Human eye is one of the most challenging organs for drug delivery, thus topical treatment for ocular diseases is still the most popular and accepted way. Nevertheless, many mechanisms protect delicate structure of the eye, therefore making it impossible to achieve sufficient drug concentration. In addition, small capacity of the conjunctival sac, short residence time at the application site, small surface of absorption along with poor patient compliance result in therapy failure. Though eye drops are the most frequently used drug form in the treatment of ocular diseases, due to their short residence time on the eye surface (with over 90% of primary dose loss), weak penetration through the cornea and absorption to the systemic circulation, there are newer forms of drugs investigated, that ensure prolonged contact of the active substance with cornea, and at the same time improve its bioavailability (1-4). One of the drug forms with aforementioned features are hydrogels – carriers and matrices based on hydrophilic polymers. As the functionality of hydrogels as drug excipients strongly depends on their rheological properties (5), and all the products designed for the ophthalmic use should fulfil sterility requirement, it is essential to use the method of sterilisation that affects those properties in the slightest manner (6).

The triblock, nonionic copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), so called poloxamers, are well-known among many polymers used in the gels manufacturing (7). In poloxamer molecules, hydrophilic chains of PEO are attached to a hydrophobic fragment of PPO, providing their amphiphilic properties. Widely investigated Poloxamer 407 or Pluronic F-127 (trade mark of BASF) (EO100-PO65-EO100) contains 70% w/w of poly(ethylene oxide) and 30% w/w of poly(propylene oxide). Water solutions of poloxamers are stable in the presence of acids, bases, and metal ions (1). Pluronics are intensively investigated polymers, suitable in obtaining in situ form that releases its active substance in a prolonged manner (8). It is possible due to their reverse thermosensitive property, enabling copolymers of PEO and PPO to increase viscosity with temperature growth. The sol-gel transition takes place without any crosslinking agents as a result of the asso-
cation of copolymer amphiphilic molecules. Unimers, soluble in water at low temperature, stay in equilibrium with forming micelles even after surpassing the critical micelle concentration. With temperature increase hydrophobic fragments (PPO) connect via van der Waals’ forces between methyl groups, which leads to the maximal reduction of contact area with solvent, reduction of bound water, and increase of the solvent’s entropy at the same time. Transition into the gel phase is possible due to the aggregation of unimers into spherical micelles and their volume fraction enlargement. Above the association temperature, these spherical micelles are tightly packed, thus the gel is physically crosslinked. The gelation is reversible and the phase transition temperature strictly depends on the polymer concentration, the length of hydrophobic fragment, and chemical properties of macromolecule – the more hydrophobic it is, the bigger increase of solvent entropy, larger driving force of hydrophobic segments aggregation, and lower gelling temperature. The gel-sol transition at higher temperatures is not yet investigated and described as precisely as sol to gel, but it can be explained by shrinking of the upper parts of PEO in micelles, related to the impact of temperature on PEO solubility, and interaction of PEO with PPO core. The outcomes obtained by other authors explain the gel-sol transition at higher temperatures as the effect of conversion of spherical forms into cylindrical, which results in loosening of packed gel structure (1, 9-14). The unique sol-gel-sol transition has made pluronics attractive materials in ophthalmic drug form technology (15-17). Both in vitro and in vivo studies confirmed the prolonged maintenance of hydrogels based on Pluronic F-127 on eye bulb surface, and also their biocompatibility with living tissue (18, 19).

The purpose of this study was the evaluation of the impact of sterilisation conditions on selected rheological properties of Pluronic F-127-based ophthalmic hydrogels.

### Table 1. Formulations used in survey marked by the method of sterilisation.

| Symbol | Sterilisation method                                      |
|--------|----------------------------------------------------------|
| NS     | non-sterilised                                          |
| S1     | steam autoclave, 121°C, 20 min                           |
| S2     | steam autoclave, 105°C, 30 min                           |
| S3     | membrane filter, 0.2 µm pore size                        |
| S4     | microwave autoclave                                      |

Pluronic F-127 was purchased from Sigma Aldrich (Germany) and used as received without any further purification.

As presented in Table 1, the study was conducted in five stages with non-sterilised formulations and with those subjected to one of four types of sterilisation.

Sterility of a drug can be achieved by terminal sterilisation in its final package or at least by aseptic preparation (20). Terminal sterilisation is a method of choice because it leads to the destruction of all living microorganisms and bacterial spores in the final product, that will not be processed any further, and because of that methods S1, S2, and S4 were chosen to the study. Steam sterilisation (S1, S2) is the recommended technique, described in European Pharmacopoeia and convenient for large-scale production. Since the process is mostly carried out at the temp. 121°C for 15-20 min. (S1), it was decided to modify those crucial parameters (S2). To the contrary, microwave sterilisation (S4) is not the pharmacopoeial method, but in comparison to the traditional steam autoclave, the sterilisation process duration is reduced 5-times, and the exposure to high temperatures is significantly lower (21). As the high temperature is the poloxamer-degrading factor (22), method S3 was also introduced to the study.

### Preparation

At each stage of the study, two gels based on Pluronic F-127 were prepared – the first one with 16.5% (w/w) and the other with 17% (w/w) of polymer amount. These concentrations allow to obtain stable hydrogels of liquid consistency at room temperature, easily applicable to the eye like standard eye drops. In these concentrations the fluids are non-Newtonian over an intermediate temperature range (5, 23). The proper amount of polymer was put into a glass bottle, and then bidistilled water was added to given mass (100.0 g). So prepared formulation was stirred on magnetic stirrer at 200 rpm for 10 min, hermetically sealed, and placed into a refrigerator (4°C). Except for the time of samples examination, each gel was stored in a refrigerator (24). Every test was conducted after 7 days from the moment of preparation, just to eliminate any potential storage time influence on rheological properties. After preparation, gels indicated for sterilisation were put into the refrigerator for 24 h, then a sterilisation took place, and eventually gels were again placed into the refrigerator. All measurements were carried out after 7 days from the moment of preparation.
Sterilisation

Gels were sterilised using one of four methods: in steam autoclave Fedegari FOB3S/TS (Switzerland) at temp. 121°C for 20 min (S1), in the same autoclave at temp. 105°C for 30 min (S2), using a cellulose acetate membrane filter VWR (514-0061) with pore size 0.2 µm and diameter 25 mm (S3), in microwave autoclave Microjet (Poland) at temp. 135°C by electromagnetic waves frequency 2450 MHz (S4) – the total process time, from insertion until removal of the flask, was about 10 min, whilst the time of exposure to sterilisation temperature was 80 s.

Rheology studies: viscosity and flow curves

Rheological studies were conducted with cone/plate Brookfield RVDV-III+CP rheometer (USA) using cone CP51 (angle 1.565°, radius 1.2 cm), and the temperature was controlled with Brookfield AP7LR-20 thermostat (USA). Gel samples of 0.5 mL were applied with sterile Becton Dickinson Discardit II (2 mL) syringes.

Studies on apparent viscosity (V) and shear rate (SR) dependence were measured at temp. 37 ± 0.1°C. Based on this, hysteresis loops were designated for each formulation – at first, a pre-shearing was conducted for 3 min by rotational speed of the spindle 0.1 rpm, then the main shearing, starting from the rotational speed 0.01 rpm (SR = 0.0384 s⁻¹), increasing by 0.01 rpm every 10 s to the maximum value 0.1 rpm (SR = 0.384 s⁻¹); after that the rotational speed decreased in the same time intervals by 0.01 rpm to the initial value. This initial point by lowest rotational speed (0.01 rpm) and the one by highest (0.1 rpm) were used in statistical analysis. Shear rate was calculated from spindle rpm using equation (I):

$$ S(I) \ SR = RC \times RPM $$

where: SRC – shear rate constant for CP51 cone (3.84).

Rheology studies: sol-gel transition and temperature coefficient

The relationship between apparent viscosity (V) [Pa s] and temperature (T) [°C] was analysed – minimal temperature of phase transition TP [°C] and temperature coefficient TC [Pa s/°C] were designated. In this study the spindle was rotated at constant speed 0.05 rpm (SR = 0.192 s⁻¹) and the temperature was linearly increased with the rate of 1°C/min in the range from 23.0°C to 43.0°C. Data points were taken every 0.5°C. Based on six points on the steep part of the V(T) plot, using the method of least squares, the equation of straight line was designated. This became a foundation to calculate TP and TC parameters. Six points with the value higher than V > 5 Pa s were chosen. The equation of linear function V(T) took form (II):

$$ (II) \ V(T) = TC \times T + b $$

where: T – temperature [°C], V – apparent viscosity [Pa s], b – intercept, TC – slope, here defined as the temperature coefficient, equal to the tangent of the angle between linear function and OX axis (axis of the temperature); it defines the dynamics of the phase transition (25).

The temperature of phase transition (TP) was calculated according to the equation for a zero of a linear function (III):

$$ (III) \ TP = -\frac{b}{TC} $$

Apparent viscosity versus shear rate study was repeated six times and apparent viscosity versus temperature study was repeated three times for each formulation.

Figure 1. Force versus time plot in the filtration pressure study for 16.5% and 17% formulations sterilised with S3 method. Selected area of the plot is the steady state plateau force. Flow rate: 0.1 mL/s
Filtration pressure study

During the sterilisation phase in method S3, a filtration pressure study was performed, using Stable Micro Systems TA.XT Plus texture analyser and Becton Dickinson Discardit II (2 mL) syringes. The texture analyser was fitted with a syringe rig, allowing to measure the force needed to maintain a vertical piston movement at a given, constant speed. A syringe was filled to a volume of 2 mL, then the membrane filter was attached and the syringe was placed in the rig. The piston linear speed was set as to give an output flow rate of 0.1 mL/s. This measurement was repeated 9 times for both formulations, with the change of the filter and syringe after every 3 passes. The measurement data from every first pass through a new filter was omitted due to excessively high peaks caused by the initial resistance of a dry membrane. After the filtration phase, empty syringes were examined in order to measure their individual friction. The force recorded in empty syringes test was subtracted from the force values obtained in the filtration phase. The resulting pressure was calculated using the mean force obtained from the texture analyser during the plateau phase of extrusion and the internal sectional area of the syringe (0.61 cm²). The plateau phase, from which a mean force was calculated, was chosen as shown in Figure 1.

Statistical analysis

Obtained results became a basis for statistical analysis. Descriptive statistics were designated – mean, standard deviation and relative standard deviation. Table 2 shows that RSD did not exceed 6% in V(SR) measurements and according to Table 3, the

| Type of sterilisation | Polymer concentration | SR = 0.0384 s⁻¹ (0.01 rpm) | SR = 0.384 s⁻¹ (0.1 rpm) |
|-----------------------|-----------------------|-----------------------------|---------------------------|
|                       | V [Pa s]              | RSD                         | V [Pa s]                  | RSD |
| NS                    | 16.5%                 | 2780.59                     | 1.91%                     | 343.56                     | 0.27% |
|                       | 17%                   | 3052.43                     | 0.77%                     | 376.35                     | 0.85% |
| S1                    | 16.5%                 | 2541.54                     | 3.00%                     | 318.36                     | 0.92% |
|                       | 17%                   | 2789.22                     | 2.73%                     | 346.24                     | 0.88% |
| S2                    | 16.5%                 | 2433.66                     | 2.90%                     | 314.48                     | 1.00% |
|                       | 17%                   | 2918.67                     | 1.48%                     | 357.28                     | 0.96% |
| S3                    | 16.5%                 | 2455.24                     | 5.74%                     | 321.81                     | 1.22% |
|                       | 17%                   | 3037.76                     | 3.30%                     | 372.47                     | 0.95% |
| S4                    | 16.5%                 | 2852.22                     | 5.10%                     | 365.48                     | 1.41% |
|                       | 17%                   | 2827.19                     | 3.08%                     | 363.58                     | 2.12% |

Table 3. Mean phase transition temperatures and temperature coefficient of investigated gels with their relative standard deviations.

| Type of sterilisation | Polymer concentration | Phase transition temp. [°C] | Temperature coefficient [Pa s/°C] |
|-----------------------|-----------------------|-------------------------------|----------------------------------|
|                       | Mean                  | RSD                           | Mean                            | RSD |
| NS                    | 16.5%                 | 26.21                         | 0.32%                           | 207.08                     | 5.18% |
|                       | 17%                   | 25.32                         | 0.27%                           | 232.01                     | 5.80% |
| S1                    | 16.5%                 | 26.89                         | 0.23%                           | 167.43                     | 6.53% |
|                       | 17%                   | 25.96                         | 0.27%                           | 194.22                     | 4.55% |
| S2                    | 16.5%                 | 26.84                         | 0.11%                           | 168.48                     | 0.72% |
|                       | 17%                   | 25.45                         | 0.82%                           | 211.66                     | 4.16% |
| S3                    | 16.5%                 | 26.09                         | 0.95%                           | 211.66                     | 4.14% |
|                       | 17%                   | 25.29                         | 0.38%                           | 225.46                     | 2.20% |
| S4                    | 16.5%                 | 25.74                         | 0.16%                           | 223.55                     | 2.59% |
|                       | 17%                   | 24.72                         | 0.34%                           | 251.98                     | 1.86% |
value of the statistic in $V(T)$ studies wasn’t higher than 1% for phase transition temperatures and less than 7% for temperature coefficient.

The Shapiro-Wilk test for the evaluation of normality, then the Brown-Forsythe test for the equality of variances, both with the same p-value $p < 0.05$ were used. As both features were proven, mean values were compared with parametric tests: Student’s t-test for independent samples or ANOVA in the case of more than two grouping variables. If ANOVA was used, also post-hoc Fisher’s LSD test was applied. When equation of the linear function describing phase transition’s dynamics was designated, the correlation between $V$ and $T$ was evaluated using Pearson’s $r$ with p-value $p < 0.05$. The coefficient in every case was at least 0.99, proving a strong correlation between experimental data and straight line equation. All statistical analyses were carried out with STATISTICA 12 software.

RESULTS

Before sterilisation

In the gel phase before sterilisation (NS) all formulations reveal their pseudoplastic properties, that is their viscosity decreases when shear rate increases. They are shear-thinning as shown in

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**Figure 2. Viscosity ($V$) versus shear rate ($SR$) plot for 16.5% formulations depending on the method of sterilisation**

**Figure 3. Viscosity ($V$) versus shear rate ($SR$) plot for 17% formulations depending on the method of sterilisation**
Table 2. Average viscosity of 16.5% hydrogels by primary shear rate 0.0384 s⁻¹ (0.01 rpm) is one order of magnitude larger than by 10-times higher shear rate 0.384 s⁻¹ (0.1 rpm).

Viscosity-shear rate dependence analysis showed a shear-thinning behaviour (pseudoplastic flow) but also a time-related viscosity change in the gel phase, visible in Figure 2 and Figure 3 as a moderate hysteresis of the rheograms. The viscosity noted on descending parts of the rheograms was consequently higher than on ascending parts for the same shear rates. While the well-known phenomenon of a decrease in viscosity due to continued shearing is called thixotropy (and is visible as a negative hysteresis), here, a time-dependent increase of viscosity should be considered as a “negative thixotropy”, i.e. rheopexy (26). Both thixotropy and rheopexy are time-dependent phenomena, and should not be confused with pseudoplastic and dilatant behaviour, which are related to the increasing shear rate and not the shearing time alone. As pictured in Figure 2 and Figure 3, under increasing shear rate, the viscosity decreases to the certain point, namely at the maximum rotational speed of the spindle 0.1 min⁻¹, thereafter with decreasing shear rate the viscosity grows, taking higher values at corresponding shear rate

![Figure 4. Viscosity (V) versus temperature (T) plot for 16.5% formulations depending on the method of sterilisation. Heating rate: 1°C/min](image-url)

![Figure 5. Viscosity (V) versus temperature (T) plot for 17% formulations depending on the method of sterilisation. Heating rate: 1°C/min](image-url)
points. Ascending part of the graph does not cover the descending one (which is higher), forming a characteristic hysteresis loop on the rheogram.

Study on temperature-viscosity dependence proved reversible thermosensitivity of Pluronic F-127-based hydrogels what is shown in Figure 4 and Figure 5 as visible phase transitions.

According to Table 3, before sterilisation the mean phase transition temperature of 16.5% gels was higher than of 17% gels, and the difference was statistically significant. Phase transition temperatures in both cases are below the mean temperature of the human body surface, thus these formulations might be administered to the cornea. The temperature coefficient was also investigated. There were no statistically significant differences in this coefficient between non-sterilised formulations with different polymer concentration, though in the case of 17% gel the viscosity increased faster.

After sterilisation

After sterilisation, despite used method and polymer concentration, gels still had pseudoplastic properties and their viscosity decreased with shear rate increase, which is shown in Table 2. The viscosity of each formulation was subjected to statistical analysis for every method of sterilisation, and the concentration of Pluronic F-127 was taken as a grouping variable. Because of two variables (16.5% and 17%), Student’s t-test was performed. Specific points by maximum shear rate 0.384 s⁻¹ (0.1 rpm) were selected. Within the same method of sterilisation, gels with 17% of pluronic concentration still were more viscous than 16.5% ones at the same shear rate. These differences were statistically significant in the case of formulations sterilised in the steam autoclave (S1, S2) and with membrane filters (S3). There were no statistically significant differences between viscosity values of hydrogels sterilised in microwave autoclave (S4).

A similar analysis of gels viscosity was performed, but the method of sterilisation was taken as a grouping variable. The variance was tested with ANOVA because of five variables (NS, S1, S2, S3, S4), and statistically significant differences were investigated with Fisher’s LSD test. In the case of 16.5% gels, as shown in Figure 6A, a statistically significant decrease in formulation viscosity was demonstrated for methods: S1, S2 and S3 compared to a non-sterilised gel. Among these three methods of sterilisation, there were no statistically significant differences between S1 and S2 or S1 and S3, but they occurred between S2 and S3. Sterilisation in microwave autoclave (S4) caused statistically significant increase of viscosity to mean value 365.48 Pa s, though it could be also possible due to a slight water evaporation from hydrogel matrix. The least difference compared to non-sterilised formulation 16.5% NS, namely 21.75 Pa s, was observed for gels sterilised with the use of membrane filters. In the case of 17% formulations, as shown in Figure 6B, viscosity decrease for each method of sterilisation was observed, but only for methods S1, S2 and S4 it was statistically significant. There were no statistically significant differences for 17% S3 formulation in comparison to non-sterilised one.
None of the performed sterilisation methods affected gels rheopexy, what was pictured in Figure 2 and Figure 3. Viscosity values during shear rate increase were lower than ones seen during shear rate decrease.

As before, after sterilisation hydrogels based on Pluronic F-127 maintained their phase transition, pictured in Figure 4 and Figure 5. According to data in Table 3, more concentrated formulations (17%) underwent a sol-gel transition at lower temperatures than 16.5% formulations, and the differences in gelling temperature were statistically significant in the case of every method of sterilisation. Dynamics of phase transition, described by temperature coefficient TC, had a similar tendency as in non-sterilised gels, that is its faster growth due to temperature increase was observed for 17% formulations. There were statistically significant differences between 16.5% and 17% gels sterilised in autoclaves (S1, S2, S4), however, gels forced through membrane filters (S3), just like non-sterilised ones, did not differ significantly between each other. It is worth pointing out, that phase transition temperature decrease is accompanied by the temperature coefficient growth and the other way around.

Variance analysis, ANOVA, was performed subsequently to compare phase transition temperature with sterilisation method as a grouping variable. Statistically significant differences, if observed, were analysed with Fisher’s LSD test. In the case of 16.5% formulations sterilised in the steam autoclave (S1, S2), a statistically significant increase of phase transition temperature was noticed, comparing to non-sterilised formulations, what can be seen in Figure 7A. There was also a statistically significant decrease of phase transition temperature in the case of gel sterilised with S4 method. Only the sterilisation with membrane filters did not change the value of tested parameter significantly – the mean sol-gel transition temperature was 26.09°C, which is only 0.12°C different from non-sterilised gels. Likewise, for gels with pluronic concentration 17%, an increase of phase transition temperature was observed in the case of autoclaved formulations and that can be seen in Figure 7B. The increase was statistically significant for S1 method, but not for S2. Sterilisation in microwave autoclave (S4) caused a statistically significant decrease of phase transition temperature. Still the most similar to non-sterilised hydrogels were outcomes for filtrated formulations S3 and the mean value was 25.29°C (only 0.03°C decrease).

Statistical analysis of temperature coefficients (TC) gave similar dependence to those received in phase transition temperature study. As shown in Figure 8A, 16.5% gels sterilised in the steam autoclave (S1, S2) demonstrated a statistically significant decrease of this coefficient in comparison to non-sterilised formulations. After sterilisation in microwave autoclave (S4), the temperature coefficient increased significantly up to 223.55 Pa s/°C. Only the use of membrane filters caused irrelevant growth of temperature coefficient value to 211.66 Pa s/°C. The same relationships were observed for 17% formulations, as shown in Figure 8B. There was a statistically significant decrease of TC in gels sterilised with S1 or S2 method. Method S4 caused a statistically significant increase of the coefficient.

![Figure 7. Categorized scatter plots with error bars of 16.5% (A) and 17% (B) formulations’ phase transition temperature (T) depending on the method of sterilisation](image-url)
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up to 251.98 Pa s/°C. Again, the sterilising filtration was the only method which did not affect the TC value significantly in comparison to non-sterilised gels, namely leading to a slight decrease to 225.46 Pa s/°C.

The filtration pressure study showed that the average pressure of 0.4 ± 0.1 MPa was necessary to force both studied formulations through a 0.2 µm/25 mm membrane filter at the rate 0.1 mL/s. The empty syringe friction was responsible for less than 7% of the force measured during filtration (1.7 ± 0.1 N, which corresponds to 0.028 ± 0.002 MPa). As shown in Figure 1, the pressure obtained for 17% formulation was slightly higher, although there were no statistically significant differences (p = 0.91) between formulations due to a wide distribution of experimental data.

DISCUSSION

Sterilisation is the key step in obtaining ophthalmic drugs of standard value. There are few reports about the impact of sterilisation conditions on properties of various hydrogel drug forms, especially for ocular use. Some methods of sterilisation, such as: lyophilisation, gamma-irradiation, UV-irradiation, hydrogen peroxide or ethylene oxide may cause many undesirable effects, e.g. loss of mechanical properties due to the polymer degradation, decrease in ability to absorb water, changes of swelling coefficient, model drug release profile, and gel surface, or even an increase of free radicals content (27, 28). Though terminal sterilisation of sodium alginate-Pluronic F-68 gel wound dressings in 70% ethanol was proposed as the best method (27), one must remember, that such form of sterilisation should not be used for pluronic-based formulations, because short-chain alcohols (i.a. ethanol) impede a structural gel formation by increasing the polymer solubility and restraining its micellization (29, 30). Steam autoclaving was used for sterilisation of hydrogels based on polyacrylic acid and hypromellose (3) (121°C for 15 min); carbopol (31) (2 bar for 60 min); poloxamer, tween, and carbopol (32) (121°C for 20 min); and Pluronic F-127 (25%) (with and without the addition of methylcellulose or hydroxypropyl methylcellulose) (33) (121°C for 30 min). Those studies did not reveal any significant changes in rheological properties (e.g. flow curves, phase transition temperature) of investigated gels, except for low-concentrated (0.5%) carbopol in the phosphate buffer solution. Though steam sterilisation was considered most adequate for Pluronic F-127 formulations (7), it was reported that it could provoke chemical changes of the composition, i.a. intensifying drug degradation, hence other techniques of sterilisation must be taken into consideration (6).

On the basis of all results obtained in this study, the sterilisation with membrane filters with 0.2 µm pore size seems to be the most suitable method for Pluronic F-127-based gels sterilisation. From all proposed methods, this one affects the rheological properties of formulation or its sol-gel phase transition temperature in the slightest way. In case of gels sterilised with the abovementioned method most of the differences were statistically

Figure 8. Categorized scatter plots with error bars of 16.5% (A) and 17% (B) formulations’ temperature coefficient (TC) depending on the method of sterilisation
insignificant. Sterilising filtration is also a method of choice when thermolabile substances are used. On the other hand, this process alone requires much effort and can be laborious with larger amount of material, thus demanding automation on a bigger scale, e.g. sterilisation driven by a sterile gas source. It is, however, adequate for compounded drugs in pharmacies or laboratory research. One must remember that according to the pharmacopoeial standards, the terminal sterilisation in the final package is recommended (20). Only if it is impossible, Pharmacopoeia allows filtration through membrane filters or preparation using sterile ingredients in aseptic conditions. For these reasons, it is important to analyse outcomes received with other methods of sterilisation. Steam autoclaving caused many changes in gels properties, i.a. viscosity decrease, phase transition temperature increase, temperature coefficient decrease. They can be explained as results of polymer degradation. According to available studies, pluronic undergo severe degradation when thermally stressed in oxygen-containing environment (22). A crucial part of the degradation process is the scission of polymer chain, taking part preferably in the PPO region, which is less thermally stable than the PEO chain. The scission of pluronic molecule produces low molecular weight products (e.g. C1-C2 alcohols, aldehydes, acids, esters), and diblock copolymer molecules, consisting of a PEO chain and a part of PPO chain, both susceptible to further degradation (34). The resulting diblock copolymers do not incorporate into micelles, and do not take part in the gelation process (35), thus the gelation ability of autoclaved solutions is reduced. It can be concluded that the crucial factor in that kind of sterilisation is the temperature, not the length of the process. Samples sterilised with S1 method, that is at 121°C for 20 min, revealed more differences from non-sterilised gels that those sterilised with S2 method, that is at 105°C for 30 min. Thus, the S2 method should be taken into consideration when final sterilisation is intended. Sterilisation in microwave autoclave caused opposite changes to the one in steam autoclave. Phase transition temperature decrease and temperature coefficient increase were observed, usually with viscosity growth.

**CONCLUSION**

The purpose of the survey was to investigate the impact of sterilisation conditions on the rheological properties of thermoresponsive gels based on Pluronic F-127 in two concentrations: 16.5 and 17%. These concentrations allow to obtain stable gels, liquid at room temperature, that can be applied as traditional eye drops. On the eye surface, at higher temperature, the sol-gel transition takes place and the new, *in situ* drug form has better adhesive properties, thus longer exists on the eye bulb and can liberate active substance in a prolonged manner. Four methods of sterilisation were introduced to the study. The least changes in investigated parameters were observed after sterilisation with membrane filters, although it is not a universal method. Steam autoclaving has larger impact on rheological properties of pluronic-based hydrogels, however it is more versatile procedure with broader application spectrum. Based on received outcomes, it can be concluded that longer duration of autoclaving with lower temperature at the same time allows to obtain formulations more similar to non-sterilised ones. Results from microwave autoclave sterilisation were unclear, probably due to solvent evaporation. In this case a further research is needed.

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**REFERENCES**

1. Achouri D., Alhanout K., Piccerelle P., Andrieu V.: Drug Dev. Ind. Pharm. 39, 1599 (2013).
2. Ludwig A.: Adv. Drug Deliv. Rev. 57, 1595 (2005).
3. Rathore K.S.: Int. J. Pharm. Bio. Sci. 2, 248 (2011).
4. Weiner A.L., Gilger B.C.: Vet. Ophthalmol. 13, 395 (2010).
5. Gandra S.C., Nguyen S., Nazzal S., Alayoubi A., Jung R., Nesamony J.: Pharm. Dev. Technol. 20, 41 (2015).
6. Asasutjarit R., Thanasanchokpibull S., Fuong-fuchat A., Veeranondha S.: Int. J. Pharm. 411, 128 (2011).
7. Dumortier G., Grossiord J.L., Agnely F., Chaumeil J.C.: Pharm. Res. 23, 2709 (2006).
8. Escobar-Chávez J.J., López-Cervantes M., Naflk A., Kalia Y.N., Quintanar-Guerrero D., Ganem-Quintanar A.: J. Pharm. Pharm. Sci. 9, 339 (2006).
9. Jeong B., Kim S.W., Bae Y.H.: Adv. Drug. Deliv. Rev. 64, 154 (2012).
10. Li L., Lim L.H., Wang Q., Jiang S.P.: Polymer 49, 1952 (2008).
11. Nirmal H., Bakliwal S., Pawar S.: Int. J. Pharm. Tech. Res. 2, 1398 (2010).
12. Alexandridis P., Alan Hatton T.: Colloids Surf. A Physicochem. Eng. Asp. 96, 1 (1995).
13. Wanka G., Hoffmann H., Ulbricht W.: Macromolecules 27, 4145 (1994).
14. Wanka G., Hoffmann H., Ulbricht W.: Colloid Polym. Sci. 268, 101 (1990).
15. Lou J., Hu W., Tian R., Zhang H., Jia Y. et al.: Int. J. Nanomedicine 9, 2517 (2014).
16. Patel N., Thakkar V., Metalia V., Baldaniya L., Gandhi T., Goel M.: Drug Dev. Ind. Pharm. 42, 1406 (2016).
17. El-Kamel A.H.: Int. J. Pharm. 241, 47 (2002).
18. Al Khateb K., Ozhmukhametowa E.K., Mussin M.N., Seikhanor S.K., Rakhbeko T.K. et al.: Int. J. Pharm. 502, 70 (2016).
19. Furrer P., Plazonnet B., Mayer J.M., Gurny R.: Int. J. Pharm. 207, 89 (2000).
20. Methods of preparation of sterile products, in European Pharmacopoeia 8th Edition, 555, Strasbourg 2014.
21. Research report: Effectiveness assessment of the sterilization process using Microjet Microwave Autoclave https://dl.dropboxusercontent.com/u/15115430/ENBIO/Validation%20report%20long%20ENG.pdf (accessed on 11.04.2017).
22. Erlandsson B.: Polym. Degrad. Stab. 78, 571 (2002).
23. Guo L., Colby R.H., Lin M.Y., Dado G.P.: J. Rheol. 45, 1223 (2001).
24. Grela K.P., Marciniak D.M., Pluta J.: Acta Pol. Pharm. 71, 167 (2014).
25. Marciniak D.M., Grela K.P., Balwierz R., Jastrz jab A., Pluta J.: Pol. Pharm. 72, 8 (2016).
26. Tropea H., Yarin A.L., Foss J.N.: Thixotropy, Rheopexy, Yield Stress, in Springer Handbook of Experimental Fluid Mechanics, Springer, pp. 661-79, Springer-Verlag, Berlin, Heidelberg 2007.
27. Stoppel W.L., White J.C., Horava S.D., Henry A.C., Roberts S.C., Bhatia S.R.: J. Biomed. Mater. Res. B Appl. Biomater. 102, 877 (2014).
28. Kanjickal D., Lopina S., Evancho-Chapman M.M., Schmidt S., Donovan D.: J. Biomed. Mater. Res. A. 87, 608 (2008).
29. Kwon K.W., Park M.J., Hwang J.: Polym. J. 33, 404 (2001).
30. Pandit N.K., McIntyre H.J.: Pharm. Dev. Technol. 2, 181 (1997).
31. Weyenberg W., Todorov V., Ludwig A.: Pharmazie 59, 121 (2004).
32. Li Hong W., Xin C., Yongxue G., Yiyin B., Gang C.: Drug Dev. Ind. Pharm. 40, 1402 (2014).
33. Desai S.D., Blanchard J.: J. Pharm. Sci. 87, 226 (1998).
34. Gallet G., Carroccio S., Rizzarelli P., Karlsson S.: Polymer 43, 1081 (2002).
35. Mortensen K., Batsberg W., Hvidt S.: Macromolecules 41, 1720 (2008).

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