The present invention in particular results from the observation of the striking improvement in the purification of enveloped viruses when acid buffers are used during anion exchange chromatography. The object of the invention is therefore a process for purifying enveloped viruses comprising an anion exchange chromatography, the buffers used during said chromatography being of a pH of less than 6.

In particular, the pH may notably be comprised between 5.5 and 6. According to an alternative, the pH of the buffers used during anion exchange chromatography is greater than or equal to 6 and further comprises a polyol.

Accordingly, in one aspect, there is provided a process for purifying an enveloped virus, comprising an anion exchange chromatography step, the buffers used during said chromatography being of a pH below 6, or of a pH greater than or equal to 6 and further comprising a polyol.

The inventors were able to show that by adding a polyol in one or several of the buffers used during one or several steps of a process for purifying an enveloped virus, it was possible to obtain a substantial increase in the purification yield. In particular, the improvement of the yield of a purification comprising an ultrafiltration/diafiltration step followed by anion exchange chromatography is observed when the buffers used during this chromatography comprise a polyol.

The inventors also propose inversion of the order of the anion exchange chromatography steps and of ultrafiltration/diafiltration steps, notably a tangential flow filtration (TFF), used during the purification. The application of an ultrafiltration/diafiltration, notably a TFF, before an anion exchange chromatography allows a substantial improvement in the yield of the purification of an enveloped vector. ~~The~~ An embodiment of the disclosure ~~invention therefore also~~ relates to a process for purifying an enveloped virus, comprising, in this order, an ultrafiltration/diafiltration step, notably a TFF step, followed by an anion exchange chromatography step.

Moreover the invention relates to a process for purifying enveloped viruses, said process comprising an ultrafiltration / diafiltration step, notably a TFF step, said step being carried out by using buffers containing a polyol.

The disclosure~~invention~~ is more particularly adapted to the purification of viruses pseudotyped with a glycoprotein derived from GaLV. Until the availability of the present ~~invention~~disclosure, the viruses pseudotyped with this type of glycoproteins were considered as "non–purifiable". The different studies carried out on these viral vectors applied raw preparations of non–purified vectors because of the fragility of the GaLV pseudotyped vectors. Unexpectedly, the inventors were able to show a substantial improvement in the purification yield by means of

the disclosed process ~~of the invention~~. They were also able to show that this process also allows improvement in the yield of the purification of viruses pseudotyped with other envelope glycoproteins, notably with VSV–G and MV glycoproteins.

DETAILED DESCRIPTION OF THE INVENTION

Production of enveloped viruses and vectors

The production of enveloped viruses or vectors is well known in the state of the art. One skilled in the art may refer to general knowledge in this field, notably represented by Ansorge et al. 2010; Schweizer and Merten 2010; Rodrigues et al. 2011.

The produced virus is notably an enveloped viral vector. The viral vector is notably derived from a retrovirus, in particular a lentivirus. The produced retroviral vectors are notably derived from alpha retroviruses (such as ALV for avian leukosis virus), from beta retroviruses (such as MMTV for mouse mammary tumor virus), from gamma retroviruses (such as the different types of MLV for murine leukemia virus), from delta retroviruses (such as the different types of HTLV for human T–lymphotropic virus), from epsilon retroviruses (such as WDSV for Walleye dermal sarcoma virus), from spumavirus (such as HFV for human foamy virus and SFV for simian foamy virus), from primate lentiviruses such as the different types of human immunodeficiency viruses (HIV for human immunodeficiency virus), the different types of simian immunodeficiency viruses (SIV for simian immunodeficiency virus), or from non–primate mammal lentiviruses such as the equine infectious anemia virus (EIAV for equine infectious anemia virus), from the feline immunodeficiency virus (FIV for feline immunodeficiency virus), the caprine arthritis–encephalitis virus (CAEV for caprine arthritis–encephalitis virus), or the ovine visna–maedi virus (VMV for visna maedi virus).

According to a particular embodiment, the enveloped virus, notably the retroviral vector, in particular lentiviral vector, is pseudotyped, i.e. it comprises an envelope glycoprotein derived from a virus different from the virus from which is derived the retroviral particle, a modified envelope glycoprotein or a chimeric envelope glycoprotein. According to a particular embodiment, the retroviral vector is pseudotyped with an envelope glycoprotein derived from the vesicular stomatitis virus (VSV–G), from the measles virus (MV for measles virus), or modified MV virus (notably modified with an anti–CMHII antibody), from the Baboon