BATCH CHROMATOGRAPHY USING CAPTO CORE 700 FOR INFLUENZA VIRUS PURIFICATION

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Vaccination is the most effective protective measure against aggressive forms of influenza virus infections. The most common technology to is the one that uses embryonated chicken eggs for viral multiplication, despite it's drawbacks, like the ovalbumin content, which poses a risk for allergic reactions. Therefore, ovalbumin reduction is a crucial step in the egg-based technology. It is usually done by ultracentrifugation in a density gradient, followed by a filtration technique. In this study, we tested the ovalbumin reduction efficiency of Capto Core 700 resin in batch chromatography, compared to isopycnic ultracentrifugation.

Keywords: Influenza, Ovalbumin, Isopycnic ultracentrifugation, Batch chromatography.

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1. Introduction

Influenza virus is one of the most common causes for respiratory infections, being a constant health threat, because of its seasonal character [1], antigenic protein variability [2] and the tendency to suffer genetic mutations, like shifting and drifting [3]. It poses a high risk for epidemics and even pandemics. Globalization and the climate changes [4], especially the rapid weather changes we face today, also contribute to the probability of often outbreaks. This increasing risk makes it crucial to have a fast, safe and efficient response, through vaccination, combined with a cost-effective technology [5].

The most widespread technology is the egg-based one [5], [6], that has been used for decades, thus its effectiveness and general response have been studied widely over the years [7]. A modern approach is the cell-based technology, that has been already adopted vaccine manufacturers [8], thanks to the shorter period of production and the lack of ovalbumin, the main drawback [9] in the egg-based technology, since it can produce allergic reactions. Although the viral multiplication using cell cultures is an important step in modernizing the vaccine manufacturing technologies [10], the egg-based one is still the reference in flu vaccine production. The ovalbumin is reduced by a series of purification steps, usually consisting in density gradient ultracentrifugation [11], followed by filtration or it can be reduced by using chromatographic techniques[12], that usually involves the use of chromatographic column, beads and resin [13].

Capto Core 700 (CC700) is a chromatographic medium, used to purify large molecules and virus particles [14]. It is composed of beads with an inactive outer layer and an active multimodal core, with pores with an exclusion limit of 700 kDa, permitting small molecules, like ovalbumin to enter its core, while the viral particles remain in suspension. It's efficiency in ovalbumin reduction has been previously proven in flowthrough chromatography, compared to gradient density gradient ultracentrifugation [15].

In this study, isopycnic ultracentrifugation (IUC) combined with batch chromatography using Capto Core 700 was established for the whole live virion as the purification method in the downstream influenza vaccine processing. Batch chromatography represents a first step in implementing a chromatographic purification method. During this process, the resin's behavior in contact with the viral solution is observed and it's protein retaining capacity is evaluated through comparison with the viral suspension purified by density gradient centrifugation. In order to determine the efficiency of the resin on ovalbumin reduction, protein concentration, hemagglutinin and ovalbumin concentrations were evaluated and compared.

2. Materials and Methods

2.1. Virus production

Three batches, each of 10000 embryonated chicken eggs were inoculated, separately, with H1N1, H3N2 and B strains, using the RAME-HART automatic inoculation machine. After inoculation, the eggs were incubated for 72 h, at 34 ºC, with 50%-60% humidity. Viral multiplication was stopped using thermic shock, placing the eggs at 4 ºC for 24 h. An automatic machine was used to harvest the infected allantoic fluid, that was then filtered before clarification, using a two-filter system with membrane pores of 50 µm and 5 µm. Clarification was performed by centrifugation at 15000 RPM, using a centrifuge from Powerfuge Systems.

2.2. Isopycnic centrifugation

A first purification was performed by isopycnic centrifugation at 35000 RPM, using a continuous flux KII ultracentrifuge from Alfa Wassermann. The density gradient was formed using a 60% sucrose solution and PBS (Phosphate-Buffered Saline). After ultracentrifugation, the fractions were collected based on their density and were combined with an equal volume of PBS, resulting in a volume of 3000 mL. A part of this volume was later processed in the size-exclusion chromatography experiment.

2.3. Batch chromatography

Batch chromatography was performed using Capto Core 700, in three different proportions: 1:5, 1:10 and 1:20. The viral suspension previously purified and inactivated acts as control. The resin used was commercialized with 20% ethanol as a preservative, which was discarded before use and the resin was washed 3 times with PBS. Three glass beaks were prepared, with 33 mL of washed resin in each one, adding the viral suspension as follows: in the first beak, corresponding to 1:20, were added 500 mL, in the 1:10 beak were added 250 mL and 125 mL were added to the 1:5 beak, as can be seen in Table 1.

Table 1

Resin distribution and concentration

Proportion	Resin volume (mL)	Purified viral suspension (mL)
1:20	33	500
1:10	33	250
1:5	33	125

The beaks were kept at room temperature for 2 h, with gentle agitation, to keep the resin from sedimentation without deteriorating it. After 2 h, the agitation was stopped, and approximately 10 minutes after, the resin hardened. The purified viral suspension washed with Capto Core 700 was collected using a vacuum flask.

The resin was collected and washed and the purified suspension was inactivated with a formaldehyde solution, before entering the diafiltration step.

Diafiltration was performed using tangential flow filtration (TFF), with a 80 kDa cut-off hollow fiber cartridge.

2.4. Analysis

All analytical determinations (protein content, ovalbumin concentration and hemagglutinin quantification) were performed on three batches, one for each strain. Egg-based influenza vaccine manufacturing inherently produces one biological lot per strain, and downstream purification steps apply to this unique batch. Therefore, the three Capto Core 700 conditions and the IUC control represent parallel processing variants of the same batch, not independent biological replicates.

Protein concentration, ovalbumin and hemagglutinin concentration were determined for all three variants of the CC700 experiment and for the ultracentrifuged suspension. The analysis was done for all three of the viral strains used. Protein concentration was determined using the Lowry protein assay. Both the ovalbumin and hemagglutinin concentrations were determined by Single Radial ImunoDiffusion (SRID) according to the European Pharmacopoeia guidelines [16].

B strain purification was evaluated based on the ovalbumin concentration, total protein concentration and hemagglutinin titer, measured using the hemagglutination assay.

The ovalbumin concentration was determined for the diafiltered suspension, to determine the impact of the chromatography on the next step of the downstream processing.

3. Results

Because all measurements originate from a single production batch per strain, each value represents a unique analytical result for that batch and cannot be averaged. Independent biological replicates would require the production of additional vaccine lots, which was beyond the scope of this study. The comparisons shown represent analytical differences between purification variants and the efficacy of batch chromatography between the three strains, not statistical differences across multiple batches. Best ovalbumin reduction in H1N1 was observed in the 1:5 variant, approximately 3 μg/mL, with 1:10 being slightly increased (3.72 μg/mL). 1:20 registered a concentration of 6.01 μg/mL. In comparison, the viral suspension purified by ultracentrifugation had an ovalbumin concentration of 16.32 μg/mL. For H3N2, the lowest ovalbumin concentration was also in the 1:5 variant, approximately 10.16 μg/mL, while the IUC suspension has a concentration of 13.53 μg/mL. A lower concentration, compared to the control, was registered in the 1:10 variant, with 10.29 μg/mL and 1:20, that registered a concentration of 12.82 μg/mL. For the B strain, the ovalbumin reduction started

from the 16.95 µg/mL, the ovalbumin concentration in the IUC suspension. From the Capto Core 700 variations, the lowest ovalbumin concentration was observed in the 1:5 variant, approximately 4.7 µg/mL. 1:10 registered a concentration of 5.6 µg/mL, while 1:20 registered a concentration of 8.46 µg/mL.. A comparative graphic can be seen in Fig.1

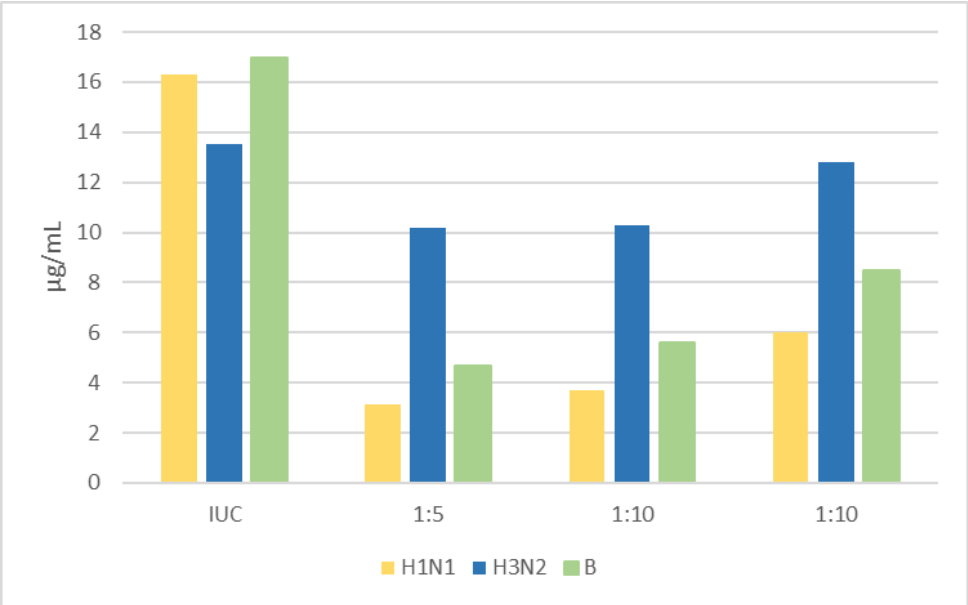

Fig. 1 Ovalbumin concentration - H1N1, H3N2 and B

Total protein concentration was evaluated and Capto Core 700 was compared to the viral suspension purified by isopycnic ultracentrifugation (IUC). The lowest ovalbumin concentration was registered in the 1:5 variant for all three strains. For H1N1, the protein content is decreasing gradually from the IUC purified suspension, to the 1:5 CC700. IUC protein concentration was 1665.95 µg/mL. 1:20 registered a protein concentration of 1254.59 µg/mL, followed by 1:10, with 1028.38 µg/mL and 1:5, with 795.68 µg/mL. (Fig. 2 below).

For H3N2, IUC suspension protein concentration was 2276.22 µg/mL. 1:20 registered a protein concentration of 1930 µg/mL, followed by 1:10, with 1793.24 µg/mL and 1:5, with 1684.05µg/mL. (Fig.3 below).

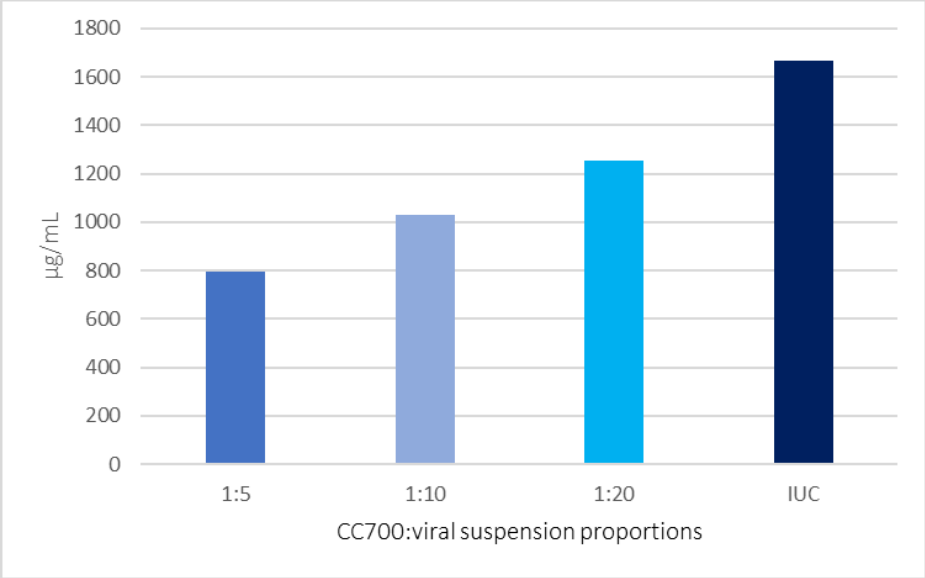

Fig. 2 Total protein concentration - H1N1

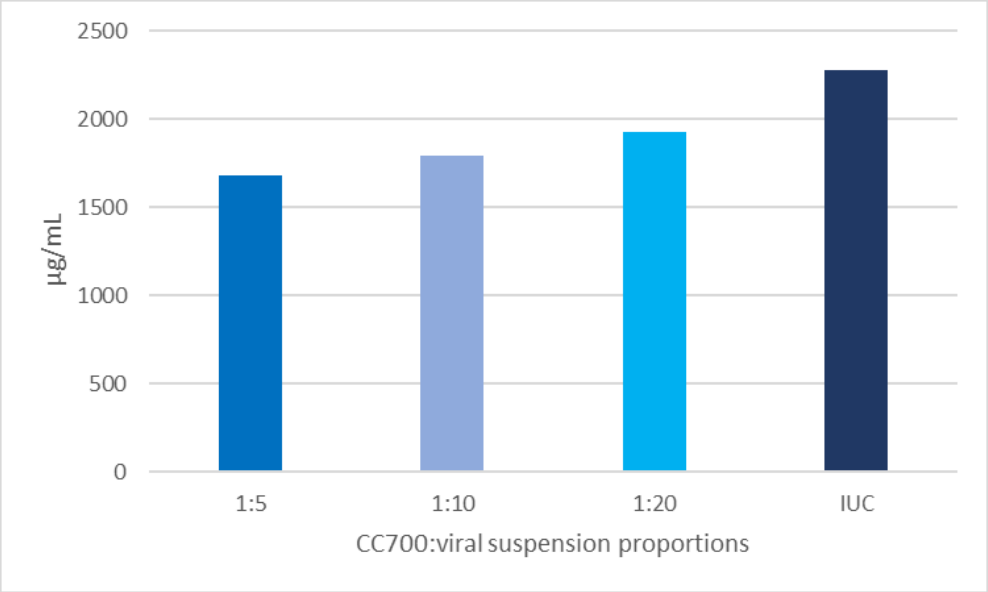

Fig. 3 Total protein concentration - H3N2

For B, the lowest ovalbumin concentration, corresponding to 1:5, was 1162.43 µg/mL. 1:20 registered a protein concentration of 1335.41 µg/mL, followed by 1:10, with 1265.14 µg/mL. IUC suspension registered a protein concentration of 1517.84 µg/mL. (Fig.4 below).

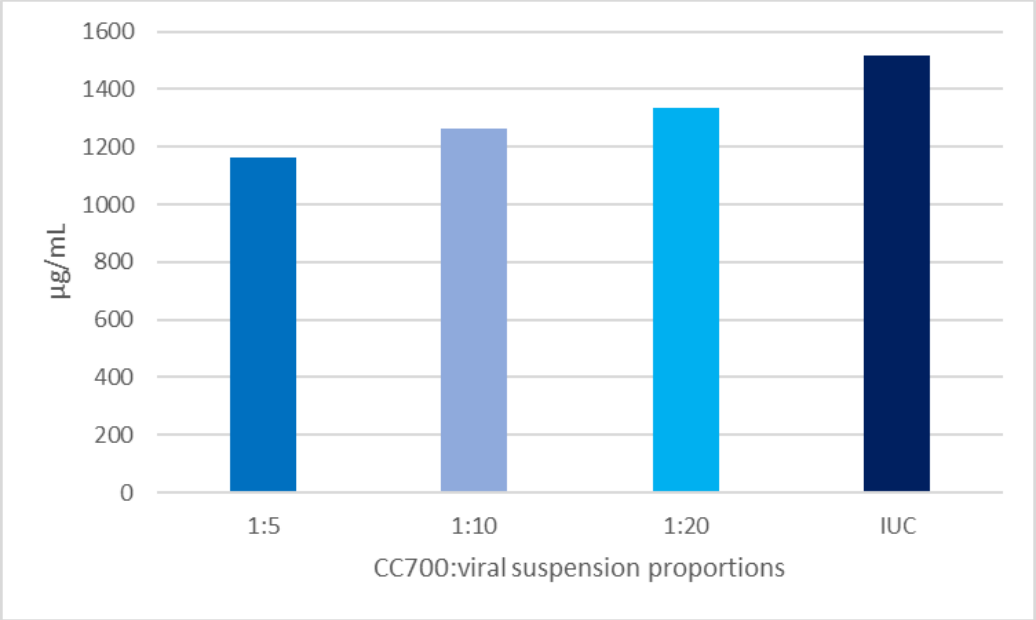

Fig. 4 Total protein concentration - B

Total protein assay and ovalbumin concentration indicated 1:5 to be the best experimental variant. Hemagglutinin content was evaluated, to determine the loss of antigenic protein in both of the purification methods and between experimental variations and strains. Hemagglutinin concentration was determined for the IUC and 1:5 variant. For H1N1, about 28% of the initial hemagglutinin was lost during chromatography, from 157.08 µg/mL (IUC), to 112.92 µg/mL (1:5). For H3N2, about 28% of the initial hemagglutinin was lost during chromatography, from, 522.78 µg/mL (IUC), with 348.85 µg/mL (1:5).

Hemagglutination assay was conducted to determine the hemagglutinin titer of the 1:5 experimental variant and control, for the B strain. The titer was constant throughout the variants and the control, with 4096 UHA/50µl for each one.

After the suspension was filtered using TFF, the ovalbumin concentration was determined for the control and 1:5 variant. For all of the strains, diafiltered 1:5 registered a low concentration of ovalbumin, not detectable using the SRID assay. For H3N2, ovalbumin concentration of the IUC viral suspension decreased from 13.53 µg/mL to 6.58 µg/mL after diafiltration (51% decrease). For H1N1, the

ovalbumin concentration of the suspension purified by ultrafiltration decreased from 16.32 µg/mL to 16.15 µg/mL after diafiltration (1% reduction). For B, diafiltration lead to a decrease of 26% in ovalbumin concentration, from 16.95 µg/mL to 12.53 µg/mL.

4. Discussion

This study was conducted to determine the ovalbumin reduction capacity of CC700 in batch chromatography, compared to IUC. Although little data is available on batch chromatography as a viral purification method, it represents a starting point in implementing a continuous, flowthrough chromatography protocol, and the results obtained are extremely valuable. Literature data found on ovalbumin reduction using flowthrough chromatography are consistent with the results obtained in this study, using batch chromatography, but with a higher reduction rate of the ovalbumin. Flowthrough chromatography, applied on clarified allantoic fluid showed a ninefold ovalbumin reduction, compared to ultracentrifugation [15]. In comparison, batch chromatography applied on the ultracentrifuged suspension resulted in a five-times lower ovalbumin concentration, compared to IUC. Moreover, some studies indicate that flowthrough chromatography can improve the hemagglutinin recovery rate, compared to ultracentrifugation techniques [17].

5. Conclusions

This study demonstrates that Capto Core 700 batch chromatography, applied after isopycnic ultracentrifugation, substantially improves ovalbumin reduction for all influenza strains tested. The 1:5 resin-to-suspension ratio proved the most efficient, achieving up to fivefold reduction compared to IUC, while maintaining acceptable hemagglutinin recovery. The improved purity also enhanced the efficiency of subsequent diafiltration, lowering ovalbumin to levels undetectable by SRID.

H1N1 and B were significantly improved by the additional purification step, with ovalbumin concentration in 1:5 being 5 times lower than that of IUC for H1N1 and almost 4 times lower than IUC for B. H3N2 registered the lowest impact in ovalbumin reduction of the three strains. The chromatography impacts the diafiltration process, leading to a further ovalbumin reduction, to a level undetectable by the SRID assay, in 1:5. Although the ovalbumin reduction is the most effective with the 1:5 proportion, it is associated with the loss of hemagglutinin.

Additional data is needed on both batch chromatography, as well as flowthrough chromatography, in order to create an effective and robust purification process that can positively impact the product’s quality.

Overall, the results show that CC700 batch chromatography is a practical and high-performance alternative for impurity removal in egg-based influenza vaccine production, offering a clear advantage over density-gradient ultracentrifugation alone.

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R E F E R E N C E S

[1] *G. Neumann and Y. Kawaoka*, "Seasonality of influenza and other respiratory viruses," *EMBO Mol Med*, vol. **14**, no. 4, Apr. 2022, doi: 10.15252/emmm.202115352.

[2] *J. Chen, Y. M. Deng*, "Influenza virus antigenic variation, host antibody production and new approach to control epidemics," *Virol J*, vol.**6**, 2009, doi:10.1186/1743-422X-6-30.

[3] *H. Kim, R. G. Webster, and R. J*. Webby, "Influenza Virus: Dealing with a Drifting and Shifting Pathogen," *Viral Immunol*, vol. **31**, no. 2, pp. 174–183, Mar. 2018, doi: 10.1089/vim.2017.0141.

[4] *W. Ruan, Y. Liang, Z. Sun, and X. An*, "Climate warming and influenza dynamics: the modulating effects of seasonal temperature increases on epidemic patterns," *NPJ Clim Atmos Sci*, vol. **8**, no. 1, Dec. 2025, doi: 10.1038/s41612-025-00968-3.

[5] *D. Gupta and S. Mohan*, "Influenza vaccine: a review on current scenario and future prospects," Dec. 01, 2023, *Springer Science and Business Media Deutschland GmbH*. doi: 10.1186/s43141-023-00581-y.

[6] *S. S. Wong and R. J. Webby*, "Traditional and new influenza vaccines," *Clin Microbiol Rev*, vol. **26**, no. 3, pp. 476–492, Jul. 2013, doi: 10.1128/CMR.00097-12.

[7] *Y. H. Kim, K. J. Hong, H. Kim, and J. H. Nam,* "Influenza vaccines: Past, present, and future," Jan. 01, 2022, *John Wiley and Sons Ltd*. doi: 10.1002/rmv.2243.

[8] *S. Rajaram, C. Boikos, D. K. Gelone, and A. Gandhi*, "Influenza vaccines: the potential benefits of cell-culture isolation and manufacturing," 2020, *SAGE Publications Ltd*. doi: 10.1177/2515135520908121.

[9] *I. Manini, C. M. Trombetta, G. Lazzeri, T. Pozzi, S. Rossi, and E. Montomoli,* "Egg-independent influenza vaccines and vaccine candidates," Jul. 18, 2017, *MDPI AG*. doi: 10.3390/vaccines5030018.

[10] *T. Zinnecker, U. Reichl, and Y. Genzel*, "Innovations in cell culture-based influenza vaccine manufacturing–from static cultures to high cell density cultivations," 2024, *Taylor and Francis Ltd.* doi: 10.1080/21645515.2024.2373521.

[11] *C. B. Reimer,', R. S. Baker, R. M. Vanfrank*, *T. E. Newlin, G. B. Cline, and N. G. Anderson*, "Purification of Large Quantities of Influenza Virus by Density Gradient Centrifugation," 1967. [Online]. Available: https://journals.asm.org/journal/jvi

[12] *T. Weigel, T. Solomaier, A. Peuker, T. Pathapati, M. W. Wolff, and U. Reichl*, "A flow-through chromatography process for influenza A and B virus purification," *J Virol Methods*, vol. **207**, pp. 45–53, Oct. 2014, doi: 10.1016/j.jviromet.2014.06.019.

[13] *Yu-Fen Tseng, Tsai-Chuan Weng, Chia-Chun Lai, Po-Ling Chen, Min-Shi Lee, and Alan Yung-Chih Hu*, "A fast and efficient purification platform for cell-based influenza viruses by

flow-through chromatography", *Vaccine*, vol. **36**, no. 22, May 2018, doi: 10.1016/j.vaccine.2017.03.016.

[14] *K. T. James et al.,* "Novel High-throughput Approach for Purification of Infectious Virions," *Sci Rep*, vol. **6**, Nov. 2016, doi: 10.1038/srep36826.

[15] *H. Blom, A. Åkerblom, T. Kon, S. Shaker, L. van der Pol, and M. Lundgren*, "Efficient chromatographic reduction of ovalbumin for egg-based influenza virus purification," *Vaccine*, vol. **32**, no. 30, pp. 3721–3724, Jun. 2014, doi: 10.1016/j.vaccine.2014.04.033.

[16] "*European Pharmacopoeia* (Ph. Eur.)," 7.0., 2011, ch. 2.7. "General Analitycal Methods".

[17] *N.N. Asanzhanova, Sh. Zh. Ryskeldinova, O. V. Chervyakova, B. M. Khairullin, M. M. Kasenov and K. K. Tabynov*, "Comparison of Different Methods of Purification and Concentration in Production of Influenza Vaccine," *Bulletin of Experimental Biology and Medicine,* vol. **164**, no. 2, Dec. 2017, doi: 0.1007/s10517-017-3964-y