



(86) Date de dépôt PCT/PCT Filing Date: 2003/02/14
(87) Date publication PCT/PCT Publication Date: 2003/08/21
(45) Date de délivrance/Issue Date: 2013/04/23
(85) Entrée phase nationale/National Entry: 2005/08/16
(86) N° demande PCT/PCT Application No.: GB 2003/000703
(87) N° publication PCT/PCT Publication No.: 2003/068188
(30) Priorités/Priorities: 2002/02/18 (GB0203773.7);
2002/02/18 (GB0203830.5); 2002/05/17 (GB0211413.0);
2002/10/18 (GB0224328.5)

(51) Cl.Int./Int.Cl. *A61K 31/737* (2006.01),
A61K 31/56 (2006.01), *A61K 31/726* (2006.01),
A61K 31/727 (2006.01), *A61K 31/728* (2006.01),
A61K 45/06 (2006.01), *A61K 47/36* (2006.01),
A61K 9/00 (2006.01), *A61K 9/12* (2006.01),
A61P 11/00 (2006.01), *A61P 11/12* (2006.01)

(72) Inventeurs/Inventors:
SHUTE, JANIS KAY, GB;
CARROLL, MARY PATRICIA, GB;
CONWAY, JOY HELEN, GB;
HOCKEY, PETER MOREY, GB

(73) Propriétaire/Owner:
OCKHAM BIOTECH LIMITED, GB

(74) Agent: NORTON ROSE CANADA

(54) Titre : THERAPIE COMBINATOIRE UTILISANT LES GLYCOSAMINOGLYCANES ET DES AGENTS
THERAPEUTIQUES POUR LE TRAITEMENT DE TROUBLES RESPIRATOIRES

(54) Title: COMBINATION THERAPY USING GLYCOSAMINOGLYCANS AND THERAPEUTIC AGENTS FOR THE
TREATMENT OF RESPIRATORY DISORDERS

(57) Abrégé/Abstract:

A glycosaminoglycan, or a physiologically acceptable salt thereof, may be used to increase the efficacy of delivery of an agent to, or via, the lung and airways of a subject. In particular the agent may be used to increase the efficacy of delivery of a therapeutic agent.



(74) **Agent(suite/continued):** S.E.N.C.R.L.,S.R.L./LLP

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 August 2003 (21.08.2003)

PCT

(10) International Publication Number
WO 03/068188 A1

(51) International Patent Classification⁷: **A61K 9/12**,
47/36, 31/727, A61P 11/00

Block, Southampton General Hospital, Tremona Road,
Southampton SO16 6YD (GB). **HOCKEY, Peter, Morey**
[GB/GB]; 7 Ferrypoint, Undershore Road, Lymington,
Hampshire SO41 5TB (GB).

(21) International Application Number: PCT/GB03/00703

(22) International Filing Date: 14 February 2003 (14.02.2003)

(74) Agent: **WOODS, Geoffrey, Corlett**; J.A. Kemp & Co., 14
South Square, Gray's Inn, London WC1R 5JJ (GB).

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0203830.5 18 February 2002 (18.02.2002) GB
0203773.7 18 February 2002 (18.02.2002) GB
0211413.0 17 May 2002 (17.05.2002) GB
0224328.5 18 October 2002 (18.10.2002) GB

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE,
SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.

(71) Applicant (*for all designated States except US*): **UNI-
VERSITY OF SOUTHAMPTON** [GB/GB]; Highfield,
Southampton_SO17 1BJ (GB).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **SHUTE, Ja-
nis, Kay** [GB/GB]; 11 Widley Court Drive, Cosham,
Portsmouth, Hampshire P06 2QP (GB). **CARROLL,
Mary, Patricia** [GB/GB]; Adult Cystic Fibrosis Unit, IDU
Ward, Southampton General Hospital, Tremona Road,
Southampton SO16 6YD (GB). **CONWAY, Joy, Helen**
[GB/GB]; Respiratory Cell Molecular Biology, University
of Southampton, School of Medicine, Level D Centre

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: COMBINATION THERAPY FOR RESPIRATORY DISORDERS

(57) Abstract: A glycosaminoglycan, or a physiologically acceptable salt thereof, may be used to increase the efficacy of delivery of an agent to, or via, the lung and airways of a subject. In particular the agent may be used to increase the efficacy of delivery of a therapeutic agent.



WO 03/068188 A1

**COMBINATION THERAPY USING GLYCOSAMINOGLYCANS AND
THERAPEUTIC AGENTS FOR THE TREATMENT OF RESPIRATORY
DISORDERS**

Field of Invention

The present invention relates to the delivery of therapeutic and non-
5 therapeutic agents to, and via, the lung and its airways.

Background of Invention

Therapeutic agents are often administered directly to the respiratory tract via
such methods as inhalation, installation and intranasal delivery. This means that in
10 respiratory disorders the therapeutic agent can be targeted directly to the site where it
is needed. The respiratory route is often also used to deliver drugs to subjects with
non-respiratory disorders and to healthy subjects. The respiratory route may also be
use to administer non-therapeutic agents.

Administration of agents via the respiratory route has the advantage that it is
15 typically quick and painless. Respiratory delivery also has the advantage that a
therapeutic effect can be quickly achieved, whilst the potential for adverse effects is
often lower than via other routes of administration. Furthermore, the subject can
often deliver the agent themselves via this route and repeated doses can be given.
Such delivery means are also relatively inexpensive.

20 A further advantage of pulmonary drug delivery is that following lung
circulation, the majority of blood travels through the whole body before reaching the
liver. Drugs administered orally are circulated much more quickly to the liver. This
means that drugs administered via inhalation, intranasally or installation have more
opportunity to reach the whole of the body before their breakdown in the liver and
25 hence can reach a wider range of targets at an effective concentration.

Summary of the Invention

The present invention is based on the finding that glycosaminoglycans can
improve the delivery of an agent delivered via the inhaled, intranasal or installed
30 routes. The agent may be a therapeutic agent or a non-therapeutic agent. In particular,
the invention is of use where the agent is being administered to a subject who is

suffering from a condition characterised by elevated mucus viscosity and/or hypersecretion, but applies for subjects suffering from non-respiratory disorders and also healthy individuals. This unexpected effect means that less agent is necessary, i.e. has to be administered, to achieve the same effect in a given subject and also that
5 effectively more agent can be delivered to the target site using, i.e. administering, the same amount of agent.

The higher level of bioavailability achievable with the same amount of agent administered may mean that conditions or individuals previously refractory to treatment with a particular therapeutic agent administered via inhalation, intranasal
10 delivery or installation, or which derived minimal benefit from such treatment, may now be treatable. It may also mean that less therapeutic agent has to be used for a given therapy and hence reduce the cost of the therapy. In cases where the agent is a non-therapeutic agent, again less agent has to be administered to get the same effect or alternatively a greater effect can be achieved using the same amount of agent.

15 The use of heparin as the glycosaminoglycan is especially preferred. Delivery via inhalation and/or intranasally is especially preferred. The use of the invention in the delivery of a therapeutic agent is also especially preferred. In addition, the use of the invention to treat subjects with a respiratory disorder is also especially preferred.

Accordingly, the present invention provides for:

- 20 - use of a glycosaminoglycan, or a physiologically acceptable salt thereof, in the manufacture of a medicament for treating a subject, wherein the subject is also being treated with a therapeutic agent and the glycosaminoglycan or salt and the therapeutic agent are to be administered via inhalation, intranasally and/or via installation;
- 25 - use of a therapeutic agent, in the manufacture of a medicament for treating a subject, wherein the subject is also being treated with a glycosaminoglycan or a physiologically acceptable salt thereof and both the glycosaminoglycan or salt and the therapeutic agent are to be administered via inhalation, intranasally and/or via installation;
- 30 - a composition comprising a glycosaminoglycan, or a salt thereof and a therapeutic or non-therapeutic agent; and

- products comprising a glycosaminoglycan, or a physiologically salt thereof and a therapeutic agent for simultaneous, separate or sequential use in the treatment of a disorder wherein the glycosaminoglycan or salt and the therapeutic agent are to be administered via inhalation, intranasally and/or via installation.

5 The present invention further provides for a method of improving the efficacy of the treatment with a therapeutic agent of a subject, which method comprises the steps of:

(a) administering to said subject a glycosaminoglycan, or a physiologically acceptable salt thereof to the subject by inhalation, intranasally and/or via
10 installation; and

(b) administering to said subject a therapeutic agent via inhalation, intranasally and/or via installation.

The present invention further provides:

- an agent for treating a subject, the agent comprising a
15 glycosaminoglycan, or a physiologically acceptable salt of either thereof, wherein the said subject is also being treated with a therapeutic agent and the glycosaminoglycan or salt and the therapeutic agent are administered by inhalation, intranasally and/or via installation; and

- an agent for treating a subject, the agent comprising a therapeutic agent
20 wherein the said subject is being treated with a glycosaminoglycan, or a physiologically acceptable salt thereof and the glycosaminoglycan or salt and the therapeutic agent are administered by inhalation, intranasally and/or via installation.

The invention further provides a kit comprising:

- a glycosaminoglycan, or a physiologically acceptable salt thereof;
25 - instructions for the administration of the glycosaminoglycan or salt to a subject by inhalation, intranasally and or via installation, where the subject is being treated with a therapeutic agent which is also being administered by inhalation, intranasally and or via installation; and

- packaging.

30 The invention also provides a kit comprising:

- a therapeutic agent;

- instructions for the administration of the therapeutic agent to a subject by inhalation, intranasally and or via installation, where the subject is also being treated with a glycosaminoglycan, or a physiologically acceptable salt thereof which is being administered by inhalation, intranasally and or via installation; and

5 - packaging.

The invention also provides a nebuliser or other liquid aerosol device, dry powder inhaler or metered dose inhaler comprising a pharmaceutical composition according to the invention.

The invention also provides for the use of a glycosaminoglycan, or a
10 physiologically acceptable salt thereof, to increase the delivery of a non-therapeutic agent to, or via, the respiratory tract of a subject, where the glycosaminoglycan or physiologically acceptable salt thereof and the agent are administered to the subject via inhalation, installation and/or intranasally.

Statement of invention

15 According to one aspect of the present invention, there is provided use of a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof in the manufacture of a medicament for treating a mammalian subject with a respiratory disorder or disease which comprises
20 increased levels and/or viscosity of mucus, wherein:

- the subject is being treated with a therapeutic agent;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- 25 - the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent to the
30 mammalian subject

4a

According to another aspect of the present invention, there is provided use of a therapeutic agent in the manufacture of a medicament for treating a mammalian subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, wherein:

- 5 - the subject is also being treated with a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof;
- 10 - the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- 15 - the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent to the mammalian subject.

According to still another aspect of the present invention, there is provided a pharmaceutical system comprising: (i) a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate and keratan sulphate, and a physiologically salt thereof and (ii) a therapeutic agent for simultaneous, separate or sequential use in the treatment of a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, wherein:

- 25 - the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;
- the glycosaminoglycan is defined herein and have an average molecular weight of from 12 to 18 kd;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- 30 - the mucus contains extracellular genomic DNA which is compromising lung function; and

4b

- the glycosaminoglycan increases delivery of the therapeutic agent to the human subject.

According to yet another aspect of the present invention, there is provided a pharmaceutical composition for treating a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, the pharmaceutical composition comprising (i) a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate and keratan sulphate and a physiologically acceptable salt thereof and (ii) an excipient, wherein:

- the subject is being treated with a therapeutic agent;
- the therapeutic agent is adapted for administration by at least one of inhalation, intranasally and instillation;
- the glycosaminoglycan is defined herein and the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the pharmaceutical composition is formulated to be delivered to the respiratory tract,
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent.

According to a further aspect of the present invention, there is provided a pharmaceutical composition for treating a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, the pharmaceutical composition comprising a therapeutic agent and an excipient, wherein:

- the subject is being treated with a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;
- the glycosaminoglycan is defined herein and the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;

4c

- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent..

5 According to yet a further aspect of the present invention, there is provided a kit comprising:

- a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate, and a physiologically acceptable salt thereof;
- 10 - instructions for the administration of the glycosaminoglycan to a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, where the administration is by at least one of inhalation, intranasally and instillation, where the subject is being treated with a therapeutic agent which is being administered by at least
- 15 one of inhalation, intranasally and instillation; and
- packaging,

wherein:

- the glycosaminoglycan is defined herein;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- 20 - the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent.

According to still a further aspect of the present invention, there is provided a kit comprising:

- 25 - a therapeutic agent;
- instructions for the administration of the therapeutic agent to a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, by at least one of inhalation, intranasally and instillation where the subject is being treated with a
- 30 glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate, and a physiologically acceptable salt thereof which is

4d

being administered by at least one of inhalation, intranasally and instillation; and

- packaging,
- wherein:
- the glycosaminoglycan is defined herein;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent.

According to another aspect of the present invention, there is provided a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof for the treatment of a mammalian subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus or other pulmonary secretions, wherein:

- the subject is being treated with a therapeutic agent;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and installation;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the mucus or the other pulmonary secretions contains extracellular genomic DNA which is compromising lung function;
- the glycosaminoglycan increases delivery of the therapeutic agent; and
- the therapeutic agent is not a bronchodilator.

According to yet another aspect of the present invention, there is provided a therapeutic agent for the treatment of a mammalian subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus or other pulmonary secretions, wherein:

- the subject is also administered a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin

4e

sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt of such a glycosaminoglycan;

- the glycosaminoglycan and the therapeutic agent are adapted for administration by inhalation, intranasally and installation;
- 5 - the therapeutic agent is formulated to be delivered to the respiratory tract;
- the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the mucus or the other pulmonary secretions contains extracellular genomic DNA which is compromising lung function;
- 10 - the glycosaminoglycan increases delivery of the therapeutic agent; and
- the therapeutic agent is not a bronchodilator.

Brief Description of the Figures

Figure 1: Effect of mucolytic agents on CAL/CF sputum (n=3). As described in Example 1, CAL (chronic airflow limitation) or CF (cystic fibrosis) sputum was homogenised and mucolytic added on a 10 % v/w basis. Mixing was performed using a 19-gauge needle. Carboxylate-modified fluorescent beads were added to sputum sample in a 1:1 ratio (v/w). After vortexing, 20 µl of sample was added to each upper well of a micro-boyden chamber. The upper and lower wells of the chamber were separated by an 8 µm polycarbonate filter, and the lower wells contained 25 µl PBS. The chamber was centrifuged for five minutes at 1000 rpm to remove air bubbles prior to incubation at 37°C, 900 rpm, for four hours in the dark. Following incubation the chamber was dismantled and the fluorescence of the solution in the lower wells measured. The results for PBS, heparin, DNase and dextran sulphate (a non-glycosaminoglycan control) for CAL sputum are shown in the top panel of Figure 1. The results for a mixture of chondroitin sulphates A and C for CF sputum are shown in the bottom panel.

Figure 2: Atomic Force Microscopy (AFM) imaging of DNA. As described in Example 2, calf thymus DNA (0.1 mg/ml) was treated with heparin (0.1

mg/ml) and AFM was performed in air under ambient conditions using a TopoMetrix TMX2000 Scanning Probe Microscope (ThermoMicroscopes, Bicester, UK). Panel A shows untreated DNA and Panel B shows DNA treated with heparin.

Figure 3: Atomic Force Microscopy (AFM) imaging of DNA. Further

5 Atomic Force Micrographs of DNA (100 µg/ml) treated with lower concentrations of heparin. Panel A shows the results for untreated DNA; Panel B for DNA treated with 0.1 µg/ml heparin; Panel C for DNA treated with 1 µg/ml heparin; and Panel D for DNA treated with 10 µg/ml heparin.

10

Detailed Description of the Invention

The present invention is based on the finding that a glycosaminoglycan, or a physiologically acceptable salt thereof, can enhance the delivery of an agent to, or via, the respiratory tract. The activity of the agent is thus potentiated. The amount of agent delivered to the target site is therefore higher per unit of agent administered. A synergistic effect can be observed. In an especially preferred embodiment of the invention the agent is a therapeutic agent. Alternatively, the agent may be a non-therapeutic agent.

The glycosaminoglycan or salt and the agent may be administered in the same or separate compositions and at the same time, simultaneously or sequentially.

Synergy of glycosaminoglycan with therapeutic agents

Unexpectedly, glycosaminoglycans are capable of increasing the delivery of a therapeutic agent or non-therapeutic agent. That is that the same molar amount of agent administered results in a higher concentration of the agent being delivered to the target site when a glycosaminoglycan is also administered. In effect, the bioavailability of the agent at the target cells in the lung and/or its airways is increased. Glycosaminoglycans can also facilitate delivery intranasally via the nasal membranes. The glycosaminoglycan is thought to act by reducing the barrier function of the sputum, and other secretions of the lung and its airways, and hence allow the agent to reach the target cells in the airway more easily.

The amount of agent, and in particular therapeutic drug, passing through the mucus, or at the target cells, may be elevated by from one, two, three, ten, twenty or more fold by the addition of the glycosaminoglycan. The increase of the amount of the drug or non-therapeutic agent reaching the target cells, or in the mucus next to the target site, may typically be from 5% to 500%, preferably from 50 to 250%, and still more preferably from 50 to 100%.

The target for delivery of the drug or non-therapeutic agent may be any part of the respiratory tract including the nasal mucosal membranes. The agent may be administered via inhalation, installation and/or intranasally and hence any part of the respiratory tract which these agents provide access to can be targeted. In some embodiments of the invention the target cells may be present in other parts of the body and the agent passes via the respiratory tract or nasal mucosal membranes to the blood and then onto the target cells. In such embodiments, any of the levels of increase or the amount of agent available, or present, specified herein may be seen in the blood stream and/or at, or in the vicinity of, the target cells. In some embodiments the aim may be to deliver the agent to the whole body or a specific organ, such as for example the brain.

The presence of the glycosaminoglycan may effectively increase bioavailability of the agent by more than one fold, double, treble, five fold, ten fold, twenty fold or more. Typically, the presence of the glycosaminoglycan may effectively reduce the molar amount, or amount by weight, of the agent necessary to achieve a given effect. The reduction may, for example, be by half, a quarter, a tenth, or more. It may reduce it by from 50 to 500%, preferably from 75 to 250%, more preferably by from 100 to 125%. Thus the amount of agent which has to be administered to achieve the same measurable effect may be decreased, for example, by any of these factors or by more than 10%, preferably more than 20%, more preferably more than 40%, even more preferably by more than 100%. The amount necessary to achieve the effect may be reduced by a factor of one, two, three, five, fifty, one hundred or more fold.

The amount of glycosaminoglycan added will typically be enough to cause a synergistic effect to increase the bioavailability of the agent. Thus the amount of

glycosaminoglycan used may typically be the amount necessary to reduce the amount of agent which has to be administered to achieve a given effect. The reduction may be by any of the factors discussed herein. In particular, the effect measured may be a therapeutic effect.

5 The ratio of the agent to glycosaminoglycan by weight, or alternatively by units, may, for example, be from 1:50,000 to 1000:1, preferably from 1:10,000 to 100:1, more preferably from 1:5,000 to 50:1, still more preferably from 1:1,000 to 25:1. The ratio may, for example, be from 1:500 to 1:20, preferably be from 1:150 to 1:5, more preferably be from 1:50 to 1:2 and even more preferably be from 1:10 to
10 1:1. The two may, for example, be in equal amounts or the agent may be present in two fold, five fold, ten fold or greater excess or alternatively the glycosaminoglycan may be present in similar excess.

For a particular agent methods will be known to assess its efficacy and the effect that it has on a subject. Such methods may be employed in the production and
15 assessment of the medicaments of the invention. Such methods may be used to determine the reduction in the amount of agent which may be administered, whilst still achieving the same effect, when a glycosaminoglycan is employed.

The elevation in bioavailability achievable using the invention may be assessed by measuring one or more of the effects of a given agent. These results may
20 be used to decide on the necessary reduction in dose of agent to be administered and/or the increase in delivery rate achievable. Such methods may be used to decide on the ratio of agent to glycosaminoglycan and the amounts of each which will be present in the medicaments or compositions of the invention or administered in the methods of the invention. A number of *in vitro* methods may also be used to assess
25 this, including the barrier method discussed herein. Various labelled compounds may be used to measure the increase in delivery of the agent seen, for example, a labelled version of a therapeutic agent, such as a fluorescent or radioactively labelled version may be employed.

The increase in delivery of the agent achievable may be measured in terms of
30 the concentration of the agent reaching, or at, the target site or through measuring a given effect of the agent. Any effect of a particular agent may be measured and in

particular any effect whose magnitude is in relation to the amount of agent reaching the target site. In some cases, the size of the effect measured may be proportional to the amount of agent reaching the target site. In other cases, the effect measured may be a qualitative, rather than a quantitative one and the amount of agent required to generate it in the presence or absence of a particular glycosaminoglycan may be measured and preferably titrated. Standard curves of the effect achieved against the amount of agent administered in the presence or absence of a specific concentration of glycosaminoglycan may be generated. In a preferred embodiment, where the agent is therapeutic, the assessment carried out will involve determining whether an effect is achieved which will be of benefit in treating the subject. In particular, an effect equivalent to that achieved when the recommended dose of an agent is administered in the absence of a glycosaminoglycan will be the target.

The effect may be a physiological or pharmacological one. The effect may be in any of the parameters described herein. The effect measured may be an improvement in the condition of a patient, such as a reduction in one or more symptom(s) of a disorder that they are suffering from, including those of any of the disorders mentioned herein. The effect measured may be the ability to image a particular area or cell type using a labeled agent and in particular the quality and intensity of the image achieved.

Any of the parameters mentioned herein may be assessed. In particular, parameters associated with lung function, such as FEV_1 , may be assessed. Mucus viscosity may be assessed. FEV_1 is defined as the maximal forced volume which can be expired in one second starting from maximum inspiration (European Resp. Journal, 1993; 6: Suppl. 16 and Coates, Lung Function: Assessment and Applications In Medicine, 4th Edition, Oxford, Blackwell, 1969). It can be measured by standard techniques well known in the art i.e. by spirometry. Mucus viscosity may be determined by any of several different methods known in the art, including a sputum compaction assay (see for example WO 94/10567), assays using a torsion pendulum (Janmey, J. Biochem. Biophys. Methods 22:41-53 1991) or other suitable rheological methodologies.

FVC and in particular the ratio of FEV_1/FVC may also be assessed. FVC

(forced vital capacity) corresponds to the maximal volume of air forcibly exhaled from the point of maximal inhalation and can be measured using standard spirometry.

In one embodiment the effect measured may be the change in blood glucose levels where the agent administered is insulin. In others the presence of an immune response may be measured, where the agent administered is an immunogen. In others, where the agent administered is a contraceptive, pregnancy rate may be examined. In cases where the agent is a gene, and in particular a therapeutic gene, transformation efficiency may be measured. The efficiency of gene expression. at the RNA and/or protein level may be measured.

Therapeutic agents

The therapeutic agent for use in the invention may be any therapeutic agent suitable for delivery via inhalation, intranasally or via installation as the invention will typically involve such delivery routes. In particular, the agent may be one suitable for delivery by inhalation and/or intranasally.

Typically, the therapeutic agent will be an agent that is suitable for treating one of the disorders named herein. Alternatively, it may be an agent administered to a healthy subject such as, for example, a contraceptive or smoking suppressant. The agent may be being administered prophylactically to prevent a disease. The agent may be one designed to reduce, eliminate or prevent worsening of one of the symptoms of a disorder, such as, for example, a symptom of any of the diseases mentioned herein. It may be one designed to prevent the development of such a symptom.

The therapeutic agent may be, for example, a bronchodilator, steroid, antimicrobial agent, antiviral agent, decongestant, or gene therapy vector. Examples of possible bronchodilators include salbutamol, salmeterol, ipratropium bromide, terbutaline sulphate, eformoterol and xanoate. Preferred steroids include beclomethasone dipropionate (BDP), budesonide, and fluticasone. Preferred antimicrobials include antibiotics (for example colistin), antivirals (such as relenza) and antiretrovirals (such as the HAART - highly active antiretroviral therapy - group

of drugs), or antifungals (for example fluconazole and intraconazole).

The antiviral agent may be one effective against a virus with a double stranded or single stranded genome, such as a DNA or RNA virus. It may be an antiviral agent effective against any pathogenic virus, but in particular against one affecting the respiratory tract, or at least having part of its life cycle in the respiratory tract. It may be an ant-retroviral drug and in particular one against HIV (human immunodeficiency virus). The agent may be one against respiratory syncytial virus (RSV) such as, for example, ribavirin. The antiviral agent may be one against an influenza virus or a rhinovirus.

In the case of an antibacterial agent, the agent may be one against any pathogenic bacterium and in particular those affecting the respiratory tract. Examples of bacteria that the agent may be effective against include those responsible for pneumonia such as *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *Legionella pneumophila*, *Chlamydia pneumoniae* or *Chlamydia psittaci*. The agent may be one effective against *Bordetella pertussis*, *Pseudomonas aeruginosa* or *Haemophilus influenzae*. It may be an agent effective against *Moraxella catarrhalis*. The agent may be active against more than one bacterium. For example, it may be active against two or more of any of the bacterium mentioned herein.

Gene delivery vectors for which the invention may be used to facilitate delivery include viral gene delivery vectors and, for example, gene delivery by adenovirus and adeno-associated virus. The vectors may include genes therapeutic for any of the conditions mentioned herein such as, for example, the CFTR gene or the α_1 -antitrypsin gene. The methods of the invention may be used to increase the efficiency of other delivery methods. These include liposome, naked DNA, polycation complexes and peptide based delivery systems.

In cases where the agent is a liposome it may, for example, comprise a cationic lipid such as DOTAP (N-1-(2,3-dioleoyloxy) propyl)-N,N,N-trimethyl ammonium methyl sulphate) or DOTMA (N-(1-(2,3-dioleoyloxyl) propyl N,N,N-trimethyl ammonium chloride). The liposome may also comprise a neutral lipid, such as, DOPE (dioleoylphosphatidylethanolamine). In some embodiments of the

invention the agent may be a liposome/polycation/nucleic acid complex. Such complexes may include polycations such as polylysine.

The agent may be a nucleic acid associated with a peptide to facilitate its delivery or packaging. For example, the agent may comprise the tetra-peptide
5 sequence serine-proline-lysine-lysine, or repeats thereof, to facilitate condensation of the nucleic acid. The agent may comprise the peptide sequence tyrosine-lysine-alanine-(lysine)₈-tryptophan-lysine to facilitate condensation of the nucleic acid. The agent may comprise a nucleic acid-peptide complex associated with a molecule which results in specific binding, and preferably entry, into a particular cell type. For
10 example, the complex may be conjugated with a ligand for a receptor and in particular one found on a cell type in the respiratory tract.

The therapeutic agent may be an ion channel modifier such as, for example, amiloride, UTP, ATP. Other possible secondary agents administered as the therapeutic agent include mucolytics, hormones, surfactants, proteins and peptides
15 for systemic delivery, anti-anginals, vasopressin, analgaesic, contraception, HRT (hormone replacement therapy) or nicotine replacement therapy. In some embodiments of the invention, the therapeutic agent is, or will comprise, a bronchodilator, preferably a β_2 agonist, such as salmeterol and formoterol or an M₁ and/or M₃ antagonist, such as revatropate and triotropium bromide. The
20 bronchodilator may be any of those used to treat asthma or chronic airflow limitation disorder (CAL).

In some embodiments of the invention, the therapeutic agent is a bronchodilator, preferably a β_2 agonist, such as salmeterol and formoterol, or an M₁ and M₃ antagonist, such as revatropate and triotropium bromide. The bronchodilator
25 may be any of those used to treat asthma or chronic airflow limitation disorder. In further embodiments of the invention, the therapeutic agent may be an anti-inflammatory, preferably an LTD₄ antagonist, an LTB₄ antagonist, such as LY 293111, SC-53228, CP-105,696, SB 201146 and BIIL284, a 5'-lipoxygenase inhibitor, such as zileuton, a chemokine inhibitor, such as an interleukin 8 inhibitor
30 or a MCP-1 antagonist, a TNF α inhibitor, such as a monoclonal TNF antibody, a soluble TNF receptor and a TNF convertase inhibitor, an antioxidant, such as N-

acetylcysteine, a stable glutathione analog and a nitrone, a prostanoid inhibitor, such as a COX-2 inhibitor, a thromboxane antagonist and an isoprostane receptor antagonist, theophylline, a phosphodiesterase-4-inhibitor, such as SB 207499, CP 80633 and CDP-840, a NF-kB inhibitor, an adhesion molecule inhibitor, such as a
5 monoclonal antibody to CD18, ICAM-1 and E-selectin, interleukin 10, a p38 MAP kinase inhibitor, such as SB 203580, SB 220025 and RWJ 67657, colchicine or a selective EP₂ agonist, such as misoprostil and butaprost.

In yet further embodiments of the invention, the therapeutic agent is a protease inhibitor, preferably a neutrophil elastase inhibitor, such as ICI 200355,
10 ONO-5046, MR-889 and L 658,758, a cathepsin inhibitor, such as suramin, a matrix metalloproteinase inhibitor, such as batimastat and marimastat, an α_1 -antitrypsin, a serum protease inhibitor (serpin), such as elafin or a secretory leukoprotease inhibitor (SLPI). In further embodiments of the invention, the therapeutic agent is a mucoregulator, preferably a tachykinin agonist, such as CP-99,994 and SR 140333, a
15 sensory neuropeptide release inhibitor, such as BW443 and levcromakalim, a cyclooxygenase inhibitor, such as indomethacin, a mucin (MUC) gene suppressor, such as a corticosteroid, a mucolytic agent or erythromycin.

In other embodiments of the invention, the therapeutic agent is an antibiotic, preferably a macrolide antibiotic, such as erythromycin, clarithromycin or
20 roxithromycin. In further embodiments of the invention, the therapeutic agent is an antifungal agent, preferably amphotericin B or an azole, such as clotrimazole, ketoconazole, miconazole, fluconazole or itraconazole. In other embodiments of the invention, the therapeutic agent is a smoking cessator, preferably zyban. The therapeutic agent may be an appetite supresant. The agent may be one designed to
25 treat diabetes such as insulin.

The agent may be an immunogen designed to produce a therapeutic immune response, for example, the therapeutic agent may be a vaccine against a pathogen including any of those mentioned herein. The agent may, for example, comprise a protein, carbohydrate, and/or nucleic acid. The agent may comprise a small organic
30 molecule. The agent may be an agonist and/or an antagonist and in particular may be an agonist, antagonist or modulator of a molecule which plays a role in any of the

conditions mentioned herein. The agonistic or antagonistic effect of the agent may be responsible for the therapeutic effect of the agent. The agent may be an enzyme. In some embodiments the agent may be a prodrug which is activated following administration.

5 In cases where the therapeutic agent is a polypeptide it may be one which has been chemically modified to alter its properties such as, for example, its biological half-life. To achieve this covalent modifications may be introduced by reacting targeted amino acid residues of the native or variant polypeptide with an organic derivatising agent that is capable of reacting with selected amino acid side-chains or
10 N- or C-terminal residues. Suitable derivatising agents and methods are well known in the art.

Residues which in particular may be derivatised include cysteinyl residues (most commonly by reaction with α -haloacetates), histidyl residues (by reaction with diethylpyrocarbonate at pH 5.5-7.0), lysinyl and amino terminal residues (by reaction
15 with succinic or other carboxylic acid anhydrides), arginyl residues (by reaction with reagents such as phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin). Carboxyl side groups (aspartyl or glutamyl) may be selectively modified by reaction with carbodiimides or may be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions. The covalent attachment of agents such as
20 polyethylene glycol (PEG) or human serum albumin to the therapeutic agents may reduce their immunogenicity and/or toxicity of the variant and/or prolong its half-life and hence may be used in the invention. In some embodiments of the invention the therapeutic agent may be directly conjugated to the glycosaminoglycan or joined through an intermediate molecule. It may be coated with a composition comprising
25 the heparin.

In some embodiments of the invention more than one therapeutic agent may be used. For example, a glycosaminoglycan may be used to facilitate the delivery of two, three, four or more agents to the same subject. These may be present in the same or different compositions and be administered at the same time, in sequence or
30 separately.

The target site which the therapeutic agent is to act on is preferably a cell type

in the respiratory tract. However, it may be any, or all, cell types in the body. The therapeutic agent may be delivered from the lung to other sites via the blood system.

Non-therapeutic agents

5 In some embodiments of the invention, the agent administered may be a non-therapeutic agent. The methods of the invention can improve the delivery of an agent regardless of whether it is a therapeutic or non-therapeutic molecule. In some cases a particular molecule may have both therapeutic and non-therapeutic utilities. For example, pregnancy is a natural biological process and the use of contraceptives to
10 control whether or not pregnancy occurs may not be regarded as a therapeutic method. However, in some cases contraception may be of therapeutic benefit, for example where a pregnancy may put the health of a female subject at risk. In either case the methods of the invention may be employed to facilitate the delivery of a contraceptive regardless of whether the purpose is therapeutic or not.

15 In general, the methods of the invention may be used to facilitate the delivery of any suitable agent whether therapeutic or non-therapeutic and whether the purpose of administration is therapeutic or non-therapeutic including all of the agents mentioned herein.

Non-therapeutic agents may also include labeled agents. For example, a non-
20 therapeutic agent may be an agent which is a radioactive, fluorescent or other labeled molecule and the purpose of the method may be to image a particular structure or tissue or monitor the transport of an agent. In one embodiment, labeled versions of any of the therapeutic agents mentioned herein may be administered, typically to allow the transport and breakdown of the agent to be monitored.

25

Glycosaminoglycans

The medicaments and methods of the invention employ glycosaminoglycans. Glycosaminoglycans are linear heteropolysaccharides possessing characteristic disaccharide repeat sequences that are typically highly N- and O-sulphated at D-
30 glucosamine, galactosamine and uronic acid residues. These sulphate moieties introduce a high degree of negative charge along the glycosaminoglycan polymer

chain and add to the heterogeneity of these macromolecules.

Any suitable glycosaminoglycan may be employed in the invention.

Preferably, the glycosaminoglycan will be in a form suitable for administration via inhalation, intranasally or via installation as typically the medicaments of the invention will be delivered via such routes. Preferably, the glycosaminoglycan employed in the invention will be any of chondroitin sulphates A to E, heparin, heparin sulfate, heparan, heparan sulphate, hyaluronic acid, keratan sulphate, a derivative of any thereof or a mixture or any two thereof. Chondroitin sulphate B is sometimes referred to as dermatan sulphate. In a more preferred embodiment of the invention the glycosaminoglycan will be any of chondroitin sulphates A, C, D or E, heparin, heparin sulfate, heparan, heparan sulphate, hyaluronic acid, keratan sulphate, a derivative of any thereof or a mixture of any two thereof. In a particularly preferred embodiment the glycosaminoglycan will be chondroitin sulphate A, chondroitin sulphate C, heparin, heparin sulfate, heparan, heparan sulphate, a derivative of any thereof or a mixture of any two thereof. More preferably, the glycosaminoglycan will be chondroitin sulphate A, chondroitin sulphate C, heparin, a derivative of any thereof or a mixture of any two thereof. In an even more preferred embodiment of the invention the glycoaminoglycan will be heparin or a derivative thereof. In some embodiments of the invention the glycosaminoglycan employed will be a mixture of more than two glycosaminoglycans from one of the above mentioned groups, such as a mixture of three, four or five of the glycosaminoglycans.

In embodiments of the invention where a mixture of two glycosaminoglycans is employed the two may, for example, be present in the ratio 1:1, 1:2, 1:4, 1:10 or 1:100. The ratio may be 90:10, 80:20, 70:30, or 60:40. Any suitable ratio may be employed and either glycosaminoglycan may be at the higher concentration. The ratio may be the same as the ratio in which the two are isolated when they are recovered from a common tissue using standard techniques. In a preferred embodiment of the invention a mixture of chondroitins A and C will be employed and in particular at a ratio of 80:20, preferably 75:25 and even more preferably 70:30 with chondroitin sulphate A being present at the higher level.

Typically, the glycosaminoglycan will not have been subjected to

fragmentation to reduce its molecular weight. Usually, the glycosaminoglycan will not have been subjected to depolymerisation, such as by chemical or enzymatic means, to reduce its molecular weight. The average number of saccharide units in the polysaccharide chains of the glycosaminoglycan may, for example, be from 18 to 5 100, preferably from 30 to 80, more preferably from 40 to 60 and still more preferably from 5 to 60 units.

The glycosaminoglycan may be any suitable commercially available glycosaminoglycan and may, for example, be an unfractionated glycosaminoglycan. The glycosaminoglycan will have typically been isolated from a natural sources such 10 as from an animal. In some cases, the glycosaminoglycan may have been synthesised rather than be a naturally occurring molecule.

In some cases the glycosaminoglycan may have been isolated from an animal, and in particular from animal tissues such as those of pigs or cattle. The glycosaminoglycan may have been obtained from tissues such as the lung, liver, or 15 gut of an animal and in particular from beef lung or pork intestinal mucosa. The glycosaminoglycan may have been obtained from the skin of such an organism.

In some embodiments, the glycosaminoglycan may have been isolated from a cartilagenous fish or other sea or freshwater organism. In some cases the glycosaminoglycan may have been isolated from a shark or squid and in particular 20 from the cartilage of such an organism. The glycosaminoglycan may have been isolated from a sturgeon and in particular from a sturgeon notochord.

Glycosaminoglycans and glycosaminoglycan salts suitable for use in the present invention will preferably have an average molecular weight of from 8 to 40 kd, preferably from 10 to 30 kd, more preferably from 12 to 20 kd. In particular, the 25 glycosaminoglycan or salt may have an average molecular weight of from 14 to 18 kd, preferably from 15 to 17 kd and more preferably from 16 to 17 kd. In some embodiments all, or substantially all, of the glycosaminoglycan molecules or glycosaminoglycan salt molecules will have a molecular weight falling within the ranges specified above. Thus from 50 to 100%, preferably from 75 to 100%, more 30 preferably from 90 to 100%, still more preferably 95 to 100% of the molecules may have such a molecular weight. In some cases at least 95%, preferably 97.5%, more

preferably 99%, still more preferably 99.5% and even more preferably 99.9% may have a molecular weight falling within the range. The glycosaminoglycan or salt may be present in a range of molecular weight sizes and typically the most commonly occurring molecular weight size will fall within one of the above specified molecular weight ranges.

One of the specific glycosaminoglycans mentioned above may have been modified to generate a derivative of the glycosaminoglycans. Thus such derivatives may be used in the invention as long as they retain the ability to facilitate delivery of the therapeutic agent and in particular to have a synergistic effect on the delivery. Thus in the case of heparin, the heparin may have been subjected to O-desulphation such as at least at the 2-O and 3-O positions. The same or equivalent modifications may be made to other glycosaminoglycans to generate derivatives for use in the present invention.

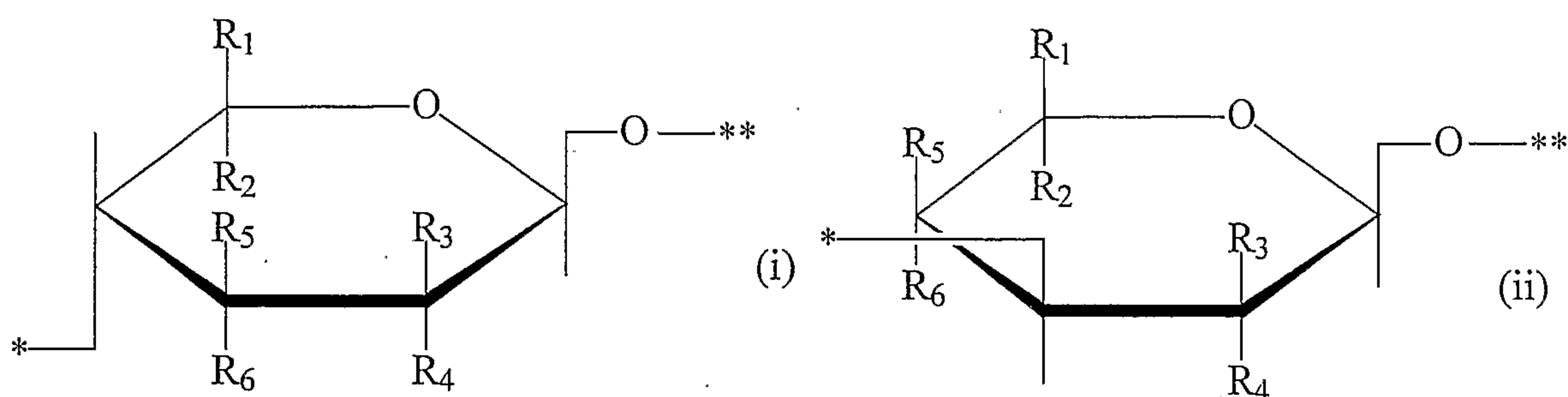
The glycosaminoglycan may have been subjected to acetylation, deacetylation, oxidation and/or decarboxylation such as, for example, periodate oxidation to generate a derivative. Heparinoids may be used in the invention.

Typically, the active compound used in the present invention comprises is a glycosaminoglycan or a physiologically acceptable salt thereof comprising repeating disaccharide units of general formula (1)



wherein:

each A is the same or different and represents a moiety of formula (i) or (ii)

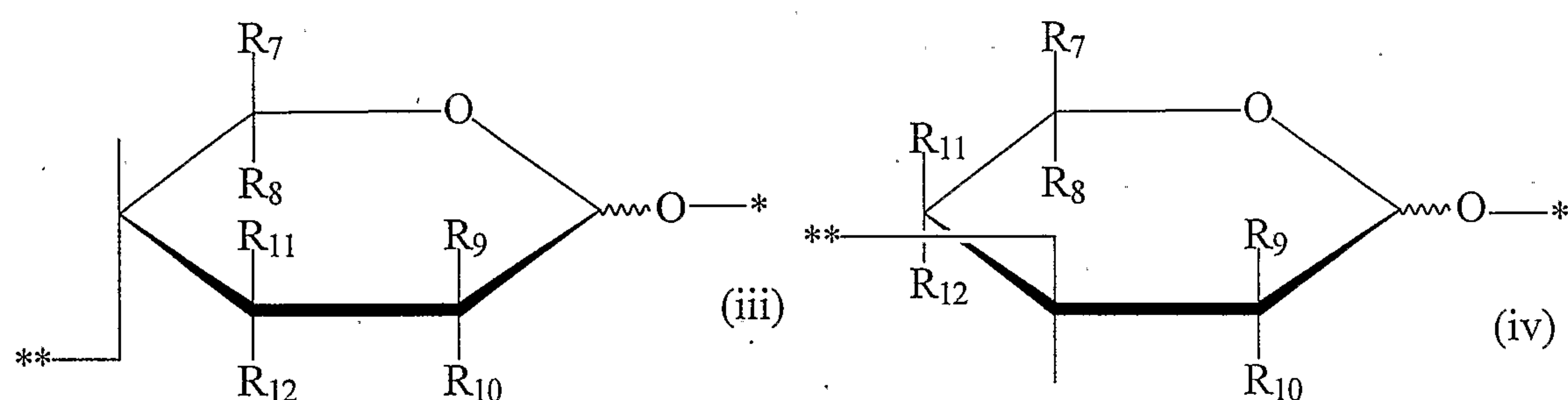


25

wherein:

- one of R_1 and R_2 is hydrogen, and the other is $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$ or $-\text{CH}_2\text{OR}$ wherein R is hydrogen or $-\text{SO}_3\text{H}$;
 - one of R_3 and R_4 is hydrogen, and the other is $-\text{OR}$ wherein R is hydrogen or $-\text{SO}_3\text{H}$;
 - 5 - one of R_5 and R_6 is hydrogen, and the other is $-\text{OH}$;
 - * represents a direct bond to an adjacent hydrogen atom or B moiety; and
 - ** represents a direct bond to an adjacent B moiety;
- each B is the same or different and represents a moiety of formula (iii) or (iv);

10



wherein:

- one of R_7 and R_8 is hydrogen and the other is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$;
- one of R_9 and R_{10} is hydrogen and the other is $-\text{NHAc}$, $-\text{NH}_2$ or $-\text{NH}\text{SO}_3\text{H}$;
- 15 - one of R_{11} and R_{12} is hydrogen and the other is $-\text{OH}$ or $-\text{OSO}_3\text{H}$;
- * represents a direct bond to a hydrogen atom or an adjacent A moiety;
- ** represents a direct bond to an adjacent A moiety; and
- indicates a bond in either stereochemical orientation;

or a physiologically acceptable salt thereof.

20 The formulae herein adopt standard practice in depicting sugars. According to this practice, the formulae include vertical lines through each of the cyclic carbon atoms. This does not, of course, mean that methyl groups are attached at each position, or that methylene groups are present as part of the link between adjacent cyclic moieties.

25 Preferably, each A moiety in the glycosaminoglycan of general formula (1) is the same. Preferably, each B moiety in the glycosaminoglycan of general formula

(1) is the same.

Preferably, each A moiety in the glycosaminoglycan of general formula (1) is a moiety of general formula (i).

Typically, one of R_1 and R_2 is hydrogen, and the other represents $-\text{CO}_2\text{H}$ or $-\text{CH}_2\text{OR}$, wherein R is hydrogen or $-\text{SO}_3\text{H}$. Preferably, one of R_1 and R_2 is hydrogen and the other represents $-\text{CO}_2\text{H}$.

Typically, R_3 is hydrogen and R_4 is $-\text{OR}$, wherein R represents hydrogen or $-\text{SO}_3\text{H}$.

Typically R_5 is $-\text{OH}$ and R_6 is hydrogen.

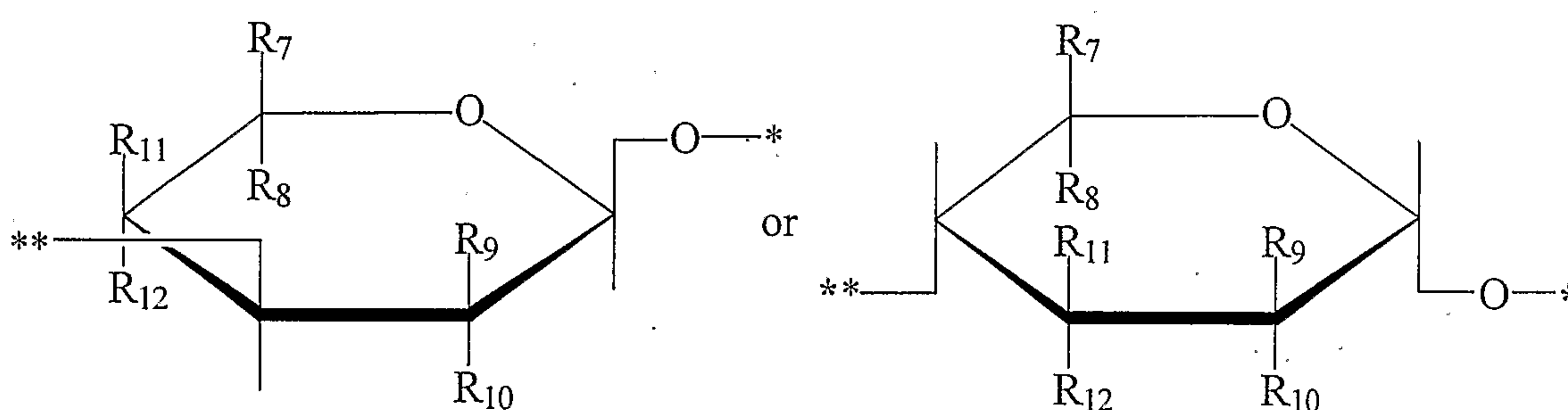
Typically each A is the same or different and represents a moiety of formula (i).

Typically R_7 is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$ and R_8 is hydrogen.

Typically R_9 is hydrogen and R_{10} is $-\text{NHAc}$, $-\text{NH}_2$ or $-\text{NHSO}_3\text{H}$.

Typically R_{11} is $-\text{OSO}_3\text{H}$ or $-\text{OH}$ and R_{12} is hydrogen.

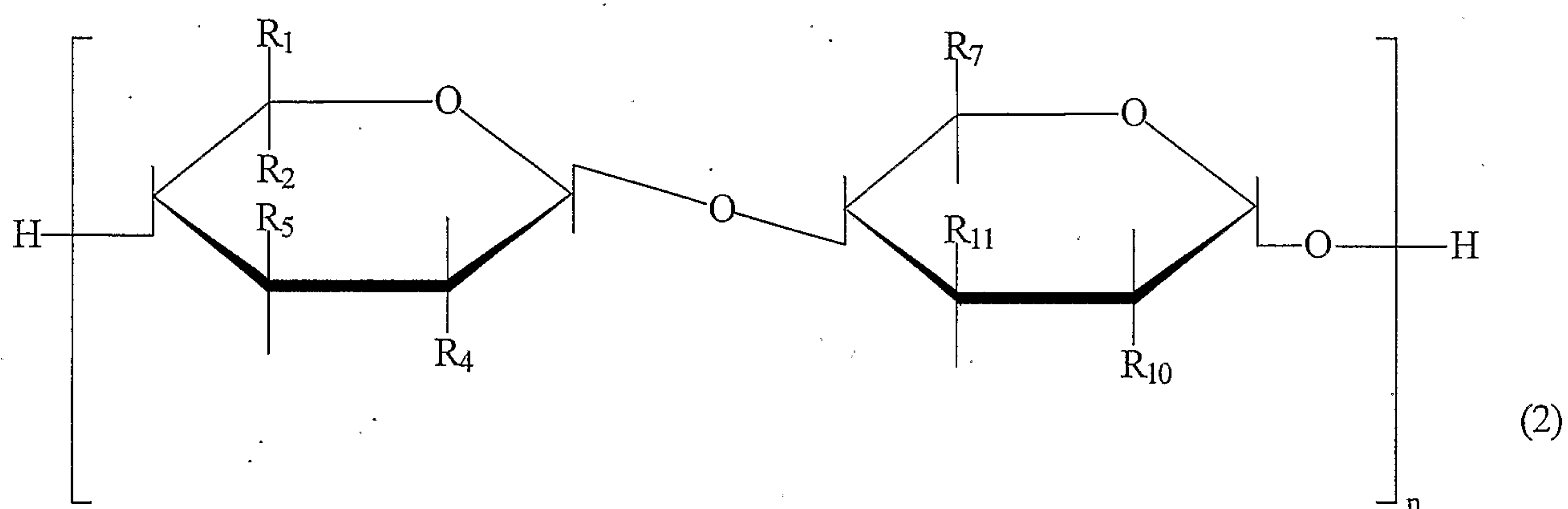
Typically each B is the same or different and represents



wherein R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} * and ** are as described above.

Preferably R_1 is not hydrogen in an A moiety which is adjacent to a moiety (iv) in which R_{12} is hydrogen.

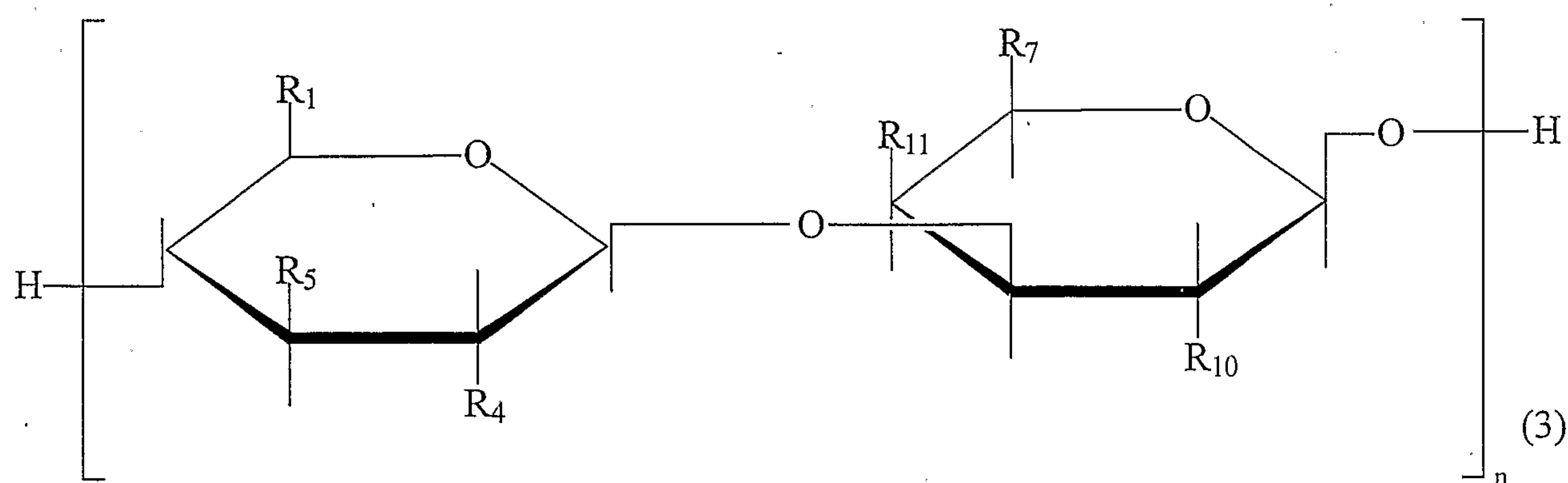
Typically the glycosaminoglycan of general formula (1) is a glycosaminoglycan of general formula (2)



wherein:

- one of R_1 and R_2 is hydrogen and the other is $-\text{CO}_2\text{H}$;
- 5 - R_4 is $-\text{OH}$ or $-\text{OSO}_3\text{H}$;
- R_5 is $-\text{OH}$;
- R_7 is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$;
- R_{10} is $-\text{NH}_2$, $-\text{NHSO}_3\text{H}$ or $-\text{NHAc}$; and
- R_{11} is $-\text{OSO}_3\text{H}$ or $-\text{OH}$.

10 Typically the glycosaminoglycan of general formula (1) is a glycosaminoglycan of general formula (3)



wherein:

- 15 - R_1 is $-\text{CO}_2\text{H}$;
- R_4 is $-\text{OH}$;

- R₅ is -OH;
- R₇ is -CH₂OH or -CH₂OSO₃H;
- R₁₀ is -NHAc; and
- R₁₁ is -OH or -OSO₃H.

5 Any suitable physiologically acceptable glycosaminoglycan salt may be employed in the invention and in particular a metallic salt such, for example a sodium salt, an alkali metal or an alkaline earth metal salt. Other salts include calcium, lithium and zinc salts. Ammonium salts may also be used. The salt may be a sodium glycosaminoglycanate or glycosaminoglycan sulphate. Salts of derivatives
 10 of specific glycosaminoglycans mentioned herein may also be used in the invention. In the present application where mention of a glycosaminoglycan is made, such mention also includes physiologically acceptable salts thereof.

Typically a physiologically acceptable salt is a salt with a physiologically acceptable acid or base. Preferred salts are salts with physiologically acceptable
 15 bases. Typically, such salts are compounds wherein the acidic hydrogen atom of a -CO₂H and/or -OSO₃H group is replaced with a cation, for example an alkali metal (e.g. sodium or potassium) or alkali earth metal (e.g. calcium or magnesium) cation. Such salts can be prepared, for example, by reaction with an appropriate hydroxide.

The number of disaccharide units present in the glycosaminoglycan or salt
 20 thereof employed in the invention will preferably be such that the molecular weight of the glycosaminoglycan or salt is from 8 to 40 kd, preferably from 10 to 30 kd, more preferably from 12 to 20 kd. In particular, it may be such that the glycosaminoglycan has a molecular weight of from 14 to 18 kd, preferably from 15 to 17 kd and more preferably from 16 to 17 kd. The number of disaccharide units
 25 present in the glycosaminoglycan may be represented by the number n, where n is any integer. Typically, n will be an integer of value such that the glycosaminoglycan has a molecular weight falling within any of the above mentioned molecular weight ranges.

Thus the glycosaminoglycan or salt employed may be represented by the
 30 general formula:

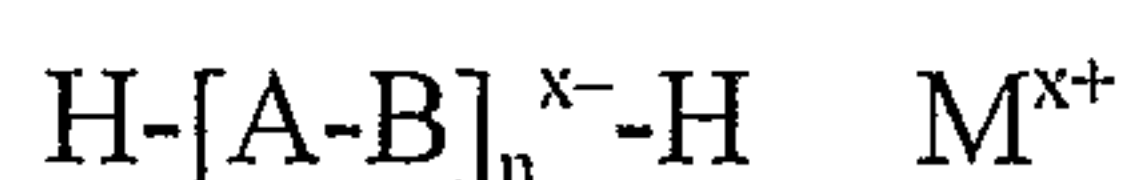


wherein -A-B is any of the disaccharides mentioned above and n is an integer.

Typically n will be an integer of value such that the glycosaminoglycan or salt thereof has a molecular weight falling within the above specified molecular weight ranges. The value of n may be, for example, from 30 to 55 and more preferably from 35 to 50.

The glycosaminoglycan employed in the invention will typically comprise more than one length chain. Hence n for some of the glycosaminoglycan chains present may be a lower or higher integer than an integer which, on its own, would give a chain of molecular weight size falling within one of the above specified ranges. Thus the average value of n of the glycosaminoglycans present in the medicaments of the invention may be any of the values specified for n herein and in particular a value of n which gives a molecular weight glycosaminoglycan or salt falling within one of the molecular weight ranges specified herein. The average value of n for the glycosaminoglycans in the medicaments of the invention may not necessarily be an integer due to a range of size chains being present, but in such cases will still fall within one of the above specified ranges.

Particularly preferred salts for use in the invention are salts of formula:



wherein $x \leq 4n$ and M represents a physiologically acceptable cation or a mixture thereof. Most preferably, $x \leq 2n$.

In a particularly preferred embodiment of the invention the glycosaminoglycan employed will be a heparin, derivative thereof or a physiologically acceptable salt thereof. Heparin is a naturally occurring mucopolysaccharide present in a variety of organs and tissues, particularly liver, lung, and the large arteries. Heparin is a polymer of alternating α -D-glucosamine and hexuronate residues joined by (1,4) glycosidic linkages. When glycosaminoglycans are synthesised in nature, typically they are conjugated to a central protein core. However, preferably the glycosaminoglycans employed in the invention will lack such a central core. Typically, glycosaminoglycan preparations will lack a core and may be employed or, if present, the core can be removed.

Commercially available preparations of glycosaminoglycans will usually lack the core and may be employed.

Heparin is clinically used as an anti-coagulant, where it is thought to exert its effects through interaction with anti-thrombin III (AT-III) and heparin co-factor II and other coagulation factors. Typically the heparin will retain some anticoagulant activity i.e. be able to increase clotting time in an individual. Thus preferably the heparin will be able to bind anti-thrombin III (AT-III) and/or heparin co-factor II (HCII) and hence inhibit clotting. Preferably it will be able to form a complex with AT-III, thrombin and a clotting factor. However, in some embodiments a heparin which lacks anti-coagulant activity or which has reduced anti-coagulant activity may also be employed. Thus the heparin may have been modified so that it has from 0 to 80%, preferably from 5 to 60%, more preferably from 10 to 40% and even more preferably from 10 to 30% of the activity of the unmodified form or in comparison to unmodified heparin. Other glycosaminoglycans, in particular dermatan sulphate, also possess anticoagulant activity. Preferably, therefore, the glycosaminoglycans and their derivatives employed will retain some anti-coagulant activity, as discussed above for heparin and its derivatives.

Subjects

The subject may be healthy or suffering from a disorder. The subject may be suffering from any disorder where delivery to, or via, the respiratory tract is appropriate for the therapeutic agent being administered. In a preferred embodiment the subject to be treated will typically have a respiratory disease or a disorder which affects the respiratory system. In a particularly preferred embodiment of the invention the subject will suffer from, or be at risk of, a respiratory disorder where the viscosity of mucus, or of other pulmonary secretions, is elevated or increased. This increase may typically be by a factor of from one, two, three, four, five or more fold. It may be by more than 20%, more preferably by more than 50%, still more preferably by more than 80%. The increase may be due to the presence of genomic DNA in the respiratory tract, such as that from necrotic inflammatory cells.

In cases where extracellular DNA is responsible for, or contributes to, an

increase in mucus viscosity in the subject, it will typically be of high molecular weight. Thus it may be, for example, that it has an average fragment size, fragment size range or predominate fragment size in the range of from 100 bases to 1 megabase, preferably from 1 kb to 500 kb, more preferably from 5 kb to 250 kb in length, still more preferably from 25 kb to 100 kb and even more preferably from 25 kb to 50 kb in length. The DNA will typically be wholly, or partially free of, histones or will comprise regions which lack histones. It may be bound by cationic factors or factors with cationic groups such as inflammatory mediators and/or proteases. Thus for example it may be complexed or associated with elastase, cathepsins and/or IL-8.

10 The presence of the DNA will typically increase the viscosity of the mucus, or other secretion, that it is present in.

Typically, the presence of DNA may increase the viscosity of the mucus by from 10 to 5000%, preferably from 50 to 2500%, more preferably from 100 to 1000%, still more preferably from 200 to 800% and even more preferably from 400 to 600%. The viscosity of the solution may typically be doubled, tripled, quadrupled or increased by five fold or more in comparison to the viscosity of the solution in the absence of the DNA. The viscosity of the DNA may be such that it may compromise a function in the subject such as, for example, lung function. It may cause airflow limitation and/or promote the occurrence of infections.

20 The increase in mucus viscosity or mucus levels may be due, or exacerbated by, the presence of factors which increase the secretion of mucus or other secretions, such as any of the irritants and pollutants discussed herein, and in particular tobacco smoke.

The respiratory disorder which the subject is suffering from may be, for example, an acute or chronic bronchial pulmonary disease, such as infectious pneumonia, bronchitis or tracheobronchitis, bronchiectasis, cystic fibrosis (CF), asthma, tuberculosis, and/or fungal infection. The subject may have a respiratory tract infection. The subject may have sinusitis, sinus congestion or viral infections which infect, or affect, the respiratory system such as a cold or flu. In a particularly preferred embodiment of the invention the subject will have cystic fibrosis, asthma or chronic airflow limitation disorder (CAL). The subject may have an infection of the

25

30

respiratory tract. The subject may have pneumonia.

The disorder to be treated may involve infiltration of inflammatory cells to the lung and airways. These cells may be macrophages, granulocytes, lymphocytes or other white blood cells. They may be T cells or B cells. The granulocytes may be eosinophils, basophils or neutrophils and in particular will be neutrophils. The number of inflammatory cells present in the lung or airways, or at a particular site in the subject, may be increased by from 1 to 10,000 fold, preferably, from 10 to 5,000 fold, more preferably from 20 to 1,000 fold, still more preferably by from 50 to 500 fold and even more preferably from 100 to 200 fold. The proportion and number of given white cell types may be determined by standard staining techniques well known in the art.

Typically, many of the infiltrating inflammatory cells will be undergoing necrosis and/or lysis to release genomic DNA into the mucus of the individual. The lysis of such cells may also release other substances capable of increasing mucus viscosity. For example, actin, and in particular polymeric actin, may be present. The number and/or proportion of necrotic cells may be elevated typically by from 10 to 5000 fold, more preferably by from 50 to 2000 fold, still more preferably from 100 to 1000 fold and most preferably from 200 to 600 fold. In particular the necrotic infiltrating cells will be granulocytes and especially neutrophils and may be elevated by any of the levels mentioned herein. Their number may be increased by double, increased by ten, 100, 200 or 1000 fold.

The infiltrating inflammatory cells may be the source of genomic DNA responsible for the increase in mucus viscosity. In embodiments where the disorder involves the presence of genomic DNA which causes the increase in mucus viscosity it may be predominately in the form of long strands of viscous genomic DNA. Proteins from the inflammatory cells such as inflammatory mediators and anti-bacterial proteins, such as proteases like neutrophil elastase and/or cathepsins, may also be present in the airways of the subject. As well as genomic DNA other polymers may also be present in the mucus further contributing to its viscosity.

A contributing factor to the migration of inflammatory cells to the lungs in the subject may be the presence of pathogens, irritants, allergens or pollutants in the

lung. For example, and in particular in cystic fibrosis, the subject may have infections such as *Pseudomonas*, *Pneumococcus*, *Staphylococcus*, *Burkholderia*, *Haemophilus* and/or *Aspergillus* infections and in particular may display infection with *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*,
5 and/or *Burkholderia cepacia*. These infections may be longstanding, occur periodically or be new. They may be acute or chronic. In some cases the pathogen will display antibiotic resistance due to prolonged exposure of antibiotics during treatment. In such cases the therapeutic agent will typically be an antibiotic which the pathogen is not resistant to.

10 Subjects may also, or alternatively, have a history of exposure to pollutants such as tobacco smoke or allergens such as pollen and these may contribute to the influx of inflammatory cells into the lung and/or the increase in mucus viscosity and levels. The presence of pathogen, allergens and/or pollutants may also promote the infiltrating cells to undergo necrosis, rather than be disposed of via processes such as
15 apoptosis where genomic DNA is not believed to be released into the lung in substantial amounts and hence increase viscosity.

The subject may have a history of exposure to pollutants and/or chemicals and in particular to those in an inhaleable form. In particular, the subject may be a tobacco smoker or a former tobacco smoker. Typically, the subject may be, or have
20 been, a heavy smoker, smoking from 10 to 100, preferably from 20 to 60, more preferably from 25 to 50 and even more preferably from 30 to 40 cigarettes per day. The subject may have done so for several years such as from 5 to 50, preferably from 10 to 40, more preferably from 15 to 30 and even more preferably from 20 to 25 years. The individual may have been, or be, a pipe or cigar smoker. The individual
25 may have chewed tobacco or products containing tobacco. In some cases the individual may have been passively exposed to tobacco smoke rather than smoke tobacco themselves. Thus the subject may, for example, have been cumulatively exposed to passive tobacco smoke for long periods due to their work, leisure and/or home environments. The subject may use inhaled narcotic such as, for example,
30 marijuana or other narcotics which are typically mixed with tobacco prior to smoking.

The subject may have additionally been, or alternatively be, exposed to other chemical or environmental pollutants. Thus the subject may work, or have worked, in an environment which exposes them to chemicals and/or pollutants. Thus, for example, the subject may be a factory worker or miner such as a coal miner. The subject may work in the construction industry. The subject may have been exposed to high levels of pollution, such as exhaust emissions from vehicles or other engines. The subject may have been exposed to smog or sulphur dioxide containing emissions. The subject may have a genetic predisposition to developing a disorder and in particular a respiratory disorder.

10 The subject may have a condition predisposing them to a disease which affects the respiratory system. For example, the subject may have α_1 -antitrypsin deficiency. The subject may have cystic fibrosis and in particular possess one or two copies of the $\Delta 508$ mutation. The subject may have Chronic Airflow Limitation (CAL) disorder. The subject may have a mutated gene which affects mucus viscosity
15 and/or the levels of mucus secretion or be infected with a pathogen which has such an effect

Although sputum is typically less of a barrier in respiratory disorders not involving increased mucus levels and viscosity and in subjects not suffering from a respiratory disorder, it still has significant barrier function. The subject may therefore
20 be one who is suffering from a respiratory disorder not characterised by an elevation in mucus levels and/or viscosity. The subject may be one suffering from a non-respiratory disorder. Non respiratory disorders that the subject may be suffering from include, in particular, diabetes and obesity.

In some cases, the subject may be a healthy individual. In cases where the
25 subject is a healthy subject, not suffering from a disorder which the agent being administered is designed to treat, the agent may be a therapeutic or non-therapeutic agent. For example, the agent may be a vaccine or other agent designed to raise an immune response in the subject.

In cases where the agent being administered is a non-therapeutic agent, the
30 subject may be a healthy subject or one suffering from a disorder, including any of those mentioned herein.

In one of the preferred embodiments of the invention the subject will have chronic airflow limitation (CAL). In one embodiment of the invention the subject may have chronic airflow limitation (CAL). CAL is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both
5 progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. In particular, although not exclusively, CAL is associated with smoking.

For the purposes of the present invention CAL may be defined as a condition where there is a progressive decline in lung function, with the affected subject having
10 an FEV₁ of less than 80% of that predicted for an individual of that age/race and/or height and/or who displays a FEV₁/FVC ratio of less than 70%. In an especially preferred embodiment, the subject to be treated with CAL will have an FEV₁ of less than 75% of that predicted. Typically the reduction of FEV₁ is only partially reversible. In particular the reduction in FEV₁ is only partially reversible by treatment
15 with bronchodilators such as, for example, β 2 adrenergic agonists and in particular salbutamol.

The FEV₁ for an individual with CAL may be from 10 to 80% of that predicted. Typically, the FEV₁ of the subject will be from 10 to 75% of the predicted value. Preferably the subject may have a FEV₁ of from 60 to 75% the predicted
20 value; more preferably from 40 to 60% of predicted and even more preferably a value below 40% of that predicted. The subject with CAL may have an FEV₁ of less than 70%, preferably less than 60%, more preferably less than 50% and even more preferably less than 40% of that predicted. The FEV₁ value for the subject will normally be measured against predicted values and adjusted for age/sex/race and/or
25 height. Predicted values may be those taken from Coates (*supra*). The expected value, which the value obtained for the subject with CAL may be compared to, may be the average expected value for smokers, or non-smokers, or both groups combined, preferably the expected value will be that for non-smokers not suffering from CAL (Coates, *supra*).

30 The reduction in FEV₁ in the subject with CAL will only be partially reversible and in particularly only be partially reversible on administration of a

bronchodilator. Thus, for example, an increase in FEV₁ over the base-line value for the subject (i.e. that prior to administration of the bronchodilator) of more than 15%, preferably more than 20% and even more preferably over 25% will be regarded as reversibility. The increase may begin from 5 to 30, preferably from 10 to 25, more preferably over a period of from 15 to 20 minutes after the administration of the bronchodilator. Preferably the increases will begin from 15 minutes after the administration of the bronchodilator. The increases persist typically from 3 to 6 hours, preferably from 4 to 5 hours and more preferably 4 hours. Typically the bronchodilator used in assessing reversability will be a β_2 adrenergic agonist such as salbutamol, or ipratropium. In one embodiment of the invention the reduction in FEV₁ may be totally, or almost totally refractory to treatment with bronchodilators.

The subject with CAL may also show similar minimal increases in FEV₁ with steroid drugs such as BecotideTM or PrednisoloneTM although typically response to such agents will not be used to define reversibility. The increases will also occur over a longer time period such as after 2 to 3 days and, if the steroid drugs are continually administered, persist. If steroids are stopped the improvement may persist for from 12 to 48 hours, or for days, weeks or even months, such as from six hours to six weeks, preferably from 1 day to 3 weeks.

Tests to assess reversibility of reduction of FEV₁ will typically be performed when the subject is clinically stable and free from infection. The subject should not have taken, or have had administered to them, inhaled short-acting bronchodilators in the previous six hours, long-acting β agonists in the previous 12 hours or sustained release theophyllines in the preceding 24 hours.

In the diagnosis of CAL spirometric values should typically be measured before and after an adequate dose of inhaled bronchodilator is given to the subject. The dose should preferably be selected to be high on the dose/response curve and usually will be given by nebuliser to be certain it has been inhaled. A similar dose may be given with multiple inhalations from a metered dose inhaler and large volume spacer, but this is less preferred. A typical dosage/measurements protocol for a human subject would be:

- before and 15 minutes after 2.5 to 5mg nebulised salbutamol or 5 to 10

mg terbutaline;

- before and 30 minutes after 500 µg nebulised ipratropium bromide; or
- before and 30 minutes after both in combination.

Equivalent protocols may be used for non-human subjects.

5 The FVC of the subject may also be measured in the diagnosis of CAL. The ratio of FEV₁ to FVC can be used in the diagnosis of CAL. Subjects to be treated who have CAL will typically have an FEV₁/FVC value of less than 70%. The ratio of FEV₁/FVC may be below 65%, preferably below 60%, more preferably below 55% and even more preferably below 55%. In an especially preferred embodiment, a
10 subject will have a FEV₁/FVC of below 70% and also have a FEV₁ value of 80% or less of that predicted.

FVC (forced vital capacity) corresponds to the maximal volume of air forcibly exhaled from the point of maximal inhalation and can be measured using standard spirometry. In particular, the above specified values for FEV₁/FVC will be those
15 after administration of a bronchodilator as outlined above. The reduction in FEV₁/FVC will typically show the same lack of reversibility as FEV₁ in subjects with CAL.

Spirometric assessment is the most preferred method for diagnosing CAL and hence method for identification of subjects who may be treated. Accordingly, in an
20 preferred embodiment of the invention spirometric assessment will be used in the diagnosis of a subject who has CAL and hence is treatable using the invention. In addition, the symptoms displayed by the subject may also be assessed to help confirm a diagnosis of CAL. Typically diagnosis will involve spirometric assessment in combination with assessment of the symptoms of a subject as well as elucidating
25 whether the subject has a history of exposure to risk factors. In some situations spirometric assessment may not be possible, particularly in situations where resources are limited, and CAL will be diagnosed by alternative means such as by looking for the symptoms of CAL listed herein and a history of exposure to risk factors for CAL. Although chest X-rays are not typically indicative of whether or not
30 a subject has CAL, they may be used to diagnose other respiratory disorders, such as TB, and hence rule out CAL.

The subject with CAL will show, or have previously shown, an accelerated rate of decline of lung function compared to the average expected for an equivalent individual not suffering from CAL and in particular for an equivalent non-smoking individual. The subject may display shortness of breath and in particular may do so
5 after physical exertion such as on exercise. Typically, this will not be induced by exposure to an allergen. The subject may also show increased incidence of bacterial or viral infection and this may exacerbate the condition.

The individual with CAL may show a rate of decrease in FEV_1 one, two, three, four or more times greater than the average annual value expected for an
10 equivalent individual not suffering from CAL. For instance a subject over thirty may show an annual reduction of from 50 to 100, preferably from 50 to 80 and more preferably from 60 to 70 ml FEV_1 /yr compared to a reduction of from 10 to 40 and typically of 20 to 40 ml of FEV_1 /yr in the equivalent non-smoker. These values may also apply to non smoking sufferers of CAL such as where the disorder is caused by
15 pollutants.

Subjects with CAL may display one or more, and sometimes all, of cough, increased sputum production, dyspnea, and/or a history of exposure to risk factors for the disease. In the case of cough, increased sputum and dyspnea these may have been present for extended periods of time such as at least a month, preferably six
20 months, more preferably at least a year and still more preferably for at least two years. Chronic cough and sputum production often precede the development of CAL and may be indicative of individuals for which the invention can be used prophylactically to prevent the development of CAL.

Subjects with CAL may have a history of exposure to pollutants, including
25 any of those mentioned herein and at any of the levels specified herein. In particular, the subject with CAL will have a history of exposure to tobacco and/or industrial pollutants.

The subject with CAL will typically be a mature adult. For example, the subject may be from 21 to 85, preferably from 25 to 70, more preferably from 30 to
30 60 and even more preferably from 40 to 50 years of age. The onset of any of, or a particular, symptom mentioned herein in association with CAL, will typically have

been in adulthood. For example, the subject may have been at least 20, more preferably at least 25, still more preferably at least 30 and even more preferably at least 35 years of age before they experienced a particular symptom. In particular, the symptoms associated with more advanced stages of CAL, such as any of those mentioned herein, may have their onset at such later stages of life. Subjects with a genetic predisposition to developing CAL, such as those with α_1 -antitrypsin deficiency, may develop CAL earlier. For example, they may display one or more, or a particular, symptom at from 10 to 21, preferably from 12 to 18, or more preferably from 14 to 16 years of age. Alternatively, they may first show the symptom at any of the age ranges mentioned herein. The subject may have been diagnosed at any of the ages, or within any of the age ranges, specified herein. These ranges may also apply to any disorder which the subject may have and in particular those mentioned herein.

The subject may have a genetic predisposition to developing CAL and may display a family history of the condition. For example, the subject may have α_1 -antitrypsin deficiency and hence be predisposed to developing CAL. Subjects at risk of developing CAL may have had low birth weights and/or a history of exposure to pollutants in the womb or in early life. The mother of the subject may be a smoker and may have continued to smoke during pregnancy. The subject may have had a history of severe childhood respiratory infection. Reduced maximal attained lung function, as measured by spirometry, may identify individuals who are at increased risk of developing CAL.

The subject may have an early stage of CAL in which the symptoms are generally moderate and may have periods of normality or of at least reduced symptoms. Alternatively, the subject may have a more developed stage of CAL in which the symptoms, and particular the reduction in FEV_1 is more pronounced. Preferably, the methods and medicaments of the invention are used, or administered at, an early stage of CAL so that they can arrest, slow or regress the increased rate in FEV_1 decline at as early a stage as possible.

CAL is typically a progressive disorder with the severity of CAL and the extent to which it has an impact on the sufferer increasing over time. Thus there may be a progressive manifestation in the symptoms of the disorder. Chronic cough is

usually the first symptom to develop. It may initially be intermittent, but later may be present every day. The cough will typically be present throughout the day, rather than just at night and in the morning. In some cases, significant airflow limitation may develop without the presence of a cough. Small amounts of tenacious sputum are
5 commonly raised after coughing bouts.

As CAL progresses the subject may experience dyspnea. The onset of dyspnea will often be one of the reasons why a human subject will first consult a physician as it can be debilitating and also induce anxiety. As the lung function of the subject deteriorates further, breathlessness becomes more intrusive. The subject
10 may display wheezing and chest tightness. The dyspnea may become worse during or after exercise. Respiratory infections may also exacerbate the condition and cause increased dyspnea. Human subjects may indicate that the dyspnea progressively impairs their ability to carry out physical labour and to exert themselves.

CAL sufferers are sometimes split into categories which reflect the stage and
15 severity of the disease. This can help define the needs of a particular subject and what course of treatment should be administered. In one classification (GOLD Executive Summary, supra) subjects are split into the following categories:

Category 0 – At risk

20 Lung function, as measured by spirometry, is normal.
Chronic symptoms (cough, sputum, production).
Typically, human subjects will be unaware of abnormal lung function.

Category I: Mild CAL

25 Mild airflow limitation.
 $FEV_1/FVC < 70\%$.
 FEV_1 greater than, or equal to, 80% of that predicted.
With or without chronic symptoms (cough, sputum, production).
Human subjects may be unaware that their lung function is abnormal:

30

Category II: Moderate CAL

Worsening airflow limitation.

FEV₁/FVC < 70%.

FEV₁ from 30 to 80% of that predicted (category can be subdivided into: IIA - FEV₁ from 50 to 80% of that predicted; and IIB - FEV₁ from 30 to 50% of that predicted).

With, or without, chronic symptoms (cough, sputum, production, dyspnea). The
5 symptoms may typically become more pronounced on exertion. Human subjects are likely to be aware of the condition and have sort medical advice.

Category III: Severe CAL

Severe airflow limitation.

10 FEV₁/FVC < 70%

FEV₁ less than 30% predicted or alternatively an FEV₁ less than 50% of that predicted in conjunction with respiratory failure or clinical signs of right heart failure (respiratory failure: arterial partial pressure of oxygen (PaO₂) less than 8.0 kPa (60 mm Hg) with or without arterial partial pressure of CO₂ (PaCO₂) greater than 6.7 kPa
15 (50 mm Hg) while breathing air at sea level).

Subject to be treated using the invention who have CAL may fall within any of the above categories. In particular, the subject may be one in categories I to III, preferably in categories II to III, and even more preferably in category III. The
20 invention also encompasses the treatment of individuals at risk of CAL (category 0) and with an early stage of the disorder (category I).

Some of the symptoms of CAL are displayed by sufferers of other diseases. For example, a number of respiratory disorders other than CAL compromise lung function. However, these diseases may be distinguished from CAL by a full
25 assessment of the subject and preferably by applying the relevant available guidelines for the diagnosis of CAL. The British Thoracic Society have published a set of Guidelines (Thorax 1997, 52 (Suppl. 5): S1-28) and in a preferred embodiment a subject to be treated will fall within the definition of the disorder provided by these Guidelines. In addition, the Global initiative for chronic Obstructive Lung Disease
30 (GOLD) has published an Executive Summary (2001) outlining a strategy for the diagnosis, management and prevention of the disorder. In a preferred embodiment

the subjects to be treated who have CAL will fall within the definition of the disorder provided by the Executive Summary.

CAL does not include cystic fibrosis (CF), although the invention can be used to treat both CAL and CF. Typically a subject with CAL will have a least one functional copy of the CFTR gene (unless they have been unfortunate enough to develop both CF and CAL). CAL typically has a much later onset than Cystic Fibrosis, where a sufferer is born with the defect. In CAL the decline in lung function and the onset of symptoms will occur progressively over time. In CF the decline in lung function will have a more immediate onset, earlier on in life. A subject who has CAL will often be in their thirties, forties or even fifties before they become aware that they are suffering from the disorder. The major exception to the later onset of CAL, is in those sufferers who have α_1 anti-trypsin deficiency. Such subjects will display an earlier onset of CAL, such as in their teens or twenties when CAL is diagnosed, as they will be born with a genetic condition predisposing them to CAL. Subjects who have α_1 antitrypsin deficiency and CF can readily be distinguished from each other by biochemical and genetic tests to identify the nature of the disorder.

The subject will be a vertebrate animal and preferably will be a mammal. Typically the subject will be human. However, the invention also encompasses the treatment of animals with conditions which are the same as, or equivalent to, the human conditions mentioned herein. The animal condition may have either an underlying pathology similar to, or the same as, that of the human condition. The animal may have one or more similar symptoms or characteristics of the human condition and in particular one or more of those listed above. Preferably, the animal will suffer from a condition which falls within the definition of any of these conditions listed above. The animal condition may, for example, be any condition which alters mucus viscosity and/or levels in the animal. In some case, the animal may be a healthy animal not suffering from a disorder, and in particular where a non-therapeutic agent is being administered.

In cases where the subject is not human it may be a domestic animal or an agriculturally important animal. The animal may, for example, be a sheep, pig, cow,

bull, poultry bird or other commercially farmed animal. In particular the animal may be a cow or bull and preferably is a dairy cow. The animal may be a domestic pet such as a dog, cat, bird, or rodent. In a preferred embodiment the animal may be a cat or other feline animal. The animal may be a monkey such as a non-human primate. 5 For example, the primate may be a chimpanzee, gorilla, or orangutan. In a preferred embodiment of the invention the animal may be a horse and, for example, may be a racehorse. The animal may be a sports animal such as, for example, a greyhound.

Subject Assessment

10 The present invention provides a way to improve the delivery of inhaled, nasally administered or installed agent and in particular a therapeutic agent delivered via such a route. Thus by using a glycosaminoglycan the same amount of agent may be administered to the subject, but a higher concentration of it can be achieved at the target site. This means that less agent needs to be administered and/or a higher 15 concentration of the agent can be achieved at the target site for a given amount of agent administered.

Subject assessment may be used to determine optimal ratios of glycosaminoglycan to agent and the increase in bioavailability. It may also be used to monitor the improvement of the condition of the subject. It may be used to tailor the 20 particular therapeutic or non-therapeutic regimen to the particular needs of the subject. It may be possible to do this so that at times of particular severity of a disorder, or particular circumstances, that the treatment regimen can be adjusted appropriately. Subject assessment can be used to confirm the factor by which a particular glycosaminoglycan increases delivery of the agent.

25 The assessment made will be suitable for the disorder and/or the agent being administered. Ways of characterising particular disorders will be well known in the art and any suitable means for a particular disorder may be used. In the case where medicaments are to be administered to a healthy subject the particular parameter which the agent is designed to modulate may be assessed rather than the treatment of 30 a specific condition. A number of parameters may be useful for several different disorders, such as spirometry to measure FEV_1 , FVC, and/or FVC/FEV_1 in the

subject and hence assess airflow limitation. In particular spirometry may be used to assess FEV_1 . Any effect of the agent on the subject and/or the disease state may be measured to confirm, and preferably quantify, the increased delivery of the drug. In particular, one of the symptoms of the disorder may be measured/assessed. In cases
5 where the agent administered is a non-therapeutic agent any effect of that agent may be assessed and in particular the effect which it is desired to achieve as the purpose of the administration.

The medicaments and methods of the invention preferably induce an improvement in the condition of the subject where the agent administered is a
10 therapeutic agent. The medicaments and methods may therefore be used to manage a patient suffering from, or prone to, any of the disorders mentioned herein. They may prevent, ameliorate, improve or cure the condition. They may prevent, reduce or reverse one or more of the symptoms and manifestations associated with the disorder. They will preferably also increase the feeling of well being in the subject and their
15 quality of life. The effect of a medicament will depend on the specific therapeutic agent, but a number of examples may be provided. A medicament or method of the invention may, for example, reduce or eliminate one or more of:

- an elevated decline in forced expiratory volume (FEV_1)
- mucus hypersecretion;
- 20 - inflammation; and/or
- damage to the structure of the lung.

The ratio of FEV_1/FVC may also be improved, or any deterioration slowed or halted.

The medicaments and methods may reduce mucus plugging. They may have a
25 mucolytic activity and/or reduce secretion and exorption of mucus. They may reduce the breakdown of the structure of the lung, such as the degradation of elastin in the airways and in the aveoulus and hence the loss of lung elasticity. They may reduce or prevent the collapse of portions of the lung and/or the development of enlarged airspaces in which air can become trapped.

30 In some embodiments of the invention, and in particular those where the agent is administered to a respiratory disorder such as chronic airflow limitation

(CAL) the medicaments and methods may typically reduce the decline in FEV₁ by from 10 to 100%, preferably from 20 to 80%, more preferably from 30 to 60% and even more preferably from 40 to 50%. They may reduce the annual decline in FEV₁ by from 10 to 100 ml, preferably from 20 to 60 ml and even more preferably from 30 to 40ml per year. In some cases on treatment the subject will display an improvement of FEV₁ so that FEV₁ is from 25 to 100%, preferably from 40 to 100%, more preferably from 60 to 100% and even more preferably from 80 to 100% of the predicted value.

The methods and medicaments of the invention may reduce inflammation and the influx of inflammatory cells to the airways and lungs. They may also ameliorate the effects of the inflammatory cells present in the lung. Thus differential cell counts on sputum and/or lavages from the subject may display normal levels, or less elevated levels, of inflammatory cells such as lymphocytes, macrophages and/or neutrophils and in particular of neutrophils.

They may reduce migration of inflammatory cells such as neutrophils to the airways and lung. They may also reduce one or more of neutrophil functions/characteristics such as priming, activation, chemotaxis, extravasation, degranulation, respiratory burst, phagocytosis, apoptosis and/or necrosis. This may also mean that there are reduced amount of neutrophil proteins in the lung and reactive oxygen species. There may also be reduced levels of inflammatory mediators in subjects following treatment and in particular IL-8 levels. They may also cause changes in the lung such as the down regulation of adhesion molecules so that neutrophils cannot enter the lung.

The subject may also display decreased inflammation following treatment. There may be decreased levels of inflammatory mediators present in the airways of the subject. These may include proteases such as matrix metallo proteases (MMPs), cathepsins and elastase. They may also display decreased myelo-peroxidase, activated complement, MCP-1, interferons (such as, in particular, IFN- γ) and interleukins such as, in particular, IL-12. Typically, IL-8 and in particular decreased IL-8 levels will be present.

In cases where the agent administered is an enzyme inhibitor, enzyme

activity, compared to that in the absence of administration of an agent of the invention, may drop by 10%, preferably 20%, and more preferably 50%. It may be reduced at least two fold, preferable four fold and still more preferably at least ten fold. Conversely, where the agent is an enzyme, increased levels of enzyme, and
5 hence enzyme activity, may be seen at the target site. For example, the increase may be two fold, three fold, five fold, ten fold or more. The increase may be from 10 to 100%, preferably from 20 to 80% and more preferably from 40 to 60%.

The lungs of the subject, or regions of them, may have decreased numbers of inflammatory cells following treatment. In particular, there may be a reduction in the
10 number of neutrophils and/or CD8⁺ T cells moving into the airways and lung. There may also be changes in the bronchial and pulmonary vasculature, such as downregulation of adhesion molecules involved in leukocyte extravasation or decreased expression of inflammatory cytokines which can recruit inflammatory cells such as neutrophils, monocytes and T cells.

15 In embodiments where the therapeutic agent is a mucolytic, mucus viscosity, may be determined. Again, preferably the situation is compared prior to and post treatment. Any of the techniques discussed herein for measuring viscosity may be used such as, for example, techniques like compaction assays and assays using contortion pendulums or any other suitable rheological methods. Examples of
20 sputum compaction assays which can be employed are described in WO94/10567 and may be employed. Examples of an assay using a torsion pendulum are described in Janmey, J. Biochem., Biophys, Methods 22:41-53, 1991, and these may also be employed to assess viscosity.

The administration of the medicaments may typically produce an
25 improvement in the status of the subject. For example, they may result in a reduction of airway obstruction, mucus plugging, foaming and/or bubbling. The subject may show an increase in FEV₁ returning it to a value closer to that predicted for an equivalent subject not suffering from respiratory disorder. This may be accompanied by the subject experiencing increased ease in breathing, promoted expiration of
30 mucus and reduced coughing. The subject may show increased ability to perform exercise and to physically exert themselves.

In embodiments of the invention where the medicament is an antipathogenic agent administration may decrease the incidence of respiratory infections. Thus the subject may show a decrease in infections with pathogens such as, for example, *Pseudomonas*, *Pneumococcus*, *Staphylococcus*, *Burkholderia*, *Haemophilus* and/or *Aspergillus*. In particular, the patient may show reduced incidence of infection with *Haemophilus influenza*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and/or *Burkholderia cepacia*. Subjects where such infections were chronic may no longer be infected or have decreased load of pathogen following treatment. Pathogenic load and the nature of pathogens can be assessed using various microbiological techniques well known in the art such as staining, culturing and plating. Viral load may be decreased. Pathogenic load may be decreased by at least 10%, preferably at least 25%, more preferably by at least 50% and even more preferably by at least 75%. In some cases the agent may decrease the production of pathogenic toxins such as, for example, of endotoxin and using the medicaments of the invention a bigger decrease may be seen.

Where the agent is a gene the efficiency of delivery and expression may be assessed by well known molecular biology techniques. The proportion of cells expressing the gene may be measured and/or the proportion comprising a specific sequence. Techniques such as northern and Southern blotting may be used to detect specific sequences and their expression, as may RT-PCR and PCR. *In situ* PCR may be used. Sequences, such as actin, may be measured/detected as controls. Western blotting and antibody staining techniques may be used to detect therapeutic proteins and in particular such proteins expressed from administered nucleic acids.

In cases where the agent is insulin and the subject is diabetic, blood glucose levels may return to normal, or at least closer to normal, following administration. The subject may show a reduction or elimination of symptoms associated with hyperglycaemia or hypoglycaemia. In cases where the agent is a smoking cessator or appetite suppressant the subject will preferably show decreased desire for cigarettes and food respectively. In the cases of appetite suppressants the subject may display weight loss and decreased obesity. The subject may show increase ability for physical activity. In cases where the agent is a painkiller the subject will show a

reduction in the amount of pain felt or in the duration that pain is experienced for.

The medicaments and methods of the invention when used to administer a therapeutic agent will typically prevent or treat the disorder in question. They may ameliorate its severity and/or eliminate or reduce symptoms associated with the disorder. In particular, the therapeutic agent will have such an effect.

The medicaments may increase life expectancy such as by, for example, 1 to 2 years, preferably from 2 to 4 years and even more preferably by more than 4 years. Due to the increase in the efficiency of the delivery of the therapeutic agent the methods of the invention may mean that individuals or conditions who/which showed no improvement, or only minimal improvement, with the therapeutic agent on its own, may show improvement, or elevated improvement, when the methods of the invention are employed to increase delivery.

The effect of the therapeutic agent will depend on the nature of the particular therapeutic agent and the assessment protocol will typically be based on what the effect of the agent is known to be. Similarly, where the agent is non-therapeutic an appropriate assessment based on the effects of the agent will be employed.

In some embodiments, labeled molecules may be administered to help assess the efficacy of the methods of the invention. For example, fluorescently or radioactively labeled molecules may be employed. In some cases, the labeled molecule may not be a therapeutic agent. In others, a labeled version of the therapeutic agent may be employed. Such methods may be used to assess delivery in both *in vitro* model systems and *in vivo* and may be employed to decide on the amount and/or ratio of therapeutic agent and glycosaminoglycan. They may also be used in imaging.

Compositions

The present invention also provides a composition comprising a glycosaminoglycan, or a salt thereof and an agent. In particular, the agent will be a therapeutic agent. In such cases the composition will typically be a pharmaceutical composition. These may typically be in a solid or liquid form. It may be in the form of a powder. It may be lyophilised or frozen. It may also comprise other components

such as water or a buffer. The composition may take the form of an aqueous solution comprising both the agent and the glycosaminoglycan or salt. The composition may comprise an aqueous suspension with either, or both, the glycosaminoglycan or salt and the agent being present in, or as, the particles of the suspension. In compositions
5 where both a glycosaminoglycan or salt and an agent are present, they may be present as separate particles or in the same particles.

The medicaments of the invention may take a variety of forms. They may be in the form of powders, powder microspheres, solutions, suspensions, gels, nano-particle suspensions, liposomes, emulsions or microemulsions. The compositions of
10 the invention may comprise a glycosaminoglycan or salt and/or an agent dissolved or suspended in liquids other than water, for example, they may be dissolved or suspended in solvents such as CFC or HFA.

The composition may also comprise stabilising agents or preservatives. It may comprise agents which allow the product to be frozen without losing enzyme activity
15 such as glycerol. The composition may be formed, for example, by mixing powders of glycosaminoglycan and the therapeutic agent. The therapeutic agent and the glycosaminoglycan or salt may be any of those mentioned herein.

Administration and Pharmaceutical compositions

20 The glycosaminoglycan or physiologically acceptable salt may be administered simultaneously, separately or sequentially to the therapeutic agent. Thus the two may be administered in the same medicament or as two separate medicaments and may be given at the same time, one after the other or separately. In
embodiments where the two are to be administered separately, preferably the
25 glycosaminoglycan, or salt will be administered prior to, or at the same time, as the therapeutic agent.

The glycosaminoglycan and therapeutic agent may typically be administered from one week to one minute apart, preferably from two days to one hour apart, even more preferably from 24 hours to 2 hours apart and still more preferably from 12 to 4
30 hours apart. The two may be administered from one minute to one hour apart, preferably from 10 minutes to 30 minutes apart and even more preferably from 15 to

25 minutes apart. The two may be administered, for example, from one minute to fifteen minutes apart or at a shorter interval such as from 10 seconds to one minute and preferably from 30 seconds to one minute apart. The two may be administered in sequence, one immediately after the other.

5 The two will preferably be administered by inhalation, installation and/or intranasally preferably via the same route. In an especially preferred embodiment of the invention both will be administered via inhalation. The two may be administered together or separately. Preferably the two will be administered by the same route either as separate medicaments or in the same medicament, typically they will be in
10 separate medicaments. The glycosaminoglycan or salt and the therapeutic agent may be provided in two separate compositions, but may, for example, be mixed prior to addition to a delivery device or in the delivery device. The delivery device may have means to regulate the amount of each delivered to the subject. It may have means to mix the two or deliver each separately.

15 In some embodiments of the invention one or both of the glycosaminoglycan and agent may be administered via installation. Typically, installation will involve the insertion of an artificial airway through which a composition of the invention is installed. In such cases, typically the composition will be a liquid composition. Administration will typically involve drawing up the composition into a syringe or
20 similar device and then expelling the medicament through the artificial airway into the pulmonary tract of the subject. Usually, installation will be used in an emergency situation and/or where the subject is unconscious. Typically volumes such as from 1 to 20 ml, preferably from 2 to 10 ml, and even more preferably from 3 to 6 ml will be installed. Any method for delivery into the pulmonary tract may be used to deliver
25 the medicaments to the invention.

 The medicaments and compositions of the present invention may be prepared by formulating the active agents, i.e the glycosaminoglycan or salt and/or the therapeutic agent, with a standard pharmaceutically acceptable carrier and/or excipient as is routine in the pharmaceutical art. The exact nature of the formulation
30 will depend upon several factors including the particular glycosaminoglycan, salt or therapeutic agent employed and the desired route of administration. Suitable types of

formulation are fully described in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Eastern Pennsylvania, USA.

The necessary dose to be administered will normally be determined by a physician. The dose of glycosaminoglycan administered may, for example, 0.01mg to 5g, preferably from 0.1 mg to 2.5g, more preferably from 1 mg to 1g, even more preferably from 10 mg to 500mg, still more preferably from 50 mg to 250 mg and even more preferably from 100 mg to 200 mg. These doses will typically be given once, twice or three times a day and will preferably be given once or twice a day and more preferably will be given twice a day. For a heparin, derivative thereof or salt the dose will typically be in the range of from 10 to 10,000 units per/kg body weight, preferably 100 to 10,000 units per/kg body weight, more preferably from 200 to 5000 units/kg body weight, even more preferably from 500 to 2000 units per/kg, and still more preferably from 1,000 to 1,500 units per/kg.

Typically the dose of the therapeutic agent will either be the normal amount of the therapeutic agent which is typically administered in the absence of glycosaminoglycan if it is desired to have an overall higher amount of agent delivered to the target or will be less than the normal amount. Thus it may typically be one half, quarter, fifth, tenth or less of the amount which would have been administered in the absence of glycosaminoglycan. It may, for example, be less than 60%, preferably less than 30%, more preferably less than 15% and even more preferably less than 5% than the amount which would have been given in the absence of glycosaminoglycan. The amount of agent may, for example, be the same as glycosaminoglycan. The number of times a day, week or month that the therapeutic agent is administered in comparison to without glycosaminoglycan may be also be reduced such as by a half, a quarter or more.

A therapeutically effective amount of the therapeutic agent will be administered. Thus taking into account the presence of the glycosaminoglycan the amount of therapeutic agent will be therapeutically effective. The dose of the therapeutic agent may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be

treated; the route of administration; and the required regimen. A physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg of body weight, according to the activity of the specific therapeutic agent, the age, weight and
5 conditions of the subject to be treated, the type of disorder. Preferably, daily dosage levels are from 5 mg to 2 g. However, dosages will depend on the particular therapeutic agent.

The length of treatment may typically be from a day, one week, two weeks, a month, six months a year or more. In many cases the subject will remain on the
10 medicaments of the invention permanently or for extended periods. This may in particular be the case where the subject has a genetic defect, such as where the subject has cystic fibrosis or α_1 -antitrypsin inhibitor deficiency, and hence permanently has the condition. This may also be the case where one of the causative factors in the disorder to be treated is something that the subject is continuously
15 exposed to and cannot avoid, or does not wish to minimise exposure to. The subject may be a tobacco smoker, but not give up smoking tobacco. In cases where the therapeutic agent is insulin or a contraceptive it may also may be administered for extended periods.

In cases where the respiratory disorder is more short lived, or occurs
20 periodically, the length of administration may be shorter and may be linked to the duration of the disorder. Thus, for example, where the condition is a cold or the flu, once it has been resolved administration of the medicament may be ceased or reduced. The administration schedule may be coordinated so that higher levels of the medicaments are administered, or initiation of administration begins, at times when
25 the disorder has increased severity. Thus in respiratory disorders such as, for example, cystic fibrosis or pneumonia this may be when the subject has an increase in airway obstruction, infection and/or inflammation. The medicament may also be given prior to exercise or other physical exertion.

Preferably the medicaments of the invention may be administered via
30 inhalation and hence will be given by the nose or mouth. Inhaled delivery includes intra-nasal delivery. They also may be administered via installation, such as through

or artificial airway. Suitable methods for formulating and preparing medicaments to be administered via inhalation, installation and intranasally are well known in the art and may be employed in the present invention. For inhalation, intranasal or installation therapy the medicament may be in a solution useful for administration by nebuliser or other liquid aerosol device, metered dose inhalers, or in a form suitable for a dry powder inhaler. The medicament may be present in a blister pack or breakable capsule. The devices suitable for administering the medicaments of the invention include In some cases, for example where the subject is a child, a spacer may be used in conjunction with the inhaler to help ensure effective delivery.

10 In some preferred embodiments, the medicaments of the present invention may be formulated as aerosols. The formulation of pharmaceutical aerosols is routine to those skilled in the art, see for example, Sciarra, J. in Remington's Pharmaceutical Sciences (*supra*). The agents may be formulated as solution aerosols, dispersion or suspension aerosols of dry powders, emulsions or semisolid preparations. The aerosol may be delivered using any propellant system known to those skilled in the art. The aerosols may be applied to the upper respiratory tract, for example by nasal inhalation, or to the lower respiratory tract or to both. The glycosaminoglycan, salt or therapeutic agent may delivered using liposomes and nano-particle delivery methods. Liposomes, particularly cationic liposomes, may be used in carrier formulations.

15 20 Where the therapeutic agent is intended for gene therapy purposes it may, for example, take the form of a virus, liposome or other suitable vector.

In cases where the glycosaminoglycan and/or therapeutic agent are administered in the form of particles or droplets, the particle/droplet size and/or other properties of the particle/droplet may be chosen to ensure that the particles are delivered to a particular region of the respiratory tract. For example, they may be designed to reach only the upper or lower parts of the respiratory tract. In cases where the glycosaminoglycan, salt or therapeutic agent are delivered in an aqueous form preferably the solution will be isotonic to help ensure effective delivery to the subject.

30 In many embodiments, the particle size of the medicament may be chosen on the basis of the desired part of the respiratory tract which it is desired to administer

the medicament to. In particular particles with a diameter of 10 μM are thought to be effective in reaching the lower parts of the respiratory tract and hence may be employed where such a site is the desired target for the medicaments. In embodiments, where it is desired to deliver the medicament to the lower parts of the respiratory tract, such as alveoli for example, the diameter of the particles administered may be less than 10 μM , preferably less than 8 μM , more preferably less than 6 μM and even more preferably less than 4 μM . In a preferred embodiment the particles may have a diameter of 3 μM or less and more preferably may have a diameter of 2 μM or less. In an especially preferred embodiment the particles will have a diameter of from 3 to 5 μM . In some cases the particles administered may be less than 1000 nm, preferably less than 500 nm, more preferably less than 250 nm and still more preferably less than 100 nm in diameter. The sizes may refer to particles of solid matter or droplets of solutions and suspensions.

The size of particles necessary to penetrate to a specific part of the respiratory tract will be known in the art and hence the particle size can be chosen to suit the target size. Techniques such as milling may be used to produce the very small particles necessary. In some cases the desired part of the respiratory tract may be the upper respiratory tract and hence larger particles sizes may be employed. The density of the particles and their shape may also be chosen to facilitate their delivery to the desired site.

In cases where the medicament is to be administered via the nose it may be, for example, in the form of nasal spray. The spray may be administered, for example, using an atomizer or nebulizer. In some cases the medicament may be in the form of nasal drop. These may be administered using a via a medicine dropper. For further discussion on nasal dosage forms see Remington's Pharmaceutical Sciences (*Supra*). Medicaments administered via the nose may include pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, gelling agents, or buffering agents. Most preferably the nasal dosage form will be isotonic with nasal secretions.

Medicaments for use in accordance with the present invention, may include, in addition to the active ingredients, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. In

particular they may include a pharmaceutically acceptable excipient. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration. Suitable pharmaceutical carriers are described in

5 Remington's Pharmaceutical Sciences (*supra*).

The medicaments may include various constituents to optimise their suitability for the particular delivery route chosen. The viscosity of the medicaments may be maintained at a desired level using a pharmaceutically acceptable thickening agent. Thickening agents that can be used include methyl cellulose, xanthan gum,
10 carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof. The concentration of the thickening agent will depend upon the agent selected and the viscosity desired.

In some embodiments, and in particular where intranasal delivery is to be used, the medicaments may comprise a humectant. This may help reduce or prevent
15 drying of the mucus membrane and to prevent irritation of the membranes. Suitable humectants include sorbitol, mineral oil, vegetable oil and glycerol; soothing agents; membrane conditioners; sweeteners; and combinations thereof.

The medicaments may comprise a surfactant. Suitable surfactants include non-ionic, anionic and cationic surfactants. Examples of surfactants that may be used
20 include, for example, polyoxyethylene derivatives of fatty acid partial esters of sorbitol anhydrides, such as for example, Tween 80, Polyoxyl 40 Stearate, Polyoxyethylene 50 Stearate, fusieates, bile salts and Octoxynol.

The medicaments of the present invention may be delivered by any device adapted to introduce one or more therapeutic composition(s) into the upper and/or
25 lower respiratory tract. In some preferred embodiments, the devices of the present invention may be metered-dose inhalers. The devices may be adapted to deliver the therapeutic compositions of the invention in the form of a finely dispersed mist of liquid, foam or powder. The device may use a piezoelectric effect or ultrasonic vibration to dislodge powder attached on a surface such as a tape in order to generate
30 mist suitable for inhalation. The devices may use any propellant system known to those in the art including, but not limited to, pumps, liquefied-gas, compressed gas

*Trade-mark

and the like.

Devices of the present invention typically comprise a container with one or more valves through which the flow of the therapeutic composition travels and an actuator for controlling the flow. Suitable devices for use in the present invention
5 may be seen in, for example, in Remington's Pharmaceutical Sciences (*supra*). The devices suitable for administering the medicaments of the invention include inhalers and nebulisers or other liquid aerosol devices such as those typically used to deliver steroids to asthmatics.

Various designs of inhalers are available commercially and may be employed
10 to deliver the medicaments of the invention. These include the Accuhaler*, Aerohaler*, Aerolizer*, Airmax*, Autohaler*, Clickhaler*, Diskhaler*, Easi-breathe* inhaler, Fisonair, Integra*, Jet inhaler, Miat-haler*, Novolizer inhaler, Pulvinal inhaler, Rotahaler*, Spacehaler, Spinhaler*, Synchroner inhaler and Turbohaler devices. A number of formulation techniques which produce particularly desirable particles are known in
15 the art and may be employed. For example the nanocrystal, pulmosol and pulmosphere technologies may be employed.

The present invention also provides a pharmaceutical composition comprising:

- a glycosaminoglycan, or a physiologically acceptable salt thereof;
- a therapeutic agent; and
- 20 - a pharmaceutically acceptable excipient, carrier or diluent.

The excipient may be any of those mention herein, or any of the carriers. The composition may take the form of any of the medicaments discussed herein or any of the characteristics or features specified herein for them. The therapeutic agent and glycosaminoglycan or salt may be any of those discussed herein.

25 All methods of formulation, administration, delivery *etc* described in this section in relation to embodiments where the agent is a therapeutic agent are typically equally applicable to embodiments where the medicament is a non-therapeutic agent. Indeed given that the invention can equally be used to deliver therapeutic and non-therapeutic agents parameters described in respect to one herein
30 will typically be equally applicable to the other and *vice versa*.

***Trade-mark**

Kits

The present invention provides a kit comprising:

- a glycosaminoglycan, or a physiologically acceptable salt thereof;
- 5 - instructions for the administration of the glycosaminoglycan or salt to a subject by inhalation, intranasally and/or via installation, where the subject is being treated with a therapeutic agent which is also being administered by inhalation, intranasally and/or via installation; and
- packaging.

10 The invention also provides a kit comprising:

- a therapeutic agent;
- instructions for the administration of the therapeutic agent to a subject by inhalation, intranasally and/or via installation where the subject is also being treated with a glycosaminoglycan, or a physiologically acceptable salt thereof which is being
- 15 administered by inhalation, intranasally and/or via installation; and
- packaging.

The present invention also provides a kit comprising:

- a glycosaminoglycan, or a physiologically acceptable salt thereof;
- instructions for the administration of the glycosaminoglycan or salt to a
- 20 subject by inhalation, intranasally and/or via installation where the subject is being treated with a therapeutic agent which is being administered by inhalation, intranasally and/or via installation and the two are administered sequentially, simultaneously or separately; and
- packaging.

25 The present invention also provides a kit comprising:

- a therapeutic agent;
- instructions for the administration of the therapeutic agent to a subject by inhalation, intranasally and/or via installation where the subject is being treated with a glycosaminoglycan, or a physiologically acceptable salt thereof which is being
- 30 administered by inhalation, intranasally and/or via installation and the two are administered sequentially, simultaneously or separately; and

- packaging.

In an especially preferred embodiment the subject will be suffering from a respiratory disorder. Alternatively, the subject may be suffering from a non-respiratory disorder or may be a healthy subject.

5 The invention also includes kits where the agent in the kit, or which the subject is having administered to them, is a non-therapeutic agent.

The instructions of the kits may take the form of a label on the packing. The kit may additionally comprise means for administering the glycosaminoglycan or salt and/or the agent such as any of those discussed herein. The agent, subject, method of
10 administration and any of the other parameters of the kit may be as described elsewhere herein.

The following Examples illustrate the invention.

Example 1: Barrier Assay on Victor Fluorometer

15 CAL sputum samples were thawed at room temperature and homogenised using a syringe (no needle). Samples were split and portions (0.12-0.20 g) weighed in small round-bottomed tubes. Tubes were centrifuged in a microfuge to sediment sputum. Mucolytic was added to sputum on a 10% volume to weight basis (e.g. 13 μ l/0.13 g sputum). The mucolytic was therefore diluted by a factor of 10 upon
20 addition to sputum. The mucolytics assessed were DNase (at a concentration of 2.9 μ g/ml of a stock of activity 2500 kunits per mg) and unfractionated heparin (at a concentration of 10 mg/ml of a stock of 160 USP/mg – a sodium heparin salt from porine intestinal mucosa from Calbiochem*, Catalogue number 375095, was used). A control portion of sputum was treated with PBS on a 10% volume to weight basis. A
25 further control with the non-glycosaminoglycan dextran sulphate was also performed (at a concentration of 10 mg/ml – a sodium dextran salt from sigma, Catalogue number D-7307, was used). The mucolytic was thoroughly mixed with the sputum by vortexing and passing up and down a 19-gauge needle.

The lower wells of a Transwell chamber contained 25 μ l of phosphate
30 buffered saline and this was separated from the upper wells by an 8 μ m pore-size polycarbonate filter (placed shiny side down). The upper section of the chamber was

*Trade-mark

placed on top of the filter and screwed down tightly. Carboxylate-modified fluorescent beads (F-8811, Molecular Probes) were sonicated for 5 mins to disperse them and beads added to sputum samples in a 1:1 ratio, e.g. 150µl beads added to 0.15g sputum sample. The mixture was vortexed to mix and 20µl of each sample
5 added to the upper wells of the chamber. Each sample was added to the chamber in quadruplicate. The chamber was centrifuged for 5 mins at 1000 rpm to remove air bubbles, then placed inside a black plastic bag and incubated in a humid chamber at 37°C, 900rpm, for 4 hrs.

Following incubation, the chamber was centrifuged for 5 mins at 1000rpm to
10 force migrated beads to the bottom of the lower wells. The upper chamber and gasket were removed in one piece to minimise leakage. The contents of the lower wells (25µl) were transferred to a Fluoro-NUNC plate and 175µl PBS added per well. The plate was read using Victor fluorometer with the 'Fluospheres' protocol (excitation 485nm, emission 535nm).

15 The results obtained are shown in Figure 1. The mucolytic effects of heparin were greater and less variable than those of DNase. A Bonferri multiple comparisons test showed that the effect of 10 mg/ml heparin was significantly greater than that of the PBS control ($P < 0.01$). However, the effects of DNase and 10 mg/ml dextran sulphate (negative control) were not significantly different when compared to the
20 PBS control ($P > 0.05$).

The effect of chondroitin sulphate on microsphere transport in CF sputum was also assessed using the same conditions as those described above. A 70:30 mixture of chondroitins A and C was tested at concentrations of 0, 0.1, 1 and 10 mg/ml (a sodium salt of chondroitin sulphate A and C from bovine trachea obtained
25 from sigma, Catalogue number C-3788 was used). A control containing sputum treated with PBS alone was again run. The results obtained are shown in the bottom panel of Figure 1. Chondroitin sulphate was found to significantly increase transport of the microspheres.

30 **Example 2: Atomic Force Microscopy (AFM) imaging of DNA**

Calf thymus DNA (0.1 mg/ml) was treated with heparin at various

concentrations (0.1, 1, 10 and 100 $\mu\text{g/ml}$). Solutions were prepared in 0.2 μm filtered, DNase and RNase free water. 10 μl of sample was added to freshly cleaved ruby muscovite mica and dried prior to AFM imaging. AFM was performed in air under ambient conditions using a TopoMetrix TMX2000 Scanning Probe

5 Microscope (ThermoMicroscopes, Bicester, UK) with a 70'70'12 mm tripod piezoelectric scanner. Topography measurements, in contact mode, were made using a "V"-shaped silicon nitride cantilever (length 200 nm, nominal spring constant (K) 0.21 Nm^{-1} ; Part. No. 1530-00, ThermoMicroscopes, Santa Clara, California, USA) bearing an integrated standard profile tip.

10 The results are shown in Figures 2 and Figure 3. In Figure 2, Panel A = 0.1 mg/ml DNA and Panel B = 0.1 mg/ml DNA + 0.1 mg/ml heparin. The addition of heparin to DNA was shown to alter the structure of the DNA network, increasing the average pore size from 180 (SD 30) μm to 780 (SD 150) μm .

Figure 3 shows results with lower concentrations of heparin. Panel A shows
15 the results for untreated DNA: Panel B for DNA treated with 0.1 $\mu\text{g/ml}$ heparin; Panel C for DNA treated with 1 $\mu\text{g/ml}$ heparin; and Panel D for DNA treated with 10 $\mu\text{g/ml}$ heparin.

CLAIMS

1. Use of a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof in the manufacture of a medicament for treating a mammalian subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, wherein:

- the subject is being treated with a therapeutic agent;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent to the mammalian subject.

2. Use of a therapeutic agent in the manufacture of a medicament for treating a mammalian subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, wherein:

- the subject is also being treated with a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;

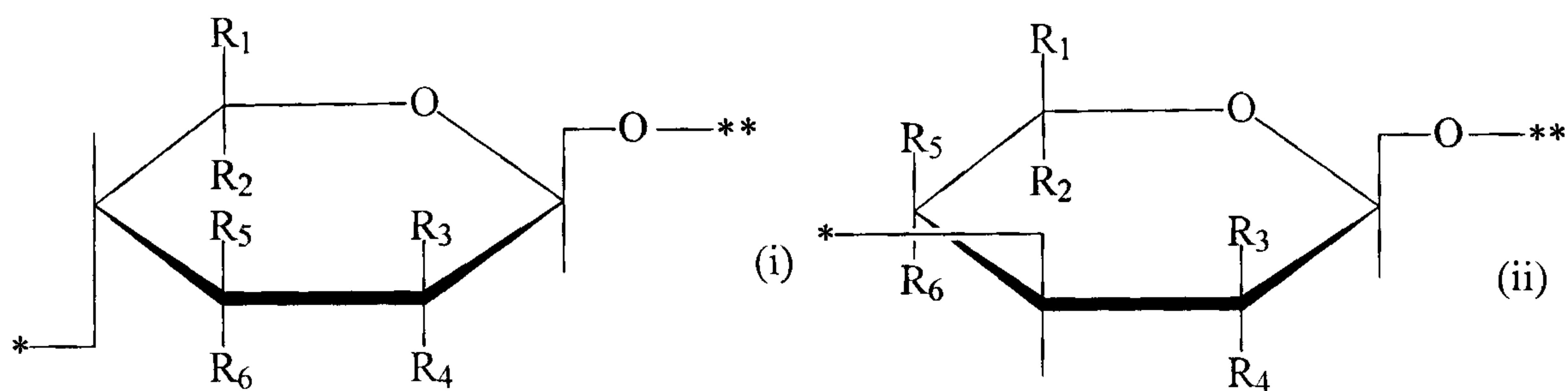
- the therapeutic agent is formulated to be delivered to the respiratory tract;
 - the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
 - the mucus contains extracellular genomic DNA which is compromising lung function; and
 - the glycosaminoglycan increases delivery of the therapeutic agent to the mammalian subject.
3. Use according to claim 1 or 2, wherein the subject has a respiratory tract infection.
 4. Use according to claim 3, wherein the infection is a bacterial or viral infection.
 5. Use according to claim 4, wherein the viral infection is influenza or a cold.
 6. Use according to any one of claims 1 to 5, wherein the therapeutic agent is not a bronchodilator.
 7. Use according to any one of claims 1 to 6, wherein the disease or disorder is selected from the group consisting of chronic airflow limitation (CAL), pneumonia, sinusitis, sinus congestion, cystic fibrosis and asthma.
 8. Use according to any one of claims 1 to 6, wherein the disease or disorder is chronic airflow limitation (CAL).
 9. Use according to any one of claims 1 to 8, wherein the mammalian subject is a human.

10. Use according to any one of claims 1 to 9, wherein the glycosaminoglycan or the physiologically acceptable salt comprises repeating disaccharide units of general formula (1)



wherein:

- each A is the same or different and represents a moiety of formula (i) or (ii)



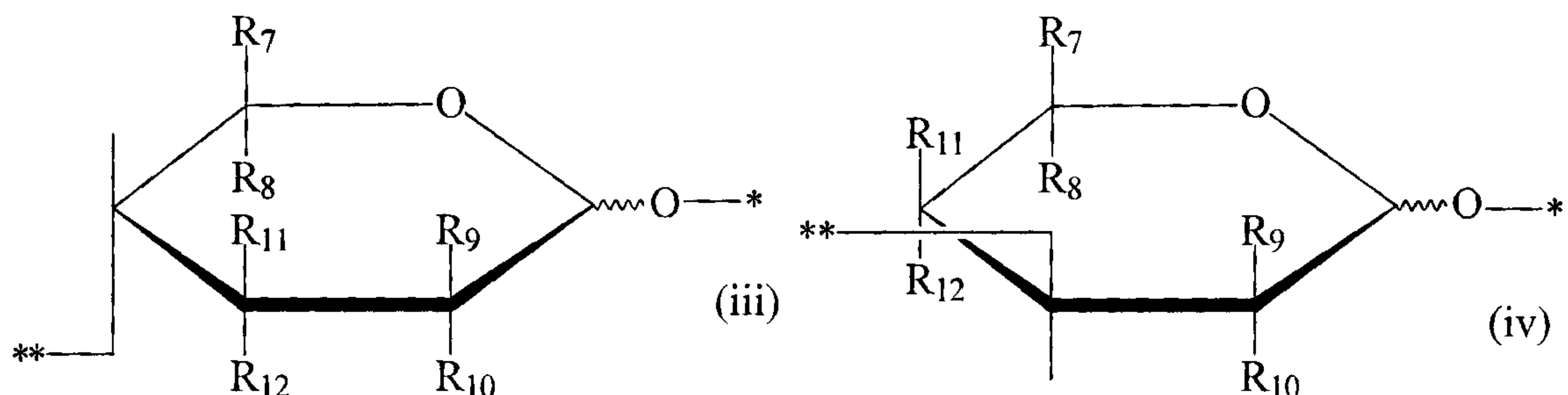
wherein:

- one of R_1 and R_2 is hydrogen and the other is $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$ or $-\text{CH}_2\text{OR}$

wherein R is hydrogen or $-\text{SO}_3\text{H}$;

- one of R_3 and R_4 is hydrogen and the other is $-\text{OR}$ wherein R is hydrogen or SO_3H ;
- one of R_5 and R_6 is hydrogen and the other is $-\text{OH}$;
- * represents a direct bond to an adjacent hydrogen atom or B moiety; and
- ** represents a direct bond to an adjacent B moiety;

each B is the same or different and represents a moiety of formula (iii) or (iv)



wherein:

- one of R₇ and R₈ is hydrogen and the other is -CH₂OH or -CH₂OSO₃H;
- one of R₉ and R₁₀ is hydrogen and the other is -NHAc, -NH₂ or -NHSO₃H;
- one of R₁₁ and R₁₂ is hydrogen and the other is -OH or -OSO₃H;
-  indicates a bond in either stereochemical orientation;
- * represents a direct bond to a hydrogen atom or an adjacent A moiety;
- ** represents a direct bond to an adjacent A moiety;

or a physiologically acceptable salt thereof.

11. Use according to any one of claims 1 to 10, wherein the glycosaminoglycan is:

- (a) heparin or a derivative thereof; or
- (b) chondroitin sulphate A, chondroitin sulphate C, chondroitin sulphate D, chondroitin sulphate E, keratan sulphate, heparan sulphate or a derivative of any thereof.

12. Use according to any one of claims 1 to 11, wherein the glycosaminoglycan is the sodium salt of heparin or heparin sulphate.

13. Use according to any one of claims 1 to 12, wherein the glycosaminoglycan:

- (a) retains anti-coagulant activity; and/or
- (b) has not been subjected to fragmentation and/or depolymerisation.

14. Use according to any one of claims 1 to 13, wherein the ratio of the amount of glycosaminoglycan to the amount of therapeutic agent administered is from 500:1 to 1:500 by weight.

15. Use according to claim 14, wherein the ratio is from 25:1 to 1:25 by weight.

16. Use according to any one of claims 1 to 15, wherein:

- (a) the glycosaminoglycan and the therapeutic agent are formulated for administration at the same time;
- (b) the glycosaminoglycan is formulated for administration before or after the therapeutic agent; and/or
- (c) the glycosaminoglycan and the therapeutic agent are formulated for administration as separate medicaments.

17. Use according to any one of claims 1 to 16, wherein the therapeutic agent is selected from the group consisting of a bronchodilator, a mucolytic, a mucokinetic, an anti-inflammatory, a protease inhibitor, α 1-antitrypsin, a mucoregulator, an anti-pathogenic agent, a gene therapy vector, an antioxidant and a steroid.

18. Use according to claim 17, wherein the anti-pathogenic agent is selected from the group consisting of an antibiotic, an antiviral agent and an antifungal agent.

19. Use according to claim 17, wherein the protease inhibitor is selected from the group consisting of an inhibitor of elastase, a cathepsin and a matrix metallo proteinase (MMP).

20. Use according to any one of claims 1 to 19, wherein the subject has an FEV1 of less than 75% of the expected value for an equivalent subject not having the disorder.

21. Use according to any one of claims 1 to 20, wherein the medicament is a dry powder.

22. A pharmaceutical system comprising: (i) a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate and keratan sulphate, and a physiologically salt thereof and (ii) a therapeutic agent for simultaneous, separate or sequential use in the treatment of a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, wherein:

- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;
- the glycosaminoglycan is defined in any one of claims 1 and 10 to 13 and have an average molecular weight of from 12 to 18 kd;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent to the human subject.

23. A pharmaceutical composition for treating a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, the pharmaceutical composition comprising (i) a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate and keratan sulphate and a physiologically acceptable salt thereof and (ii) an excipient, wherein:

- the subject is being treated with a therapeutic agent;
- the therapeutic agent is adapted for administration by at least one of inhalation, intranasally and instillation;
- the glycosaminoglycan is defined in any one of claims 1 and 10 to 13 and the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the pharmaceutical composition is formulated to be delivered to the respiratory tract,
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent.

24. A pharmaceutical composition for treating a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, the pharmaceutical composition comprising a therapeutic agent and an excipient, wherein:

- the subject is being treated with a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and instillation;
- the glycosaminoglycan is defined in any one of claims 1 and 10 to 13 and the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the mucus contains extracellular genomic DNA which is compromising lung function; and

- the glycosaminoglycan increases delivery of the therapeutic agent.

25. A kit comprising:

- a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate, and a physiologically acceptable salt thereof;
- instructions for the administration of the glycosaminoglycan to a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, where the administration is by at least one of inhalation, intranasally and instillation, where the subject is being treated with a therapeutic agent which is being administered by at least one of inhalation, intranasally and instillation; and
- packaging,

wherein:

- the glycosaminoglycan is defined in any one of claims 1 and 10 to 13;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent.

26. A kit comprising:

- a therapeutic agent;
- instructions for the administration of the therapeutic agent to a human subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus, by at least one of inhalation, intranasally and instillation where the subject is being treated with a glycosaminoglycan selected

from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate, and a physiologically acceptable salt thereof which is being administered by at least one of inhalation, intranasally and instillation; and

- packaging,

wherein:

- the glycosaminoglycan is defined in any one of claims 1 and 10 to 13;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the mucus contains extracellular genomic DNA which is compromising lung function; and
- the glycosaminoglycan increases delivery of the therapeutic agent.

27. A pharmaceutical system according to claim 22, a pharmaceutical composition according to claim 23 or 24 or a kit according to claim 25 or 26, wherein at least one of:

- the therapeutic agent is defined in any one of claims 17 to 19; and
- the ratio of glycosaminoglycan to therapeutic agent is defined in claim 14 or 15.

28. A pharmaceutical system according to claim 22, a pharmaceutical composition according to claim 23 or 24 or a kit according to claim 25 or 26, wherein the subject is defined in any one of claims 3 to 5 or 7 to 9.

29. A glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt thereof for the treatment of a mammalian subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus or other pulmonary secretions, wherein:

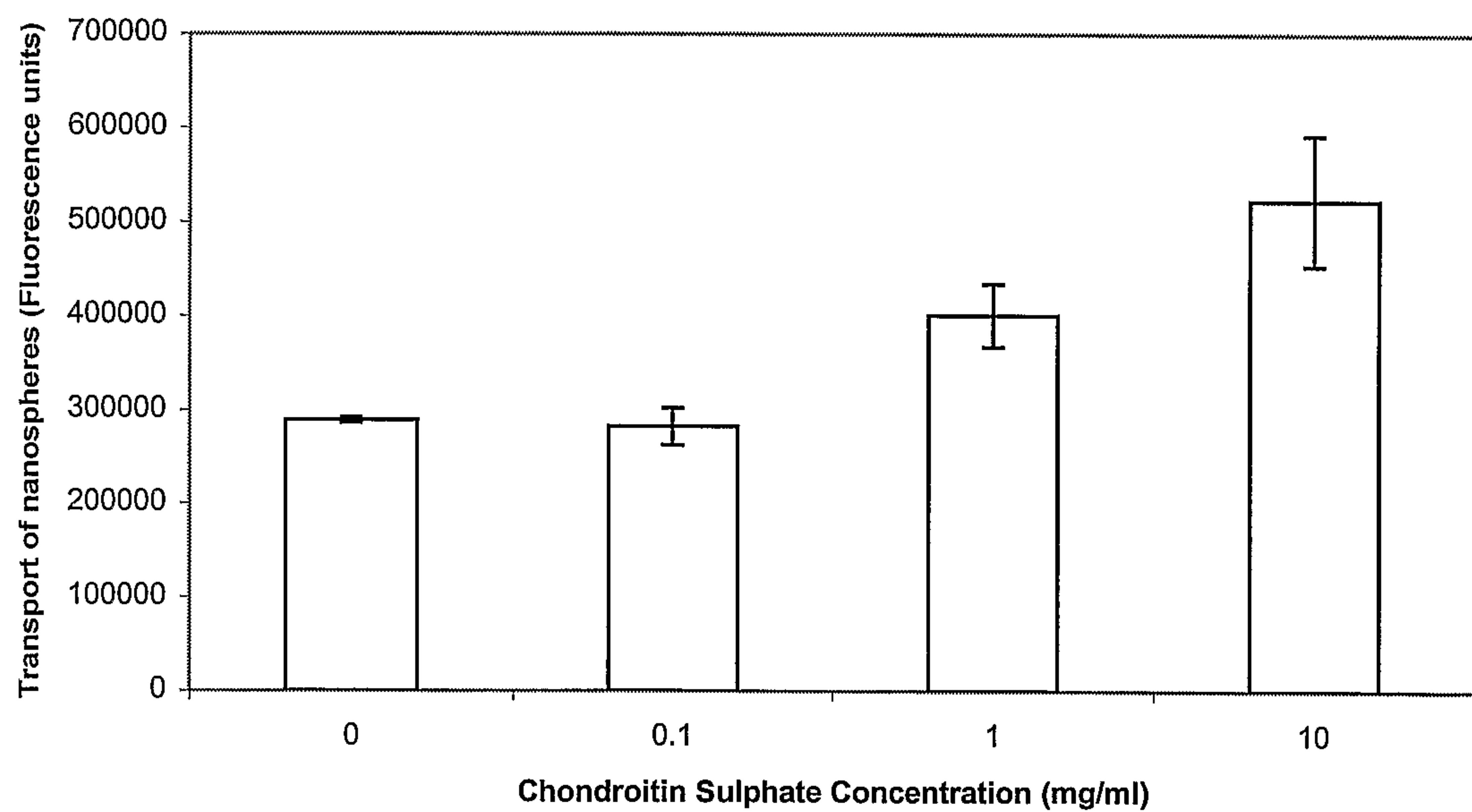
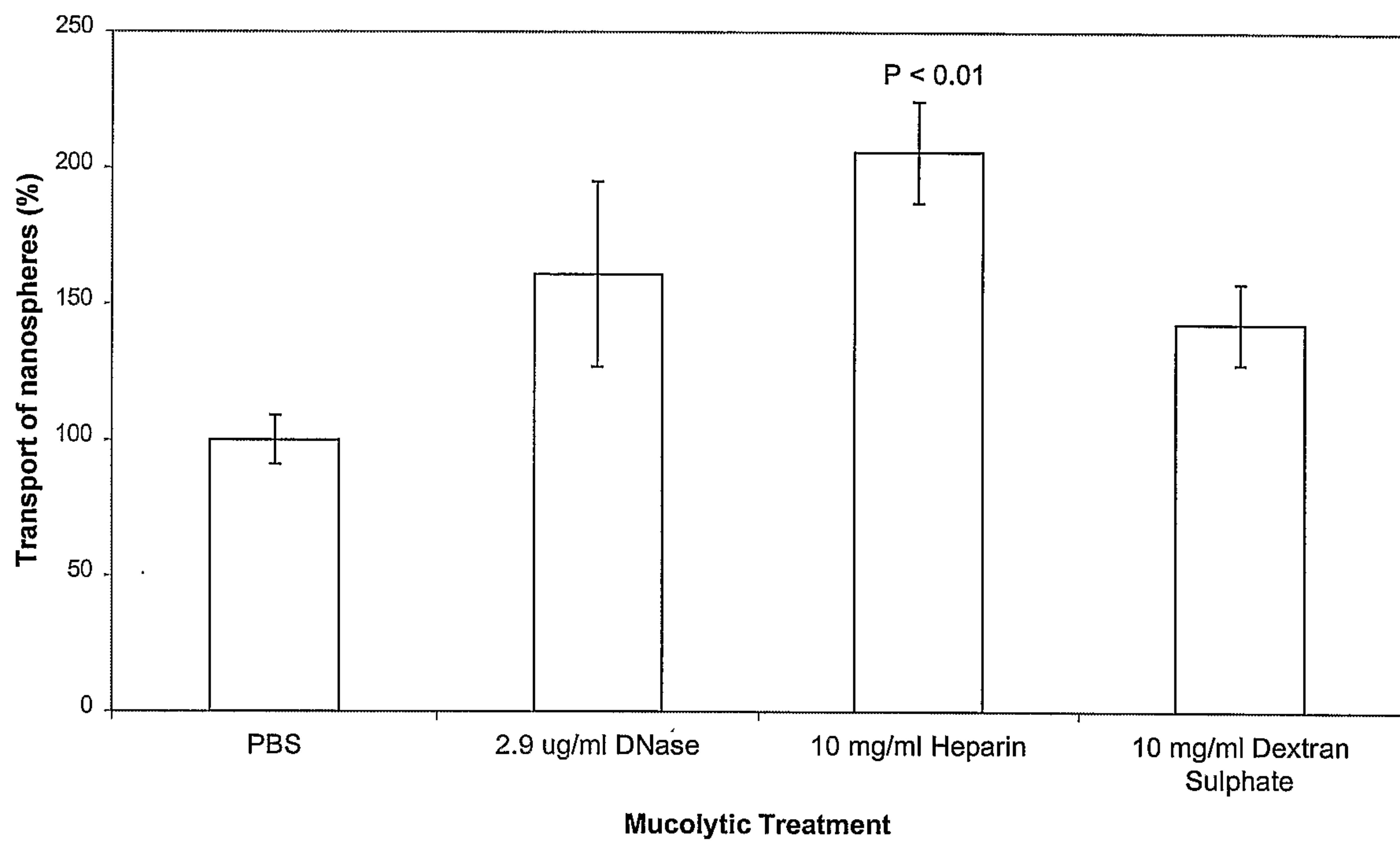
- the subject is being treated with a therapeutic agent;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by at least one of inhalation, intranasally and installation;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the mucus or the other pulmonary secretions contains extracellular genomic DNA which is compromising lung function;
- the glycosaminoglycan increases delivery of the therapeutic agent; and
- the therapeutic agent is not a bronchodilator.

30. A therapeutic agent for the treatment of a mammalian subject with a respiratory disorder or disease which comprises increased levels and/or viscosity of mucus or other pulmonary secretions, wherein:

- the subject is also administered a glycosaminoglycan selected from the group consisting of chondroitin sulphates A to E, heparin, heparin sulphate, heparan, heparan sulphate, keratan sulphate and a physiologically acceptable salt of such a glycosaminoglycan;
- the glycosaminoglycan and the therapeutic agent are adapted for administration by inhalation, intranasally and installation;
- the therapeutic agent is formulated to be delivered to the respiratory tract;
- the glycosaminoglycan has an average molecular weight of from 12 to 18 kd;
- the mucus or the other pulmonary secretions contains extracellular genomic DNA which is compromising lung function;

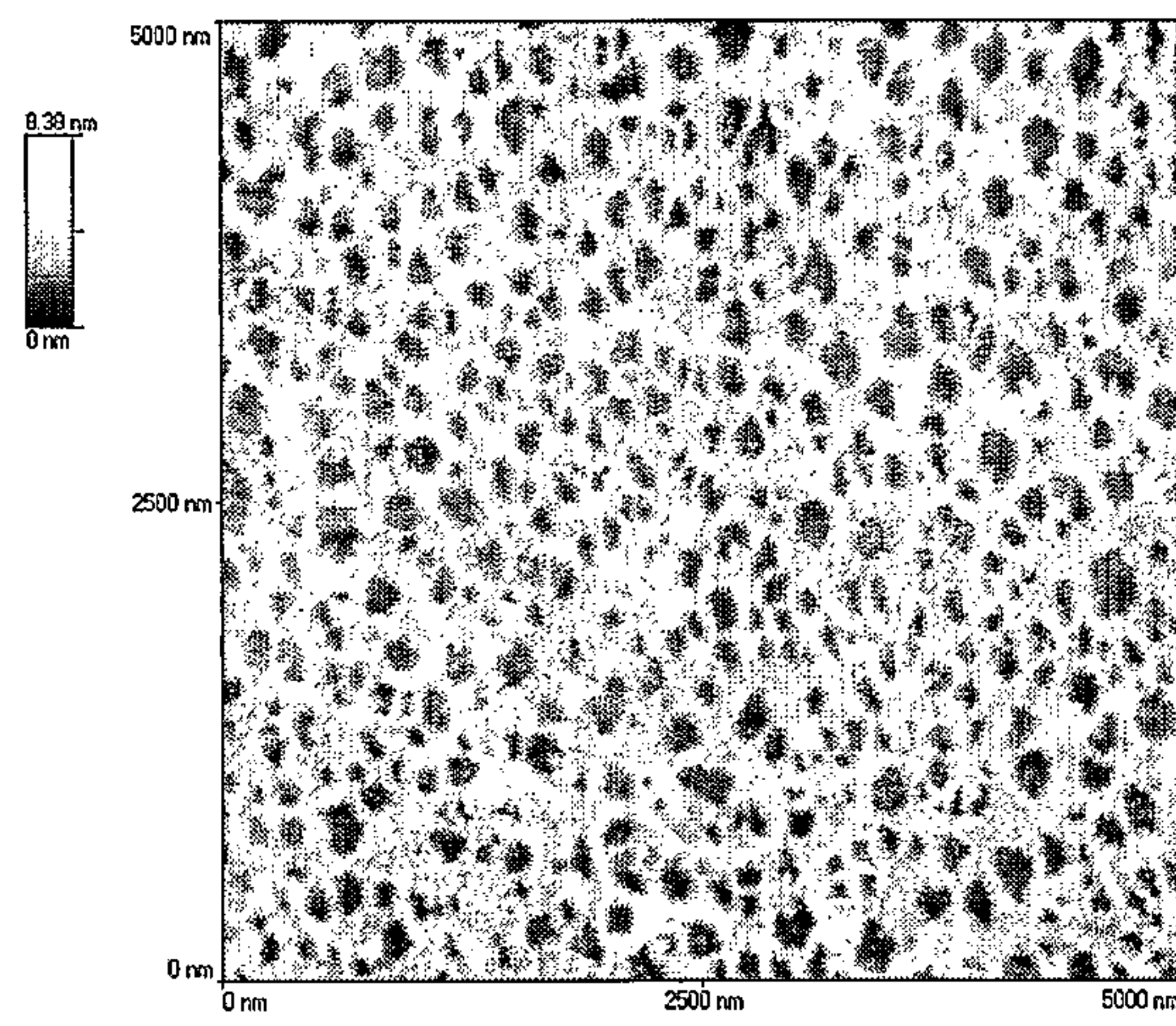
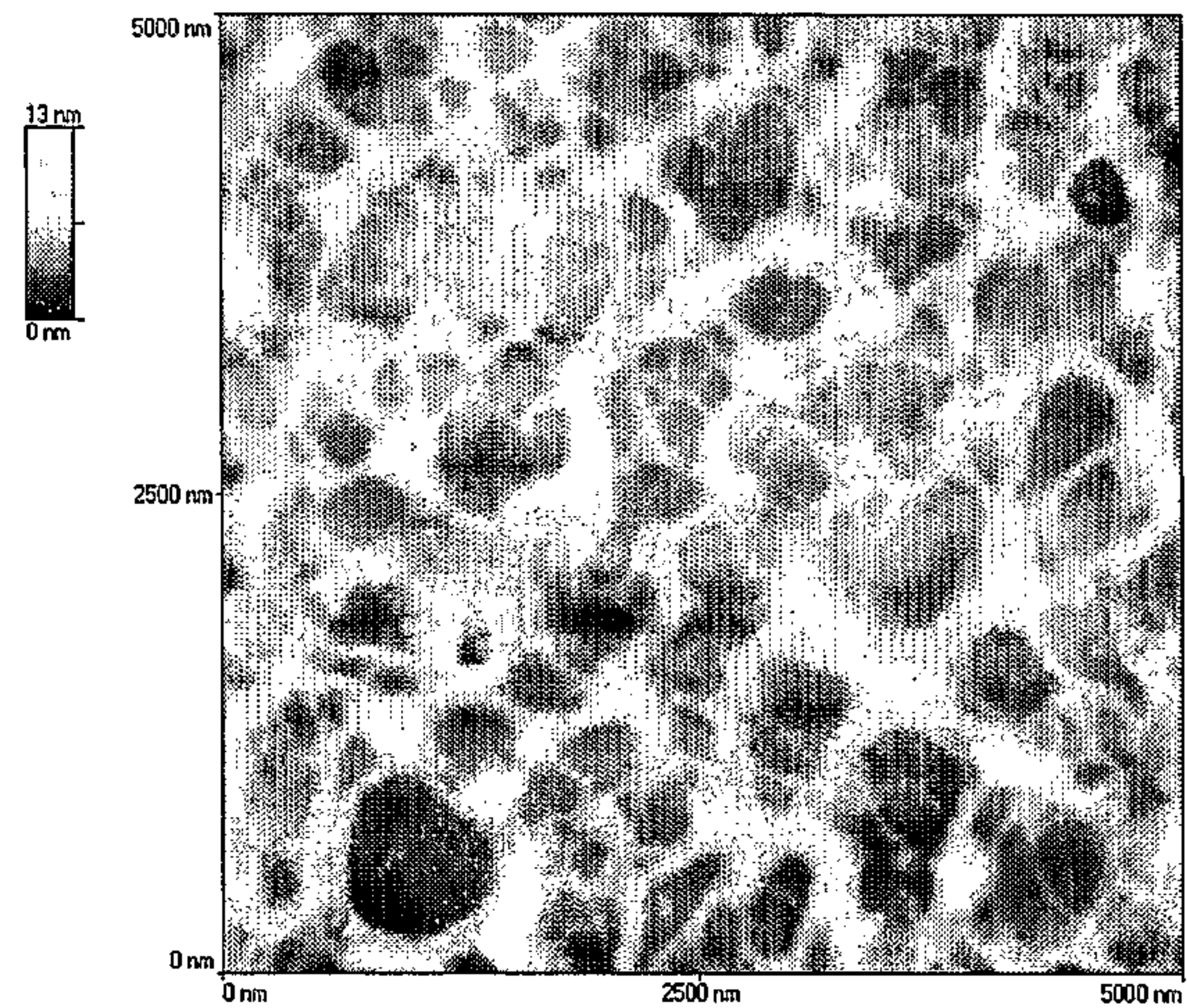
- the glycosaminoglycan increases delivery of the therapeutic agent; and
- the therapeutic agent is not a bronchodilator.

1/3

**FIGURE 1**

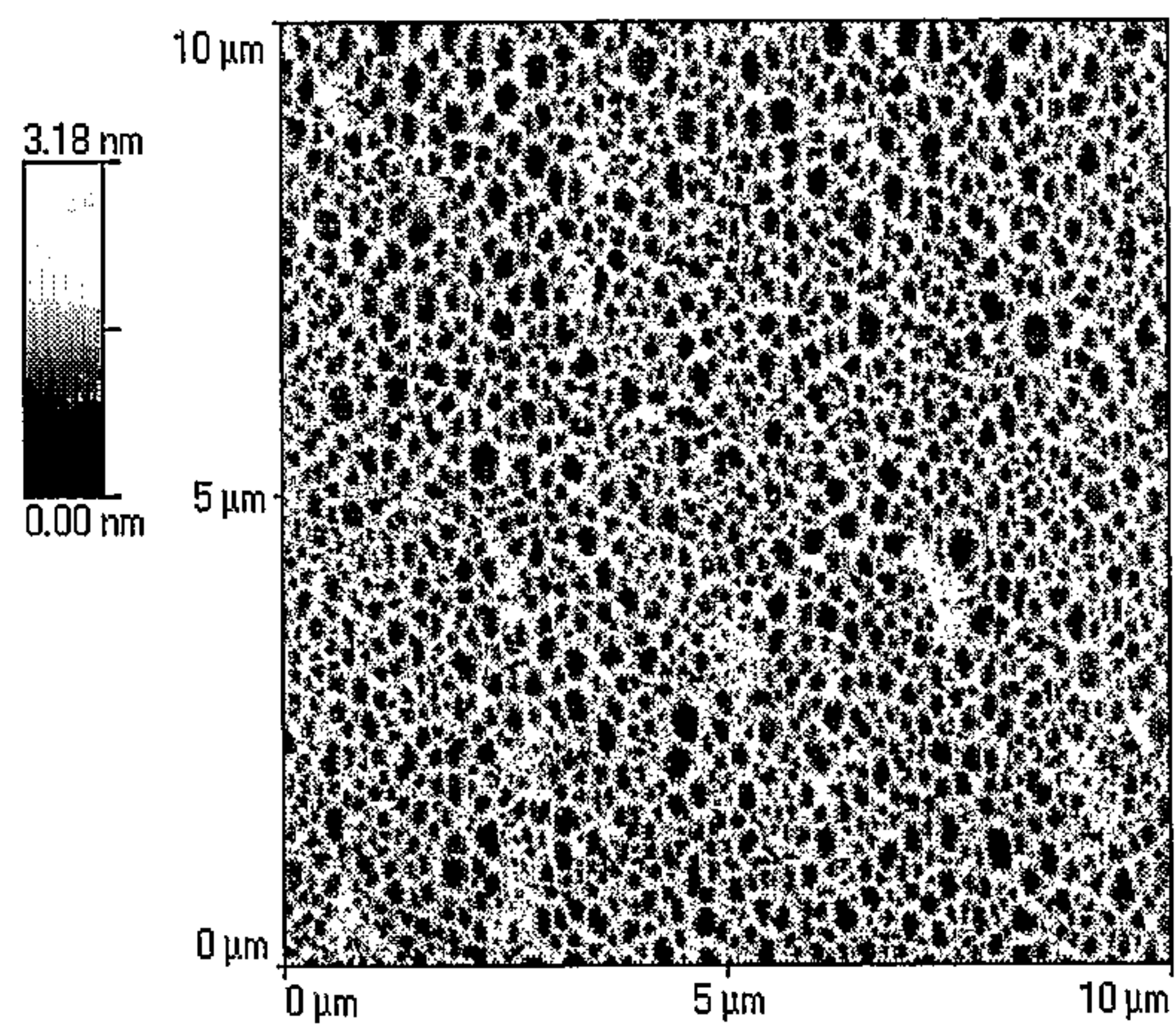
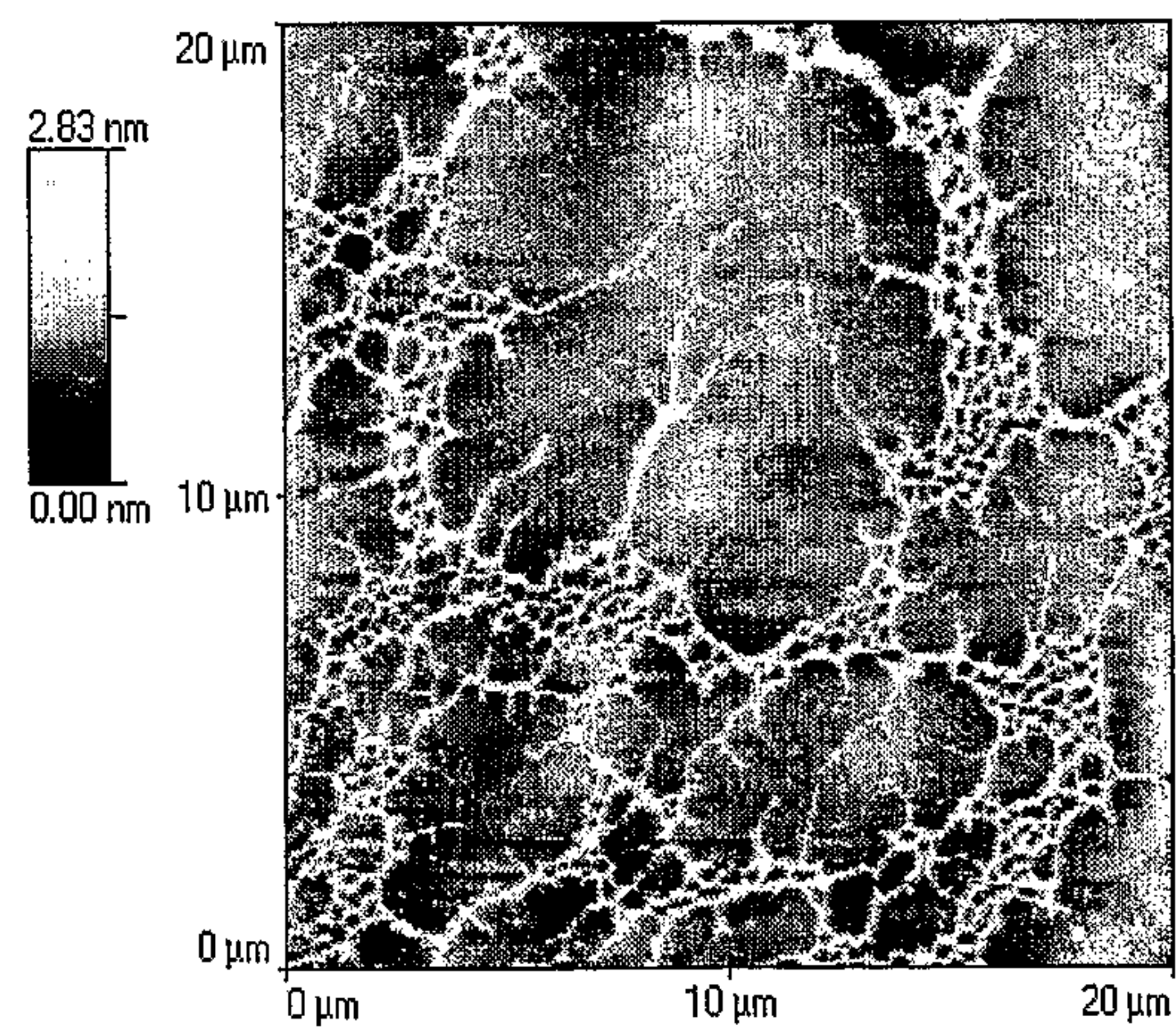
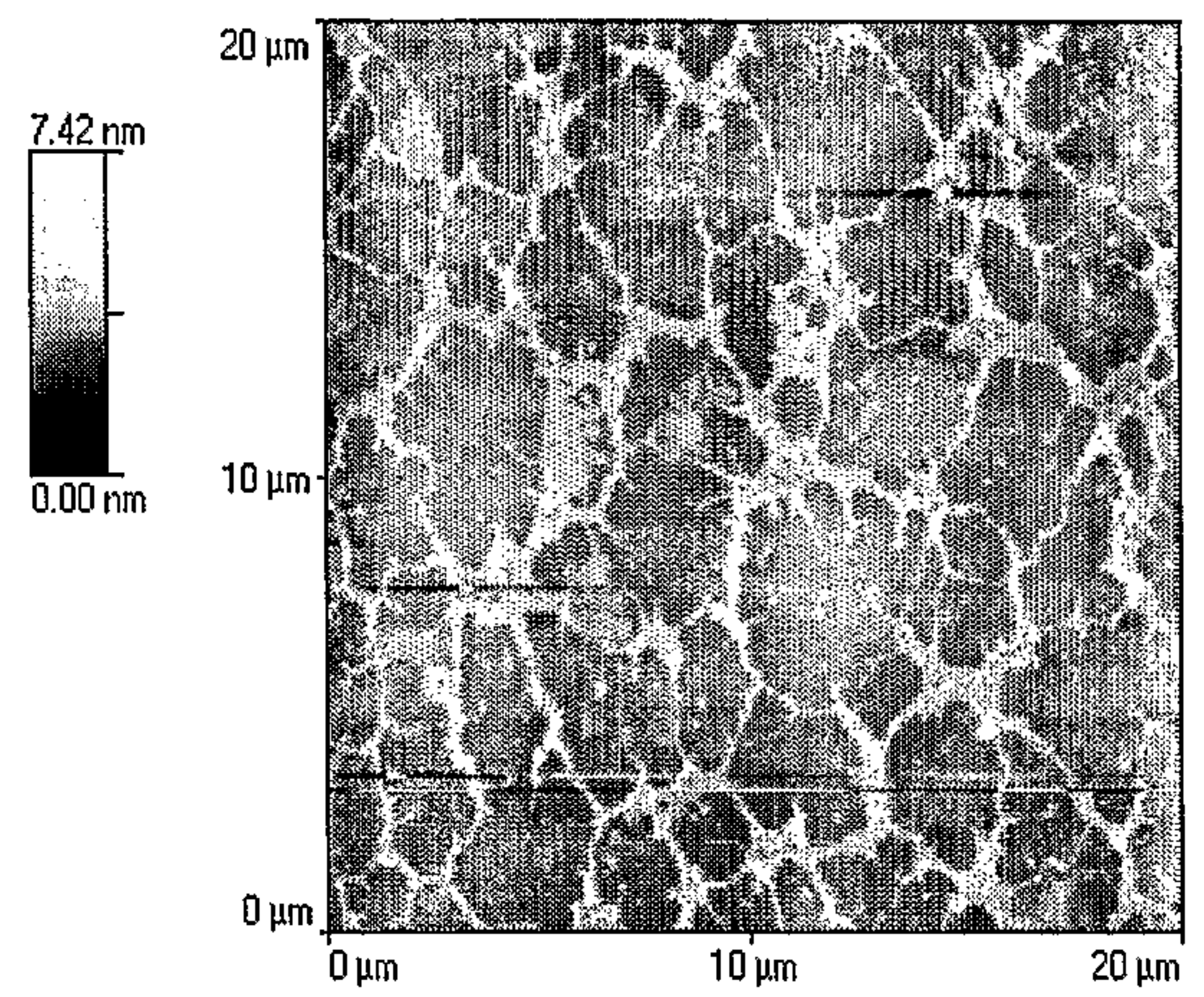
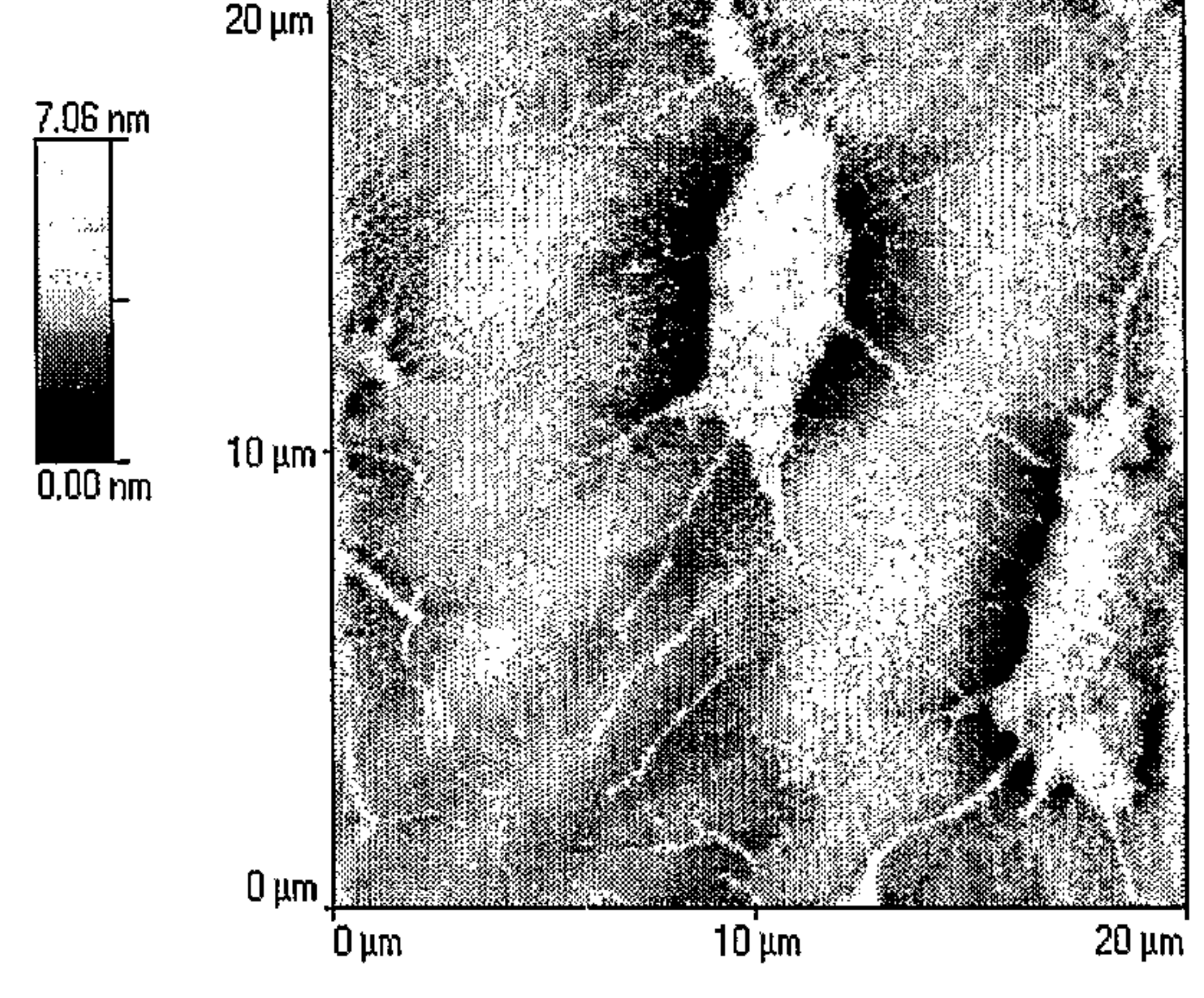
2/3

FIGURE 2

A.**B.**

3/3

FIGURE 3

A.**B.****C.****D.**