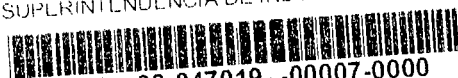


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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 03-047019- -00007-0000

Fecha 2008-10-23 15:22:23 Dep 2020 NULCRACION
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Act 444 RLSPULSIARLOUL Folios 8

2563/P

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. **03.047.019**

Solicitud de patente a nombre de **NOVARTIS AG**

Yo, JIMENA ESCOBAR URIBE, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 No. 86-53, piso 6, obrando como apoderada de la sociedad **NOVARTIS AG**, domiciliada en Basilea, Suiza, solicitante de la patente de la referencia, en respuesta al oficio No. 9866 notificado por fijación en lista el 01 de agosto de 2008, relacionado con el concepto técnico de fondo No. 1171, me permito manifestar:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial un nuevo capítulo reivindicatorio, en el cual se realizan las modificaciones más abajo enunciadas.

1.1 Se corrigen la reivindicación 1 para especificar la naturaleza del agente farmacológicamente activo y del agente de entrega.

1.2 Se corrige el capítulo reivindicatorio para eliminar las reivindicaciones afectadas por excepciones a la patentabilidad.

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de superar las objeciones de claridad planteadas por el examinador en su concepto técnico.

Para este efecto, me permito acompañar el recibo de pago No. 08-77,831 del 30 de septiembre de 2008, con el cual queda cubierta la tasa correspondiente a la modificación de una solicitud de patente en cuanto a su capítulo reivindicatorio.

2. Claridad de la solicitud

El examinador objeta la claridad de la descripción argumentando que "[...] es necesario que el interesado señale cual es la composición de las formulaciones que fueron utilizadas como referencia incorporando las denominaciones comunes de los excipientes y las

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proporciones usada, para tal efecto se recomienda evitar el uso de denominaciones comerciales que no permiten comprender la clase de coadyuvante usado en la formulación". Al respecto el solicitante quisiera anotar que no es clara la objeción del examinador puesto que en las páginas 18 a 24 de la solicitud se describe de manera precisa como se elaboran las diferentes composiciones, con cantidades, tiempos y resultados específicos. A modo de ejemplo se cita el método de obtención del ejemplo comparativo B:

"Ejemplo Comparativo B"

Se combinan **14 gramos** de la sal disódica de 5-CNAC y **0.56 gramos** de CabOSil, y se tamizan a través de un tamiz de malla 40. Se combinan **0.3 gramos** la mezcla de sal disódica de 5-CNAC/CabOSil, **0.028 gramos** de calcitonina de salmón, previamente tamizada a través de un tamiz de malla 40, y **0.56 gramos** de Ac-Di-Sol, previamente tamizado a través de un tamiz de malla 30, en una mezcladora-V de 1 cuarto. La mezcla se bate durante **2 minutos**. Se agregan aproximadamente **14.3 gramos** de la mezcla de sal disódica de 5-CNAC/Cab-O-Sil geométricamente a la mezcladora-V, y se mezclan durante **2 minutos** después de cada adición (se agregan en sucesión aproximadamente **0.8, 1.7, 3.2, y 8.6 gramos**). Se agregan **12.43 gramos** de Avicel PH 102 y **0.42 gramos** de estearato de magnesio, previamente tamizados a través de un tamiz de malla 40, a la mezcladora-V, y se mezclan durante **5 minutos**. Luego se tamiza la mezcla final a través de un tamiz de malla 40, y se comprime en tabletas utilizando, por ejemplo, una prensa Manesty F3. Los **pesos de las tabletas** son de aproximadamente **400 miligramos**."

Para el técnico con habilidad ordinaria en la materia la composición del ejemplo comparativo B es clara y específica, hasta el peso de las tabletas fabricadas es evidente. De manera similar sucede con los demás ejemplos comparativos, por lo que la objeción del examinador carece de fundamento.

En cuanto al uso de términos comerciales, una lectura simple de la invención le permite al técnico con habilidad ordinaria en la materia encontrar que en la página 15 de la descripción se encuentra el significado de 5-CNAC, en la página 16 se encuentra definido el Avicel PH 102 y en la página 18 se define el Ac-Di-Sol, por lo que no resulta muy clara la objeción del examinador, pues todos y cada uno de los componentes utilizados en los ejemplos comparativos se encuentran definidos en la descripción y resultan perfectamente claros para dicho técnico con habilidad ordinaria en la materia. Con el fin de atender de la mejor manera posible las objeciones del examinador, el solicitante respetuosamente le requiere mayor especificidad respecto a aquellos apartes de la descripción que no resultan del todo claros para ese Despacho, pues se ha tratado de ubicar el origen de las objeciones, pero no ha sido posible identificar dentro del texto de la

descripción los fragmentos pertinentes.

En cuanto a la claridad de las reivindicaciones, el solicitante presenta un nuevo capítulo reivindicatorio en el cual se corrige la reivindicación 1 para especificar la naturaleza del agente farmacológicamente activo y limitar el agente de entrega empleado.

3. Excepciones a la patentabilidad

El examinador objeta las reivindicaciones 12 a 14 por referirse a un método de tratamiento terapéutico, lo cual no es patentable en Colombia. Por lo tanto, el solicitante retira dichas reivindicaciones.

4. Novedad

El examinador objeta la novedad a la luz de lo divulgado por las anterioridades D1 – D4, en conjunto. Al respecto el solicitante quisiera anotar que el examinador esta cometiendo un error al tomar diferentes documentos para objetar la novedad de diferentes aspectos de la invención. Es necesario citar el Manual Andino de Patentes, página 59, en el cual se establece que: “*Para el análisis de la novedad no se pueden combinar diferentes documentos del estado de la técnica*”.

De acuerdo con lo expresado por el Manual Andino sumado a la práctica generalizada de esa oficina, la novedad se evalúa a partir de un único documento y solo se puede ver afectada si la totalidad de la invención se encuentra contenida en dicho documento. En consecuencia, el hecho que el examinador cite diferentes documentos para cada aspecto de la invención implica, en un comienzo, que la solicitud posee diferencias respecto a cada uno de los documentos citados. La presencia de estas diferencias, por pequeñas que sean, es sin lugar a dudas un indicio claro e irrefutable de novedad de la presente solicitud por sobre el arte previo.

Siendo así, es evidente que el análisis realizado por la SIC ha sido errado y la afirmación sobre la falta de novedad de la invención es equivocada.

Lo anterior se ve reforzado por dicho Manual Andino de Patentes, el cual en la página 60 dice que: “*La impugnación de la novedad deberá hacerse a partir de una misma divulgación, teniendo en cuenta que no se podrá combinar distintas fuentes de referencia*”.

Contrario a esta indicación, el examinador busca cada una de las características relevantes de la invención en diferentes documentos y combina varias fuentes

para evaluar la novedad de la solicitud. Como resultado de estos errores, la conclusión del examinador sobre la falta de novedad es incorrecta.

5. Nivel Inventivo

El examinador objeta la altura inventiva de la solicitud argumentando que la combinación de la anterioridad D1 con cualquiera de las anterioridades D2 a D4 le permite al técnico con habilidad ordinaria en la materia llegar a lo propuesto por la presente solicitud.

El solicitante quisiera llamar la atención del examinador a los ejemplos de la presente solicitud, los cuales demuestran diferencias significativas sobre la biodisponibilidad de un agente activo de péptido, en particular la calcitonina, cuando se entrega con una formulación que contiene crosprovidona contra composiciones comparativas que contiene desintegrantes alternativos, a saber: Avicel y CabOSil.

Los resultados en la tabla 1 muestran una $C_{\max}$ en plasma para calcitonina que es 6 o 7 veces mayor para una composición de la invención que comprende crosprovidona que las de los ejemplos comparativos. Este es un efecto enteramente inesperado y sorprendente.

No hay una razón particular para que una persona con habilidad en la materia hubiera elegido la crosprovidona en lugar de cualquiera de los demás desintegrantes empleados en el arte previo y nuevamente se hace énfasis en que ciertamente no hay ninguna expectativa de que la crosprovidona lleve a una biodisponibilidad oral significativamente mejor que la de los demás excipientes/desintegrantes.

Es claro, entonces, que el técnico con habilidad ordinaria en la materia no podría derivar del arte previo la actividad mejorada que se obtiene al combinar crosprovidona con calcitonina ya que ninguno de dichos documentos sugiere tal combinación.

Cabe anotar que el técnico con habilidad en la materia reconocerá que combinar crosprovidona con otras proteínas no indica que la combinación específica de la presente solicitud sea obvia y que el resultado obtenido sea evidente.

En resumen, me permito decir que gracias a las modificaciones expuestas en el punto 1 de este memorial y los argumentos anteriormente mencionados, la presente solicitud cumple cabalmente con los requisitos de claridad y altura inventiva respecto a la formulación expuesta en el capítulo reivindicatorio. Por lo tanto, solicito comedidamente a ese despacho se sirva reconocer estos requisitos

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y conceder el privilegio de patente de invención a la solicitud en estudio.

Señor Superintendente,


JIMENA ESCOBAR URIBE
CC. 39.779.452 de Usaquén
TP 68228
Cód. 5376

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 77,831
FECHA : SEPTIEMBRE 30 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 03-047019- -00007-0000

Fecha: 2008-10-23 15:22:23 Dep: 2020 NUL CREACION
Tra: 11 PATENTILYII Lve: 379 FASE NACIONALII
Act: 444 RESPUESTA RLOUL Folios: 8

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CLARKE MODET Y CO. COLOMB	CONSIGNACION	BANCO DE BOGOTA	062754387	92970393	30/09/2008	51,690,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	103,000.00
		TOTAL :	\$ 103,000.00

SON: CIENTO TRES MIL PESOS

RESPONSABLE : _____

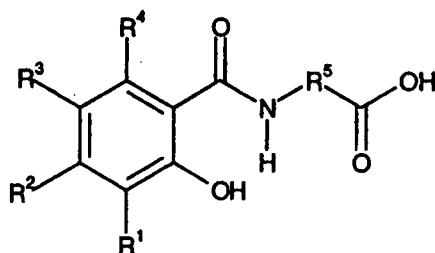
RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

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REIVINDICACIONES

1- Una composición farmacéutica sólida apropiada para la entrega oral de un agente farmacéuticamente activo que comprende:

- (a) una cantidad terapéuticamente efectiva de un agente péptido farmacológicamente activo; calcitonina
- (b) una crospovidona o povidona; y
- (c) un agente de entrega para dicho agente farmacológicamente activo que comprende el siguiente compuesto de fórmula I: 5-CNAC.



en donde:

R^1 , R^2 , R^3 y R^4 son independientemente hidrógeno, -OH, - NR^6R^7 , halógeno, C_1 - C_4 alquilo o C_1 - C_4 alcoxi;

R^5 es C_2 - C_{16} alquilenos sustituido o sin sustituir, C_2 - C_{16} alquenileno sustituido o sin sustituir, C_1 - C_{12} alquil(arileno) sustituido o sin sustituir o aril (C_1 - C_{12} alquilenos) sustituido o sin sustituir; y

R^6 y R^7 son independientemente hidrógeno, oxígeno, o C_1 - C_4 alquilo; e

hidratos y solvatos de alcohol del mismo.

2- Una composición de acuerdo con la reivindicación 1 en donde el agente activo es un péptido seleccionado del grupo que consiste de calcitonina, hormona del crecimiento humano, hormona paratiroide y fragmentos de hormona paratiroide.

3- Una composición de acuerdo con la reivindicación 1 en donde el péptido es una calcitonina.

4- Una composición de acuerdo con la reivindicación 3 en donde la calcitonina es calcitonina de salmón.



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United States Patent [19]

Conte et al.

[11] **Patent Number:** 5,464,633[45] **Date of Patent:** Nov. 7, 1995

[54] **PHARMACEUTICAL TABLETS RELEASING THE ACTIVE SUBSTANCE AFTER A DEFINITE PERIOD OF TIME**

[75] Inventors: **Ubaldo Conte**, Busto Arsizio; **Aldo La Manna**; **Lauretta Maggi**, both of Pavia, all of Italy

[73] Assignee: **Jagotec AG**, Hergiswill, Switzerland

[21] Appl. No.: **248,232**

[22] Filed: **May 24, 1994**

[51] Int. Cl.⁶ **A61K 9/30**; A61K 9/32; A61K 9/34; A61K 9/36

[52] U.S. Cl. **424/480**; 424/474; 424/475; 424/476; 424/481; 424/482; 424/468

[58] Field of Search 424/464, 474, 424/475, 480, 482

[56] **References Cited**

U.S. PATENT DOCUMENTS

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4,756,911 7/1988 Drost et al. 424/468

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Techniques of Solubilization of Drugs, S. H. Yalkowsky, Ed. Marcel Dekker Inc. NY (1985) pp. 1,15,91, 135 & 159.

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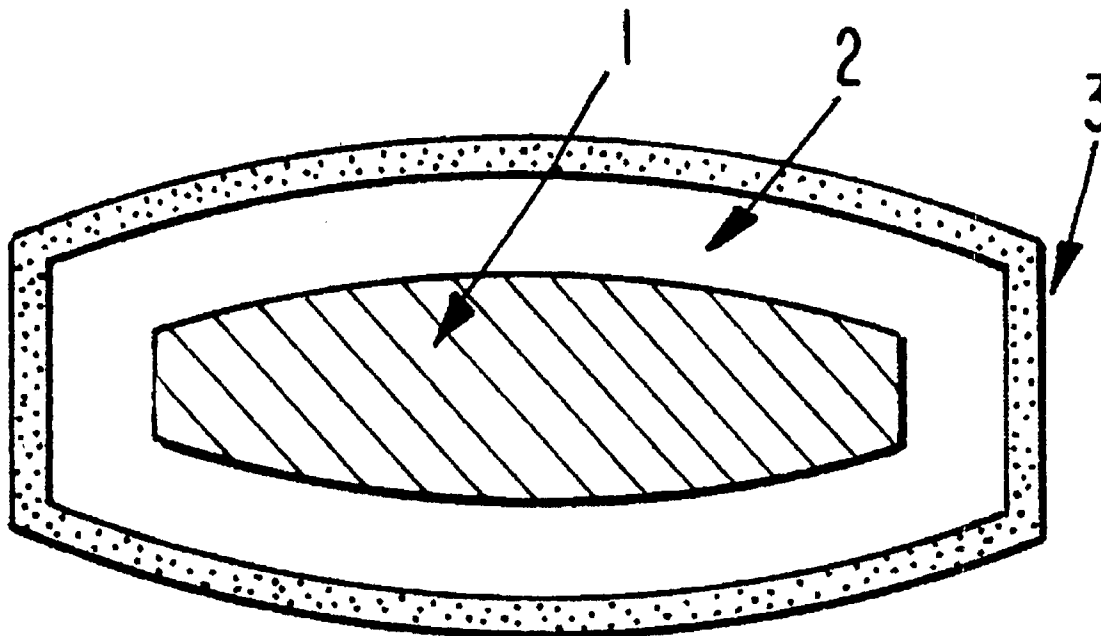
Primary Examiner—Thurman K. Page

Assistant Examiner—James M. Spear

[57]

ABSTRACT

Pharmaceutical tablets releasing the active substance after a definite period of time, consisting of: a core containing the active substance and polymeric substances which swell and/or gel and/or erode on contact with water or with aqueous liquids; a layer applied externally to said core, suitable to prevent the immediate release of the active substance contained in the core and to allow the release of said active substance after a definite period of time, consisting of natural and/or synthetic polymeric materials of the class of the hydrophilic polymers which erode and/or gel and/or dissolve in an aqueous medium; a possible gastroresistant and enterosoluble coating.

15 Claims, 1 Drawing Sheet

administration.

Said pharmaceutical tablet consists of:

a core containing the active substance or substances to be released in the gastric or intestinal tract, polymeric substances which swell and/or gel and/or erode on contact with water or aqueous liquids and adjuvant and excipient substances normally used in the pharmaceutical technique;

a layer applied externally to said cope, preferably by compression, suitable to allow the release of the active substance or substances contained in the core after a definite period of time, and consisting of natural and/or synthetic polymeric materials of the class of hydrophilic polymers which erode and/or gel and/or dissolve in an aqueous medium and adjuvant and excipient substances normally used in the pharmaceutical technique. Such a layer does not contain active substance but acts only as a releasing modulator allowing the release of the active substance contained in the core after a definite period of time which can be controlled by proper in vitro tests;

a possible gastroresistant and enterosoluble coating applied on said external layer.

A new pharmaceutical form is obtained which does not release the active substance contained in the core for a definite period of time. In fact the release of the active substance after administration of the pharmaceutical form occurs only when the characteristics of said external layer are modified by the contact with the gastro-intestinal liquids in such a way to allow the active substance dissolution. The finished pharmaceutical tablet (core+ external layer) can be coated with a film of gastroresistant polymeric material in such a way to allow the system activation only when the tablet attains the duodenal tract.

DETAILED DESCRIPTION OF THE INVENTION

The characteristics and advantages of the pharmaceutical tablets with release of the active substance after a definite period of time according to the present invention, will be pointed out to a greater extent during the course of the following detailed description.

Said tablets consist of:

a core containing the active substance and polymeric substances swelling and/or gellable and/or erodible on contact with water or aqueous liquids and adjuvant substances;

a layer externally applied to said cope, able to prevent the immediate release of the active substance contained in the core and to allow the release of the said active substance after a definite period of time, consisting of natural and/or synthetic polymeric substances belonging to the class of the erodible and/or gellable and/or soluble in an aqueous medium hydrophilic polymers and adjuvant substances;

a possible gastroresistant and enterosoluble coating.

BRIEF DESCRIPTION OF THE DRAWING

Said tablet has the form shown in FIG. 1, in which the cope is indicated by (1), the layer externally applied to the cope is indicated by (2) and the possible coating is indicated by (3).

The cope (1) is prepared by compression of the relative mixture possibly pregranulated, the layer (2) is preferably applied by compression of the relative mixture possibly pregranulated and the coating (3) is preferably applied by film-coating.

One of the characteristics of the tablet according to the invention consists in that in the preparation of the cope, in addition to the active substance, also polymeric substances able to modulate (to slow and/or to speed up) the release of the active substance, are used.

An active substance very soluble in water and/or aqueous liquids independently from the pH value of the dissolution medium and/or of the gastrointestinal tract may be used or an active substance poorly soluble in the same conditions, may be used. In this case particular adjuvants may be used to facilitate the quick dissolution as, for example, described in the volume "Techniques of Solubilization of Drugs" by S. H. Jalkowsky Ed.—M. Dekker New York 1985 and the Italian patent application No. 21091A/90 of 20 Jul. 90.

Non steroid (NSAID) or steroid antiinflammatory drugs as sodium diclofenac, indomethacin, ibuprofen, ketoprofen, diflunisal, piroxicam, naproxen, flurbiprofen, sodium tolmetin, or sleep inductors and tranquilizers as diazepam, nitrazepam, flurazepam, oxazepam, chlordiazepoxide, medazepam, lorazepam or active substances for the prevention of angina attacks and hypertension as diltiazem, trapidil, urapidil, benziodarone, dipyridamole, lidoflazine, naphthydrofuryl oxalate, perhexiline maleate, oxyfedrine hydrochloride, or drugs of peptidic nature as insulin, calcitonin and somatostatin, may be used as active substances.

Crosslinked polyvinylpyrrolidone, hydroxypropylmethylcellulose, crosslinked sodium carboxymethylcellulose, carboxymethylstarch, potassium methacrylatedivinylbenzene, copolymer, polyvinylalcohols, starches, starch derivatives, betacyclodextrin and dextrans derivatives in general may be used, for example, as polymeric substances in the preparation of the said core. Said polymeric substances constitute from 1.0 to 90% of the core weight.

As far as the hydroxypropylmethylcellulose is concerned various types with different molecular weight (between 1,000 and 4,000,000) and with different substitution degree may be used.

Said types of hydroxypropylmethylcellulose present differentiated characteristics being mostly erodible or mostly gellable depending on the substitution degree (S.D.) shown in the polymer chain.

In addition adjuvant substances as natural and/or synthetic polymeric materials belonging to the class of the so called gellable hydrophilic polymers, able to slow the release of the active substance from the core, may be used.

Finally, excipients normally used in the pharmaceutical technique as mannitol, lactose, magnesium stearate, colloidal silica and others as glyceryl monostearate, hydrogenated castor oil, waxes, mono-bi-and trisubstituted glycerides, may be used.

When the penetration of the water and/or aqueous fluids in the core is to be helped, hydrophilic diluents as mannitol, lactose, starches of different origin, sorbitol and xylitol are introduced.

When the penetration of water and/or aqueous fluids in the core is to be slowed down, hydrophobic diluents as glyceryl monostearate, hydrogenated castor oil, waxes and mono-bi-trisubstituted glycerides are introduced. It is to remark that generally in the case of hydrophilic matrices, like those generally constituting the said core, particularly in the case of very soluble in aqueous liquids drugs, the "dose dumping" effect may occur, that is the release of an excessive amount of drug in a very short period of time, this fact causing the appearance of collateral effects not always easily tolerable by the patient.

The pharmaceutical tablets of the invention have the

advantage of releasing the active substance contained in the core after a definite period of time and, containing a reduced amount of drug compared to the traditional retard forms, of avoiding the dose dumping effect.

Such result is reached by completely coating said core with an external layer, preferably but not necessarily, obtained by compression.

Said layer consists of natural and/or synthetic polymeric materials belonging to the class of the gellable hydrophilic polymers and/or to the class of the erodible hydrophilic polymers and/or gellable and soluble in water and/or in the dissolution medium at a rate which can be rigorously predetermined and controlled with proper tests in vitro, the rate of erosion and/or of dissolution being determined by all the substances constituting the formulation. In addition to said polymeric materials, excipients as hydrophilic agents, so called canalizing agents, may be used in the formulation used for said layer when the erosion or gelling or dissolution process is to be accelerated, or hydrophobic and water repellent agents when said process have to be slowed down.

Other technological adjuvants able to give to the material or to the mixture the suitable characteristics of industrial workability are moreover used.

The polymeric substances used in the preparation of the external layer, can show characteristics of gastroresistance and enterosolubility allowing the activation of the system only after the tablet has reached the enteric tract and in particular formulations of said polymeric substances able to obtain tablets expressly designed to the release of the active substance at the colon level, are possible.

The polymeric substances used for the external layer preparation are selected in the class comprising hydroxypropylmethylcellulose with a molecular weight between 1,000 and 4,000,000, hydroxypropylcellulose with a molecular weight between 2,000 and 2,000,000, carboxyvinyl polymers, polyvinylalcohols, glucans scleroglucans, mannans, xanthans, carboxymethylcellulose and derivatives, ethylcellulose, methylcellulose and, in general, cellulosic derivatives.

As far as the hydroxypropylmethylcellulose is concerned the remarks expressed on this substance in the use for the core preparation, are confirmed.

Said polymeric substances may be present in a percentage between 5 and 90 per cent of the total weight of said layer but preferably between 50 and 85 per cent.

Said polymeric substances, used one by one or mixed together, are able to determinate a "retard" in the release of the active substance contained in the core in a period of time between 15 minutes and more than 8 hours, also modifying the drug release rate from the core.

Said polymeric substances may be contained in the core too, when a slowed release of the active substance is to be obtained. Said external layer has a thickness between 0.2 and 4.5 mm if it is by compression applied and between 0.1 and 4.5 mm if it is by film-coating applied.

In the case said external layer is applied by compression diluents as those traditionally used in the solid forms preparation or fatty, waxy, natural and synthetic or semisynthetic substances as glyceryl monostearate and semisynthetic triglyceride derivatives, semisynthetic glycerides, hydrogenated castor oil, glycerylpalmitostearate, glyceryl behenate and other adjuvants such as binders as polyvinylpyrrolidone, gelatin, ethylcellulose, methylcellulose, sodium carboxymethylcellulose and other natural or synthetic substances well known to the skilled in the field, may be used.

For example magnesium stearate, stearic acid, sodium stearate, talc, sodium benzoate, boric acid, polyoxyethylenglycols and colloidal silica, are used.

Moreover diluent, lubricant, antiadherent and glidant substances, hydrophilic and canalizing agents and other substances able to give to said layer the requested characteristics can be used, as the following examples will better specify.

Said external layer may be obtained also by film-coating and/or lacquering process according to methods well known to the skilled in the field.

In such case besides the basic polymeric material as before described plasticizing substances as butylphthalate, propylphthalate, diethylphthalate, zein, polyoxyethylenglycols with different molecular weight and opacity agents as titanium dioxide and other adjuvants well known to the skilled in the field, may be used.

On said layer, independently from the ways of application, a film of gastroresistant and enterosoluble polymeric material can be further applied, to allow the system activation only after the tablet has reached the duodenum-intestinal tract.

Pharmaceutical systems of the last type may be used for the preparation of tablets specifically designed and addressed to release the active substance in the last part of the intestinal tract, that is at the colon level.

As polymeric materials to obtain the gastroresistance, cellulose, acetophthalate, cellulose acetopropionate, cellulose trimellitate, acrylic and methacrylic polymers and copolymers with different molecular weight and solubility dependent on different pH values may be used.

Said materials may be applied on the finished pharmaceutical form (core+external layer) by the classic film-coating process using solutions in organic solvents or aqueous dispersions and operating by nebulization in basin or in fluidized bed.

Said gastroresistant and enterosoluble materials may also be used in association with retarding polymers.

The following examples refer to the preparation of prototypes of the realization before described and they are reported only for explanatory and not limitative purpose of the present invention.

EXAMPLE 1

PREPARATION OF TRAPIDIL BASE TABLETS

1a—Preparation of the core granulate

N° 10.000 cores each having the following unit composition, are prepared:

Trapidil (Inverni della Beffa, Milan, I)	150.0 mg
Hydroxypropylmethylcellulose (Methocel K4, colorcon, Orpington, U.K.)	30.0 mg
Mannitol (USP grade, C. Erba, Milan, I)	98.3 mg
Polyvinylpyrrolidone (Plasdone © K29-32, Gaf Corp., Wayne, NY, U.S.A.)	3.7 mg
Magnesium stearate (USP grade, C. Erba Milan, I)	2.0 mg
Colloidal silica (Syloid 244, Grace GmbH, Worms, D)	1.0 mg

The hydrophilic core manufacture process consists in the preparation of a granulate by mixing, in a sigma Erweka K5 type mod. (Frankfurt a.M. -D) mixer, the proper amounts of active substance, hydroxypropylmethylcellulose and man-

TABLE VIII

Time (h)	Drug % released from the core without external layer	Drug % released from the finished tablet
1	18.6	0.0
2	32.1	0.0
4	55.7	0.1
6	76.9	5.8
8	98.0	14.3
10	100.8	25.4
12		38.7
14		52.9
16		67.4
18		84.8
20		95.6

Also in this case it is clear that the active substance release from the finished tablet begins only after a period of about three/four hours compared to the core without external layer.

This behaviour confirms the validity of the embodiment and in this case points out that a change of the external layer leads to a change in the "time-lag" of about one/two hours compared to a lower thickness.

We claim:

1. Pharmaceutical tablet for oral administration suitable to release the active substance after a definite period of time, consisting essentially of:

a core containing the active substance to be released in the gastric or intestinal tract, a polymeric substance which swells and/or gels and/or erodes on contact with water or aqueous liquids and is selected from the group consisting of hydroxypropylmethylcellulose having a methoxyl content of 22.1% and a viscosity of 15,000 centipoises, crosslinked polyvinylpyrrolidone, crosslinked sodium carboxymethylcellulose, potassium methacrylate-divinyl-benzene copolymer, polyvinylalcohols and beta cyclodextrin and adjuvants and excipients;

a layer applied externally to said core by a compression process said layer being suitable to allow the release of the active substance contained in the core after a definite period of time and being selected from the group consisting of hydroxypropylmethylcellulose having a methoxyl content of 22.1 and a viscosity of 4,000 centipoises, carboxy vinyl polymers, glucans, mannans, xanthans and carboxymethylcellulose and adjuvants and excipients;

wherein said layer is applied externally to said core and has a thickness of 0.2-4.5 mm which allows the release of said active substance in said core after contact with water or an aqueous liquid for a period of 2 to 3 hours.

2. Tablet as claimed in claim 1, characterized in that said active substance is a non-steroid antiinflammatory drug or steroid antiinflammatory drug selected from the group consisting of sodium diclofenac, indomethacin, ibuprofen, ketoprofen, diflunisal, piroxicam, naproxen, flurbiprofen and sodium tolmetin.

3. Tablet as claimed in claim 1, characterized in that said active substance is a sleep inducer drug selected from the group consisting of diazepam, nitrazepam, flurazepam, oxazepam, chlordiazepoxide, medazepam and lorazepam.

4. Tablet as claimed in claim 1, characterized in that said active substance is an anti-hypertension drug selected from the group consisting of trapidil, urapidil, benzodiarone, dipyrindamole, diltiazem, lidoflazine, naphthodifuryl oxalate, perhexiline maleate and oxyfedrine hydrochloride.

5. Tablet as claimed in claim 1, characterized in that said active substance is a peptide drug selected from the group consisting of insulin, calcitonin, and somatostatin.

6. Tablet as claimed in claim 1, characterized in that in said core composition said polymeric substances are contained in a percentage of from 1.0 to 90.0 of said core weight.

7. Pharmaceutical tablet for oral administration suitable to release a peptide drug selected from the group consisting of insulin, calcitonin and somatostatin after a definite period of time, said tablet consisting of:

core containing a peptide drug selected from the group consisting of insulin, calcitonin and somatostatin, a polymeric substance which swells and/or gels and/or erodes on contact with water or aqueous liquids and is selected from the group consisting of hydroxypropylmethylcellulose having a methoxyl content of 22.1% and a viscosity of 15,000 centipoises, crosslinked polyvinylpyrrolidone, crosslinked sodium carboxymethylcellulose, potassium methacrylate-divinyl-benzene copolymers, polyvinylalcohols and beta cyclodextrin and adjuvants and excipients;

a layer applied externally to said core by a compression process said layer being suitable to allow the release of said peptide drug contained in the core after a definite period of time and being selected from the group consisting of hydroxypropylmethylcellulose having a methoxyl content of 22.1 and a viscosity of 4,000 centipoises, carboxy vinyl polymers, glucans, mannans, xanthans and carboxymethylcellulose and adjuvants and excipients;

wherein said layer is applied externally to said core and has a thickness of 1.0-1.50 mm which allows the release of said active substance in said core after contact with water or an aqueous liquid for a period of 2 to 3 hours.

8. Tablet as claimed in claim 1, characterized in that said adjuvant substances of the core are hydrophilic diluents selected from the group consisting of mannitol, lactose, starches of different source, sorbitol, xylitol.

9. Tablet as claimed in claim 1, characterized in that said adjuvant substances of the core are hydrophobic diluents selected from the group consisting of glyceryl monostearate, hydrogenated castor oil, waxes and glycerides.

10. Pharmaceutical tablet for oral administration as defined in claim 1 wherein the external layer is about 1.0 mm to 1.5 mm thick.

11. Pharmaceutical tablet for oral administration as defined in claim 1 wherein the external layer is about 1.0 mm thick.

12. Pharmaceutical tablet for oral administration as defined in claim 1 wherein the external layer is about 1.5 mm thick.

13. Pharmaceutical tablet as claimed in claim 1, characterized in that said active substance is selected from the group consisting of diflunisal, piroxicam, flurbiprofen, sodium tolmetin, nitrazepam, flurazepam, oxazepam, chlordiazepoxide, medazepam, lorazepam, trapidil, urapidil, benzodiarone, dipyrindamole, diltiazem, lidoflazine, naphthodifuryl oxalate, perhexiline maleate, oxyfedrine hydrochloride, insulin, calcitonin and somatostatin.

14. Tablet as claimed in claim 1, characterized in that said adjuvant and excipient substances used for the preparation of the external layer are selected from the group consisting of glyceryl monostearate, semisynthetic glycerides, hydrogenated castor oil, glyceryl palmitostearate, glyceryl behenate, polyvinylpyrrolidone, gelatin, ethylcellulose, methylcellulose, sodium carboxymethylcellulose, magnesium stearate, stearic acid, sodium stearate, talc, sodium benzoate, boric acid, polyoxyethylene glycol and colloidal silica.

15. Pharmaceutical tablet for oral administration suitable to release the active substance after a definite period of time, consisting essentially of:

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a core containing the active substance to be released in the gastric or intestinal tract, a polymeric substance which swells and/or gels and/or erodes on contact with water or aqueous liquids and is selected from the group consisting of hydroxypropylmethylcellulose having a methoxyl content of 22.1% and a viscosity of 15,000 centipoises, crosslinked polyvinylpyrrolidone, crosslinked sodium carboxymethylcellulose, potassium methacrylate-divinyl-benzene copolymer, polyvinylalcohols and beta cyclodextrin and adjuvants, and excipients;

a layer applied externally to said core by a compression process said layer being suitable to allow the release of the active substance contained in the core after a definite period of time and being selected from the group consisting of hydroxypropylmethylcellulose

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having a methoxyl content of 22.1 and a viscosity of 4,000 centipoises, carboxyl vinyl polymers, glucans, mannans, xanthans and carboxymethylcellulose and adjuvants and excipients;

wherein said layer is applied externally to said core and has a thickness of 0.2-4.5 mm which allows the release of said active substance in said core after contact with water or an aqueous liquid for a period of 2 to 3 hours wherein said pharmaceutical tablet has a gastroresistant and enterosoluble coating which consists of polymeric materials selected from the group consisting of cellulose acetophthalate, cellulose acetopropionate, cellulose trimellitate, acrylic polymers, acrylic copolymers, methacrylic polymers and methacrylic copolymers.

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United States Patent [19]

Stern et al.

[11] **Patent Number:** 5,912,014[45] **Date of Patent:** Jun. 15, 1999[54] **ORAL SALMON CALCITONIN
PHARMACEUTICAL PRODUCTS**[75] **Inventors:** William Stern, Tenaflly; James P. Gilligan, Union, both of N.J.[73] **Assignee:** Unigene Laboratories, Inc., Fairfield, N.J.[*] **Notice:** This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).[21] **Appl. No.:** 08/616,250[22] **Filed:** Mar. 15, 1996[51] **Int. Cl.⁶** A61K 9/28; A61K 9/30[52] **U.S. Cl.** 424/474; 424/475; 424/477;
424/480; 424/491; 424/457; 424/459; 424/461;
424/426; 514/12[58] **Field of Search** 424/426, 474,
424/475, 477, 480, 491, 457, 459, 461;
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[57]

ABSTRACT

Bioavailability of salmon calcitonin to be administered orally is enhanced by a pharmaceutical composition providing targeted release of the peptide to the intestine, together with an absorption enhancer and a sufficient amount of a pH-lowering agent to lower local intestinal pH. Specific concentrations and classes of these agents are disclosed to account for the particular characteristics of salmon calcitonin.

32 Claims, No DrawingsD₂
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ORAL SALMON CALCITONIN PHARMACEUTICAL PRODUCTS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to oral salmon calcitonin pharmaceuticals, to methods of enhancing bioavailability of orally administered salmon calcitonin, and to methods of treating bone and calcium disorders in humans by orally administering salmon calcitonin in accordance with the invention.

2. Description of the Related Art

Salmon calcitonin is a peptide hormone which decreases uptake of calcium from bone. When used to treat bone-related diseases and calcium disorders (such as osteoporosis, Paget's disease, hypercalcemia of malignancy, and the like), it has the effect of helping maintain bone density. Many types of calcitonin have been isolated (human calcitonin, salmon calcitonin, eel calcitonin, elkatoinin, porcine calcitonin, and chicken calcitonin). There is significant structural non-homology among the various calcitonin types. For example, there is 50% percent identity between the amino acids making up human calcitonin and those making up salmon calcitonin.

Salmon calcitonin used in the prior art has usually been administered by injection or by nasal administration. However, these modes of administering the calcitonin are significantly less convenient than, and involve more patient discomfort than, oral administration. Often this inconvenience or discomfort results in substantial patient noncompliance with a treatment regimen. However, the prior art is not believed to have reported an ability to achieve reproducible blood levels of salmon calcitonin when administered orally. This is believed to be because salmon calcitonin lacks sufficient stability in the gastrointestinal tract, and tends to be poorly transported through intestinal walls into the blood.

Proteolytic enzymes of both the stomach and intestines may degrade salmon calcitonin, rendering it inactive before the calcitonin can be absorbed into the bloodstream. Any amount of salmon calcitonin that survives proteolytic degradation by proteases of the stomach (typically having acidic pH optima) is later confronted with proteases of the small intestine and enzymes secreted by the pancreas (typically having neutral to basic pH optima). Other difficulties arising from the oral administration of salmon calcitonin involve the relatively large size of the molecule, and the charge distribution it carries. This may make it more difficult for salmon calcitonin to penetrate the mucus along intestinal walls or to cross the intestinal brush border membrane into the blood. These additional problems may further contribute to the limited bioavailability of salmon calcitonin.

SUMMARY OF THE INVENTION

It is accordingly an object of the present invention to provide a therapeutically effective oral pharmaceutical composition for reliably delivering salmon calcitonin.

It is a further object of the invention to provide therapeutic methods for enhancing the bioavailability of salmon calcitonin.

It is a further object of the invention to provide methods of treating bone-related diseases and calcium disorders by administering salmon calcitonin orally.

In one aspect, the invention provides a pharmaceutical composition for oral delivery of salmon calcitonin comprising

(A) a therapeutically effective amount of said salmon calcitonin;

(B) at least one pharmaceutically acceptable pH-lowering agent;

(C) at least one absorption enhancer effective to promote bioavailability of said salmon calcitonin; and

(D) an enteric coating capable of (i) conducting said salmon calcitonin, absorption enhancer and pH-lowering agent through a patient's stomach while protecting said salmon calcitonin from degradation by stomach proteases, and (ii) releasing said salmon calcitonin, absorption enhancer and pH-lowering agent together into the intestine of said patient;

wherein said pH-lowering agent is present in said pharmaceutical composition in a quantity which, if added to 10 milliliters of a 0.1M aqueous sodium bicarbonate solution, would be sufficient to lower the pH of said solution to no higher than 5.5.

In another aspects the invention provides a method for enhancing the bioavailability of salmon calcitonin delivered orally, said method comprising selectively releasing said salmon calcitonin, together with at least one pH-lowering agent and at least one absorption enhancer, into a patient's intestine following passage of said salmon calcitonin pH lowering agent, and absorption enhancer through the patient's mouth and stomach under protection of an enteric coating which substantially prevents contact between stomach proteases and said salmon calcitonin;

wherein said pH-lowering compound is released by said vehicle into said intestine in an amount which, if added to 10 milliliters of 0.1M aqueous sodium bicarbonate solution, would be sufficient to lower pH of said solution to no higher than 5.5.

The present invention is believed to reduce the likelihood of proteolytic degradation of the salmon calcitonin by simultaneously protecting salmon calcitonin from proteolytic attack by (1) stomach proteases which are typically most active at acidic pH) and (2) intestinal or pancreatic proteases (which are typically most active at basic to neutral pH).

Then the invention is believed to speed the process by which salmon calcitonin crosses the intestinal brush border membrane into the blood, while continuing to protect the salmon calcitonin from proteolytic degradation.

An enteric coating, or the like, protects the peptide active agent from the acid-acting proteases of the stomach. Significant quantities of acid (with which the active agent is intermixed) then reduce the activity of neutral to basic-acting proteases in the intestine (e.g. luminal or digestive protease and proteases of the brush border membrane) by lowering pH out of the optimal activity range of these intestinal proteases. Absorption enhancers of the invention may be used to enhance transport of the peptide agent through intestinal mucous layers, through the brush border membrane and into the blood.

The simultaneous use of absorption enhancers together with a pH-lowering compound, in accordance with the invention, provides a surprisingly synergistic effect on the bioavailability of salmon calcitonin relative to absorption enhancer alone, or pH-lowering compound alone. Compare Table 4 (infra), formulation I (salmon calcitonin alone), Table 3, formulation I (salmon calcitonin and pH-lowering compound) and Table 4, formulation II (salmon calcitonin and absorption enhancer) with Table 4 formulation III (salmon calcitonin, pH-lowering compound, and absorption enhancer).

Other features and advantages of the present invention will become apparent from the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention, patients in need of treatment with salmon calcitonin are provided with an oral pharmaceutical composition containing the salmon calcitonin in tablet or capsule form of an ordinary size in the pharmaceutical industry. The dosages and frequency of administering the products are discussed in more detail below. Patients who may benefit are any who suffer from calcium disorders or bone diseases. The invention may be used, for example, to treat osteoporosis, Paget's disease, hypercalcemia of malignancy and the like.

Salmon calcitonin is a preferred calcitonin for use in accordance with the invention because it provides a number of advantages over human calcitonin, even though used as a pharmaceutical agent for human patients. Among the advantages provided by utilizing salmon calcitonin instead of human calcitonin for the treatment of human osteoporosis are increased potency, analgesia and increased half-life. Salmon calcitonin is more effective than natural human calcitonin in treatment, since lower dosages are necessary than with human calcitonin. There is substantial non-homology between salmon and human calcitonin, with only 50% identity in the amino acid sequences of the two calcitonins.

The pharmaceutical composition of the invention overcomes a series of different and unrelated natural barriers to bioavailability. Various components of the pharmaceutical compositions act to overcome different barriers by mechanisms appropriate to each, and result in synergistic effects on the bioavailability of salmon calcitonin. As discussed below, inherent physical and chemical properties of salmon calcitonin make certain absorption enhancers more effective than others in boosting bioavailability.

The salmon calcitonin of the invention may be administered orally. In accordance with the invention, proteolytic degradation of the salmon calcitonin by stomach proteases (most of which are active in the acid pH range) and intestinal or pancreatic proteases (most of which are active in the neutral to basic pH range) is reduced. Solubility enhancers aid passage of the peptide active agent through the intestinal epithelial barrier.

Without intending to be bound by theory, it appears that, in accordance with the present invention, the salmon calcitonin is transported through the stomach under the protection of a coating or other appropriate vehicle for substantially preventing contact between the salmon calcitonin and any stomach proteases capable of degrading it. Once the pharmaceutical composition of the invention passes through the stomach and enters the intestinal region where basic to neutral pH predominates, and where proteases tend to have basic to neutral pH optima, the enteric coating or other vehicle releases the salmon calcitonin and acid (in close proximity to each other).

The acid is believed to lower the local intestinal pH, where the active agent has been released to levels below the optimal range for many intestinal proteases. This decrease in pH reduces the proteolytic activity of the intestinal proteases, thus affording protection to the peptide from potential degradation. The activity of these proteases is diminished by the temporarily acidic environment provided by the invention. It is preferred that sufficient acid be provided that local intestinal pH is lowered temporarily to 5.5 or below, especially a range between about 4.0 and 5.5. The sodium bicarbonate test described below (in the section captioned "the pH-Lowering Agent") is indicative of the

required acid amount. Preferably, conditions of reduced intestinal pH persist for a time period sufficient to protect the peptide agent from proteolytic degradation until at least some of the peptide agent has had an opportunity to cross the intestinal wall into the bloodstream. Experiments have, in fact, demonstrated T_{max} of 5-15 minutes for salmon calcitonin in the blood when the active components are injected directly into the duodenum, ilium or colon. The absorption enhancers synergistically speed salmon calcitonin into the blood while conditions of reduced proteolytic activity prevail.

The mechanism by which the invention is believed to accomplish the goal of enhanced bioavailability is aided by having active components of the pharmaceutical composition released together as simultaneously as possible. To this end, it is preferred to keep the volume of enteric coating as low as possible consistent with providing protection from stomach proteases. Thus enteric coating is less likely to interfere with salmon calcitonin release, or the release of the other components in close time proximity with the salmon calcitonin. Preferably, the enteric coating adds less than 20% to the weight of the remainder of pharmaceutical composition (i.e., before enteric coating). More preferably, the coating adds between 5 and 15% to the weight of the uncoated ingredients.

The absorption enhancer which may be a solubility enhancer and/or transport enhancer (as described in more detail below) aid transport of the peptide agent from the intestine to the blood, and may speed the process so that it better occurs during the time period of reduced intestinal pH and reduced intestinal proteolytic activity. Many surface active agents may act as both solubility enhancers and uptake enhancers. Again without intending to be bound by theory, it is believed that enhancing solubility provides (1) a more simultaneous release of the active components of the invention into the aqueous portion of the intestine, (2) better solubility of the salmon calcitonin in, and transport through, a mucous layer along the intestinal walls. Once salmon calcitonin reaches the intestinal walls, an uptake enhancer provides better transport through the brush border membrane of the intestine into the blood, via either transcellular or paracellular transport. As discussed in more detail below, many preferred compounds may provide both functions. In those instances, preferred embodiments utilizing both of these functions may do so by adding only one additional compound to the pharmaceutical composition.

Each of the preferred ingredients of the pharmaceutical composition of the invention is separately discussed below. Combinations of multiple pH-lowering agents, or enhancers can be used as well as using just a single pH-lowering agent and single enhancer. Some preferred combinations are discussed below.

The Salmon Calcitonin

The salmon calcitonin preferably comprises from 0.02 to 0.2 percent by weight relative to the total weight of the overall pharmaceutical composition (exclusive of enteric coating). Salmon calcitonin is commercially available (for example, from BACHEM, Torrance, Calif.). Alternatively it may be synthesized by known methods, some of which are discussed briefly below.

Salmon calcitonin precursors may be made by either chemical or recombinant syntheses known in the art. However, the latter is believed significantly more cost effective. Precursors are converted to active salmon calcitonin by amidation reactions that are also known in the art.

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For example, enzymatic amidation is described in U.S. Pat. No. 4,708,934 and European Patent Publications 0 308 067 and 0 382 403. Recombinant production is preferred for both the salmon calcitonin precursor and the enzyme that catalyzes the conversion of the precursor to salmon calcitonin. Such recombinant production is discussed in *Biotechnology*, Vol. 11 (1993) pp. 64-70, which further describes a conversion of precursor to recombinant salmon calcitonin product. The recombinant product is identical to natural salmon calcitonin and salmon calcitonin produced using solution and solid phase chemical peptide synthesis.

The production of recombinant salmon calcitonin (rsCT) may proceed, for example, by producing glycine-extended salmon calcitonin precursor in *E. coli* as a soluble fusion protein with glutathione-S-transferase. The glycine-extended precursor has a molecular structure that is identical to active salmon calcitonin except at the C-terminal (where salmon calcitonin terminates -pro-NH₂, while the precursor terminates -pro-gly. An α -amidating enzyme described in the publications above catalyzes conversion of precursors to salmon calcitonin. That enzyme is preferably recombinantly produced, for example, in Chinese Hamster Ovary (CHO) cells) as described in the *Biotechnology* article cited above.

The pH-Lowering Agent

The total amount of the pH lowering compound to be administered with each administration of salmon calcitonin should preferably be an amount which, when it is released into the intestine, is sufficient to lower the local intestinal pH substantially below the pH optima for proteases found there. The quantity required will necessarily vary with several factors including the type of compound used (discussed below) and the equivalents of protons provided by a given compound in practice, the amount required to provide good bioavailability for the administered salmon calcitonin is an amount which, when added to a solution of 10 milliliters of 0.1 M sodium bicarbonate, lowers the pH of that sodium bicarbonate solution to no higher than 5.5, and preferably to between 4.0 and 5.5.

The pH-lowering agent of the invention may be any pharmaceutically acceptable compound that is not toxic in the gastrointestinal tract and is capable of either delivering hydrogen ions (a traditional acid) or of inducing higher hydrogen ion content from the local environment. It may also be any combination of such compounds. Examples of compounds inducing higher hydrogen ion content include aluminum chloride and zinc chloride. Pharmaceutically acceptable traditional acids include, but are not limited to acid salts of amino acids (e.g. amino acid hydrochlorides) or derivatives thereof. Examples of these are acid salts of acetylglutamic acid, alanine, arginine, asparagine, aspartic acid, betaine, carnitine, carnosine, citrulline, creatine, glutamic acid, glycine, histidine, hydroxylysine, hydroxyproline, hypotaurine, isoleucine, leucine, lysine, methylhistidine, norleucine, ornithine, phenylalanine, proline, sarcosine, serine, taurine, threonine, tryptophan, tyrosine and valine.

Other examples of useful pH-lowering compounds, include carboxylic acids such as acetylsalicylic, acetic, ascorbic, citric, fumaric, glucuronic, glutaric, glyceric, glycolic, glyoxylic, isocitric, isovaleric, lactic, maleic, oxaloacetic, oxalosuccinic, propionic, pyruvic, succinic, tartaric, valeric, and any combination thereof that achieves the required pH level of no higher than 5.5 in the sodium bicarbonate test discussed above.

Other useful pH-lowering agents that might not usually be called "acids" in the art, but which may nonetheless be

useful in accordance with the invention are phosphate esters (e.g., fructose 1, 6 diphosphate, glucose 1, 6 diphosphate, phosphoglyceric acid, and diphosphoglyceric acid). CAROPOL® (Trademark B F Goodrich) and polymers such as polycarbophil may also be used to lower pH.

The Absorption Enhancer

The absorption enhancers are preferably present in a quantity that constitutes from 0.1 to 20.0 percent by weight, relative to the overall weight of the pharmaceutical composition (exclusive of the enteric coating) Preferred absorption enhancers are surface active agents which act both as solubility enhancers and uptake enhancers. Generically speaking, "solubility enhancers" improve the ability of the active components of the invention to be solubilized in either the aqueous environment into which they are originally released or into the lipophilic environment of the mucous layer lining the intestinal walls, or both. "Uptake enhancers" (which are frequently the same surface active agents used as solubility enhancers) are those which facilitate the ease by which peptide agents cross the intestinal wall.

~~One or more absorption enhancers may perform one function only (e.g., solubility), or one or more absorption enhancers may perform the other function only (e.g., uptake),~~ within the scope of the invention. It is also possible to have a mixture of several compounds some of which provide improved solubility, some of which provide improved uptake and/or some of which perform both. Without intending to be bound by theory, it is believed that uptake enhancers may act by (1) increasing disorder of the hydrophobic region of the membrane exterior of intestinal cells, allowing for increased transcellular transport; or (2) leaching membrane proteins resulting in increased transcellular transport; or (3) widening pore radius between cells for increased paracellular transport.

Surface active agents are believed to be useful both as solubility enhancers and as uptake enhancers. For example, detergents are useful in (1) solubilizing all of the active components quickly into the aqueous environment where they are originally released, (2) enhancing lipophilicity of the components of the invention, especially the peptide active agent aiding its passage into and through the intestinal mucus, (3) enhancing the ability of the normally polar peptide active agent to cross the epithelial barrier of the brush border membrane; and (4) increasing transcellular or paracellular transport as described above.

When surface active agents are used as the absorption enhancers, it is preferred that they be free flowing powders for facilitating the mixing and loading of capsules during the manufacturing process. Because of inherent characteristics of salmon calcitonin (e.g., its isoelectric point, molecular weight, amino acid composition, etc.) certain surface active agents are preferred. Indeed, some can undesireably interact with the charged portions of salmon calcitonin and prevent its absorption, thus undesireably resulting in decreased bioavailability. It is therefore preferred, when trying to increase the bioavailability of salmon calcitonin, that any surface active agent used as an absorption enhancer be selected from the group consisting of (i) anionic surface active agents that are cholesterol derivatives (e.g., bile acids), (ii) cationic surface agents (e.g., acylcarnitines, phospholipids and the like), (iii) nonionic surface active agents, and (iv) mixtures of anionic surface active agents (especially those having linear hydrocarbon regions) together with negative charge neutralizers for said salmon calcitonin.

Negative charge neutralizers include but are not limited to acylcarnitines, cetyl pyridinium chloride, and the like. It is also preferred that the absorption enhancer be soluble at acid pH, particularly in the 3.0 to 5.0 range. One especially preferred combination mixes cationic surface active agents with anionic surface active agents that are cholesterol derivatives, both of which are soluble at acid pH. A particularly preferred combination is an acid soluble bile acid together with a cationic surface active agent. When a single absorption enhancer is used alone, it is preferred that it be a cationic surface active agent. It is the intent of these preferences to avoid interactions with salmon calcitonin that interfere with absorption of salmon calcitonin into the blood.

To reduce the likelihood of side effects, preferred detergents are either biodegradable or reabsorbable (e.g. bile acids, phospholipids, and/or acylcarnitines). Acylcarnitines are believed particularly useful in enhancing paracellular transport. When a bile acid (or another anionic detergent lacking linear hydrocarbons) is used in combination with a cationic detergent, salmon calcitonin is better transported both to and through the intestinal wall.

Preferred absorption enhancers include: (a) salicylates such as sodium salicylate, 3-methoxysalicylate, 5-methoxysalicylate, and homovanilate; (b) bile acids such as taurocholic, taurodeoxycholic, deoxycholic, cholic, glycholic, lithocholate, chenodeoxycholic, ursodeoxycholic, ursocholic, dehydrocholic, fusidic, etc.; (c) non-ionic surfactants such as polyoxyethylene ethers (e.g. Brij 36T, Brij 52, Brij 56, Brij 76, Brij 96, Texaphor A6, Texaphor A14, Texaphor A60 etc.), p-t-octyl phenol polyoxyethylenes (Triton X-45, Triton X-100, Triton X-114, Triton X-305 etc.) nonylphenoxypolyoxyethylenes (e.g. Igepal CO series), polyoxyethylene sorbitan esters (e.g. Tween-20, Tween-80 etc.); (d) anionic surfactants such as dioctyl sodium sulfosuccinate; (e) lyso-phospholipids such as lysolecithin and lyso-phosphatidylethanolamine; (f) acylcarnitines, acylcholines and acyl amino acids such as lauroylcarnitine, myristoylcarnitine, palmitoylcarnitine, lauroylcholine, myristoylcholine, palmitoylcholine, hexadecahoyllysine, N-acylphenylalanine, N-acylglycine etc.; (g) water soluble phospholipids such as diheptanoylphosphatidylcholine, dioctylphosphatidylcholine etc.; (h) medium-chain glycerides which are mixtures of mono-, di- and triglycerides containing medium-chain-length fatty acids (caprylic, capric and lauric acids); (i) ethylenediaminetetraacetic acid; (j) cationic surfactants such as cetylpyridinium chloride; (k) fatty acid derivatives of polyethylene glycol such as Labrasol, Labrafac, etc.; and (l) alkylsaccharides or acyl saccharides such as lauryl maltoside, lauryl sucrose, myristoyl sucrose, palmitoyl sucrose, etc.

In some preferred embodiments, cationic detergents are included to provide solubility enhancement by another mechanism. In particular, prevents the binding of salmon calcitonin to mucus. Preferred cationic ion exchange agents include protamine chloride or any other polycation.

Other Optional Ingredients

In some preferred embodiments, a protein (such as albumin, casein, soy protein, other animal or vegetable proteins and the like) is included to reduce non-specific adsorption (e.g., binding of salmon calcitonin to the intestinal mucus barrier, thereby lowering the necessary concentration of expensive salmon calcitonin. When added, the protein is preferably from 1.0 to 10.0 percent by weight relative to the weight of the overall pharmaceutical composition (exclusive of enteric coating).

All pharmaceutical compositions of the invention may optionally also include common pharmaceutical diluents, glycant, lubricants, gelatin capsules, preservatives, colorants and the like in their usual known sizes and amounts. In some embodiments peptides that may act as substrates for intestinal proteases are added (preferably from 1.0 to 10.0 percent by weight relative to the weight of the overall pharmaceutical composition (exclusive of enteric coating).

The Carrier or Vehicle

Any carrier or vehicle that protects the salmon calcitonin from stomach proteases and which releases active components of the invention in the intestine is suitable. Many enteric coatings are known in the art, and are useful in accordance with the invention. Examples include cellulose acetate phthalate, hydroxypropylmethylethylcellulose succinate, hydroxypropylmethylcellulose phthalate, carboxymethylethylcellulose and methacrylic acid-methyl methacrylate copolymer.

Suitable enteric coatings for protecting the peptide agent from stomach proteases may be applied to capsules, with the remaining active components of the invention loaded within the capsule. In other embodiments, enteric coating is coated on the outside of a tablet or coated on the outer surface of particles of active components which are then pressed into tablet form, or loaded into a capsule, which is itself preferably enteric coated. In other embodiments, active components of the invention are included in a sufficiently viscous protective syrup to permit protected passage of the active components through the stomach. The term "active components" includes all compounds pertinent to the invention (for example, salmon calcitonin, absorption enhancers such as solubility and/or uptake enhancer(s) and pH-lowering compound(s).

It is very desirable that all of the active components be released from the carrier or vehicle, and solubilized in the intestinal environment as simultaneously as possible. It is preferred that the vehicle or carrier release the active components in the small intestine where uptake enhancers that increase transcellular or paracellular transport are less likely to cause undesirable side effects than if the same uptake enhancers were later released in the colon. It is emphasized, however, that the present invention is believed effective in the colon as well as in the small intestine. Numerous vehicles or carriers, in addition to the ones discussed above, are known in the art. It is desirable (especially in optimizing how simultaneously the components of the invention are released) to keep the amount of enteric coating low. Preferably, the enteric coating adds no more than 20% to the weight of the remainder of pharmaceutical composition (exclusive of enteric coating). More preferably, it adds from 5 to 15 percent to the weight of the uncoated composition.

A preferred pharmaceutical composition of the invention includes a size 0 gelatin capsule filled with 0.25 mg. of salmon calcitonin, 400 mg. of granular citric acid (available for example from Archer Daniels Midland Corp.), 100 mg. of taurodeoxycholic acid (available for example from SIGMA), 100 mg. lauroylcarnitine (SIGMA), 40 mg. albumin and 10 mg. silica (as a desiccant).

All of the foregoing ingredients are for eventual insertion into the gelatine capsule, and are powders which may be added to a blender in any order. Thereafter, the blender is run for about five minutes until the powders are thoroughly intermixed. Then the mixed powders are loaded into the large end of the gelatine capsules. The other end of the capsule is then added, and the capsule snapped shut. 500 or

more such capsules may be added to a coating device (e.g., Vector LDCS 20/30 Laboratory Development Coating System (available from Vector Corp., Marion, Iowa)).

An enteric coating solution is made as follows. Weigh 500 grams of EUDRAGIT L30 D-55 (a methacrylic acid copolymer with methacrylic acid methyl ester, an enteric coating available from RÖHM Tech Inc., Maiden, Mass.). Add 411 grams distilled water, 15 grams triethyl citrate and 38 grams talc. This amount of coating will be sufficient to coat about 500 size 0 capsules.

The capsules are weighed and placed into the drum of the coating machine. The machine is turned on to rotate the drum (now containing capsules) at 24–28 rpm. The temperature of inlet sprayer is preferably about 45° C. Exhaust temperatures are preferably about 30° C. Uncoated capsule temperature is preferably about 25° C. Air flow is about 38 cubic feet per minute.

A tube from the machine is then inserted into the coating solution prepared as discussed above. The pump is then turned on for feeding solution into the coating device. Coating then proceeds automatically. The machine can be stopped at any time to weigh capsules to determine if the coating amount is sufficient. Usually coating is allowed to proceed for 30 to 60 minutes. The pump is then turned off for about five minutes while the machine is still running to help dry the coated capsules. The machine can then be turned off. The capsule coating is then complete, although it is recommended that the capsules be air dried for about two days.

Because of the enhanced bioavailability provided by the present invention, the concentration of expensive salmon calcitonin in the pharmaceutical preparation of the invention may be kept relatively low.

Treatment of Patients

Treatment of osteoporosis with salmon calcitonin proceeds best with periodic administration. Salmon calcitonin is metabolized quickly with a half-life of only 20–40 minutes following subcutaneous administration in man. However, its beneficial effect on osteoclasts is much longer lasting, and may last for more than 24 hours notwithstanding rapid decrease in blood levels. There is usually no-detectable blood levels more than two hours after injection of salmon calcitonin at usual dosages. Accordingly periodic administration of one dose about 5 days per week is preferred. Subcutaneous administration of salmon calcitonin (100 International units) has frequently resulted in peak serum concentration of about 200 picograms per milliliter. Nasally administered salmon calcitonin (200 International units) has proven effective against osteoporosis at peak levels as low as 10 picograms per milliliter. Some patients report some gastrointestinal distress at high peak levels (e.g. at 200 picograms per milliliter). Accordingly, it is preferred that serum salmon calcitonin peak between 10 and 150 picograms per milliliter, more preferably between 10 and 50 picograms per milliliter. The serum levels may be measured by radioimmunoassay techniques known in the art. The attending physician may monitor patient response, salmon calcitonin blood levels, or surrogate markers of bone disease (such as urinary pyridinoline or deoxypyridinoline), especially during the beginning of treatment. He may then alter the dosage somewhat to account for individual patient metabolism and response.

The bioavailability achievable in accordance with the present invention permits oral delivery of salmon calcitonin into the blood at the above-identified preferred concentration

levels while using only 100–1000 micrograms of salmon calcitonin per capsule, preferably 100–400 micrograms, especially between 100 and 200 micrograms.

It is preferred that a single capsule be used at each administration because a single capsule best provides simultaneous release of the polypeptide, pH-lowering agent and absorption enhancers. This is highly desirable because the acid is best able to reduce undesirable proteolytic attack on the polypeptide when the acid is released in close time proximity to release of the polypeptide. Near simultaneous release is best achieved by administering all components of the invention as a single pill or capsule. However, the invention also includes dividing the required amount of acid and enhancers among two or more capsules which may be administered together such that they together provide the necessary amount of all ingredients.

Set forth below are a series of tables showing the effect on bioavailability caused by varying certain parameters.

TABLE 1

Effect of Buffer pH on the Absorption of
Salmon Calcitonin from the Duodenum of the Rat

Formulation	pH*	Peak Plasma Calcitonin ng/ml	Absolute Bioavailability percent
I. Citrate/Citric acid (77 mg) Calcitonin (0.1 mg)	5	0.4	0.02
II. Citrate/Citric acid (77 mg) Calcitonin (0.1 mg)	4	1.9	0.10
III. Citrate/Citric acid (77 mg) Calcitonin (0.1 mg)	3	4.1	0.64
IV. Citrate/Citric acid (77 mg) Calcitonin (0.1 mg)	2	4.8	0.69

*buffer pH

Method

Female Wistar rats (250–275 g) (n=3 for each formulation) were anesthetized with ketamine and xylazine prior to the insertion of a cannula in the carotid artery. The cannula was fitted to a three way valve through which blood was sampled and replaced with physiological saline. A midline incision was made in the abdominal cavity and 0.5 ml of formulation was injected directly into the exposed duodenum. The pH of the formulation was adjusted by mixing varying amounts of equal molar concentrations of citric acid and sodium citrate. Blood (0.5 ml) was collected before and at 5, 15, 30, 60 and 120 minutes after the administration of the formulation. Samples of blood were centrifuged for 10 minutes at 2600 g and the resulting plasma supernatant was stored as –20° C. The concentration of calcitonin in plasma was determined by a competitive radioimmunoassay. The absolute bioavailability (i.e., relative to an intravenous dose of calcitonin) was calculated from the areas under the curve obtained from plots of the plasma concentration of calcitonin as a function of time.

Results and Discussion

When the pH of the buffer was reduced from 5 (formulation I) to 4 (formulation II) the absolute bioavailability increased 5 fold from 0.02% to 0.1%. When the pH was reduced to 3 (formulation III) the absolute bioavailability increased an additional 6.4 fold. There was very little increase in the bioavailability of calcitonin when the pH was reduced to 2. The overall bioavailability of calcitonin increased 32 fold when the pH of the buffer was reduced from 5 to 3.

TABLE 2

Effect of Citric Acid Concentration on the Bioavailability of Salmon Calcitonin from the Duodenum of the Rat		
Formulation	Peak Plasma Calcitonin ng/ml	Absolute Bioavailability percent
I. Citric acid (9.6 mg) Taurodeoxycholic acid (5 mg) Mannitol (22 mg) Calcitonin (0.1 mg)	3.65	0.25
II. Citric acid (48 mg) Taurodeoxycholic acid (5 mg) Mannitol (22 mg) Calcitonin (0.1 mg)	17.44	2.43

Method

Formulations consisting of a constant amount of taurodeoxycholic acid and 2 different amounts of citric acid in a total volume of 0.5 ml were administered into the duodenum of anesthetized rats as described in the legend to Table 1. Mannitol was included in formulations as a marker to measure paracellular transport. Samples of blood were removed at various times and analyzed for calcitonin as described previously.

Results and Discussion

The bioavailability of salmon calcitonin administered in the presence of 9.6 mg citric acid (I) was 0.25%, whereas in the presence of 48 mg citric acid (II) the bioavailability was 2.43%. In the presence of a fixed amount of taurodeoxycholic acid the bioavailability of salmon calcitonin increased nearly 10 fold when the amount of citric acid in the formulation was increased only 5 fold.

TABLE 3

Effect of Enhancers in the Presence of Citric Acid on the Absorption of Salmon Calcitonin from the Duodenum of the Rat		
Formulation	Peak Plasma Calcitonin ng/ml	Absolute Bioavailability percent
I. Citric acid (77 mg) Calcitonin (0.1 mg)	4.8	0.69
II. Citric acid (48 mg) Taurodeoxycholic acid (5 mg) Calcitonin (0.1 mg)	26.59	3.03
III. Citric acid (48 mg) Taurodeoxycholic acid (5 mg) Cetylpyridinium chloride (5 mg) Calcitonin (0.1 mg)	36.48	4.54
IV. Citric acid (48 mg) Tween-20 (5 mg) Calcitonin (0.1 mg)	15.50	3.10
V. Citric acid (48 mg) Sucrose ester-15 (5 mg) Mannitol (22 mg) Calcitonin (0.1 mg)	38.93	5.83
VI. Citric acid (48 mg) Lauroylcarnitine chloride (5 mg) Calcitonin (0.1 mg)	38.89	4.53
VII. Citric acid (48 mg) Diheptanoylphosphatidylcholine (5 mg) Calcitonin (0.1 mg)	20.93	2.97

Method

Formulations consisting of citric acid, calcitonin and various classes of enhancers in a total volume of 0.5 ml were administered into the duodenum of anesthetized rats as

described in the legend to Table 1. Mannitol was included in formulation V as a marker to measure paracellular transport. Samples of blood were removed at various times and analyzed for calcitonin as described previously.

Results and Discussion

In the absence of an enhancer, the absolute bioavailability of calcitonin was 0.69%. The inclusion of a water soluble phospholipid (formulation VII) increased the bioavailability 4.3 fold to 2.97%. The most effective enhancer was the sugar ester class (formulation V) in which the calcitonin bioavailability was 5.83%. The use of a mixture of bile acid and a cationic detergent (formulation III) a non-ionic detergent (formulation IV) and an acylcarnitine (formulation VI) resulted in intermediate bioavailabilities ranging from 3.03% to 4.53%. The differences in the bioavailabilities of calcitonin in the presence of various classes of enhancers are minor compared to that observed when only citric acid and no enhancer is present in the formulation.

TABLE 4

Effect of Lauroylcarnitine in the Presence of Various Additives on the Absorption of Salmon Calcitonin from the Duodenum of the Rat		
Formulation	Peak Plasma Calcitonin ng/ml	Absolute Bioavailability percent
I. Calcitonin (1 mg)	9.44	0.096
II. Lauroylcarnitine chloride (5 mg) Calcitonin (0.1 mg)	2.27	0.17
III. Laurylcarnitine chloride (5 mg) Citric acid (48 mg) Calcitonin (0.1 mg)	38.89	4.53
IV. Laurylcarnitine chloride (1 mg) Citric acid (48 mg) Calcitonin (0.1 mg)	27.72	4.81
V. Laurylcarnitine chloride (5 mg) Diheptanoylphosphatidylcholine (5 mg) Citric acid (48 mg) Calcitonin (0.1 mg)	44.89	6.45
VI. Lauroylcarnitine chloride (5 mg) Bovine Serum Albumin (25 mg) Calcitonin (0.1 mg)	4.58	0.42

Method

Formulations consisting of lauroylcarnitine, calcitonin and various other compounds in a total volume of 0.5 ml were administered into the duodenum of anesthetized rats as described in the legend to Table 1. Samples of blood were removed at various times and analyzed for calcitonin as described previously.

Results and Discussion

In the absence of citric acid or any enhancer (formulation I), the absolute bioavailability of calcitonin was 0.096%. In the presence of 5 mg lauroylcarnitine chloride (formulation II), the bioavailability increased 1.8 fold to 0.17%. When citric acid was included with lauroylcarnitine (formulation III), the bioavailability increased an additional 27 fold to 4.53%. A 5 fold reduction in the amount of lauroylcarnitine but not citric acid (formulation IV), did not significantly reduce the bioavailability of salmon calcitonin. The inclusion of 5 mg diheptanoylphosphatidylcholine to formulation III to produce formulation V increased the bioavailability slightly (1.4 fold). The substitution of 25 mg bovine serum albumin for citric acid (formulation VI) reduced the bioavailability from 4.53% (formulation III) to 0.42%. These results taken together show the synergistic effect between a pH-lowering substance like citric acid and an enhancer like lauroylcarnitine.

TABLE 5

Effect of Formulation on the Absorption of Salmon Calcitonin from the Duodenum of the Dog		
Formulation	Peak Plasma Calcitonin ng/ml	Absolute Bioavailability percent
I. Calcitonin (25 mg)	1.15	0.015
II. Citric acid (192 mg) Calcitonin (10 mg)	10.65	0.37
III. Citric acid (192 mg) Taurodeoxycholic acid (20 mg) Calcitonin (5 mg)	14.99	0.81

Method

Modified vascular access ports were surgically implanted into the duodenum, ileum and colon of male beagle dogs. The septum/reservoir bodies of the ports were implanted under the skin and were used as sites for the administration of calcitonin formulations. Before and after the administration of calcitonin formulations into conscious dogs, the ports were flushed with 2 ml of the formulation without calcitonin. Blood (2 ml) was collected through angiocatheter tubes in the leg vein at t=30, 15 and 0 before administration of calcitonin and at 5, 10, 20, 30, 40, 50, 60 and every 15 minutes thereafter for 2 hours. Samples of blood were centrifuged for 10 minutes at 2600 g and the resulting plasma supernatant was stored at -20° C. The concentration of calcitonin in plasma was determined by a competitive radioimmunoassay. The absolute bioavailability (i.e. relative to an intravenous dose of calcitonin) was calculated from the areas under the curve obtained from plots of the plasma concentration as a function of time obtained.

Results and Discussion

The absolute bioavailability of calcitonin administered in water (I) was 0.015%. In the presence of 192 mg citric acid (II) the bioavailability of calcitonin increased 25 fold. The inclusion of 20 mg taurodeoxycholic acid in the formulation (III) produced an additional 2.2 fold increased in absolute bioavailability to 0.81%. The combination of a pH-lowering compound, citric acid, and an enhancer, taurodeoxycholic acid, resulted in overall 54 fold increased in the absolute bioavailability of salmon calcitonin.

TABLE 6

Effect of Dosage Form and Formulation on the Absolute Bioavailability of Salmon Calcitonin Administered Orally to Dogs					
Capsule	Dissolution in HCl min	Formulation	Peak Plasma Calcitonin ng/ml	Peak Plasma Calcitonin min	Absolute Bioavailability percent
I. Starch	10	Citric acid (100 mg) Taurodeoxycholic acid (100 mg) Calcitonin (10 mg)	0.98	10-30	0.07
II. Gelatin	30	Citric acid (100 mg) Taurodeoxycholic acid (100 mg) Calcitonin (10 mg)	5.79	10-30	0.26
III. Gelatin	30	Citric acid (600 mg) Taurodeoxycholic acid (80 mg) Calcitonin (5 mg)	6.92	10-30	0.62
IV. Gelatin	>60	Citric acid (600 mg) Taurodeoxycholic acid (80 mg) Calcitonin (5 mg)	7.79	90	1.48

Method

Starch and gelatin capsules were filled with the indicated formulations and coated for 60 min with either hydroxypropylmethylcellulose phthalate 50 (I,II,III) (1% weight gain) or Eudragit L 30 D-55 (IV) (10% weight gain) in a pan coater. The stability of the capsules in 0.1N HCl were determined in a dissolution bath using the "basket method." At least 2 dogs were given each of the indicated capsules by mouth and blood was sampled and analyzed for salmon calcitonin as previously described.

Results

The bioavailability of 10 mg calcitonin mixed with 100 mg citric acid and 100 mg taurodeoxycholic acid and delivered in a starch capsule (I) was 0.07%. When the same formulation was given to dogs in a gelatin capsule (II), the bioavailability of salmon calcitonin increased to 0.26%. A six fold increase in the amount of citric acid and a 50% reduction in the amount of calcitonin (III) resulted in a nearly 3 fold increase in calcitonin bioavailability.

When the enteric coat was changed from hydroxypropylmethylcellulose phthalate 50 to Eudragit L 30 D-55, a methacrylate polymer and the formulation was kept the same (IV), the bioavailability of salmon calcitonin increased from 0.62% to 1.48%. Changing the enteric coat from hydroxypropylmethylcellulose phthalate 50 to Eudragit L 30 D-55 resulted in increased stability of the capsule in 0.1N HCl. This increased stability resulted in peak calcitonin levels appearing at a later time point in the dog's blood. The instability of capsules I, II and III in HCl suggests that these capsules were potentially opening in the dogs' stomachs, whereas the improved stability of capsule IV suggests that it was completely stable in the stomachs of dogs and was opening in the intestines of dogs. This indicates that a certain minimum enteric coating amount is preferred. At the same time, too much coating can delay release of calcitonin behind release of other important components (e.g., acid and detergent). Preferably, enteric coating adds 5 to 15% to the weight of the uncoated pharmaceutical.

TABLE 7

PHARMACOKINETICS OF ORAL CALCITONIN (10.5 mg) IN HUMANS						
Time min	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Mean
Plasma Calcitonin (pg/ml)						
0	0	0	0	0	0	0
15	34	0	0	0	1	7
30	497	91	206	0	70	173
40	327	86	99	26	35	114
50	173	114	78	117	26	102
60	87	106	40	180	20	87
70	72	108	64	63	35	68
80	27	85	54	30	25	44
90	43	102	46	19	14	45
100	40	89	28	11	28	41
110	0	91	16	13	0	24
120	49	117	34	0	6	41
180	34	107	0	0	16	41
Bioavailability (%)	.06	0.94	0.03	0.02	0.02	0.03

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Methods

Starch capsules were filled with 138 mg citric acid, 105 mg taurodeoxycholic acid and 10.5 mg salmon calcitonin. The capsules were coated for 20 min with hydroxypropylmethylcellulose phthalate 50 in a pan coater and stored at 4° C. Fasted subjects were given 1 capsule followed by a glass of orange juice in the morning of the study. Samples of blood were taken 15 minutes before taking the capsules and at the indicated times after taking the calcitonin capsule. The concentration of calcitonin in blood was determined by competitive radioimmunoassay. The absolute bioavailability (i.e., relative to an intravenous dose of calcitonin) was calculated from the areas under curve obtained from plots of the plasma concentration of calcitonin as a function of time. Results

When 10.5 milligrams of salmon calcitonin alone was administered to humans, no detectable serum levels of salmon calcitonin resulted. However, when individuals were given the composition of the invention as described in Table 7, maximum levels of calcitonin were detected in the blood between 30 and 60 minutes after the individuals took the capsule. The maximum concentration of calcitonin in the blood was between 70 and 497 pg/ml. The mean peak concentration of calcitonin for the 5 individuals was 173 pg/ml at t=30 min. The absolute bioavailability ranged from 0.02 to 0.06% with a population mean of 0.03%.

Although the present invention has been described in relation to particular embodiments thereof many other variations and modifications and other uses will become apparent to those skilled in the art. The present invention therefore is not limited by the specific disclosure herein, but only by the appended claims.

What is claimed is:

1. A pharmaceutical composition for oral delivery of salmon calcitonin comprising:

- (A) a therapeutically effective amount of said salmon calcitonin;
- (B) at least one pharmaceutically acceptable pH-lowering agent;
- (C) at least one absorption enhancer effective to promote bioavailability of said salmon calcitonin; and
- (D) an enteric coating;

wherein said pH-lowering agent is present in said pharmaceutical composition in a quantity which, if added to 10 milliliters of 0.1M aqueous sodium bicarbonate solutions would be sufficient to lower the pH of said solution to no higher than 5.5.

2. The pharmaceutical composition of claim 1, wherein said enteric coating is present at a weight which is no more than 20% of the weight of the remainder of said pharmaceutical composition excluding said enteric coating.

3. The pharmaceutical composition of claim 1, wherein said enteric coating is present at a weight which is no more than 5-15% of the weight of the remainder of said pharmaceutical composition excluding said enteric coating.

4. The pharmaceutical composition of claim 1, wherein said absorption enhancer is a surface active agent.

5. The pharmaceutical composition of claim 4, wherein said surface active agent is absorbable or biodegradable.

6. The pharmaceutical composition of claim 5, wherein said surface active agent is selected from the group consisting of acylcarnitines, phospholipids and bile acids.

7. The pharmaceutical composition of claim 1, wherein said absorption enhancer is a surface active agent selected from the group consisting of (i) an anionic agent that is a cholesterol derivative, (ii) a mixture of a negative charge neutralizer and an anionic surface active agent, (iii) nonionic surface active agents, and (iv) cationic surface active agents.

8. The pharmaceutical composition of claim 1, wherein said absorption enhancer is selected from the group consisting of a cationic surfactant and an anionic surfactant that is a cholesterol derivative.

9. The pharmaceutical composition of claim 1, wherein said pharmaceutical composition includes at least two absorption enhancers, one of which is a cationic surface active agent, and another of which is an anionic surface active agent that is a cholesterol derivative.

10. The pharmaceutical composition of claim 9, wherein said anionic surface active agent is an acid-soluble bile acid.

11. The pharmaceutical composition of claim 10, wherein said enteric coating is present at a weight that is no more than 20% of the combined weight of the remainder of said pharmaceutical composition excluding said enteric coating.

12. The pharmaceutical composition of claim 11, wherein all of said absorption enhancers are absorbable or biodegradable.

13. The pharmaceutical composition of claim 7, wherein said enteric coating is present at a weight which is no more than 20% of the combined weight of the remainder of the pharmaceutical composition excluding said enteric coating.

14. The pharmaceutical composition of claim 1, further comprising an amount of a protein effective to reduce non-specific adsorption of said salmon calcitonin.

15. The pharmaceutical composition of claim 14 wherein said protein is albumin, casein, or soy protein.

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16. The pharmaceutical composition of claim 1 further comprising a peptide that is susceptible to degradation by intestinal proteases.

17. A method for enhancing the bioavailability of salmon calcitonin delivered orally, said method comprising selectively releasing said salmon calcitonin, together with at least one pH-lowering agent and at least one absorption enhancer, into a patient's intestine following passage of said salmon calcitonin, pH-lowering agent and absorption enhancer through said patient's mouth and stomach under protection of an enteric coating which substantially prevents contact between stomach proteases and said salmon calcitonin;

wherein said pH-lowering agent is released into said intestine in a quantity which, if added to 10 milliliters of 0.1M aqueous sodium bicarbonate solution, would be sufficient to lower pH of said solution to no higher than 5.5.

18. The method of claim 17, wherein said enteric coating is present at a weight which is no more than 20% of the weight of the remainder of all components administered to said patient exclusive said enteric coating.

19. The method of claim 17, wherein said enteric coating is present at a weight which is no more than 5-15% of the weight of the remainder of all components administered to said patient exclusive of said enteric coating.

20. The method of claim 17, wherein said absorption enhancer is a surface active agent.

21. The method of claim 20, wherein said surface active agent is absorbable or biodegradable.

22. The method of claim 21, wherein said surface active agent is selected from the group consisting of acylcarnitines, phospholipids and bile acids.

23. The method of claim 17, wherein said absorption enhancer is a surface active agent selected from the group

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consisting of (i) an anionic agent that is a cholesterol derivative, (ii) a mixture of a negative charge neutralizer and an anionic surface active agent, (iii) a nonionic surface active agent, and (iv) a cationic surface active agent.

24. The method of claim 17, wherein said absorption enhancer is selected from the group consisting of a cationic surface active agent and an anionic surface active agent that is a cholesterol derivative.

25. The method of claim 24, comprising administering at least two absorption enhancers, one of which is a cationic surface active agent, and another of which is an anionic surface active agent that is a cholesterol derivative.

26. The method of claim 25, wherein said anionic surface active agent is an acid-soluble bile acid.

27. The method of claim 26, wherein said enteric coating is present at a weight that is no more than 20% of the combined weight of all components administered to said patient, exclusive of said enteric coating.

28. The method of claim 27, wherein all absorption enhancers that are administered are absorbable or biodegradable.

29. The method of claim 23, wherein said enteric coating is present at a weight which is no more than 20% of the combined weight of the remainder of all components administered to said patient exclusive of said enteric coating.

30. The method of claim 17, wherein a protein is administered together with said salmon calcitonin in an amount sufficient to reduce non-specific adsorption of said salmon calcitonin.

31. The method of claim 30 wherein said protein is albumin, casein or soy protein.

32. The method of claim 17 further comprising a peptide that is susceptible to degradation by intestinal proteases.

* * * * *

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US005866536A

United States Patent [19]**Leone-Bay et al.**[11] **Patent Number:** **5,866,536**[45] **Date of Patent:** **Feb. 2, 1999**D₃

ISS

[54] **COMPOUNDS AND COMPOSITIONS FOR DELIVERING ACTIVE AGENTS**[75] Inventors: **Andrea Leone-Bay**, Ridgefield, Conn.; **Koc-Kan Ho**, Monmouth Junction, N.J.; **Donald J. Sarubbi**, Bronxville; **Sam J. Milstein**, Larchmont, both of N.Y.[73] Assignee: **Emsphere Technologies, Inc.**, Tarrytown, N.Y.[21] Appl. No.: **798,033**[22] Filed: **Feb. 6, 1997****Related U.S. Application Data**

[63] Continuation of PCT/US96/04580 Apr. 1, 1996 which is a continuation-in-part of Ser. No. 414,654, Mar. 31, 1995, Pat. No. 5,650,386.

[60] Provisional application Nos. 60/003,111 Sep. 1, 1995 and 60/017,902 Mar. 29, 1996.

[51] **Int. Cl.⁶** **A61K 31/725**; A61K 38/00; A61K 47/12; C07C 229/06[52] **U.S. Cl.** **514/2**; 514/56; 514/784; 554/11; 562/444[58] **Field of Search** 424/85.1, 85.2, 424/85.4, 141.1, 184.1; 514/2.3, 8, 11, 12, 21, 56, 456, 616, 558, 559, 563, 784; 554/111; 562/444[56] **References Cited****U.S. PATENT DOCUMENTS**Re. 24,899 11/1960 Green 514/784
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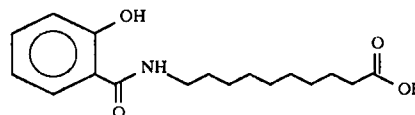
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[57]

ABSTRACT

Carrier compounds, compositions, and dosage unit forms therefor which are useful in the delivery of active agents are provided. The present invention provides a compound having the formula:



or a salt thereof, wherein the compound may be used in a composition or dosage unit form for delivery of at least one active agent, including a peptide, mucopolysaccharide, carbohydrate, or a lipid. Methods of administration and preparation of the compounds and compositions of the invention are provided as well, including oral administration. Further, the compositions of the invention may be prepared by mixing at least one active agent, at least one carrier compound, and, optionally, a dosing vehicle.

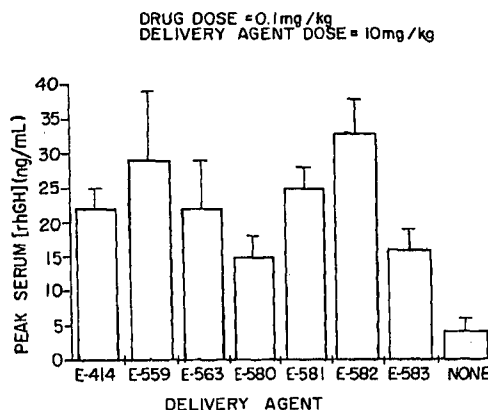
23 Claims, 3 Drawing Sheets

TABLE 7-continued

Delivery Agent Enhancement of Parathyroid Hormone (PTH) Oral Bioavailability.					
Exam- ple	Carrier	Carrier Dose mg/kg	Method of Administration	Active Agent Dose (μ g/kg)	Peak Serum [PTH] (pg/ml.)
54	CVIII	100	intracolonic	25	115 $\pm$ 20
55	LXXXVI	100	intracolonic	25	40 $\pm$ 12
56	LVIII	100	intracolonic	25	145 $\pm$ 25
57	CXIV	100	intracolonic	25	65 $\pm$ 15
58	LXXXIX	100	intracolonic	25	70 $\pm$ 15

The above mentioned patents, applications, test methods, and publications are hereby incorporated by reference in their entirety.

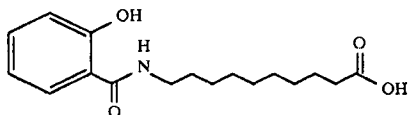
Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. All such obvious variations are within the full intended scope of the appended claims.

What is claimed is:

1. A composition comprising:

(A) at least one active agent; and

(B) a compound having the following formula



or a salt thereof.

2. A composition as defined in claim 1, wherein said active agent is selected from the group consisting of a biologically active agent, a chemically active agent, and a combination thereof.

3. A composition as defined in claim 2, wherein said biologically active agent comprises at least one peptide, mucopolysaccharide, carbohydrate, or lipid.

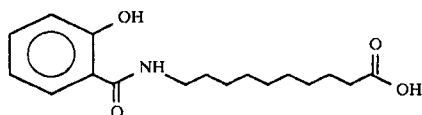
4. A composition as defined in claim 2, wherein said biologically active agent comprises heparin.

5. A composition as defined in claim 2, wherein said biologically active agent comprises low molecular weight heparin.

6. A composition comprising:

(a) at least one active agent; and

(b) a carrier comprising a compound having the following formula:



or a salt thereof, wherein said carrier is selected from the group consisting of a poly(amino acid) and a polypeptide.

7. A composition as defined in claim 6, wherein said carrier comprises a polypeptide.

8. A dosage unit form comprising:

(A) a composition as defined in claim 1; and

(B) (a) an excipient,

(b) a diluent,

(c) a disintegrant,

(d) a lubricant,

(e) a plasticizer,

(f) a colorant,

(g) a dosing vehicle, or,

(h) any combination thereof.

9. A dosage unit form as defined in claim 8, wherein said active agent is selected from the group consisting of a biologically active agent, a chemically active agent, and a combination thereof.

10. A dosage unit form as defined in claim 9, wherein said active agent is selected from the group consisting of at least one peptide, mucopolysaccharide, carbohydrate, and a combination thereof.

11. A dosage unit form as defined in claim 9 wherein said biologically active agent comprises heparin.

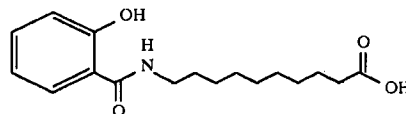
12. A dosage unit form as defined in claim 9 wherein said biologically active agent comprises low molecular weight heparin.

13. A dosage unit form as defined in claim 8, comprising a tablet, a capsule, or a liquid.

14. A dosage unit form as defined in claim 13, wherein said dosing vehicle is selected from the group consisting of water, 1,2-diol, ethanol, and any combination thereof.

15. A method for administering a biologically active agent to an animal in need of said agent, said method comprising administering orally to said animal a composition as defined in claim 2.

16. A compound having the formula



or a salt thereof.

17. A method for preparing a composition, said method comprising mixing:

(A) at least one active agent;

(B) at least one compound as defined in claim 16; and

(C) optionally, a dosing vehicle.

18. The composition as defined in claim 3, wherein said biologically active agent is selected from the group consisting of: human growth hormone, bovine growth hormone, growth hormone-releasing hormone, an interferon, interleukin-II, insulin, heparin, low molecular weight heparin, calcitonin, erythropoietin, atrial natriuretic factor, an antigen, a monoclonal antibody, somatostatin, adrenocorticotropin, gonadotropin-releasing hormone, oxytocin, vasopressin, cromolyn sodium, vancomycin, desferrioxamine (DFO), and any combination thereof.

19. A composition as defined in claim 18, wherein said biologically active agent comprises an interferon, interleukin-II, insulin, heparin, low molecular weight heparin, calcitonin, oxytocin, vasopressin, vancomycin, DFO, or combinations thereof.

20. A composition as defined in claim 2, wherein said biologically active agent comprises recombinant human growth hormone.

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21. The dosage unit form as defined in claim 10, wherein said biologically active agent is selected from the group consisting of human growth hormone, bovine growth hormone, growth hormone-releasing hormone, an interferon, interleukin-II, insulin, heparin, low molecular weight heparin, calcitonin, erythropoietin, atrial natriuretic factor, an antigen, a monoclonal antibody, somatostatin, adrenocorticotropin, gonadotropin releasing hormone, oxytocin, vasopressin, cromolyn sodium, vancomycin, desferrioxamine (DFO), and any combination thereof.

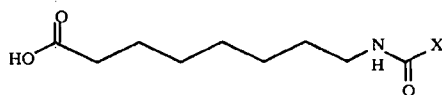
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22. A dosage unit form as defined in claim 21, wherein said biologically active agent comprises an interferon, interleukin-II, insulin, heparin, low molecular weight heparin, calcitonin, oxytocin, vasopressin, vancomycin, DFO, or combinations thereof.

23. A dosage unit form as defined in claim 9, wherein said biologically active agent comprises recombinant human growth hormone.

* * * * *

-continued



Compound

X

CXX
CXXI
CXXII
CXXIII

1-morpholino
O-t-Butyl
CH(CH₂Ph)NC(O)O-t-Bu
2-hydroxyphenyl

It has been discovered that organic acid compounds, and their salts, having an aromatic amide group, having a hydroxy group substituted in the ortho position on the aromatic ring, and a lipophilic chain with from about 4 carbon atoms to about 20 atoms in the chain are useful as carriers for the delivery of active agents. In a preferred form the lipophilic chain can have from 5 to 20 carbon atoms.

Compositions comprising the carrier compounds discussed above and active agents have been shown effective in delivering active agents to selected biological systems. These compositions include at least one active agent which is preferably a biologically or chemically active agent, and at least one carrier compound having the formula



wherein

Ar is a substituted or unsubstituted phenyl or naphthyl;

R⁷ is selected from the group consisting of C₄ to C₂₀ alkyl, C₄ to C₂₀ alkenyl, phenyl, naphthyl, (C₁ to C₁₀ alkyl) phenyl, (C₁ to C₁₀ alkenyl) phenyl, (C₁ to C₁₀ alkyl) naphthyl, (C₁ to C₁₀ alkenyl) naphthyl, phenyl (C₁ to C₁₀ alkyl), phenyl (C₁ to C₁₀ alkenyl), naphthyl (C₁ to C₁₀ alkyl), and naphthyl (C₁ to C₁₀ alkenyl);

R⁸ is selected from the group consisting of hydrogen, C₁ to C₄ alkyl, C₁ to C₄ alkenyl, hydroxy, and C₁ to C₄ alkoxy;

R⁷ is optionally substituted with C₁ to C₄ alkyl, C₁ to C₄ alkenyl, C₁ to C₄ alkoxy, —OH, —SH and —CO₂R⁹ or any combination thereof;

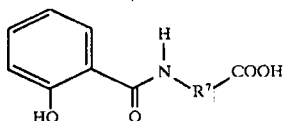
R⁹ is hydrogen, C₁ to C₄ alkyl or C₁ to C₄ alkenyl;

R⁷ is optionally interrupted by oxygen, nitrogen, sulfur or any combination thereof;

with the proviso that the compounds are not substituted with an amino group in the position alpha to the acid group, or salts thereof.

The preferred R⁶ groups are of C₄ to C₂₀ alkyl and C₄ to C₂₀ alkenyl. The most preferred R⁶ groups are C₅ to C₂₀ alkyl and C₅ to C₂₀ alkenyl.

A preferred carrier compound can have the formula



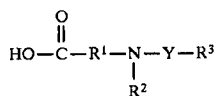
wherein R⁷ is defined above.

Further contemplated by the present invention are dosage unit forms that include these compositions.

Also contemplated is a method for preparing these compositions which comprises mixing at least one active agent with at least one compound as described above, and optionally, a dosing vehicle.

In an alternative embodiment, these non-toxic compounds are orally administered to animals as part of a delivery system by blending or mixing the compounds with an active agent prior to administration.

Further provided is a method for the preparation of a compound having the formula



CXXIV

wherein Y is



or SO₂;

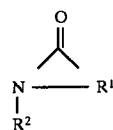
R¹ is C₃–C₂₄ alkyl, C₂–C₂₀ alkenyl, C₂–C₂₀ alkyne, cycloalkyl, or aromatic;

R² is hydrogen, C₁–C₄ alkyl, or C₂–C₄ alkenyl; and

R³ is C₁–C₇ alkyl, C₃–C₁₀ cycloalkyl, aryl, thienyl, pyrrolo, or pyridyl, where R³ is optionally substituted by one or more C₁–C₅ alkyl group, C₂–C₄ alkenyl group, F, Cl, OH, SO₂, COOH, or SO₃H;

said method comprising

(a) reacting in water and the presence of a base, a compound having the formula



CXXV

with a compound having the formula



wherein

Y, R¹, R², and R³ are as above and X is a leaving group.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graphic illustration of the results of subcutaneous injection of rhGH composition in rats.

FIG. 2 is a graphic illustration of the results of Sublingual (SL), intranasal (IN), and intracolonic (IC) dosing of rhGH in rats.

FIG. 3 is a graphic illustration of the results of intracolonic dosing of delivery of heparin with compound XXXI carrier.

DETAILED DESCRIPTION OF THE INVENTION

The specific compositions of the present invention include an active agent and a modified amino acid. These compo-

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sitions may be used to deliver various active agents through various biological, chemical, and physical barriers and are particularly suited for delivering active agents which are subject to environmental degradation. The compositions of the subject invention are particularly useful for delivering or administering biologically or chemically active agents to any animals such as birds; mammals, such as - primates and particularly humans; and insects.

Other advantages of the present invention include the use of easy to prepare, inexpensive raw materials. The compositions and the formulation methods of the present invention are cost effective, simple to perform, and amenable to industrial scale up for commercial production.

Subcutaneous, sublingual, and intranasal coadministration of an active agent, such as recombinant human growth hormone (rhGH), and the delivery agents, and particularly proteins, described herein results in an increased bioavailability of the active agent compared to administration of the active agent alone. A similar result is obtained by coadministration of salmon calcitonin with the delivery agents, in rats. Data supporting these findings are presented in the examples.

Active Agents

Active agents suitable for use in the present invention include biologically or chemically active agents, chemically active agents, including, but not limited to, fragrances, as well as other active agents such as, for example, cosmetics.

Biologically or chemically active agents include, but are not limited to, pesticides, pharmacological agents, and therapeutic agents. For example, biologically or chemically active agents suitable for use in the present invention include, but are not limited to, peptides, and particularly small peptides; hormones, and particularly hormones which by themselves do not or only a fraction of the administered dose passes through the gastro-intestinal mucosa and/or are susceptible to chemical cleavage by acids and enzymes in the gastrointestinal tract; polysaccharides, and particularly mixtures of muco-polysaccharides; carbohydrates; lipids; or any combination thereof. Further examples include, but are not limited to, human growth hormones; bovine growth hormones; growth releasing hormones; interferons; interleukin-1; insulin; heparin, and particularly low molecular weight heparin; calcitonin; erythropoietin; atrial natriuretic factor; antigens; monoclonal antibodies; somatostatin; adrenocorticotropin, gonadotropin releasing hormone; oxytocin; vasopressin; cromolyn sodium (sodium or disodium chromoglycate); vancomycin; desferrioxamine (DFO); parathyroid hormone anti-microbials, including, but not limited to anti-fungal agents; or any combination thereof.

Modified Amino Acids

The terms modified amino acid, modified poly amino acid, and modified peptide are meant to include amino acids which have been modified, or poly amino acids and peptides in which at least one amino acid has been modified, by acylating or sulfonating at least one free amine group with an acylating or sulfonating agent which reacts with at least one of the free amine groups present.

Amino acids, poly amino acids, and peptides, in modified form, may be used to deliver active agents including, but not limited to, biologically or chemically active agents such as for example, pharmacological and therapeutic agents.

An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids.

Poly amino acids are either peptides or two or more amino acids linked by a bond formed by other groups which can be linked, e.g. an ester, anhydride, or an anhydride linkage.

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Peptides are two or more amino acids joined by a peptide bond.

Peptides can vary in length from dipeptides with two amino acids to poly peptides with several hundred amino acids. See *Chambers Biological Dictionary*, editor Peter M. B. Walker, Cambridge, England: Chambers Cambridge, 1989, page 215. Special mention is made of di-peptides, tri-peptides, tetra-peptides, and penta-peptides.

Although compounds I-CXXIII above have been found to act as carriers for the oral delivery of biologically or chemically active agents, special mention is made of compounds I-XXXI above.

Modified amino acids are typically prepared by modifying the amino acid or an ester thereof. Many of these compounds are prepared by acylation or sulfonation with agents having the formula



wherein:

R^4 is the appropriate radical to yield the modification indicated in the final product,

Y is



or SO_2 , and X is a leaving group. Typical leaving groups include, but are not limited to, halogens such as, for example, chlorine, bromine, and iodine. Additionally, the corresponding anhydrides are modifying agents.

Many of the compounds of the present invention can be readily prepared from amino acids by methods within the skill of those in the art based upon the present disclosure. For example, compounds I-VII are derived from aminobutyric acid; Compounds VIII-X and XXXII-XXXV are derived from aminocaproic acid; and Compounds XI-XXVI and XXXVI are derived from aminocaprylic acid. For example, the modified amino acid compounds above may be prepared by reacting the single amino acid with the appropriate modifying agent which reacts with free amino moiety present in the amino acids to form amides. Protecting groups may be used to avoid unwanted side reactions as would be known to those skilled in the art.

The amino acid can be dissolved in aqueous alkaline solution of a metal hydroxide, e.g., sodium or potassium hydroxide, and heated at a temperature ranging between about 5° C. and about 70° C., preferably between about 10° C. and about 40° C., for a period ranging between about 1 hour and about 4 hours, preferably about 2.5 hours. The amount of alkali employed per equivalent of NH_2 groups in the amino acid generally ranges between about 1.25 and about 3 mmole, preferably between about 1.5 and about 2.25 mmole per equivalent of NH_2 . The pH of the solution generally ranges between about 8 and about 13, preferably ranging between about 10 and about 12.

Thereafter, the appropriate amino modifying agent is added to the amino acid solution while stirring. The temperature of the mixture is maintained at a temperature generally ranging between about 5° C. and about 70° C., preferably between about 10° C. and about 40° C., for a period ranging between about 1 and about 4 hours. The amount of amino modifying agent-employed in relation to the quantity of amino acid is based on the moles of total free NH_2 in the amino acid. In general, the amino modifying



US005773647A

United States Patent [19]**Leone-Bay et al.**[11] **Patent Number:** **5,773,647**[45] **Date of Patent:** **Jun. 30, 1998**D4
160[54] **COMPOUNDS AND COMPOSITIONS FOR DELIVERING ACTIVE AGENTS**[75] **Inventors:** **Andrea Leone-Bay**, Ridgefield, Conn.; **Eric Wang**, Yonkers, N.Y.; **Donald J. Sarubbi**, Bronxville, N.Y.; **Harry Lelpold**, Elmsford, N.Y.[73] **Assignee:** **Emisphere Technologies, Inc.**, Hawthorne, N.Y.[21] **Appl. No.:** **796,337**[22] **Filed:** **Feb. 7, 1997**[51] **Int. Cl.⁶** **C07C 229/00**[52] **U.S. Cl.** **562/444; 252/182.31; 514/563**[58] **Field of Search** **562/444; 252/182.31; 514/563**[56] **References Cited****U.S. PATENT DOCUMENTS**

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Primary Examiner—Gary Geist
Assistant Examiner—Brian J. Davis
Attorney, Agent, or Firm—Darby & Darby

[57] **ABSTRACT**

Carrier compounds and compositions therewith which are useful in the delivery of active agents are provided. Methods of administration and preparation are provided as well.

23 Claims, No Drawings

COMPOUNDS AND COMPOSITIONS FOR DELIVERING ACTIVE AGENTS

FIELD OF THE INVENTION

The present invention relates to compounds for delivering active agents, and particularly, biologically or chemically active agents. These compounds are used as carriers to facilitate the delivery of a cargo to a target. The carrier compounds are well-suited to form non-covalent mixtures with biologically active agents for oral administration to animals. Methods for the preparation and administration of such compositions are also disclosed.

BACKGROUND OF THE INVENTION

Conventional means for delivering active agents are often severely limited by biological, chemical, and physical barriers. Typically, these barriers are imposed by the environment through which delivery occurs, the environment of the target for delivery, or the target itself. Biologically or chemically active agents are particularly vulnerable to such barriers.

For example in the delivery to animals of biologically active or chemically active pharmacological and therapeutic agents, barriers are imposed by the body. Examples of physical barriers are the skin and various organ membranes that must be traversed before reaching a target. Chemical barriers include, but are not limited to, pH variations, lipid bi-layers, and degrading enzymes.

These barriers are of particular significance in the design of oral delivery systems. Oral delivery of many biologically or chemically active agents would be the route of choice for administration to animals if not for biological, chemical, and physical barriers such as varying pH in the gastrointestinal (GI) tract, powerful digestive enzymes, and active agent impermeable gastro-intestinal membranes. Among the numerous agents which are not typically amenable to oral administration are biologically or chemically active peptides, such as calcitonin and insulin; polysaccharides, and in particular mucopolysaccharides including, but not limited to, heparin; heparinoids; antibiotics; and other organic substances. These agents are rapidly rendered inef-

fective or are destroyed in the gastro-intestinal tract by acid hydrolysis, enzymes, or the like.

Earlier methods for orally administering vulnerable pharmacological agents have relied on the co-administration of adjuvants (e.g., resorcinols and non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation.

Liposomes have also been described as drug delivery systems for insulin and heparin. See, for example, U.S. Pat. No. 4,239,754; Patel et al. (1976), *FEBS Letters*, Vol. 62, pg. 60; and Hashimoto et al. (1979), *Endocrinology Japan*, Vol. 26, pg. 337.

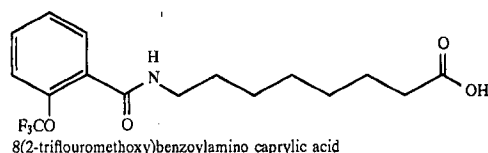
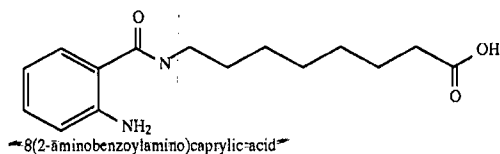
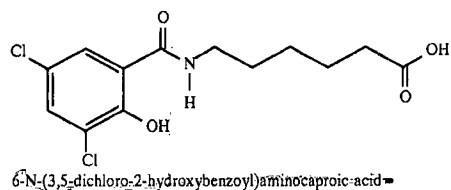
However, broad spectrum use of such drug delivery systems is precluded because: (1) the systems require toxic amounts of adjuvants or inhibitors; (2) suitable low molecular weight cargos, i.e. active agents, are not available; (3) the systems exhibit poor stability and inadequate shelf life; (4) the systems are difficult to manufacture; (5) the systems fail to protect the active agent (cargo); (6) the systems adversely alter the active agent; or (7) the systems fail to allow or promote absorption of the active agent.

More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals. For example, U.S. Pat. No. 4,925,673 describes drug-containing proteinoid microsphere carriers as well as methods for their preparation and use. These proteinoid microspheres are useful for the delivery of a number of active agents.

There is still a need in the art for simple, inexpensive delivery systems which are easily prepared and which can deliver a broad range of active agents.

SUMMARY OF THE INVENTION

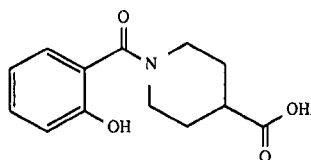
Compounds and compositions which are useful in the delivery of active agents are provided. These compositions include at least one active agent, preferably a biologically or chemically active agent, and at least one of the following compounds 1-193, or salts thereof.



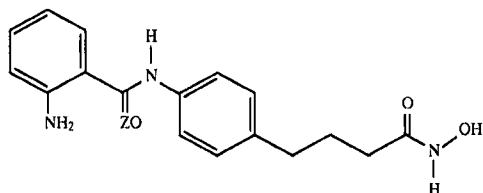
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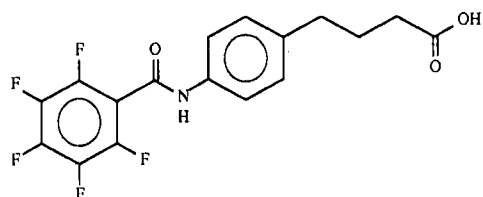
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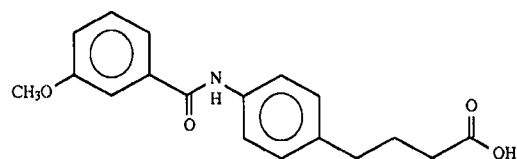
N-(2-hydroxybenzoyl)isonipecotic acid



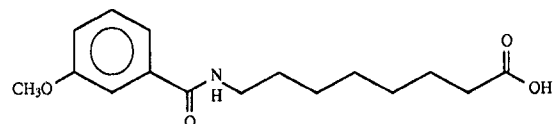
4-[4-(2-aminobenzoylamino)phenyl]butyrylhydroxamic acid



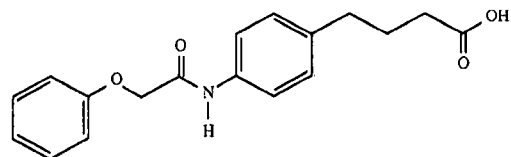
4-(4-(pentafluorobenzoyl)aminophenyl)butyric acid



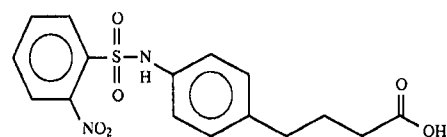
4-(4-(3-anisoyl)aminophenyl)butyric acid



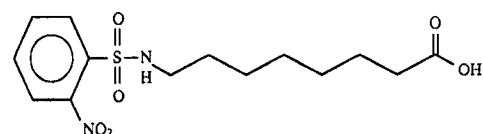
8-(3-anisoyl)aminocaprylic acid



4-(4-(phenoxyacetyl)aminophenyl)butyric acid



4-(4-(2-nitrobenzenesulfonyl)aminophenyl)butyric acid



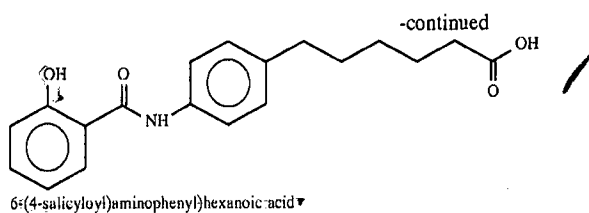
8-(2-nitrobenzenesulfonyl)aminocaprylic acid

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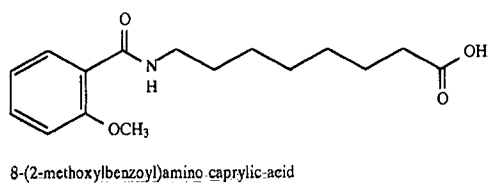
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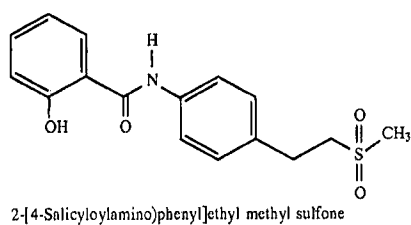
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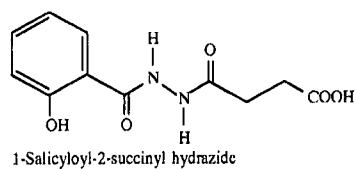
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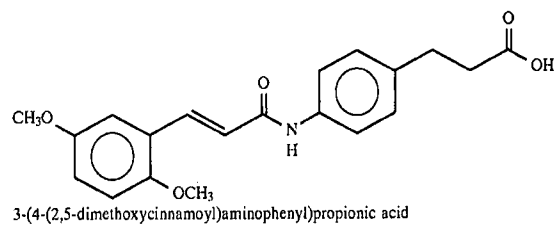
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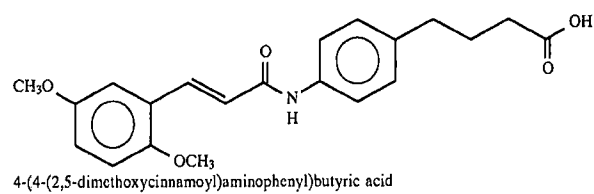
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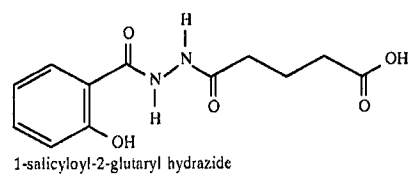
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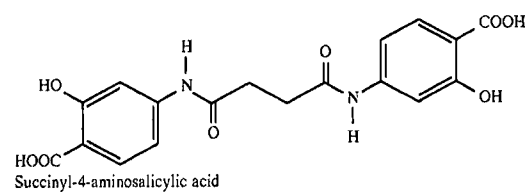
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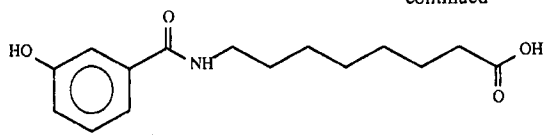


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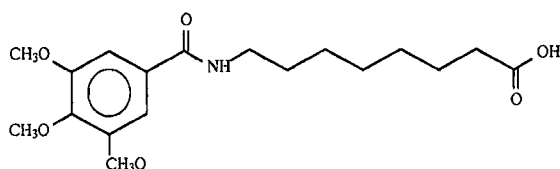
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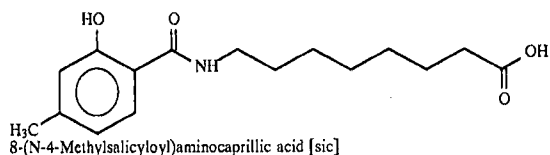
8-(2-Hydroxybenzoyl)aminocaprylic acid,

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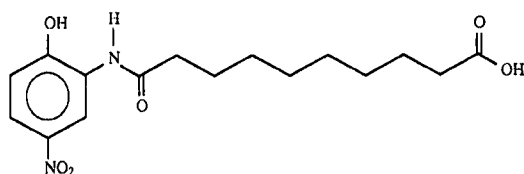
8-(3,4,5-Trimethoxybenzoyl)aminocaprylic acid

190



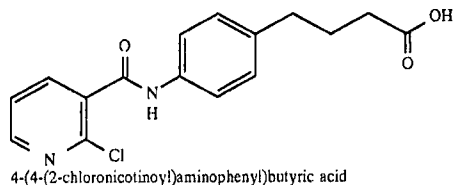
8-(N-4-Methylsalicyloyl)aminocaprillic acid [sic]

191



N-10-(2-hydroxy-5-nitroanilino)decanoic acid

192



4-(4-(2-chloronicotinoyl)aminophenyl)butyric acid

193

Compositions comprising the carrier compounds discussed above and active agents are effective in delivering active agents to selected biological systems. 40 described herein result in an increased bioavailability of the active agent compared to administration of the active agent alone.

DETAILED DESCRIPTION OF THE INVENTION

The specific compositions of the present invention include an active agent and a carrier. These compositions may be used to deliver various active agents through various biological, chemical, and physical barriers and are particularly suited for delivering active agents which are subject to environmental degradation. The compositions of the subject invention are particularly useful for delivering or administering biologically or chemically active agents to any animals such as birds including, but not limited to, chickens; mammals, such as primates and particularly humans; and insects.

Other advantages of the present invention include the use of easy to prepare, inexpensive raw materials. The compositions and the formulation methods of the present invention are cost effective, simple to perform, and amenable to industrial scale up for commercial production.

Subcutaneous, sublingual, and intranasal coadministration of an active agent, such as, for example, recombinant human growth hormone (rhGH), salmon calcitonin, heparin, including, but not limited to, low molecular weight heparin, parathyroid hormone, and compounds in compositions as

Active Agents

45 Active agents suitable for use in the present invention include biologically or chemically active agents, chemically active agents, including, but not limited to, fragrances, as well as other active agents such as, for example, cosmetics.

Biologically or chemically active agents include, but are not limited to, pesticides, pharmacological agents, and therapeutic agents. For example, biologically or chemically active agents suitable for use in the present invention include, but are not limited to, peptides, and particularly small peptides; hormones, and particularly hormones which by themselves do not or only a fraction of the administered dose passes through the gastro-intestinal mucosa and/or are susceptible to chemical cleavage by acids and enzymes in the gastro-intestinal tract; polysaccharides, and particularly mixtures of muco-polysaccharides; carbohydrates; lipids; or any combination thereof. Further examples include, but are not limited to, human growth hormones; bovine growth hormones; growth releasing hormones; interferons; interleukin-1; insulin; heparin, and particularly low molecular weight heparin; calcitonin; erythropoietin; atrial natriuretic factor; antigens; monoclonal antibodies; somatostatin; adrenocorticotropin, gonadotropin releasing hormone; oxytocin; vasopressin; cromolyn sodium (sodium or disodium

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These carrier compounds or poly amino acids, and peptides, including the amino acids, may be used to deliver active agents including, but not limited to, biologically or chemically active agents such as for example, pharmacological and therapeutic agents.

An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids.

Poly amino acids are either peptides or two or more amino acids linked by a bond formed by other groups which can be linked, e.g. an ester, anhydride, or an anhydride linkage.

Peptides are two or more amino acids joined by a peptide bond. Peptides can vary in length from dipeptides with two amino acids to poly peptides with several hundred amino acids. See *Chambers Biological Dictionary*, editor Peter M. B. Walker, Cambridge, England: Chambers Cambridge, 1989, page 215. Special mention is made of di-peptides, tri-peptides, tetra-peptides, and penta-peptides.

Salts such as, for example, sodium salt of these carrier compounds can be used as well.

Many of the compounds described herein are derived from amino acids.

Many of the compounds of the present invention can be readily prepared from amino acids including, but not limited to, aminocaproic acid, butyrylhydroxamic acid, aminophenylbutyric acid, aminophenylhexanoic acid, aminophenylpropionic acid, amino salicylic acid, aminophenylsuccinic acid, aminononanoic acid, aminonicotinic acid, amino valenic acid, aminophenylacetic acid, aminocaproic acid, aminoundecanoic acid, aminoheptanoic acid, amino-hydroxybenzoic acid, and aminodecanoic acid by methods within the skill of those in the art based upon the present disclosure and the methods described in U.S. patent application Ser. Nos. 60/017,902, filed Mar. 29, 1996; 08/414,654, filed Mar. 31, 1995; 08/335,148, filed Oct. 25, 1994; and 60/003,111, filed Sep. 1, 1995.

For example, these compounds may be prepared by reacting the single acid with the appropriate agent which reacts with free amino moiety present in the amino acids to form amides. Protecting groups may be used to avoid unwanted side reactions as would be known to those skilled in the art.

The carrier compound may be purified by recrystallization or by fractionation on solid column supports. Suitable recrystallization solvent systems include acetonitrile, methanol and tetrahydrofuran. Fractionation may be performed on a suitable solid column supports such as alumina, using methanol/n-propanol mixtures as the mobile phase; reverse phase column supports using trifluoroacetic acid/acetonitrile mixtures as the mobile phase; and ion exchange chromatography using water as the mobile phase. When anion exchange chromatography is performed, preferably a subsequent 0-500 mM sodium chloride gradient is employed.

Delivery Systems

The compositions of the present invention may include one or more active agents.

In one embodiment, compounds or salts of compounds 1-193 or poly amino acids or peptides that include at least one of these compounds or salts may be used directly as a delivery carrier by simply mixing one or more compound or salt, poly amino acid or peptide with the active agent prior to administration.

The administration mixtures are prepared by mixing an aqueous solution of the carrier with an aqueous solution of

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the active ingredient, just prior to administration. Alternatively, the carrier and the biologically or chemically active ingredient can be admixed during the manufacturing process. The solutions may optionally contain additives such as phosphate buffer salts, citric acid, acetic acid, gelatin, and gum acacia.

Stabilizing additives may be incorporated into the carrier solution. With some drugs, the presence of such additives promotes the stability and dispersibility of the agent in solution.

The stabilizing additives may be employed at a concentration ranging between about 0.1 and 5% (W/V), preferably about 0.5% (W/V). Suitable, but non-limiting, examples of stabilizing additives include gum acacia, gelatin, methyl cellulose, polyethylene glycol, carboxylic acids and salts thereof, and polylysine. The preferred stabilizing additives are gum acacia, gelatin and methyl cellulose.

The amount of active agent is an amount effective to accomplish the purpose of the particular active agent. The amount in the composition typically is a pharmacologically, biologically, therapeutically, or chemically effective amount. However, the amount can be less than a pharmacologically, biologically, therapeutically, or chemically effective amount when the composition is used in a dosage unit form, such as a capsule, a tablet or a liquid, because the dosage unit form may contain a multiplicity of carrier/biologically or chemically active agent compositions or may contain a divided pharmacologically, biologically, therapeutically, or chemically effective amount. The total effective amounts can then be administered in cumulative units containing, in total, pharmacologically, biologically, therapeutically or chemically active amounts of biologically or pharmacologically active agent.

The total amount of active agent, and particularly biologically or chemically active agent, to be used can be determined by those skilled in the art. However, it has surprisingly been found that with some biologically or chemically active agents, the use of the presently disclosed carriers provides extremely efficient delivery, particularly in oral, intranasal, sublingual, intraduodenal, or subcutaneous systems. Therefore, lower amounts of biologically or chemically active agent than those used in prior dosage unit forms or delivery systems can be administered to the subject, while still achieving the same blood levels and therapeutic effects.

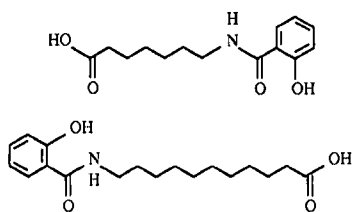
The amount of carrier in the present composition is a delivery effective amount and can be determined for any particular carrier or biologically or chemically active agent by methods known to those skilled in the art.

~~Dosage unit forms can also include any of: excipients; diluents; disintegrants; lubricants; plasticizers; colorants; and dosing vehicles, including, but not limited to water, 1,2-propanediol, ethanol, olive oil, or any combination thereof.~~

Administration of the present compositions or dosage unit forms preferably is oral or by intraduodenal injection.

The delivery compositions of the present invention may also include one or more enzyme inhibitors. Such enzyme inhibitors include, but are not limited to, compounds such as actinonin or epiactinonin and derivatives thereof. These compounds have the formulas below:

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These dosing solutions are designated H-35A-DS, H-35B-DS, and H-109A-DS, respectively.

EXAMPLES 8

In Vivo Evaluation of Heparin in Rats

The dosing solutions of Example 7 were administered to fasted rats either by oral gavage or intracolonic instillation.

Blood samples were collected by cardiac puncture following the administration of ketamine (44 mg/kg). Heparin activity was determined by utilizing the activated partial thromboplastin time (APTT) according to the method of Henry, J. B., Clinical Diagnosis and Management by Laboratory Methods; Philadelphia, Pa; W. B. Saunders (1979).

Results are illustrated in Table 4, below.

Comparative Examples 8A

In Vivo Evaluation of Heparin in Rats

The dosing solutions of Comparative Example 7A were administered to fasted rats by intracolonic instillation. Blood samples were collected and heparin activity was determined by the method of Example 8.

Results are illustrated in Table 4, below.

Comparative Example 8B

In Vivo Evaluation of Heparin in Rats

An intracolonic dosing solution of 25 mg/kg of heparin and an oral gavage dosing solution of 100 mg/kg of heparin were administered to fasted rats. These dosage solutions were designated H-ØA-DS and H-ØB-DS, respectively.

Blood samples were collected, and heparin activity was determined by the methods of Example 8.

Results are illustrated in Table 4, below.

TABLE 4

In Vivo Evaluation of Heparin in Rats	
Dosing Solution	Heparin APTT (sec)
H-9-DS	48 ± 18 (IC)
H-35-DS	54 ± 27 (PO), 177 ± 85 (IC)
H-47-DS	30 ± 14 (IC)
H-50-DS	40 ± 22 (IC)
H-58-DS	24 ± 4 (IC)
H-62-DS	37 ± 13 (IC)
H-64-DS	59 ± 28 (PO), 168 ± 75 (IC)
H-67-DS	76 ± 36 (IC)
H-76-DS	63 ± 27 (PO)
H-96-DS	36 ± 8 (IC)
H-102-DS	111 ± 108 (IC)
H-109-DS	56 ± 28 (IC)
H-110-DS	37 ± 9 (IC)
H-111-DS	71 ± 39 (IC)
H-117-DS	140 ± 128 (IC)

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TABLE 4-continued

In Vivo Evaluation of Heparin in Rats	
Dosing Solution	Heparin APTT (sec)
H-122-DS	49 ± 21 (IC), 207 ± 7 (PO)
H-123-DS	42 ± 14 (PO)
H-139-DS	31 ± 11 (IC)
H-141-DS	59 ± 26 (IC)
H-144-DS	26 ± 3 (IC)
H-35A-DS	61 ± 29 (IC)
H-35B-DS	51 ± 30 (IC)
H-169-DS	23 ± 2 (IC)
H-ØA-DS	23 ± 2 (PO)
H-ØB-DS	33 ± 6 (IC)

The above mentioned patents, applications, test methods, and publications are hereby incorporated by reference in their entirety.

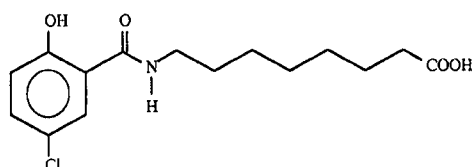
Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. All such obvious variations are within the full intended scope of the appended claims.

What is claimed is:

1. A composition comprising:

(A) at least one active agent; and

(B) a compound having the following formula



or a salt thereof.

2. A composition as defined in claim 1, wherein said active agent is selected from the group consisting of a biologically active agent, a chemically active agent, or a combination thereof.

3. A composition as defined in claim 2, wherein said biologically active agent comprises at least one peptide, mucopolysaccharide, carbohydrate, or lipid.

4. A composition as defined in claim 2, wherein said biologically active agent is selected from the group consisting of human growth hormone, bovine growth hormone, growth hormone-releasing hormone, an interferon, interleukin-II, insulin, heparin, low molecular weight heparin, calcitonin, erythropoietin, atrial natriuretic factor, an antigen, a monoclonal antibody, somatostatin, adrenocorticotropin, gonadotropin releasing hormone, oxytocin, vasopressin, cromolyn sodium, vancomycin, parathyroid hormone, desferrioxamine (DFO), or any combination thereof.

5. A composition as defined in claim 4, wherein said biologically active agent comprises an interferon, interleukin-II, insulin, heparin, low molecular weight heparin, calcitonin, oxytocin, vasopressin, vancomycin, DFO, parathyroid hormone, and combinations thereof.

6. A composition as defined in claim 2, wherein said biologically active agent comprises recombinant human growth hormone.

7. A composition as defined in claim 2, wherein said biologically active agent comprises heparin.

8. A composition as defined in claim 2, wherein said biologically active agent comprises low molecular weight heparin.

9. A composition as defined in claim 1, wherein said carrier comprises a poly(amino acid).