Insulin aggregation assessment by size-exclusion chromatography and capillary gel electrophoresis

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1) INTRODUCTION

Insulin aggregates could be formed during production and storage of insulin formulation and may lead to complications in insulin therapy. Size-exclusion chromatography (SEC) is the method of choice for the analysis of protein aggregates of biopharmaceuticals. Currently the **United Sates and European Pharmacopoeias** use a **SEC method with acidic mobile phase** to quantify those aggregates in insulin formulations. Changes in aggregates assessment have already been reported when the mobile phase composition differs from sample dissolution medium. For that reason, the influence of mobile phase in SEC and of sieving gel in capillary gel electrophoresis (CGE) during the analysis of insulin aggregates was investigated.

2) RESULTS

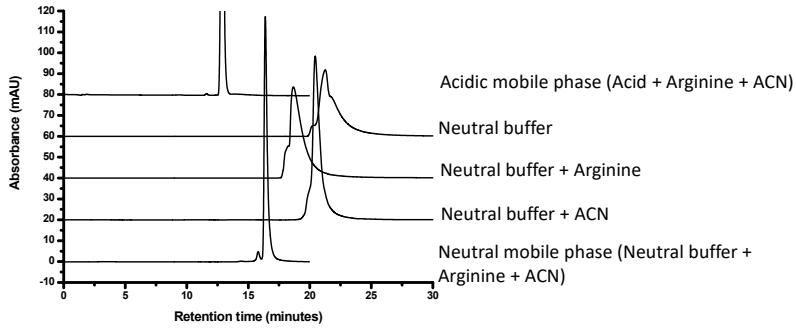

Fig. 1. Influence of mobile phase composition on SEC profiles

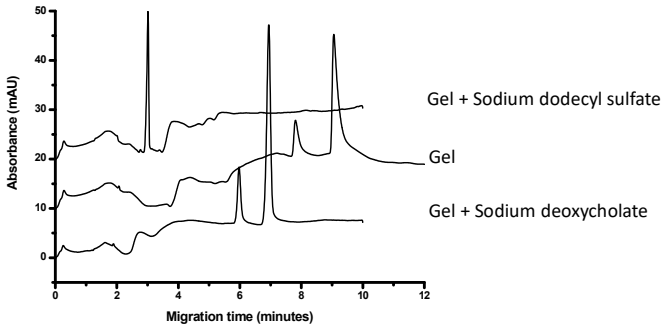

Fig. 2. Influence of sieving gel on CGE profile

The influence of mobile phase adjuvants on SEC profile (**Fig. 1.**) was observed by injecting the same sample using five mobile phases. The adjuvants added to neutral buffer were responsible for changes in profile and in aggregates percentage. In CGE (**Fig. 2.**), SDS was found to disrupt non-covalent aggregates. The addition of sodium deoxycholate was useful to reduce migration time and improve peak shape.

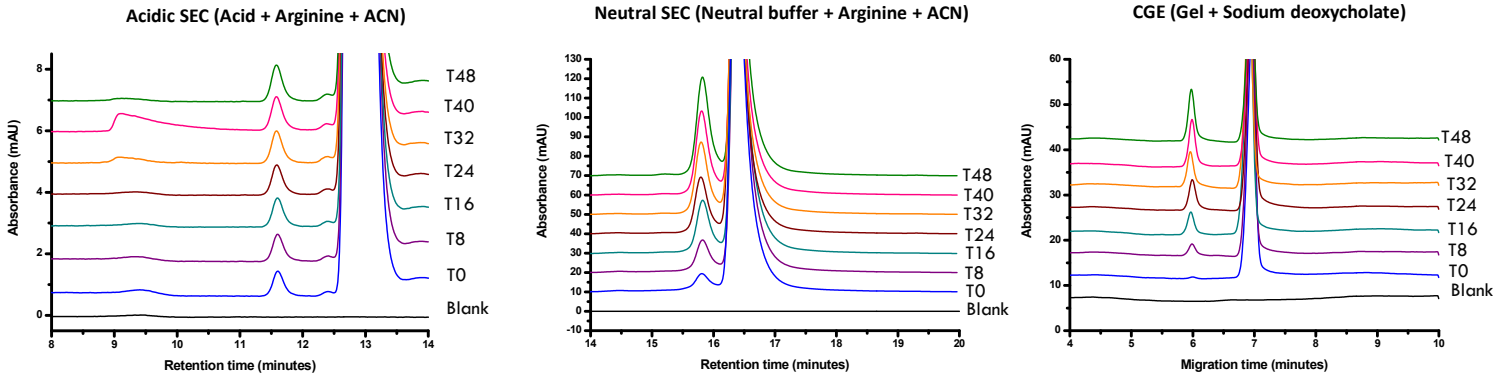

Fig. 3. Insulin before and after 8,16,24,32,40 and 48 hours of agitation analyzed by acidic SEC, neutral SEC and CGE

Insulin aggregation was studied by SEC in neutral and acidic mobile phase conditions and by CGE (**Fig. 3.**). A similar increase of dimers percentage with incubation time was noticed by both neutral SEC and CGE, while no significant increase of dimers content was observed by acidic SEC. However, an insulin polymeric complex was observed for some samples with acidic SEC.

3) CONCLUSIONS

- In SEC, the impact of mobile phase composition on aggregation profile was confirmed.
- In CGE, the disruption of insulin aggregates by sodium dodecyl sulfate was observed. A fast CGE method using sodium deoxycholate for the determination of insulin aggregates was developed.
- **A clear difference in aggregates assessment between acidic and neutral SEC was observed.** This could be explained by the difference in pH or ionic strength of the mobile phase.
- Neutral SEC gave similar results to CGE, a complementary technique.

Those results raise question on the adequacy of the actual acidic pharmacopoeias method to analyze neutral insulin formulations.

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