

## Suivi en ligne d'une étape de précipitation sur équipement petite échelle par conductivité et modèle mathématique

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### Résumé

Dans le cadre de ses activités d'amélioration continue de production de produits pharmaceutiques, les équipes MSAT d'industrialisation de Sanofi suivent en permanence les procédés de production pouvant être améliorés. Une étape de précipitation a été identifiée comme une opération unitaire qui induit une variabilité sur les caractéristiques physicochimiques d'un produit fabriqué par Sanofi.

Cette précipitation est opérée en ajoutant un non-solvant en semi-batch dans une cuve agitée en chambre froide. Son mécanisme d'action est basé sur des interactions électrostatiques entre le cation d'un sel présent dans le milieu et le produit d'intérêt, un polyoside de charge négative. La précipitation du produit est obtenue par la différence de solubilité générée par l'action du non-solvant ajouté et la neutralisation de la charge du polyoside avec le contre-ion dérivé de la dissociation du sel. Ces molécules forment un complexe instable qui s'agrège et sédimente, permettant ainsi la séparation des phases. Le produit d'intérêt est alors récupéré pour être traité dans la suite du procédé de production, la phase liquide est éliminée.

La conception d'un modèle réduit (*scaled-down*) de 2 L représentatif de l'installation de production, un plan d'expérience, le choix d'une technologie de suivi du procédé, ainsi qu'une modélisation de la cinétique de la réaction ont été conçus dans le but d'investiguer l'impact de deux paramètres opératoires sur la vitesse de précipitation : le débit et la température d'alimentation du non-solvant.

La dynamique du système a été suivie avec l'intégration des sondes de conductivité électrique et de température in-situ. Etant donné que le non-solvant ne libère pas d'ions, et que la concentration ionique dans le milieu diminue sous la double action de la dilution générée par le volume de non-solvant ajouté et de la consommation des ions qui précipitent, la conductivité au sein du milieu diminue au cours de la réaction.

Pour la construction du modèle mathématique, on considère que le taux de conversion calculé à partir de la conductivité est liée à l'avancement de la transformation de phase. Ensuite, on utilise le modèle cinétique d'Avrami (ou modèle de JMAK), pour réaliser l'identification des paramètres cinétiques,  $k$  et  $n$ .

La valeur de  $n$  pour l'intégralité des tests réalisés nous permet d'identifier qu'il s'agit d'un taux de transformation constant de premier ordre, quel que soit le scénario. On observe que la température d'introduction du non-solvant ne semble pas avoir d'impact sur l'avancement de la transformation de phase parmi la plage de températures testés. De fait, ces 5 essais indépendants confortent la reproductibilité du modèle à petite échelle.  $T_1 < T_2 < T_3 < T_4 < T_5$ , et  $T_1 = T_5 - 20^\circ$ .

En revanche, étant donné que le débit d'alimentation du non-solvant est directement proportionnel à la vitesse de précipitation, on a pu trouver une corrélation linéaire entre le débit et le paramètre de vitesse  $k$  du modèle d'Avrami. De cette façon, la transition de phase en fonction du débit pourrait être représentée comme une extension du modèle d'Avrami.

## Online monitoring of a precipitation step on scale down model by conductivity and mathematical model

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### **Abstract**

As part of its continuous improvement of pharmaceutical production activities, Sanofi MSAT teams continuously monitor production processes that can be improved. A precipitation step has been identified as a unit operation that induces variability on the physicochemical characteristics of a product manufactured by Sanofi.

This semi-batch precipitation is carried out by adding a non-solvent in a stirred tank in a cold room at a regulated temperature. Its mechanism of action is based on electrostatic interactions between the cation of a salt present in the medium and the product of interest, a polysaccharide of negative charge. The precipitation of the product is obtained by the difference in solubility generated by action of the non-solvent added and the charge neutralization of the polysaccharide by consuming its counterion derived from the complete dissociation of the salt. These molecules form an unstable complex that aggregates and sediments, thus allowing the separation of phases. The product of interest is then recovered to be processed in the rest of the production process; the liquid phase is eliminated.

The design of a 2 L scaled-down model representative of the industrial scale, a design of experiences, the choice of an analytical monitoring technology, as well as the modeling of the reaction kinetics, were designed to investigate the impact of two operating parameters on the precipitation rate: the flow rate and supply temperature of the non-solvent.

The dynamics of the system were monitored with the integration of both in-situ an electrical conductivity and temperature probes. Since the non-solvent does not release ions, and since the medium's ionic concentration decreases under the dual action of the dilution brought by increasing volume of non-solvent added and the consumption of precipitating ions, the conductivity within the medium decreases during the reaction.

For the construction of the mathematical model, it is considered that the conversion rate calculated from conductivity is directly related to the progress of the phase transformation. The Avrami kinetic model (or JMAK model) was used to identify the kinetic parameters,  $k$  and  $n$ .

The value of the order of the reaction,  $n$ , for all the experiences carried out allows us to identify a first-order transformation rate. It was observed that the non-solvent supply temperature does not seem to have an impact on the progress of the phase transformation among the temperature range tested. In fact, these 5 independent experiences reinforce the reproducibility of the scaled-down model.  $T_1 < T_2 < T_3 < T_4 < T_5$ , and  $T_1 = T_5 - 20^\circ$ .

On the other hand, the feed rate of non-solvent has a greater impact. Since the feed rate is directly proportional to the precipitation rate, a linear correlation between the flow rate and the parameter  $k$  was found. Therefore, the phase transition could be represented as an extension of the Avrami model by introducing the non-solvent feed rate.

## **Introduction**

The heterogeneous precipitation equilibrium involves the transfer of mass from the liquid phase to the solid phase. The initial liquid phase contains a complete dissociation of a salt, providing ions of  $M_{(aq)}^+$  to the medium and a dissolved polysaccharide, providing an ionic group  $N_{(aq)}^-$  per monosaccharide.

Even with a high excess of  $M_{(aq)}^+$ , during this initial aqueous solution, the necessary conditions for precipitating the polysaccharide are not met. Thus, the presence of a non-solvent is required to modify the dielectric permittivity of the medium and favor the ionic interaction between the polysaccharide and its counterion. At this stage of the manufacturing process, there is no analytical characterization of polysaccharide (molecular weight distribution, polydispersity, etc.). Hence, the solubility of the polysaccharide in the aqueous phase or in presence of non-solvent is not known.

The reaction can be represented by a theoretical stoichiometry of 1:1.



The sense of equilibrium depends on the molecular weight and concentration of the polysaccharide, the concentration of salt, the ratio of non-solvent (%vol/vol) and the temperature. In our reaction conditions, the temperature of the reactor walls is regulated by a double envelope during the whole reaction and  $M_{(aq)}^+$  is in excess relative to the polysaccharide.

The driving force of the precipitation is mainly related to the difference in solubility and electrostatic forces. In this context, two process parameters were targeted:

- The non-solvent supply temperature,  $T_s$ : Even if the temperature of the reactor walls is regulated by a double envelope, the temperature of the medium varies according to the enthalpy of the non-solvent supply flow rate, plus the non-solvent– aqueous medium mixture enthalpy, as well as the reaction enthalpy related to ionic interactions. The temperature profile could potentially alter the precipitation kinetics.
- The non-solvent flow rate,  $D_s$ : The non-solvent is added at a constant flow rate up to a given concentration in %vol/vol. Since the precipitation rate depends on the interaction between the non-solvent and the medium, the attributes of the solid phase may differ at different precipitation rates.

## **Experimental method**

### **I. Choice of the analytical technology**

The dynamics of the system were monitored with the integration of in-situ electrical conductivity and temperature probes. Since the non-solvent does not release ions, and since the medium's ionic concentration decreases under the dual effect of the dilution brought by increasing non-solvent volume and the consumption of precipitating ions, the conductivity within the medium decreases during the reaction. The electrical conductivity probe was chosen based on the work carried out by Villar-Cocina, E., Valencia-Morales, E., González-Rodríguez, R., y Hernández-Ruiz, J. in 2003.

### **II. Analytical measurements.**

After each experiment, a sample of the precipitate was analyzed by elementary Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The polysaccharide contains a specific element that can be quantified per each monosaccharide. Considering the mass ratio between this specific element and the monosaccharide, the recovery of the polysaccharide in the solid phase can be estimated. Moreover, the precipitated counterion is also analyzed by ICP-OES.

### III. Calibration

During the precipitation reaction, the temperature varies between 2 and 5°C inside the reactor. A calibration was performed between the conductivity of the medium (in the absence of non-solvent) and the temperature at constant volume.

This linear correlation allowed us to characterize experimentally the variation in the conductivity of this specific medium with respect to the variation in temperature,  $\frac{d\sigma}{dT}$ . Therefore, a reference state of the initial conductivity could be established throughout the reaction considering the temperature variation. It is to be noted that this assumes that the medium conductivity is specific to the electrolytes and polysaccharide in aqueous solution.

$$\sigma_0(T) = \sigma_{0,T_{ref}} + \frac{d\sigma}{dT} \cdot (T - T_{ref}) \quad (2)$$

### IV. Scaled-down model:

The scientific approach applied for scaling down is based on a macroscopic perspective related to mixing characteristics such as:

- The Reynolds number. Transposes the ratio between inertial forces to viscous forces. It reflects the flow rate of a fluid.
- Power dissipated per unit of volume. Relates the mechanical energy transmitted to the liquid medium from the stirrer.
- The mixing blades' speed and mixing time.
- The geometry of the stirrer.
- The pumping flow rate.
- The ratio between the height and the diameter of the H/D tank.
- The ratio between the diameter of the stirrer and the diameter of the d/D tank.

In the industrial scale, this unit operation is carried out in a cold room at a regulated temperature. Taking into account this cooling environment, a fluid at a regulated temperature is circulated in the double envelope of the 2 L stirred tank in the scaled down model. It is considered that keeping the reactor walls at the temperature of the industrial scale cold room was adequate to simulate its cooling effect.

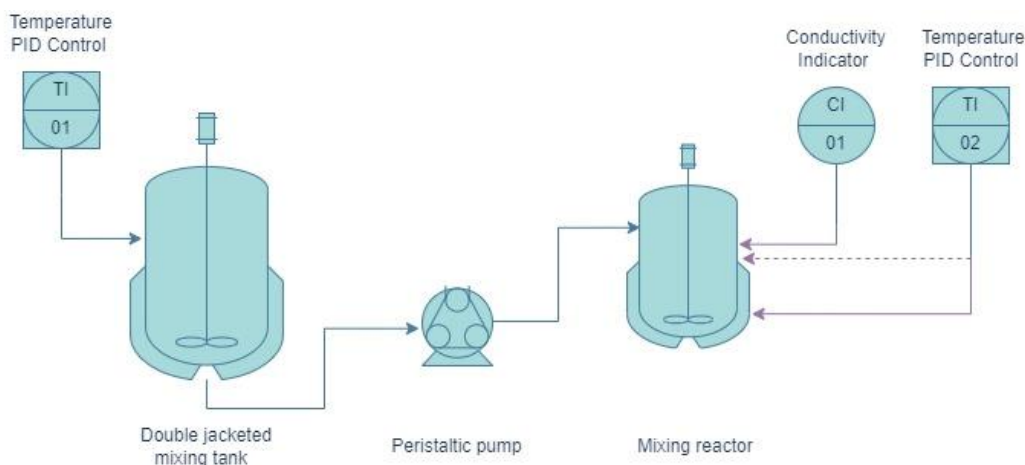


Figure 1. Scale down model schema.

Before feeding the non-solvent, the polysaccharide and the salt are completely dissolved and mixed in an aqueous medium at a regulated temperature. At the same time, in another tank, the non-solvent temperature is regulated at the desired supply temperature. When the temperatures profiles are stable in both tanks, the reaction starts. The reactor begins to be fed at constant volumetric flow rate of non-solvent by using a peristaltic pump. The end of the reaction is determined by the fixed non-solvent concentration in %vol/vol.

### Modeling and estimation of kinetic parameters

The conversion of the reaction is related to the difference in superficial conductance,  $\sigma_s$ , between what was measured by the conductivity probe and the reference state.

$$\sigma_s [s.m^2] = \sigma \left[ \frac{S}{m} \right] \cdot V_{rxn} [m^3] \quad (3)$$

The reaction volume,  $V_{rxn}$ , is estimated from the initial volume,  $V_0$ , reaction time,  $t$ , and the non-solvent feed rate,  $D_s$ .

$$V_{rxn} = V_0 + D_s \cdot t \quad (4)$$

The definition of a reference state is estimated in relation to the temperature variation throughout the reaction and the initial volume. See Figure 2A.

$$\sigma_{s,0} = \sigma_0(T) \cdot V_0 \quad (5)$$

The theoretical dilution of the initial conductivity can be simulated according to the volume variation rate to observe the conductivity profile without the ions consumption. See Figure 2A.

$$\sigma_{s,Dilution} = \sigma_{s,0} \cdot \left( \frac{V_0}{V_{rxn}} \right) \quad (6)$$

The conversion is based on the reference state, conductivity medium measurement and reaction volume.

$$X(t) = \frac{\sigma_{s,0} - \sigma(t) \cdot V_{rxn}(t)}{\sigma_{s,0}} \quad (7)$$

By establishing that the progress of phase transition,  $Y_{rxn}$ , is equivalent to the relative conversion. The Avrami model or JMAK model was used to identify the kinetic parameters,  $k$  and  $n$ . The interpretation for the exponent  $n$  is related to the order of nucleation and growth kinetics. While parameter  $k$  is proportional to the rate of nucleation and growth. By Shirzad, K., y Viney, C. in 2023.

$$\frac{X(t)}{X_{\%vol/vol}} = Y_{rxn} = 1 - \exp(-k \cdot t^n) \quad (8)$$

The parameter identification of  $k$  and  $n$  was carried out by optimizing the RSME in MATLAB.

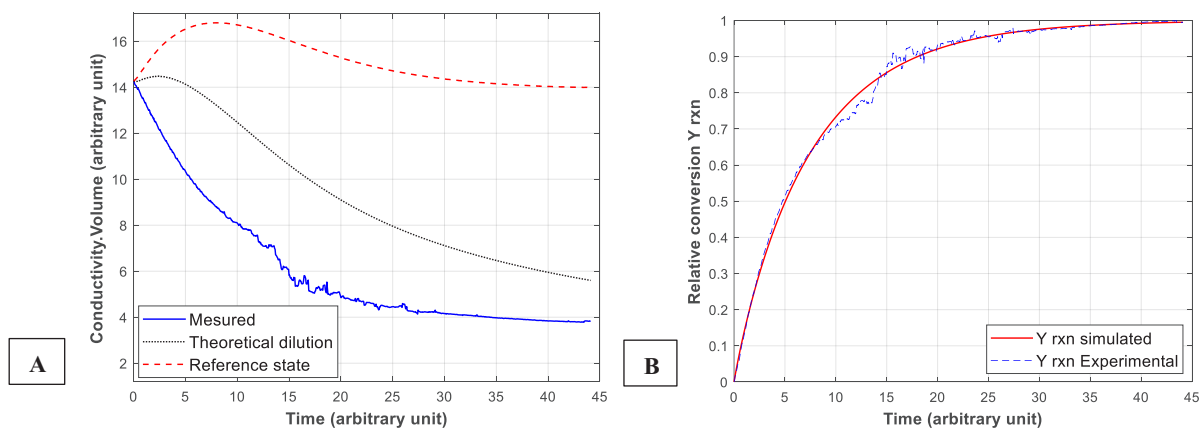


Figure 2. A. Conductivity profile of the reference state, the theoretical dilution and the conductivity measured for one of the tests performed. B. Profile of the relative conversion from raw conductivity data and simulation from the kinetic coefficients of the Avrami model.

## Results and discussion

Considering the ICP-OES analysis of the precipitate, a recovery of more than 96% of the dissolved polysaccharide and the stoichiometry of its monosaccharide and its counterion of 1:1 under all conditions tested were confirmed. Thus, relative conversion determined from conductivity can be used to calibrate Avrami kinetic parameters of the global transition phase.

### I. The effect of the non-solvent supply temperature

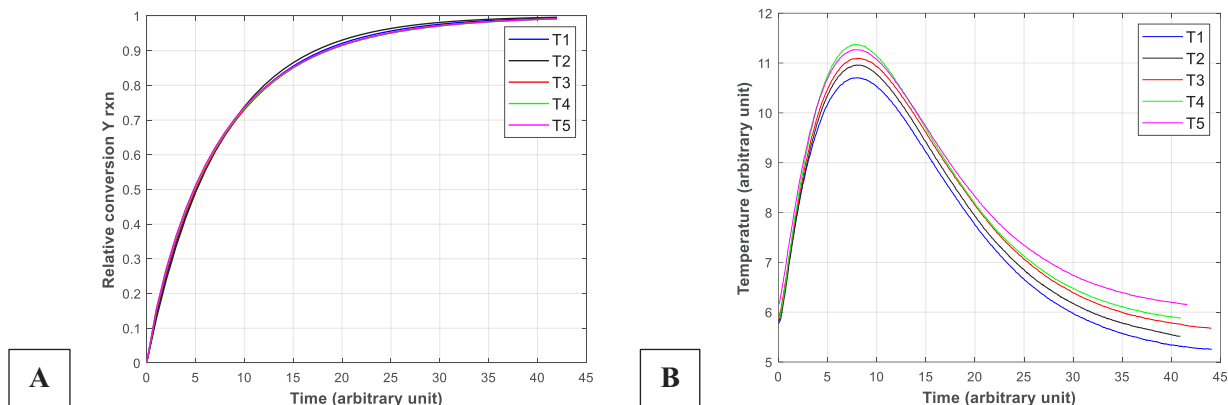


Figure 3A. Simulation of the relative conversion from the Avrami model with different non-solvent supply temperatures. 3B. Experimental temperature profile in the tank during the reaction.  $T1 < T2 < T3 < T4 < T5$ , and  $T1 = T5 - 20^\circ$

The non-solvent was added at the same constant flow rate up to a fixed concentration in %vol/vol for the range of non-solvent supply temperature tested. The temperature and conductivity profile inside the tank was similar for all the non-solvent supply temperatures tested.  $T1 < T2 < T3 < T4 < T5$ , and  $T1 = T5 - 20$ . The identification of kinetic parameters shows a first-rate phase transition reaction and similar constant rate.

### II. The effect of the non-solvent supply flow rate

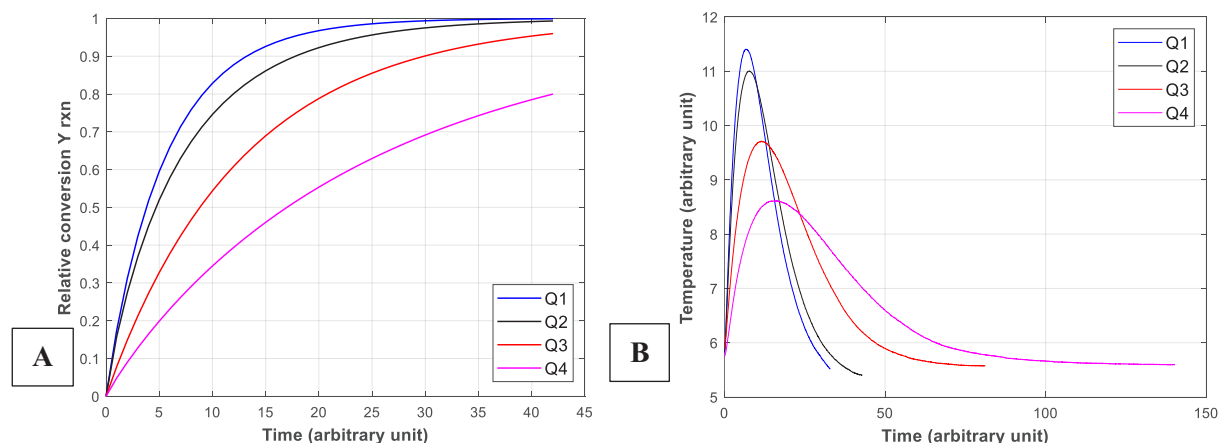


Figure 4. A. Simulation of the relative conversion from the Avrami model with different feed rates. B. Experimental temperature profile in the tank during the reaction. ( $Q1 > Q2 > Q3 > Q4$ )

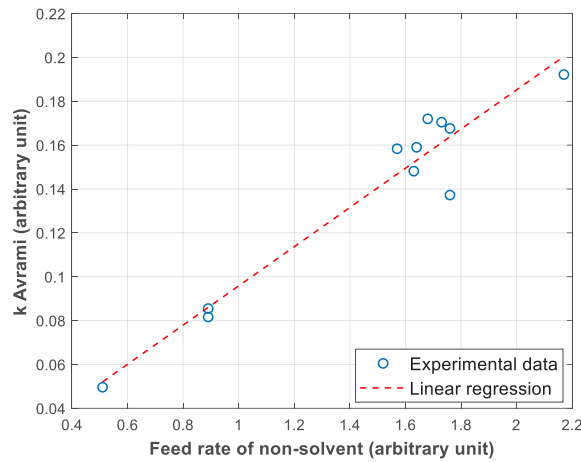


Figure 5. Linear regression between the coefficient  $k$  and the non-solvent flow rate.

Four feed rates were tested until the fixed concentration of non-solvent in %vol/vol was reached. The non-solvent was added at the same temperature supply. In each of these tests, the same amount of non-solvent had been added to the tank by the end of the feed. However, the system dynamics were different, and both the temperature and conductivity profiles varied. For example, the temperature increase for the lowest and highest flow rates were 2.5°C and 5°C, respectively.

Since the concentration and temperature vary according to the operating flow rate, the precipitation rate may influence the solid phase characteristics. A rapid rate of precipitation could potentially form branched polysaccharide aggregate populations while a slower rate form more compacted structures of aggregates.

The identification of kinetic parameters shows a first-order phase transition reaction. A linear correlation with the flow rate was identified for the rate parameter,  $k$ . The simulated kinetics from these parameters are shown in Figure 4A.

The phase transformation based on the non-solvent flow rate could be represented as an extension of the Avrami model. With  $D_s$  the non-solvent flow rate,  $\left(\frac{dk}{dD_s}\right)$  the slope of the trend line and  $k_0$  the intersection with the Y-axis. Figure 5.

$$Y_{rxn}(t) = 1 - \exp \left[ - \left( \left( \frac{dk}{dD_s} \right) \cdot D_s + k_0 \right) \cdot t \right] \quad (9)$$

### III. Interpretation of kinetic parameters

The order of the reaction. The possible interpretation of a first-order reaction can be expressed from two phenomena involved in the precipitation reaction: nucleation and growth. In this case, the growth is not an ordered crystalline growth but one that is related to the aggregation of the polysaccharide.

$$n = n_{Nucléation} + d \cdot n_{Growth} \quad (10)$$

For a first-order transformation, the different possible meanings are listed in Table 1, according to the analyses carried out by Blázquez, J., Romero, F., Conde, C., y Conde, A. in 2022.

Table 1. Meaning of a first-rate phase transformation from the Avrami model.

<b>Nucleation order</b> <i>n<sub>Nucleation</sub></i>	<b>Growth order</b> <i>n<sub>Growth</sub></i>	<b>Dimensionality of growth (d)</b>
1 : Constant nucleation rate	0 : No growth	0
0.5 : Nucleation rate decreases over time	0.5 : Growth limited by the diffusion of solute <sup>a</sup> .	1
0 : No nucleation	0.5 : Growth limited by the diffusion of solute <sup>a</sup>	2
0 : No nucleation	1 : Growth limited by the interphase <sup>b</sup> .	1

<sup>a</sup> Growth limited by diffusion: Growth depends particularly on the interactions between the different clusters (non-soluble polysaccharide) distributed within the tank. Growth is therefore limited by the mobility of these clusters in the liquid phase.

<sup>b</sup> Growth limited by interphase: This mechanism involves processes that take place in the immediate vicinity of the interface. The growth rate depends only on the attachment of the molecules from the parent phase to the new phase at the interface.

According to this interpretation, kinetics could have two mechanisms:

- Either it is a simultaneous effect in which, at the beginning of the reaction, there is only a phenomenon of constant nucleation and no growth. Then, aggregation will depend on whether the cluster growth is limited by the interphase or by the mobility of other clusters.
- Either the nucleation rate decreases over time and the aggregation rate is limited by the mobility of other clusters.

#### IV. Rheology of the liquid phase

An experimental determination of the dynamic viscosity of the medium (salt, polysaccharide, non-solvent) was also characterized at the operating temperature according to the concentration in % vol/vol of the non-solvent as shown in Figure 6. The reference of the sinusoidal vibration viscosity measuring device is the SV-A 42. All measurements of the samples were carried out at a regulated process temperature.

The rate of nucleation and aggregation could potentially be affected by system rheology. The increase in the dynamic viscosity of the liquid medium decreases the mobility of reacting ions and clusters, neutral complex polysaccharide. This rheology delays the rate of aggregation.

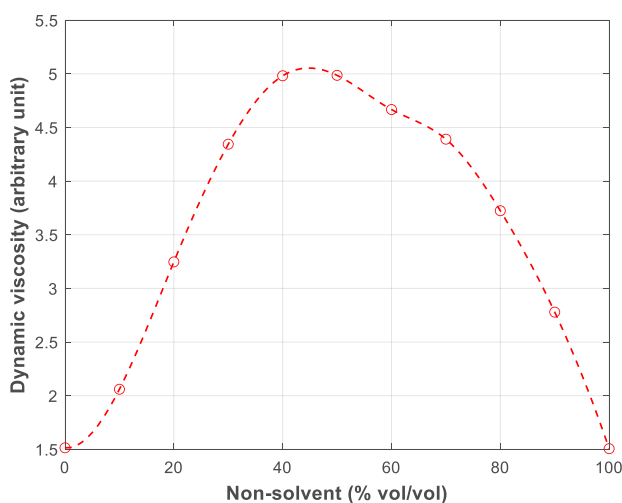


Figure 6. Dynamic viscosity profile of the liquid medium according to the concentration in % vol/vol of non-solvent.



## **Conclusion**

The kinetic parameters of the Avrami model were determined to investigate the effect of two process parameters:

- The non-solvent supply temperature. The kinetics parameters identified seem to show the same phase transformation profile for the range of temperatures tested.
- The non-solvent supply flow rate. The phase transformation order remains the same for all tested flow rates. However, the constant rate,  $k$ , is directly related and can even be expressed in terms of the non-solvent flow rate. A high non-solvent feed rate represents a rapid precipitation rate that could potentially promote polysaccharide aggregate formation in a branched structure while a slow reaction by the action of a low flow rate rate could promote a compact structure aggregation.

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All authors are Sanofi employees and may hold shares and/or stock options in the company.

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