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(54) **EPINEPHRINE NANOPARTICLES, METHODS OF FABRICATION THEREOF, AND METHODS FOR USE THEREOF FOR TREATMENT OF CONDITIONS RESPONSIVE TO EPINEPHRINE**

(75) Inventors: **Mutasem Rawas-Qalaji**, Fort Lauderdale, FL (US); **Enrique Nieves**, Fort Lauderdale, FL (US); **Keith John Simons**, Winnipeg (CA); **Frances Estelle Reed Simons**, Winnipeg (CA); **Ousama Rachid**, Winnipeg (CA)

(73) Assignees: **UNIVERSITY OF MANITOBA**, Winnipeg, Manitoba (CA); **NOVA SOUTHEASTERN UNIVERSITY**, Fort Lauderdale, FL (US)

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(52) **U.S. Cl.** **514/653**; 977/773; 977/788; 977/900; 977/915

(57) **ABSTRACT**

The invention provides a composition including epinephrine nanoparticles and methods for therapeutic use of the composition in the treatment of conditions responsive to epinephrine such as a cardiac event or an allergic reaction, particularly anaphylaxis. The epinephrine nanoparticles can be incorporated into orally-disintegrating and fast-disintegrating tablet pharmaceutical formulations and can significantly increase the sublingual bioavailability of epinephrine, and thereby reduce the epinephrine dose required. Additionally, the invention provides methods for fabrication of stabilized epinephrine nanoparticles for use in the described compositions.

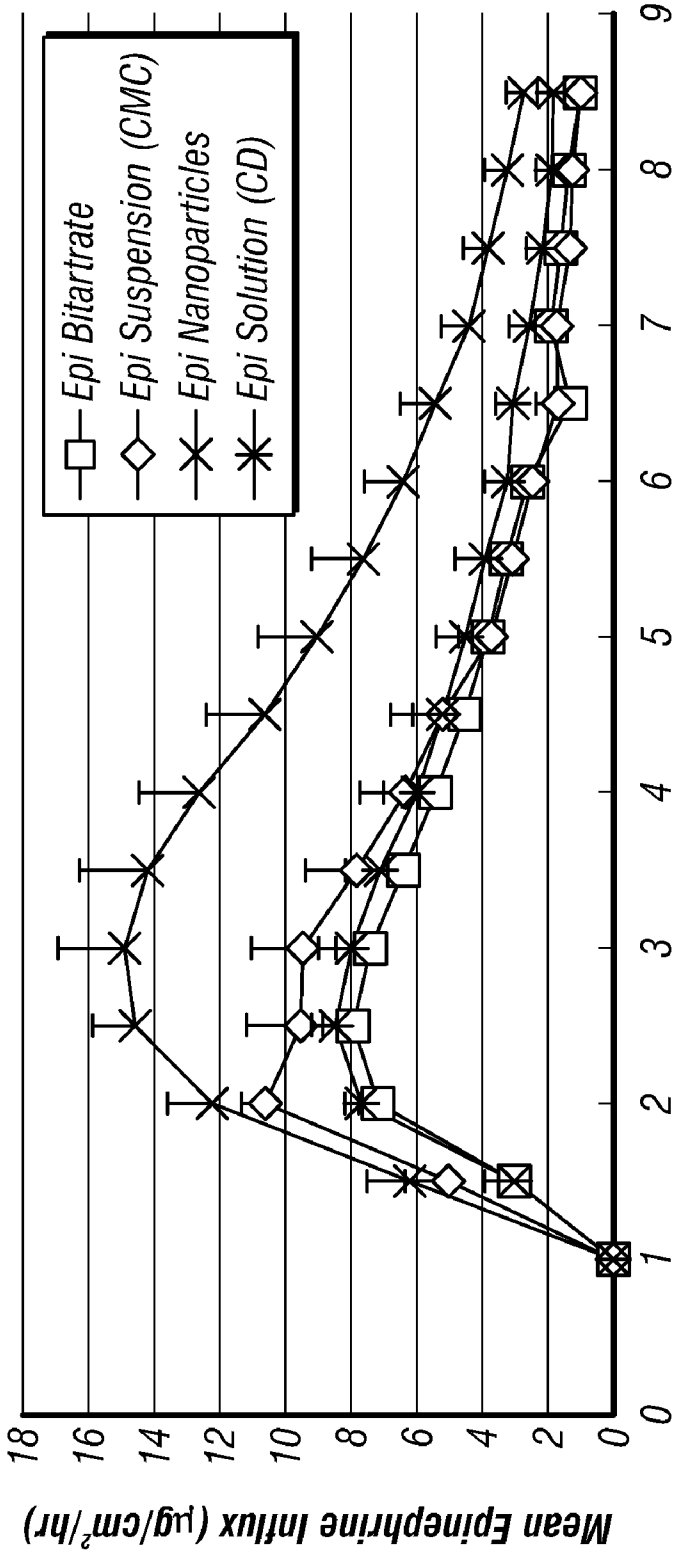
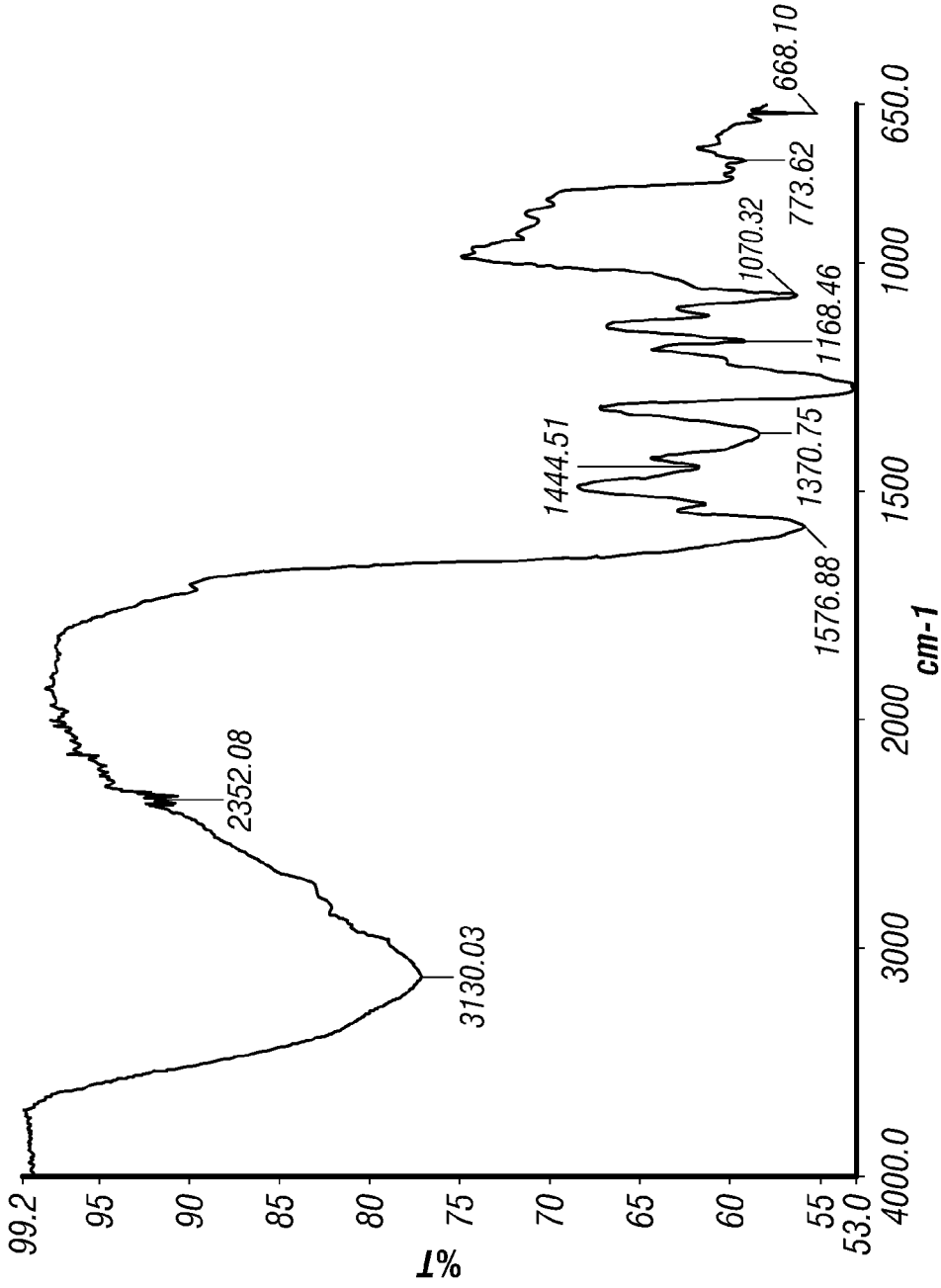

Epi Bitartrate
Epi Suspension (CMC)
Epi Nanoparticles
Epi Solution (CD)
Mean Epinephrine Influx (µg/cm²/hr)
Time (hrs)
0
2
4
6
8
10
12
14
16
18
1
3
5
7
9

FIG. 1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95
90
85
80
75
70
65
60
55
53.0
%T
4000.0
3000
2000
1500
1000
650.0
cm-1
3130.03
2352.08
1576.88
1444.51
1370.75
1168.46
1070.32
773.62
668.10

FIG. 2A

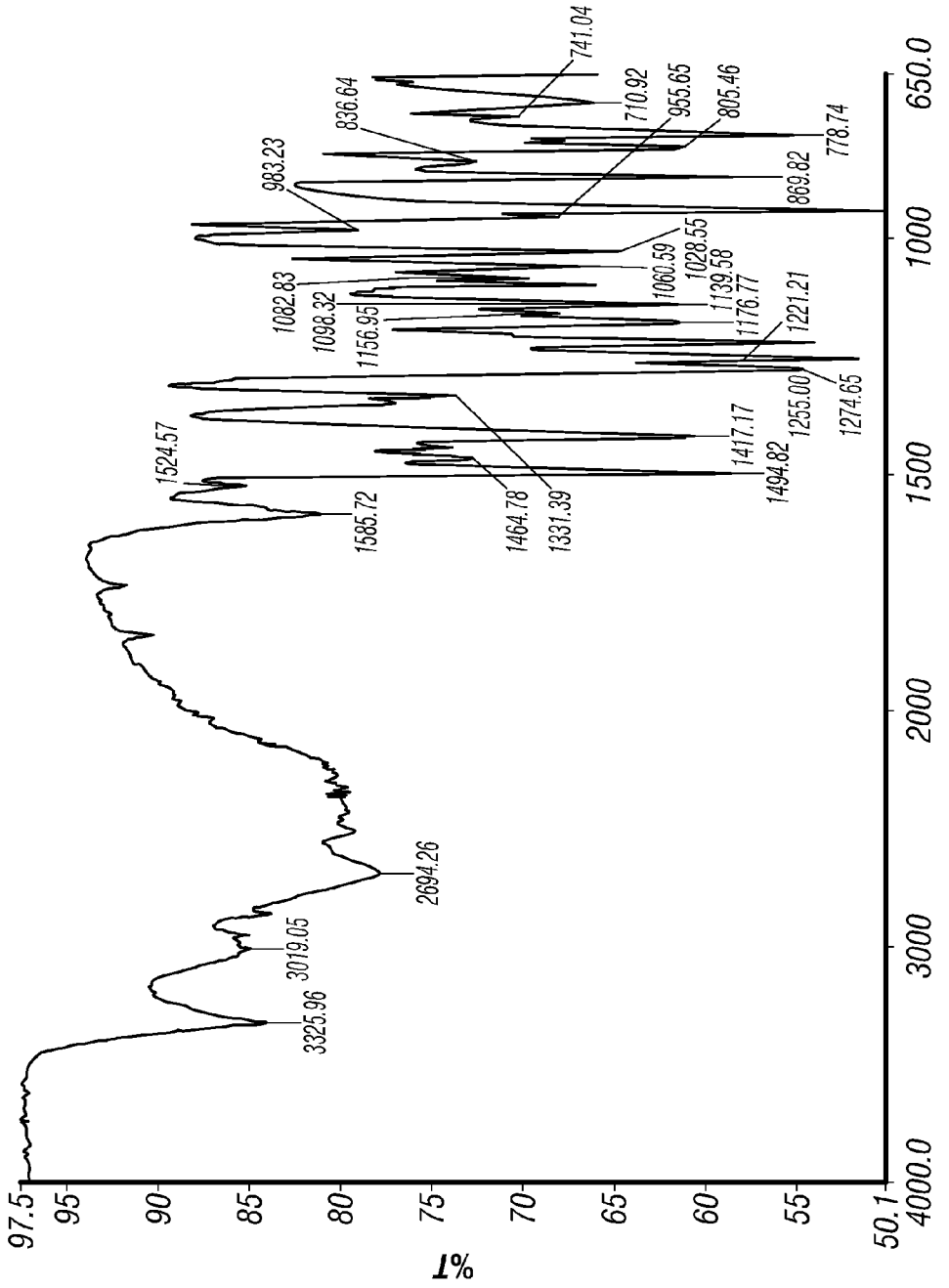
%T
97.5
95
90
85
80
75
70
65
60
55
50.1
4000.0
3000
2000
1500
1000
650.0
cm-1
3325.96
3019.05
2694.26
1585.72
1524.57
1464.78
1331.39
1494.82
1417.17
1274.65
1255.00
1221.21
1176.77
1139.58
1028.55
1060.59
1156.95
1098.32
1082.83
983.23
869.82
836.64
805.46
778.74
741.04
710.92
955.65

FIG. 2B

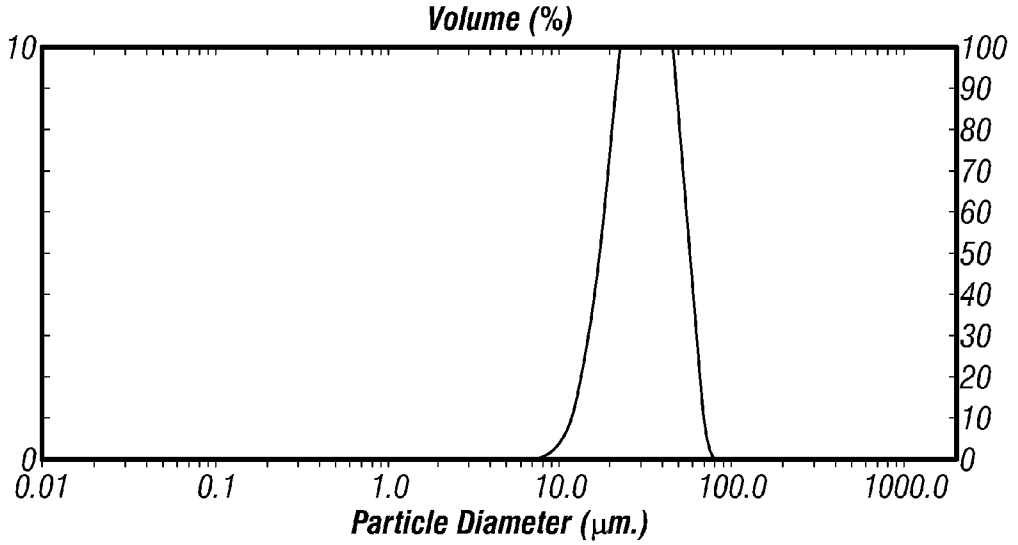
Volume (%)
10
0
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1.0
10.0
100.0
1000.0
Particle Diameter (μm.)

FIG. 3

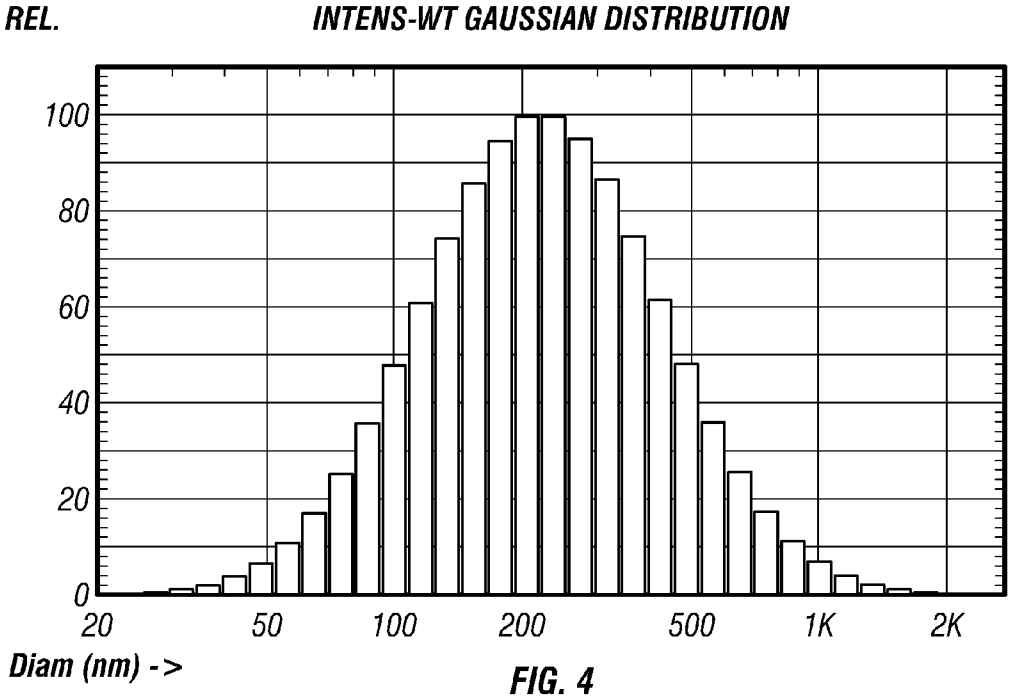
REL.
INTENS-WT GAUSSIAN DISTRIBUTION
100
80
60
40
20
0
20
50
100
200
500
1K
2K
Diam (nm) ->

FIG. 4

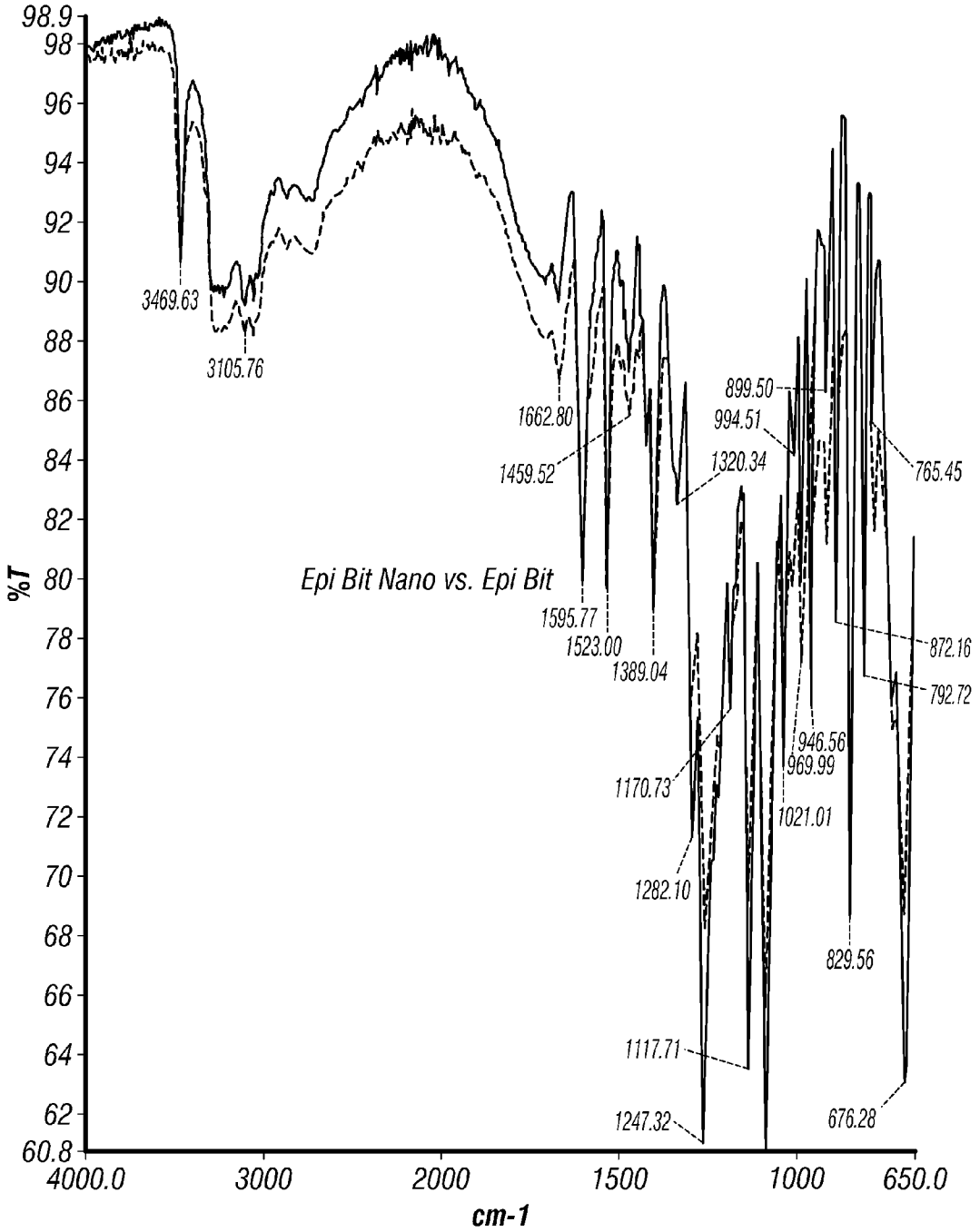
98.9
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
68
66
64
62
60.8
%T
4000.0
3000
2000
1500
1000
650.0
cm-1
Epi Bit Nano vs. Epi Bit
3469.63
3105.76
1662.80
1459.52
1595.77
1523.00
1389.04
1320.34
899.50
994.51
765.45
872.16
792.72
946.56
969.99
1021.01
1170.73
1282.10
829.56
1117.71
1247.32
676.28

FIG. 5

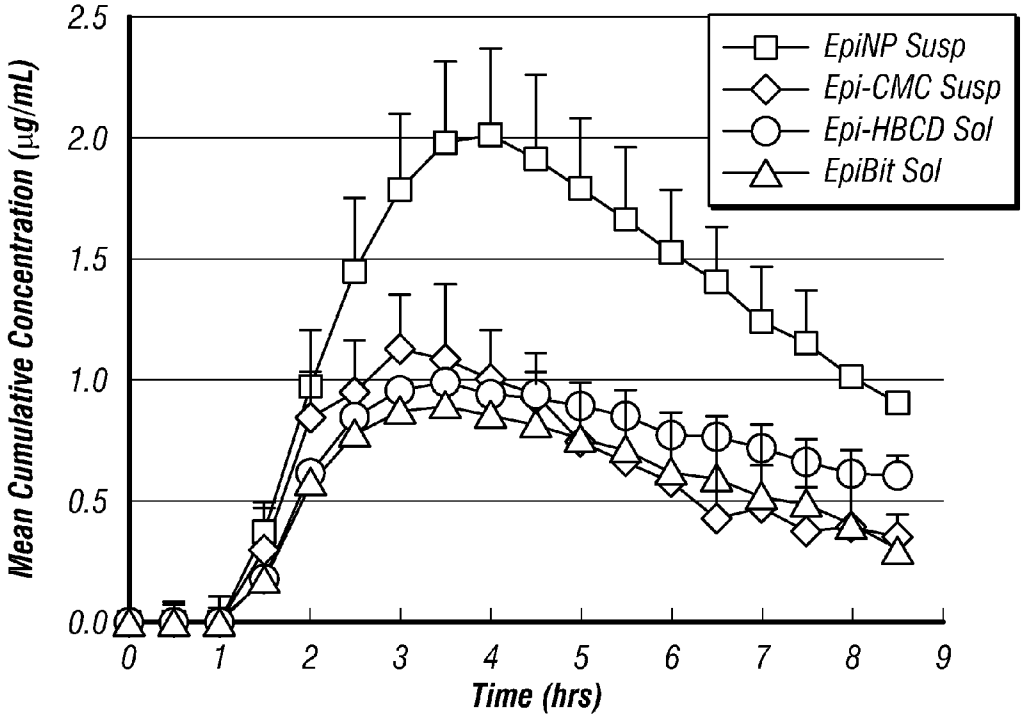
Mean Cumulative Concentration (μg/mL)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
9
Time (hrs)
EpiNP Susp
Epi-CMC Susp
Epi-HBCD Sol
EpiBit Sol

FIG. 6A

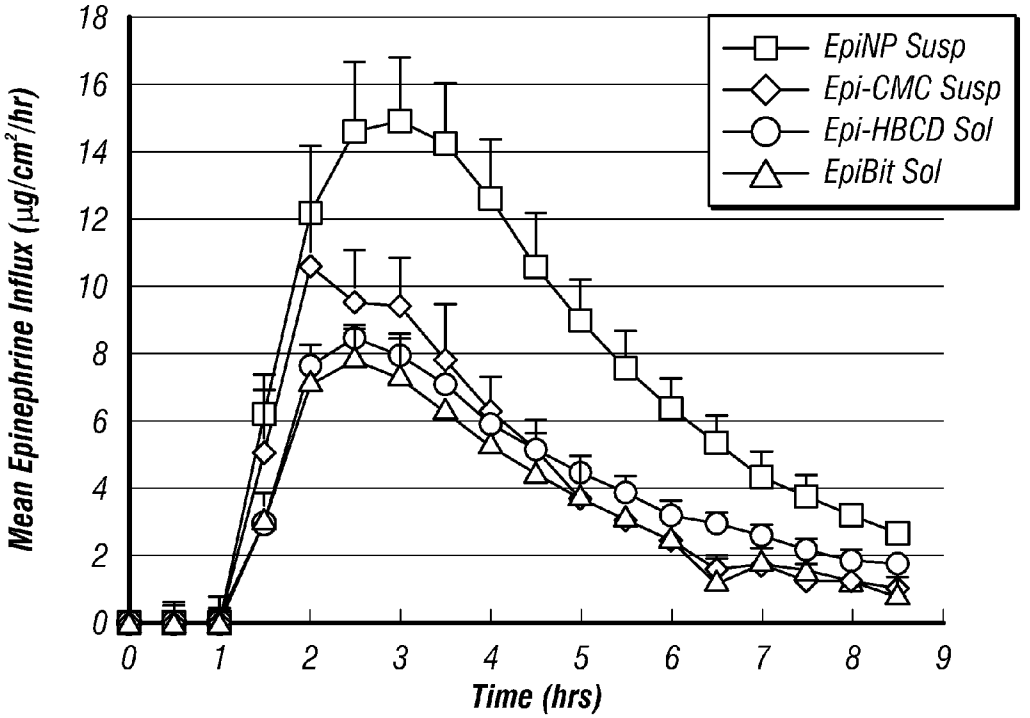
Mean Epinephrine Influx (μg/cm²/hr)
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
8
9
Time (hrs)
EpiNP Susp
Epi-CMC Susp
Epi-HBCD Sol
EpiBit Sol

FIG. 6B

EPINEPHRINE NANOPARTICLES, METHODS OF FABRICATION THEREOF, AND METHODS FOR USE THEREOF FOR TREATMENT OF CONDITIONS RESPONSIVE TO EPINEPHRINE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 61/309,136, filed on Mar. 1, 2010, the content of which is hereby incorporated by reference in its entirety.

[0002] This application is related to U.S. Provisional Patent Application No. 60/715,180, filed on Sep. 9, 2005, and U.S. Provisional Patent Application No. 60/759,039, filed on Jan. 17, 2006. This application is also related to U.S. Utility patent application Ser. No. 11/672,503, filed on Feb. 7, 2007, now abandoned, which is a continuation-in-part of U.S. Utility patent application Ser. No. 11/530,360, filed on Sep. 8, 2006, now abandoned. The aforementioned provisional and utility applications are hereby incorporated by reference in their entireties. The information incorporated is as much a part of the instant application as filed as if the text was repeated in the application, and should be treated (the incorporated information) as part of the text of the application as filed.

FIELD OF THE INVENTION

[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 nanoparticles for sublingual administration in treatment of conditions responsive to epinephrine.

BACKGROUND

[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.

[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).

[0006] Likewise, due to high buccal vascularity, buccally-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 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 mucosa has 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).

[0007] The buccal mucosa is 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 mucosa, 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 route could be useful for drug administration to unconscious patients, patients undergoing an anaphylactic attack, or patients who sense the onset of an anaphylactic attack.

[0008] Epinephrine (EP) 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 only as an injectable dosage form in ampoules or in autoinjectors. 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). Feasibility studies in humans and animals have shown that EP 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 EP 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 EP in a suitable carrier. Based on historical and anecdotal evidence, an approximately 0.3 mg dose of EP, 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, EP 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)).

[0009] As stated above, epinephrine (EP) is typically administered either subcutaneously or intramuscularly by injection. Thus, EP injections are the accepted first aid means of delivering EP 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 EP injectors in a convenient place at all times.

[0010] Given the difficulties associated with manual subcutaneous or intramuscular administration of EP, such as patient apprehension related to injections or the burden of an at risk person having to always maintain an EP injector close at hand, there exists a need in the art for more convenient dosage forms which can provide immediate administration of EP, particularly to a person undergoing anaphylaxis wherein the need for injection or EP injectors is obviated.

[0011] Recently, a novel fast-disintegrating tablet suitable for sublingual (SL) administration 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 Ser. No. 11/672,503; and U.S. Utility patent application Ser. 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. SL doses ranging from 5 to 40 mg epinephrine as the bitartrate salt were studied to achieve equivalent plasma concentrations.

[0012] Without being bound by theory, it is thought that fabrication of epinephrine into nanoparticles and incorporation of the nanoparticles 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.

SUMMARY OF THE INVENTION

[0013] The invention provides a composition, including epinephrine nanoparticles, capable of enhancing the sublingual bioavailability of epinephrine, particularly in the emergency treatment of anaphylaxis.

[0014] The invention additionally provides a method for fabrication of stabilized epinephrine nanoparticles and incorporation of the fabricated nanoparticles into orally-disintegrating and fast-disintegrating tablets.

[0015] The invention also provides a pharmaceutical composition including epinephrine nanoparticles and at least one of a pharmaceutically-acceptable carrier, penetration enhancers, and mucoadhesives for buccal or sublingual administration.

[0016] The invention additionally provides a method for treatment of an allergic emergency comprising the administration of a pharmaceutical composition including epinephrine nanoparticles to a patient diagnosed with or suspected of having an allergic emergency. The allergic emergency can be anaphylaxis, asthma, or bronchial asthma.

[0017] The invention also provides a method for treatment of a cardiac event comprising the administration of a pharmaceutical composition including epinephrine nanoparticles to a patient diagnosed with or suspected of having a cardiac event. The cardiac event can be cardiac arrest.

[0018] 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.

[0019] 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

[0020] 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.

[0021] FIG. **1** is a graph showing mean epinephrine influx ($\mu g/cm^2/hr$) obtained from the tested formulations; epinephrine nanoparticles suspension (EP-NP Susp) (size 200 nm), epinephrine solution (Epi-HBCD Sol), epinephrine suspension (Epi-CMC Susp), and epinephrine bitartrate solution (Epi Bit Sol).

[0022] FIG. **2**A is a Fourier Transform Infrared (FT-IR) spectrum for epinephrine base nanoparticles after fabrication (processing).

[0023] FIG. **2**B is a FT-IR spectrum for epinephrine base nanoparticles before processing.

[0024] FIG. **3** illustrates particle size distribution of epinephrine base measured before size reduction (processing) using Mastersizer.

[0025] FIG. **4** illustrates particle size distribution of epinephrine base measured after size reduction using NiComp 370.

[0026] FIG. **5** is a FT-IR spectrum for epinephrine bitartrate nanoparticles before and after processing (nanoparticle fabrication).

[0027] FIG. **6**A is a graph showing the AUC (mean cumulative epinephrine concentration) (μg/ml) obtained from the four tested formulations; EP-NP Susp, Epi-CMC Susp, Epi-HBCD Sol, and Epi Bit Sol.

[0028] FIG. **6**B is a graph showing mean epinephrine influx ($\mu g/cm^2/hr$) obtained from the tested formulations; EP-NP Susp, Epi-CMC Susp, Epi-HBCD Sol, and Epi Bit Sol.

DETAILED DESCRIPTION OF THE INVENTION

[0029] 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.

Summary of In Vitro Diffusion Experiments and Results

[0030] The experiments described herein were carried out to assess the in vitro diffusion of epinephrine nanoparticles. The use of epinephrine nanoparticles instead of epinephrine salt was hypothesized to enhance the sublingual bioavailability of epinephrine from administration of a fast-disintegrating sublingual tablet formulation for the emergency treatment of anaphylaxis and/or treatment of other conditions responsive to epinephrine.

Methods:

[0031] The diffusion of 80 μg epinephrine from four formulations, epinephrine base nanoparticles suspension (Epi-NP Susp) (size 200 nm), epinephrine solution (Epi-HBCD Sol); epinephrine base using hydroxypropyl-β-cyclodetrin as a solubilizing agent, epinephrine suspension (Epi-CMC Susp); epinephrine base using 0.3% carboxymethyl cellulose as a suspending agent, and epinephrine bitartrate solution (Epi Bit Sol), was studied over 8.5 hours using automated flow-through Franz cell system (n=6). Cumulative epinephrine concentrations in the donor cells were measured using HPLC-UV (High Performance Liquid Chromatography sys-

tem with an ultraviolet detector). The cumulative epinephrine concentration versus time (AUC), maximum epinephrine flux (J_{max}), time to reach Jmax (Jt_{max}), and epinephrine permeation coefficient (Kp) for each formulation were calculated and statistically analysed using one-way ANOV and Tukey-Kramer tests, NCSS program, at a level of significance $p<0.05$.

Results:

[0032] The AUC and Jmax obtained from epinephrine nanoparticles (Epi-NP Susp), 10.4±1.7 μg/ml/hr and 15.1±1.9 μg/cm^2/hr respectively, were significantly higher than epinephrine suspension (Epi-CMC Susp), 5.1±1.1 μg/ml/hr and 10.4±1.6 μg/cm^2/hr, epinephrine solution (Epi-HBCD Sol), 5.5±0.5 μg/ml/hr and 8.6±0.3 μg/cm^2/hr, and epinephrine bitartrate (Epi Bit Sol), 4.6±0.9 μg/ml/hr and 7.9±1.0 μg/cm^2/hr. Jt_{max} was not significantly different between the four formulations. The Kp of epinephrine nanoparticles, 0.19±0.07 cm/hr was significantly higher than epinephrine suspension, 0.13±0.002 cm/hr, epinephrine solution, 0.11±0.04 cm/hr, and epinephrine bitartrate, 0.10±0.04 cm/hr. These results are illustrated in the graph of FIG. **1**.

Conclusions:

[0033] In these experiments, the permeation of epinephrine nanoparticles (Epi-NP Susp) was almost 2 folds higher than the epinephrine bitartrate (Epi Bit Sol) and epinephrine solution (Epi-HBCD Sol). Epinephrine nanoparticles may have the potential to enhance the sublingual bioavailability of epinephrine compared to epinephrine salt in sublingual tablet formulation. Ex vivo and in vivo studies are contemplated and will be pursued to confirm these results.

Details of Fabrication Experiments and Results

Fabrication of Nanoparticles

[0034] Nanoparticles were fabricated from epinephrine base and epinephrine bitartrate (Bit) using high energy fluidization (microfluidization) techniques. These techniques involve the use of oversaturated solutions of various solvents, particularly water and isopropanol, at various temperatures and pressures ranging from about 8,000 psi to 30,000 psi and to about 8.3° to 43.3° C. under various passes. Particle size was measured before and after size reduction using a Mastersizer (Malvern) and/or a NiComp 370 Submicron Particle Sizer (NiComp) and nano-sized particles were confirmed using laser diffraction techniques. The particles were lyophilized (freeze-dried) using a bench top lyophilizer (ART Inc.).

Solubility Studies

[0035] In order to determine suitable vehicles to suspend epinephrine base and epinephrine bitartrate (Bit) for nanoparticle fabrication, solubility studies were carried out.

TABLE 1

Solubility

Sample Name	Amount Dissolved %
Epinephrine Base solubility in water	2.67
Epinephrine Bit solubility in methanol	10.45
Epinephrine Bit solubility in isopropyl alcohol	0.62
Epinephrine Bit solubility in acetonitrile	0.77
Epinephrine Bit solubility in acetone	1.56
Epinephrine Bit solubility in hexane	0.03
Epinephrine Bit solubility in choloroform	0.09
Epinephrine Bit solubility in dichloromethane (DCM)	0.00
Epinephrine Bit solubility in tetrahydrofuran (THF)	7.76
Epinephrine Bit solubility in ethyl acetate	0.63

Fabrication of nanoparticles was first attempted using epinephrine base.

Fabrication: Epinephrine Base

[0036]

TABLE 2

Epinephrine Base

Sample	Solvent	Concentration (mg/ml)	Pressure (psi) (#passes)	Particle Size Distribution (nm) (NiComp)	Sample Temperature (° C.)	Sample Color after Processing
1	water	0.3	30,000	273.9	43.3	brown
2	water	0.308	29,000 (1)	334	18.3	brown
3	0.1% phosphoric acid	1.03	15,000 (2)	335	36.8 first pass 41.1 second pass	brown
4	1M acetic Acid	1.55	8,500 (1) 15,000 (1)	392	36.6 first pass 38.4 second pass	brown
5	water	4.03	15,000	905.9	10	brown
6	0.1 mM sodium metabisulfite in water	4.02	15,000	903.1	8.3	brown
7	0.1 mM sodium metabisulfite in 0.1M perchloric acid	12.02	15,000	903.1 Note: 111.5 nm (80)%) and 2.2 nm (20%) using Zetasizer machine	8.3	pink

TABLE 2-continued

Epinephrine Base						
Sample	Solvent	Concentration (mg/ml)	Pressure (psi) (#passes)	Particle Size Distribution (nm) (NiComp)	Sample Temperature (° C.)	Sample Color after Processing
8	water	10.0	8,000 (1) 15,000 (1)	447		

[0037] Nanoparticles of epinephrine base in various sizes were produced ranging in diameter from about 273.9 to 905.9 nm.

First Sample

[0038] The sample consisted of 30 mg epinephrine in 100 ml of distilled water. One pass at 30,000 psi was applied and a temperature of 43.3° C. was measured after the process. The sample was processed using a M-110P High Energy Fluidizer™ (Microfluidics). The particles were lyophilized using bench top lyophilizer (ART Inc.). The mean particle size obtained was 273.9 nm using the NiComp 370 Submicron Particle Size Analyzer. The sample was stored in the refrigerator.

Second Sample

[0039] This sample consisted of 30 mg epinephrine in 100 ml of distilled water. One pass at 29,000 psi was applied and a temperature of 18.3° C. was measured after the process. The homogenizer was setup using the cooling coil. Ice packs and tap water were used to cool the pressurized sample to 14° C. The mean particle size obtained was 334.3 nm using the NiComp 370 Submicron Particle Size Analyzer. The sample was stored in the refrigerator.

Third Sample

[0040] This sample was prepared in 0.1% phosphoric acid. The phosphoric acid solution was prepared by diluting 0.5 ml of phosphoric acid 85% (Mallinckrodt Chemicals, LOT H39A04, Exp. Sep. 30, 2011) in 500 ml of distilled water. The epinephrine sample was prepared by weighing 103 mg of epinephrine base into 100 ml of 0.1% phosphoric acid solution prior to sample passes. Two passes at 15,000 psi were applied to the sample. In the first pass a temperature of 36.8° C. was measured after the process and in the second pass a temperature of 41.1° C. was obtained. The mean particle size obtained was 334.6 nm using the NiComp 370 Submicron Particle Size Analyzer. The sample was stored in the refrigerator.

Fourth Sample

[0041] This sample was prepared in 1M acetic acid. The 1M acetic acid solution was prepared by diluting 27.5 ml of glacial acetic acid (BDH Aristar, ACS, USP, FCC grade, LOT 200929924) in 500 ml of distilled water. The epinephrine sample was prepared by weighing 155 mg of epinephrine base into 100 ml of 1M acetic acid solution. The M-110p was flushed with distilled water, followed by acetic acid solution prior to sample passes. Two passes were applied to the sample, in the first pass a pressure of 8,500 psi was applied and a temperature of 36.6° C. was measured in the collected sample. In the second pass a pressure of 15,000 psi was applied and a temperature of 38.4° C. was measured in the collected sample. The mean particle size obtained was 392.0 nm using the NiComp 370 Submicron Particle Size Analyzer. The sample was stored in the refrigerator.

Fifth, Sixth, and Seventh Samples

[0042] These samples were prepared in a dark room to avoid light. The homogenizer was setup using the cooling coil. Ice packs and tap water were used to cool the pressurized samples. Higher drug concentration was used in the seventh sample since the acidic solvent tends to dissolve more drug than the other previously-used solvents.

Visual Observations

[0043] The main problem was discoloration (a brown color formed) due to degradation. All samples were discolored to a pinkish color and then became dark brownish after processing, indicating epinephrine instability. The seventh sample (water+0.1 mM sodium metabisulfite+0.1 M perchloric acid) discolored to a slightly pinkish color. 0.1 mM sodium metabisulfite+0.1 M perchloric acid usually provided optimum stability for epinephrine for several months.

[0044] The FT-IR spectrum for epinephrine base before (FIG. **2**B) is different from the FT-IR spectrum after processing (FIG. **2**A), which reflects the degradation that occurs during processing. The epinephrine base required stabilization with acetic acid or phosphoric acid (in the suspension media) and cooling of the reaction chamber to minimize degradation.

Sizing

[0045] The first sample (epinephrine in water) was used.

TABLE 3

Sizes of Epinephrine Base Before and After Processing		
Sample	Before Fabrication (nm)	After Fabrication (110 F., 30 Kpsi) (nm)
1	33030	273.9
2	32530	
3	33160	
Mean	32900	
Standard Error	192.04	
Standard Deviation	332.62	179

[0046] The epinephrine particle size reduction to nanosize was successful. The mean±SD size was reduced from 32.91±0.33 μm (FIG. **3**) to 273.9±179.0 nm (FIG. **4**).

Fabrication: Epinephrine Bitartrate (Bit)

[0047] In light of the instability associated with the epinephrine base particles, fabrication using the epinephrine salt, epinephrine bitartrate, was pursued.

[0048] Isopropyl alcohol (IPO) was selected as a suspending vehicle based on its safety profile and the solubility study previously performed (see above) for several solvents.

TABLE 4

Epinephrine Bitartrate (Bit)

Sample	Solvent	Concentration (mg/ml)	Pressure (psi) (# passes)	PSD nm (NiComp)
1	IPO	7.0	15,000 (1)	43,000
2	IPO	3.5	25,000 (1)	8,766
			25,000 (1)	3,879
3	IPO	0.875	25,000 (1)	3,971
4	IPO	0.70	25,000 (6)	2,368
			25,000 (16)	1,203

Observations

[0049] Nanoparticles of epinephrine bitartrate in various sizes were produced ranging in diameter from about 43,000 to 1,203 nm.

[0050] The first sample, a suspension of 7.0 mg/ml, was used as a stock suspension and was used to prepare the other dilutions. Thus, the passes are additive and each (pass) represents an additional pass to the previous dilution.

[0051] After ten passes in the last run, includes samples one, two, three, and the first pass of sample 4, the particle size distribution (PSD) did not change (no effect after ten passes) according to NiComp readings.

[0052] The fourth sample was processed six times (6 passes in one step) followed by an additional ten passes (for a total of sixteen passes continuously).

[0053] The epinephrine bitartrate (salt form of epinephrine) was more stable than the epinephrine base, did not show any discoloration, and tolerated the fabrication conditions (nanomilling).

First Sample

[0054] The particle size distribution (PSD) of epinephrine bitartrate after processing (fabrication) using Zetasizer was 5000 nm (60%) and 500-1000 nm (30-40%). The yield of fabricated epinephrine bitartrate after drying was 68%. The Fourier Transformation Infrared (FT-IR) spectrums are similar in both epinephrine bitartrate before and after processing (FIG. **5**).

Details of In Vitro Diffusion Experiments and Results

[0055] Epinephrine diffusion was evaluated using an automated, flow through cell system (n=6) under the following parameters:

[0056] Flow rate: 50 μl/minute

[0057] Donor cell orifice area: 0.2 cm^2

[0058] Sample volume added to donor cell: 200 μl

[0059] Medium in receptor cells: phosphate buffer (pH=5.8)

[0060] Membrane: 7 Spectra/Por® dialysis membranes (1000 MWt cutoff).

[0061] Epinephrine, base or salt equivalent to 400 μg/ml epinephrine base, in the following four different formulations were used:

[0062] 1) Epinephrine base nanoparticles suspension (Epi-NP Susp).

[0063] 2) Epinephrine base suspension using 0.3% carboxymethyl cellulose as a suspending agent (Epi-CMC Susp).

[0064] 3) Epinephrine base solution using hydroxypropyl-β-cyclodetrin as a solubilizing agent (Epi-HBD Sol).

[0065] 4) Epinephrine bitartrate solution (Epi Bit Sol).

[0066] 200 μl from each of the four formulations was spiked into the donor cells. Samples were collected every 30 minutes for 8.5 hours and analyzed by High Performance Liquid Chromatography (HPLC) for epinephrine concentration.

HPLC Analysis

[0067] HPLC analysis was performed under the following parameters:

[0068] PerkinElmer HPLC system with ultraviolet (UV) detector

[0069] Column: Econspher (Alltech), C_{18} 4.6×150 mm, 3 μm

[0070] Mobile Phase: USP 26th Edition, 2003

[0071] Flow Rate: 1 ml/minute

[0072] Detection Wavelength: 280 nm

[0073] Retention Time: epinephrine 4.8 minutes

Statistical Analysis of Results

[0074] Results were statistically analyzed using one-way ANOV and Tukey-Kramer tests, NCSS program, at a level of significance $p<0.05$.

[0075] Mean±SD values of cumulative epinephrine concentration versus time (AUC), maximum epinephrine flux (JMax), time to reach JMax (tJMax), and epinephrine permeation coefficient (Kp) for each formulation was calculated.

Results

[0076] Mean±SD values of cumulative epinephrine concentration versus time (AUC), maximum epinephrine flux (JMax), and epinephrine permeation coefficient (Kp) obtained from EP-NP Susp were significantly higher than Epi-CMC Susp, Epi-HBCD Sol, and Epi Bit Sol ($p<0.05$). The time to reach JMax (tJMax) was not significantly different between the four formulations. These results are illustrated in the graphs of FIGS. **6**A-B.

TABLE 5

In Vitro Diffusion Data

Formulation:	Epi-NP Susp	Epi-CMC Susp	Epi-HBCD Sol	Epi Bit Sol
AUC (μg/ml/hr)	10.4 ± 1.7*	5.1 ± 1.1	5.5 ± 0.5	4.6 ± 0.9
JMax (μg/cm^2/hr)	15.1 ± 1.9*	10.4 ± 1.6	8.6 ± 0.3	7.9 ± 1.0

TABLE 5-continued

In Vitro Diffusion Data				
Formulation:	Epi-NP Susp	Epi-CMC Susp	Epi-HBCD Sol	Epi Bit Sol
t_{Jmax} (hr)	9.41 ± 0.26	9.41 ± 0.50	10.17 ± 0.10	10.12 ± 0.09
Kp (cm/hr)	0.19 ± 0.07*	0.13 ± 0.002	0.11 ± 0.04	0.10 ± 0.04

[0077] All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. 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 nanoparticles, pharmaceutical tablets, 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. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention. 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.

1. A pharmaceutical composition comprising stabilized epinephrine nanoparticles and at least one pharmaceutically-acceptable carrier, the composition formulated for buccal or sublingual administration.

2-3. (canceled)

4. A method for fabricating stabilized epinephrine nanoparticles, comprising:

a) combining a pre-determined amount of epinephrine and a solvent in a reaction chamber to form a mixture; and

b) exposing the mixture to at least one pass at a pre-determined pressure and a pre-determined temperature.

5. The method in accordance with claim **4**, wherein the epinephrine is an epinephrine base or an epinephrine bitartrate salt.

6. The method in accordance with claim **4**, wherein the solvent is selected from the group consisting of water, isopropyl alcohol (ISP), methanol, acetonitrile, acetone, hexan, chloroform, dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate, phosphoric acid, acetic acid, and sodium metabisulfite.

7. The method in accordance with claim **4**, wherein the pre-determined pressure ranges from about 8,000 psi to 30,000 psi.

8. The method in accordance with claim **4**, wherein the pre-determined temperature ranges from about 8.3 to 43.3° C.

9. The method in accordance with claim **4**, further including exposing the mixture to a second pass at a different pre-determined pressure and a different pre-determined temperature from that of the first pass.

10. The method in accordance with claim **4**, further including lyophilizing nanoparticles obtained by carrying out steps a) and b).

11. A stabilized epinephrine nanoparticle produced in accordance with the method of claim **10**.

12. A pharmaceutical composition comprising the stabilized epinephrine nanoparticle of claim **11** and at least one of a pharmaceutically-acceptable carrier, a penetration enhancer, and a mucoadhesive, the composition formulated for buccal or sublingual administration.

13-14. (canceled)

15. A method for treating a condition responsive to epinephrine in a subject in need thereof, comprising:

providing a composition including epinephrine nanoparticles and a pharmaceutically-acceptable carrier; and

administering the composition to the subject.

16. The method in accordance with claim **15**, wherein the condition is a cardiac event or an allergic reaction.

17. The method in accordance with claim **16**, wherein the cardiac event is cardiac arrest and the allergic reaction is anaphylaxis, asthma, or bronchial asthma.

18-33. (canceled)

34. The pharmaceutical composition in accordance with claim **1**, further comprising at least one of a penetration enhancer and a mucoadhesive.

35. The method in accordance with claim **16**, wherein the cardiac event is cardiac arrest.

36. The method in accordance with claim **15**, wherein treating includes enhancing sublingual bioavailability of epinephrine.

37. The method in accordance with claim **15**, wherein the condition is a breathing difficulty.

38. The method in accordance with claim **37**, wherein the breathing difficulty is associated with anaphylaxis, asthma, bronchial asthma, bronchitis, emphysema, or respiratory infections.

39. The method in accordance with claim **15**, wherein the condition is an allergic emergency.

40. The method in accordance with claim **39**, wherein the allergic emergency is associated with anaphylaxis, asthma, or bronchial asthma.

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