Application of AI/ML for risk assessments on Antisense Oligonucleotides (ASOs) aggregation

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Abstract

Antisense oligonucleotides (ASOs) are a growing class of pharmaceutical products. However, aggregation and particle formation of ASOs may decrease their bioactivity and negatively impact product safety. Silicone oil is widely used as a lubricant during manufacturing and packaging of biopharmaceuticals. In this study we investigated the impact of low levels of silicon oil in morpholino antisense oligonucleotides (PMO) drug products on extent of subvisible particle formation. However, the similarities of silicon oil particles to PMO aggregates and air bubbles pose a challenge to accurately assessing the impact of silicon oil on aggregation of PMOs. To this end, we developed machine learning and AI methods for classification and quantification of particles in PMO drug products.

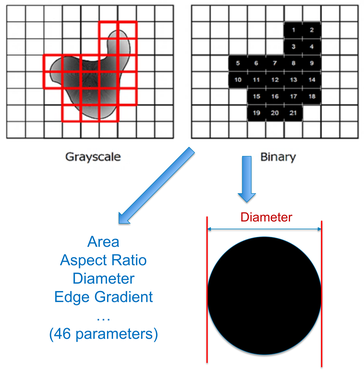
Materials and methods

AMONDYS 45 (Casimersen) was dissolved in PBS buffer, with or without 0.1% silicone oil, to prepare a 50mg/ml solution. The Interfacial properties was then measured using KRUSS drop shape Analyzer-DSA100. Next, 5 mg/ml Casimersen solution with or without 0.001% w/v of silicone oil were rotated end-to-end at 40 rpm for 2 days, followed by subvisible particles measurement by FlowCam. Then Machine learning and AI methods were developed and compared to classify the subvisible particles captured by FlowCam: Artificial Neural Network (ANN), kPCA with ANN or Logistic Regression, and Convolutional Neural Network.

Drop Shape Analyzer for Surface Property

Tube Rotator

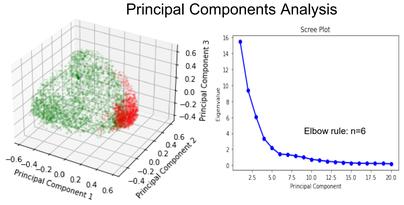
FlowCam 8000 for Particle Imaging



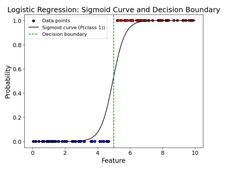
46 morphological parameters are generated by FlowCam for each particle

Area (Filled)	Aspect Ratio	Average Blue	Diameter (ESD)	Diameter (FD)	Edge Gradient
7041.8	0.25	158.28	196.1	94.69	104.43
10094.28	0.3	160.81	156.05	113.37	110.79
1708.41	0.29	168.74	95.89	46.64	107.67
548.48	0.12	166.17	91.71	26.43	99.54

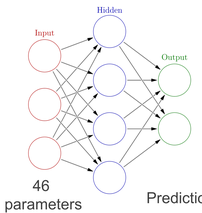
Principal Components Analysis



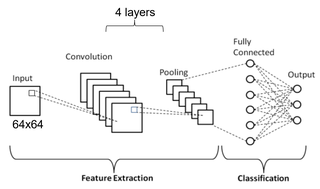
Logistic Regression



Artificial Neural Network

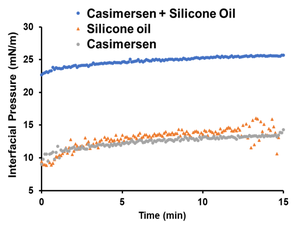


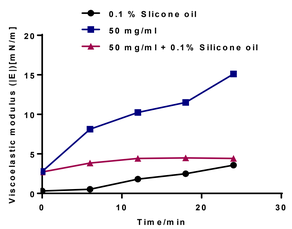
Convolutional Neural Network



Results and discussion

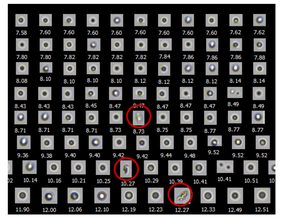
Impact of Silicone oil on Interfacial Properties





- Synergistic increase in Interfacial pressure possible due to colocalization of silicone oil and PMO (Casimersen) molecules
  - Viscoelastic modulus dominated by silicone oil
  - Lower viscoelastic modulus of mixture of silicon oil and Casimersen than Casimersen alone
  - Casimersen potentially absorbed on the surface of silicone oil

Particles Images from FlowCam



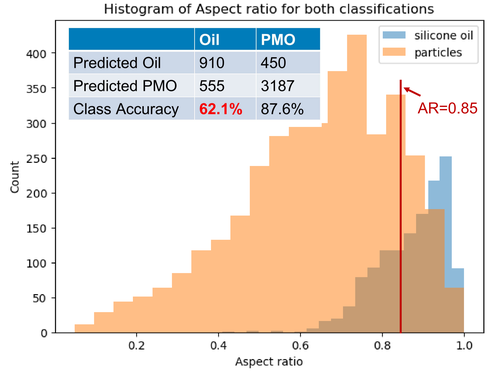
Casimersen Particles

How do we efficiently separate the Casimersen particles from silicone oil particles?

Classification by Aspect Ratio Cut-off at 0.85

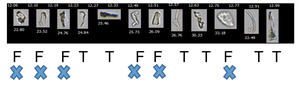
Histogram of Aspect ratio for both classifications

	Oil	PMO
Predicted Oil	910	450
Predicted PMO	555	3187
Class Accuracy	62.1%	87.6%



- Aspect ratio (AR) cut-off: particles with AR≤0.85 are predominantly Casimersen particles, while particles with AR >0.85 are mainly silicone oil particles
- Traditional and simple method inadequate due to significant overlap, there is a need for a better method for particle classification

Artificial Neural Network (ANN)



F: Predicted as oil
T: Predicted as Casimersen

kPCA with ANN:

	Particles	Oil
Predicted Particles	302	14
Predicted Silicone Oil	10	255
Class Accuracy	96.79%	94.80%

Accuracy: 95.87%

- Full connection of datasets with ANN overfitted the data
  - Not all 46 parameters are important for classification
  - Some of the parameters are linearly dependent
- Need to reduce dimensionality

kPCA with Logistic Regression

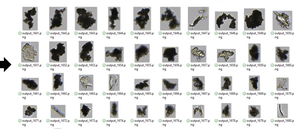
	Particles	Oil
Predicted Particles	301	13
Predicted Silicone Oil	11	256
Class Accuracy	96.47%	95.17%

Accuracy: 95.87%

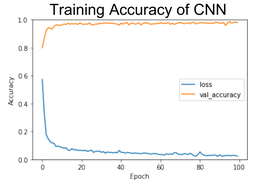
Convolutional Neural Network (CNN)

Raw Images from FlowCam

Individual Particle Images



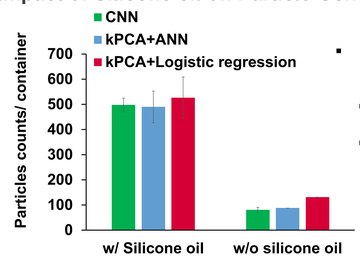
Training Accuracy of CNN



Predicted Particles	312	6
Predicted Silicone oil	6	266
Class Accuracy	98.1%	97.79%

Accuracy: 97.97%

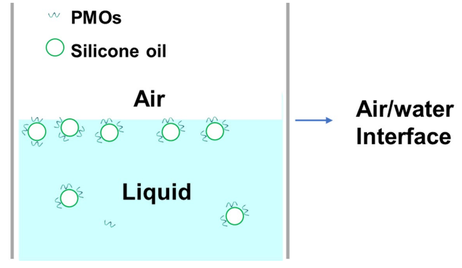
Impact of Silicone oil on Particle Generation



- Presence of low silicone oil level significantly increase particle counts in Casimersen solution
  - 0.001% silicone oil -> 4 times particles
  - Increased oil-water interfacial area only 3.4 mm^2 (2.1%)

Conclusion

- **AI and machine learning classifiers are more accurate for classification of particles in Casimersen solution for injection than the use of a single morphological parameter**
- **The presence of low levels of silicon oil in morpholino oligonucleotides drug products may lead to a minor increase in interfacial area but substantial increase the risk of subvisible particle formation**



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