

## Chemistry in life sciences

### Biocompatible excipients to enhance the stability of avian immunoglobulin Y (IgY) antibodies, envisaging their use as biopharmaceuticals

Almeida, C.A.S.<sup>a</sup>; Silva, L.C.V.<sup>a</sup>; Silva, M.C.<sup>a</sup>; Neves, M.C.<sup>a</sup>; Freire, M.G.<sup>a</sup>

a) CICECO- Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal

Email: [ac.almeida@ua.pt](mailto:ac.almeida@ua.pt)

Immunoglobulins, also termed antibodies, are glycoproteins produced by jawed vertebrates, providing them immunity against bacteria, viruses, and other foreign agents<sup>1</sup>. Within the known numerous immunoglobulins classes, avian immunoglobulin Y (IgY), that can be found in the serum of chickens and other egg laying animals and also in egg yolk, is a promising antibody<sup>2</sup>.

In contrast to its mammalian analogous immunoglobulin G (IgG), IgY exhibits several advantages, among them high immunogenicity and binding avidity, and the capacity to be recovered by a non-invasive method from egg yolk at high yields<sup>3-5</sup>. The amount of IgY antibodies obtained from an egg is equal to that from 200-300 mL of mammalians blood, being possible for a hen to lay approximately 300 eggs *per* year and to produce 17 to 35 g of total IgY<sup>3,6</sup>. Furthermore, IgY is a polyclonal antibody, being capable to recognize more epitopes on an antigen<sup>4</sup>. As such, IgY is a promising candidate to be engaged in various applications, such as in research, diagnosis, and in the treatment and prophylaxis of several diseases, for instance bacterial infections<sup>3,4</sup>. However, by being proteins present in a complex media such as egg yolk, the use of IgY antibodies as biopharmaceuticals is restricted by their recovery at high yields and high purity, together with their preservation<sup>2</sup>.

IgY antibodies were isolated from the yolk of commercial chicken eggs and then purified by two precipitation steps. The purity degree and recovery yields of the obtained IgY antibodies were evaluated by Size Exclusion- High Performance Liquid Chromatography (SEC-HPLC) and dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), being > 98% and > 85%, respectively. Several carbohydrates and polyols were studied as alternative stabilizers of IgY antibodies. The stability of IgY was evaluated in different storage time, temperature, and pH conditions by several techniques, among them SEC-HPLC and Circular Dichroism Spectroscopy (CD).

Novel bio-based compounds have been identified as promising stabilizers to improve the stability of avian immunoglobulin Y antibodies, paving the way for their usage as excipients in IgY therapeutic formulations.

#### Acknowledgements:

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Fundação para a Ciência e Tecnologia/ Ministério da Educação e Ciência (FCT/MEC) and when appropriate co-financed by Fundo Europeu de Desenvolvimento Regional (FEDER) under the PT2020 Partnership Agreement. M.G. Freire acknowledges the European Research Council (ERC) for the Grant ERC-2020-PoC-899921. Márcia C. Neves acknowledges FCT, I.P. for the research contract CEECIND/00383/2017 under the CEEC Individual 2017.

#### References:

1. Sun, Yi, Zhancai Liu, Liming Ren, Zhiguo Wei, Ping Wang, Ning Li, and Yaofeng Zhao. *Journal of Animal Science and Biotechnology*. **2012**, 3, 1-5.
2. Kovacs-Nolan, Jennifer, and Yoshinori Mine. *Avian and Poultry Biology Reviews*. **2004**, 15, 25–46.
3. Pereira, E. P.V., M. F. van Tilburg, E. O.P.T. Florean, and M. I.F. Guedes. *International Immunopharmacology*. **2019**, 73, 293–303.
4. Kovacs-Nolan, Jennifer, and Yoshinori Mine. *Annual Review of Food Science and Technology*. **2012**, 3, 163–82.
5. Zhang, Wei Wei. *Drug Discovery Today*. **2003**, 8, 364–71.
6. Larsson, Anders, Ros-mari Balow, Tomas L Lindahl, and Per-Olof Forsberg. *Poultry Science*. **1993**, 72, 1807–12.