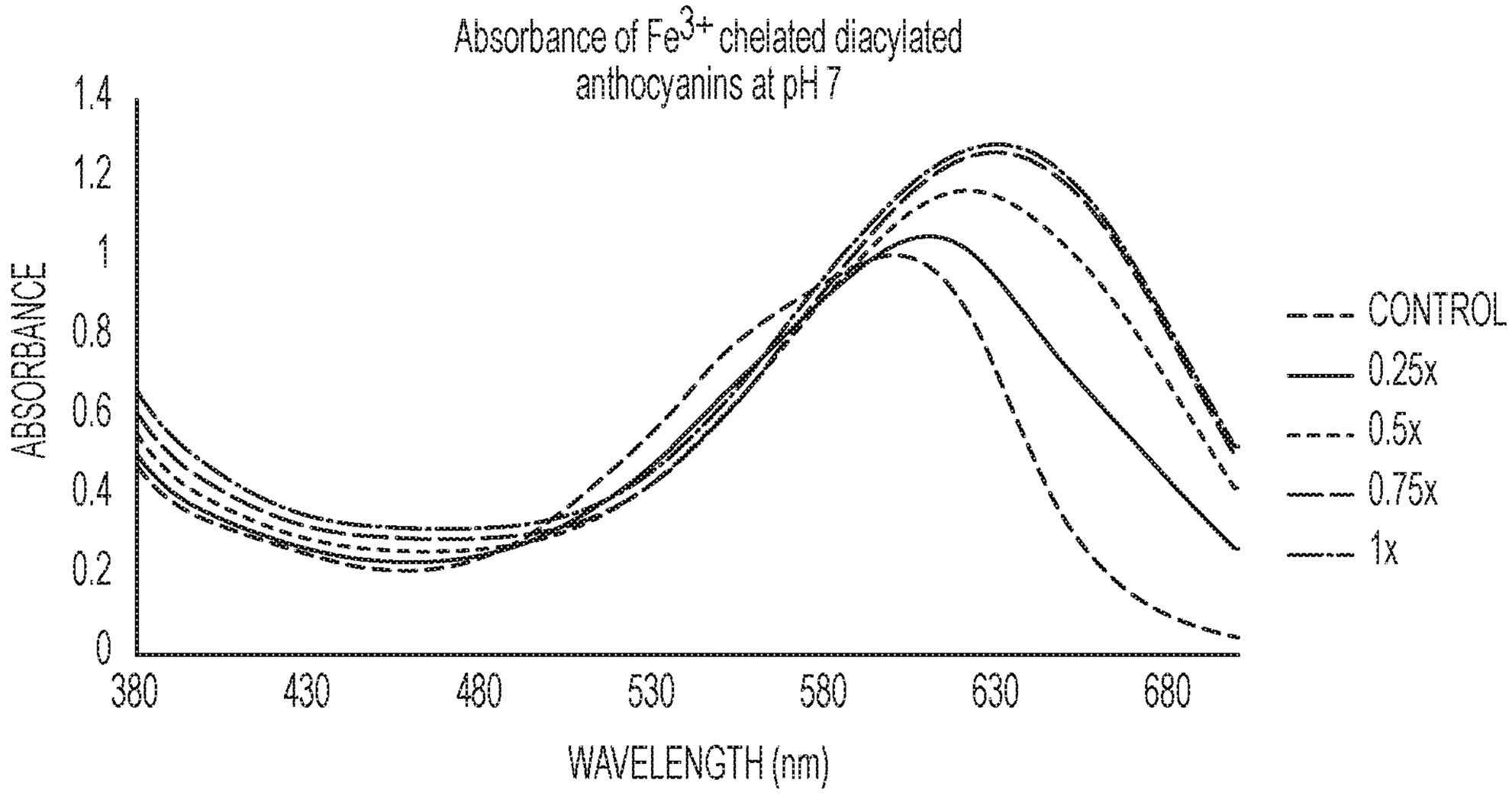

Absorbance of Fe^{3+} chelated diacylated anthocyanins at pH 7
ABSORBANCE
1.4
1.2
1
0.8
0.6
0.4
0.2
0
380
430
480
530
580
630
680
WAVELENGTH (nm)
CONTROL
0.25x
0.5x
0.75x
1x

FIG. 12

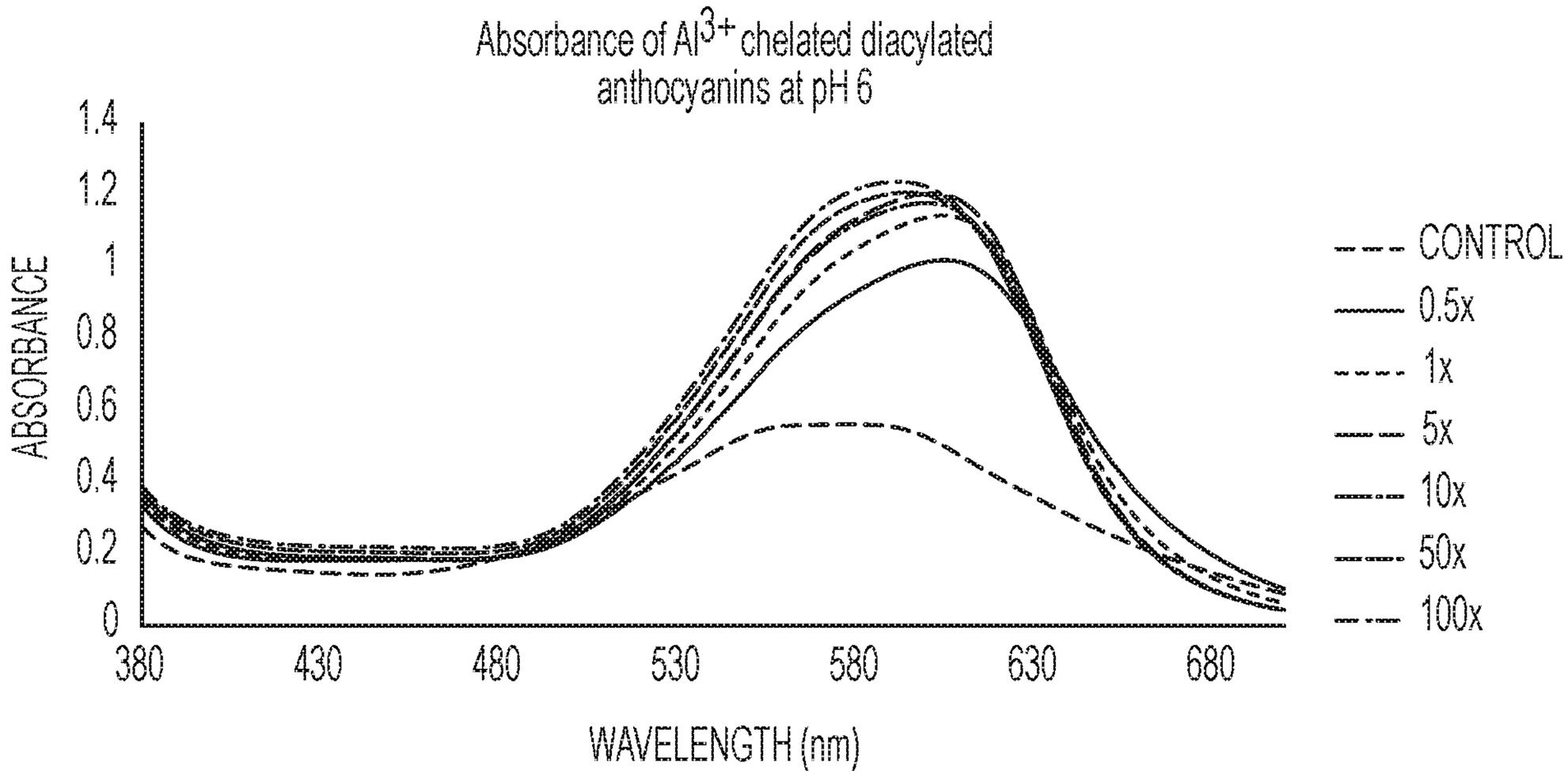

Absorbance of Al^{3+} chelated diacylated anthocyanins at pH 6
ABSORBANCE
1.4
1.2
1
0.8
0.6
0.4
0.2
0
380
430
480
530
580
630
680
WAVELENGTH (nm)
CONTROL
0.5x
1x
5x
10x
50x
100x

FIG. 13

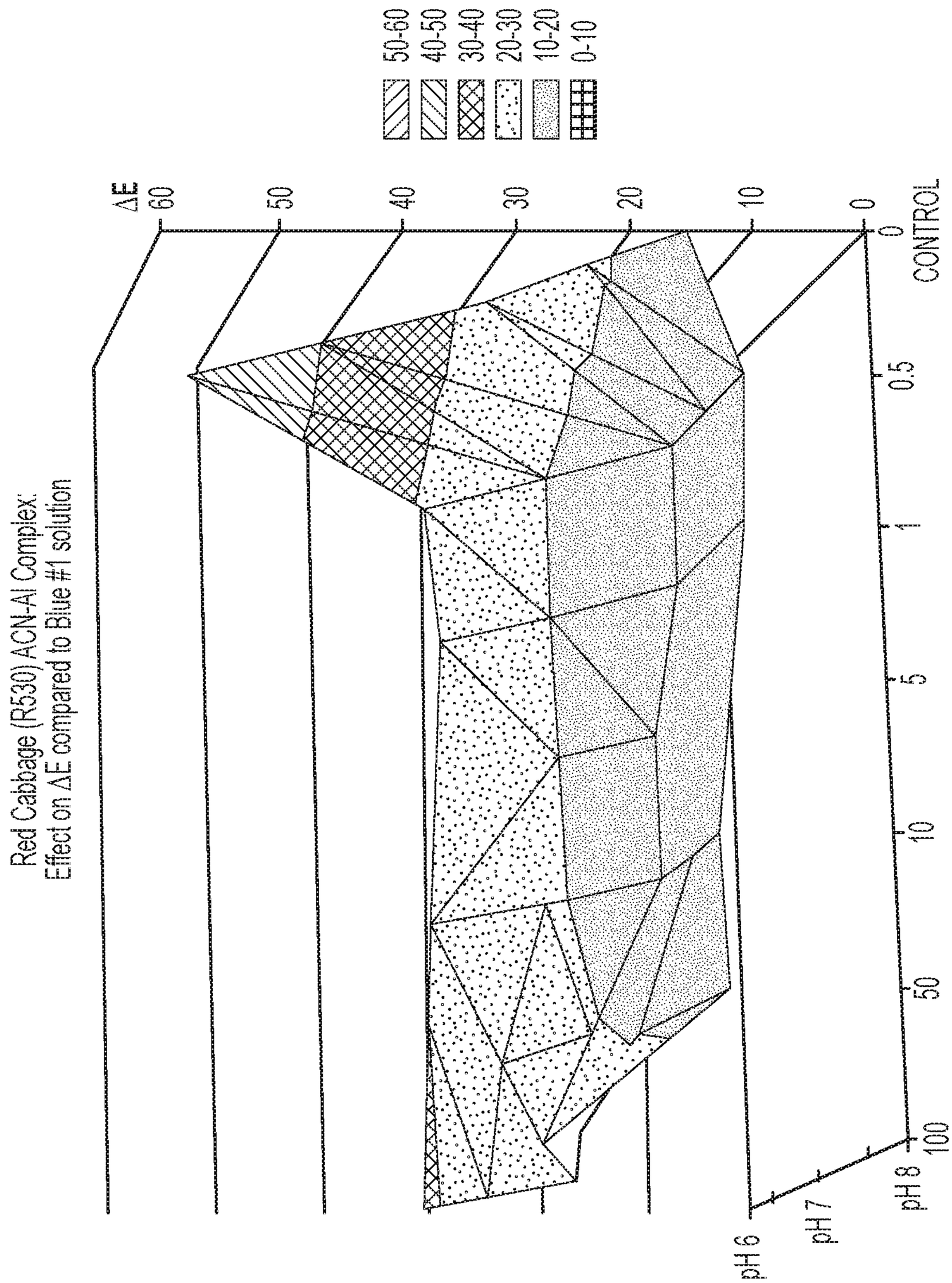

50-60
40-50
30-40
20-30
10-20
0-10
ΔE
60
50
40
30
20
10
0
CONTROL
0
0.5
1
5
10
50
100
pH 7
pH 8
pH 9
Red Cabbage (R530) ACN-Al Complex
Effect on ΔE compared to Blue #1 solution
Concentration of Metal Ions to Concentration of Anthocyanins

FIG. 14A

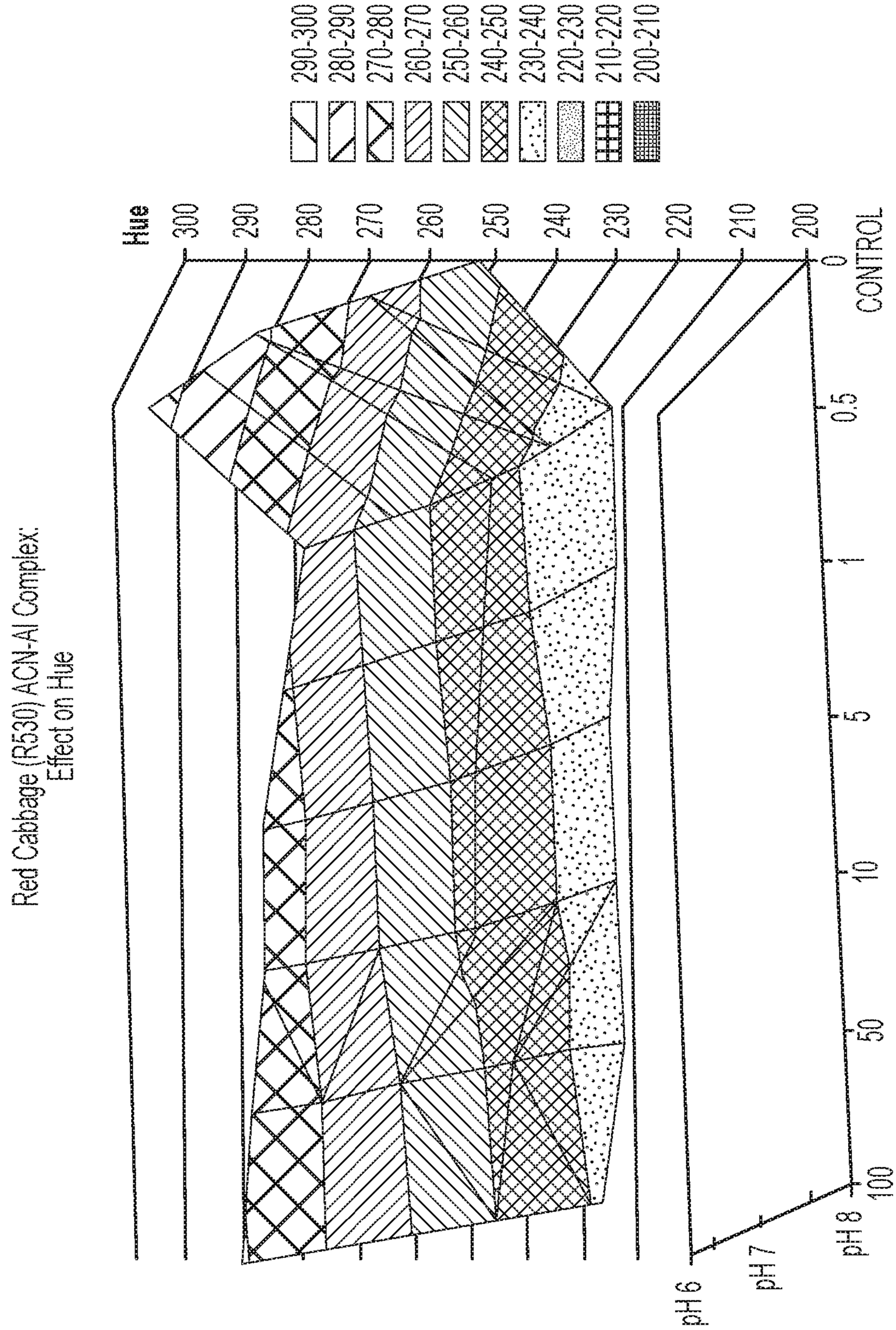

290-300
280-290
270-280
260-270
250-260
240-250
230-240
220-230
210-220
200-210
Hue
300
290
280
270
260
250
240
230
220
210
200
0
CONTROL
0.5
1
5
10
50
100
pH 8
pH 7
pH 6
Red Cabbage (R530) ACN-Al Complex:
Effect on Hue
Concentration of Metal Ions to Concentration of Anthocyanins

FIG. 14B

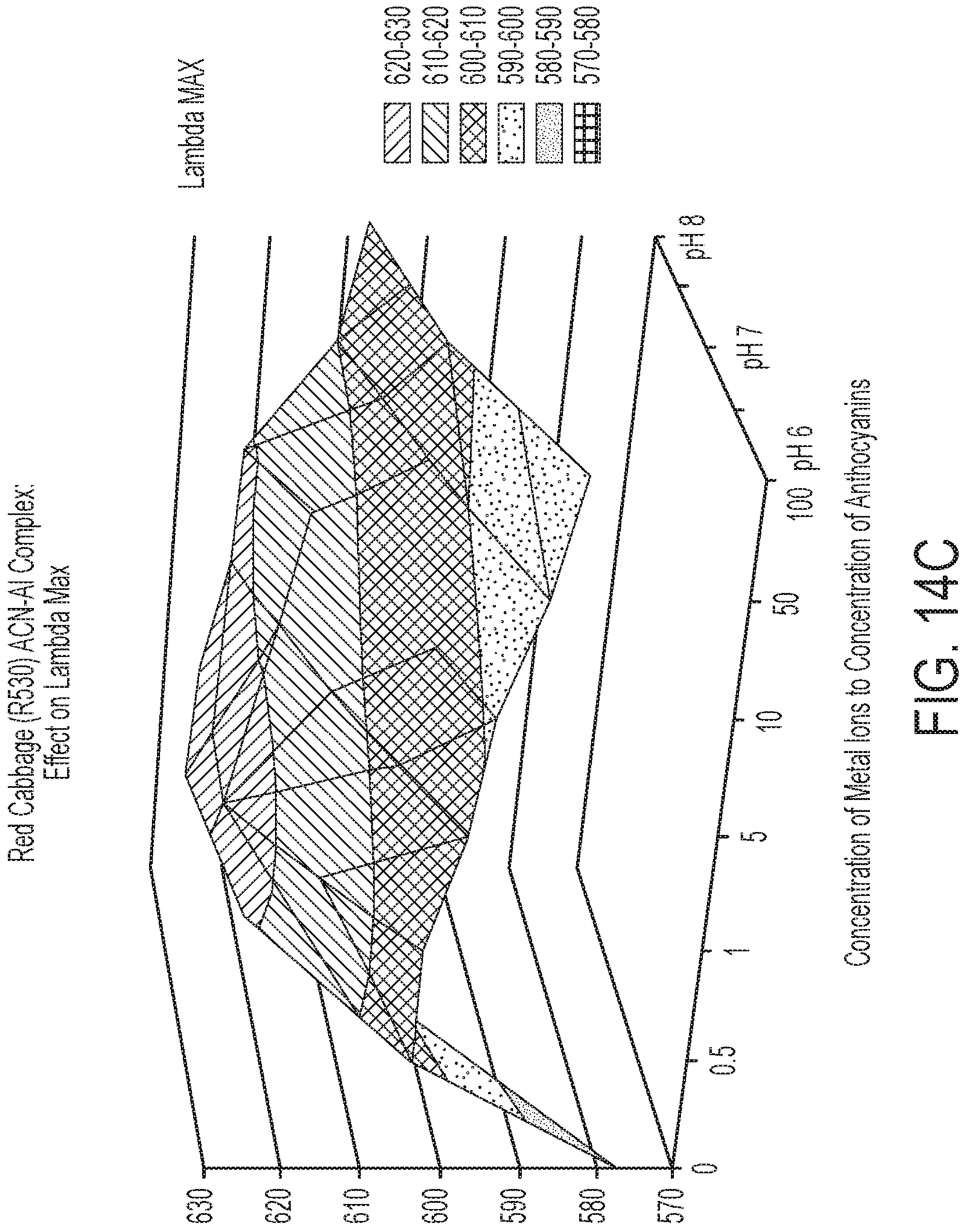

Red Cabbage (R530) ACN-Al Complex:
Effect on Lambda Max
Lambda Max
630-620
620-610
610-600
600-590
590-580
580-570
630
620
610
600
590
580
570
pH 8
pH 7
pH 6
0
0.5
1
5
10
50
100
Concentration of Metal Ions to Concentration of Anthocyanins

FIG. 14C

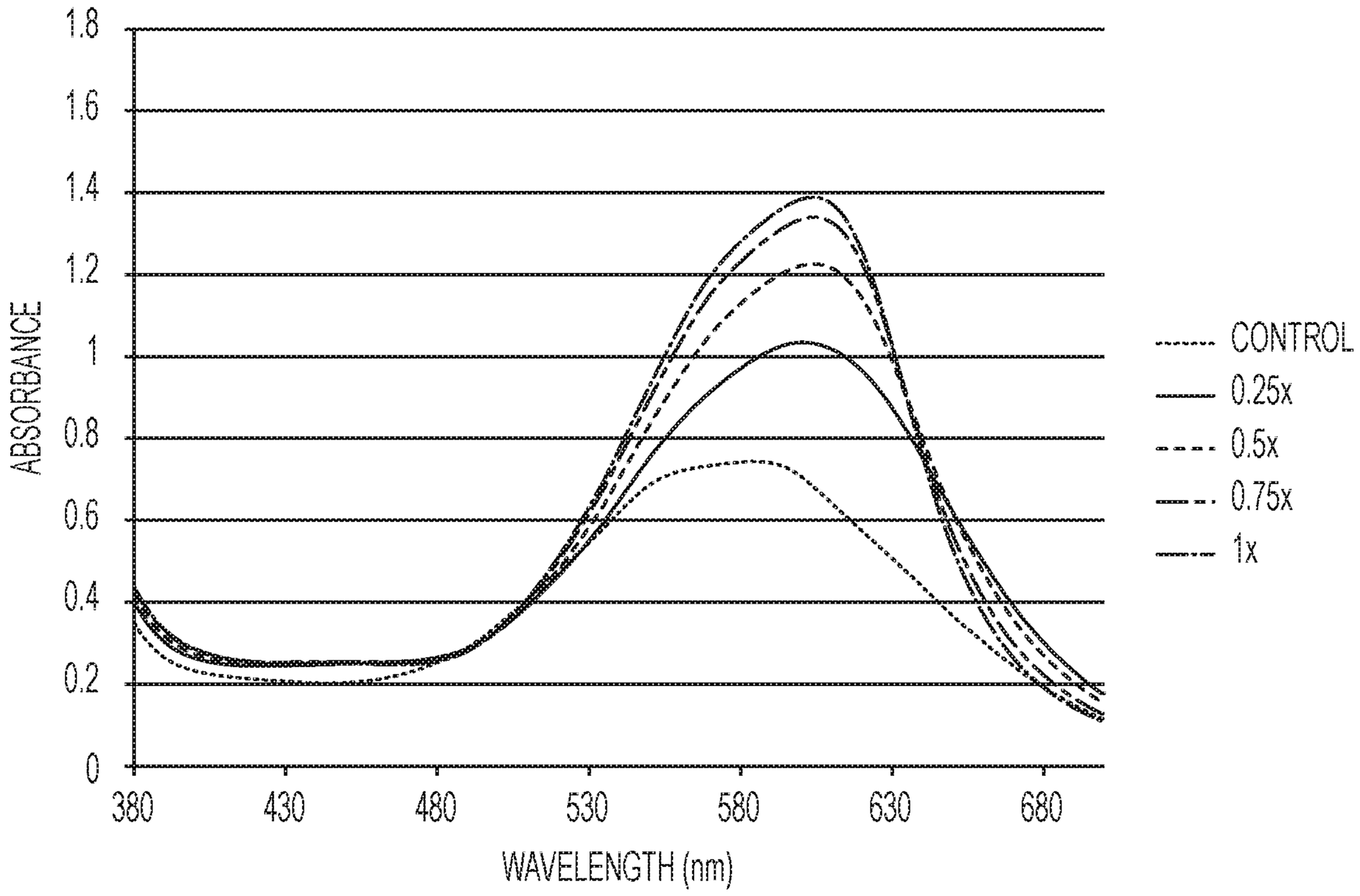


FIG. 15A

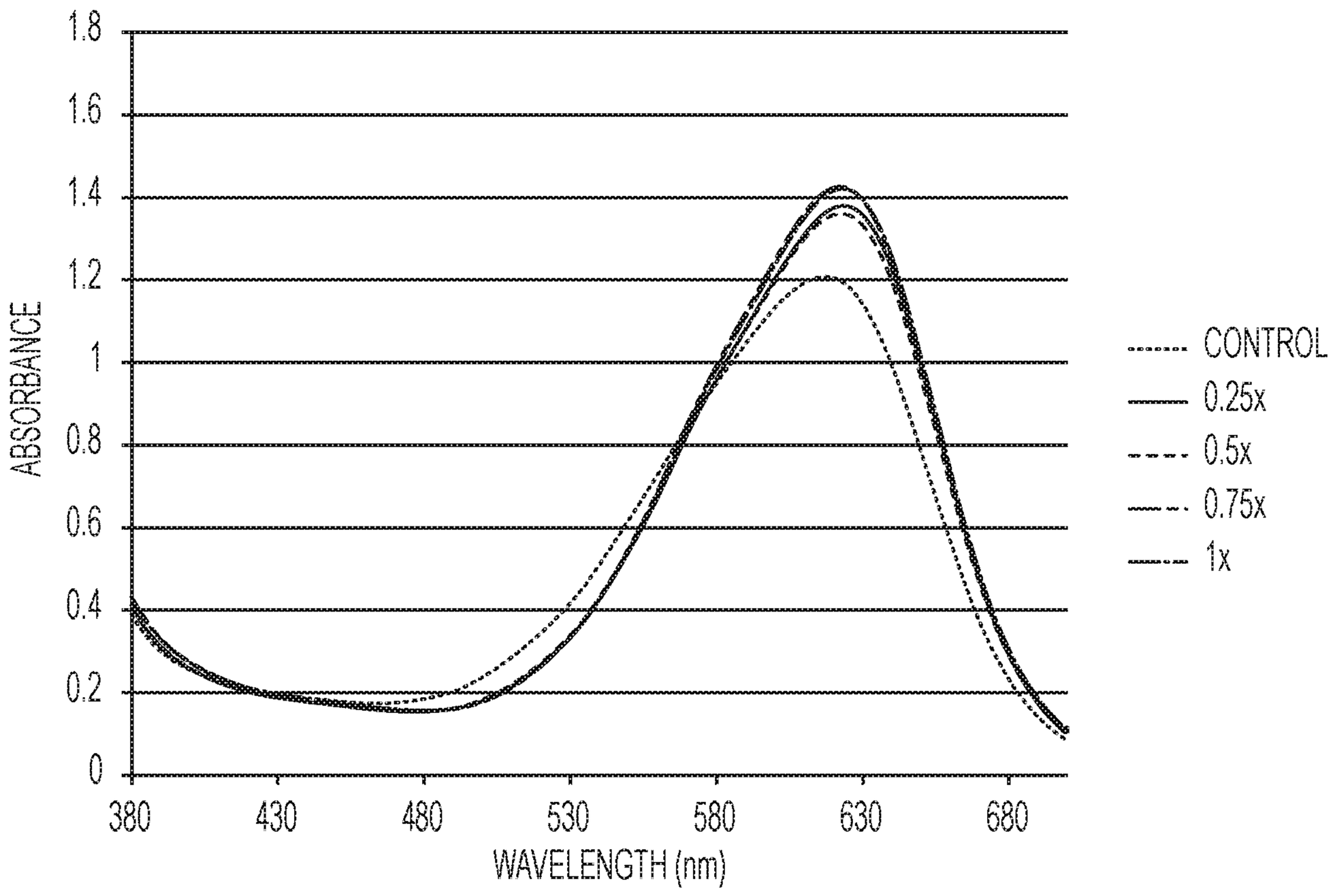


FIG. 15B

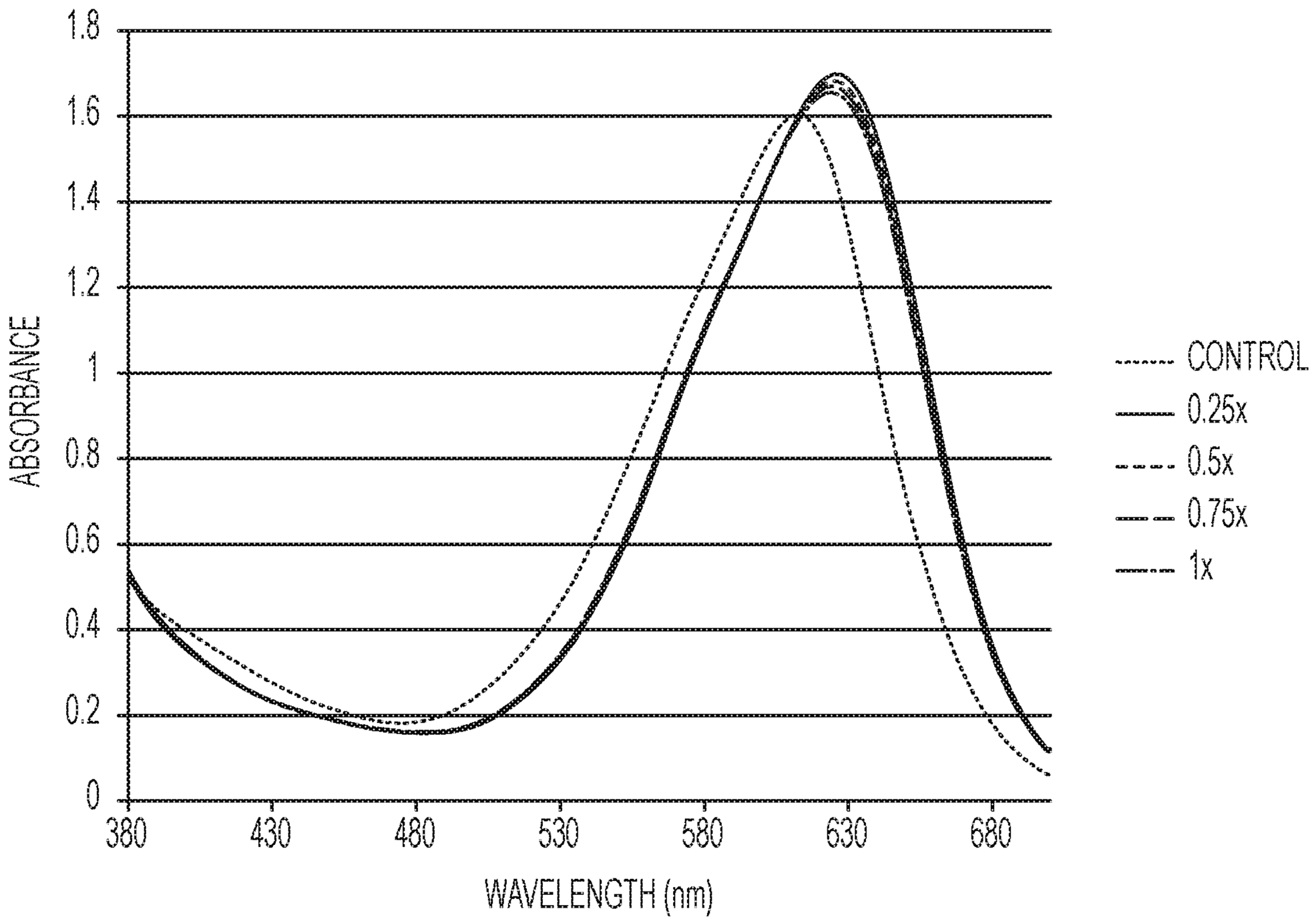

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ABSORBANCE
380
430
480
530
580
630
680
WAVELENGTH (nm)
CONTROL
0.25x
0.5x
0.75x
1x

FIG. 15C

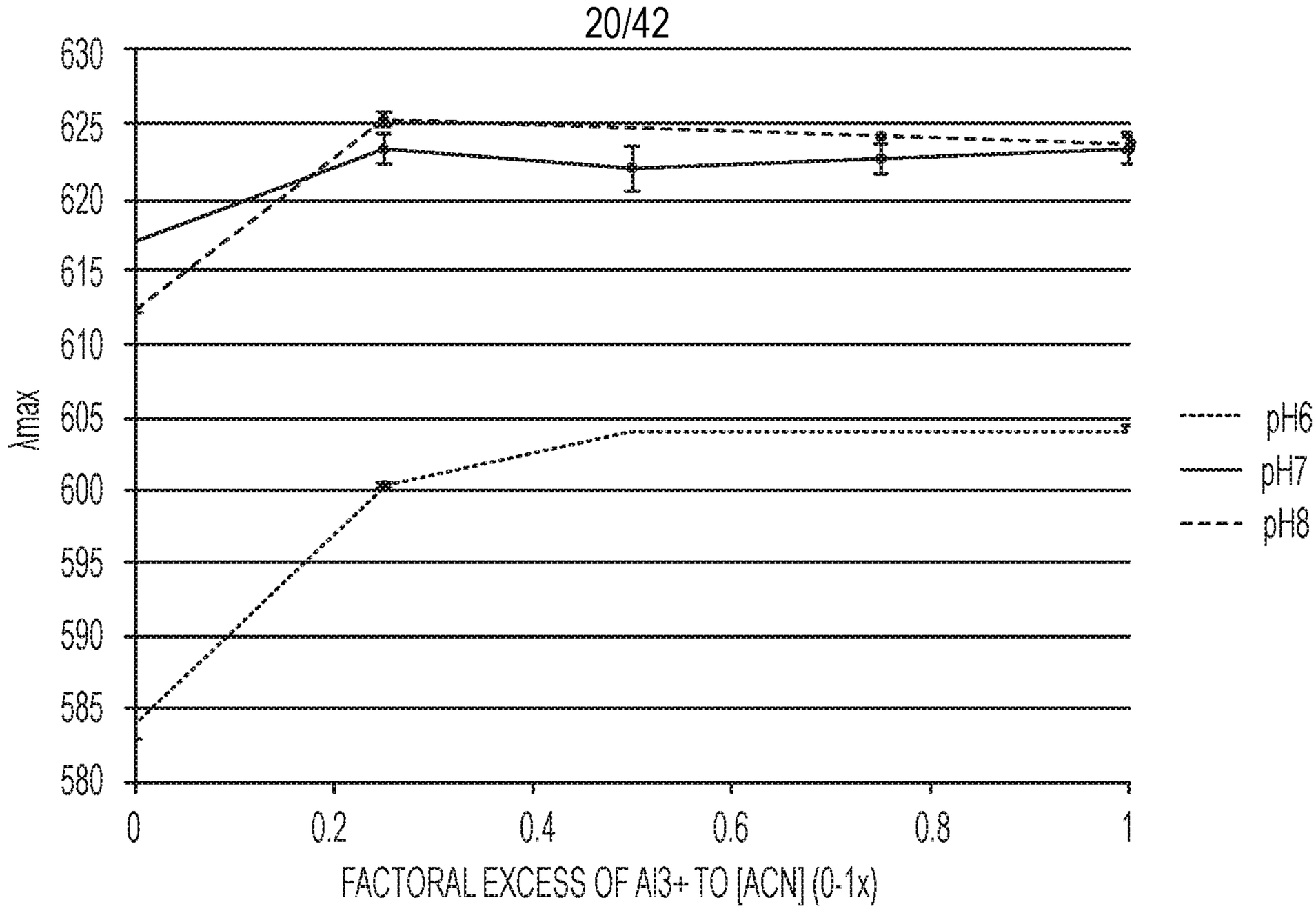


FIG. 16A

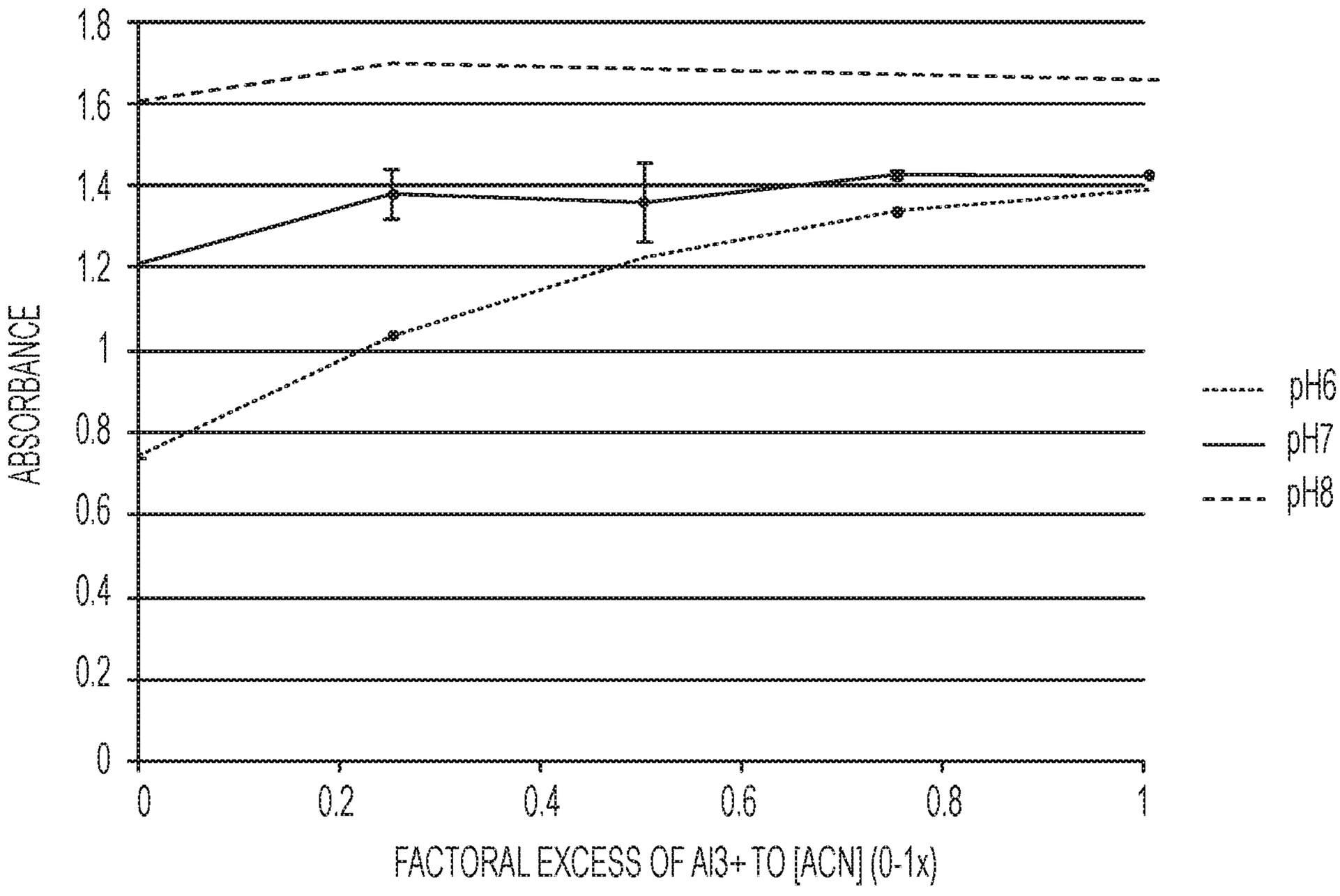


FIG. 16B

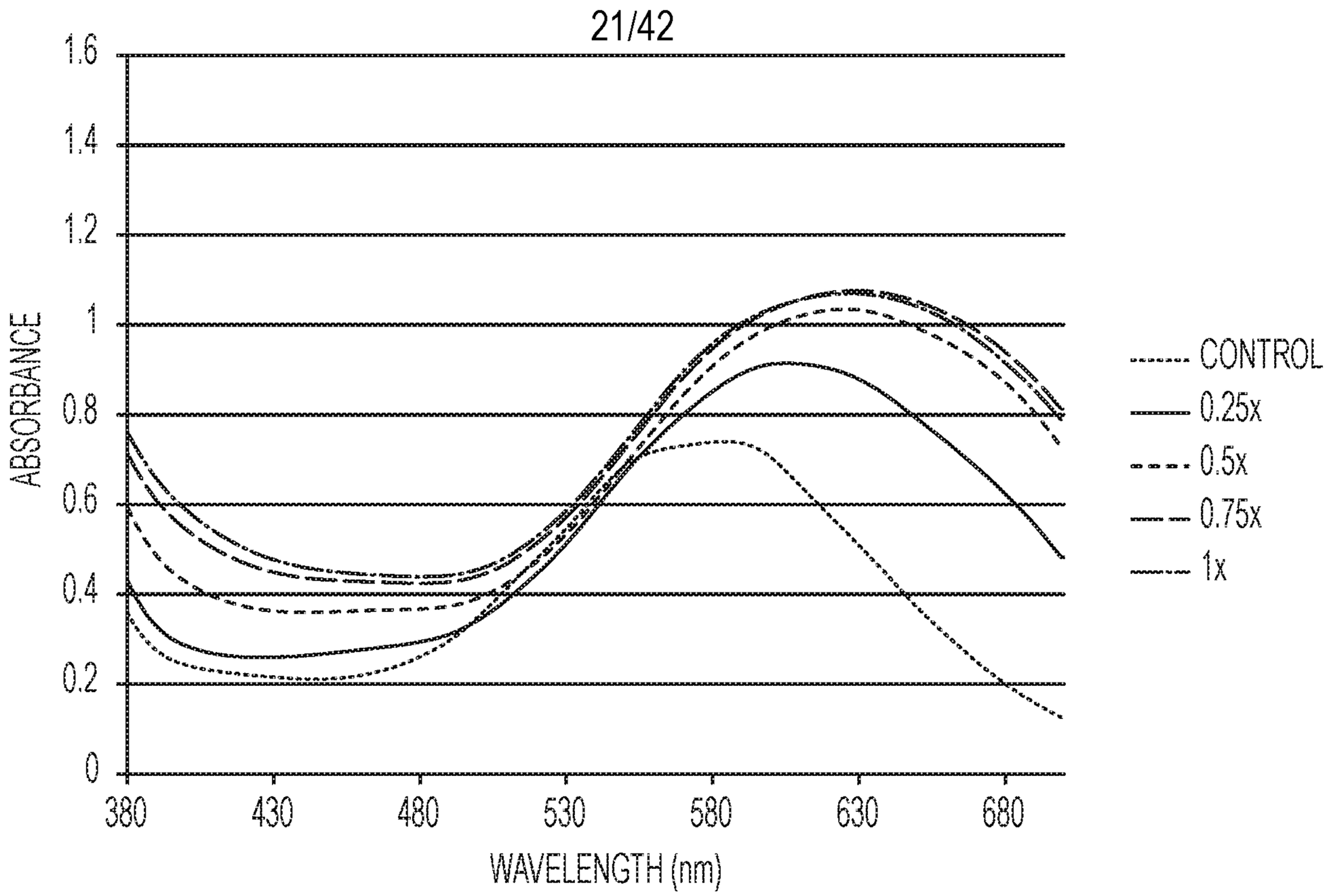


FIG. 17A

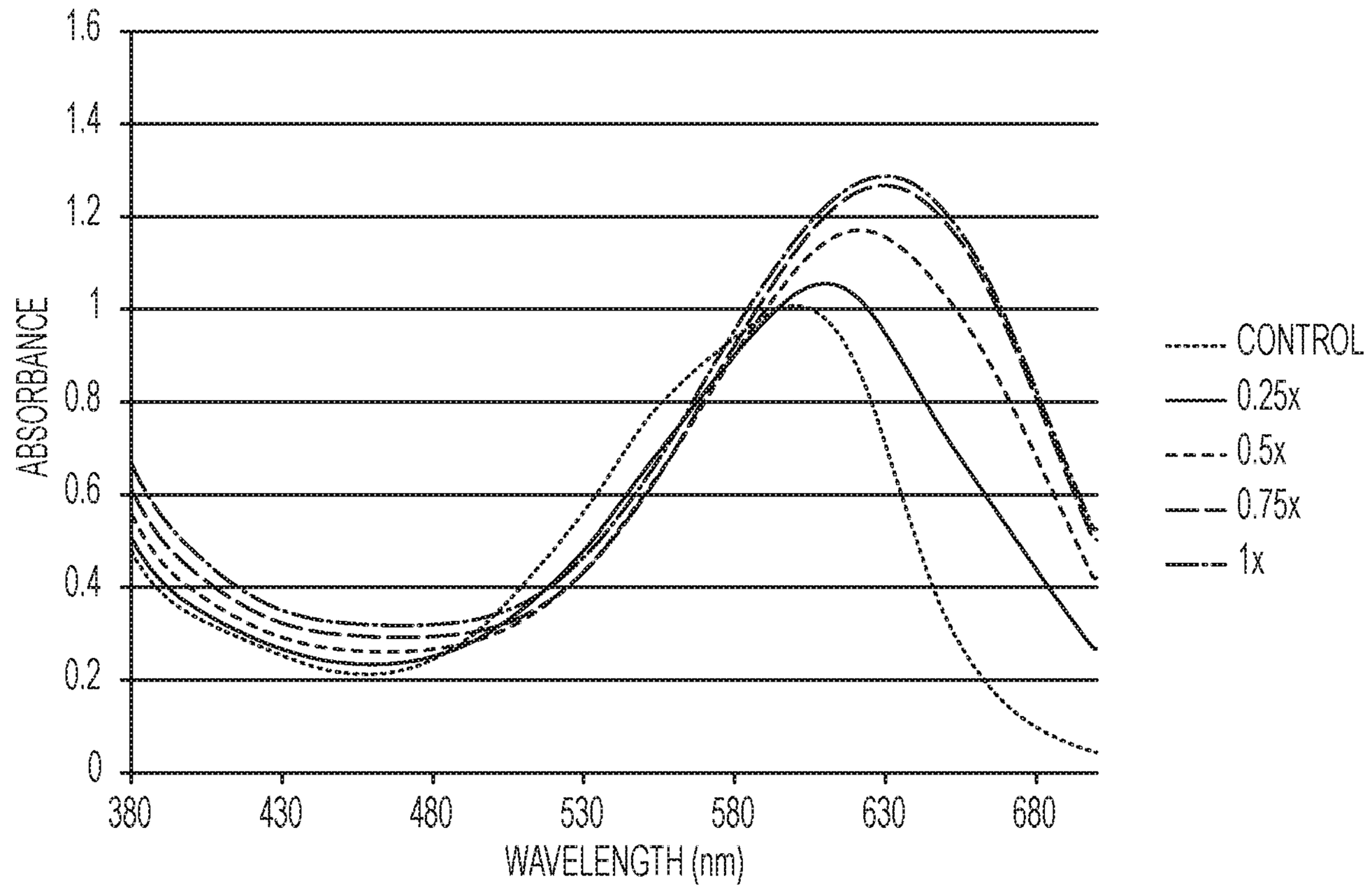


FIG. 17B

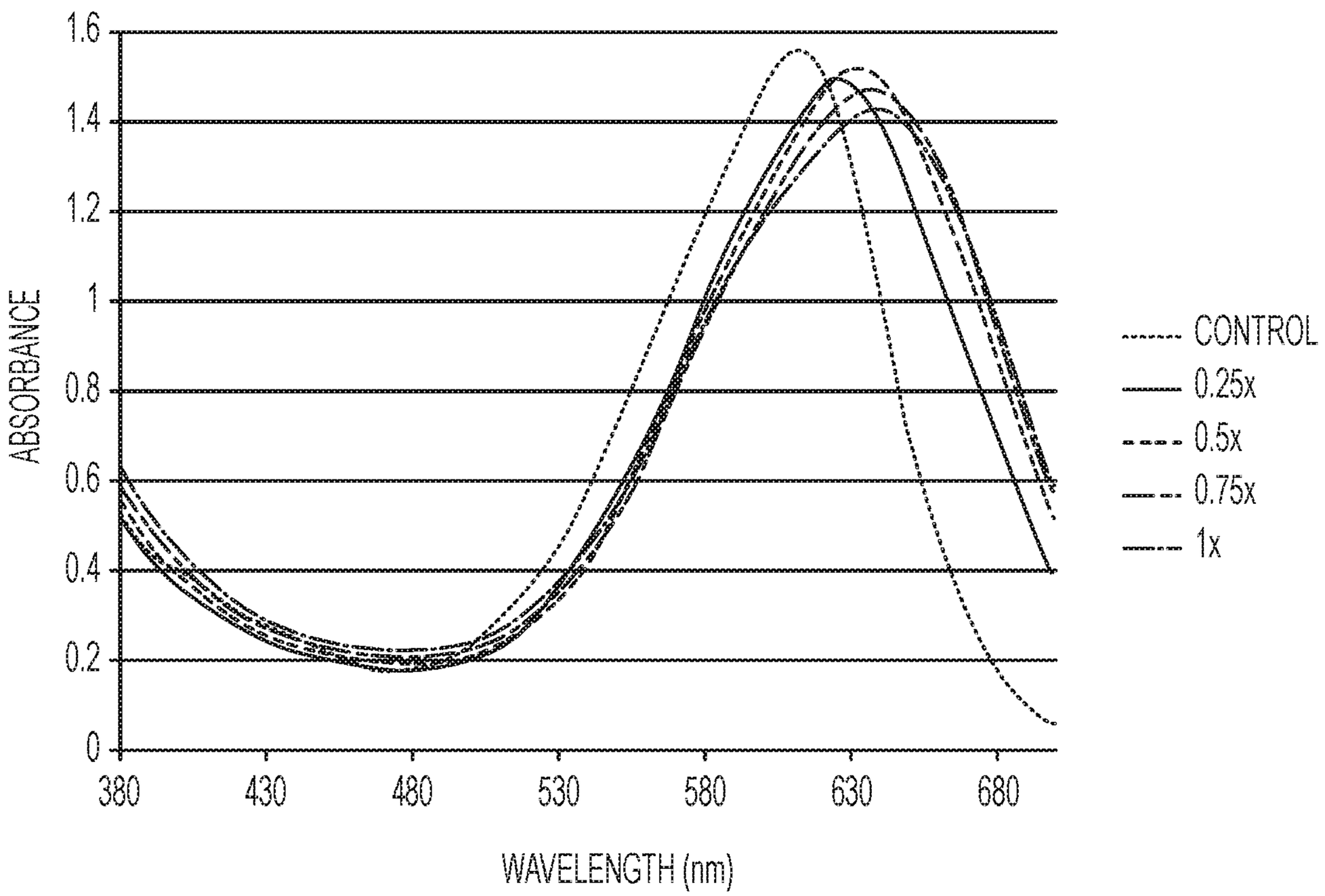

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ABSORBANCE
380
430
480
530
580
630
680
WAVELENGTH (nm)
CONTROL
0.25x
0.5x
0.75x
1x

FIG. 17C

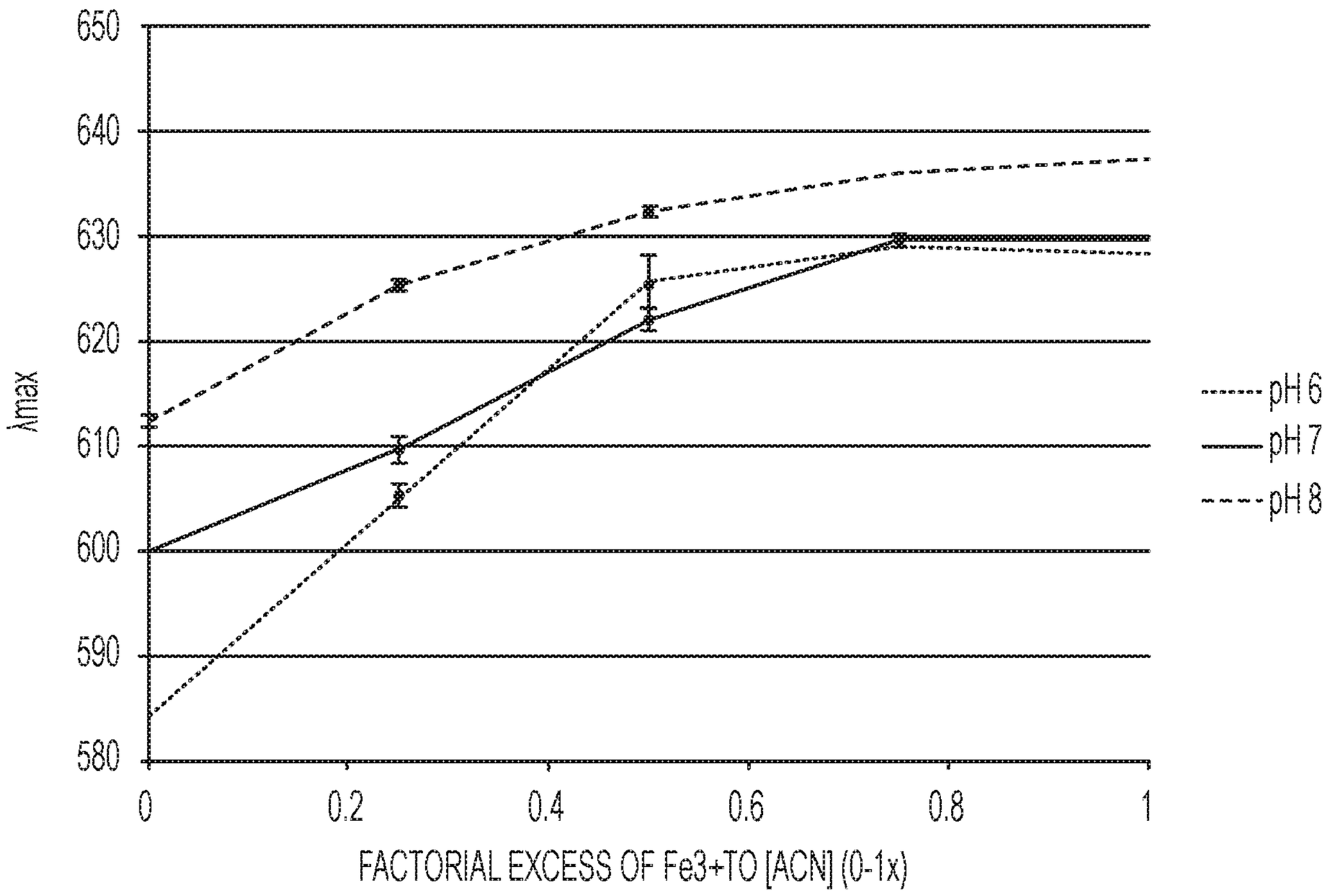


FIG. 18A

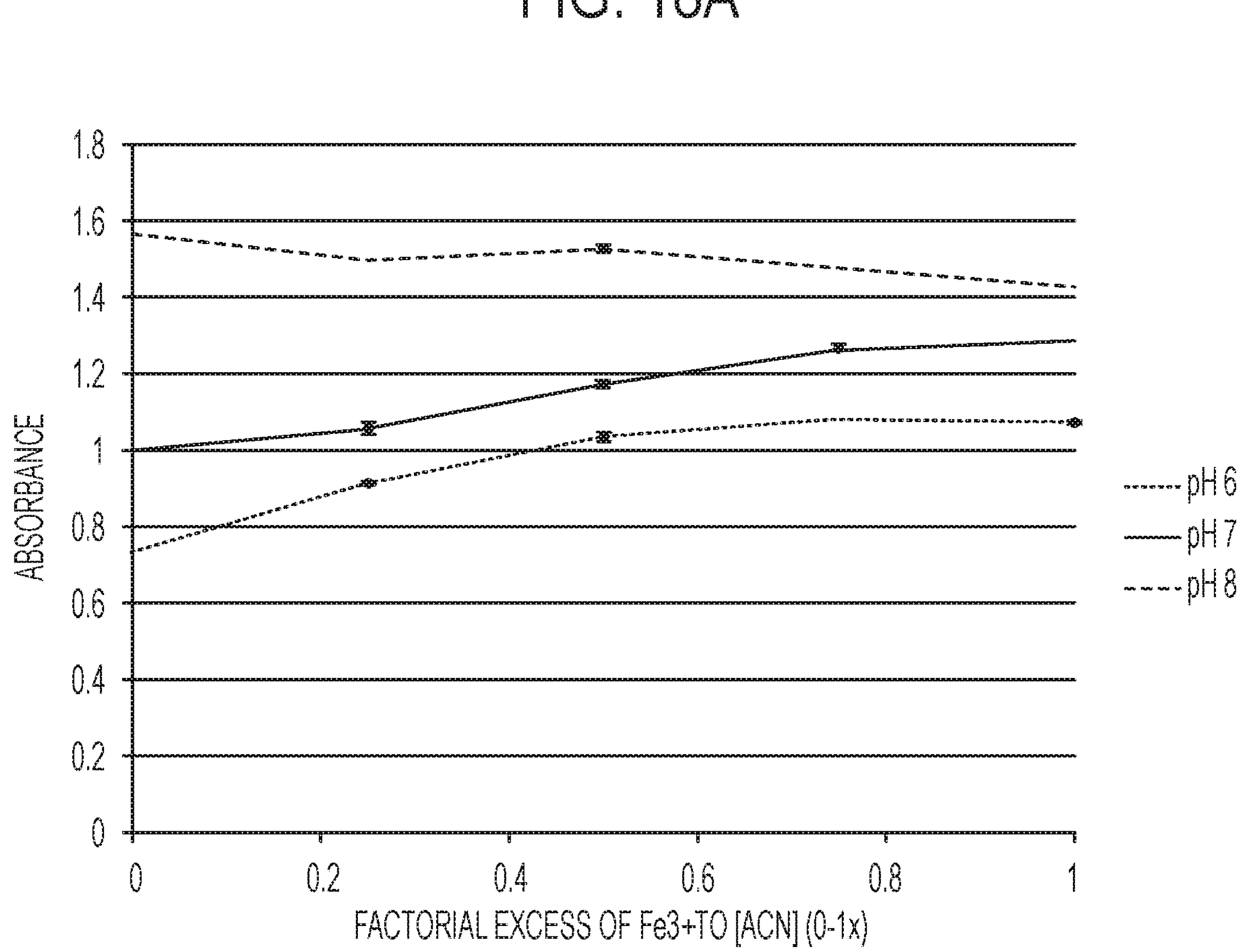


FIG. 18B

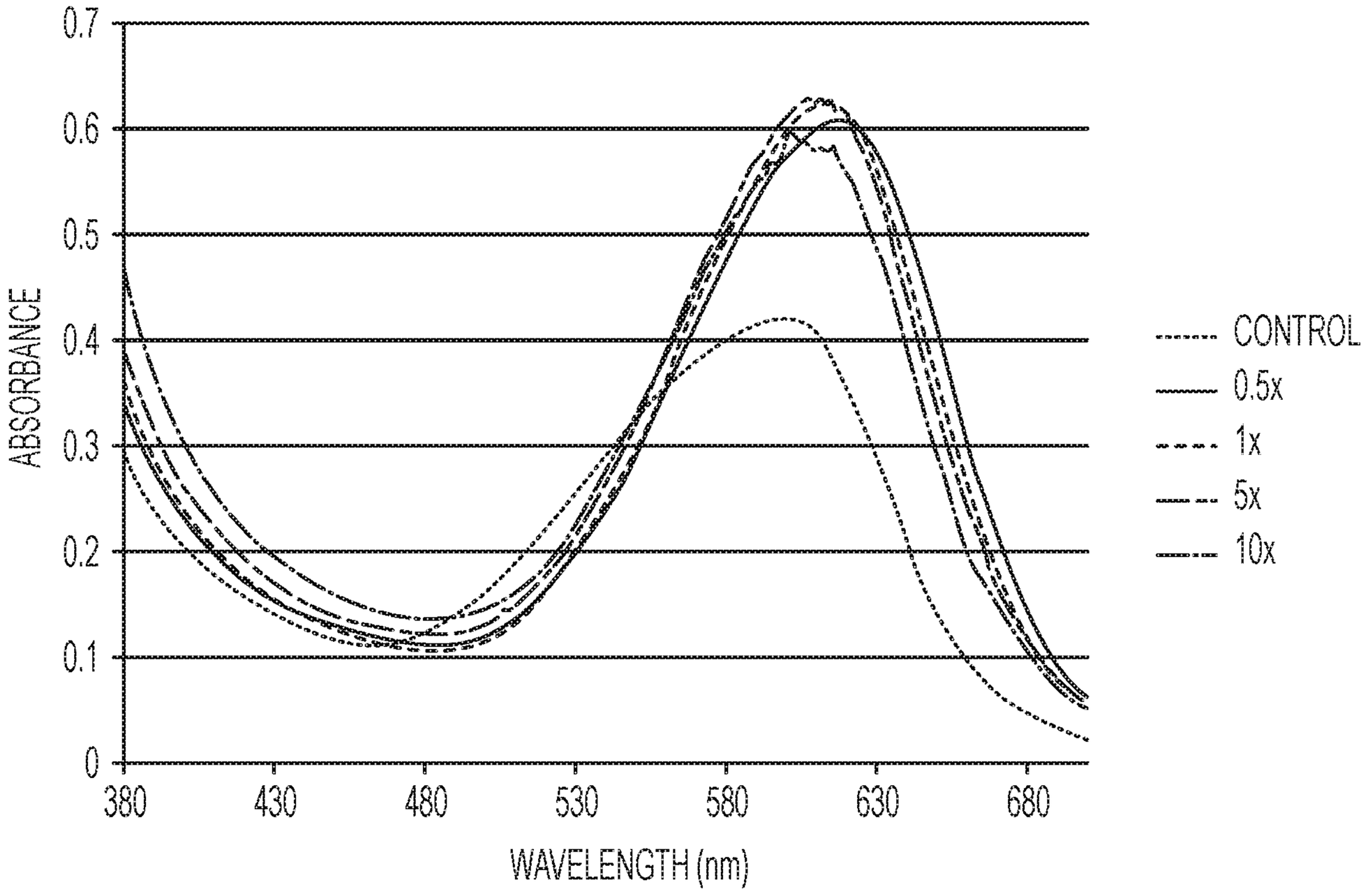


FIG. 19A

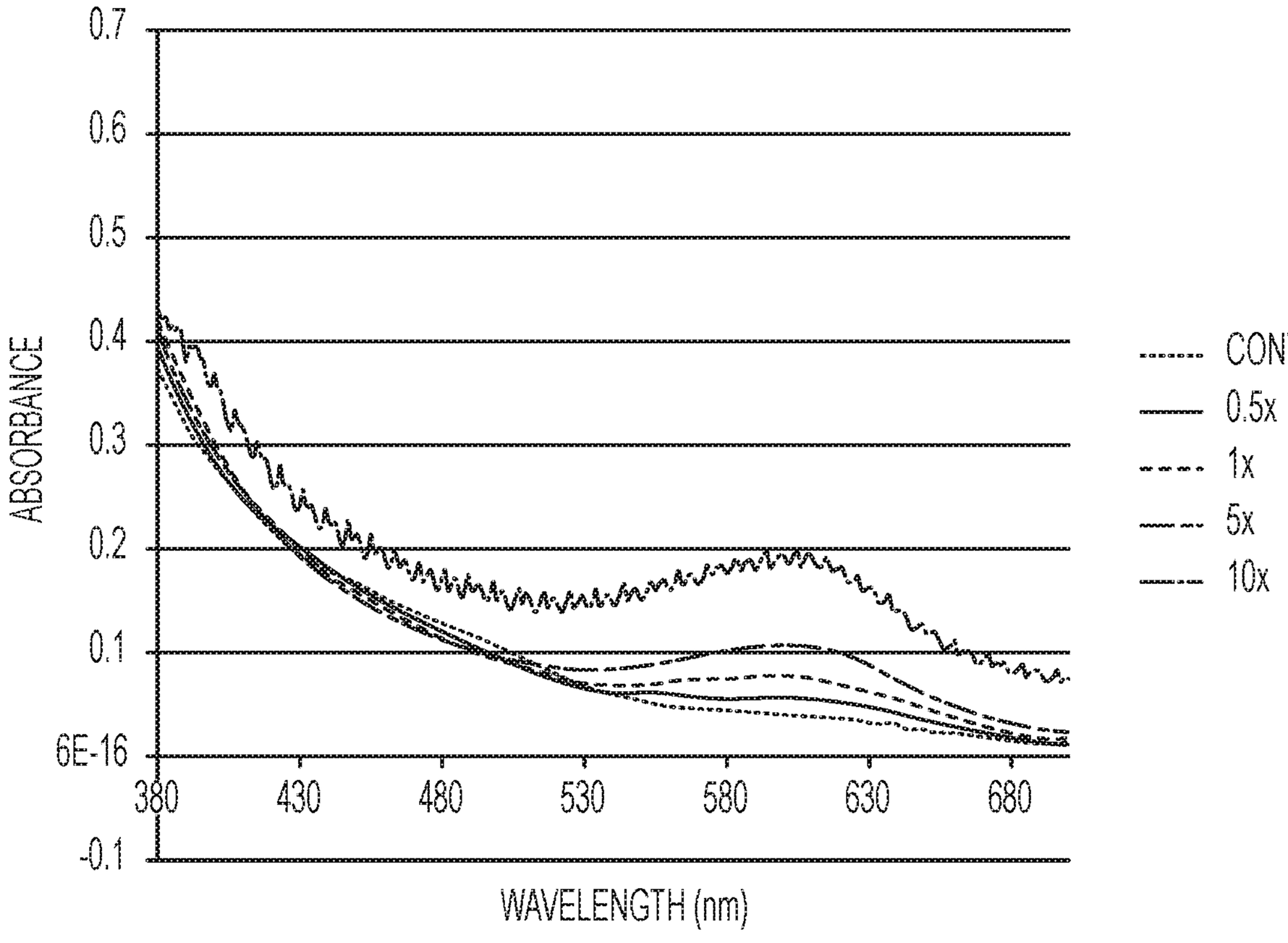


FIG. 19B

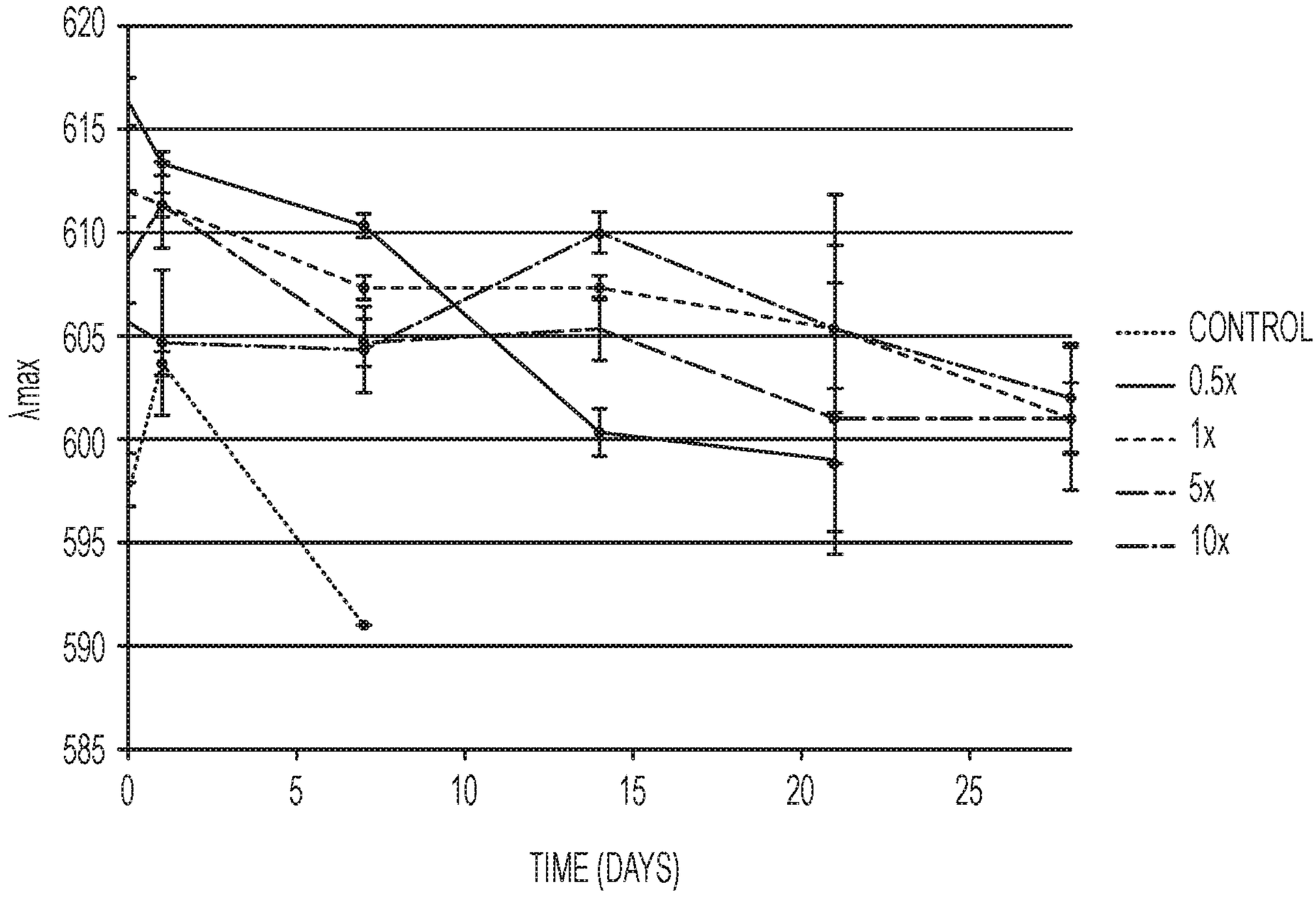


FIG. 20A

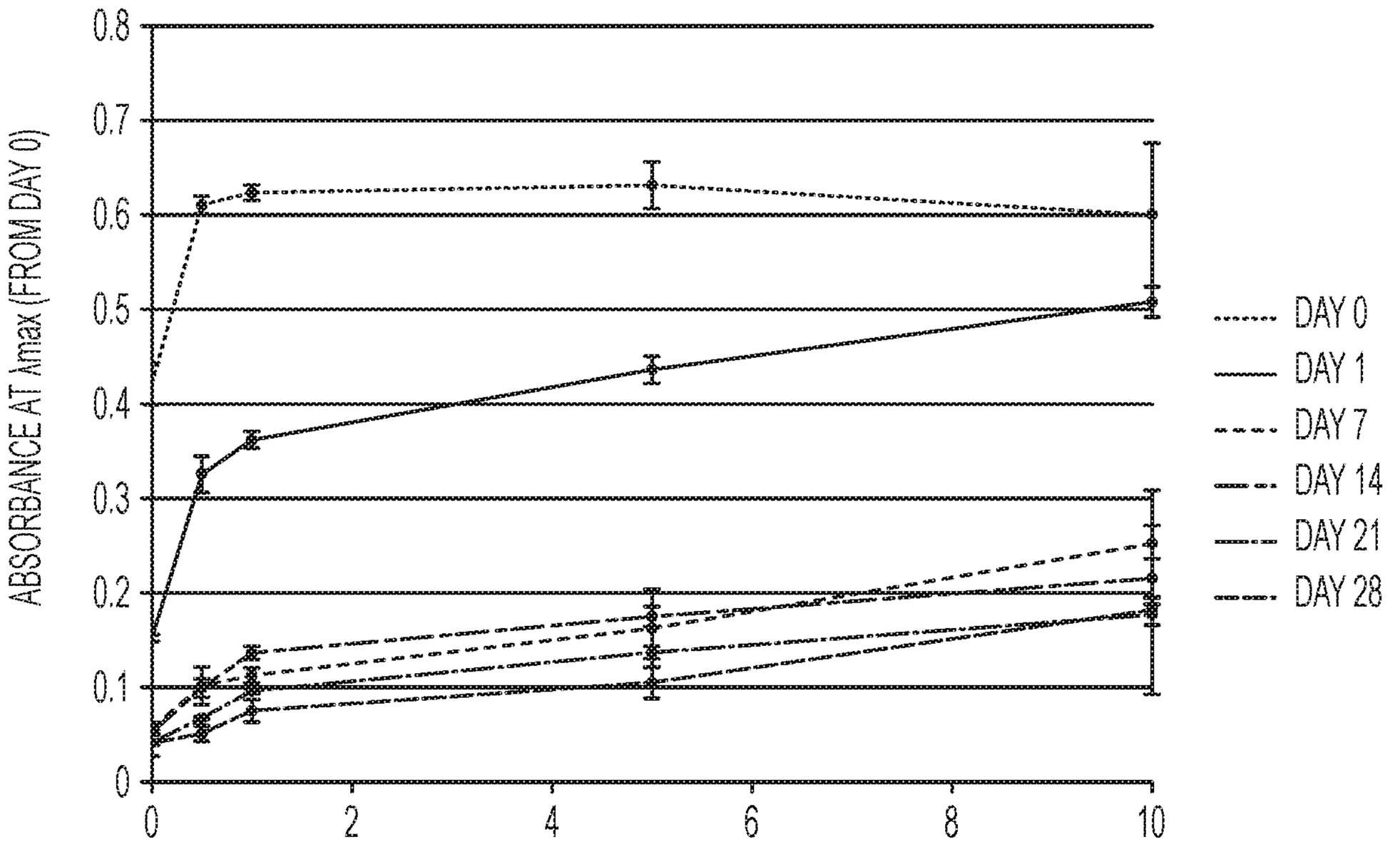


FIG. 20B

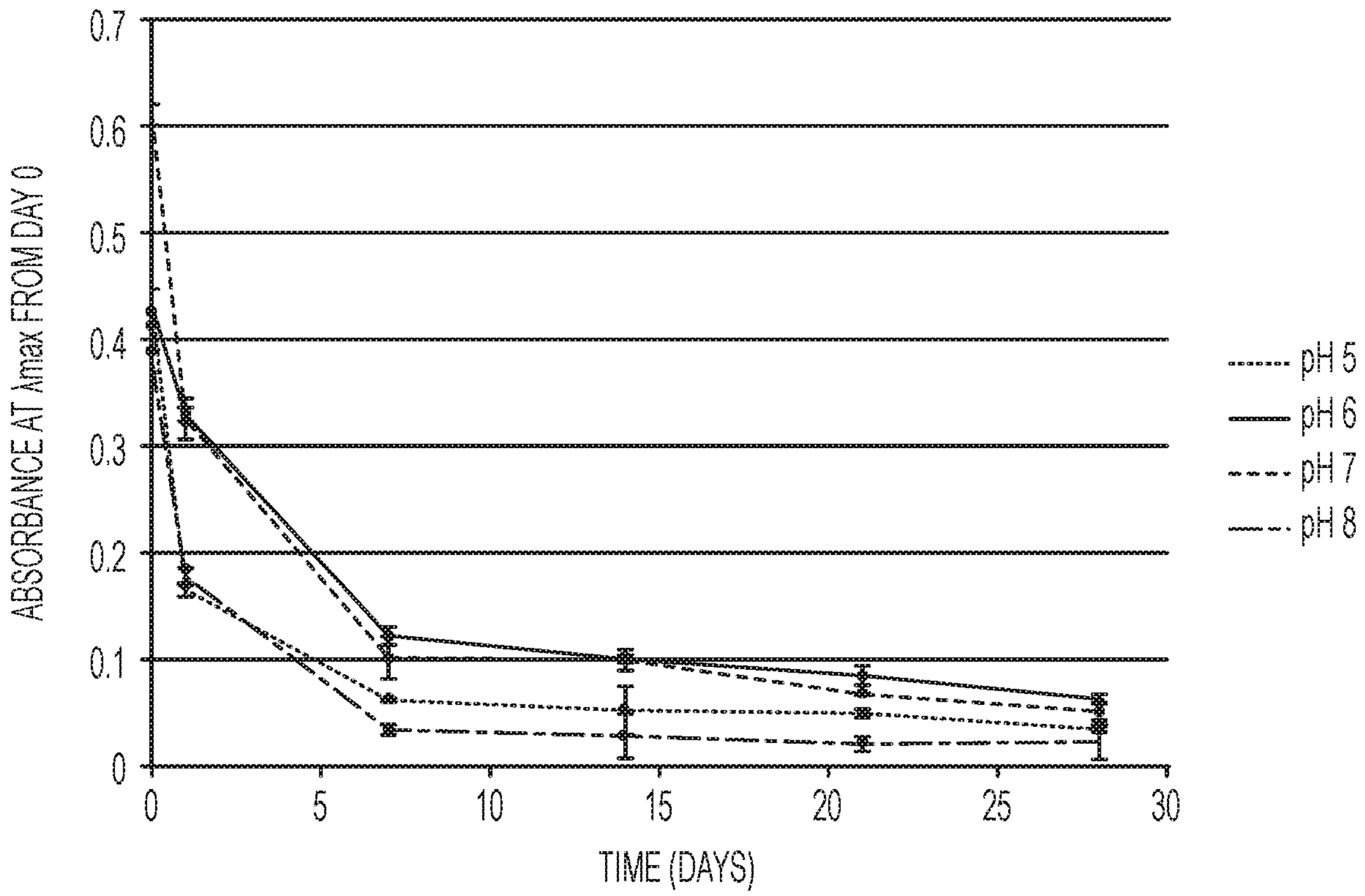


FIG. 21A

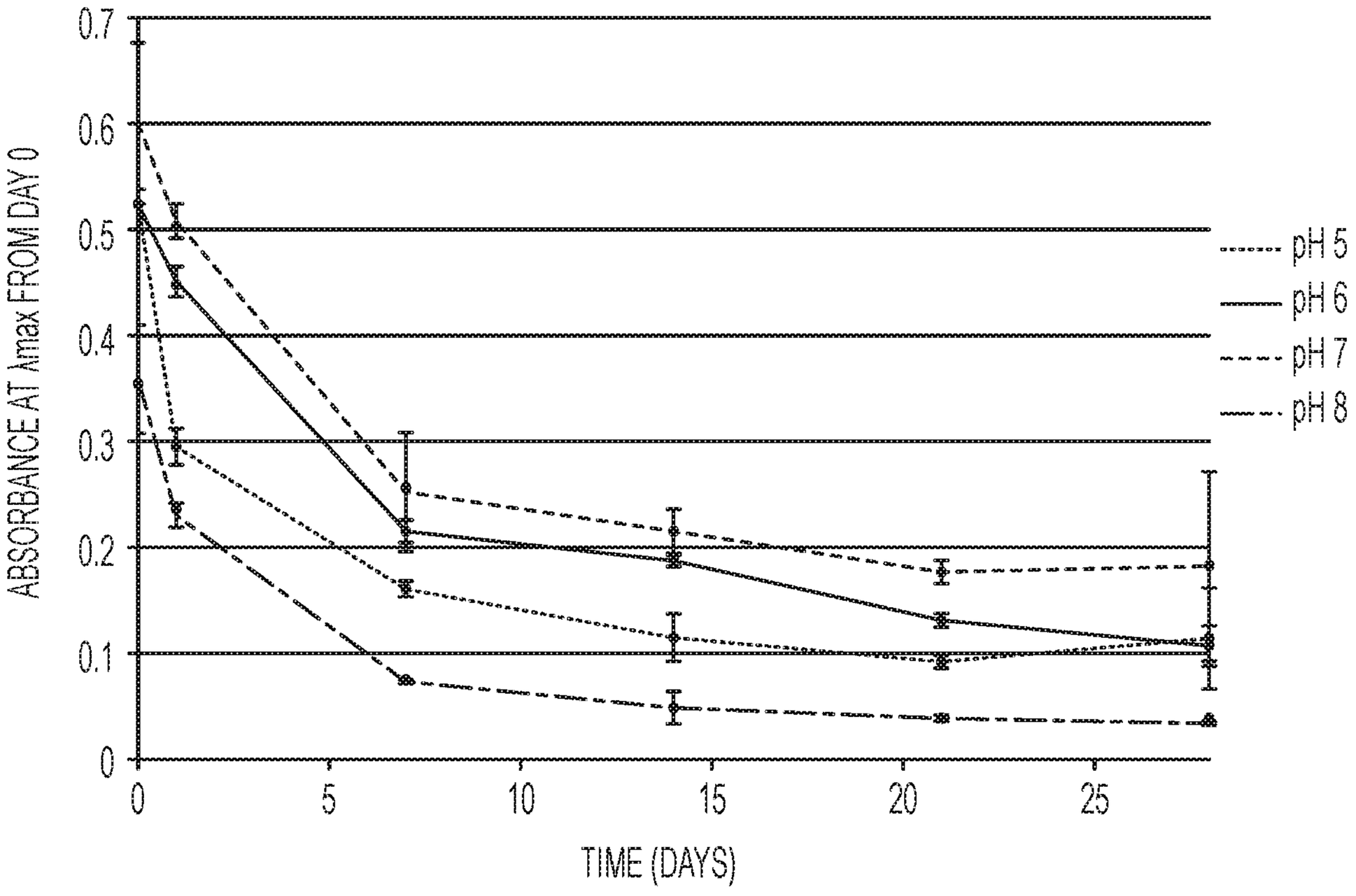


FIG. 21B

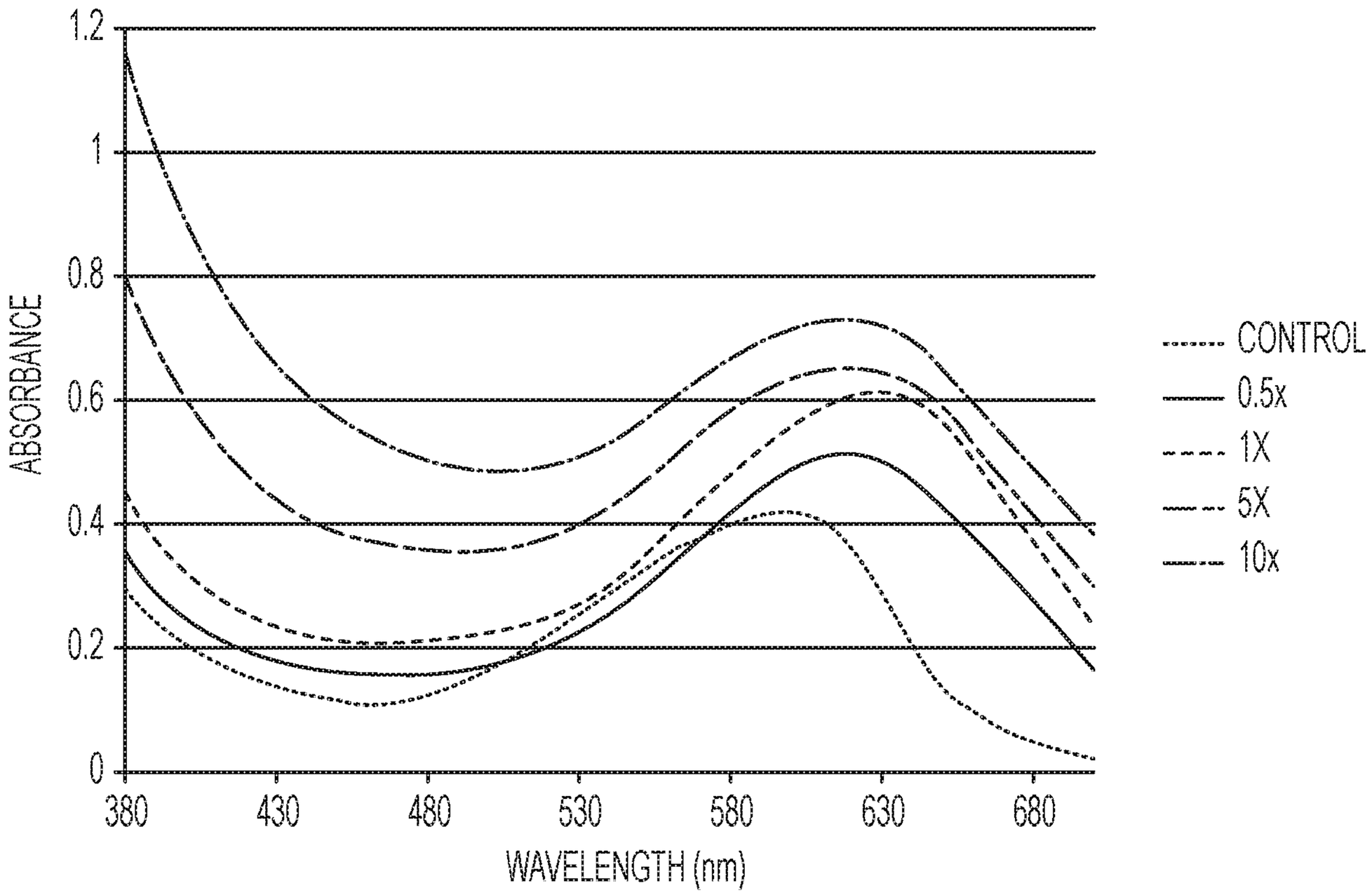


FIG. 22A

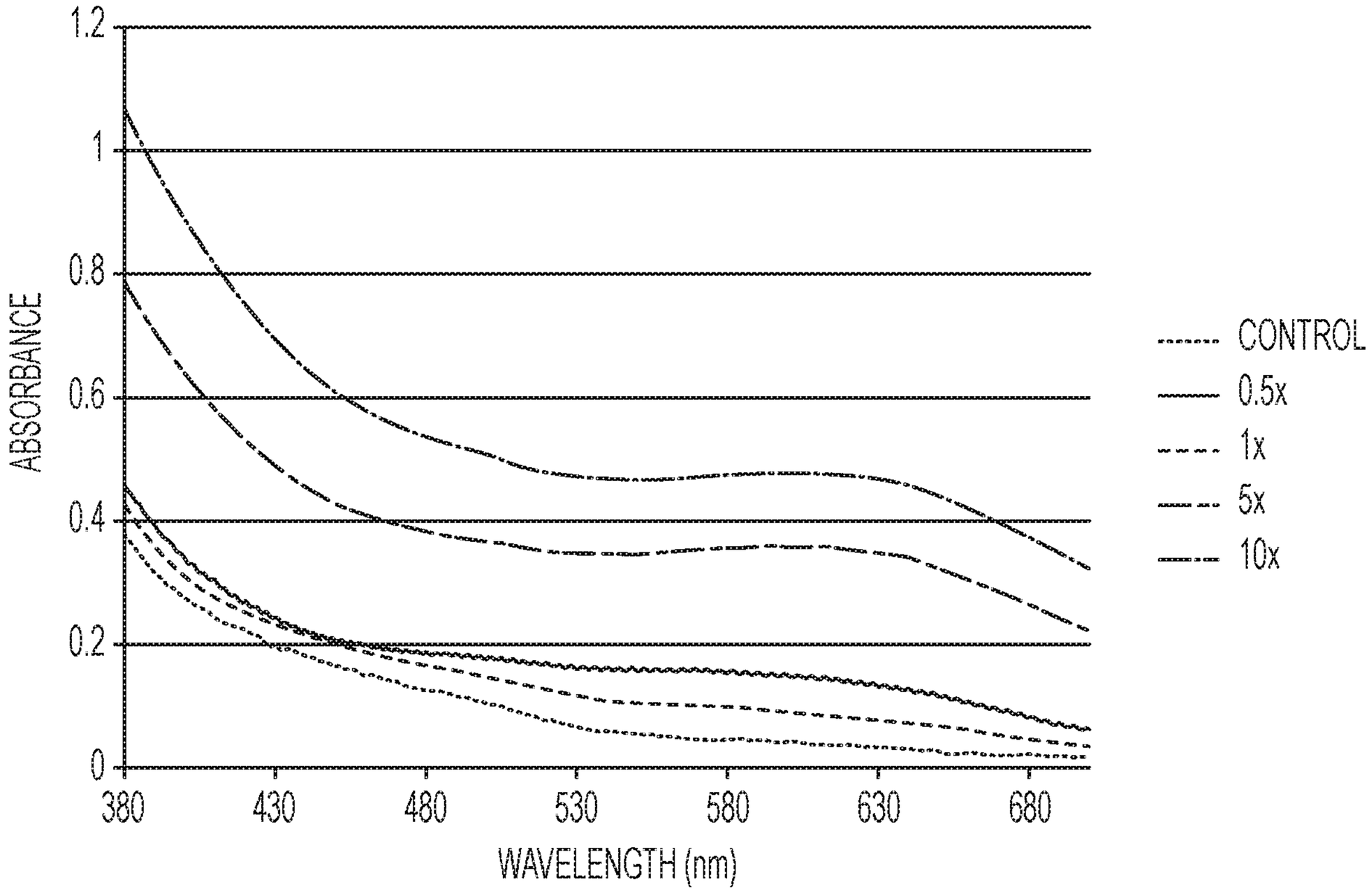


FIG. 22B

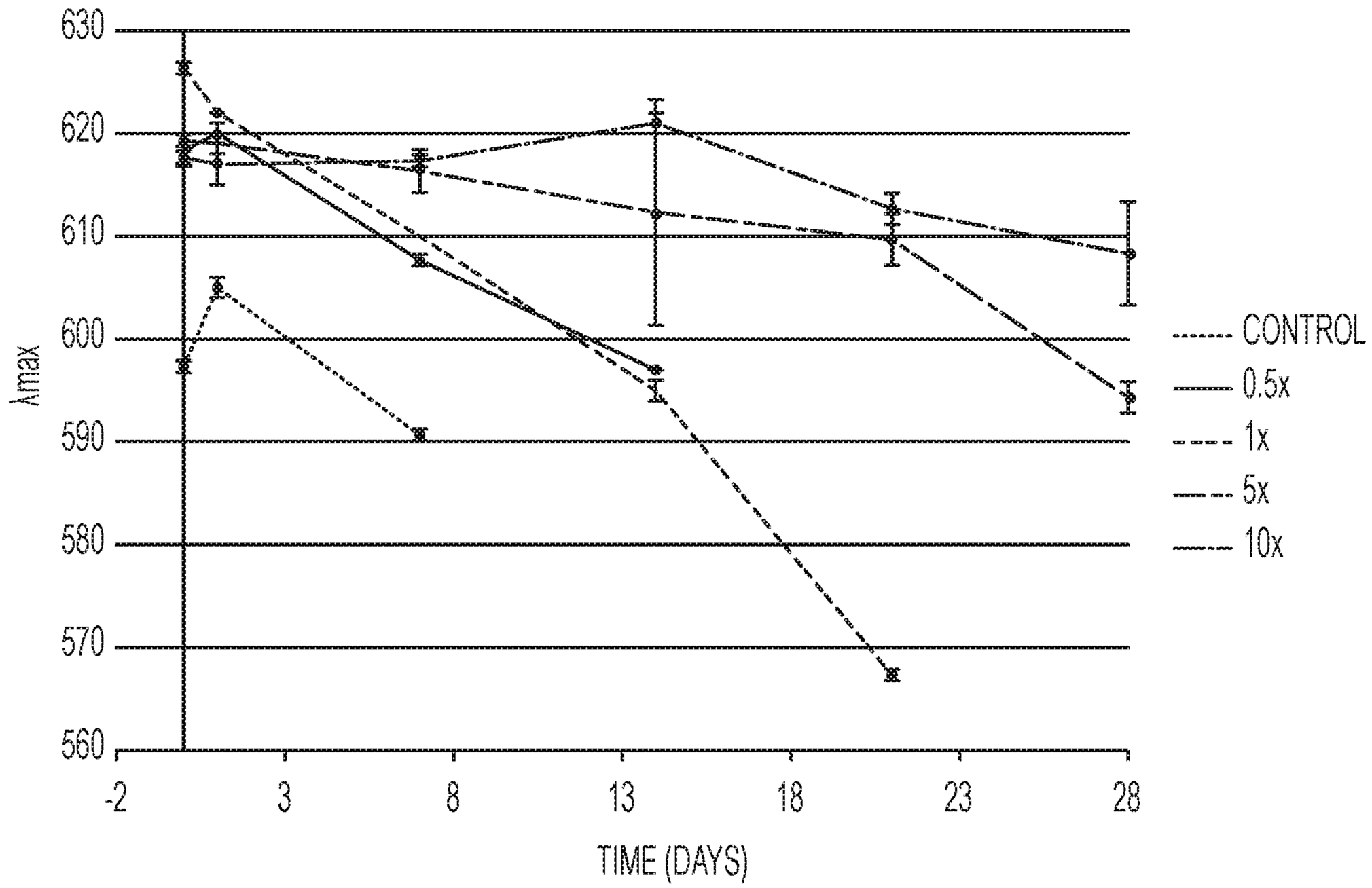


FIG. 23A

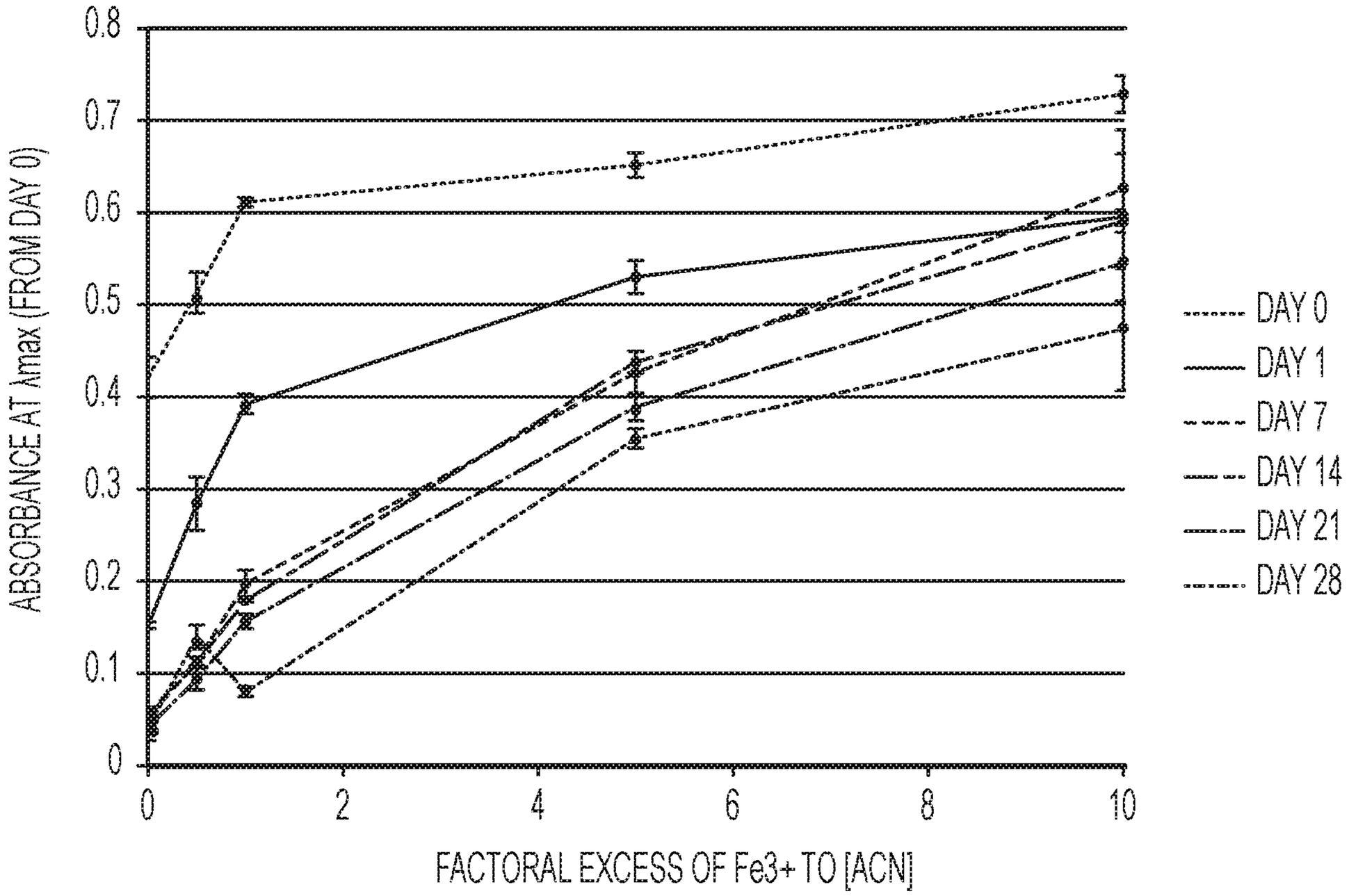


FIG. 23B

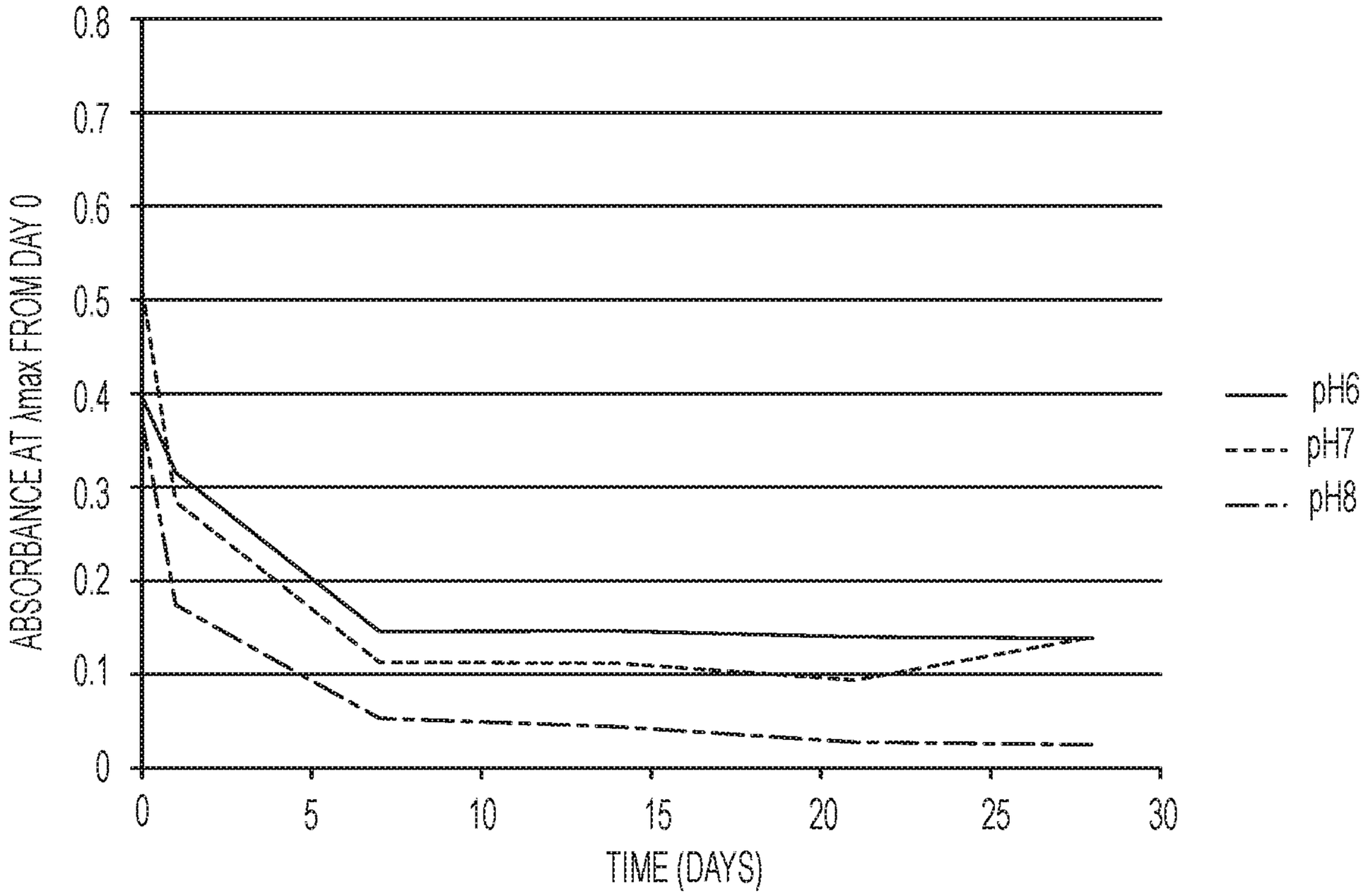


FIG. 24A

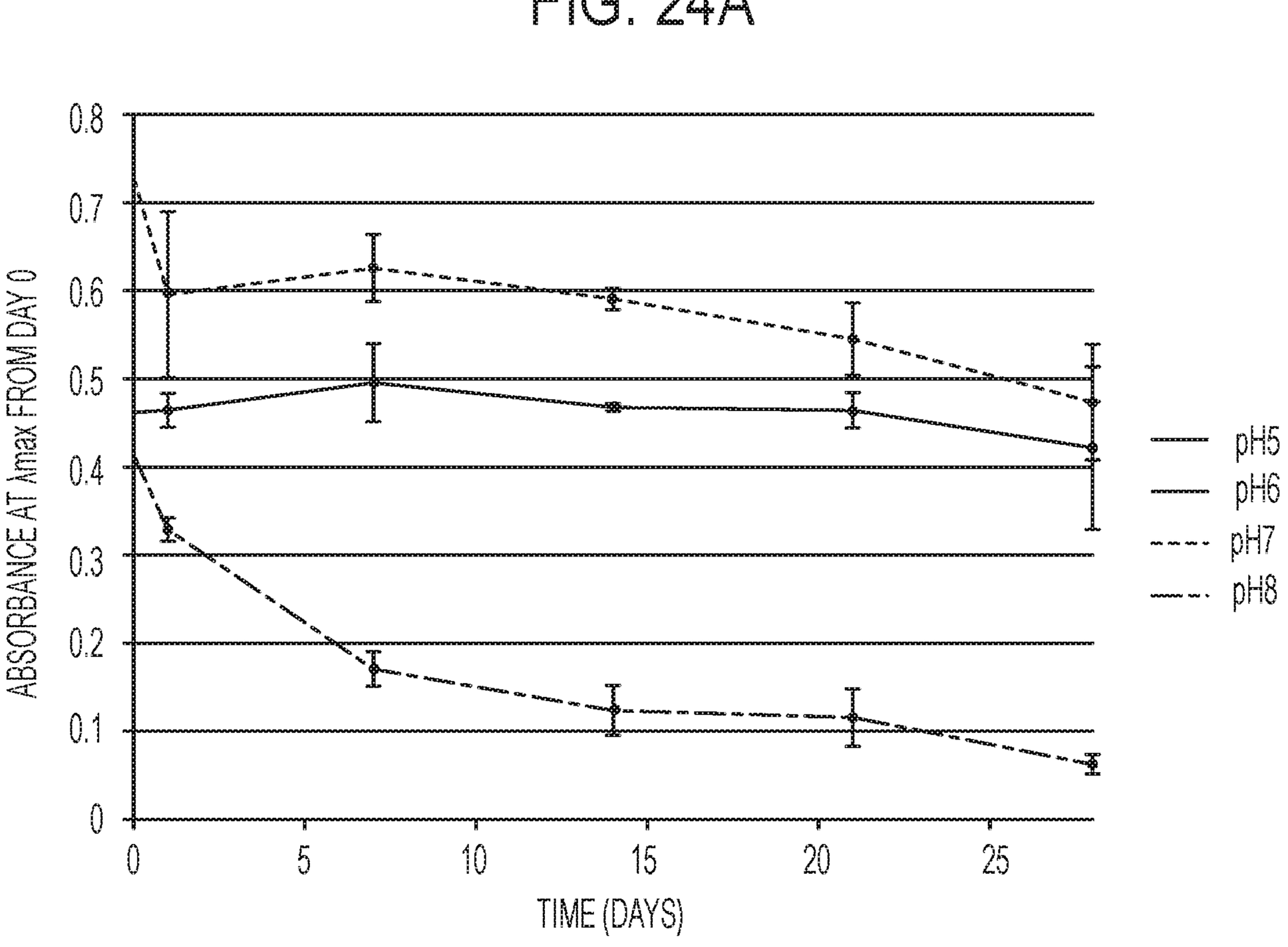


FIG. 24B

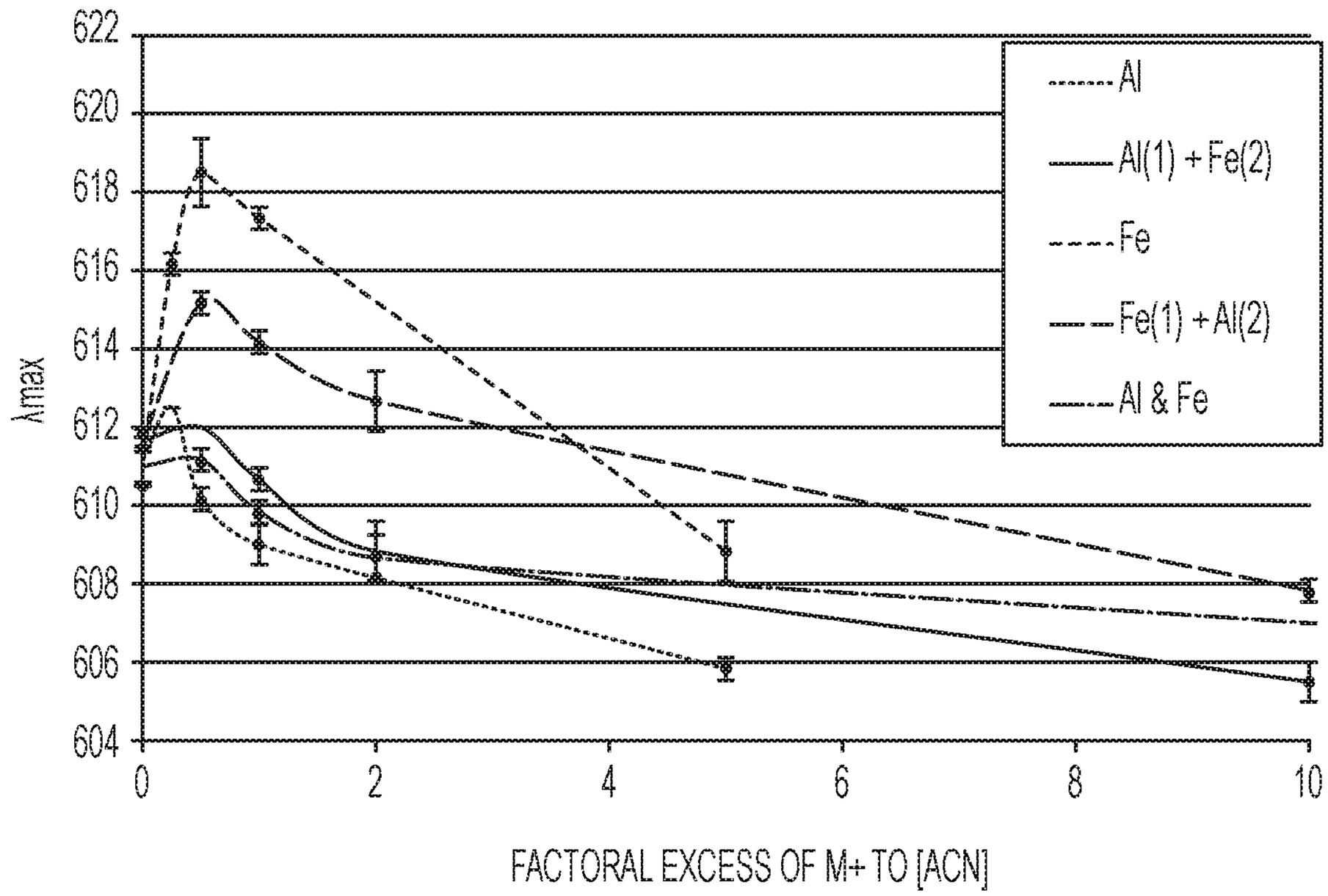


FIG. 25A

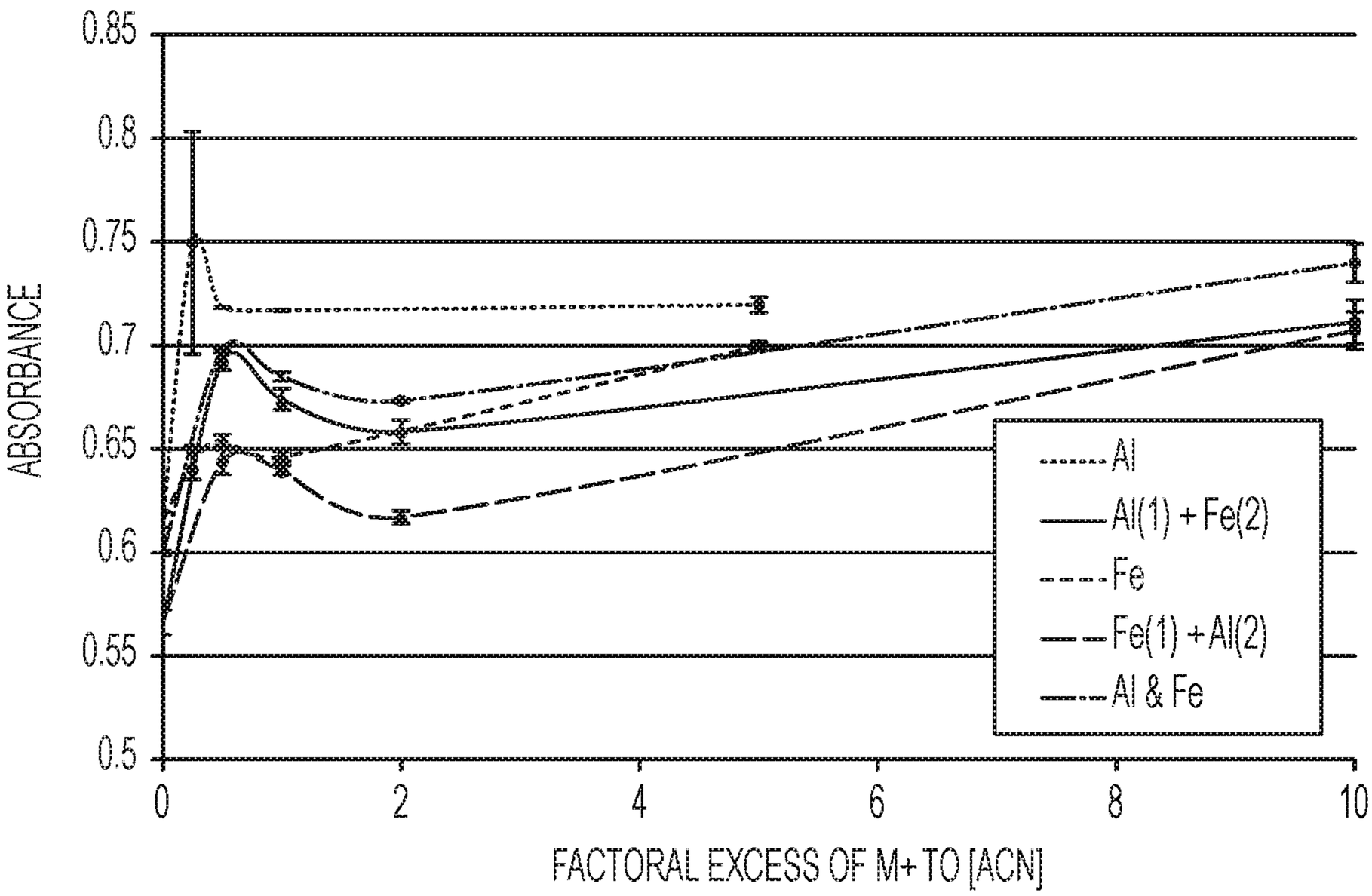


FIG. 25B

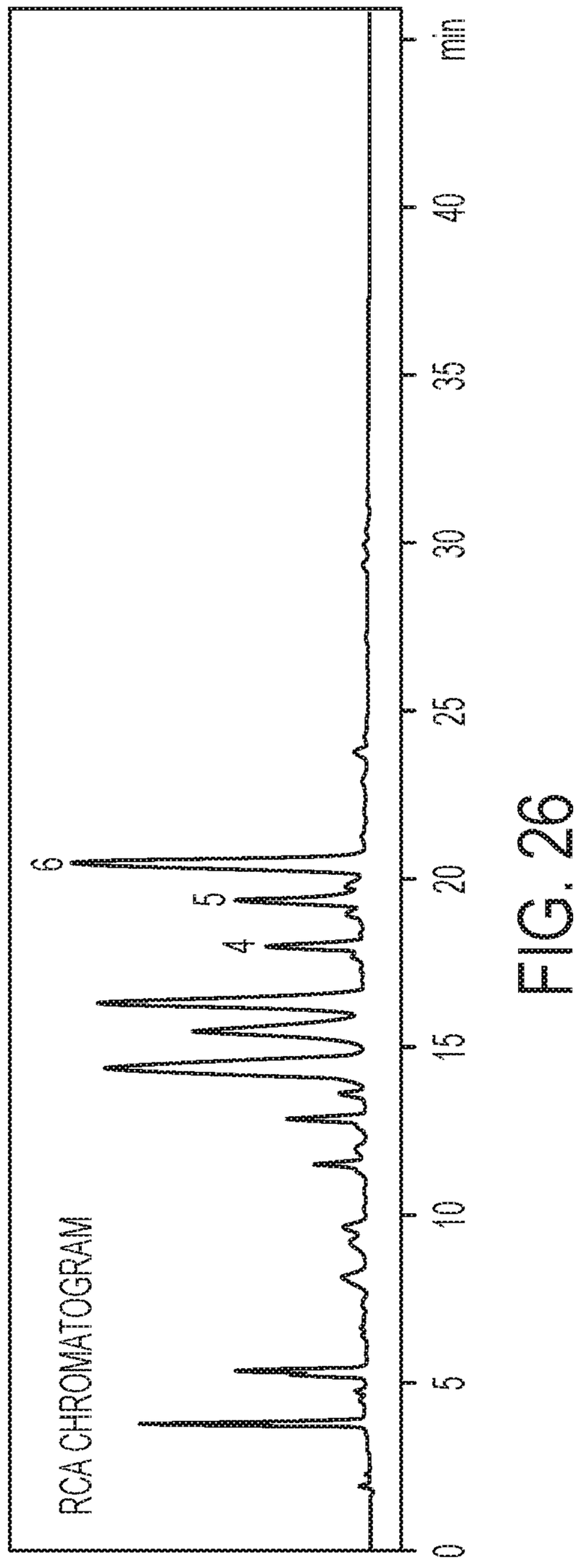

RCA CHROMATOGRAM
ABSORBANCE
0
5
10
15
20
25
30
35
40
min
1
2
3
4
5
6

FIG. 26

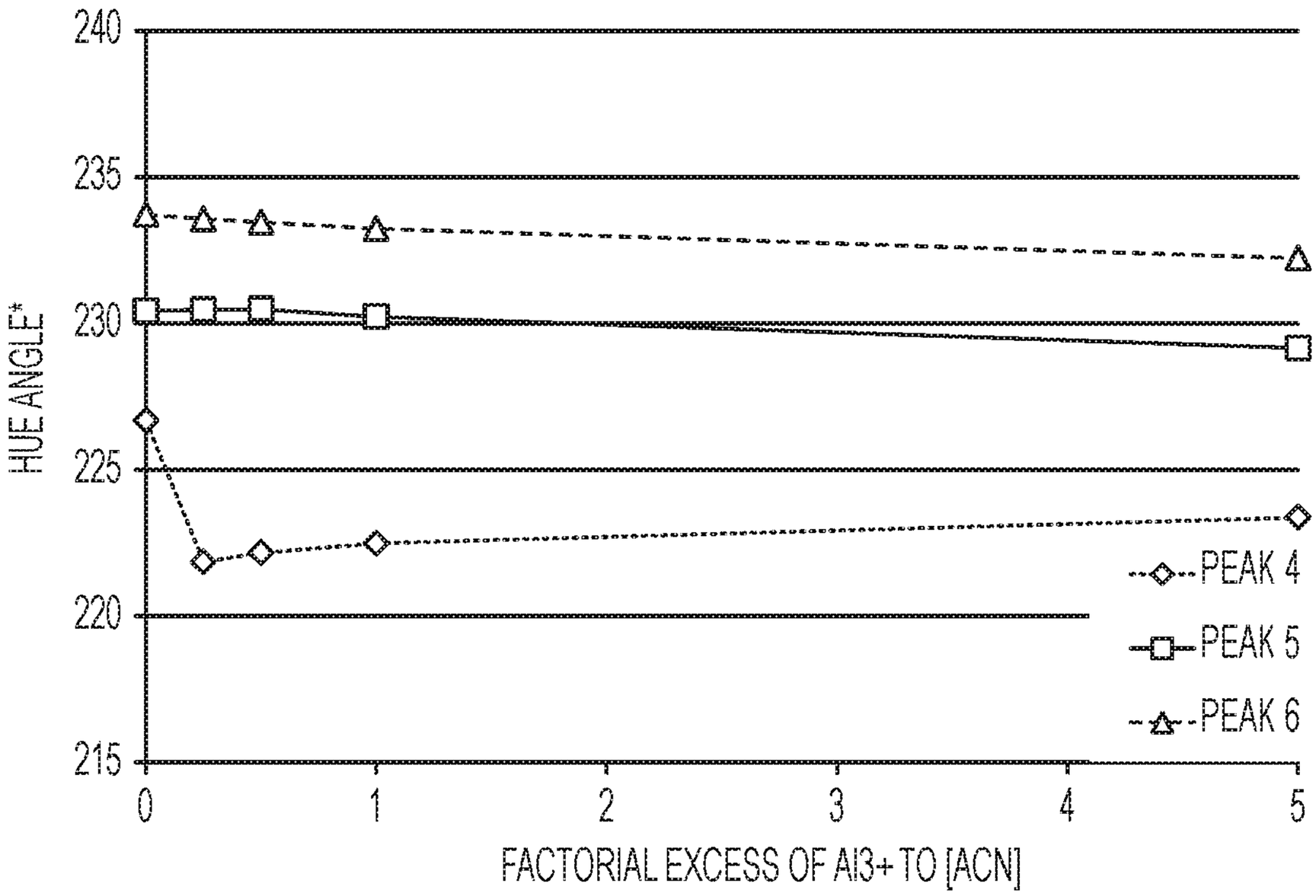


FIG. 27A

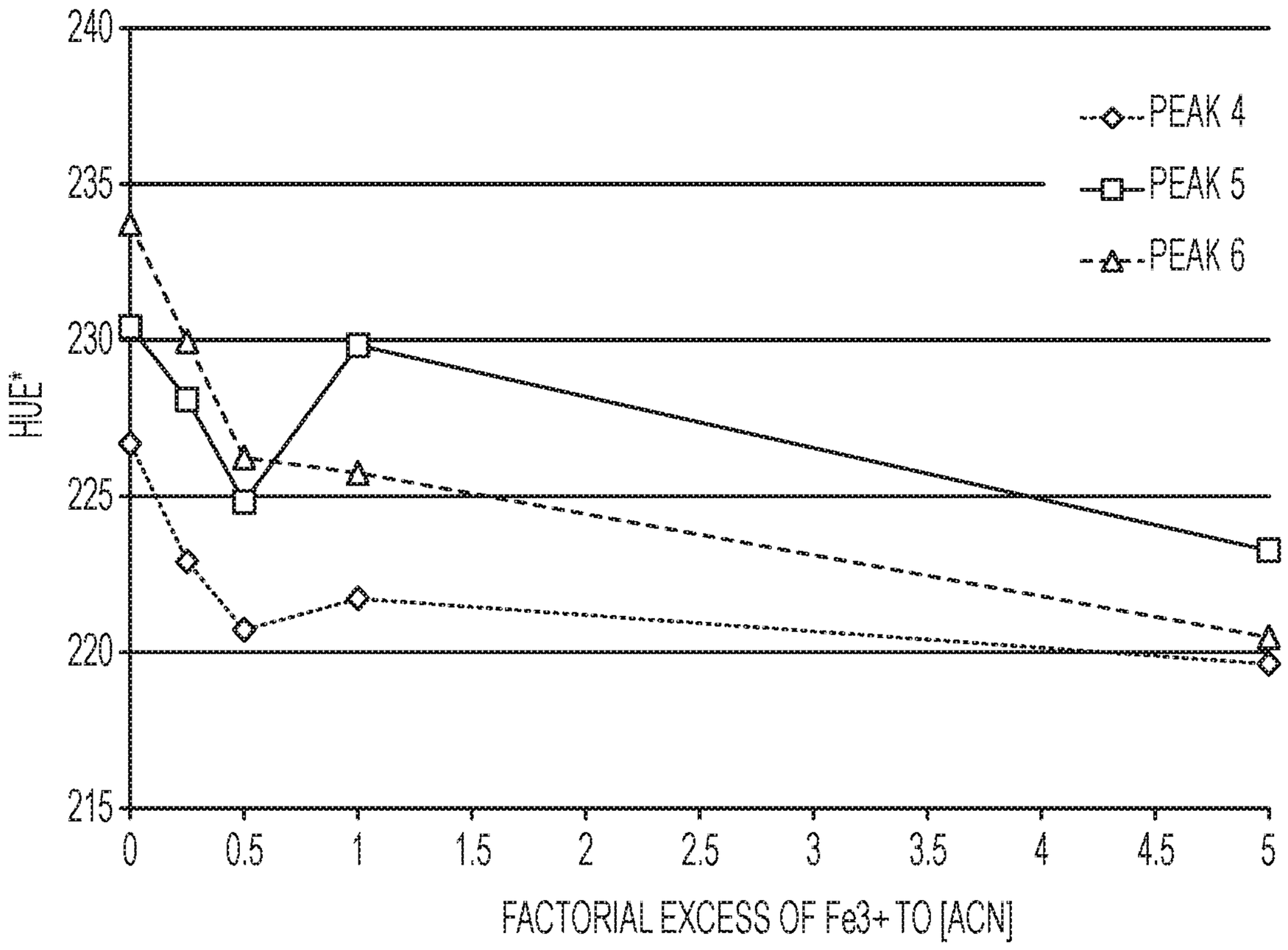


FIG. 27B

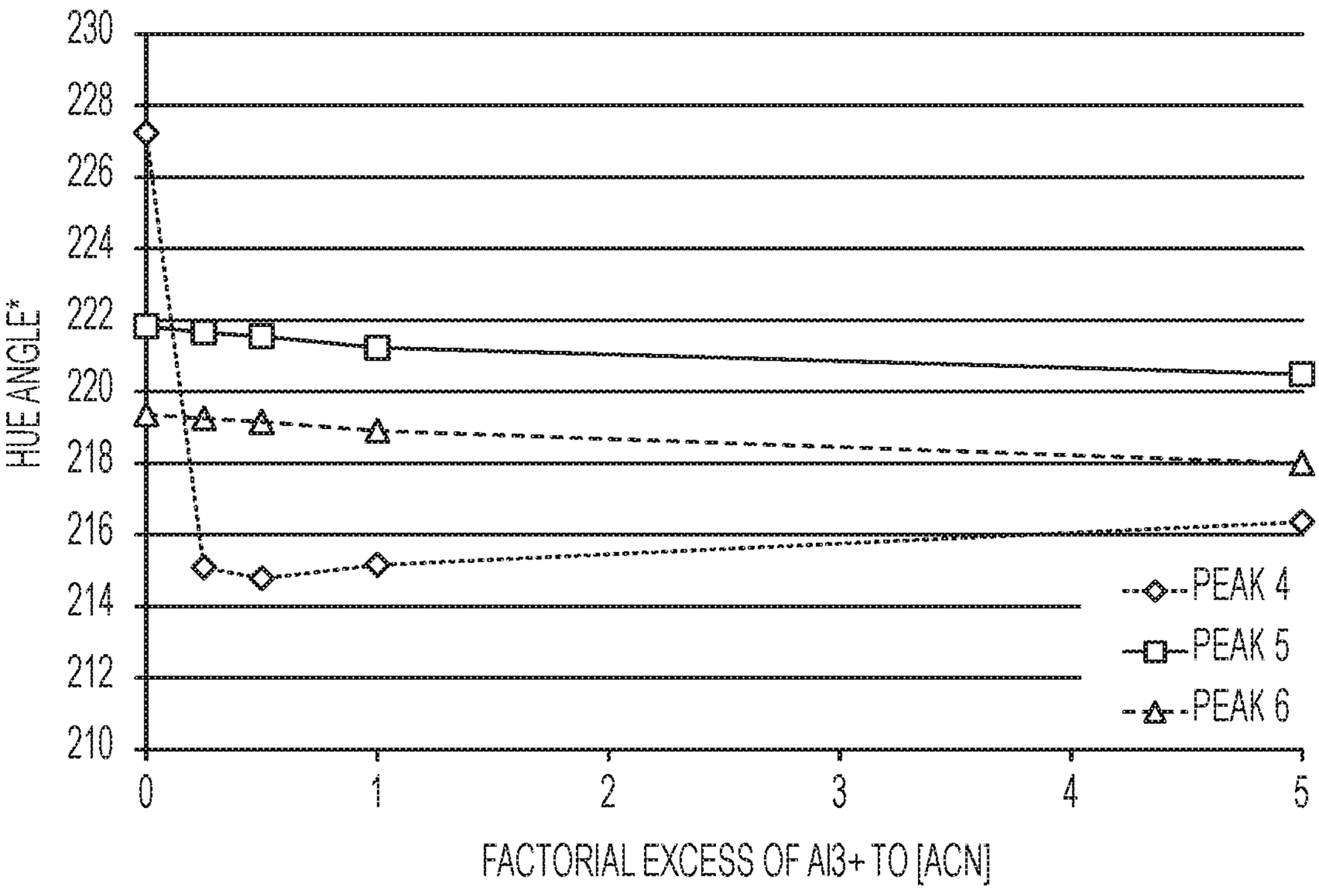


FIG. 28A

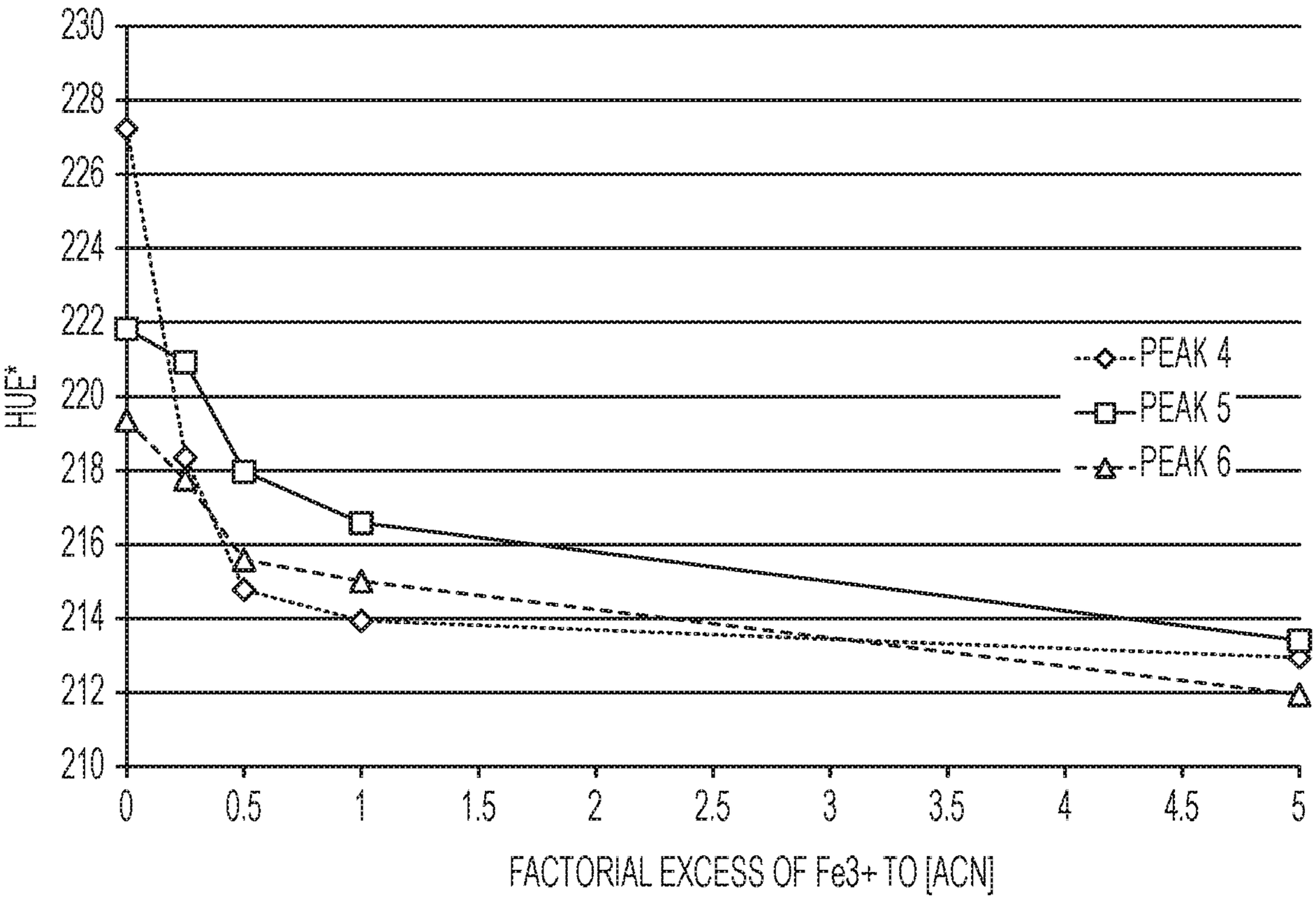


FIG. 28B

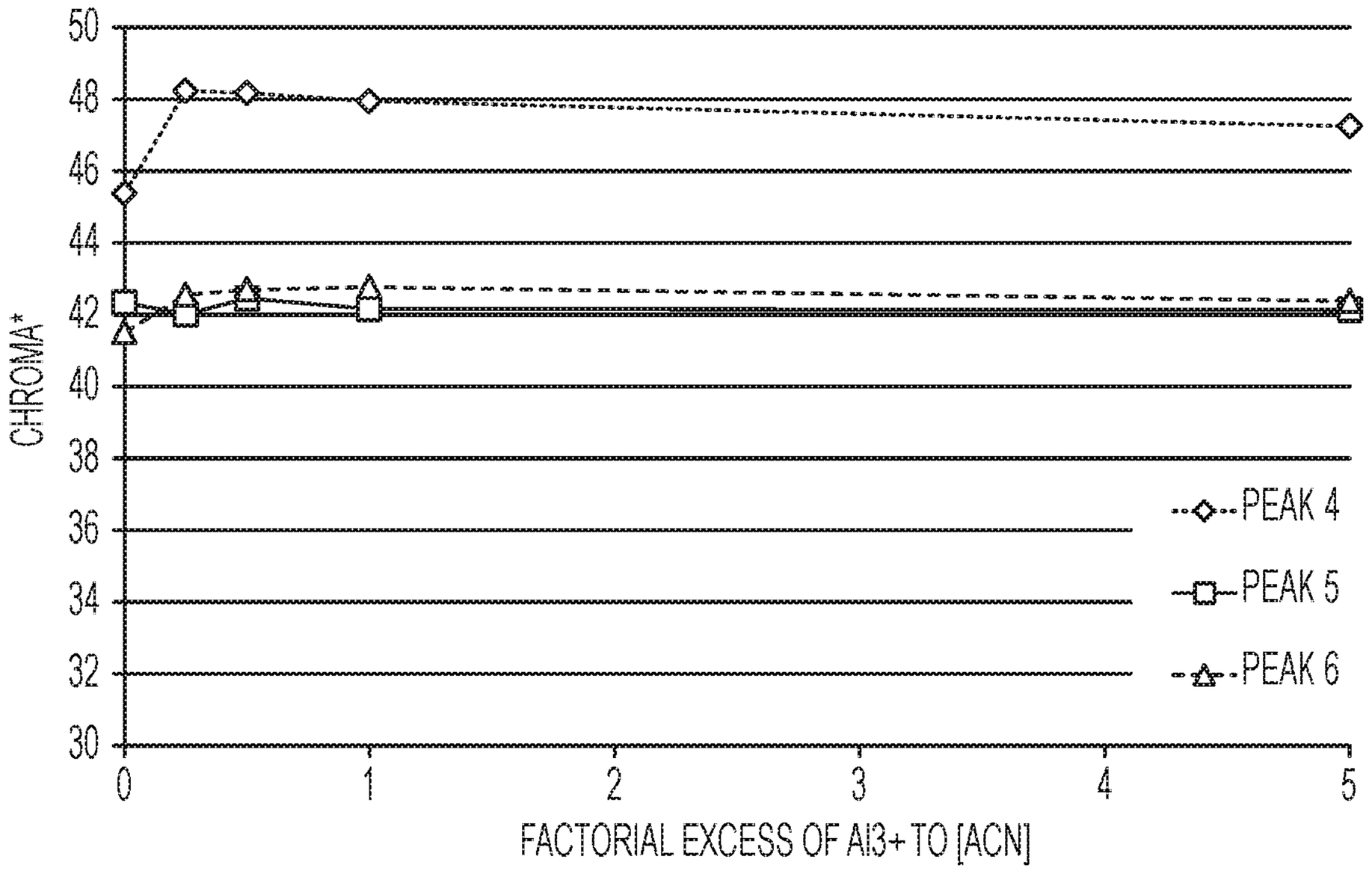


FIG. 29A

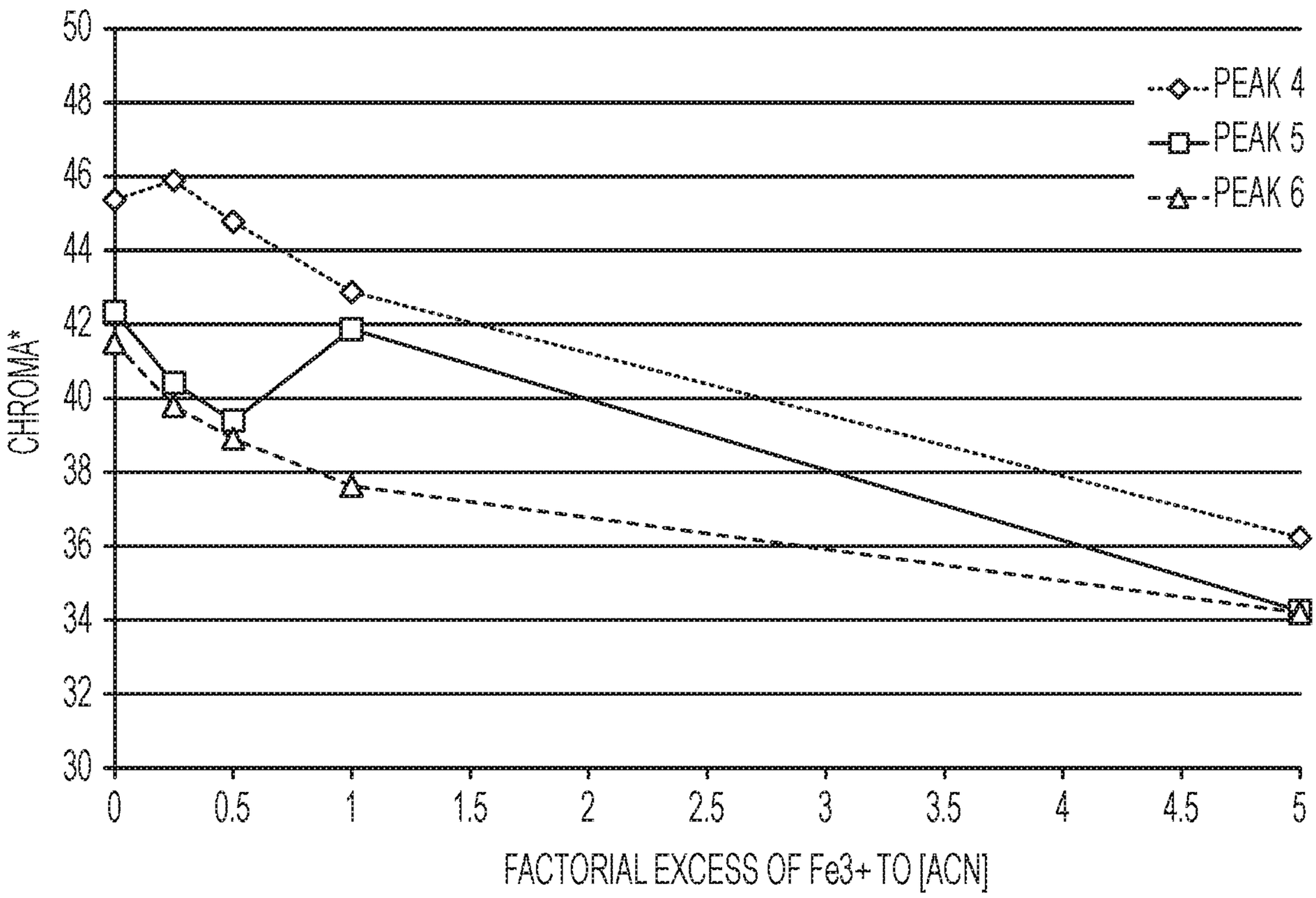


FIG. 29B

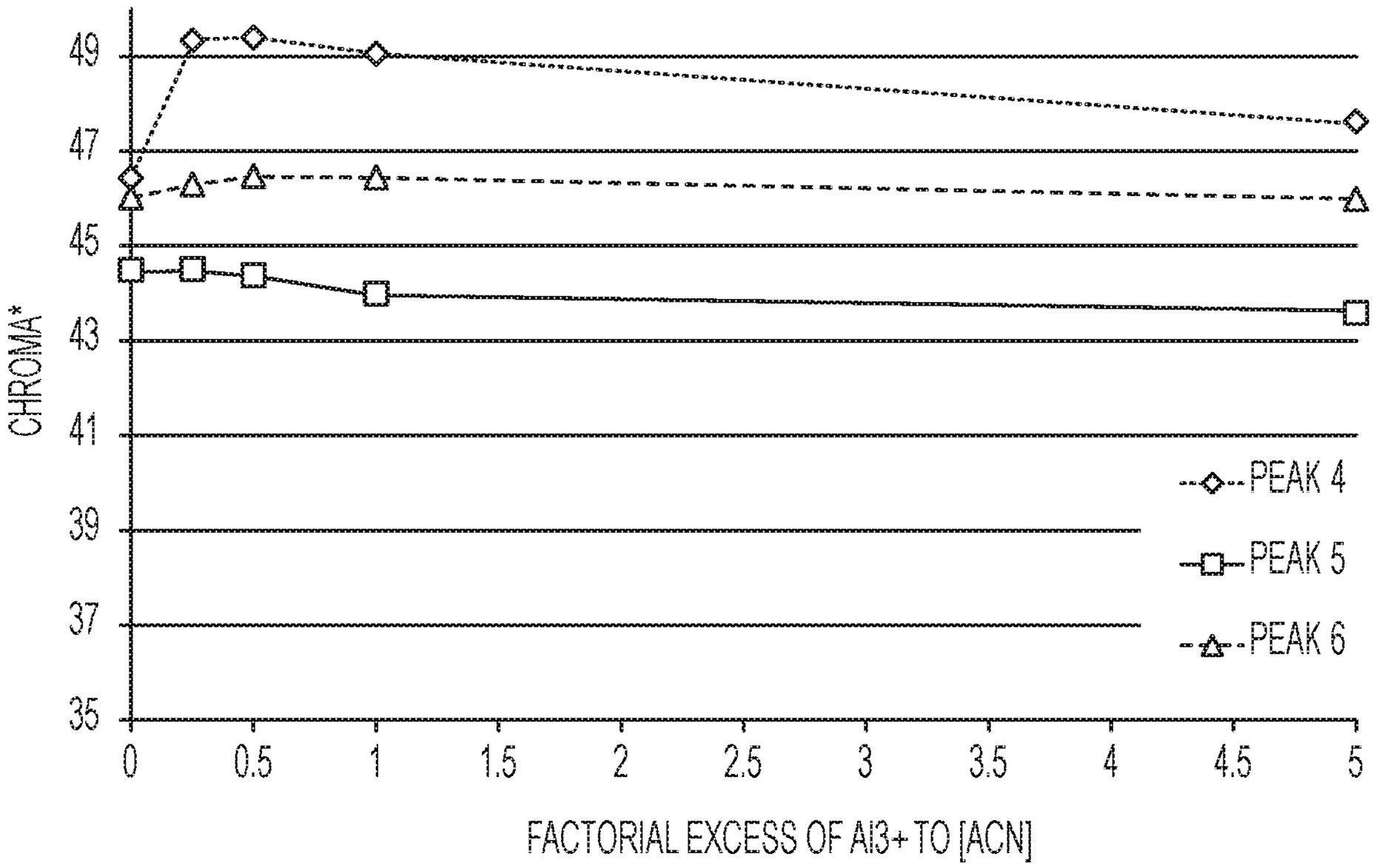


FIG. 30A

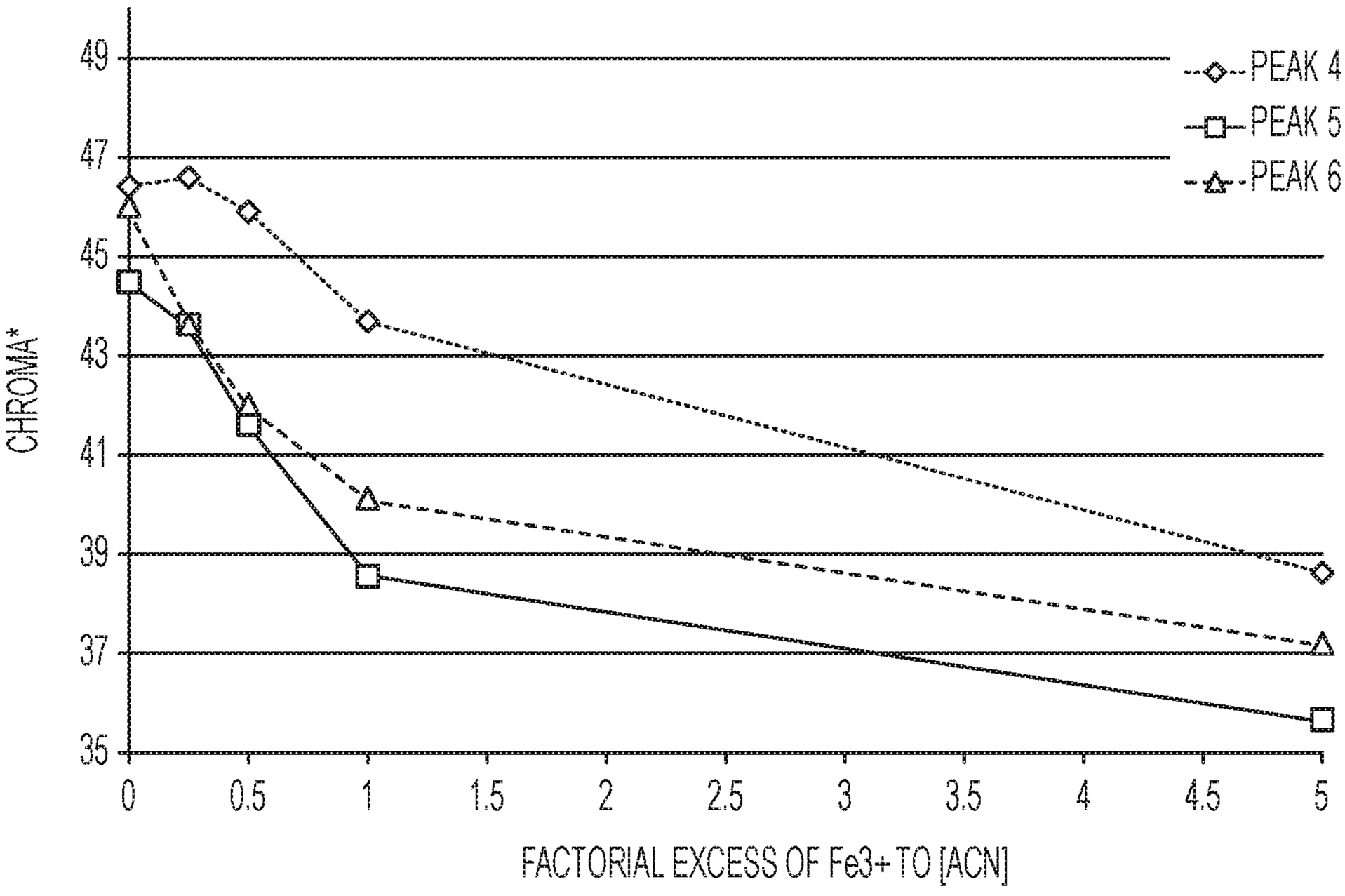


FIG. 30B

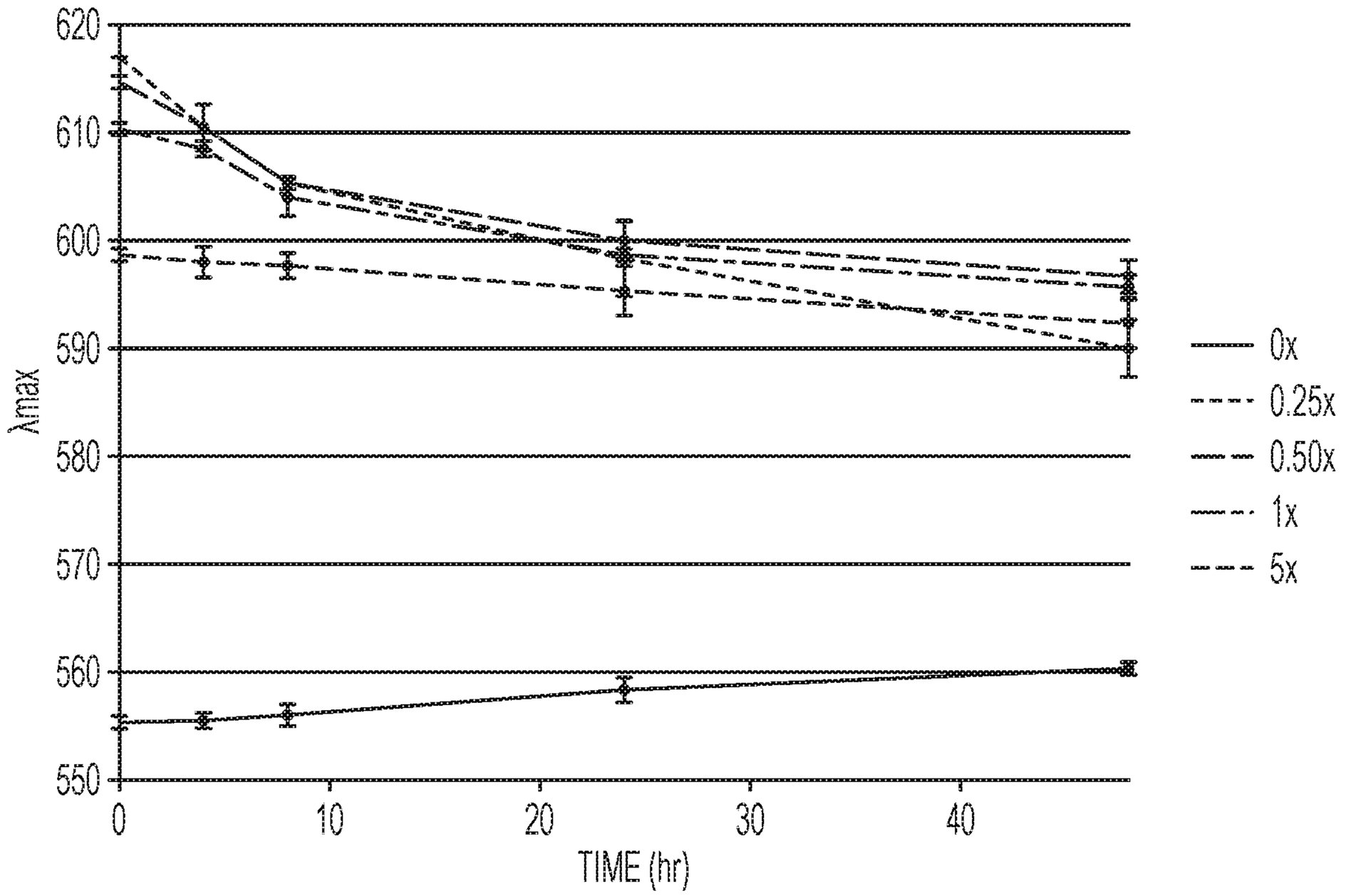


FIG. 31A

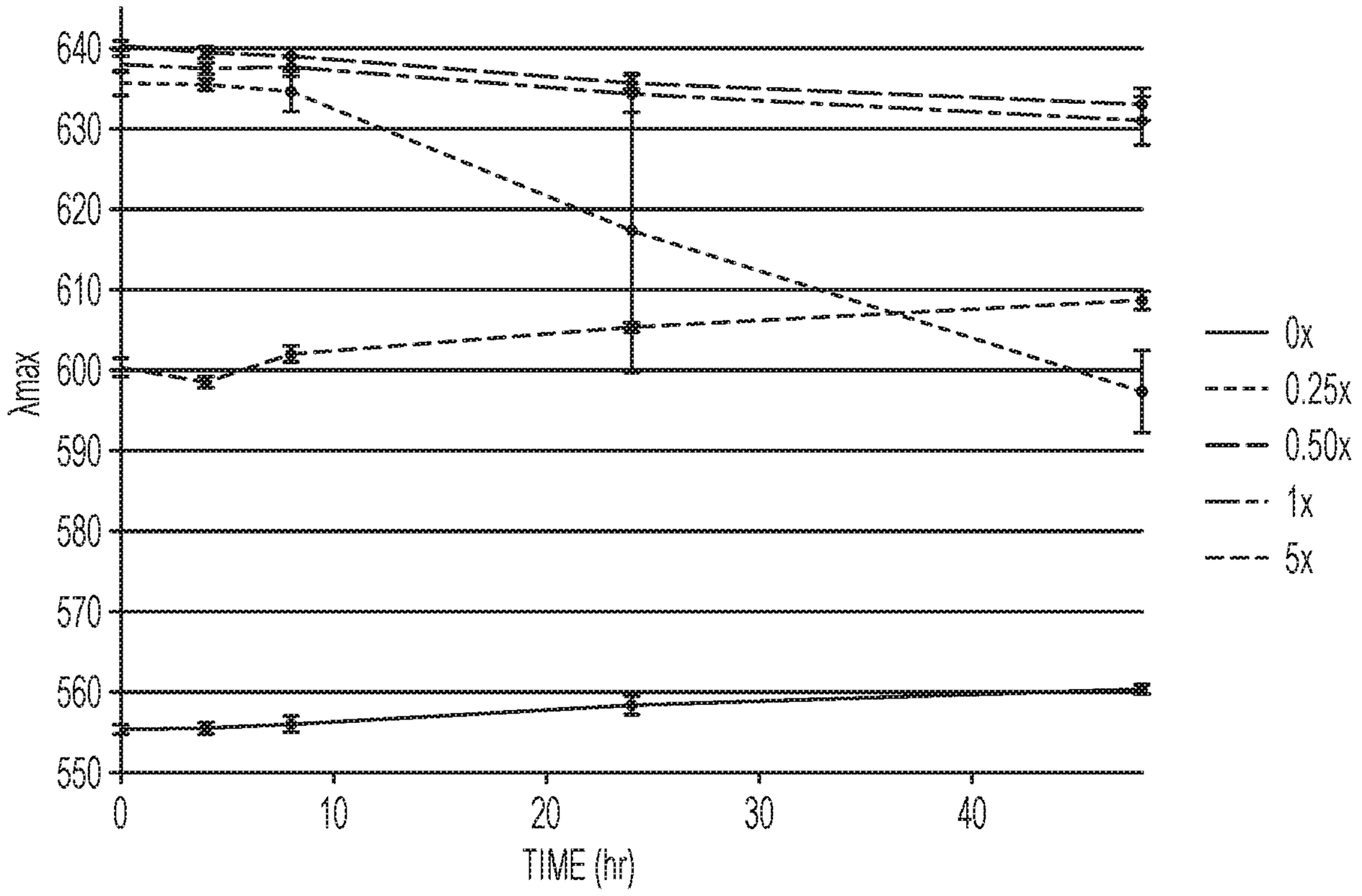


FIG. 31B

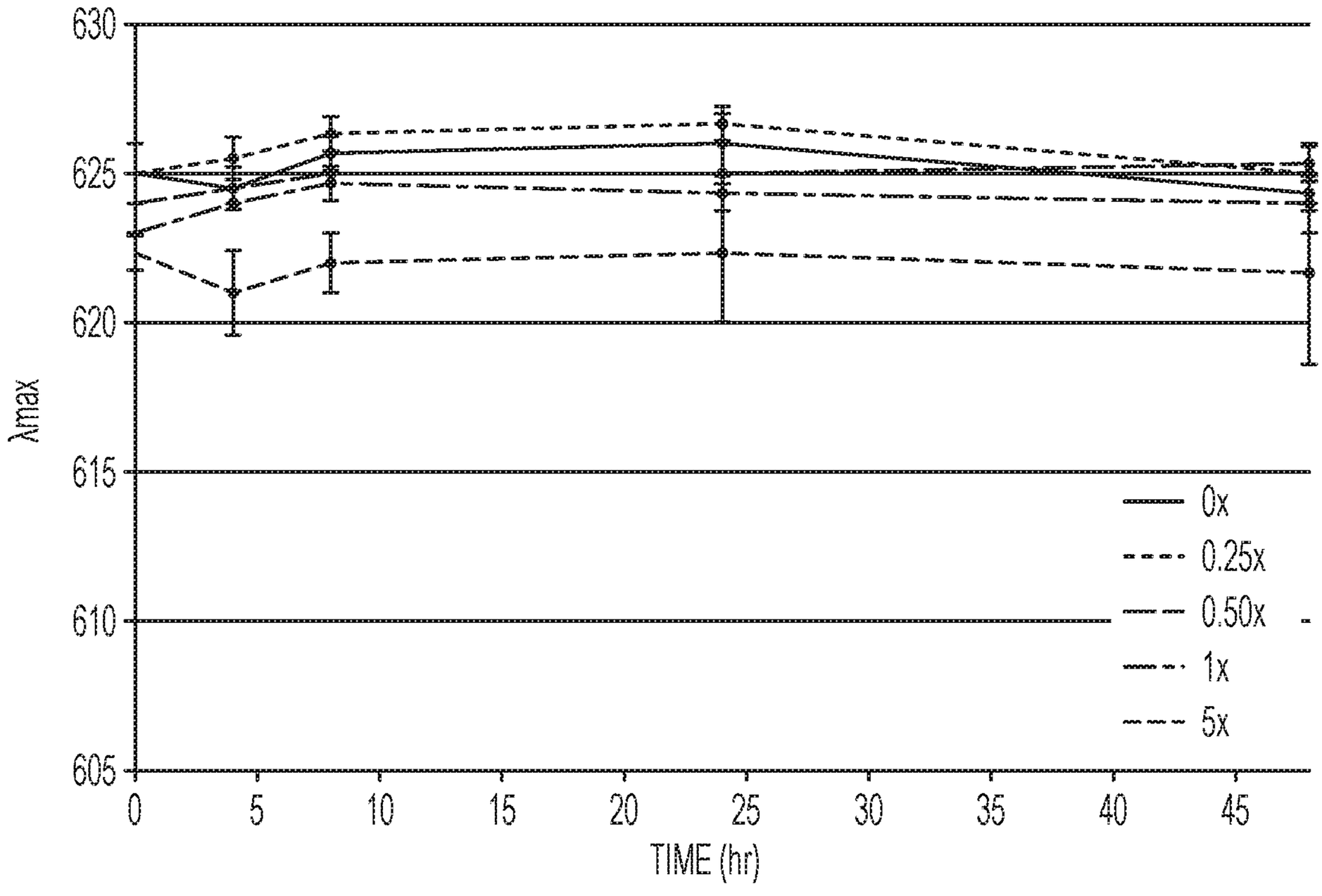


FIG. 32A

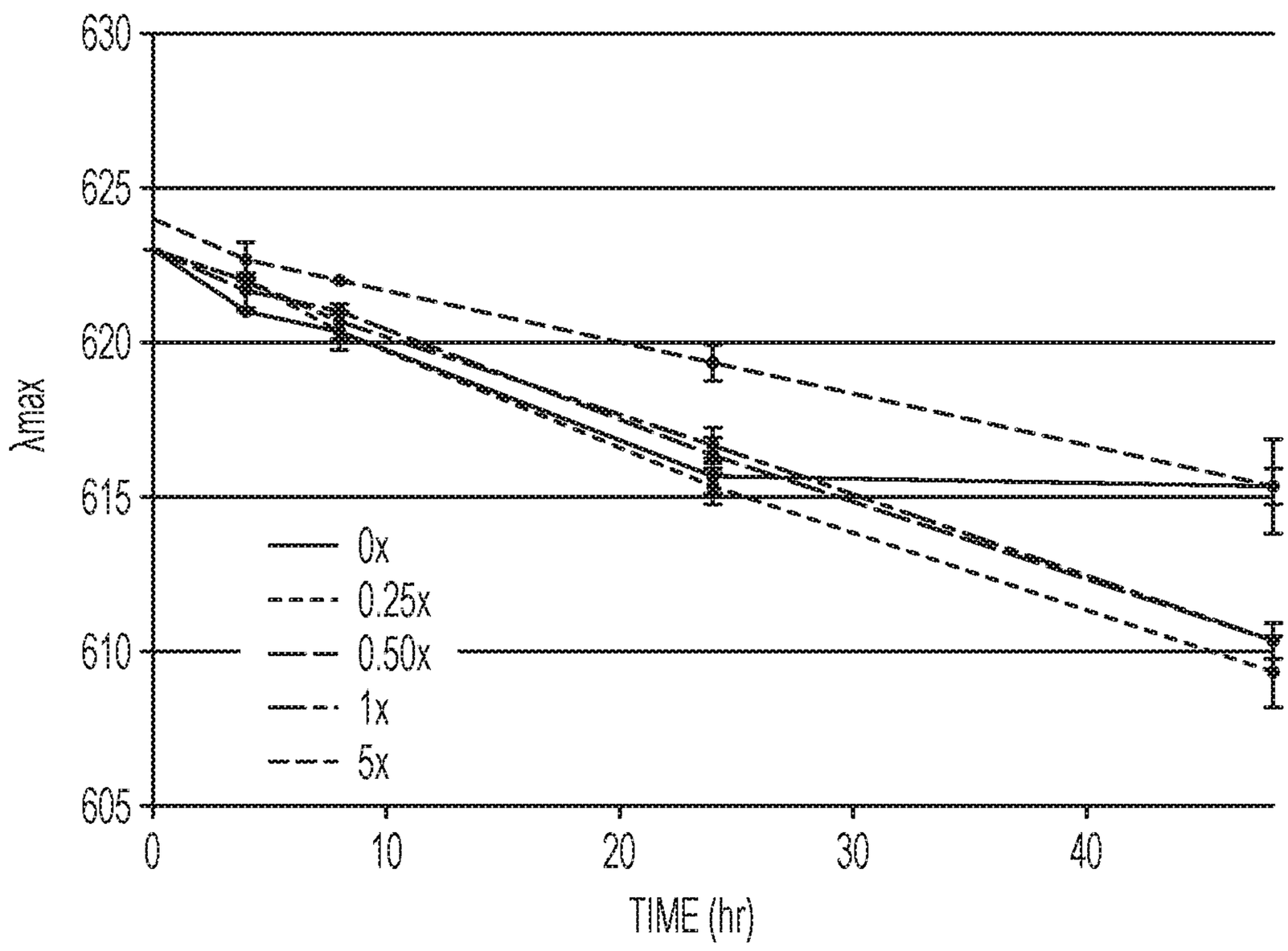


FIG. 32B

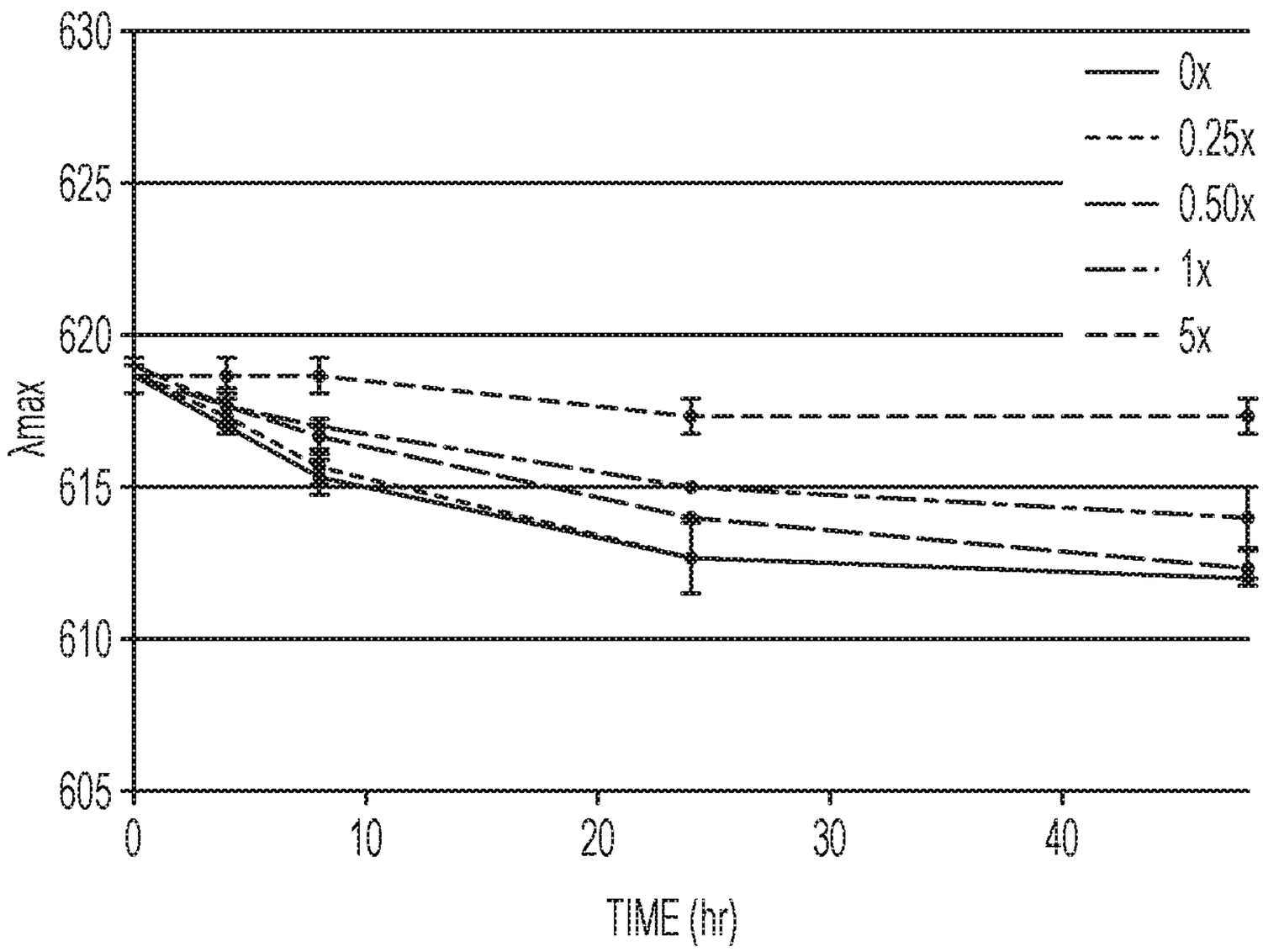


FIG. 32C

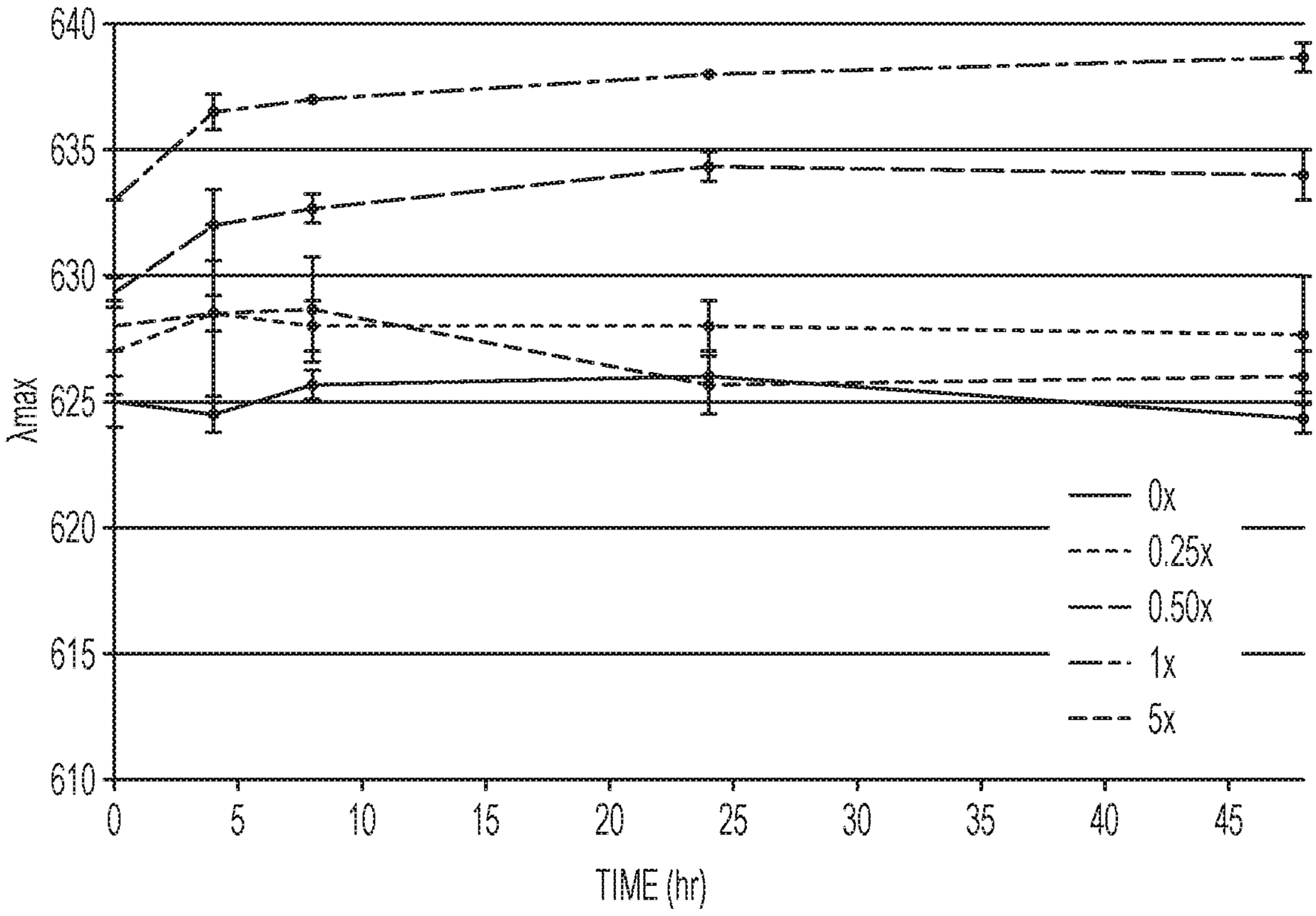


FIG. 32D

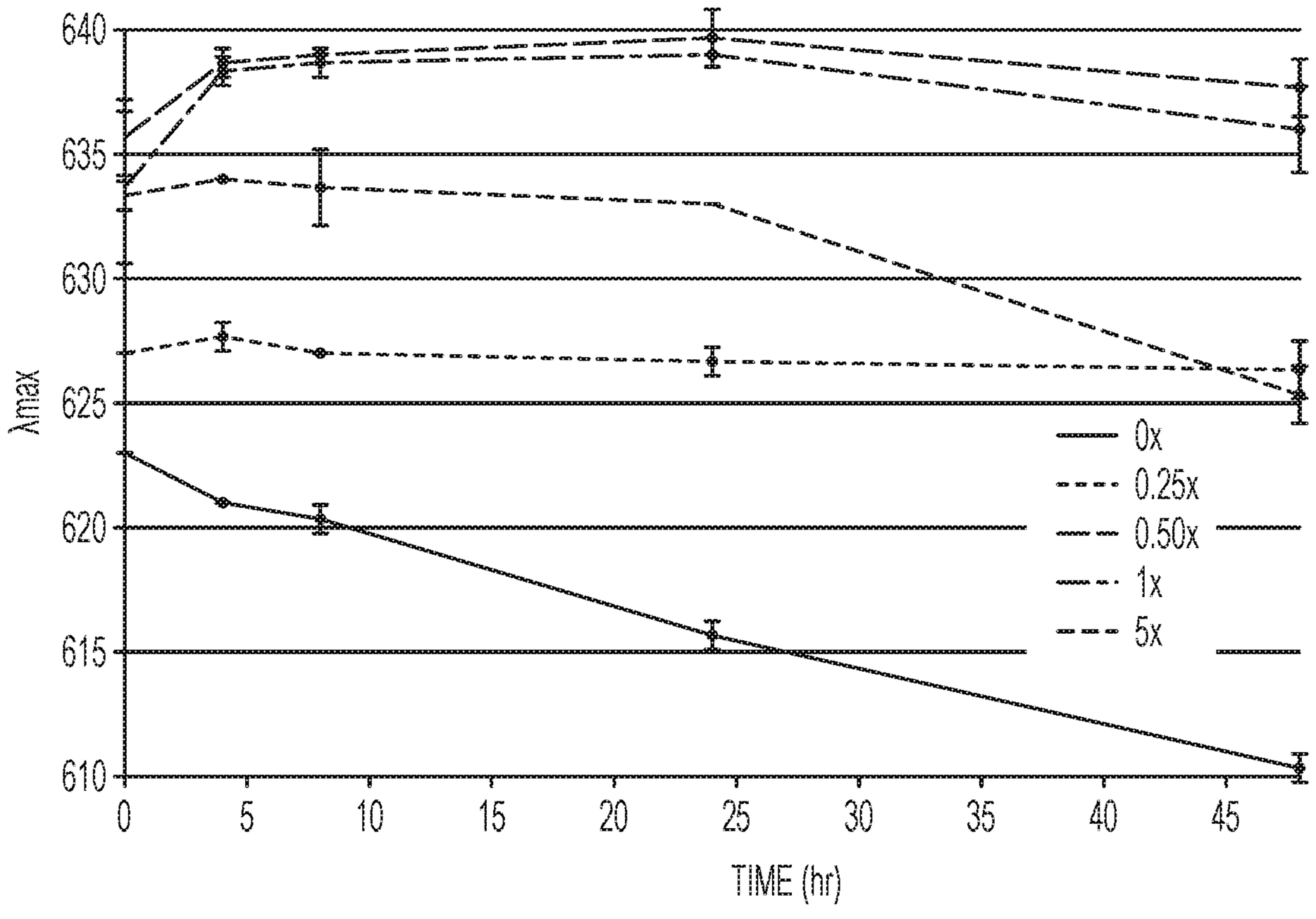


FIG. 32E

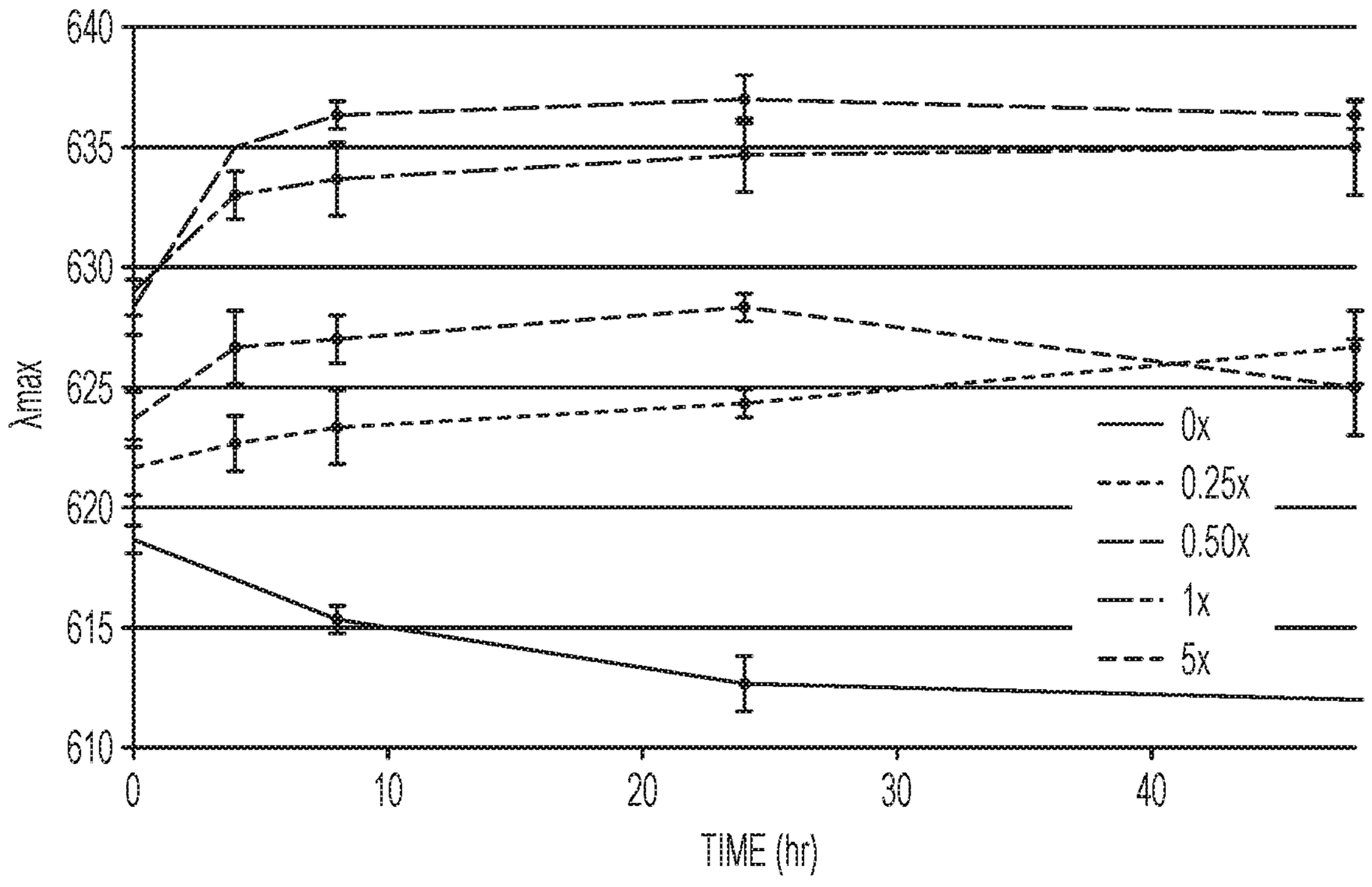


FIG. 32F

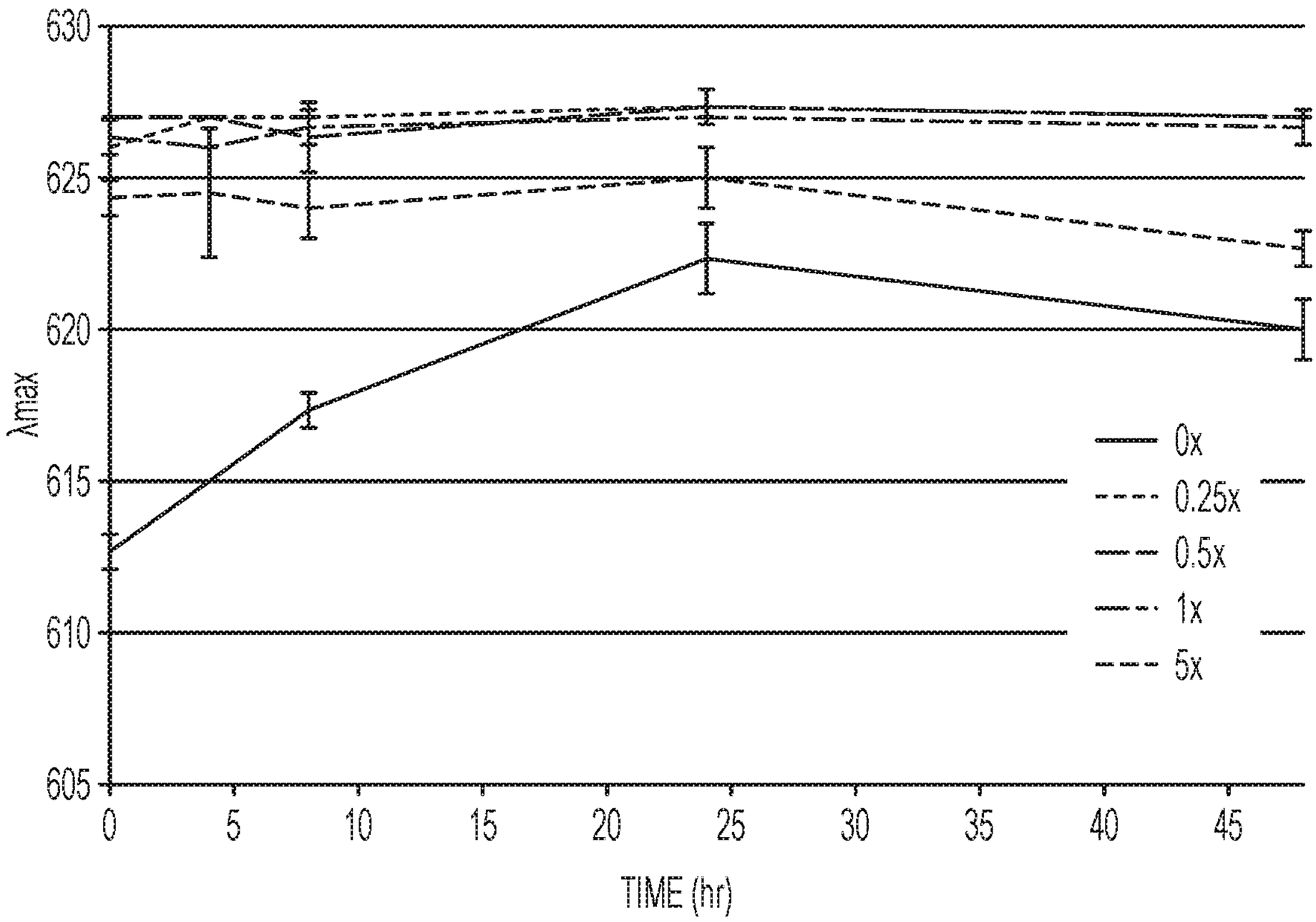


FIG. 33A

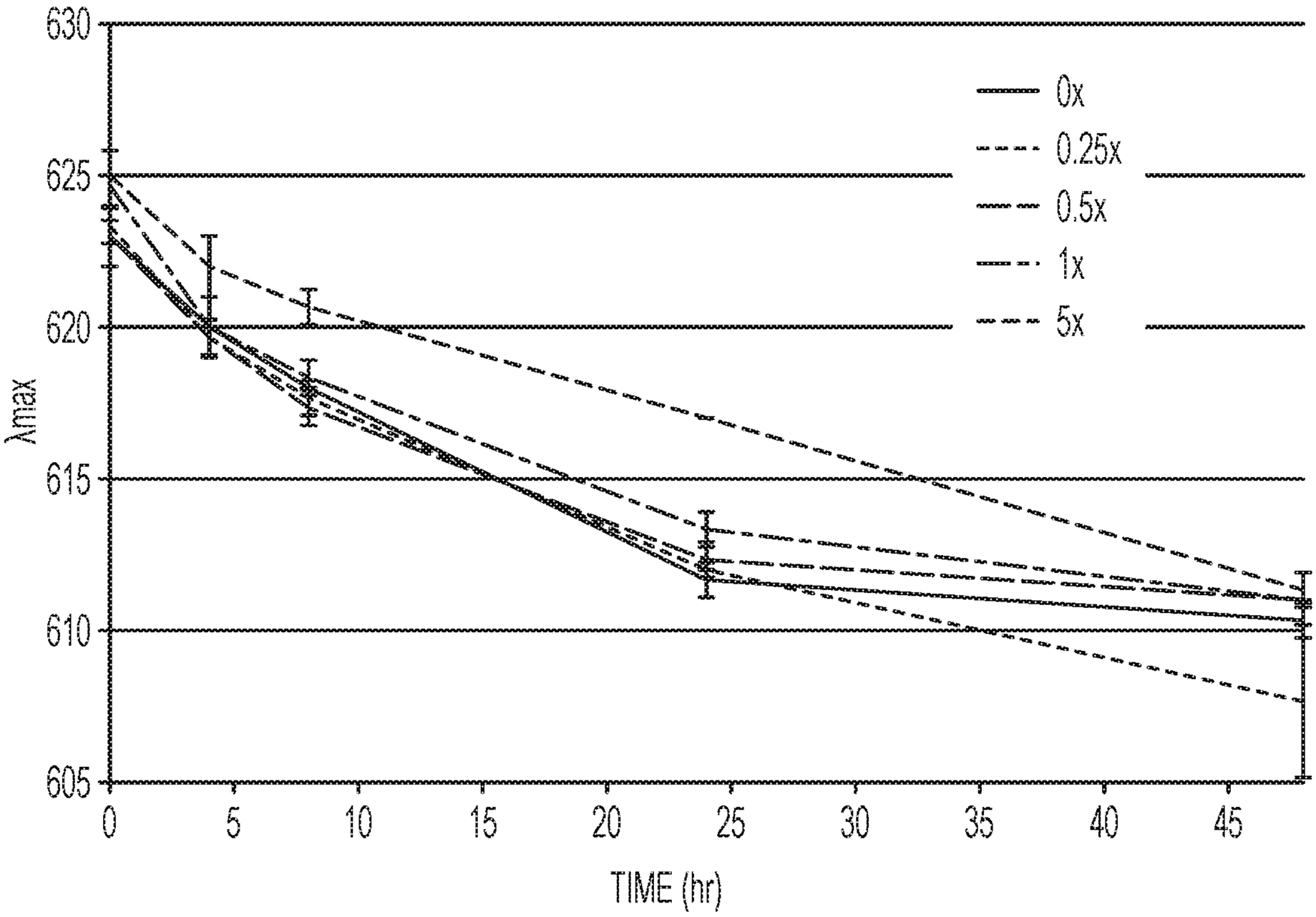


FIG. 33B

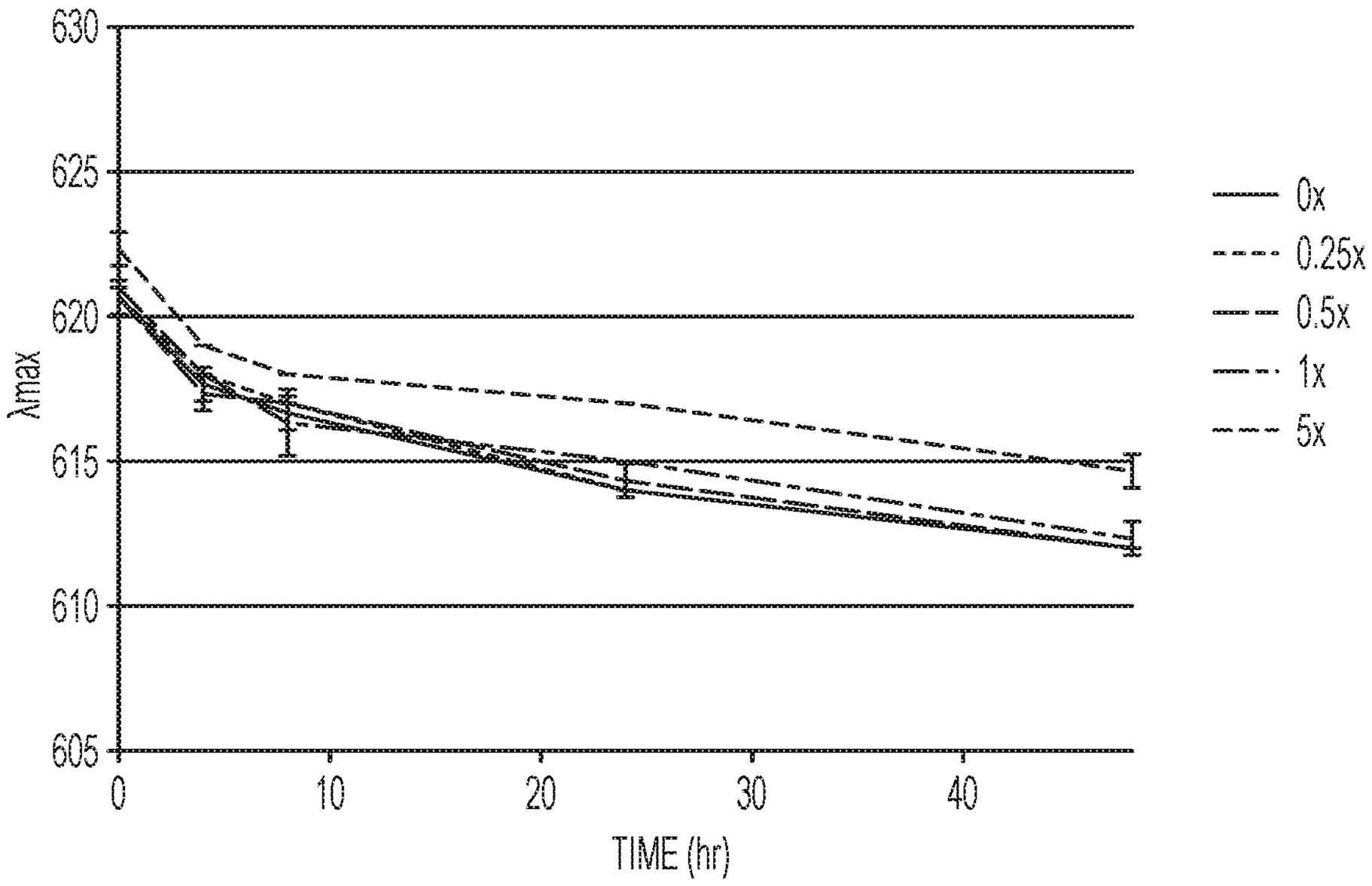


FIG. 33C

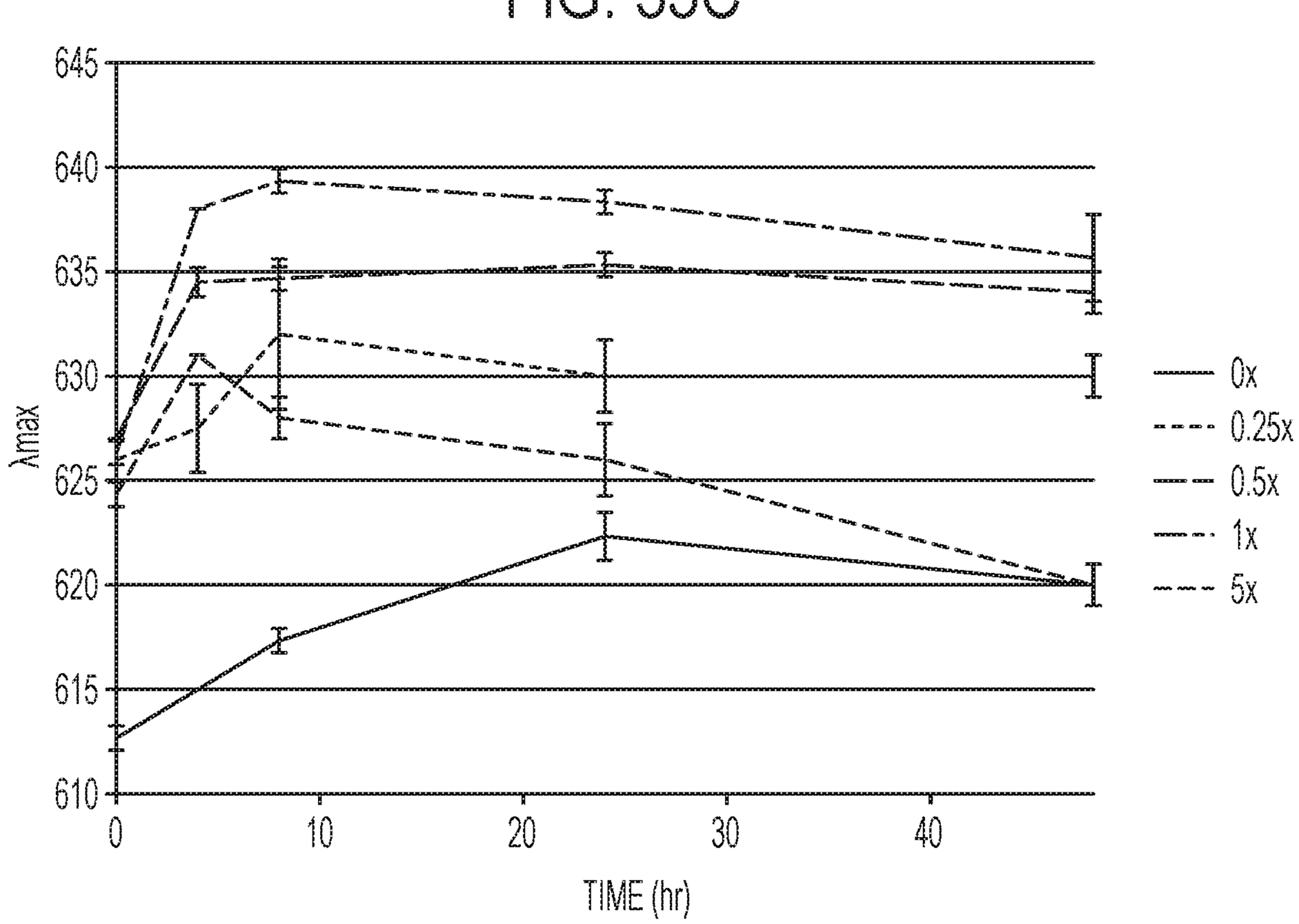


FIG. 33D

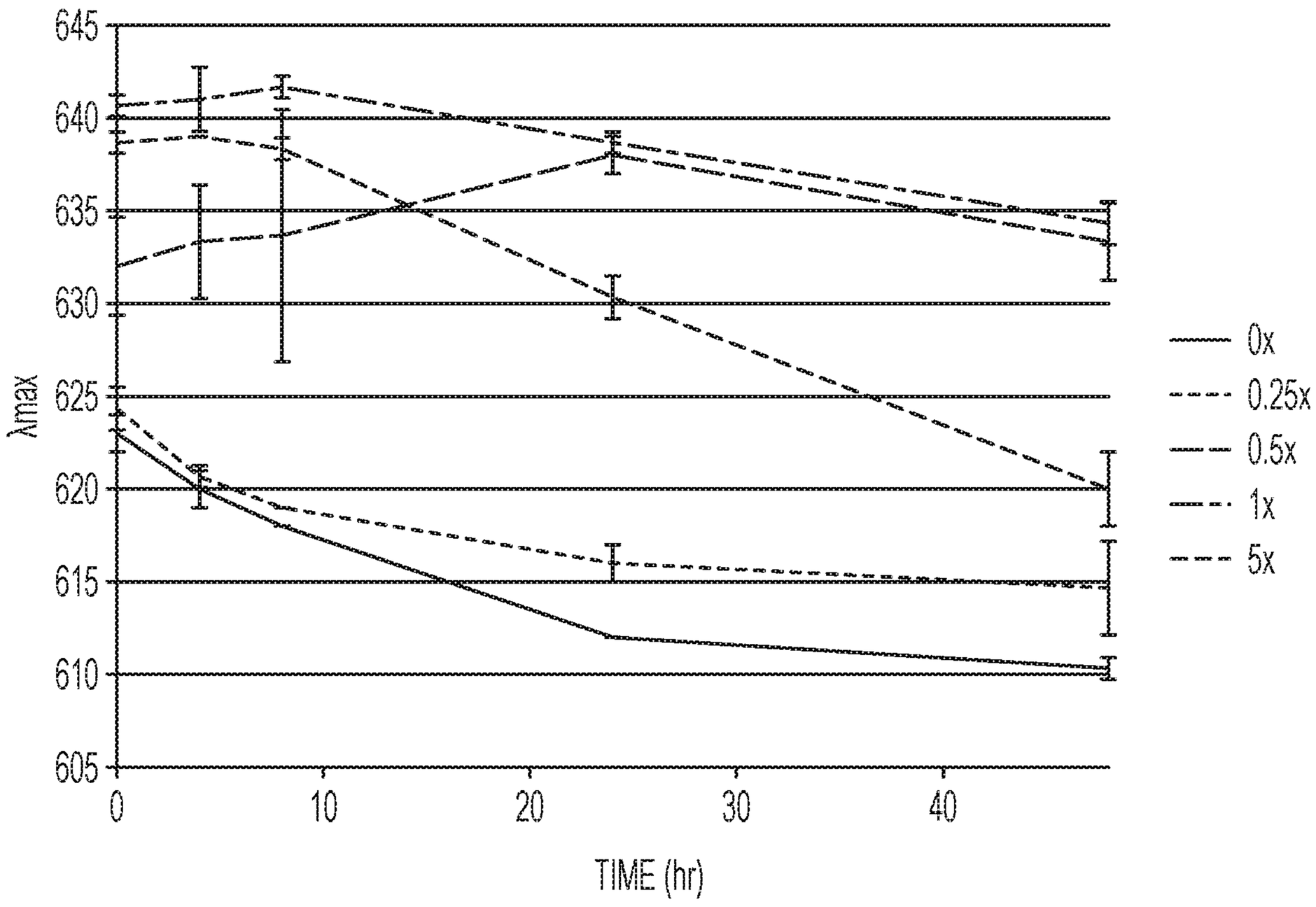


FIG. 33E

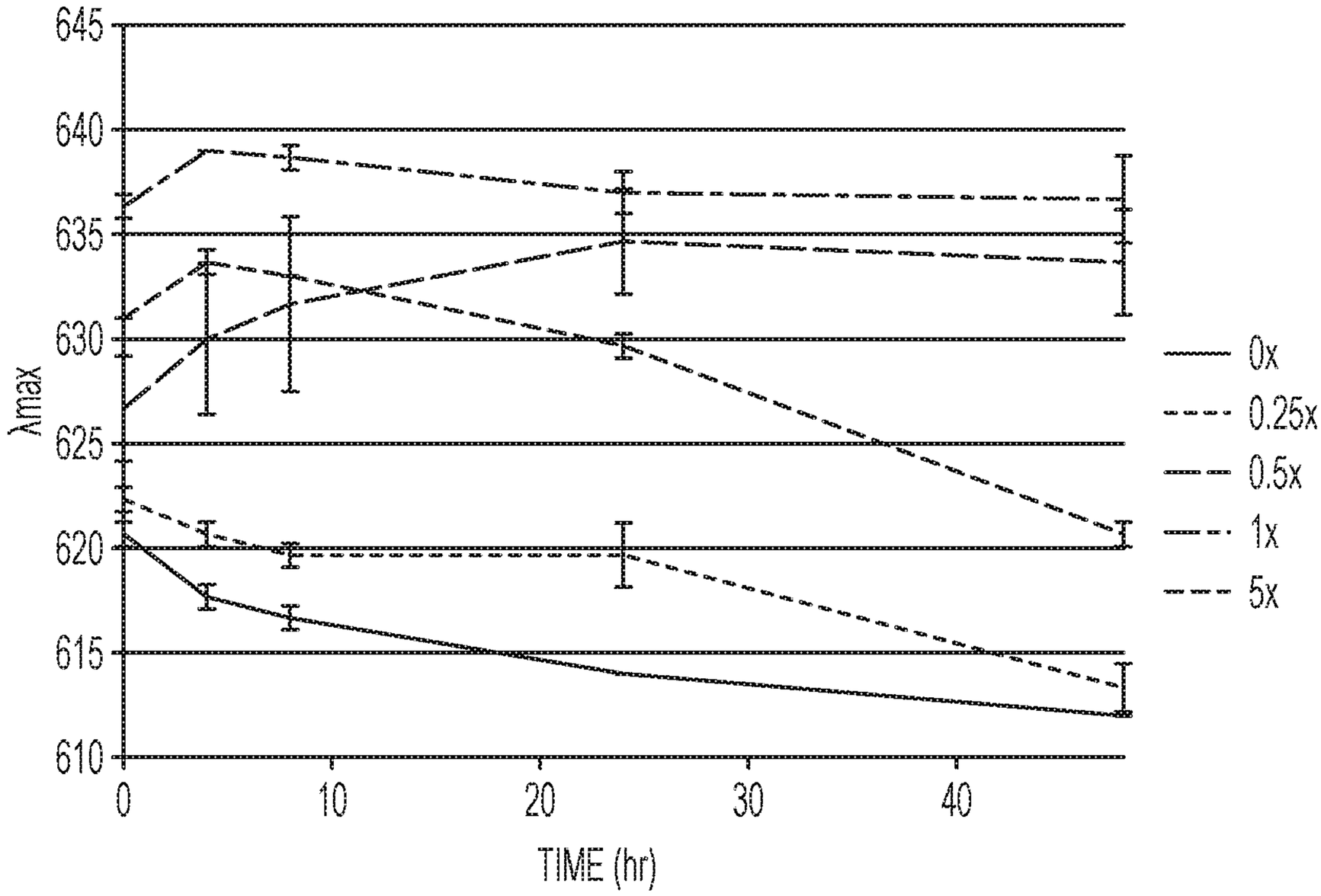


FIG. 33F

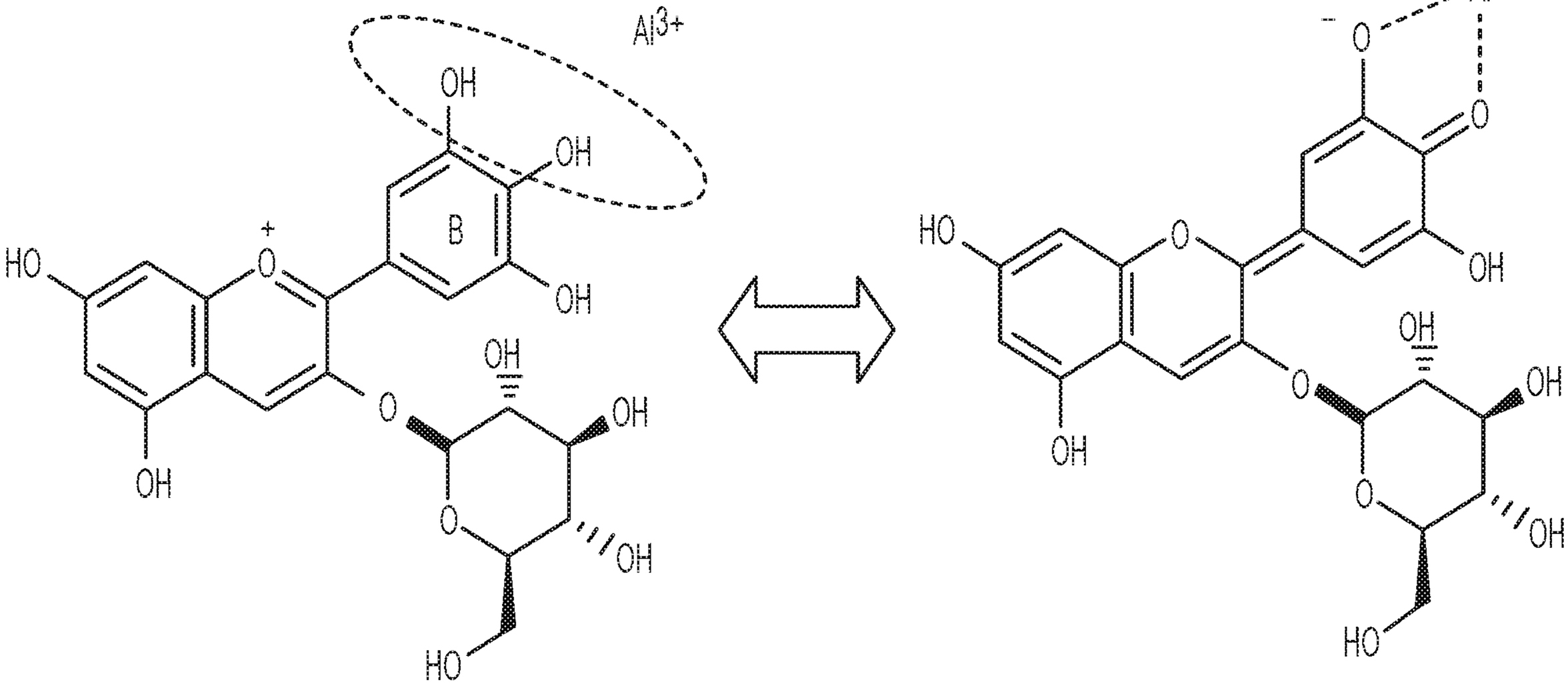


FIG. 1