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Application No. 16 703 205.1 - 1118	Ref. BET 17M2828	Date 19.07.2023
Applicant Genzyme Corporation		

Communication pursuant to Article 94(3) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 126(2) and 131(2) and (4) EPC. One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

If filing amendments, you must identify them and indicate the basis for them in the application as filed. Failure to meet either requirement may lead to a communication from the Examining Division requesting that you correct this deficiency (R. 137(4) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

Registered Letter

EPO Form 2001 11.16AUWF (14.07.2023)



Roscoe, Richard
Primary Examiner
For the Examining Division

Enclosure(s): 4 page/s reasons (EPO Form 2906)

The examination is being carried out on the **following application documents**

Description, Pages

1-74 as originally filed

Claims, Numbers

1-17 filed in electronic form on 10-01-2022

Drawings, Figures

1-16 as originally filed

1 The following documents are referred to herein:

D1 - van Tricht, 2013

D2 - Berkowitz, 2007

D3 - Xiaoyu, 2008

D4 - Wright, 2014

D5 - Burnham, 2015 (incl. ref to De La Maza that shows that AAV particles can easily be separated in CsCl density gradients (p.229, col.2)). Note: applicant encloses D5 in his letter of 10.01.2022, yet it was already present as the fifth citation in the SR (hence D5).

D6 - WO2014/125101

EP3247793 is the publication number of present application and is repeatedly referred to in the 3rd party observations.

2 Application presently comprises a single independent claim:

1. A method of measuring the relative amount of capsid particles comprising variant recombinant viral genomes or empty viral capsid particles in a preparation of recombinant adeno-associated virus (AAV) particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),
c) integrating the area under each peak in the C(s) $[[C(S)]]$ distribution to determine the relative concentration of each species of recombinant viral particles,
d) comparing the amount of recombinant AAV particles having an S values that do not correspond to recombinant AAV particles comprising intact viral genomes to the amount of recombinant AAV particles having an S value that corresponds to recombinant viral particles comprising intact AAV genomes or to the total amount of recombinant AAV particles in the preparation.

The following analysis will focus on this independent claim.

3 Novelty (Art.54 EPC)

D6 discloses AUC based analysis of AAV viral particles. A detailed summary of D6 is found in the 3rd party observations filed on 23.07.2020. In his letter of 10.01.2022, applicant argues that D6 (erroneously referred to as D4) does not teach or suggest analytical ultracentrifugation under boundary sedimentation conditions. The 3rd party observation of 07.03.2023 contests this statement. AUC operates under either sedimentation velocity (SV) conditions (as claimed) or sedimentation equilibrium (SE) conditions. Application itself teaches that "*sedimentation velocity conditions*" or "*boundary sedimentation velocity conditions*" may refer to any experimental conditions under which a sample solution is subjected to sedimentation velocity analysis ([0076]), thus SV involves use of boundary conditions. SV is used for establishing sedimentation coefficients, whilst SE is used to derive molecular weights. As D6 clearly reports derivation of sedimentation coefficients (p.11, l.9; p.13, para.1) AUC in D6 must have been performed under SV conditions.

Unless, applicant can prove this analysis to be flawed, claim 1 must be considered as lacking novelty over D6.

4 Inventive Step (Art.56 EPC)

D1 discloses the assessment of the suitability of several techniques for the characterization of virus particle (in particular adenovirus) aggregation or integrity, including AUC under boundary conditions. Data analysis using SEDFIT/Lamb is shown just as in present application (see e.g. Fig.7 (p.23) - shows monomer v. dimer distribution). After assessing several techniques, the author of D1 concludes that AUC in SV mode (boundary conditions) is suitable

for the assessment of aggregation, but also the integrity (incompletes, empties) of viral particles, and is capable of both identifying and quantifying such aberrant particles (see Table 3).

D2 discloses monitoring of the heterogeneity of adenovirus preparations using AUC under boundary conditions. The method is used to assess lot-to-lot consistency of pharmaceutical preparations. On p.17 (col.2), examples of the kind of information that can be obtained is listed. Data analysis is by SEDFIT using c(s) or ls-g(s) methods. Fig.8C shows a data analysis performed in exactly the same manner as in present application with a c(s) v S plot. In the example provided, the plot identifies various multimeric forms of adenovirus particles in a preparation.

D3 is a further document applying AUC to determine the presence of empty capsids of adenovirus, in particular Ad5. The document states that AUC in CsCl density gradients should become a routine tool to evaluate the quality of rAd preparations (p.747, top. col.2). Empty capsids were determined by absorbance measurements at 320 / 260nm at 3000 / 40000rpm. Further optimization of centrifugation parameters was performed to maximize resolution between virus particle forms with similar size densities (i.e. slight different genome size) (see p.751, col.2). Since the data was not analysed by plotting c(s) v S, D3 is not considered novelty-destroying.

Applicant is considered to be addressing the problem of the provision of a method for the qualitative / quantitative assessment of particle heterogeneity (at aggregate or genome level) in AAV preparations. A skilled person starting with e.g. D1 or D2 as the closest prior art is well aware of the utility of AUC under boundary conditions for assessment of viral preparations. Both D1 and D2 actually show how aggregate compositions can be effectively determined using the methodology and D1 additionally states that integrity (incompletes / empties) can also be qualitatively and quantitatively assessed by the method. This is further shown in D3, which although not applying the same algorithm to the data analysis clearly shows that the techniques resolution is sufficient for resolving even small differences in genome size (using the example of two different adenovirus vectors with genomes of slightly differing sizes). The differences to the prior art are essentially only explicit listing of obvious features not specifically addressed but obviously within the realms of the disclosures in the prior art. These differences do not establish inventive activity. The fact that the method can achieve all that is claimed is thus immediately obvious to a skilled person viewing the prior art. Choice of exact algorithms or data analysis forms can not render the claimed subject-matter inventive, neither can the wording of

an intention falling within the generic characterization of particles which is well known to be within the capabilities of the technique. Applicant is deploying known publically available software using algorithms developed by other people. He has made no contribution in this respect. Further, it is obvious that various means of tracking movement of particles in the centrifuge are available (e.g. different speeds, wavelengths etc.) or that the methodology can apply to any type of particle (since the method obviously is for distinguishing particle size / density and this would not be expected to work any differently for other viruses). This also applies to the application to viruses made by different recombinant techniques. Hence, application totally devoid of inventive subject-matter.

Applicant argues that due to the fact that AAV is far smaller (5-10x) than Adenovirus, a skilled person would not expect the method applicable to Adenovirus in D1-D3 to function for AAV. However, D6 appears to directly teach that AUC under boundary conditions works for AAV.

He also argues that the level of accuracy achieved is surprising. The level of accuracy is however merely the inevitable result of using the method. Further, and most critically, using applicants post-published document D5 as technical evidence one finds that a 1980 paper by De La Maza is referenced that shows that AAV particles can easily be separated in CsCl density gradients (p.229, col. 2). Even genomes of varying sizes could be distinguished so it is clear that full and empty particles would be very easy to distinguish on this basis. Any virus particles that can be separated by CsCl density gradient obviously can be resolved by AUC since the method effectively is a modern real-time form of readout of the movement of particles in a density gradient. Hence, the question is merely whether a skilled person would consider applying the claimed methods to AAV. The answer is clearly yes, since distinguishing empty from full virus particles is important for quality control in any virus type and rAAV is one of the primary virus types currently being developed for gene delivery and also suffers from this issue (see e.g. D4).