

APPLICATION OF SAM-FLEX Technology for protein adsorption on aluminum gels (vaccine Production)

Vaccine adjuvants are essential for the effectiveness of many vaccines. In fact, in their absence, the injected antigens, for example peptides or haptens, do not generate a sufficiently large immune response.
Ones of the most widely used adjuvants are aluminum salts such as aluminum oxyhydroxide (AlOOH).

When manufacturing vaccines, proper adsorption of the hapten-carrying proteins on the aluminum gel is essential. Measurement of this parameter will have several advantages. The two main ones are :

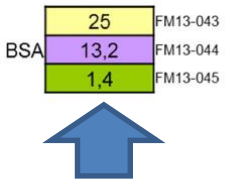
- The guarantee of optimal efficiency during the injection
- Limitation of aluminum salts concentration in the final product.

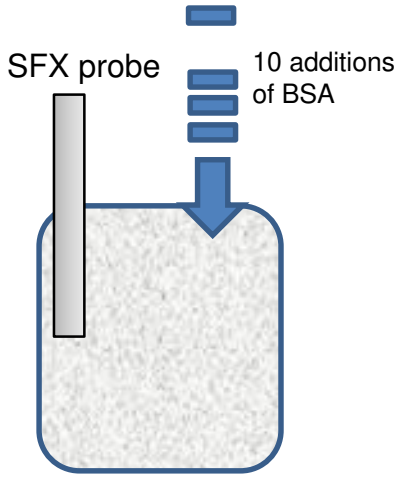
SCOPE of the study

Develop a method based on SAM-Flex technology in order to predict adsorption of protein model BSA on ALOOH. Several factors have been taken into account. :

- Water for injection concentration
- ALOOH concentration
- Protein concentration (0 à 400µg/ml)
- Temperature (+5 à 25°C step by 5°C)

T°	Exp	AlOOH (ml)	Eau (ml)	BSA (ml) total (volume cumulé des 10 ajouts)	Volume pour 1 ajout de BSA (ml)
+15°C	1	379,9	120,1	117,4	11,7
+15°C	2	379,9	120,1	117,4	11,7
+25°C	3	269,61	230,39	785,7	78,6
+5°C	4	490,2	9,8	1428,6	142,9
+25°C	5	490,2	9,8	80	8
+15°C	6	379,9	120,1	117,4	11,7
+5°C	7	269,61	230,39	44	4,4





BSA was added with different concentration levels. Each step = 40µg/ml.

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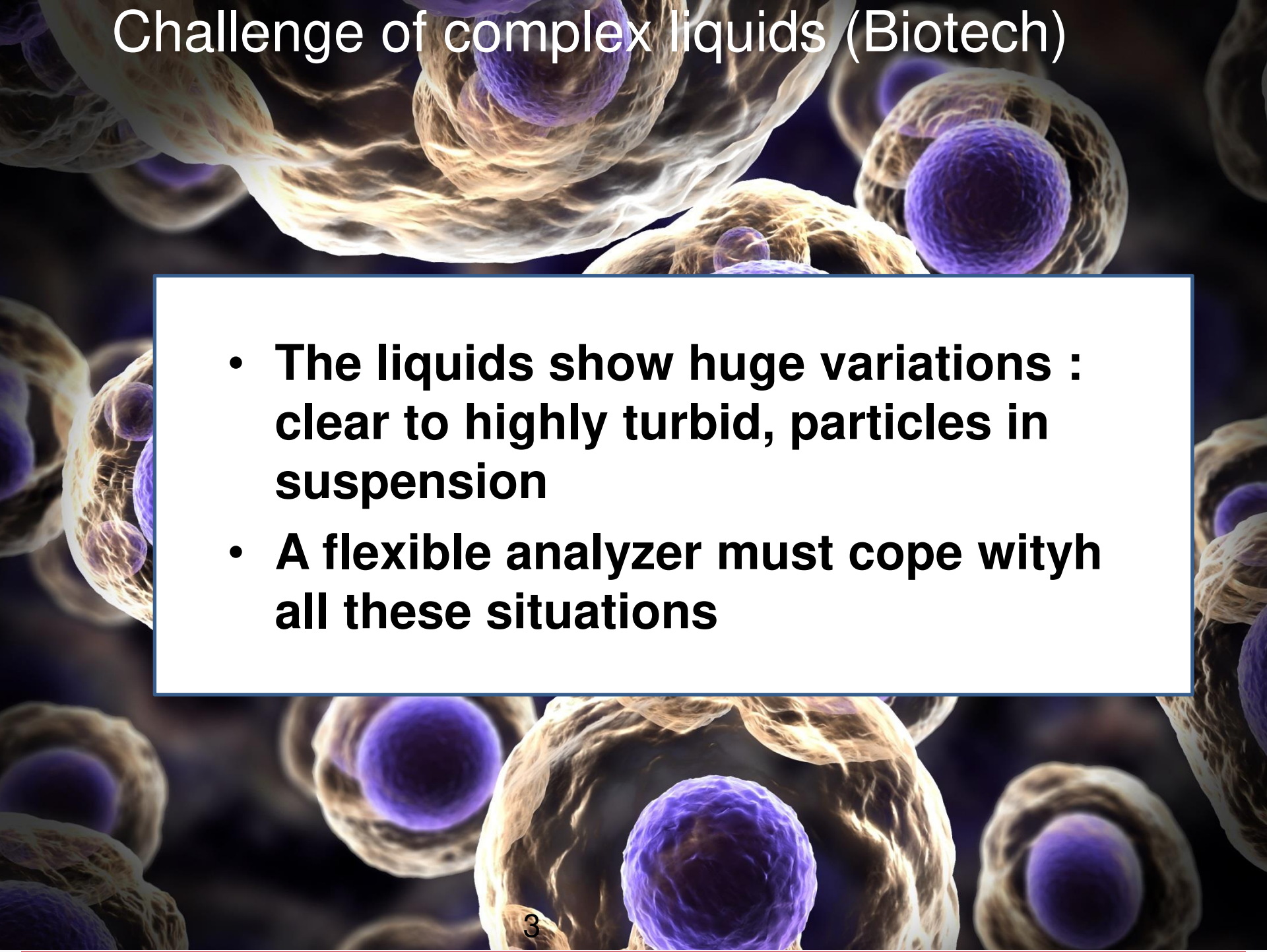
Challenge of complex liquids (Biotech)



- **The liquids show huge variations : clear to highly turbid, particles in suspension**
- **A flexible analyzer must cope wityh all these situations**

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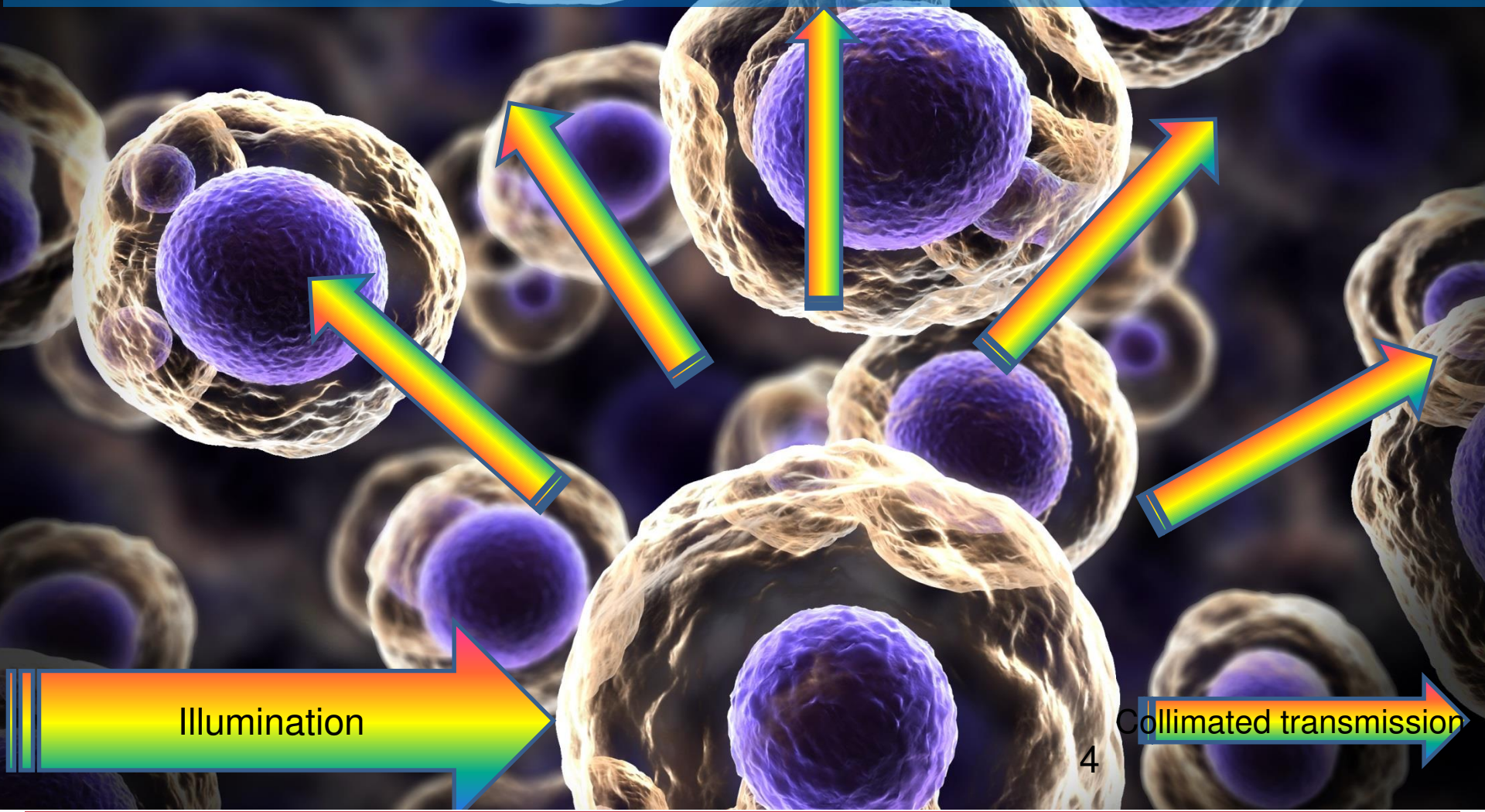
Challenge of complex liquids (Biotech)

- **Many theories on particle characterization for all measurements at different angles are necessary**
- **The probe can be designed according to this approach**



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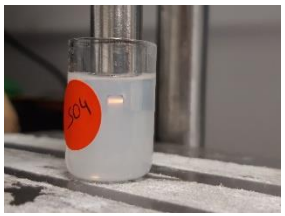

SAM-FLEX
12mm
3 mm path length



Asuryan
UV-VIS – 200nm-600nm
3 Channels (simultaneous)

In biotech, the physical effects are critical.
Examples :

- Cell culture : when cells die, their membranes ares modified
- Vaccine : Adsorption of protein on adjuvant
- Encapsulation : Some proteins need to be encapsulated into lipids (nanoglobule)
- Some cells need to be fixed on microcarriers in suspension to develop properly

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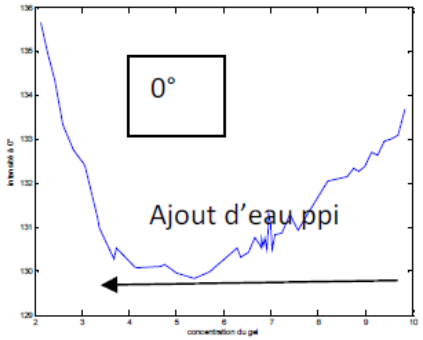
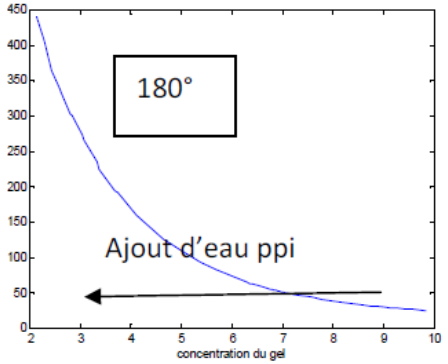
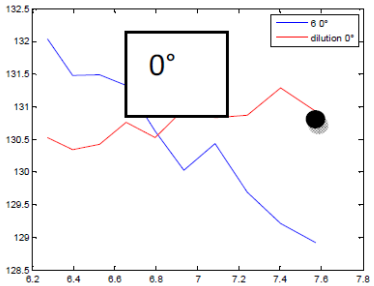
Signal related to BSA versus basic dilution

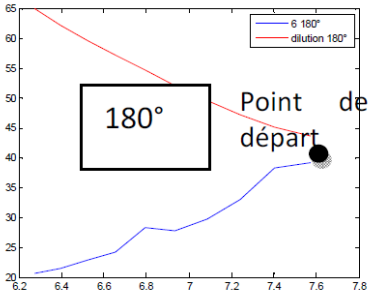
Water addition





BSA addition

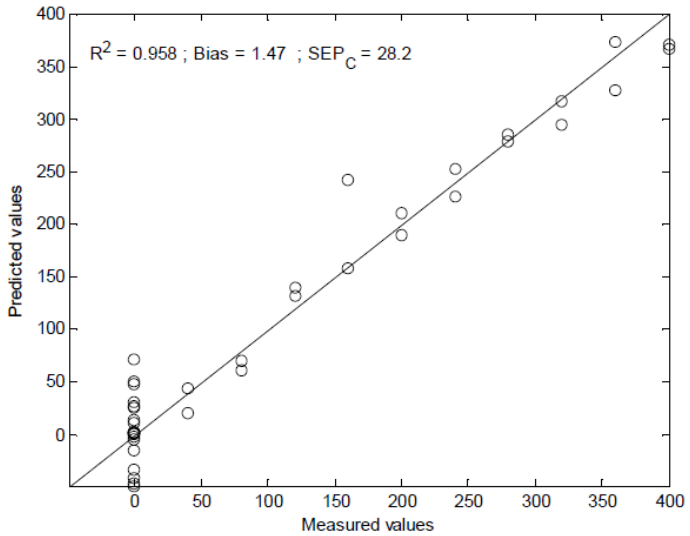




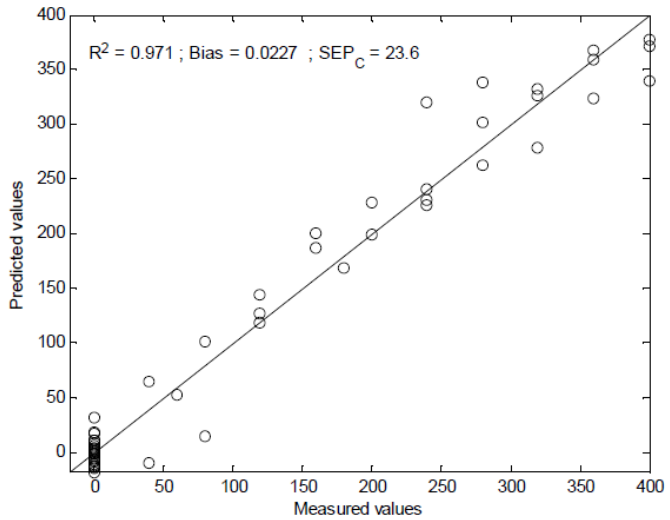
Modelling Performance based on 3 angles

Prediction of BSA concentration on Aluminum

Highly diluted addition



Concentrate addition



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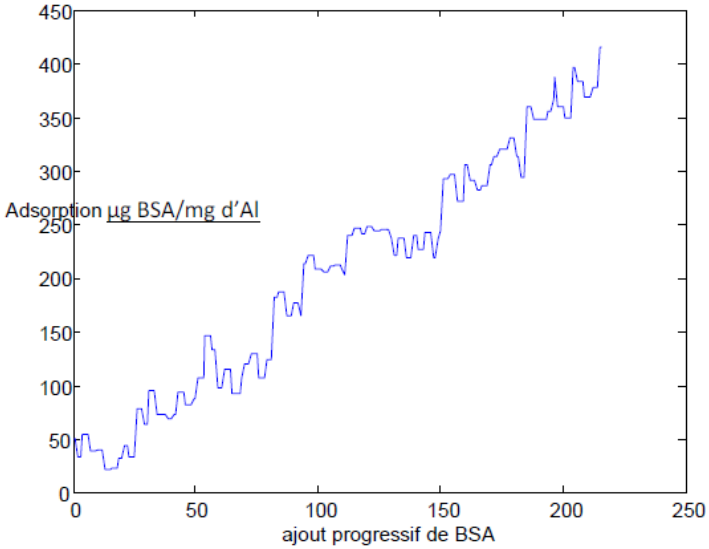
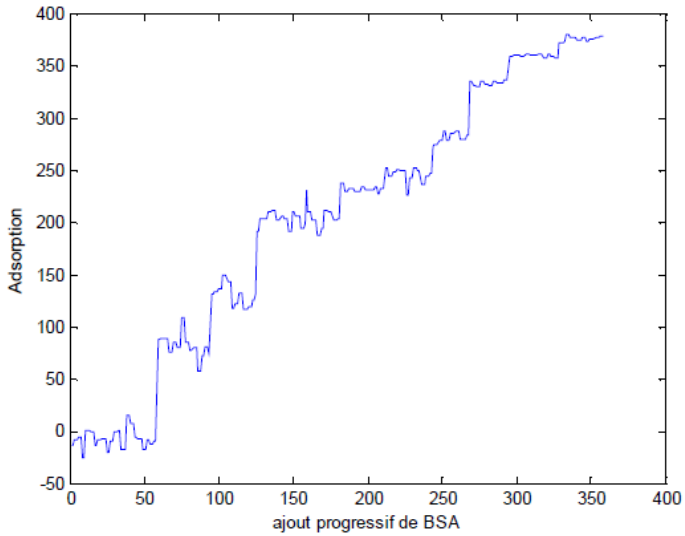
Evolution of BSA concentration during production





Conclusion

The best performance were obtain with two PLS models (one dedicated for highly diluted samples)
In order to improve the result a normalization was performed at the starting of the batch (before addition of BSA)

Performances reached are around 30µg/mg of Aluminum. These performances are acceptable for production requirements.

SVM will be investigated in order to use only one modelling
New production entities have been tested
Test with different polarities have been done to validate the difference in signal if adsorption does not occur.

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