

Validation of method for rheological characterization of poloxamer 407 hydrogels used for 3D bioprinting

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Introduction

In recent years, the research and development of new dosage forms manufactured by 3D bioprinting technique (3DP) has been increasing. The polymeric materials used in 3DP (bioinks) should possess certain biological as well as mechanical and rheological characteristics. Their rheological behaviour, viscosity, elasticity and plasticity significantly influence the manufacturing process. The determination of rheological properties is essential from the point of view of uniform dispersion of the active substances, stress during controlled extrusion, liquefaction of bioinks during extrusion through the small orifices of printing nozzles, viscoelastic properties for protection against shear stress, thixotropy, the possibility of gelation, etc. Poloxamer 407 (P407) is widely used in different pharmaceutical products, but also shows great potential in 3DP as bioink due to its thermogelling characteristics (Dumortier et al., 2006).

The aim of this study was to establish and validate a method for the rheological characterization of P407 hydrogel, as one of the most commonly used biopolymers for 3DP. The paper of Simões et al. (2020) was used as a guide for the validation.

Materials and methods

Preparation of biopolymer solution: Cold method was applied for preparation of 30% (m/V) solution of P407 (BASF Schweiz AG, Germany). Briefly, appropriate amount of P407 was added in cold distilled water (4 °C)

and stirred on magnetic stirrer (250 rpm, 25 °C, Variomag, Multipoint HP 15, Germany) until homogenous mixture was formed. Afterwards, the mixture was kept at 4-8 °C until homogenous solution was obtained.

Rheological characterization: The rheological profile of the prepared P407 biopolymeric solution was determined with modular compact rheometer equipped with Peltier temperature device (MCR 92 Anton Paar Rheometer, Austria). Apparent viscosity was used as indicator of the rheological behaviour of the solution and it was measured with the rotational viscosity flow method. The measurements were performed on 25 mL cold 30% P407 solution, using cylinder (B-CC27) and measuring cup (C-CC27/T200/XL/AL) with 5 mm space between them. When cone (CP50-1) geometry system was used the zero gap was set to 0.104 mm. The analyses were conducted at 25 ± 0.1 °C, by logarithmic increase of the shear rate from 0.1 to 100 s⁻¹, measuring 21 points in 30 seconds. For the validation of the rheological method precision, accuracy, sensitivity, specificity, selectivity (discriminatory power) and robustness as validation parameters were assessed.

Determination of apparent viscosity of 6 individual samples of 30% P407 solution carried out in a single day, calculation of average value and relative standard deviation (RSD) were used to assess the method precision.

Method accuracy was determined by calculation of Bias (%). First Bias was calculated by subtracting the reference value from the measured values. The Bias (%) was calculated by dividing the Bias value by the reference value and multiplying by 100. Measurements of apparent viscosity of 30% P407 samples were conducted on two

separate days (at least 6 individual samples at each day). The average value from the first day was used as reference. Bias (%) was computed for each measurement and Bias (%) average was calculated from at least 6 Bias (%) individual values.

Method sensitivity was assessed using 3 different concentrations of P407 solutions - 25%, 30% and 35%. At least 6 individual samples from each solution were measured in a single day. One-way ANOVA single factor ($p < 0.05$) and post hoc LSD ($p < 0.05$) (STATGRAPHICS Centurion XVI evaluation, StatPoint technologies Inc., USA) were employed.

Average values of apparent viscosity obtained from P407 solutions with different concentration (25%, 30% and 35%) were plotted and specificity of the method was assessed by determination of correlation coefficient (R^2).

Method selectivity (discriminatory power) was determined by *t-Test*: paired two sample for means ($p < 0.05$) in Microsoft Excel program, using the apparent viscosity values of at least 6 individual samples from each P407 solutions with different concentration (*t-Test* for 25% and 30%; 25% and 35% as well as 30% and 3 %) determined in single day.

The robustness of the method was evaluated by assessing the influence of the temperature (23 ± 0.1 °C, 25 ± 0.1 °C and 27 ± 0.1 °C), sample application method (direct pouring into the measuring cup versus spoon) and geometry system (cylinder and cone)). At least 6 individual samples from 30% P407 were measured at each condition in a single day. Average values were calculated and relative standard deviation (RSD) was used to evaluate the method robustness for each condition.

Acceptance criteria for all tested validation parameters were as follows: RSD < 15% for precision and robustness, Bias (%) < 15 for accuracy, $p < 0.05$ for sensitivity and selectivity and $R^2 > 0.9$ for specificity of the method.

Results and discussion

Biopolymeric solution of 30% P407 shows Non-Newton behaviour, i.e. shear thinning properties with pseudoplastic flow.

Assessment of the method precision conducted as repeatability testing indicated that the average apparent viscosity of 30% P407 solution was 596198.34 mPa·s with RSD of 0.90%. Calculated average Bias (%) value of 4.64 showed method accuracy thus representing the agreement (closeness) between the value that is accepted as the true (reference) value and the other measured values. Sensitivity of the method shows the possibility of the method to detect the difference in viscosity between formulations with different concentration. Results from one way ANOVA ($p < 0.05$) and post hoc LSD ($p < 0.05$) disclosed statistically significant difference among P407

samples with different concentration (25%, 30% and 35%) thus indicating method sensitivity. The relation between viscosity and P407 concentration could be best described by second-order polynomial regression model (Eq. 1) with $R^2=1$ thus pointing to method specificity.

$$y = -1103.2x^2 + 112321x - 2 \cdot 10^6 \text{ (Eq. 1)}$$

t-Test: paired two sample for means ($p < 0.05$) recognized the difference between apparent viscosity of 25% and 30%, 25% and 35% and 30 and 35% P407 biopolymeric solutions thus confirming the method selectivity. The method was robust in terms of temperature (RSD values of 0.49%, 0.9% and 1.72% for 23 °C, 25 °C and 27 °C consequently), sample application method (RSD values of 0.9% and 1.65% for direct pouring and spoon, accordingly) and geometry system (RSD values of 0.9% and 1.72% for cylinder and cone geometry, respectively).

Conclusion

Considering that all validation parameters were with lower values than set acceptance criteria it can be concluded that method used for rheological characterization of P407 hydrogel was precise, accurate, sensitive, specific, selective and robust thus demonstrating its validity.

Acknowledgments: The authors would like to acknowledge the project "Improving the technical competence of the Center for pharmaceutical nanotechnology by introducing standard analytical procedures in the field of rheometry and 3D bioprinting" financed by Ministry of education and science, North Macedonia.

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