



# THE STUDY OF VIRUS-ANTIBODY INTERACTION BY CAPILLARY ELECTROPHORESIS (CE)



S. S. Beloborodov,<sup>a</sup> V. G. Panferov,<sup>b</sup> I. V. Safenkova,<sup>b</sup> S. M. Krylova,<sup>a</sup> B. B. Dzantiev,<sup>b</sup> and S. N. Krylov<sup>a</sup>



<sup>a</sup> Department of Chemistry, York University, Toronto, ON, Canada

<sup>b</sup> Russian Academy of Sciences, A.N. Bach Institute of Biochemistry, Moscow, Russian Federation



## Overview

In this work, complex formation between potato virus X (PVX) and monoclonal antibodies (mAb) was studied by CE with light-absorption detection (Figure 1). The determined  $EC_{50}$  value was around 15 nM of PVX (at 6.4  $\mu$ M mAb). The maximum binding stoichiometry was found to be in a range of 320-250 mAb units per virus particle which is close to the value obtained for the similar Tobacco Mosaic Virus (TMV).<sup>1</sup> The study of PVX-mAb complexes by Transmission Electron Microscopy (TEM) revealed that the complexes constituted large agglomerates of PVX-virions crosslinked with mAb. This data is in agreement with the published structure of TMV-Ab complexes.<sup>2</sup> Unexpectedly, it was observed that the PVX-mAb complexes migrated slower than the unbound PVX and mAb, which is atypical in CE.

## Introduction

Potato virus X is a member of the genus Potexvirus, family Flexiviridae, containing about 40 species. Potexvirus virions are flexible cylinders of various lengths (470-580 nm) and a diameter of 13 nm. The molecular weight of virions is around 35,000 kDa. RNA/Protein weight ratio is 6/94. Each virion contains around 1,300 identical coat protein (CP) subunits (8.9 CP subunits per turn of primary helix). In this study, we used PVX as a model to investigate the interaction between a virus and an antibody with CE.

## Methods

All CE experiments were done with a P/ACE MDQ instrument from Beckman Coulter (Fullerton, CA, U.S.) with light-absorption detection at 260 and 280 nm using a PDA detector. An uncoated fused silica capillary (total length of 80 cm) was used. The samples (PVX and mAb alone as well as PVX-mAb mixtures) were injected by pressure (0.5 psi for 5 s) and separated at an electric field of 310 V/cm at 25 °C. 50 mM Tris-HCl, pH 7.5 was used as a running and dilution buffer. Originally PVX and mAb stored in PBS buffer (with addition of 50%<sup>vol</sup> glycerol in a case of PVX).

## Results and Discussion

Under the influence of an electric field the virus and antibodies (mAb) migrated as individual zones with close values of electrophoretic mobilities ( $\mu$ ). Multiple complexes formed by mixing antibodies with PVX migrated slower than unbound PVX and mAb (complexes had a greater  $\mu$  value, Figure 1).

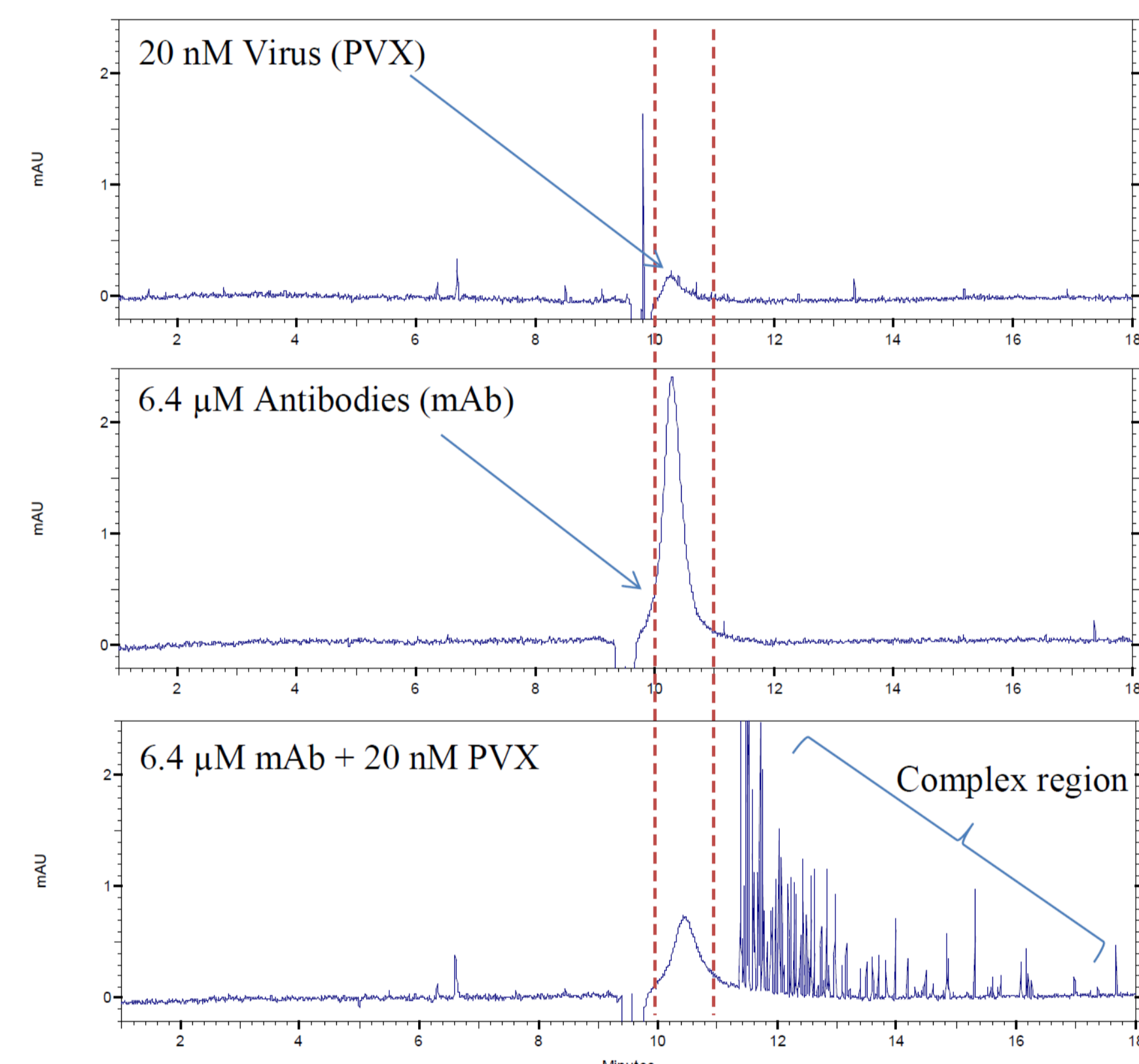


Figure 1. Migration of the virus (PVX), antibodies (mAb) and PVX-mAb complexes in CE.

This is unexpected, because usually the electrophoretic mobility of the complex lies between motilities of the components involved in complex formation. It can be illustrated in the example showing the interaction between the spherical particles (Figure 2):

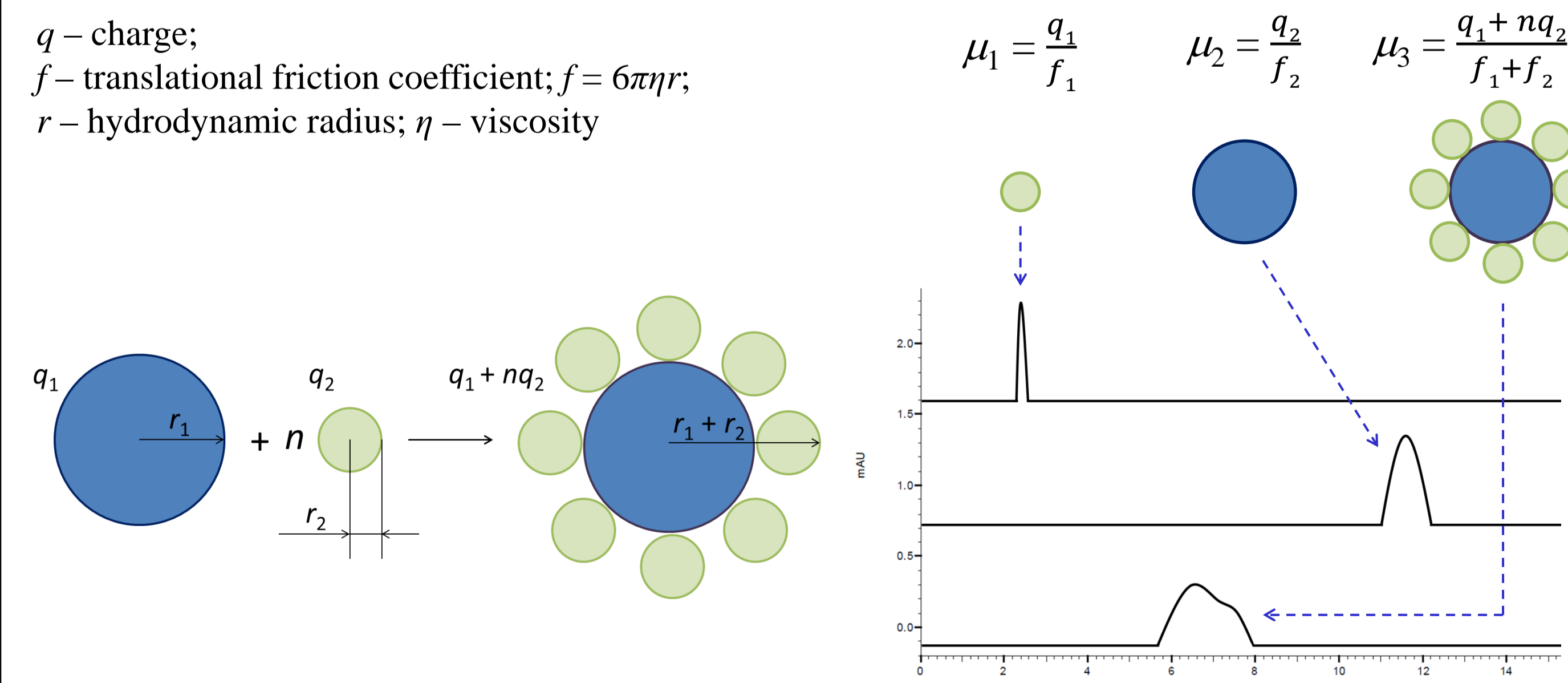


Figure 2. Electrophoretic mobility as the ratio of the total charge to the sum of frictional coefficients.

However, in a case of cylindrical particles, the frictional coefficient  $f$  depends on parameter  $p$  (ratio of cylinder length ( $L$ ) to its diameter ( $d$ )):<sup>3</sup>

$$f = 6\pi\eta_0 L (3/16p^2)^{1/3} (1.009 + 1.395 \times 10^{-2} (\ln p) + 7.880 \times 10^{-2} (\ln p)^2)$$

From this dependence, the frictional coefficient grows with increasing  $p$ ; accordingly, the electrophoretic mobility decreases with increasing  $p$ . Let's assume the mechanism of aggregation in which virus particles stick together forming cylinders with lower  $p$  than PVX by itself (Figure 3). Such kind of complexation should result in an increase in electrophoretic mobility of the complexes and the peak order in electropherogram presented in Figure 1.

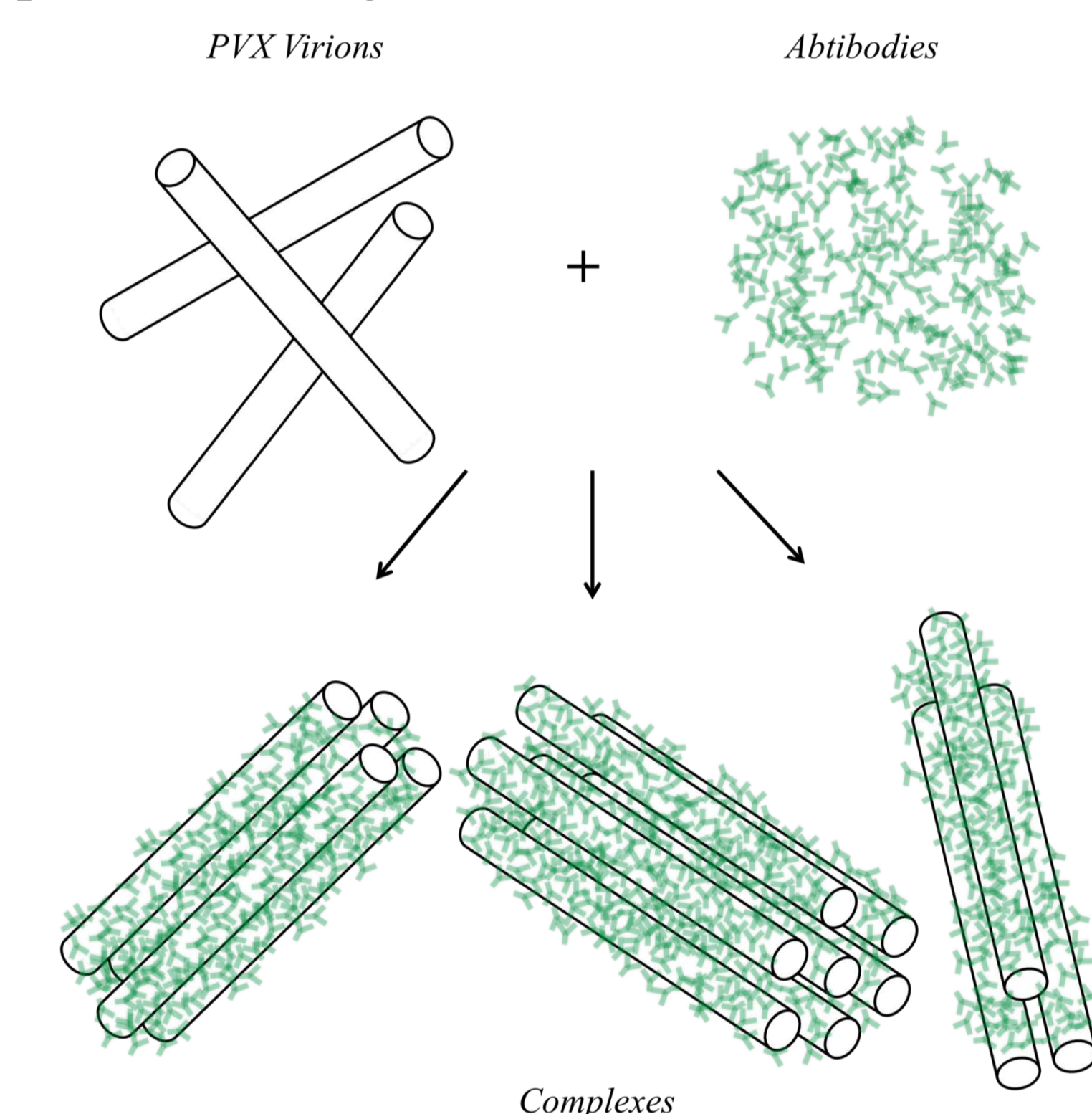


Figure 3. The formation of cylinder-type complexes between PVX and mAb.

## Conclusion

We found an explanation for the unusual peak order in the complex formation electropherogram which is in consistent with previously published theory that predicts behaviour of cylinder particles in a hydrodynamic flow.

## References

1. J. L. Pellequer and M. H. V. Regenmortel. *J. Imm. Met.* 1941, 166: 133-143
2. T. F. Anderson and W. M. Stanley. *J. Biol. Chem.* 1941, 139: 339-344
3. A. Ortega and J. Garcia de la Torre *J. Chem. Phys.* 2003, 119: 9914 – 9919