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(54) **EPINEPHRINE FINE PARTICLES AND METHODS FOR USE THEREOF FOR TREATMENT OF CONDITIONS RESPONSIVE TO EPINEPHRINE**

FEINE EPINEPHRINTEILCHEN UND VERFAHREN ZUR VERWENDUNG DAVON ZUR BEHANDLUNG VON AUF EPINEPHRIN REAGIERENDEN ZUSTÄNDEN

PARTICULES FINES D'ÉPINÉPHRINE ET MÉTHODES D'UTILISATION DE CELLES-CI POUR LE TRAITEMENT D'ÉTATS PATHOLOGIQUES RÉAGISSANT À L'ÉPINÉPHRINE

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- (56) References cited:
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- **RAWAS-QALAJI M M ET AL: "Fast-disintegrating Sublingual Tablets: Effect of Epinephrine Load on Tablet Characteristics", AAPS PHARMSCITECH, SPRINGER NEW YORK LLC, US, vol. 7, no. 2, 1 April 2006 (2006-04-01), pages E1-E7, XP003009194, ISSN: 1530-9932, DOI: 10.1208/PT070241**

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- **RAWAS-QALAJI ET AL: "Sublingual epinephrine tablets versus intramuscular injection of epinephrine: Dose equivalence for potential treatment of anaphylaxis", JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 117, no. 2, 1 February 2006 (2006-02-01), pages 398-403, XP005275425, ISSN: 0091-6749, DOI: 10.1016/J.JACI.2005.12.1310**

Description

CROSS REFERENCE TO RELATED APPLICATIONS

5 **[0001]** This application claims the benefit of priority to U.S. Provisional Patent Application No. 61/804,519 (filed on March 22, 2013) and U.S. Provisional Patent Application No. 61/804,892 (filed on March 25 2013).

10 **[0002]** This application is related to U.S. Provisional Patent Application No. 61/660,273, filed on June 15, 2012. This application is related to U.S. Provisional Patent Application No. 61/550,359, filed on October 21, 2011. This application is related to International Application No. PCT/US2011126604, filed on March 1, 2011, which claims the benefit of U.S. Provisional Patent Application No. 61/309,136, filed on March 1, 2010. This application is related to U.S. Provisional Patent Application No. 60/715,180, filed on September 9, 2005, and U.S. Provisional Patent Application No. 60/759,039, filed on January 17, 2006. This application is related to U.S. Utility Patent Application No. 11/672,503, filed on February 7, 2007, now abandoned, which is a continuation-in-part of U.S. Utility Patent Application No. 11/530,360, filed on September 8, 2006, now abandoned.

FIELD OF THE INVENTION

20 **[0003]** The invention generally relates to compositions and methods for treatment of conditions responsive to epinephrine (also known as adrenaline), particularly to compositions and methods for emergency treatment of conditions responsive to epinephrine, and most particularly to compositions including epinephrine fine particles, including epinephrine nanoparticles or nanocrystals and epinephrine microparticles or microcrystals, for sublingual administration in treatment of conditions responsive to epinephrine.

BACKGROUND

25 **[0004]** Tablets that disintegrate or dissolve rapidly in the patient's mouth without the use of water are convenient for the elderly, young children, patients with swallowing difficulties, and in situations where water is not available. For these specially designed formulations, the small volume of saliva that is available is sufficient to disintegrate or dissolve a tablet in the oral cavity. The drug released from these tablets can be absorbed partially or entirely into the systemic circulation from the buccal mucosa or sublingual cavity, or can be swallowed as a solution to be absorbed from the gastrointestinal tract.

30 **[0005]** The sublingual route usually produces a faster onset of action than traditional orally-administered tablets and the portion absorbed through the sublingual blood vessels bypasses the hepatic first pass metabolic processes (Birudaraj et al., 2004, J Pharm Sci 94; Motwani et al., 1991, Clin Pharmacokinet 21: 83-94; Ishikawa et al., 2001, Chem Pharm Bull 49: 230-232; Price et al., 1997, Obstet Gynecol 89: 340-345; Kroboth et al., 1995, J Clin Psychopharmacol 15: 259-262; Cunningham et al., 1994, J Clin Anesth 6: 430-433; Scavone et al., 1992, Eur J Clin Pharmacol 42: 439-443; Spenard et al., 1988, Biopharm Drug Dispos 9: 457-464).

35 **[0006]** Likewise, due to high buccal and sublingual vascularity, buccally- or sublingually-delivered drugs can gain direct access to the systemic circulation and are not subject to first-pass hepatic metabolism. In addition, therapeutic agents administered via the buccal or sublingual route are not exposed to the acidic environment of the gastrointestinal tract (Mitra et al., 2002, Encyclopedia of Pharm. Tech., 2081-2095). Further, the buccal and sublingual mucosae have low enzymatic activity relative to the nasal and rectal routes. Thus, the potential for drug inactivation due to biochemical degradation is less rapid and extensive than other administration routes (de Varies et al., 1991, Crit. Rev. Ther. Drug Carr. Syst. 8: 271-303).

40 **[0007]** The buccal and sublingual mucosae are also highly accessible, which allows for the use of tablets which are painless, easily administered, easily removed, and easily targeted. Because the oral cavity consists of a pair of buccal mucosae, tablets, such as fast disintegrating tablets, can be applied at various sites either on the same mucosa or, alternatively, on the left or right buccal mucosa (Mitra et al., 2002, Encyclopedia of Pharm. Tech., 2081-2095). In addition, the buccal and sublingual routes could be useful for drug administration to unconscious patients, patients undergoing an anaphylactic attack, or patients who sense the onset of an anaphylactic attack.

45 **[0008]** Anaphylaxis is a sudden, severe systemic allergic reaction, which can be fatal within minutes. Epinephrine (Epi) is the drug of choice for the treatment of anaphylaxis worldwide (Joint Task Force on Practice Parameters, 2005, J Allergy Clin Immunol 115: S483-S523; Lieberman, 2003, Curr Opin Allergy Clin Immunol 3: 313-318; Simons, 2004, J Allergy Clin Immunol 113: 837-844). It is available as an injectable dosage form in ampoules or in autoinjectors, however these are underused when anaphylaxis occurs (Simons, F.E.R. J Allergy Clin Immunol 124(4):625-636 2009; Simons, F.E.R. J Allergy Clin Immunol 125:S161-181 2010). The drawbacks of Epi auto-injectors include high cost, perceived large size and bulkiness, limitations on repeated dosing (if required), fear and anxiety associated with the use of needles (especially in children), and dosing errors caused by incorrect techniques of administration (Simons, K.J. et al. Current

Opinion in *Clinical Immunology* 10:354-361 2010). Furthermore, in aqueous solutions, epinephrine is unstable in the presence of light, oxygen, heat, and neutral or alkaline pH values (Connors et al., 1986, in *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists*. Wiley-Interscience Publication: New York) and thus has limited shelf-life; approximately one year.

[0009] The sublingual route of administration is a promising alternative route for epinephrine administration. The formulation of sublingual tablets of epinephrine would enable the development of tablets with a range of epinephrine doses to match the population on an mg/kg basis. Sublingual tablets of epinephrine would be easy to carry and self-administer eliminating the fear and anxiety associated with needles used in autoinjectors for young children, as well as readily providing the capability of multiple doses. Feasibility studies in humans and animals have shown that epinephrine can be absorbed sublingually (Gu et al., 2002, *Biopharm Drug Dispos* 23: 213-216; Simons et al., 2004, *J Allergy Clin Immunol* 113: 425-438). The recommended dose of epinephrine for the treatment of anaphylaxis is about 0.01 mg/Kg: usually about 0.2 mL to about 0.5 mL of a 1:1000 dilution of epinephrine in a suitable carrier. Based on historical and anecdotal evidence, an approximately 0.3 mg dose of epinephrine, by subcutaneous (SC) or intramuscular (IM) injection into the deltoid muscle, has been agreed upon as the dose required for the emergency treatment of anaphylaxis. Recent studies have demonstrated that if the approximately 0.3 mg dose is administered IM into the laterus vascularis (thigh) muscle, Epi plasma concentrations are higher and occur more quickly than SC or IM administration into the deltoid muscle. (Joint Task Force on Practice Parameters, 2005, *J Allergy Clin Immunol* 115: S483-S523; Lieberman, 2003, *Curr Opin Allergy Clin Immunol* 3: 313-318; Simons, 2004, *J Allergy Clin Immunol* 113: 837-844)).

[0010] As stated above, epinephrine (Epi) is typically administered either subcutaneously (SC) or intramuscularly (IM) by injection. Thus, Epi injections are the accepted first aid means of delivering Epi and are administered either manually or by automatic injectors. It is recommended that persons at risk of anaphylaxis, and persons responsible for children at risk for anaphylaxis, maintain one or more automatic Epi injectors in a convenient place at all times.

[0011] Given the difficulties associated with manual subcutaneous (SC) or intramuscular (IM) administration of Epi, such as patient apprehension related to injections or the burden of an at risk person having to always maintain an Epi injector close at hand, there exists a need in the art for more convenient dosage forms which can provide immediate administration of Epi, particularly to a person undergoing anaphylaxis wherein the need for injection or Epi injectors is obviated.

[0012] Recently, a novel fast-disintegrating tablet suitable for sublingual (SL) administration of Epi was developed. See related U.S. applications: U.S. Provisional Patent Application No. 60/715,180; U.S. Provisional Patent Application No. 60/759,039; U.S. Utility Patent Application No. 11/672,503; and U.S. Utility Patent Application No. 11/530,360. Sublingual administration of 40 mg epinephrine as the bitartrate salt using these novel tablets resulted in a rate and an extent of epinephrine absorption similar to that achieved following intramuscular injections of 0.3 mg epinephrine in the thigh. Sublingual doses ranging from 5 to 40 mg epinephrine as the bitartrate salt were studied to achieve equivalent plasma concentrations. In an animal model, it was determined that a 40 mg epinephrine dose administered sublingually as a bitartrate salt in tablet form resulted in plasma epinephrine concentrations similar to those achieved by 0.3 mg epinephrine intramuscular (IM) injection (Rawas-Qalaji et al. *J Allergy Clin Immunol* 117:398-403 2006).

[0013] Without being bound by theory, it is thought that fabrication of epinephrine into fine particles, including epinephrine nanoparticles or nanocrystals and epinephrine microparticles or microcrystals, and incorporation of the epinephrine fine particles into a tablet formulation with pharmaceutically-acceptable carriers, penetration enhancers, and mucoadhesives will significantly increase the absorption of SL-administered epinephrine and will result in the reduction of SL epinephrine dose required.

[0014] US 2007 059361 A1 discloses in paragraph [0002] formulations for fast-disintegrating epinephrine tablets.

[0015] WO 2007 143674 A2 discloses in paragraph [0002] formulations for epinephrine tablets.

[0016] Rawas-Qalaji M.M. et al., *AAPS PHARMSCITECH*, vol. 7, no. 2, 2006, pages E1-E7, and Rawas-Qalaji M.M. et al., *Journal of Allergy and Clinical Immunology*, vol. 117, no. 2, 2006, pages 398-403 are journal articles disclosing the fast-disintegrating epinephrine tablets as described in US 2007 059361 A1.

[0017] Further prior art could be found in the references listed below:

18. USP/NF. *Physical Tests: Uniformity of Dosage Units* (905). 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008.

19. USP/NF. *Official Monograph: Epinephrine Injection*. 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008,

20. USP/NF. *Physical Tests: Tablet Friability* (1216). 31/26 ed. Rockville, MD: United States Pharmacopeial Convention, Inc.; 2008.

21. Olfert ED, Cross BM, McWilliam AA. *Guide to the care and use of experimental animals*. 2 ed. Ottawa: Canadian Council on Animal Care; 1993.

SUMMARY OF THE INVENTION

5 [0018] Epinephrine (Epi) is life-saving in the treatment of anaphylaxis. In community settings, a first-aid dose of epinephrine in an amount of 0.15 mg or 0.3 mg is injected into the mid-outer thigh by patients or caregivers using an auto-injector such as an EpiPen® (epinephrine auto-injector 0.3 or 0.15 mg, Mylan Inc., Basking Ridge, NJ). Epi auto-injectors are under-used because of needle phobia, bulky size, and high cost; additionally, there are only two fixed doses, shelf-life is only 12-18 months, and unintentional injection and injury sometimes occur.

10 [0019] The instant invention circumvents the aforementioned problems by providing a fast-disintegrating epinephrine tablet formulation for anaphylaxis treatment. Although this formulation was designed with regard to anaphylaxis, it is equally effective and contemplated for use in treatment of any condition responsive to epinephrine such as cardiac events, *i.e.* cardiac arrest, and breathing difficulties, *i.e.* asthma, bronchial asthma, bronchitis, emphysema, and respiratory infections.

15 [0020] In a validated rabbit model, this fast-disintegrating epinephrine tablet formulation resulted in plasma epinephrine concentrations similar to those achieved after a 0.3 mg epinephrine intra-muscular injection (Rawas-Qalaji et al. J Allergy Clin Immunol 117:398-403 2006). Furthermore, epinephrine was stable in these fast-disintegrating tablets for at least seven years.

20 [0021] One of the most common approaches to enhance the rate of drug dissolution and absorption is to significantly reduce its particle size to the micro- or nano-size range. Drug nanocrystals (NC) or microcrystals (MC) are advantageous due to the minimal required excipients and almost 100% of the pure drug is produced during the fabrication process¹⁷. Also, the collected dried drug NC or MC can be formulated into various dosage forms.

[0022] The phrase "epinephrine fine particles" refers to epinephrine particles of about 2.5 μm or less to about 100 nm in size and includes epinephrine nanoparticles or nanocrystals and epinephrine microparticles or microcrystals.

[0023] In one aspect, the disclosure provides epinephrine fine particles.

25 [0024] In one aspect, the disclosure provides epinephrine nanoparticles. The epinephrine can be either an epinephrine base or an epinephrine bitartrate salt.

[0025] In another aspect, the disclosure provides epinephrine nanocrystals. A nanocrystal is a nanoparticle having a crystalline structure. The term "nanocrystal" is a more specific term for describing a nanoparticle. A drug nanocrystal contains almost 100% pure drug, thus an epinephrine nanocrystal contains almost 100% pure epinephrine. A drug nanoparticle can include nanocrystals or a drug encapsulated within a polymer at different ratios. One example is the epinephrine nanoparticles comprising chitosan and tripolyphosphate (TPP) described in the previously-filed related application WO2013059629A1.

[0026] In another aspect, the disclosure provides a composition including epinephrine nanoparticles or nanocrystals capable of enhancing the sublingual bioavailability of epinephrine for the emergency treatment of anaphylaxis.

35 [0027] In another aspect, the disclosure provides "oral disintegrating tablets (ODTs)" including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals.

[0028] As described herein, buccal or sublingual oral disintegrating tablets (ODTs) are distinguished from conventional sublingual tablets, lozenges, or buccal tablets by the ODTs' ability to fully dissolve or disintegrate in less than about one minute in the mouth.

40 [0029] The disclosure also provides pharmaceutical compositions including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals in ODT form.

[0030] The disclosure also provides a pharmaceutical composition including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals and a pharmaceutically-acceptable carrier for buccal or sublingual administration.

45 [0031] The phrase "pharmaceutically-acceptable carrier" refers to an inactive and non-toxic substance used in association with an active substance, *i.e.* epinephrine, especially for aiding in the application of the active substance. Non-limiting examples of pharmaceutically-acceptable carriers are diluents, binders, disintegrants, flavorings, fillers, and lubricants. Pharmaceutically-acceptable carriers can have more than one function, *i.e.* a filler can also be a disintegrant. Additionally, pharmaceutically-acceptable carriers may also be referred to as non-medicinal ingredients (NMIs).

50 [0032] The disclosure also provides a pharmaceutical composition, for buccal or sublingual administration, including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals and at least one of a pharmaceutically-acceptable carrier, a surfactant, a penetration enhancer, and a mucoadhesive. The pharmaceutical composition can further include at least one of a taste enhancer and a sweetening agent and mouthfeel enhancer. A non-limiting example of a taste enhancer is citric acid. Citric acid masks the bitter taste of epinephrine. A non-limiting example of a sweetening agent and mouthfeel enhancer is mannitol. The pharmaceutical composition can further include at least one of a filler, a lubricant, and a disintegrant. Non-limiting examples include microcrystalline cellulose (filler), magnesium stearate (lubricant), and hydroxypropyl ethers of cellulose (disintegrant).

55 [0033] Additionally, the disclosure provides a pharmaceutical composition including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals, in which the bitter taste of the epinephrine is masked by a

taste enhancer. A non-limiting example of a taste enhancer is citric acid.

[0034] In another aspect, the disclosure provides a method for enhancing sublingual bioavailability of epinephrine in a subject in need thereof including steps for providing a composition including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. The described fast-disintegrating epinephrine tablets enhance bioavailability of epinephrine by releasing epinephrine within sixty seconds of administration.

[0035] In another aspect, the disclosure provides a method for treating a condition responsive to epinephrine in a subject in need thereof including steps for providing a composition including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. Conditions responsive to epinephrine react to administration of epinephrine. Non-limiting examples of conditions responsive to epinephrine include a cardiac event, *i.e.* cardiac arrest, or an allergic reaction, *i.e.* anaphylaxis, asthma, or bronchial asthma.

[0036] The phrase "effective amount" refers to the amount of a composition necessary to achieve the composition's intended function.

[0037] The phrase "pharmaceutically-effective dose" refers to the amount of a composition necessary to achieve a desired pharmaceutical effect. It is often desirable to use the smallest effective dose of a drug. One example of a dose range for the described epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals is approximately 10 mg to 40 mg epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals.

[0038] The phrase "therapeutically-effective amount" refers to the amount of a composition required to achieve the desired function, *i.e.* treatment of the condition responsive to epinephrine.

[0039] In another aspect, the disclosure provides a method for treating a breathing difficulty in a subject in need thereof including steps for providing a composition including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. Breathing difficulties responsive to epinephrine include, but are not limited to, breathing difficulties associated with anaphylaxis, asthma, bronchial asthma, bronchitis, emphysema, and respiratory infections.

[0040] The disclosure additionally provides a method for treatment of an allergic emergency in a subject diagnosed with or suspected of having an allergic emergency including steps for providing a composition including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. Non-limiting examples of allergic emergencies are anaphylaxis, asthma, and bronchial asthma.

[0041] In an additional aspect, the disclosure provides a method for treatment of a cardiac event in a subject diagnosed with or suspected of having a cardiac event including steps for providing a composition including epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals and at least one pharmaceutically-acceptable carrier and administering the composition to the subject. A non-limiting example of a cardiac event is cardiac arrest.

[0042] Any of the above-described epinephrine fine particles (including epinephrine nanoparticles or nanocrystals and epinephrine microparticles or microcrystals), compositions, and pharmaceutical compositions can be formulated for buccal or sublingual administration, particularly those epinephrine fine particles (including epinephrine nanoparticles or nanocrystals and epinephrine microparticles or microcrystals), compositions, and pharmaceutical compositions intended for use in emergency situations.

[0043] In another aspect, any of the above-described epinephrine fine particles (including epinephrine nanoparticles or nanocrystals and epinephrine microparticles or microcrystals) can be used in the manufacture of any of the above-described compositions and pharmaceutical compositions.

[0044] The present invention relates to a pharmaceutical composition as defined in claims 1 to 15.

[0045] Other objectives and advantages of this invention will become apparent from the following description taken in conjunction with the accompanying drawings, wherein are set forth, by way of illustration and example, certain embodiments of this invention. The drawings constitute a part of this specification and include exemplary embodiments of the present invention and illustrate various objects and features thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] A more complete understanding of the present invention may be obtained by references to the accompanying drawings when considered in conjunction with the subsequent detailed description. The embodiments illustrated in the drawings are intended only to exemplify the invention and should not be construed as limiting the invention to the illustrated embodiments.

FIG. 1 is an FTIR spectra of epinephrine bitartrate dried particles before and after processing of a 2.8 mg/mL sample processed at 30 KPsi for 1 pass (cycle).

FIG. 2 is an FTIR spectra of epinephrine bitartrate dried particles after processing of a 2.8 mg/mL sample processed

at 30 KPsi for 1 pass (cycle) and isopropyl alcohol.

FIG. 3 is a Differential Scanning Calorimetry (DSC) spectrum of epinephrine bitartrate (EpiBit) before processing.

FIG. 4 is a Differential Scanning Calorimetry (DSC) spectrum of epinephrine bitartrate (EpiBit) after processing.

FIGS. 5A-D: FIG. 5A is another view of the DSC spectrum of epinephrine bitartrate (EpiBit) before processing. FIG.

5 5B is another view of the DSC spectrum of epinephrine bitartrate (EpiBit) after processing. FIG. 5C is a Scanning Electron Microscopy (SEM) image of epinephrine bitartrate (EpiBit) before processing. FIG. 5D is a Scanning Electron Microscopy (SEM) image of epinephrine bitartrate (EpiBit) after processing.

FIG. 6 shows the mean \pm SD (n=4) cumulative diffused epinephrine per dialysis membrane area versus time.

FIG. 7 shows the mean \pm SD (n=4) percentage of diffused epinephrine through dialysis membrane versus time.

10 FIG. 8 shows the mean \pm SD (n=4) of epinephrine influx (J) through dialysis membrane.

FIG. 9 shows the mean \pm SD (n=4) of epinephrine permeability (P) through dialysis membrane.

FIG. 10 shows the mean \pm SD (n=4) cumulative diffused epinephrine per sublingual mucosa area versus time.

FIG. 11 shows the mean \pm SD (n=4) percentage of diffused epinephrine through sublingual mucosa versus time.

FIG. 12 shows the mean \pm SD (n=4) of epinephrine influx (J) through sublingual mucosa.

15 FIG. 13 shows the mean \pm SD (n=4) of epinephrine permeability (P) through sublingual mucosa.

FIG. 14 shows the mean \pm SD plasma epinephrine concentration versus time plots (n=5) after administration of epinephrine by intramuscular (IM) injection, epinephrine microcrystals sublingual (SL) tablets, epinephrine sublingual (SL) tablets, or placebo sublingual tablets.

20 FIG. 15 shows the correlation between the cumulative diffused epinephrine per area through dialysis and excised sublingual membranes.

DETAILED DESCRIPTION OF THE INVENTION

25 **[0047]** For the purpose of promoting an understanding of the principles of the invention, reference will now be made to embodiments illustrated herein and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended. Any alterations and further modification in the described compositions and methods and any further application of the principles of the invention as described herein, are contemplated as would normally occur to one skilled in the art to which the invention relates.

30 **[0048]** Epinephrine (Epi) 0.3 mg IM injection in the thigh is the drug of choice and the only available dosage form for the treatment of anaphylaxis in community settings. Previously, the instant inventors were able to develop and evaluate rapidly-disintegrating sublingual epinephrine tablets. These studies showed that sublingually administered epinephrine is absorbed and bioequivalent to 0.3 mg IM Injection in a rabbit animal-model.

35 **[0049]** For the study described herein, it was hypothesized that formulating Epi as nanocrystals (NC) or microcrystals (MC) would significantly enhance its sublingual diffusion. The objectives were to prepare Epi NC or Epi MC and formulate them into rapidly-disintegrating sublingual tablets (ODT) to be tested for their *in vitro* diffusion, *ex vivo* diffusion, and *in vivo* absorption using dialysis membranes, excised sublingual porcine mucosal membranes, and validated rabbit's animal model, respectively.

40 **[0050]** Epi NC or Epi MC were prepared by top-bottom technique using LV-1 Microfluidizer as described in a previously-filed patent application; U.S. Provisional Patent Application Serial No. 61/660,273, filed on June 15, 2012. ODTs were manufactured by direct compression using our previously developed and published formulation. The *in vitro* and *ex vivo* diffusion of 10, 20, and 40 mg Epi ODT, and 10, 20 mg Epi MC ODT (n=4) were evaluated using static vertical Franz cells. Epi 10 mg solution was used as a control. Mean \pm SD JAUC₀₋₉₀ of diffused Epi, J_{max}, and Epi influx (J) from 40 mg Epi ODT and 20 mg Epi MC ODT were not significantly different from each other both *in vitro* and *ex vivo* (p > 0.05).

45 **[0051]** The *in vivo* absorption of 40 mg Epi ODT and 20 mg Epi MC ODT (n=5) were evaluated in a validated rabbits animal-model. Epi 0.3 mg IM injection in the thigh was used as a positive control and placebo ODT was used as a negative control. The mean \pm SD AUC₀₋₆₀ and C_{max} from 20 mg Epi MC ODT and 40 mg Epi ODT did not differ significantly (p > 0.05) from Epi 0.3 mg IM. However, the mean \pm SD AUC₀₋₆₀ and C_{max} of exogenous epinephrine administered through either the sublingual or intramuscular routes differed significantly (p < 0.05) from placebo sublingual tablets, endogenous epinephrine.

50 **[0052]** These micro-sized Epi ODT improved Epi diffusion by two folds and have the potential to reduce the bioequivalent dose of sublingually administered Epi by 50%. These micro-sized Epi ODT have the potential for the first-aid treatment of anaphylaxis in community settings are suitable for phase I studies in humans.

55 **[0053]** For the emergency treatment of anaphylaxis, prompt intramuscular injection of epinephrine (Epi) in the thigh muscle is the drug of choice. Epi auto-injectors such as EpiPen®, EpiPen Jr® (Mylan Inc, Basking Ridge, NJ), Twinject 0.3 mg®, and Twinject 0.15® (Shionogi Pharma, Inc. Atlanta, GA) are commonly prescribed and the only available dosage form for the first-aid emergency treatment of anaphylaxis in a community setting. However, self-injectable epinephrine is underutilized when anaphylaxis occurs due to several drawbacks.

[0054] The sublingual route is a promising alternative route for Epi administration. Drugs that can be absorbed sub-

lingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form. Epi is extensively metabolized after oral administration by the catechol-O-methyltransferase in the gastrointestinal tract and by monoamine oxidase in the gastrointestinal tract and in the liver.

5 **[0055]** The high vascularity of the sublingual mucosa and the low molecular weight of Epi facilitate its rapid absorption directly into the venous circulation through the sublingual and frenular veins. The described rapidly-disintegrating sublingual 40 mg Epi tablets, which retain sufficient hardness to withstand shipping and handling and disintegrate to release Epi rapidly (≤ 30 sec), have shown to be bioequivalent to the adult dose of Epi IM injection, 0.3 mg, in a validated rabbit model. This high dose was essential to create the required concentration gradient that promotes Epi absorption across

10 the sublingual membrane and results in therapeutic plasma drug concentrations.
[0056] One of the most common approaches to enhance the rate of drug dissolution and absorption is to significantly reduce its particles size to the micro- or nano-size range. Drug nanocrystals (NC) or microcrystals (MC) are advantageous due to minimal required excipients and almost 100% of the pure drug is produced during the fabrication process. Also, the collected dried drug NC or MC can be formulated into various dosage forms.

15 **[0057]** In designing the experiments described herein, it was hypothesized that using reduced particle size of Epi instead of regular raw Epi crystals will significantly increase Epi dissolution rate and absorption. Also, they would reduce the required bioequivalent dose to Epi 0.3 mg IM injections.

[0058] In the study described herein, the *in vitro* and *ex vivo* diffusion of epinephrine bitartrate microcrystals (EpiBit MC) against regular epinephrine bitartrate (EpiBit) crystals formulated into our rapidly-disintegrating tablets (ODT) was tested to evaluate the permeability of these micro-sized Epi ODT before performing *in vivo* studies.

20 **[0059]** In the *in vivo* study, the absorption of epinephrine bitartrate microcrystals (EpiBit MC) and regular epinephrine bitartrate (EpiBit) crystals formulated into our rapidly-disintegrating tablets (ODT) was tested against the standard Epi 0.3 mg IM injection in the thigh. The aim was to establish a significantly lower bioequivalent sublingual dose of Epi than the one previously achieved.

25 **[0060]** These rapidly-disintegrating sublingual epinephrine tablets will have the potential as user-friendly, non-invasive alternative for the first-aid emergency treatment of anaphylaxis in a community setting.

MATERIALS

30 **[0061]** These materials are useful for the *in vitro* and *ex vivo* diffusion studies described below and for the fabrication of epinephrine fine particles and tablets.

[0062] (-)-Epinephrine (+) bitartrate was purchased from Sigma-Aldrich (St. Louis, MO). Ceolus® PH-301 (microcrystalline cellulose) with a mean particle size of 50 μm was supplied by Asahi Kasei Chemicals Corp (Tokyo, Japan) and low-substituted hydroxypropyl cellulose (LH11) with a mean particle size of 50 μm was supplied by Shin-Etsu Chemical Co (Tokyo, Japan). Magnesium stearate was purchased from Mallinckrodt Baker (Phillipsburg, NJ). Isopropyl alcohol, 99.5%, was purchased from BDH (VWR, West Chester, PA). Spectra/Por® 7 dialysis membranes with 1000 Dalton MWCO were purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Potassium phosphate monobasic was purchased from Sigma-Aldrich (St. Louis, MO) and sodium hydroxide was purchased from J.T. Baker (Phillipsburg, NJ).

40 Fabrication and Characterization of Epinephrine Fine Particles Using High Shear Fluid Processor (Microfluidizer)-Homogenixation Method

45 **[0063]** Epinephrine bitartrate fine particles were fabricated, developed, and characterized as described in the previously-filed related application; U.S. Provisional Patent Application Serial No. 61/660,273, filed on June 15, 2012.

PREPARATION OF EPINEPHRINE BITARTRATE NANOCRYSTALS

50 **[0064]** The EpiBit NC (or EpiBit MC) was prepared by a top-bottom technique using LV-1 High Sheer Fluid Processor "Microfluidizer" (Microfluidics, Newton, MA) equipped with G10Z reaction chamber. Briefly, epinephrine bitartrate (2.8 mg/mL), (with and without the use of any excipients), was suspended in 6 mL isopropyl alcohol, sonicated for 30 seconds and injected into the system. The suspension was processed at 30,000 Psi for one cycle. The microfluidizer-receiving coil was immersed in ice to reduce the heat produced during the process. The nanosuspension was centrifuged using Avanti J-25 centrifuge (Beckman Coulter, Inc, Miami, FL) at 15,000 rpm and 15°C for 30 minutes. The upper clear solvent

55 was removed by aspiration and the remaining particles were dried by vacuum concentrator at room temperature.

CHARACTERISTICS OF THE EPINEPHRINE BITARTRATE NANOCRYSTALS

Particle Size and Zeta Potential Measurement

5 **[0065]** The average particles size (by volume) of EpiBit before processing was measured using laser diffraction technique using Mastersizer (Malvern Instruments Inc, Westborough, MA). D (0.1), D (0.5) or median, D (0.9), and D (4, 3) or mean volume are shown in Table 1.

10 **[0066]** Mean \pm SD particles size distribution (by volume) of EpiBit crystals before processing was $131.8\pm 10.5 \mu\text{m}$ (n=6). The 10th percentile (Dv0.1), median (Dv0.5), and 90th percentile (Dv0.9) were $39.8 \pm 3.0 \mu\text{m}$, $113.6 \pm 9.1 \mu\text{m}$, and $254.8 \pm 20.1 \mu\text{m}$, respectively.

Table 1: Particles Size Distribution (by Volume) of EpiBit Before Processing

Before Fabrication (μm)				
Sample #	D (4,3)	D (0.1)	D (0.5)	D (0.9)
1	147.1	44.4	128.0	282
2	129.5	40.27	111.4	249.6
3	121.6	37.4	105.2	234.7
4	136.0	41.0	117.5	262
5	137.2	40.25	116.1	269.6
6	119.2	35.7	103.3	230.7
Mean	131.8	39.8	113.6	254.8
Standard Deviation	10.5	3.0	9.1	20.1

30 **[0067]** The Z-average particles size (by intensity) and the average zeta potential of EpiBit after processing were measured using light scattering technique using Zetasizer ZS90 (Malvern Instruments Inc, Westborough, MA). Z-average with polydispersity index (Pdi) and zeta potential are shown in Table 2.

[0068] Mean (\pm SD) particles size distribution by intensity and by volume, Pdi, and zeta potential (n=3) of EpiBit crystals after processing using the microfluidizer for one cycle at 30,000 Psi were $2.4\pm 0.4 \mu\text{m}$, $2.5\pm 0.4 \mu\text{m}$, 0.185 ± 0.019 , and $-4.5\pm 1.4 \text{mV}$, respectively.

35 **[0069]** The processing of EpiBit results in fine particles with a mean particle size at the low end of the micro-size range but approaching the nano-size range. The particles of this size range were used for diffusion studies and *in vivo* animal studies.

Table 2: Particles Size Distribution (by intensity) and zeta potential of EpiBit After Processing

After Fabrication			
Sample #	Z-average (d.nm)	Pdi	Z-potential (mV)
1	2649	0.187	-6.0
2	1958	0.165	-3.4
3	2615	0.202	-4.0
Mean	2407.3	0.185	-4.5
Standard Deviation	389.5	0.019	1.4

Fourier Transformation InfraRed (FT-IR)

55 **[0070]** The processed EpiBit were tested for stability and removal of isopropyl alcohol using FT-IR spectrometer, spectrum 100 (PerkinElmer, Waltham, MA) scanned from $4000\text{-}650 \text{cm}^{-1}$. The FT-IR spectrum of EpiBit before and after processing is shown in FIG. 1. There was no evidence of EpiBit degradation after processing as the spectra before and after processing were similar.

[0071] The FT-IR spectrum of isopropyl alcohol and EpiBit after processing is shown in FIG. 2. The isopropyl alcohol

peaks are missing, which indicates successful removal of the isopropyl alcohol. Thus, there was no evidence of isopropyl alcohol remaining in the EpiBit particles after drying as shown in the spectrum of processed EpiBit.

Differential Scanning Calorimetry (DSC)

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 [0072] Also, the processed EpiBit were tested for purity, stability, and crystallinity changes using Differential Scanning Calorimetry (DSC) 4000 (PerkinElmer, Waltham, MA) that was calibrated using an indium standard and heated from 30 to 300 °C at rate of 10 °C/min and with a nitrogen purge of 20 mL/min. The DSC spectra of EpiBit before and after processing are shown in FIGS. 3 and 4, respectively. There was no evidence of EpiBit degradation or crystallinity change after processing. FIG. 5A shows another view of a DSC spectrum of EpiBit before processing and FIG. 5B shows another view of a DSC spectrum after processing. These spectra (FIGS. 5A and 5B) are similar before and after processing.

Scanning Electron Microscopy (SEM)

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 [0073] The morphologies of EpiBit before and after processing were examined using Quanta 200 Environmental Scanning Electron Microscope (FEI, Hillsboro, OR) operated at an accelerating voltage of 20 kV. Fresh suspension of processed EpiBit and a fresh dispersion of unprocessed EpiBit were deposited on an aluminum stub following the evaporation of isopropyl alcohol and sputter coated with gold using Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, England). The Scanning Electron Microscopy (SEM) images of EpiBit before and after processing are shown in FIGS. 5C and 5D, respectively. There was a morphological change in the EpiBit crystals from a rectangular shape before processing to a smaller, spherical shape after processing.

RAPIDLY-DISINTEGRATING EPINEPHRINE SUBLINGUAL TABLET FORMULATION

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 [0074] Rapidly-disintegrating tablets for sublingual administration were developed and evaluated as described in the previously-filed related applications; U.S. Utility Patent Application Serial No. 11/672,503, filed on February 7, 2007 and U.S. Utility Patent Application Serial No. 11/530,360, filed on September 8, 2006. A range of epinephrine (Epi) doses were formulated as rapidly-disintegrating tablets using equivalent amounts of regular L-epinephrine bitartrate (EpiBit) obtained from Sigma-Aldrich or nanocrystals (NC) or microcrystals (MC) of EpiBit fabricated as previously described. Tablets containing 10, 20, and 40 mg Epi and 10 and 20 mg Epi MC were manufactured using equivalent amounts of EpiBit.

Manufacturing and Quality Control of Tablets for *In Vitro* and *Ex Vivo* Diffusion Studies

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 [0075] Five ODT formulations containing EpiBit equivalent to 10 mg, 20 mg, and 40 mg, epinephrine and EpiBit MC equivalent to 10 mg and 20 mg epinephrine were manufactured by direct compression. These tablets were formulated using microcrystalline cellulose, low-substituted hydroxypropyl cellulose, and magnesium stearate as described in our previous studies. The tablet weight was 150 mg. All excipients were used as supplied and kept under low humidity condition before mixing. The mixing process was performed in a nitrogen-preflushed opaque glass container using three-dimensional manual mixer (Inversina, Bioengineering AG, Wald, Switzerland). The powder mixture of the five tablet formulations was compressed right after mixing using 4-stations Colton rotary press (Key Industries, Englishtown, NJ) at a pre-selected compression force for each tablet formulation, based on our previous results to ensure sufficient hardness to withstand shipping and handling while maintaining rapid tablet disintegration.

[0076] All tablet formulations were tested for quality control as follows:

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Dimensions: Six tablets were randomly selected from each formulation. The diameter and the thickness of rapidly-disintegrating Epi tablets were measured using digital caliper with a range of 0-100 mm and accuracy of 0.02 (Harbor Freight Tools, Camarillo, CA). The mean \pm SD (mm) and RSD% of tablets' diameters and thicknesses are shown in Table 3.

Hardness: Six tablets were randomly selected from each formulation. The hardness or the breaking force of rapidly-disintegrating Epi tablets was measured using Hardness Tester LIH-3 (Vanguard, Spring, TX). The mean \pm SD (Kgf) and RSD% of hardness for various tablet formulations are shown in Table 3.

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Disintegration Time: Six tablets were randomly selected from each formulation. The disintegration time of rapidly-disintegrating Epi tablets was measured using a previously developed and published method to discriminate between the disintegration times of rapidly-disintegrating tablets or orally disintegrating tablets. The mean \pm SD (Sec) and RSD% of disintegration time for various tablet formulations are shown in Table 3.

USP Weight Variation Test: Tablet weight variation was measured using the USP methods and criteria¹⁸. The mean \pm SD (%) and RSD% of weight variation for various tablet formulations are shown in Table 3.

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USP Content Uniformity Test: Tablet drug content uniformity was measured using the USP methods and criteria¹⁸. Drug content was analyzed using a High Performance Liquid Chromatography (HPLC) system with ultraviolet detection (UV) (PerkinElmer, Waltham, MA) according to USP¹⁹. The mean \pm SD (%) and RSD% of content uniformity for various tablet

formulations are shown in Table 3.

USP Friability Test: The friability of rapidly-disintegrating Epi tablets was measured using USP Friability Tester LIC-1 (Vanguard, Spring, TX) according to USP methods and criteria¹⁸. The mean tablets weight loss (%) for various tablet formulations are shown in Table 3.

Mean \pm SD hardness, disintegration time, weight variation, content uniformity, and friability for 10 mg, 20 mg, and 40 mg Epi, and 10 mg and 20 mg Epi MC tablets are shown in Table 3. All tablet formulations were within UDP criteria for weight variation, drug content uniformity, and friability^{18,20}.

Table 3: The mean \pm SD hardness (n=6), disintegration time, weight variation, content uniformity, tablet diameter, tablet thickness, and friability for 10 mg, 20 mg, and 40 mg tablet formulations*

Formulations	Tablets Characteristics*						
	H	DT	WV (RSD%)	CU (RSD%)	D	T	F
10 mg Epi Tablets	1.7 \pm 0.3	16.3 \pm 0.3	100.0 \pm 0.0 (0.0)	100.6 \pm 4.0 (4.0)	7.9 \pm 0.0	3.5 \pm 0.0	0.4
20 mg Epi Tablets	1.6 \pm 0.1	15.8 \pm 0.4	99.9 \pm 0.7 (0.7)	97.7 \pm 2.7 (2.7)	7.9 \pm 0.0	3.9 \pm 0.0	0.5
40 mg Epi Tablets	1.7 \pm 0.2	31.3 \pm 0.4	100.0 \pm 0.6 (0.6)	95.6 \pm 2.4 (2.5)	7.9 \pm 0.0	3.4 \pm 0.0	0.6
10 mg Epi MC Tablets	2.3 \pm 0.0	5.5 \pm 0.7	99.7 \pm 1.2 (1.2)	92.9 \pm 0.3 (0.3)	8.0 \pm 0.1	3.7 \pm 0.0	NA
20 mg Epi MC Tablets	2.5 \pm 0.1	8.7 \pm 0.3	98.3 \pm 1.7 (1.7)	92.2 \pm 4.2 (4.5)	8.0 \pm 0.1	NA	NA

*H indicates tablet hardness (kgf); DT, disintegration time (sec); WV, weight variation (%); CU, content uniformity (%); RSD, relative standard deviation (%); D, tablet diameter (mm); T, tablet thickness (mm); F, Friability (%).

Manufacturing and Quality Control of Tablets for *In Vivo* Absorption Studies

Additionally, five ODT formulations containing EpiBit equivalent to 0 mg and 40 mg Epi and EpiBit MC equivalent to 20 mg Epi were manufactured by direct compression. These tablets were formulated and manufactured using the same excipients and method in our previous studies. All tablet formulations were tested for tablet weight variation, drug content uniformity, and friability using the harmonized USP methods and criteria^{18,20}. Also, they were tested for disintegration time using a novel *in vitro* disintegration test developed to simulate the sublingual environment. Drug content was analyzed using a high performance liquid chromatography (HPLC) system with ultra violet (UV) detection (PerkinElmer, Waltham, MA) according to USP method for Epi injections¹⁹.

These tablets did not contain lactose or bisulfite and met USP standards for tablet weight variation, content uniformity, and friability^{18,20}. They also disintegrated in less than 30 seconds.

Methods for *In Vitro* and *Ex Vivo* Diffusion Studies

The *in vitro* and *ex vivo* diffusion of EpiBit MC and EpiBit formulated into ODT were evaluated using static vertical jacketed Franz Cells with OD of 20 mm and reservoir volume of 20 \pm 1 mL (PermeGear Inc., Hellertown, PA). For *in vitro* diffusion studies, 7 Spectra/Por® dialysis membranes with 1000 Dalton MWCO (Spectrum Laboratories, Inc., Rancho Dominguez, CA) were used as the diffusion membranes. For *ex vivo* diffusion studies, sublingual mucosa (floor of the mouth) were excised from pigs and used as the diffusion membranes. Frozen pig's heads were obtained from a local abattoir and defrosted at room temperature. The porcine mucosa were excised by dissecting the sublingual mucosa and removing the underlying connective tissue using a scalpel and fine tweezers using established surgical technique. The excised mucosa were inspected for integrity and then frozen on aluminum foil at -20 °C until used (<4 weeks). The mucosal membranes were defrosted at room temperature before each experiment.

Four ODT containing EpiBit equivalent to 10, 20, and 40 mg Epi or EpiBit MC equivalent to 10, and 20 mg Epi were tested *in vitro* and *ex vivo*. EpiBit equivalent to 10 mg Epi was dissolved in 1 mL of the diffusion medium and used as a control (n=4).

The receptor chamber that has a magnetic stirrer was filled with phosphate buffer, pH 5.8 (saliva average pH), as the diffusion medium. Air bubbles were removed after mounting the membrane between the donor and receptor chambers and before the beginning of the experiment. The water bath was set at 37 °C and water was circulated in the jacketed Franz Cells. The mounted membranes were equilibrated with the diffusion medium for 30 minutes from both

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sides before the experiment and were checked for any leaks.

[0082] The tested tablet was placed at the center of the donor chamber on the membrane at T_0 and 2 mL of the diffusion medium was added to facilitate tablet disintegration and dissolution. Aliquots, 200 μL , were withdrawn from the receptor chamber using 6 inch-long needles (Popper & Sons, Inc, New Hyde Park, NY) and 1 mL syringes at 5, 10, 15, 20, 30, 45, 60, 75, and 90 min. The withdrawn volumes were replaced with fresh medium. Samples were transferred to HPLC vials for HPLC analysis using UV detector as described below.

Epinephrine HPLC Analysis

[0083] Samples from tablets for content uniformity test and from diffusion studies were analyzed for Epi content according to USP method for Epi injection analysis¹⁹ using HPLC system with UV detection (PerkinElmer, Waltham, MA). The calibration curve was linear over the range of 6.25 to 200.0 $\mu\text{g/mL}$ with correlation of coefficients (R^2) of > 0.99 ($n=5$). The coefficient of variation (RSD%) of the system reproducibility at concentrations of 6.25 and 200 $\mu\text{g/mL}$ ($n=5$ each) were 1.07% and 0.40%, respectively. The intra- and inter-assay RSD% were 0.40% and 0.70% ($n=2$) and 2.8% and 1.5% ($n=3$), respectively.

Data Analysis

[0084] The mean \pm SD cumulative diffused Epi per area ($\mu\text{g/cm}^2$) and percentage of diffused Epi for each ODT formulation were calculated. The mean \pm SD Epi influx, J ($\mu\text{g/cm}^2/\text{min}$), and lag time, t_L (min), were calculated from the slope and the intercept with the x-axis of each graph ($n=4$). Also, Epi permeability, P (cm/min), was calculated by dividing J by Epi concentration in the donor chamber at T_0 . The area under the curve of diffused Epi per area, JAUC_{0-90} ($\mu\text{g/cm}^2/\text{min}$); the maximum Epi diffused, J_{max} ($\mu\text{g/cm}^2$); and the time to reach J_{max} , T_{max} (min) were calculated using WinNonlin software (Pharsight, Mountain View, CA). Data were statistically compared by one-way ANOVA and Tukey-Kramer tests using NCSS statistical software (NCSS, Kaysville, UT). Differences were considered to be statistically significant at $p < 0.05$.

Results

1) The *In Vitro* Diffusion of Epinephrine Microcrystals Sublingual Tablets

[0085] The mean \pm SD ($n=4$) cumulative diffused Epi per area and percentage of diffused Epi for each formulation through dialysis membrane are shown in Tables 4 and 5, and illustrated in FIGS. 6 and 7, respectively.

Table 4: Mean \pm SD ($n=4$) cumulative diffused epinephrine per area ($\mu\text{g/cm}^2$) for each formulation through dialysis membrane.

Time (min)	10 mg Epi Tablet	10 mg Epi MC Tablet	20 mg Epi Tablet	20 mg Epi MC Tablet	40 mg Epi Tablet
5	62.1 \pm 9.3	99.2 \pm 30.9	456.1 \pm 130.5	735.8 \pm 101.0	835.2 \pm 107.8
10	183.9 \pm 25.0	321.8 \pm 153.9	1499.6 \pm 694.8	1642.4 \pm 370.1	1934.6 \pm 391.7
15	329.5 \pm 7.6	466.7 \pm 123.4	1764.3 \pm 337.7	2431.0 \pm 659.0	3573.7 \pm 240.0
20	436.2 \pm 142.4	668.4 \pm 262.0	2600.7 \pm 996.2	3386.2 \pm 770.8	4673.6 \pm 833.3
30	606.3 \pm 91.4	744.5 \pm 223.4	3781.5 \pm 1127.9	4112.5 \pm 1235.6	5075.7 \pm 625.2
45	731.9 \pm 90.3	873.4 \pm 339.0	3207.6 \pm 1180.6	5085.0 \pm 698.4	6504.1 \pm 105.3
60	683.2 \pm 201.9	1198.9 \pm 288.5	3739.7 \pm 1315.3	5325.4 \pm 745.5	6421.7 \pm 1041.7
75	876.3 \pm 497.1	906.7 \pm 364.6	4602.4 \pm 857.2	6568.8 \pm 755.3	7585.8 \pm 1554.4
90	888.1 \pm 149.7	1235.3 \pm 419.9	4614.7 \pm 824.0	6554.1 \pm 804.0	7337.4 \pm 725.6

Table 5: Mean \pm SD ($n=4$) percentage of diffused epinephrine (%) for each formulation through dialysis membrane.

Time (min)	10 mg Epi Tablet	10 mg Epi MC Tablet	20 mg Epi Tablet	20 mg Epi MC Tablet	40 mg Epi Tablet
5	2.0 \pm 0.3	3.1 \pm 1.0	7.2 \pm 2	11.6 \pm 1.6	6.6 \pm 0.8
10	5.8 \pm 0.8	10.1 \pm 4.8	23.5 \pm 10.9	25.8 \pm 5.8	15.2 \pm 3.1
15	10.4 \pm 2.5	14.7 \pm 3.9	27.7 \pm 5.3	38.2 \pm 10.3	28.1 \pm 1.9
20	13.8 \pm 4.6	21.0 \pm 8.2	40.8 \pm 15.6	53.2 \pm 12.1	36.7 \pm 6.5

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(continued)

Time (min)	10 mg Epi Tablet	10 mg Epi MC Tablet	20 mg Epi Tablet	20 mg Epi MC Tablet	40 mg Epi Tablet
30	19.1±2.9	23.4±7.0	59.4±17.7	64.6±19.4	39.8±4.9
45	23.1±2.8	27.4±10.6	50.4±18.5	79.8±11.0	51.1±0.8
60	21.6±6.4	37.6±9.1	58.7±20.7	83.6±11.7	50.4±8.2
75	27.7±15.8	28.5±11.4	72.3±13.5	103.1±11.9	59.5±12.2
90	28.0±4.7	38.8±13.2	72.5±12.9	102.9±12.6	57.6±5.7

[0086] The mean (\pm SD) Epi $JAUC_{0-90}$, J_{max} , T_{max} , J , P , and t_1 are shown in Table 6. Also, Epi J and P for each formulation are illustrated in FIGS. 8 and 9, respectively.

[0087] The mean (\pm SD) Epi $JAUC_{0-90}$ and J_{max} of 40 mg Epi tablets ($484184.9 \pm 29655.9 \mu\text{g}/\text{cm}^2/\text{min}$ and $7508.3 \pm 568.7 \mu\text{g}/\text{cm}^2$, respectively) and 20 mg Epi MC tablets ($402852.2 \pm 55299 \mu\text{g}/\text{cm}^2/\text{min}$ and $6727.2 \pm 736.3 \mu\text{g}/\text{cm}^2$, respectively) were not significantly different ($p > 0.05$) from each other and were significantly higher ($p < 0.05$) than the rest of the formulations (FIG. 6 and Table 6). The Epi T_{max} was not significantly different ($p > 0.05$) between all formulations (Table 6).

[0088] The mean (\pm SD) Epi J of 40 mg Epi tablets ($234.2 \pm 99.6 \mu\text{g}/\text{cm}^2/\text{min}$) and 20 mg Epi MC tablets ($172.2 \pm 49.8 \mu\text{g}/\text{cm}^2/\text{min}$) were not significantly different ($p > 0.05$) from each other and were significantly higher ($p < 0.05$) than the 10 mg Epi tablets and 10 mg Epi MC tablets (FIG. 8 and Table 6). The Epi t_L was not significantly different ($p > 0.05$) between all formulations (Table 6).

[0089] The mean (\pm SD) Epi P of 20 mg Epi MC tablets ($17.2 \pm 5.0 \text{ cm}/\text{min}$) was significantly higher ($p < 0.05$) than the rest of the formulations (FIGS. 7 and 9, and Table 6).

Table 6: Mean \pm SD (n=4) of epinephrine JAU_{C₀₋₉₀}, J_{max}, T_{max}, J, P, and t_L for each formulation through dialysis membrane.

	10 mg Epi Tablet	10 mg Epi MC Tablet	20 mg Epi Tablet	20 mg Epi MC Tablet	40 mg Epi Tablet
JAU _{C₀₋₉₀} (μ g/cm ² /min)	54604.1 \pm 11332.5	72461 \pm 21229.2	292089 \pm 58875.7	402852.2 \pm 55299	484184.9 \pm 29655.9
J _{max} (μ g/cm ²)	1070.9 \pm 384.2	1297.8 \pm 305.3	5093.8 \pm 249.5	6727.2 \pm 736.3	7508.3 \pm 568.7
T _{max} (min)	78.8 \pm 14.4	82.5 \pm 15.0	71.3 \pm 28.4	86.3 \pm 7.5	82.5 \pm 8.7
J(μ g/cm ² /min)	22.1 \pm 4.1	37.0 \pm 13.6	128.6 \pm 39.2	112.2 \pm 49.8	234.2 \pm 99.6
P(cm/min)	4.4 \pm 0.8	7.4 \pm 2.7	12.9 \pm 3.9	17.2 \pm 5.0	11.7 \pm 5.0
t _L (min)	1.4 \pm 0.9	2.0 \pm 0.8	0.5 \pm 1.0	0.0 \pm 0.0	1.6 \pm 1.4

JAU_{C₀₋₉₀}, area under the curve of diffused Epi per area versus time; J_{max}, the maximum Epi diffused; T_{max}, the time to reach J_{max}; J, Epi influx; P, Epi permeability; t_L, lag time.

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[0090] The $JAUC$, J_{max} , J , P for 20 mg Epi MC tablets was not significantly different ($p > 0.05$) from 40 mg Epi tablets *in vitro*. The reduction of EpiBit particles size close to the nano-size range increased EpiBit influx two folds, which presents a great potential for these reduced sized Epi ODT to reduce the required Epi sublingual dose by half.

5 2) The *Ex Vivo* Diffusion of Epinephrine Microcrystals Sublingual Tablets

[0091] The mean \pm SD ($n=4$) cumulative diffused Epi per area and percentage of diffused Epi for each formulation through sublingual mucosa are shown in Tables 7 and 8, and illustrated in FIGS. 10 and 11, respectively.

10 **Table 7:** Mean \pm SD ($n=4$) cumulative diffused epinephrine per sublingual mucosa area ($\mu\text{g}/\text{cm}^2$) for each formulation through sublingual mucosa.

Time (min)	10 mg Epi Solution	10 mg Epi Tablet	10 mg Epi MC Tablet	20 mg Epi Tablet	20 mg Epi MC Tablet	40 mg Epi Tablet
5	24.5 \pm 8.7	16.8 \pm 12.7	32.5 \pm 27.4	40.2 \pm 44.9	176.1 \pm 128.7	156.6 \pm 159.4
10	803 \pm 26.5	72.5 \pm 50.5	161.5 \pm 80.8	124.5 \pm 123.1	639.1 \pm 469.1	622.5 \pm 559.3
15	143.0 \pm 40.5	182.3 \pm 104.3	296.7 \pm 110.0	232.5 \pm 217.1	1211.1 \pm 808.0	1147.4 \pm 1023.4
20	198.9 \pm 56.5	248.0 \pm 116.9	401.2 \pm 110.1	341.7 \pm 302.1	1588.9 \pm 998.6	1689.4 \pm 1437.7
30	219.8 \pm 70.2	286.7 \pm 88.7	465.5 \pm 110.1	525.7 \pm 444.6	2161.7 \pm 1285.2	2415.0 \pm 1834.7
45	273.8 \pm 96.2	341.0 \pm 37.6	499.0 \pm 88.7	664.9 \pm 501.1	2628.4 \pm 1496.8	3311.4 \pm 2321.8
60	248.7 \pm 60.5	364.3 \pm 75.9	488.9 \pm 86.8	898.1 \pm 643.1	3037.6 \pm 1574.1	3989.8 \pm 2648.3
75	266.1 \pm 73.4	390.0 \pm 47.8	479.5 \pm 80.0	1072.8 \pm 733.2	3435.1 \pm 1828.8	4464.8 \pm 2928.8
90	277.2 \pm 80.8	430.1 \pm 100.1	478.4 \pm 58.9	1263.1 \pm 807.6	3496.3 \pm 1722.8	4795.7 \pm 2988.2

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Table 8: Mean \pm SD ($n=4$) percentage of diffused epinephrine (%) for each formulation through sublingual mucosa.

Time (min)	10 mg Epi Solution	10 mg Epi Tablet	10 mg Epi MC Tablet	20 mg Epi Tablet	20 mg Epi MC Tablet	40 mg Epi Tablet
5	1.1 \pm 0.7	0.5 \pm 0.4	1.0 \pm 0.9	0.6 \pm 0.7	2.8 \pm 2.0	1.2 \pm 1.3
10	3.1 \pm 1.2	2.3 \pm 1.6	5.1 \pm 2.5	2.0 \pm 1.9	10.0 \pm 7.4	4.9 \pm 4.4
15	5.0 \pm 1.5	5.7 \pm 3.3	9.3 \pm 3.5	3.7 \pm 3.4	19.0 \pm 12.7	9.9 \pm 8.0
20	6.5 \pm 1.8	7.8 \pm 3.7	12.6 \pm 3.5	5.4 \pm 4.7	24.9 \pm 15.7	13.3 \pm 11.3
30	7.1 \pm 2.3	9.1 \pm 2.8	14.6 \pm 3.2	8.3 \pm 7.0	33.9 \pm 20.2	19.0 \pm 14.4
45	8.7 \pm 3.0	10.7 \pm 1.2	15.7 \pm 2.8	10.4 \pm 7.9	41.3 \pm 23.5	26.0 \pm 18.2
60	8.0 \pm 2.0	11.4 \pm 2.4	15.4 \pm 2.7	14.1 \pm 10.1	47.7 \pm 24.7	31.3 \pm 1020.8
75	8.5 \pm 2.4	12.2 \pm 1.5	15.1 \pm 2.5	16.8 \pm 11.5	53.9 \pm 28.7	35.0 \pm 23.0
90	8.6 \pm 2.5	13.5 \pm 3.1	15.0 \pm 1.8	19.8 \pm 12.7	54.9 \pm 27.0	37.6 \pm 23.5

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[0092] The mean (\pm SD) Epi $JAUC_{0-90}$, J_{max} , T_{max} , J , P , and t_L are shown in Table 9. Also, Epi J and P for each formulation are illustrated in FIGS. 12 and 13, respectively.

[0093] The mean Epi $JAUC_{0-90}$ and J_{max} of 40 mg Epi tablets (264556.4 \pm 182820.3 $\mu\text{g}/\text{cm}^2/\text{min}$ and 4795.7 \pm 2988.2 $\mu\text{g}/\text{cm}^2$, respectively) and 20 mg Epi MC tablets (211368.5 \pm 116025.1 $\mu\text{g}/\text{cm}^2/\text{min}$ and 3526.8 \pm 1754.6 $\mu\text{g}/\text{cm}^2$, respectively) were not significantly different ($p > 0.05$) from each other and 40 mg Epi tablets was significantly higher ($p < 0.05$) than the rest of the formulations (FIG. 10 and Table 9).

[0094] The Epi J of 40 mg Epi tablets (106.0 \pm 82.4 $\mu\text{g}/\text{cm}^2/\text{min}$) and 20 mg Epi MC tablets (91.1 \pm 54.6 $\mu\text{g}/\text{cm}^2/\text{min}$) were not significantly different ($p > 0.05$) from each other but due to the high variability there were not significantly different ($p > 0.05$) from 20 mg Epi tablets (19.9 \pm 16.0 $\mu\text{g}/\text{cm}^2/\text{min}$) and 10 mg Epi tablets (24.8 \pm 6.5 $\mu\text{g}/\text{cm}^2/\text{min}$) as well (FIG. 12 and Table 9). The Epi J of 40 mg Epi tablets was only significantly higher ($p < 0.05$) than the 10 mg Epi solution (11.7 \pm 3.2 $\mu\text{g}/\text{cm}^2/\text{min}$) and 10 mg Epi tablets (17.1 \pm 6.7 $\mu\text{g}/\text{cm}^2/\text{min}$) (FIG. 12 and Table 9). The Epi t_L was not significantly different ($p > 0.05$) between all formulations (Table 9).

[0095] The Epi P of 20 mg Epi MC tablets (9.1 \pm 5.5 cm/min) and 40 mg Epi tablets (5.3 \pm 4.1 cm/min) were not significantly different ($p > 0.05$) from each other and 20 mg Epi MC tablets was significantly higher ($p < 0.05$) than 20 mg Epi tablets (2.0 \pm 1.6 cm/min) (FIGS. 11 and 13, and Table 9).

[0096] All the diffusion parameters for both 10 mg Epi solution and 10 mg Epi ODT (Table 9) were not significantly different ($p > 0.05$) from each other.

Table 9: Mean \pm SD (n=4) of epinephrine $J AUC_{0,90}$, J_{max} , T_{max} , J , P , and t_L for each formulation through sublingual mucosa.

	10 mg Epi Solution	10 mg Epi Tablet	10 mg Epi MC Tablet	20 mg Epi Tablet	20 mg Epi MC Tablet	40 mg Epi Tablet
$J AUC_{0,90}$ ($\mu\text{g}/\text{cm}^2/\text{min}$)	19325.8 \pm 5599.3	26441.6 \pm 5651.6	36799.7 \pm 7226.5	60031.0 \pm 43809.8	211368.5 \pm 116025.1	264556.4 \pm 182820.3
J_{max} ($\mu\text{g}/\text{cm}^2$)	236.4 \pm 101.9	436.7 \pm 96.9	507.2 \pm 81.4	1263.1 \pm 807.6	3526.8 \pm 1754.6	4795.7 \pm 2988.3
T_{max} (min)	75.0 \pm 21.2	86.3 \pm 7.5	48.8 \pm 18.9	90.0 \pm 0.0	82.5 \pm 8.7	90.0 \pm 0.0
J ($\mu\text{g}/\text{cm}^2/\text{min}$)	11.7 \pm 3.2	17.1 \pm 6.7	24.8 \pm 6.5	19.9 \pm 16.0	91.1 \pm 54.6	106.0 \pm 82.4
P (cm/min)	2.3 \pm 0.6	3.4 \pm 1.3	5.0 \pm 1.3	2.0 \pm 1.6	9.1 \pm 5.5	5.3 \pm 4.1
t_L (min)	2.9 \pm 0.4	5.8 \pm 2.0	3.6 \pm 1.5	5.1 \pm 2.8	3.0 \pm 2.4	5.2 \pm 2.3

$J AUC_{0,90}$, area under the curve of diffused Epi per area versus time; J_{max} , the maximum Epi diffused; T_{max} , the time to reach J_{max} ; J , Epi influx; P , Epi permeability; t_L , lag time.

[0097] The $JAUC$, J_{max} , J , P for 20 mg Epi MC tablets was not significantly different ($p > 0.05$) from 40 mg Epi tablets. The reduction of EpiBit particles size close to the nano-size range increased EpiBit influx two folds, which presents a great potential for these reduced-sized Epi ODT to reduce the required Epi sublingual dose by half.

5 In Vivo Absorption Studies

[0098] The research was conducted according to current guidelines published by the Canadian Council on Animal Care²¹ and was approved by the University of Manitoba Protocol Management and Review Committee.

10 Methods

[0099] Using a prospective, placebo-controlled, randomized, crossover study design, six New Zealand female white rabbits (mean \pm SD weight 3.6 ± 0.1 Kg) were investigated on different study days at least four weeks apart, using a protocol described previously. Each rabbit received sublingually either Epi 40 mg, Epi MC 20 mg ODT, or placebo ODT (as a negative control). Epi 0.3 mg IM injection was given in the rabbit's thigh muscle from an EpiPen® as a positive control.

[0100] For the sublingual administration of tablets, the rabbit's mouth was opened using speculum and the tablet was placed underneath the tongue using a pair of flat forceps. A 0.1-0.2 mL volume of water was administered immediately after dosing to facilitate tablet disintegration. The rabbit's tongue was gently pressed for 2 minutes to prevent the rabbit from chewing or swallowing the tablet. At the end of the 2-minute immobilization time, the mouth was rinsed with 30-40 mL of water, in order to remove any insoluble tablet residue from the oral cavity.

[0101] Epi 0.3 mg was injected IM in the thigh using an EpiPen®, after which the solution remaining in the EpiPen® was evacuated into a plastic tube and frozen at -20 °C, to be analyzed for Epi content using a reverse phase high performance liquid chromatography (HPLC) system (Waters Corp., Milford, MA) with ultra violet detection (UV) according USP method¹⁹.

25 Measurement of Plasma Epinephrine Concentrations

[0102] An indwelling catheter (22G 1", BD, Ontario, Canada) was inserted into an ear artery at least 30 minutes before dosing. A 2 mL blood sample was withdrawn immediately before dosing and at 5, 10, 15, 20, 30, 40, and 60 minutes afterwards.

[0103] All collected blood samples were transferred into Vacutainer plasma separation tubes containing EDTA (BD, Ontario, Canada), refrigerated within 1 hour of sampling, and centrifuged at 1600g, 4 °C. Plasma were transferred into appropriately labeled polypropylene tubes, and stored at -20 °C until analysis. Before analysis, plasma was thawed at room temperature and Epi was extracted by a solid-liquid extraction process, with an efficiency of 78% - 83%. Epi concentrations were measured using HPLC system (Waters Corp., Milford, MA) with electrochemical detection (EC). Two calibration curves with two different Epi concentration ranges were prepared. The low range calibration curve was linear over the range of 0.1 to 1.0 ng/ml with a coefficient of variation of 0.4% at 0.1 ng/ml and 0.1% at 1.0 ng/ml. The high range calibration curve was linear over the range of 1.0 to 10.0 ng/ml with a coefficient of variation of 0.1% at 1.0 ng/ml and 0.1% at 10.0 ng/ml.

40 Data Analysis

[0104] The maximum plasma Epi concentration (C_{max}), the time at which C_{max} was achieved (T_{max}), and the area under the plasma concentration versus time curves (AUC) were calculated from the plasma Epi concentration versus time plots of each individual rabbit using WinNonlin® 5.3 (Pharsight, Mountain View, CA). The AUC, C_{max} , and T_{max} values for each rabbit were compared using ANOVA, ANCOVA and Tukey-Kramer multiple comparison tests using NCSS Statistical Analysis Software (NCSS, Kaysville, UT). Differences were considered to be significant at $p < 0.05$.

50 Results

[0105] The mean (\pm SD) of Epi dose injected using EpiPen® auto-injectors was 0.29 ± 0.02 mg as calculated by multiplying the Epi concentration, measured in the solution remaining in the EpiPen® after injection, by the stated injected volume (0.3 mL).

[0106] Mean (\pm SD) plasma Epi concentration versus time plots after the sublingual administration of placebo ODT, Epi 40 mg ODT, and Epi MC 20 mg ODT, and the IM injection of Epi 0.3 mg using EpiPen® are shown in Figure 14. Mean (\pm SD) AUC, $C_{baseline}$ (endogenous E), C_{max} , and T_{max} values after the sublingual administration of placebo ODT, Epi 40 mg ODT, and Epi MC 20 mg ODT, and Epi 0.3 mg IM injection are shown in Table 10. No adverse effects were observed.

[0107] Mean (\pm SD) AUC after the administration of Epi MC 20 mg ODT (942.0 ± 243.7 ng/ml/min), Epi 40 mg ODT (678.0 ± 149.0 ng/ml/min), and Epi 0.3 mg IM (592.0 ± 122.3 ng/ml/min) did not differ significantly, but were significantly higher than after placebo ODT (220.1 ± 78.0 ng/ml/min).

[0108] Mean (\pm SD) C_{max} values after Epi MC 20 mg ODT (38.0 ± 9.9 ng/ml), Epi 40 mg ODT (31.7 ± 10.1 ng/ml) and Epi 0.3 mg IM (27.6 ± 7.0 ng/ml) did not differ significantly, but were significantly higher than after placebo ODT (7.5 ± 3.0 ng/ml).

[0109] Mean (\pm SD) T_{max} after the sublingual administration of placebo ODT (33.3 ± 17.5 min), Epi MC 20 mg ODT (28.0 ± 29.3 min), and Epi 40 mg ODT (20.0 ± 7.1 min), and IM injection of Epi 0.3 mg (30.0 ± 0.0 min) did not differ significantly.

Table 10: Epinephrine bioavailability after sublingual administration of placebo, epinephrine and epinephrine nanocrystals tablets and epinephrine intramuscular injection in the thigh.

Mean \pm SD*	Placebo	Sublingual ODT		IM Injection
		40 mg Epi	20 mg Epi MC	EpiPen®
Epinephrine dose (mg)	0	40.0	20.0	0.3
AUC (ng/ml/min)	220.1 ± 78.0	678.0 ± 149.0 †	942.0 ± 243.7 †	592.0 ± 122.3 †
$C_{baseline}$ (ng/ml)	1.1 ± 1.2	5.0 ± 3.0	2.9 ± 1.6	5.6 ± 1.9 ‡
C_{max} (ng/ml)	7.5 ± 3.0	31.7 ± 10.1 †	38.0 ± 9.9 †	27.6 ± 7.0 †
T_{max} (min)	33.3 ± 17.5	20.0 ± 7.1	28.0 ± 29.3	30.0 ± 0.0

*n=5

† $p < 0.05$ from placebo tablet but not from each others.

‡ $p < 0.05$ from placebo tablet but not from others.

AUC: area under the plasma concentration versus time curve; $C_{baseline}$: Baseline plasma concentration (endogenous epinephrine); C_{max} : maximum plasma concentration (mean \pm SD of individual C_{max} values from each rabbit, regardless of the time at which C_{max} was achieved); T_{max} : time at which maximum plasma epinephrine concentration was achieved (mean \pm SD of individual T_{max} values from each rabbit).

Discussion of Experiments

[0110] Previously, the Epi was delivered sublingually using rabbit's animal model. It was determined that 40 mg Epi, using EpiBit, is the bioequivalent sublingual dose using the novel ODT tablets to the recommended IM injection of 0.3 mg Epi given in the thigh muscle for adults. Also, the ODT formulations were developed to taste mask the bitter taste of Epi and this ODT formulation was evaluated using electronic tongue. This new taste-masked, sublingually administered 40 mg Epi ODT formulation was bioequivalent to 0.3 mg Epi IM injection as well.

[0111] In order to enhance the sublingual bioavailability of Epi, the particles size of EpiBit crystals were reduced up to 55 folds. Significant reduction in the drug particles' size results in increasing the saturation solubility, which increases the concentration gradients that promotes absorption, and dissolution rate of the drug that will ultimately increase its bioavailability, thus, resulting in a significant reduction in the required dose and any associated side effects. This is particularly important for the sublingual drug delivery due to the small saliva volume available for drug dissolution and the short sublingual residence time compared to the GIT.

[0112] Despite that the aim was to reduce the particles size of EpiBit to the nano-size (1000 nm or less), the size was reduced to a range that is very close to the nano-size range. It was very challenging to reach to a nanosize range while not using a surfactant, which may need to be evaluated later, and by processing EpiBit for only one cycle to reduce any potential stress on EpiBit that can influence its stability. The concentration of EpiBit suspension, the pressure applied, and the number of cycles were optimized to obtain the smallest particle size range with the lowest possible number of cycles.

[0113] The FT-IR spectra of EpiBit before and after processing for one cycle using Microfluidizer, LV-1, were similar, which indicates for the stability of the EpiBit during the particles size reduction process under these processing conditions (FIG. 1). Also, the drying step to obtain the reduced-sized EpiBit crystals was very efficient and no evidence in the FT-IR spectrum for any remaining isopropyl alcohol, which was used as a carrier to process EpiBit (FIG. 2).

[0114] The DSC spectra of EpiBit before and after processing were also similar with a single endothermic peak around 157 °C that indicates for the absence of any change in the purity and crystallinity of EpiBit (FIGS. 5A-5B).

[0115] The Scanning Electron Microscopy (SEM) images (FIGS. 5C-5D) of EpiBit before and after processing demonstrate clearly the change in EpiBit crystalline morphology from rectangular to spherical crystals with much smaller size.

[0116] The diffusion studies were conducted using dialysis membranes initially and then by using excised porcine sublingual mucosal membranes. It has been already established that the sublingual mucosa of pigs and rabbits are very similar to the human sublingual mucosa and were previously used for similar studies. Therefore, pigs' sublingual mucosa was selected for these diffusion studies and rabbits were always been selected in our previous studies for *in vivo* studies. The sublingual mucosa of pigs has bigger surface area that is easy to be excised surgically for *ex vivo* studies and rabbits are easier to handle and house for *in vivo* studies.

[0117] Results from both *in vitro* and *ex vivo* experiments were highly correlated, ($R^2 \geq 87$) (FIG. 15) and demonstrated that the percentage of Epi diffused from 20 mg Epi MC ODT was significantly higher than the rest of the formulations including 40 mg Epi ODT (FIGS. 7 and 11). This resulted in similar $JAUC_{0-90}$, J_{max} , and influx (J) for both 20 mg Epi MC ODT and 40 mg Epi ODT, despite of the non-statistically different permeability, although higher, for 20 mg Epi MC ODT (Tables 6 and 9). Also, formulating EpiBit into the ODT tablet formulation did not pose any delay nor influenced EpiBit diffusion as shown from comparing the 10 mg Epi diffusion from solution and ODT (Table 9).

[0118] The significant reduction of the particles size of EpiBit increased its influx two folds, which presents a great potential for these micro-sized Epi ODT to reduce the required Epi sublingual dosed by half. Animal studies in rabbits have shown similar results.

[0119] This study demonstrates that reducing the particles size of EpiBit to almost to the nano-size range improved its diffusion from rapidly-disintegrating tablet formulation (ODT) by two folds. These micro-sized Epi ODT tablets have the potential to reduce the bioequivalent dose of sublingually administered Epi by 50%.

[0120] All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. It is to be understood that while a certain form of the invention is illustrated, it is not intended to be limited to the specific form or arrangement herein described and shown. It will be apparent to those skilled in the art that various changes may be made without departing from the scope of the invention and the invention is not to be considered limited to what is shown and described in the specification. One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objectives and obtain the ends and advantages mentioned, as well as those inherent therein. The compositions, epinephrine fine particles, epinephrine nanoparticles, epinephrine nanocrystals, epinephrine microparticles, epinephrine microcrystals, pharmaceutical tablets, pharmaceutically-effective doses of epinephrine nanoparticles or nanocrystals or epinephrine microparticles or microcrystals, methods, procedures, and techniques described herein are presently representative of the preferred embodiments, are intended to be exemplary and are not intended as limitations on the scope. Although the invention has been described in connection with specific, preferred embodiments, it should be understood that the invention as ultimately claimed should not be unduly limited to such specific embodiments. Indeed various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the invention.

Claims

1. A pharmaceutical composition formulated for buccal or sublingual administration, comprising:

a pharmaceutically-effective dose of approximately 10 mg to approximately 40 mg of epinephrine bitartrate microcrystals; and
at least one of a pharmaceutically-effective carrier, surfactant, penetration enhancer, and mucoadhesive.

2. The pharmaceutical composition in accordance to Claim 1, wherein the pharmaceutically-effective dose is approximately 10 mg or approximately 20 mg of the epinephrine bitartrate microcrystals.

3. The pharmaceutical composition in accordance to Claim 1, further comprising at least one of a filler, a lubricant, a disintegrant, a sweetening agent and mouthfeel enhancer, and a taste enhancer; preferably wherein the sweetening agent and mouthfeel enhancer is mannitol and the taste enhancer is citric acid.

4. The pharmaceutical composition in accordance to Claim 1, comprising:

a pharmaceutically-effective dose of approximately 10 mg to approximately 40 mg of the epinephrine bitartrate microcrystals, preferably approximately 10 mg or approximately 20 mg of the epinephrine bitartrate microcrystals; and
a microcrystalline cellulose, a low-substituted hydroxypropyl cellulose, and magnesium stearate.

5. The pharmaceutical composition in accordance to Claim 1, wherein the epinephrine bitartrate microcrystals have a mean particle size distribution by intensity and by volume, Pdi, and zeta potential (n=3) of epinephrine bitartrate

microcrystals, after processing using a microfluidizer for one cycle at 30,000 Psi, of $2.4 \pm 0.4 \mu\text{m}$, $2.5 \pm 0.4 \mu\text{m}$, $0.185 \pm 0.019 \mu\text{m}$, and $-4.5 \pm 1.4 \text{ mV}$, respectively.

6. The pharmaceutical composition in accordance to Claim 1, comprising:

a pharmaceutically-effective dose of approximately 10 mg to approximately 40 mg of the epinephrine bitartrate microcrystals, preferably approximately 10 mg or approximately 20 mg of the epinephrine bitartrate microcrystals; wherein the epinephrine bitartrate microcrystals have a mean particle size distribution by intensity and by volume, Pdi, and zeta potential (n=3) of epinephrine bitartrate microcrystals, after processing using a microfluidizer for one cycle at 30,000 Psi, of $2.4 \pm 0.4 \mu\text{m}$, $2.5 \pm 0.4 \mu\text{m}$, $0.185 \pm 0.019 \mu\text{m}$, and $-4.5 \pm 1.4 \text{ mV}$, respectively; and a microcrystalline cellulose, a low-substituted hydroxypropyl cellulose, and magnesium stearate.

7. A pharmaceutical composition formulated for buccal or sublingual administration, comprising:

a pharmaceutically-effective dose of approximately 10 mg to approximately 40 mg of epinephrine bitartrate microcrystals; and at least one of a pharmaceutically-acceptable carrier, surfactant, penetration enhancer, and mucoadhesive; wherein the composition is for use in a method for enhancing sublingual bioavailability of epinephrine in a subject in need thereof.

8. A pharmaceutical composition formulated for buccal or sublingual administration, comprising:

a pharmaceutically-effective dose of approximately 10 mg to approximately 40 mg of epinephrine bitartrate microcrystals; and at least one of a pharmaceutically-acceptable carrier, surfactant, penetration enhancer, and mucoadhesive; wherein the composition is for use in a method for treating a condition responsive to epinephrine in a subject in need thereof.

9. The pharmaceutical composition for the use of Claim 8, wherein the condition responsive to epinephrine is an allergic reaction.

10. The pharmaceutical composition for the use of Claim 9, wherein the allergic reaction is anaphylaxis, asthma, or bronchial asthma.

11. A pharmaceutical composition formulated for buccal or sublingual administration, comprising:

a pharmaceutically-effective dose of approximately 10 mg to approximately 40 mg of epinephrine bitartrate microcrystals; and at least one of a pharmaceutically-acceptable carrier, surfactant, penetration enhancer, and mucoadhesive; wherein the composition is for use in a method for treating a breathing difficulty in a subject in need thereof; or treating an allergic emergency in a subject diagnosed with or suspected of having an allergic emergency.

12. The pharmaceutical composition for the use of Claim 11, wherein the breathing difficulty is associated with anaphylaxis, asthma, bronchial asthma, bronchitis, emphysema, or a respiratory infection; or the allergic emergency is anaphylaxis, asthma, or bronchial asthma.

13. The pharmaceutical composition for the use of Claim 7, 8, or 11, wherein the pharmaceutically-effective dose is approximately 10 mg or approximately 20 mg of the epinephrine bitartrate microcrystals.

14. The pharmaceutical composition for the use of Claim 7, 8, or 11, wherein the epinephrine bitartrate microcrystals have a mean particle size distribution by intensity and by volume, Pdi, and zeta potential (n=3) of epinephrine bitartrate microcrystals, after processing using a microfluidizer for one cycle at 30,000 Psi, of $2.4 \pm 0.4 \mu\text{m}$, $2.5 \pm 0.4 \mu\text{m}$, $0.185 \pm 0.019 \mu\text{m}$, and $-4.5 \pm 1.4 \text{ mV}$, respectively.

15. The pharmaceutical composition for the use of Claim 7, 8, or 11, comprising:

a pharmaceutically-effective dose of approximately 10 mg to approximately 40 mg of the epinephrine bitartrate microcrystals, preferably approximately 10 mg or approximately 20 mg of the epinephrine bitartrate microcrystals;

wherein the epinephrine bitartrate microcrystals have a mean particle size distribution by intensity and by volume. Pdi, and zeta potential (n=3) of epinephrine bitartrate microcrystals. after processing using a microfluidizer for one cycle at 30,000 Psi, of $2.4 \pm 0.4 \mu\text{m}$, $2.5 \pm 0.4 \mu\text{m}$, $0.185 \pm 0.019 \mu\text{m}$, and $-4.5 \pm 1.4 \text{ mV}$, respectively; and

a microcrystalline cellulose, a low-substituted hydroxypropyl cellulose, and magnesium stearate.

Patentansprüche

1. Pharmazeutische Zusammensetzung, formuliert zur bukkalen oder sublingualen Verabreichung, umfassend:

eine pharmazeutisch wirksame Dosis von etwa 10 mg bis etwa 40 mg Epinephrinbitartrat-Mikrokristalle; und mindestens einen pharmazeutisch wirksamen Träger, ein oberflächenaktives Mittel, einen Penetrationsverstärker und ein Mucoadhäsiv.

2. Pharmazeutische Zusammensetzung nach Anspruch 1, wobei die pharmazeutisch wirksame Dosis etwa 10 mg oder etwa 20 mg der Epinephrinbitartrat-Mikrokristalle beträgt.

3. Pharmazeutische Zusammensetzung nach Anspruch 1, weiter umfassend mindestens einen Füllstoff, ein Gleitmittel, ein Sprengmittel, ein Süßungsmittel und einen Mundgefühlsverstärker sowie einen Geschmacksverstärker; vorzugsweise, wobei das Süßungsmittel und der Mundgefühlsverstärker Mannit ist und der Geschmacksverstärker Zitronensäure ist.

4. Pharmazeutische Zusammensetzung nach Anspruch 1, umfassend: eine pharmazeutisch wirksame Dosis von etwa 10 mg bis etwa 40 mg der Epinephrinbitartrat-Mikrokristalle, vorzugsweise etwa 10 mg oder etwa 20 mg der Epinephrinbitartrat-Mikrokristalle; und eine mikrokristalline Cellulose, eine niedrig-substituierte Hydroxypropylcellulose und Magnesiumstearat.

5. Pharmazeutische Zusammensetzung nach Anspruch 1, wobei die Epinephrinbitartrat-Mikrokristalle eine mittlere Teilchengrößenverteilung nach Intensität und nach Volumen, Pdi, und Zetapotenzial (n=3) von Epinephrinbitartrat-Mikrokristallen nach Verarbeitung unter Verwendung eines Mikrofluidisators für einen Zyklus bei 30000 Psi von $2,4 \pm 0,4 \mu\text{m}$, $2,5 \pm 0,4 \mu\text{m}$, $0,185 \pm 0,019 \mu\text{m}$ bzw. $-4,5 \pm 1,4 \text{ mV}$ aufweisen.

6. Pharmazeutische Zusammensetzung nach Anspruch 1, umfassend:

eine pharmazeutisch wirksame Dosis von etwa 10 mg bis etwa 40 mg der Epinephrinbitartrat-Mikrokristalle, vorzugsweise etwa 10 mg oder etwa 20 mg der Epinephrinbitartrat-Mikrokristalle; wobei die Epinephrinbitartrat-Mikrokristalle eine mittlere Teilchengrößenverteilung nach Intensität und nach Volumen, Pdi, und Zetapotenzial (n=3) von Epinephrinbitartrat-Mikrokristallen nach Verarbeitung unter Verwendung eines Mikrofluidisators für einen Zyklus bei 30000 Psi von $2,4 \pm 0,4 \mu\text{m}$, $2,5 \pm 0,4 \mu\text{m}$, $0,185 \pm 0,019 \mu\text{m}$ bzw. $-4,5 \pm 1,4 \text{ mV}$ aufweisen; und eine mikrokristalline Cellulose, eine niedrig-substituierte Hydroxypropylcellulose und Magnesiumstearat.

7. Pharmazeutische Zusammensetzung, formuliert zur bukkalen oder sublingualen Verabreichung, umfassend:

eine pharmazeutisch wirksame Dosis von etwa 10 mg bis etwa 40 mg Epinephrinbitartrat-Mikrokristalle; und mindestens einen pharmazeutisch verträglichen Träger, ein oberflächenaktives Mittel, einen Penetrationsverstärker und ein Mucoadhäsiv; wobei die Zusammensetzung zur Verwendung in einem Verfahren zur Erhöhung der sublingualen Bioverfügbarkeit von Epinephrin bei einem Patienten, der dies benötigt, bestimmt ist.

8. Pharmazeutische Zusammensetzung, formuliert zur bukkalen oder sublingualen Verabreichung, umfassend:

eine pharmazeutisch wirksame Dosis von etwa 10 mg bis etwa 40 mg Epinephrinbitartrat-Mikrokristalle; und mindestens einen pharmazeutisch verträglichen Träger, ein oberflächenaktives Mittel, einen Penetrationsverstärker und ein Mucoadhäsiv; wobei die Zusammensetzung zur Verwendung in einem Verfahren zur Behandlung eines Zustands, der auf Epinephrin anspricht, in einem Patienten, der dies benötigt, bestimmt ist.

9. Pharmazeutische Zusammensetzung für die Verwendung nach Anspruch 8, wobei der auf Epinephrin ansprechende Zustand eine allergische Reaktion ist.

5 10. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 9, wobei die allergische Reaktion Anaphylaxie, Asthma oder Bronchialasthma ist.

11. Pharmazeutische Zusammensetzung, formuliert zur bukkalen oder sublingualen Verabreichung, umfassend:

10 eine pharmazeutisch wirksame Dosis von etwa 10 mg bis etwa 40 mg Epinephrinbitartrat-Mikrokristalle; und mindestens einen pharmazeutisch verträglichen Träger, ein oberflächenaktives Mittel, einen Penetrationsverstärker und ein Mucoadhäsiv;
wobei die Zusammensetzung zur Verwendung in einem Verfahren zur Behandlung einer Atembeschwerde bei einem Patienten, der dies benötigt, oder
15 Behandlung eines allergischen Notfalls bei einem Patienten, bei dem ein allergischer Notfall diagnostiziert wurde oder bei dem ein solcher vermutet wird, bestimmt ist.

20 12. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 11, wobei die Atembeschwerde mit Anaphylaxie, Asthma, Bronchialasthma, Bronchitis, Emphysem oder einer Atemwegsinfektion verbunden ist; oder der allergische Notfall Anaphylaxie, Asthma oder Bronchialasthma ist.

13. Pharmazeutische Zusammensetzung für die Verwendung nach Anspruch 7, 8 oder 11, wobei die pharmazeutisch wirksame Dosis etwa 10 mg oder etwa 20 mg der Epinephrinbitartrat-Mikrokristalle beträgt.

25 14. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 7, 8 oder 11, wobei die Epinephrinbitartrat-Mikrokristalle eine mittlere Teilchengrößenverteilung nach Intensität und nach Volumen, Pdi, und ein Zetapotenzial (n=3) der Epinephrinbitartrat-Mikrokristalle nach Verarbeitung unter Verwendung eines Mikrofluidisators für einen Zyklus bei 30000 Psi von $2,4 \pm 0,4 \mu\text{m}$, $2,5 \pm 0,4 \mu\text{m}$, $0,185 \pm 0,019 \mu\text{m}$ bzw. $-4,5 \pm 1,4 \text{ mV}$ aufweisen.

30 15. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 7, 8 oder 11, umfassend:

eine pharmazeutisch wirksame Dosis von etwa 10 mg bis etwa 40 mg der Epinephrinbitartrat-Mikrokristalle, vorzugsweise etwa 10 mg oder etwa 20 mg der Epinephrinbitartrat-Mikrokristalle; wobei die Epinephrinbitartrat-Mikrokristalle eine mittlere Teilchengrößenverteilung nach Intensität und nach Volumen, Pdi, und Zetapotenzial (n=3) von Epinephrinbitartrat-Mikrokristallen nach Verarbeitung unter Verwendung eines Mikrofluidisators für einen Zyklus bei 30000 Psi von $2,4 \pm 0,4 \mu\text{m}$, $2,5 \pm 0,4 \mu\text{m}$, $0,185 \pm 0,019 \mu\text{m}$ bzw. $-4,5 \pm 1,4 \text{ mV}$ aufweisen; und eine mikrokristalline Cellulose, eine niedrig-substituierte Hydroxypropylcellulose und Magnesiumstearat.

Revendications

40 1. Composition pharmaceutique formulée en vue d'une administration buccale ou sublinguale, comprenant :

une dose pharmaceutiquement active de 10 mg approximativement à 40 mg approximativement de microcristaux de bi-tartrate d'épinéphrine ; et
45 au moins l'un d'un milieu pharmaceutiquement actif, d'un surfactant, d'un promoteur de pénétration et d'un agent muco-adhésif.

50 2. Composition pharmaceutique selon la revendication 1, dans laquelle la dose pharmaceutiquement active est de 10 mg approximativement ou de 20 mg approximativement des microcristaux de bi-tartrate d'épinéphrine.

3. Composition pharmaceutique selon la revendication 1, comprenant, en outre, au moins l'un d'un excipient, d'un lubrifiant, d'un agent désintégrateur, d'un édulcorant et d'un rehausseur d'effet en bouche, et d'un rehausseur de goût ; de préférence, dans laquelle l'édulcorant et le rehausseur d'effet en bouche sont à base de mannitol et le rehausseur de goût est de l'acide citrique.

55 4. Composition pharmaceutique selon la revendication 1, comprenant ;
une dose pharmaceutiquement active de 10 mg approximativement à 40 mg approximativement des microcristaux de bi-tartrate d'épinéphrine, de préférence, de 10 mg approximativement ou de 20 mg approximativement des

microcristaux de bi-tartrate d'épinéphrine ; et
une cellulose micro-cristalline, une cellulose hydroxypropylique faiblement substituée et du stéarate de magnésium.

5 **5.** Composition pharmaceutique selon la revendication 1, dans laquelle les microcristaux de bi-tartrate d'épinéphrine présentent une distribution de taille de particule moyenne en intensité et en volume, un indice Pdi (polydispersity index) et un potentiel zêta (n=3) de microcristaux de bi-tartrate d'épinéphrine, après traitement en utilisant un microfluidiseur pour un cycle sous 2068 bars (30 000 PSI), respectivement de $2,4 \pm 0,4$ μm , $2,5 \pm 0,4$ μm , $0,185 \pm 0,019$ et $-4,5 \pm 1,4$ mV.

10 **6.** Composition pharmaceutique selon la revendication 1, comprenant :

15 une dose pharmaceutiquement active de 10 mg approximativement à 40 mg approximativement des microcristaux de bi-tartrate d'épinéphrine, de préférence, de 10 mg approximativement ou de 20 mg approximativement des microcristaux de bi-tartrate d'épinéphrine ; dans laquelle les microcristaux de bi-tartrate d'épinéphrine présentent une distribution de taille de particule moyenne en intensité et en volume, un indice Pdi et un potentiel zêta (n=3) de microcristaux de bi-tartrate d'épinéphrine, après traitement en utilisant un microfluidiseur pour un cycle sous 2068 bars (30 000 PSI), respectivement de $2,4 \pm 0,4$ μm , $2,5 \pm 0,4$ μm , $0,185 \pm 0,019$ et $-4,5 \pm 1,4$ mV ; et
20 une cellulose micro-cristalline, une cellulose hydroxypropylique faiblement substituée et du stéarate de magnésium.

7. Composition pharmaceutique formulée en vue d'une administration buccale ou sublinguale, comprenant :

25 une dose pharmaceutiquement active de 10 mg approximativement à 40 mg approximativement de microcristaux de bi-tartrate d'épinéphrine ; et
au moins l'un d'un milieu pharmaceutiquement actif, d'un surfactant, d'un promoteur de pénétration et d'un agent muco-adhésif ;
dans laquelle la composition est destinée à être utilisée dans un procédé d'amélioration de la biodisponibilité sublinguale de l'épinéphrine chez un sujet qui en a besoin.

30 **8.** Composition pharmaceutique formulée en vue d'une administration buccale ou sublinguale, comprenant :

35 une dose pharmaceutiquement active de 10 mg approximativement à 40 mg approximativement de microcristaux de bi-tartrate d'épinéphrine ; et
au moins l'un d'un milieu pharmaceutiquement actif, d'un surfactant, d'un promoteur de pénétration et d'un agent muco-adhésif ;
dans laquelle la composition est destinée à être utilisée dans un procédé de traitement d'une condition sensible à l'épinéphrine chez un sujet qui en a besoin.

40 **9.** Composition pharmaceutique destinée à une utilisation selon la revendication 8, dans laquelle la condition sensible à l'épinéphrine est une réaction allergique.

10. Composition pharmaceutique destinée à une utilisation selon la revendication 9, dans laquelle la réaction allergique est l'anaphylaxie, l'asthme, ou l'asthme bronchique.

45 **11.** Composition pharmaceutique formulée en vue d'une administration buccale ou sublinguale, comprenant :

50 une dose pharmaceutiquement active de 10 mg approximativement à 40 mg approximativement de microcristaux de bi-tartrate d'épinéphrine ; et
au moins l'un d'un milieu pharmaceutiquement actif, d'un surfactant, d'un promoteur de pénétration et d'un agent auto-adhésif ;
dans laquelle la composition est destinée à être utilisée dans un procédé de traitement d'une difficulté respiratoire chez un sujet qui en a besoin ; ou
de traitement une urgence allergique chez un sujet diagnostiqué en urgence allergique ou suspecté d'en présenter une.

55 **12.** Composition pharmaceutique destinée à une utilisation selon la revendication 11, dans laquelle la difficulté respiratoire est associée à l'anaphylaxie, l'asthme, l'asthme bronchique, la bronchite, l'emphysème ou à une infection

respiratoire ; ou
l'urgence allergique est l'anaphylaxie, l'asthme, ou l'asthme bronchique.

- 5
13. Composition pharmaceutique destinée à une utilisation selon la revendication 7, 8, ou 11, dans laquelle la dose pharmaceutiquement active est de 10 mg approximativement ou de 20 mg approximativement des microcristaux de bi-tartrate d'épinéphrine.
- 10
14. Composition pharmaceutique destinée à une utilisation selon la revendication 7, 8, ou 11, dans laquelle les microcristaux de bi-tartrate d'épinéphrine présentent une distribution de taille de particule moyenne en intensité et en volume, un indice Pdi et un potentiel zêta (n=3) de microcristaux de bi-tartrate d'épinéphrine, après traitement en utilisant un microfluidiseur pour un cycle sous 2068 bars (30 000 PSI), respectivement de $2,4 \pm 0,4 \mu\text{m}$, $2,5 \pm 0,4 \mu\text{m}$, $0,185 \pm 0,019$ et $-4,5 \pm 1,4 \text{ mV}$.
- 15
15. Composition pharmaceutique destinée à une utilisation selon la revendication 7, 8, ou 11, comprenant :
- 20
- une dose pharmaceutiquement active de 10 mg approximativement à 40 mg approximativement des microcristaux de bi-tartrate d'épinéphrine, de préférence, de 10 mg approximativement ou de 20 mg approximativement des microcristaux de bi-tartrate d'épinéphrine ; dans laquelle les microcristaux de bi-tartrate d'épinéphrine présentent une distribution de taille de particule moyenne en intensité et en volume, un indice Pdi et un potentiel zêta (n=3) de microcristaux de bi-tartrate d'épinéphrine, après traitement en utilisant un microfluidiseur pour un cycle sous 2068 bars (30 000 PSI), respectivement de $2,4 \pm 0,4 \mu\text{m}$, $2,5 \pm 0,4 \mu\text{m}$, $0,185 \pm 0,019$ et $-4,5 \pm 1,4 \text{ mV}$; et
- 25
- une cellulose micro-cristalline, une cellulose hydroxypropylique faiblement substituée et du stéarate de magnésium.
- 30
- 35
- 40
- 45
- 50
- 55

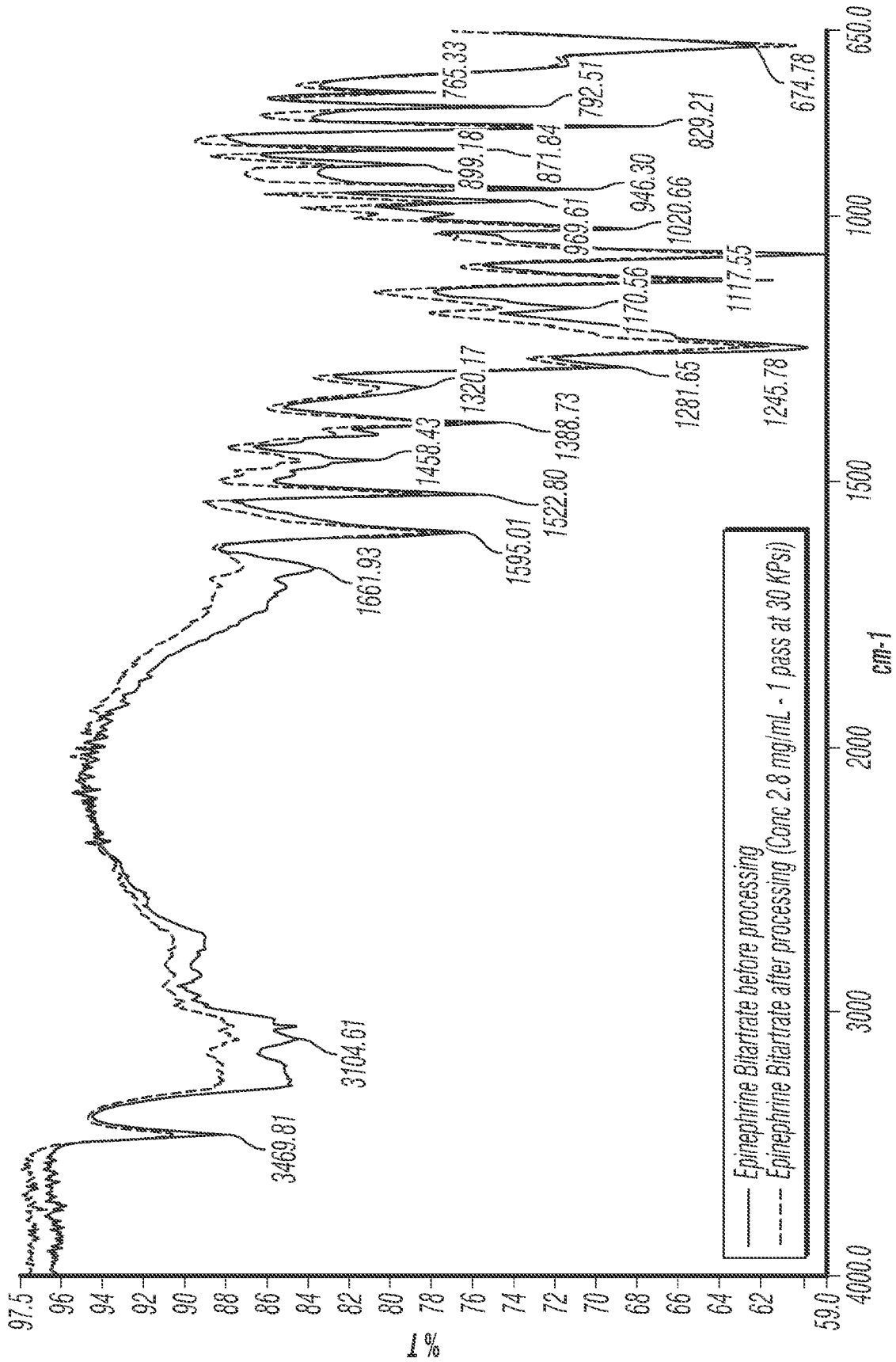


FIG. 1

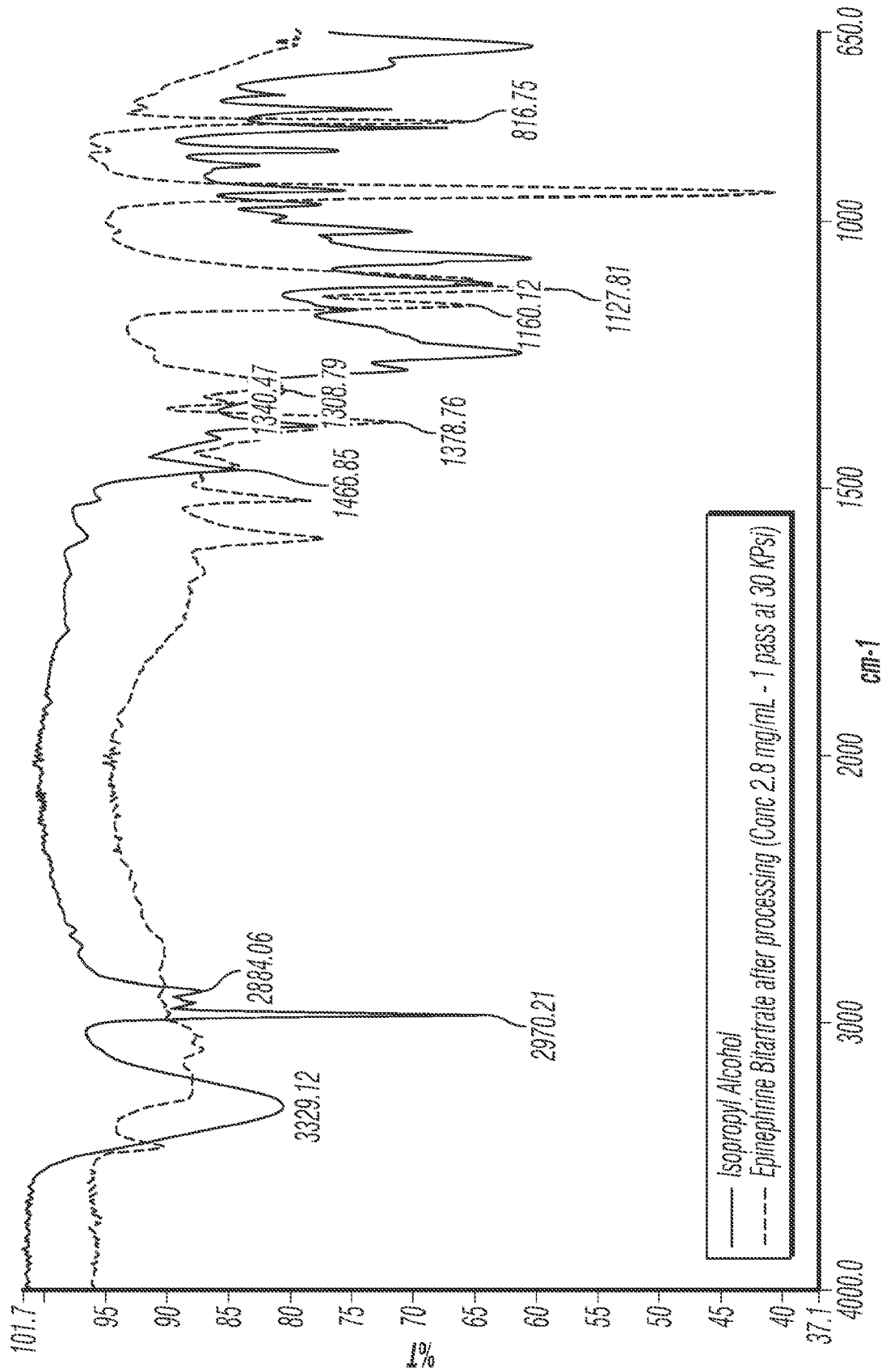


FIG. 2

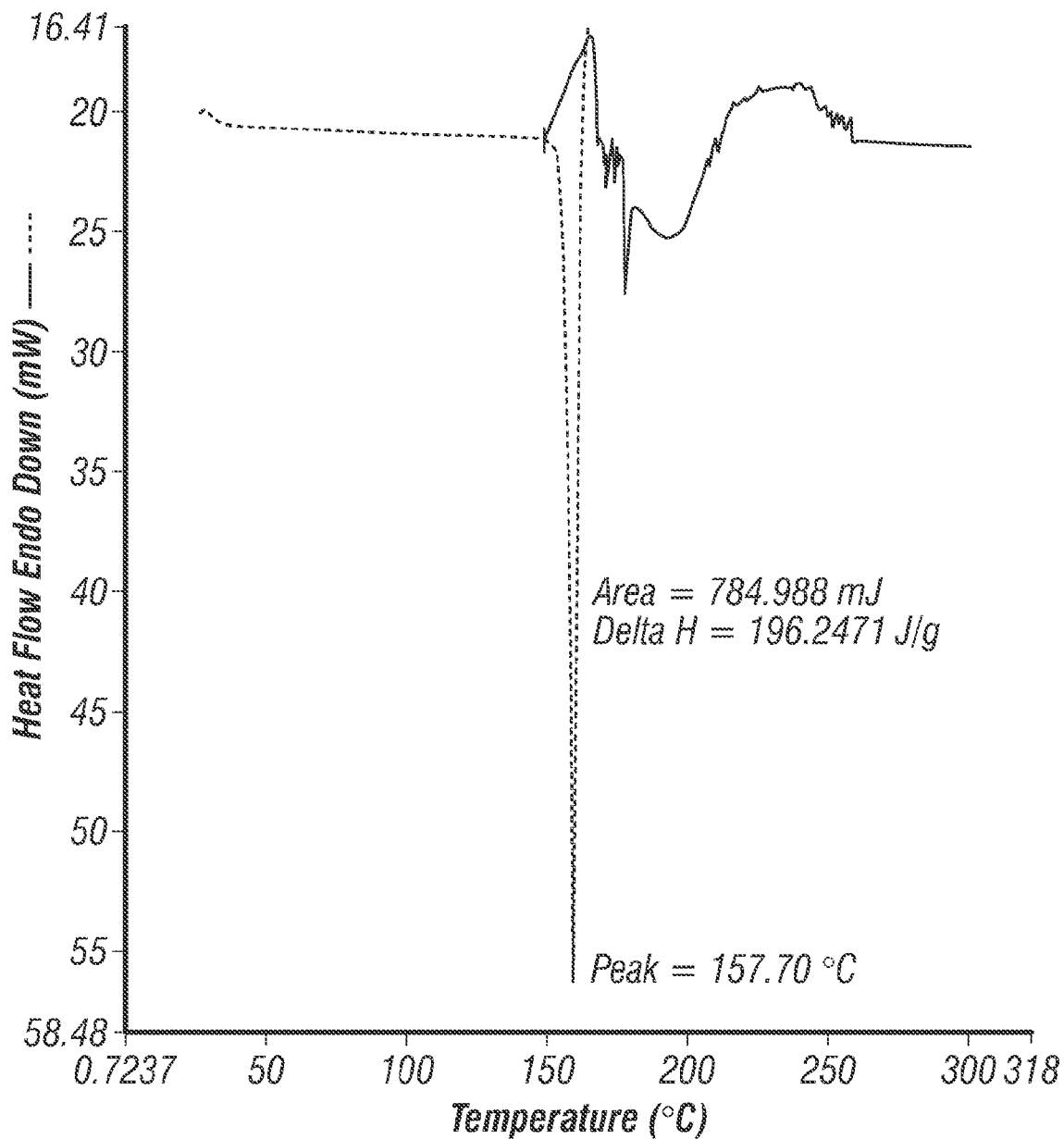


FIG. 3

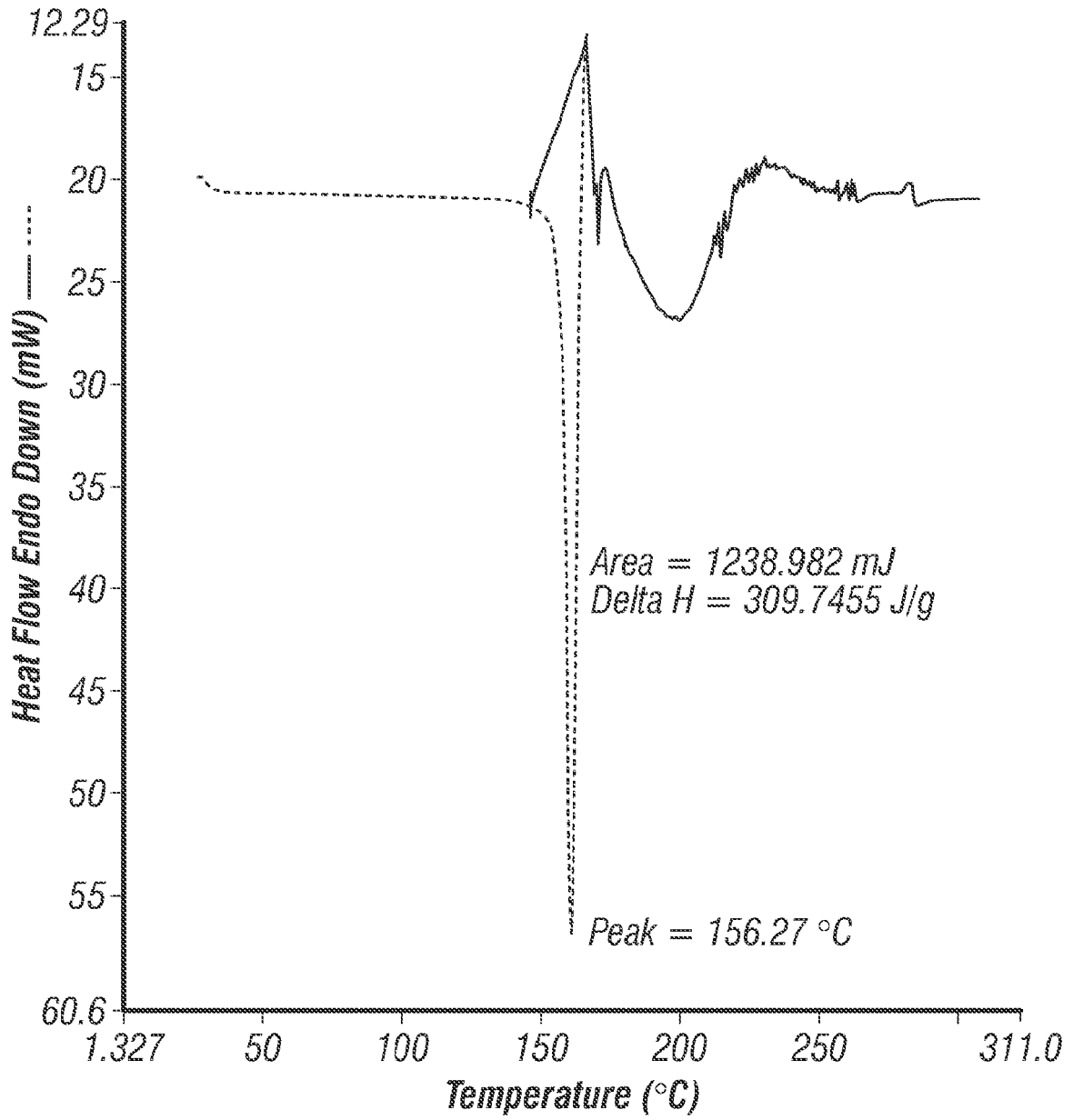


FIG. 4

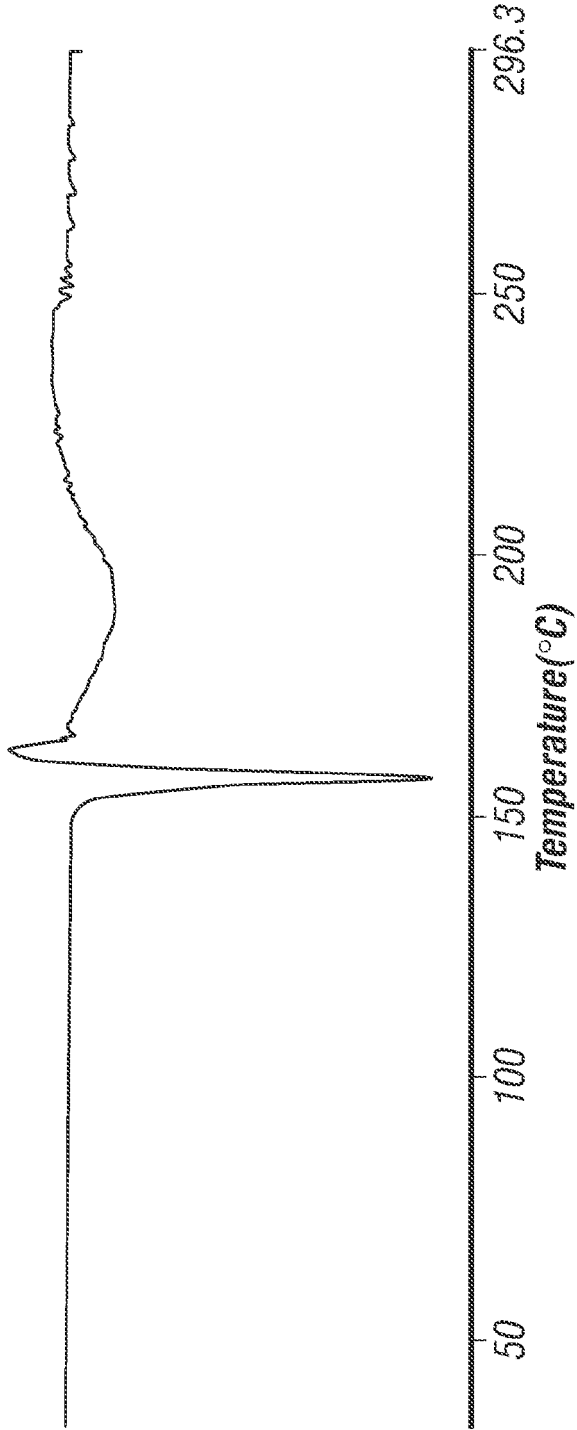


FIG. 5A

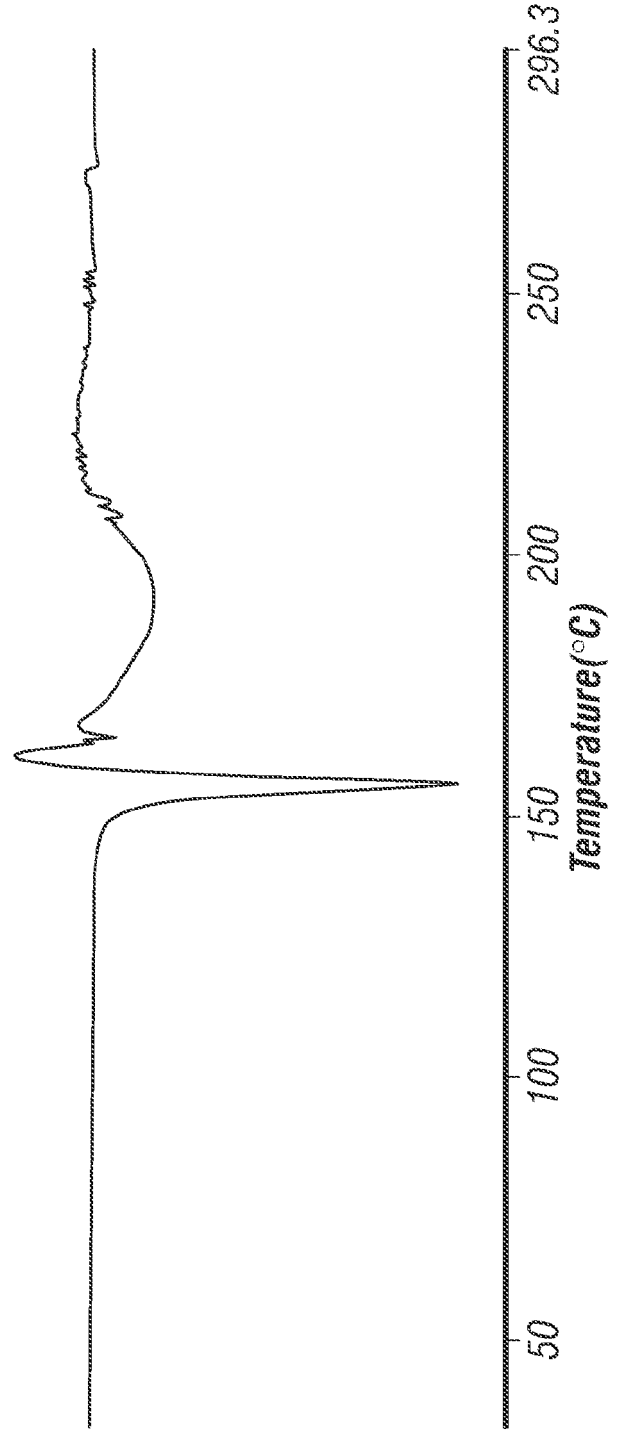


FIG. 5B

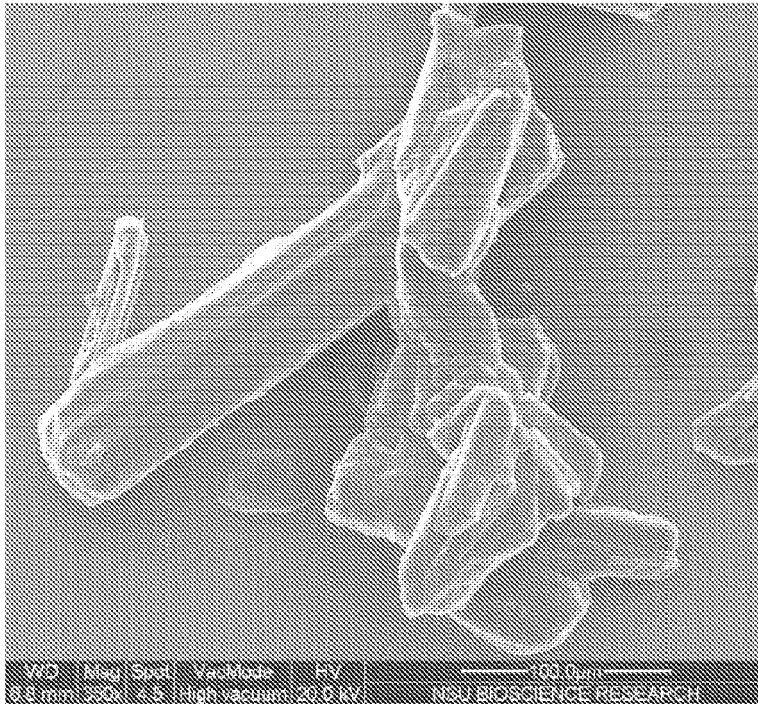


FIG. 5C

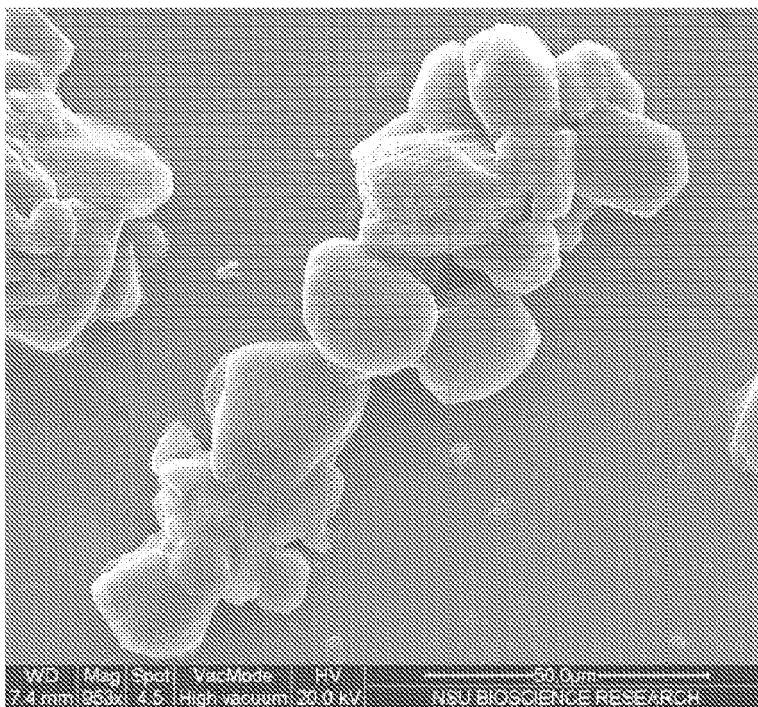


FIG 5D

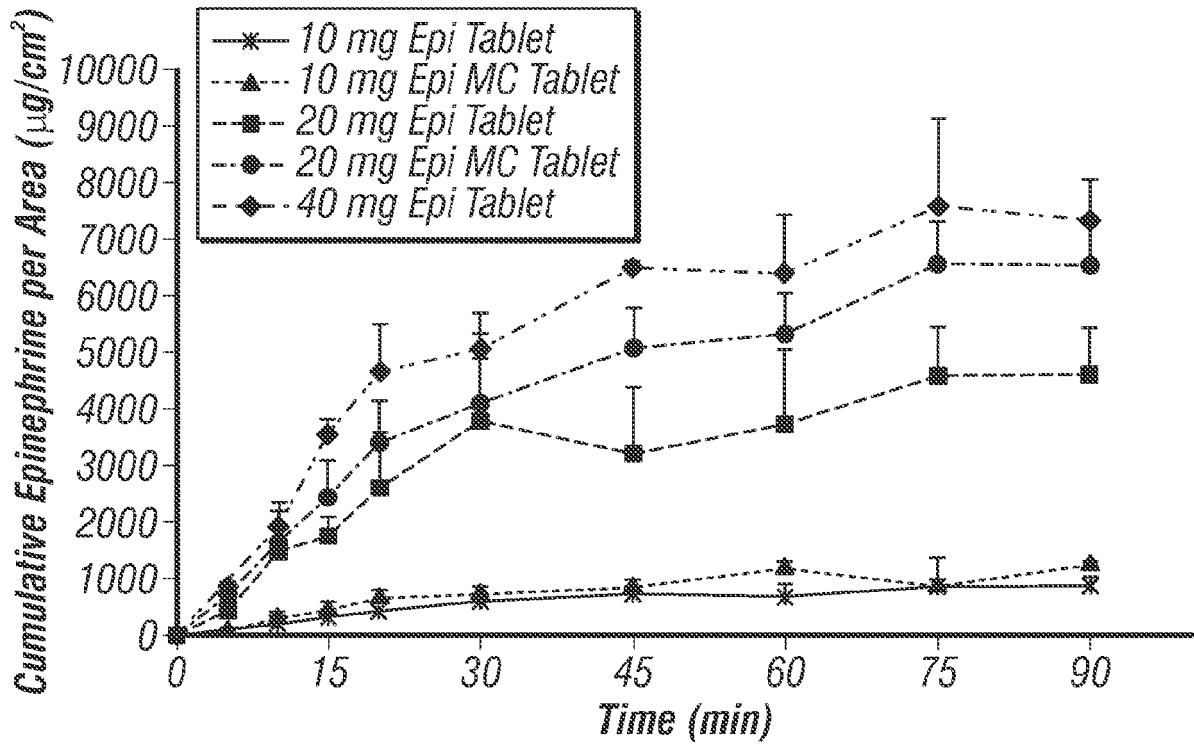


FIG. 6

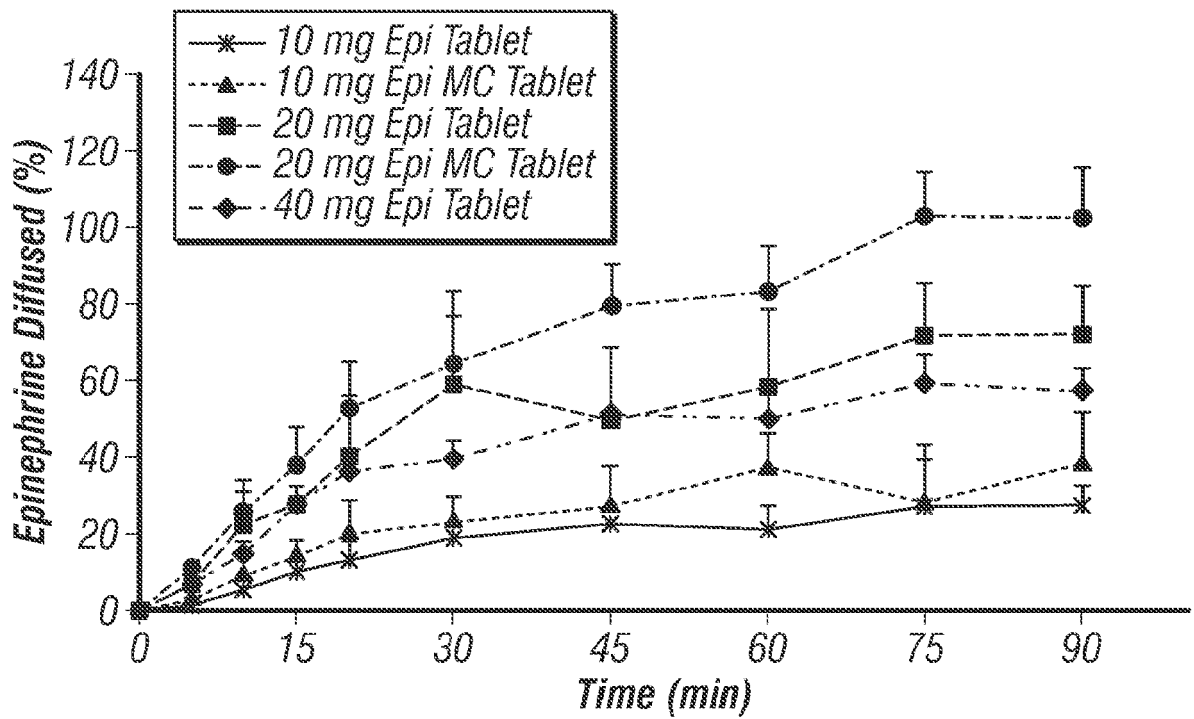


FIG. 7

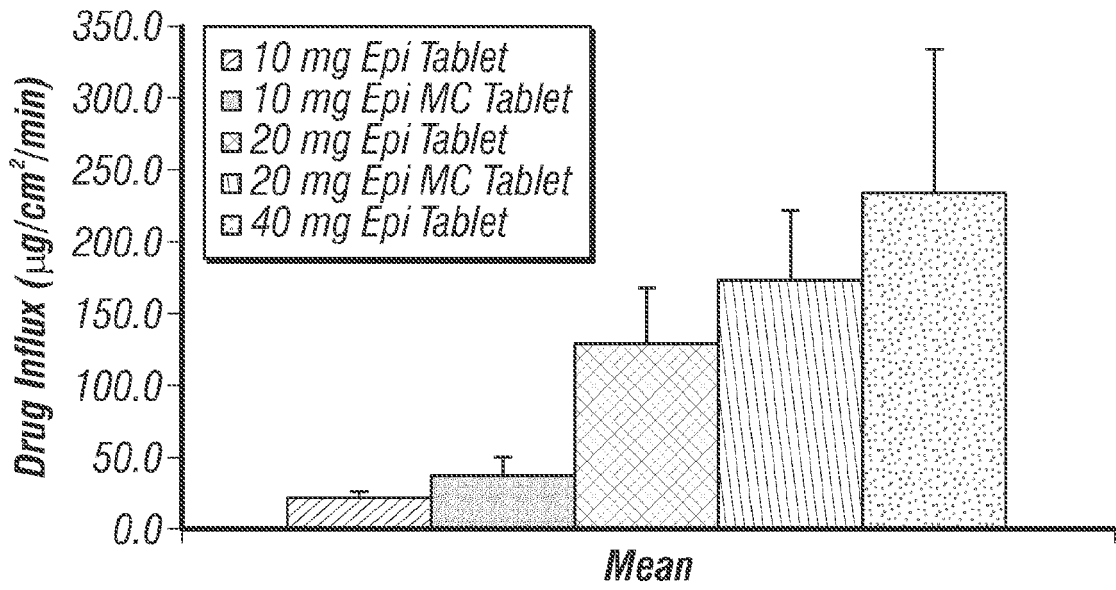


FIG. 8

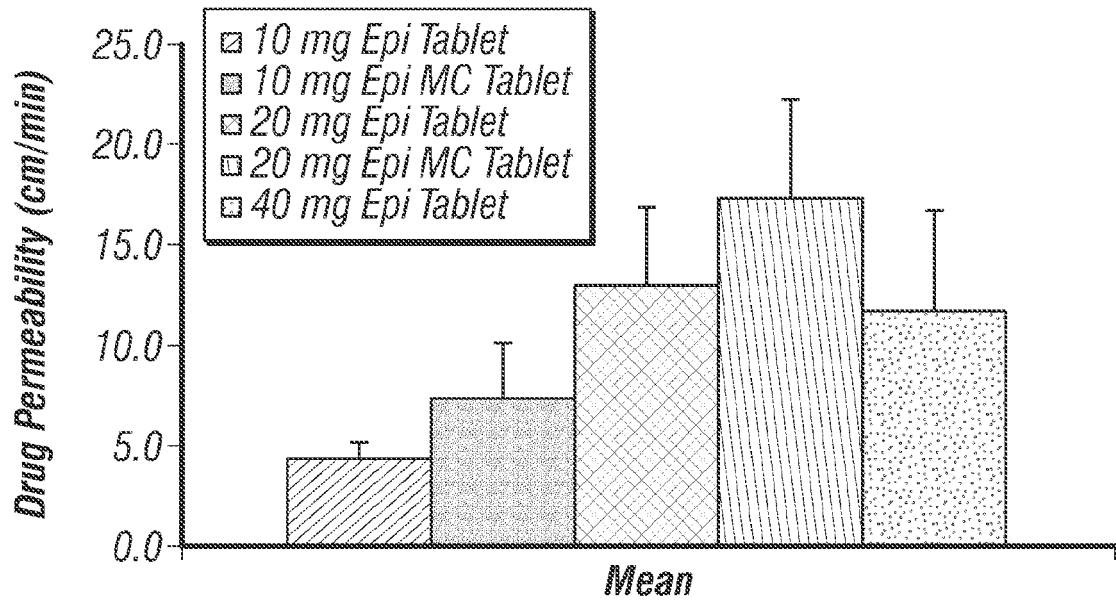


FIG. 9

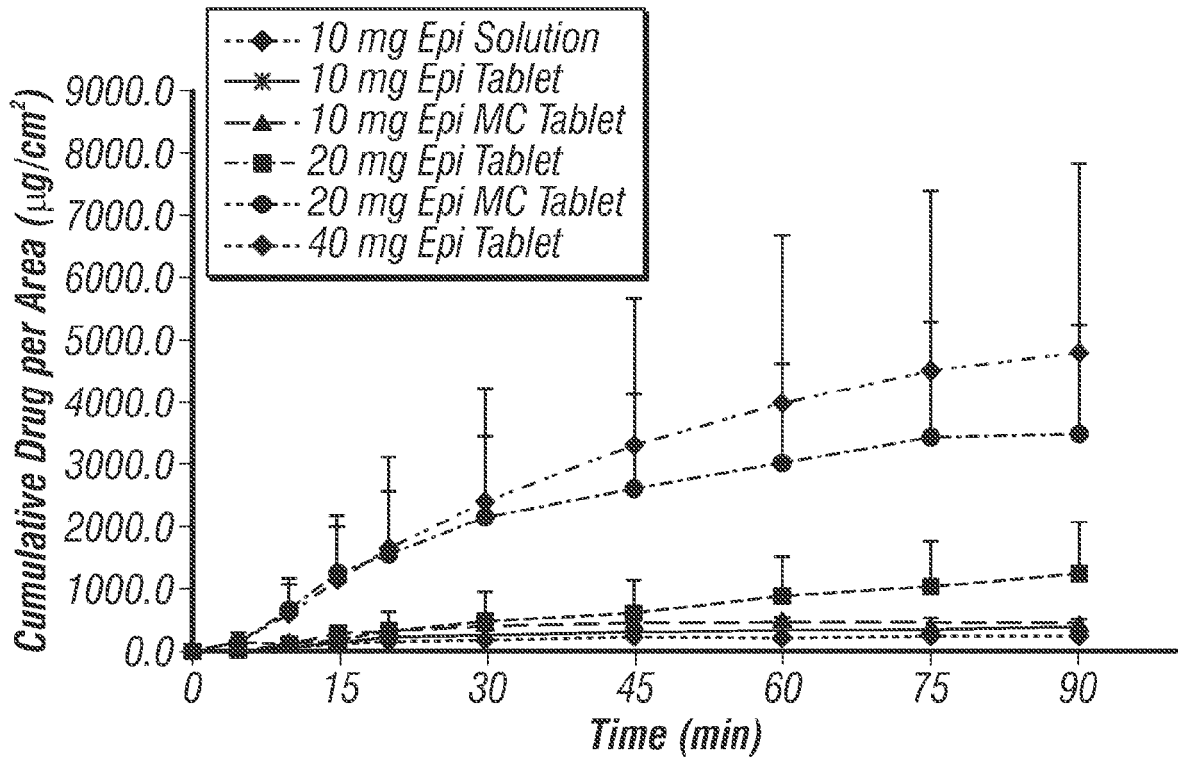


FIG. 10

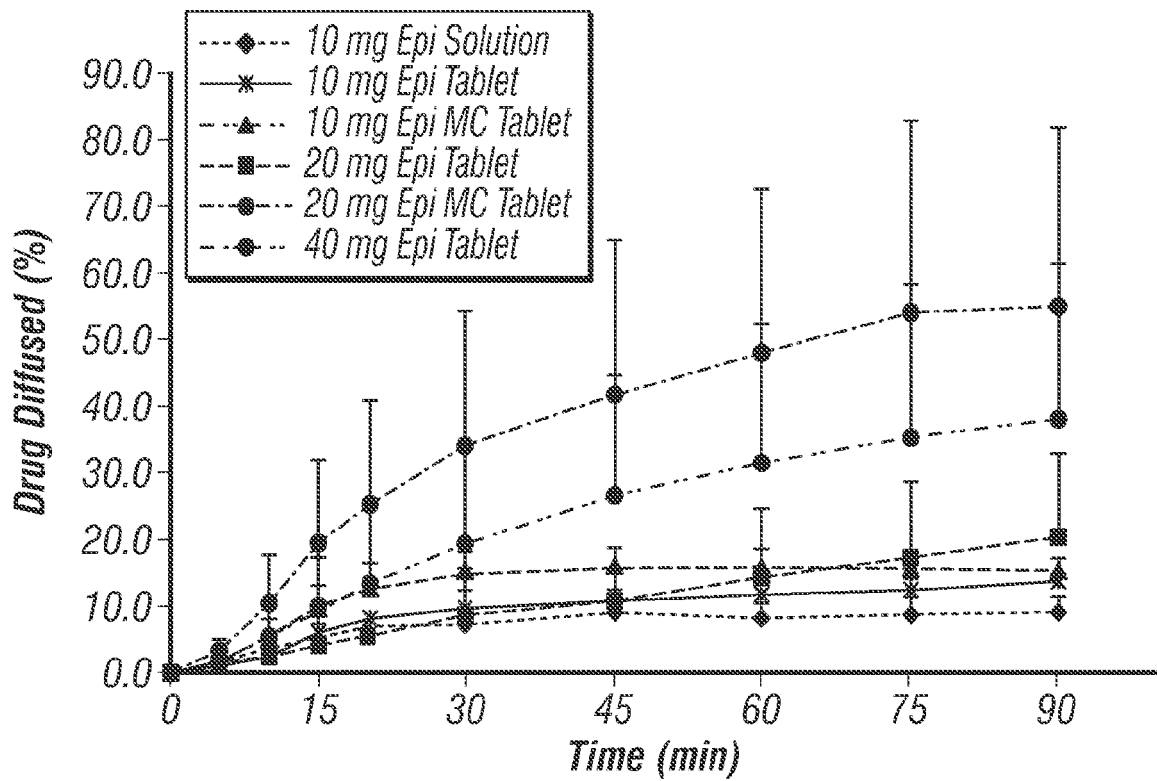


FIG. 11

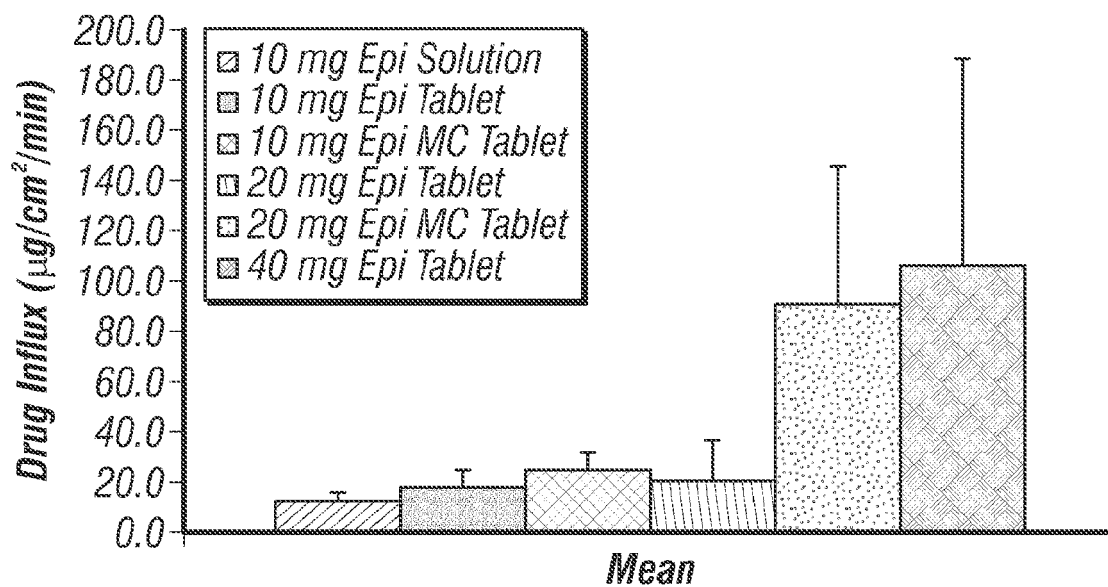


FIG. 12

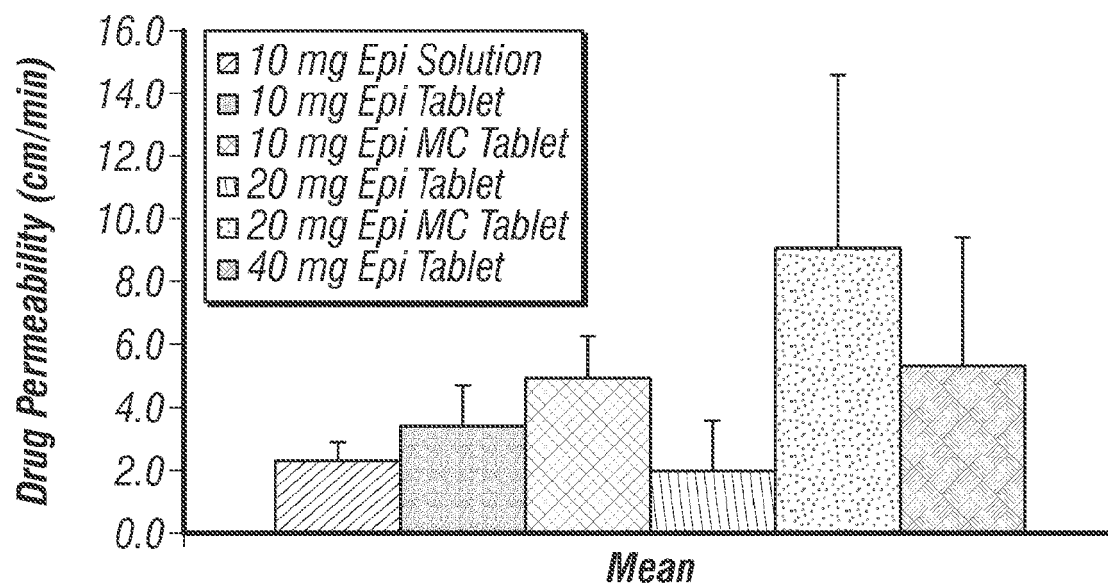


FIG. 13

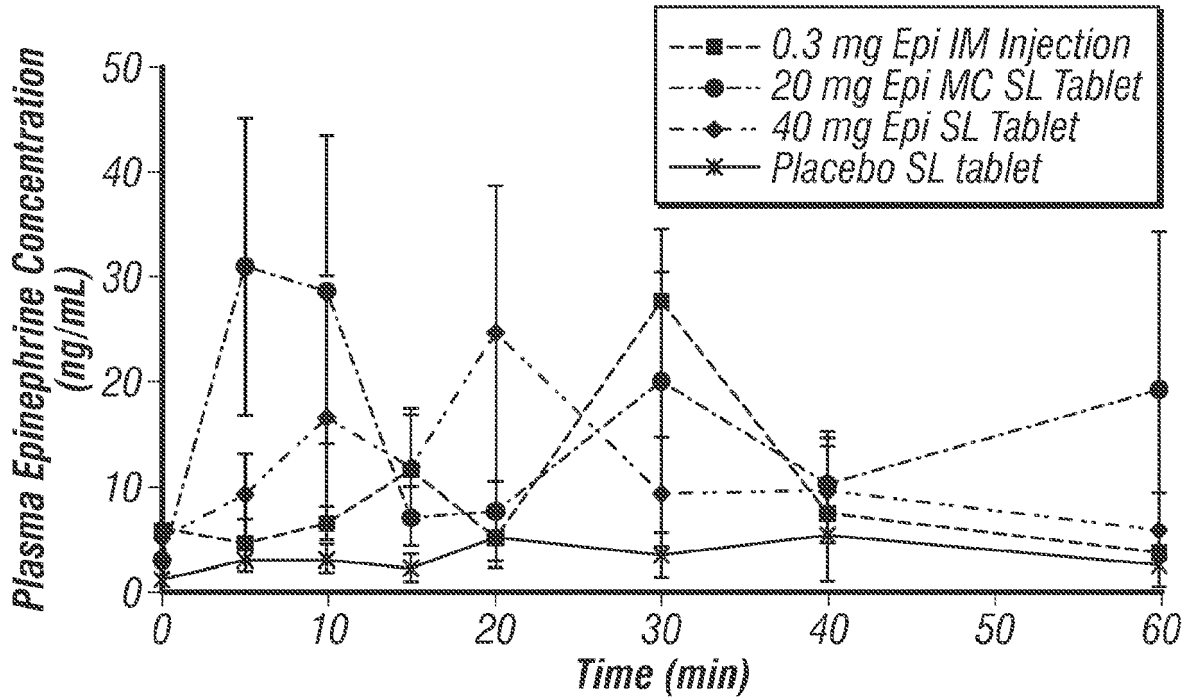


FIG. 14

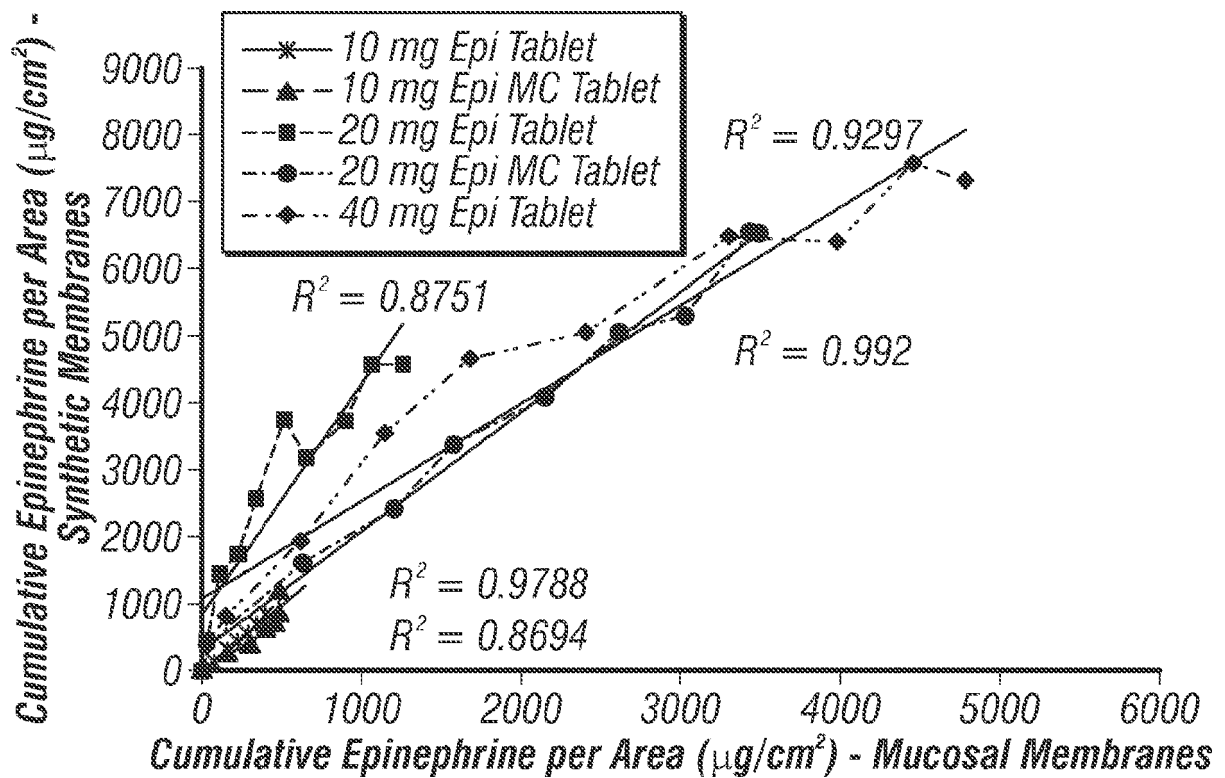


FIG. 15

REFERENCES CITED IN THE DESCRIPTION

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