properties affect the residence time distribution. The inventors surprisingly found that the mean particle diameter and particle size distribution of the beads forming the packed bed have the highest impact on residence time distribution in the tested range. Specifically, the inventors found that said packed bed of non-porous beads provides for a particularly narrow residence time distribution when the beads have a mean particle diameter in the range of 0.05 mm to 1 mm, and when the particle size distribution is narrow. Moreover, the inventors have found that larger volumes of the packed beds of non-porous beads result in narrower residence time distributions. Further, according to the disclosure, longer beds of beads (e.g. in forms of columns) also result in narrower residence time distributions.

In contrast to many currently used methods, the methods of the present disclosure can be scaled-up easily. This is because the method of the present disclosure is not very sensitive to changes in flow rates and superficial linear velocities, and because the residence time distribution gets narrower when using packed beds of non-porous beads that have larger volumes and are longer. Thus, the method of the present disclosure can easily be integrated into commercial production processes.

In one aspect, the present disclosure refers to a continuous-flow method for continuous virus inactivation, wherein the method is for incubating a mixture of at least two liquids, the method comprising: i) mixing said at least two liquids to obtain a mixture; and ii) passing said mixture through a packed bed of inert non-porous beads, thereby incubating said mixture, wherein a first of said at least two liquids is a liquid potentially containing a virus, wherein a second liquid of said at least two liquids comprises a virus-inactivating agent, and wherein the Bodenstein number of said mixture when passing through said packed bed of inert non-porous beads is equal to or higher than 50.

In another aspect, the present disclosure refers to a method for preparing a biopharmaceutical drug, the method comprising performing the method for incubating the mixture of at least two liquids as disclosed herein, wherein said first liquid comprises a biopharmaceutical drug, and recovering said biopharmaceutical drug.

In yet another aspect, the present disclosure refers to use of a device for continuous virus inactivation, wherein the device is a device for the preparation of a biopharmaceutical drug, which is a continuous-flow reactor suitable for continuous virus inactivation, the device comprising a packed bed of inert non-porous beads, wherein the device is used in any one of the methods as disclosed herein.

In a further aspect, the present disclosure refers to a method for modification of a continuous-flow virus inactivation process, wherein the modification comprises using a packed bed of inert non-porous beads for continuous-flow virus inactivation, and passing a mixture of at least two liquids through said packed bed of inert non-porous beads, thereby incubating said mixture for virus inactivation, wherein said virus inactivation process uses a virus-inactivating agent for virus inactivation, wherein a first of said at least two liquids is a liquid potentially containing a virus, wherein a second liquid of said at least two liquids comprises a virus-inactivating agent, wherein the modification comprises modifying the virus inactivation process such that the Bodenstein number of the mixture passing through said packed bed of inert non-porous beads is equal to or higher than 50, and wherein said continuous-flow virus inactivation process is optionally a process for the preparation of a biopharmaceutical drug.

Overall, the present disclosure provides improved means for incubating liquids by providing the preferred examples described below:

1. A method for incubating a mixture of at least two liquids, the method comprising:

   i) mixing said at least two liquids to obtain a mixture; and

   ii) passing said mixture through a structure having multiple interconnected channels, thereby incubating said mixture.

2. The method according to item 1, wherein the method is a continuous-flow method.

3. The method according to item 1 or 2, wherein said mixing and passing is carried out continuously.

4. The method according to any one of the preceding items, wherein the structure having multiple interconnected channels is a packed bed of non-porous beads.

5. The method according to item 4, wherein the non-porous beads are inert non-porous beads.

6. The method according to item 4 or item 5, wherein the non-porous beads are glass beads, or ceramic beads, or plastic beads such as PMMA beads, or steel beads.

7. The method according to any one of items 4 to 6, wherein the mean particle diameter of the non-porous beads is in the range of 0.05-1 mm, preferably in the range of 0.05-0.6 mm, more preferably 0.05 to 0.5 mm, and most preferably in the range of 0.05-0.3 mm.

with the step of mixing and passing according to the invention), it means that this step occurs without interruption. If the term “continuous” or “continuously” or “continuous-flow” is used herein in accordance with a method or process of the invention, it means that said method or process occurs without interruption. Preferably, where the method or process is carried out continuously, all method or process method steps are carried out continuously. Alternatively, it is also possible that only the output of the method or process is continuous, whereas parts of said method or process (e.g. particular method or process steps) are carried out discontinuously or semi-continuously. For example, a series of batch processes can deliver a continuous output over time, although the individual processes are operated discontinuously.

The term “non-porous beads” as used herein refers to any suitable non-porous beads that can be used for a packed bed of non-porous beads according to the invention. The “non-porous beads” can be spherical or irregularly shaped. In a preferred embodiment in accordance with all other embodiments of the invention, the non-porous beads are preferably spherical. The “non-porous beads” can, for instance, be made of any solid particulate material that is compatible with biopharmaceutical processing, e.g. plastics, glass or metal.

Non-porous beads are known in the art and are commercially available.

Glass beads are known in the art and can, for instance be made of silica glass. For example, glass beads can be purchased from Cospheric LLC.

Plastic beads are also known and can, for instance, be made of Poly(methyl methacrylate) (PMMA), polyethylene (PE), polypropylene (PP),or polystyrene (PS). For example, plastic beads can be purchased from Cospheric LLC, Altuglas Arkema, and Kisker Biotech.

Steel beads are also known in the art and can, for instance, be made of stainless steel. For example, steel beads can be purchased from Cospheric LLC.

The term “ceramic beads” as used herein refers to any ceramic beads that are suitable for forming a “packed bed of non-porous beads” according to the invention. For example, ceramic beads can be purchased from Kuhmichel Abrasiv GmbH.

The packed bed of non-porous beads according to the invention is not particularly limited and can, for instance, be contained in variously shaped containers, such as columns or reactors. The size of the container is not particularly limited, and can be selected based on the desired throughput and incubation time.

The term “inert” in connection with the non-porous beads of the invention has the meaning of the term that is known in the art.

Inert materials for the non-porous beads of the invention can be chosen by a person skilled in the art. In a method or process of the invention, it will be possible to select appropriate known inert materials such that they do not or not substantially (e.g. not measurably) react with the liquid or mixture of liquids that is passed through the bed of beads. The inert non-porous beads of the invention are beads that do not or do not substantially chemically react with the liquid mixture of the present invention.

The term “deviate from the mean particle diameter” by a given percentage as used herein refers to a deviation which depends on the mean particle diameter. For example, if beads with a mean particle diameter of 0.2 mm do not deviate from the mean particle diameter by more than 50%, 95% of the beads have a particle diameter of not more than 0.3 mm and not less than 0.1 mm. Similarly, if beads with a mean particle diameter of 0.2 mm do not deviate from the mean particle diameter by more than 20%, 95% of the beads have a particle diameter of not more than 0.24 mm and not less than 0.16 mm. For particles to be used in accordance with the present invention which are not spherical, the diameter refers to the longest axis of the particles.

The term “vibration treatment” as used herein refers to any treatment that involves vibration and which is suitable to increase the packing density of the packed bed of non-porous beads. For example, a vibrational device can be used for subjecting the packed bed of non-porous beads to vibration treatment.

A preferable vibrational device contains a rack to which the column is immobilized. In an example of a vibration treatment using a vibrational device, the empty column is immobilized, and the beads are added during vibration. The rack is then vibrated using a vibration motor. Said motor can, for instance, be powered electrically or pneumatically. Packed beds of non-porous beads can be packed using a vibration frequency of less than 40 kHz, preferably of 1-10 kHz, an acceleration of less than 10 g, preferably 0-5 g, and a vibration amplitude of less than 5mm, preferably up to 2mm. An illustrative example of a vibrational device used for column packing is shown in Figure 22.

The term “reactor” as used herein refers to any container or other structure that is suitable to contain fluids. The reactor can be used in order to allow the fluids to chemically react. However, in the present invention the term “reactor” also refers to reactors in which no chemical reaction occurs. It is understood that the reactor can be adjusted based on the intended use. For example, it is understood that a reactor that is used for virus inactivation will be suitable for virus inactivation. Likewise, if the reactor is used for the preparation of a biopharmaceutical drug it will be suitable for the preparation of that drug.

CLAIMS

1. A continuous-flow method for continuous virus inactivation, wherein the method is for incubating a mixture of at least two liquids, the method comprising:

   i) mixing said at least two liquids to obtain a mixture; and

   ii) passing said mixture through a packed bed of inert non-porous beads, thereby incubating said mixture,

   wherein a first of said at least two liquids is a liquid potentially containing a virus, wherein a second liquid of said at least two liquids comprises a virus-inactivating agent, and wherein the Bodenstein number of said mixture when passing through said packed bed of inert non-porous beads is equal to or higher than 50.

2. The method according to claim 1,

   wherein said mixing and passing is carried out continuously,

   and/or wherein the inert non-porous beads are glass beads, or ceramic beads, or plastic beads such as PMMA beads, or steel beads.

3. The method according to claim 1 or 2,

   wherein the mean particle diameter of the inert non-porous beads is in the range of 0.05-1 mm, or in the range of 0.05-0.6 mm or 0.05 to 0.5 mm, or in the range of 0.05-0.3 mm,

   and/or wherein 95% of the inert non-porous beads do not deviate from the mean particle diameter by more than 50%, or not more than 35%, or not more than 20%.

4. The method according to any one of claims 1 to 3, wherein the packed bed of inert non-porous beads has a length of at least 5 cm, or at least 10 cm, or at least 20 cm, or at least 30 cm, or at least 50 cm, or at least 70 cm, or at least 100 cm.

5. The method according to any one of claims 1 to 4, wherein the method is characterized by at least one of the following:

   (A) the packed bed of inert non-porous beads is obtainable by a method which comprises subjecting said inert non-porous beads to a vibration treatment,

   (B) for the packed bed of inert non-porous beads, the fraction of the volume of voids over the total volume is in the range of 0.2 to 0.45 or in the range of 0.37 to 0.42,

   (C) the packed bed of inert non-porous beads is contained in a column and/or a reactor, wherein the column has optionally a diameter of more than 5 mm, or a diameter of at least 10 mm,

and/or

(D) the void volume of the packed bed of inert non-porous beads is at least 10 mL, or at least 40 mL, or at least 150 mL, or at least 470 mL or at least 700 mL.

6. The method according to any one of claims 1 to 5, wherein said first liquid comprises a biopharmaceutical drug,

and/or wherein the method is for virus inactivation of enveloped viruses,

and/or wherein said virus is a retrovirus and/or a virus of the Flaviviridae family, wherein said retrovirus is optionally X-MuLV, and wherein said virus of the Flaviviridae family is optionally BVDV.

7. The method according to any one of claims 1 to 6,

wherein the virus-inactivating agent is a solvent/detergent mixture suitable for solvent/detergent virus-inactivating treatment, or an acidic solution suitable for low pH virus-inactivating treatment,

and/or wherein the virus-inactivating agent is a solvent/detergent mixture for solvent-detergent treatment,

and/or wherein the method achieves at least a 1 Log10 reduction value (LRV), at least a 2 LRV, at least a 4 LRV or at least a 6 LRV for at least one virus, and wherein said at least one virus is optionally a retrovirus and/or a virus of the Flaviviridae family, wherein said retrovirus is optionally X-MuLV, and wherein said virus of the Flaviviridae family is optionally BVDV.

8. The method according to any one of claims 1 to 7,

wherein the superficial linear velocity of said mixture through said packed bed of inert non-porous beads is equal to or lower than 600 cm/h, or equal to or lower than 300 cm/h, or equal to or lower than 200 cm/h, or equal to or lower than 100 cm/h, or equal to or lower than 50 cm/h, or equal to or lower than 20 cm/h,

and/or wherein the Bodenstein number of said mixture when passing through said packed bed of inert non-porous beads is equal to or higher than 300, or equal to or higher than 400, or equal to or higher than 500, or equal to or higher than 600, or equal to or higher than 800.

9. A method for preparing a biopharmaceutical drug, the method comprising performing the method of any one of claims 6 to 8, wherein said first liquid comprises a biopharmaceutical drug, and recovering said biopharmaceutical drug.

10. Use of a device for continuous virus inactivation, wherein the device is a device for the preparation of a biopharmaceutical drug, which is a continuous-flow reactor suitable for continuous virus inactivation, the device comprising a packed bed of inert non-porous beads, wherein the device is used in the method according to any one of claims 1 to 9.

11. Use of the device according to claim 10, wherein the device is characterized by at least one of the following:

(a) the non-porous beads are glass beads, or ceramic beads, or plastic beads such as PMMA beads, or steel beads,

(b) the mean particle diameter of the non-porous beads is in the range of 0.05-1 mm, or in the range of 0.05-0.6 mm, or in the range of 0.05-0.5 mm, or in the range of 0.05-0.3 mm,

(c) the non-porous beads do not deviate from the mean particle diameter by more than 50%, or not more than 35%, or not more than 20%,

(d) the packed bed of non-porous beads has a length of at least 5 cm, or at least 10 cm, or at least 20 cm, or at least 30 cm, or at least 50 cm, or at least 70 cm, or at least 100 cm,

(e) the packed bed of non-porous beads is obtainable by a method which comprises subjecting said non-porous beads to a vibration treatment,

(f) for the packed bed of non-porous beads, the fraction of the volume of voids over the total volume is in the range of 0.2 to 0.45 or in the range of 0.37 to 0.42,

(g) the packed bed of non-porous beads is contained in a column and/or a reactor, and the column optionally has a diameter of more than 5 mm or a diameter of at least 10 mm,

(h) the void volume of the packed bed of non-porous beads is at least 10 mL, or at least 40 mL, or at least 150 mL, or at least 470 mL or at least 700 mL,

(i) the device additionally comprises one or multiple mixers, which are connected to the packed bed of non-porous beads, and the mixer is optionally a static mixer such as a T-junction mixer, or the mixer is optionally a dynamic mixer such as a dynamic stirrer, and/or

(j) the device additionally comprises a filter, and the filter is optionally positioned between the packed bed of non-porous beads and a static mixer such as a T-junction mixer, and the filter has optionally a pore size of 0.2 µm.

12. A method for modification of a continuous-flow virus inactivation process, wherein the modification comprises using a packed bed of inert non-porous beads for continuous-flow virus inactivation, and passing a mixture of at least two liquids through said packed bed of inert non-porous beads, thereby incubating said mixture for virus inactivation,

wherein said virus inactivation process uses a virus-inactivating agent for virus inactivation, wherein a first of said at least two liquids is a liquid potentially containing a virus, wherein a second liquid of said at least two liquids comprises a virus-inactivating agent, wherein the modification comprises modifying the virus inactivation process such that the Bodenstein number of the mixture passing through said

packed bed of inert non-porous beads is equal to or higher than 50, and wherein said continuous-flow virus inactivation process is optionally a process for the preparation of a biopharmaceutical drug.

13. The method according to claim 12, wherein said virus inactivation process is for virus inactivation of enveloped viruses,

and/or wherein the virus-inactivating agent used in said virus inactivation process is a solvent/detergent mixture suitable for solvent/detergent virus-inactivating treatment, or an acidic solution suitable for low pH virus-inactivating treatment and the virus-inactivating agent used in said virus inactivation process is optionally a solvent/detergent mixture for solvent-detergent treatment.

14. The method according to any one of claims 12 to 13, wherein the method is characterized by at least one of the following:

(1) the modification comprises modifying the virus inactivation process to achieve at least a 1 Log10 reduction value (LRV), at least a 2 LRV, at least a 4 LRV or at least a 6 LRV for at least one virus,

(2) the modification comprises modifying the virus inactivation process such that the Bodenstein number of the mixture passing through said packed bed of inert non-porous beads is equal to or higher than 300, or equal to or higher than 400, or equal to or higher than 500, or equal to or higher than 600, or equal to or higher than 800,

(3) the modification comprises modifying the virus inactivation process such that the superficial linear velocity of the mixture through said packed bed of inert non-porous beads is equal to or lower than 600 cm/h, or equal to or lower than 300 cm/h, or equal to or lower than 200 cm/h, or equal to or lower than 100 cm/h, or equal to or lower than 50 cm/h, or equal to or lower than 20 cm/h,

and/or

(4) the modification comprises using a packed bed of inert non-porous beads as defined in any one of claims 2-5.

15. The method according to claim 14, wherein the modification comprises adjusting the flow through time of said mixture in said packed bed of inert non-porous beads to achieve said Log10 reduction value (LRV), and wherein the flow through time is adjusted by adjusting the superficial linear velocity of the mixture and/or the void volume of said packed bed of inert non-porous beads.