

***In vitro* studies of the properties of thermosensitive systems prepared on Pluronic F-127 as vehicles for methotrexate for delivery to solid tumours**

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Summary

The aim of the trial was to prepare a thermosensitive vehicles for methotrexate which, after implantation to a solid tumour, could form *in situ* a specific implant releasing the active substance at the site of application. Pluronic F-127 with certain additives, i.e. lactose, glucose, propylene glycol, glycerol and sodium phosphate was used for investigations. The sol-gel transition temperature of the obtained systems at increasing temperature and various shear rates as well as their physicochemical properties were investigated. All the prepared systems underwent sol-gel transition at physiological ranges of temperature.

Methotrexate release from selected formulations approached zero order kinetics and the investigations have shown that the use of Pluronic F-127 at concentration of 14.8% in the construction of drug carriers enables to obtain such a form that, depending on the presence of additives and their concentration, provides active substance release at any time. Analysis of physicochemical properties of the investigated formulations, i.e. pH, osmotic

pressure, density allows to believe that their administration by means of a thin needle should not be problematic and they will not irritate tissues or cause pain after application.

Key words: Sol-gel transition temperature, Intratumoral injection, Methotrexate

INTRODUCTION

Despite maintaining specific therapeutic principles, systemic chemotherapy of tumours does not usually bring satisfactory results. Numerous reports indicate that the efficacy of this method is low especially in the therapy of solid tumours, i.e. tumours of the head, liver, lung, breast, intestine and prostate [1,2]. Systemic administration of a cytotoxic drug decreases the immunological status of the patient, leads to secondary oncogenicity and mutagenicity and thus to genetic changes in the cell genotype [3, 4]. Moreover, the drugs may lose their effectiveness due to chemoresistance developed by the tumour cells. The phenomenon of resistance, characteristic for the majority of solid tumours, contributes to the fact that surgical procedure is generally considered as a radical method of choice in the treatment of the disease. However surgical removal of a tumour is possible only in cases of localized disease and in patients who are in relatively good general condition. Thus non-operable tumours, diseases recognized as non-systemic, or those which proved resistant to primary treatment still remain an unsolved problem [5-7]. Apart from surgical intervention, also radiotherapy offers a perspective for radical therapy of solid tumours. However the use of this therapeutic modality is limited by insensitivity of many tumours to irradiation

and low tolerance of irradiation of tumour surrounding tissues [8]. Low effectiveness of systemic chemotherapy is mainly associated with the complicated biology of the tumour. Lack of lymph vessels, poor and changeable blood supply to the pathologically changed tissue and increased pressure limit significantly the possibility of delivery of the drug to the tumour [1,3,9]. Delivery of the cytotoxic drug directly to the site of its action results in a better distribution of the drug within the pathological tissue and reduces adverse effects. Amelioration or cessation of adverse drug effects following local administration of the cytotoxic drug is significant especially for active substances like methotrexate, the blood level of which has to be monitored after systemic administration [10]. Decreased interaction with other drugs administered to patients for other associated conditions is an additional clinical advantage of local therapy. Modifications of the drug form without interfering with the chemical structure of the active substance give the possibility of obtaining the desired pharmacological effect and meet the requirements of an effective therapy.

The aim of the study was to evaluate the physicochemical properties of formulations produced on the basis of a thermosensitive polymer to be injected into a solid tumour. This drug form may be administered as implant enabling slow release of the active substance at the site of application. The proposed therapeutic model may be applied in the treatment of non-surgical tumours which cannot be excised in view of their localization, i.e. tumours of the liver pancreas, ovaries, skin, breast, brain, lungs, the region of trachea or when the possibility of surgical excision is largely limited due to neoplastic infiltration of several neighbouring organs. Administration of a drug in systems with thermosensitive properties is possible when the active substance carrier is applied in the form of a solution able to gelate *in situ* at physiological temperature. This form of system can be administered

by means of a thin needle used for deep injections and would not cause pain. Pluronic F-127, which was used to prepare the investigated formulations, is a biocompatible polymer undergoing bioerosion in the organism. It was used in drug form technology as parenteral carrier for proteins, antibiotics and analgesic drugs, as a polymer forming gels for nasal delivery and applied on the eyeball as well as suspension stabilizer, thickening agent, emulsifying agent and hydrophilic agent binding water in gels, emulsions and ointment vehicles [11-15]. Kabanov et al. described a new application of this block copolymer in the treatment of multidrug-resistant cancer (MDR). The polymer interacts with the tumour cells evoking their sensitivity to various chemotherapeutic agents through the effect on several distinct immunological mechanisms, mainly through inhibition of the delivery and uptake of the chemotherapeutic drug in acid base, inhibition of S-transferase glutathione detoxication system, which participates in the degeneration and excretion of the drug. All the above-described drug resistance mechanisms are energy-dependent and the depletion of ATP induced by the block co-polymer in MDR cells is believed to be the potential reason of sensitizing the tumour cells to chemotherapy [16].

MATERIALS AND METHODS

Materials

Pluronic F-127 (Sigma-Aldrich Company Ltd, USA); D(+)-Glucose monohydrate (Fluka, Farnce); D(+)-Lactose monohydrate (Fluka, Switzerland); Propylene glycol-1.2 (POCh, Gliwice, Poland); Glycerol 85% (Fluka, Italy); Banzalconium chloride (Fluka, Denmark); Di-sodium hydrogen phosphate (Riedel-de Haeen); Methotrexate 10mg/ml (EBWE).

Calibrated solution at osmotic pressure of 0 mOsm/l and 400 mOsm/l. Bidistilled water meeting the requirements of Polish Pharmacopoeia VI [17].

METHODS

Preparation of thermosensitive systems

Liquid formulations containing Pluronic F-127 were prepared using the cold method described by Schmolka in a chamber with Lamil laminar ventilation (Karstulan Metalli OY, Finlandia) [18]. All the additives, i.e. glucose, lactose, glycerol, propylene glycol, sodium diphosphate and benzalconium chloride as preservative and methotrexate were added gradually to de-ionized pre-sterilized in autoclave water at about 15°C. After dissolution of the additives, the polymer was added in increments and stirred. The mixed solution was filtered through aprotogenic membrane filters Arcodisc® Syringe Filter (Pall Gelman Laboratory, Ann Arbor, USA) to sterile vials. Ready formulations were stored in refrigerator and investigated after 72h. The composition of obtained systems is given in Table 1.

The preliminary investigations of the effect of different shear times and shear rates on viscosity of the formulation were performed on systems prepared on 14.8% and 15% Pluronic F-127 without additives.

Determination of pH

PH measurement was performed by means of multi-functional computer system Elmetron CX-742 (Zabrze Grzybkowice, Poland) for pH measurement with OSH 10-10 combined

electrode (EuroSensor, Gliwice) at $20^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The measurements were six times repeated for every formulation and mean pH was calculated.

Density measurements

Density of the obtained formulations was measured for every formulation according to a method specified in FPVI in a 10 ml picnometer with thermometer and air jacket at 20°C [18]. Density was calculated from the following formula:

$$d_{20} = m/w \times 0,997 + 0,0012 \text{ [g/ml]}$$

in which:

d_{20} – density at 20°C in g/ml; m – mass of the investigated formulation at 20°C in g; w – mass of the corresponding volume of water in air at 20°C in g; 0.997 – density of water at 20°C ; 0.0012 –allowance for weighing in air.

Every formulation was six times weighed on Sartorius analytical balance exact to 0.001 g and mean density was calculated.

Osmolarity measurements

Initial evaluation of osmolarity of the formulations was performed on an osmometer (Memory-Osmometr 020-AT, P. Z. Intech) for systems not containing methyotrexate. Individual formulations were placed in the apparatus and cooled. Decrease of temperature resulted in solidification of water and the released heat of melting increased the temperature of the solution to freezing temperature. Prior to the measurements the apparatus was scaled using reference solutions with a determined, known osmotic pressure. 100 μl of the formulation was taken by a micropipette for examination. The pressure was

read on the scale of the apparatus in mOsm/l. The osmotic pressure measurements were six times repeated for every formulation and mean values were calculated for all the systems.

Transmittation measurments

Transmittation (T) expressed in percentage determines the ratio of the intensity of monochromatic radiation at a determined wavelength (I) passing through the sample to the intensity of incident radiation (I_0). The examination was performed in quartz dish in UV/VIS spectrophotometer Cecil Instruments-Chemist-Handel (M. B. H., Austria) at 520 nm wavelength in the presence of water [18], for which the transmittation was 100%.

Studies of the rheological properties of the formulations

Investigation of the effect of shear time, shear rate and concentration of methotrexate on viscosity and sol-gel transition temperature in formulations prepared on Pluronic F-127

The measurements of structural viscosity of the investigated formulations were performed on a Brookfield rheometer type DV-III (Middleboro, USA) connected to an ultrathermostate with the use of CP-51 cone, in the viscosity range from 20.48 to 512 000 mPs.s. The effect of shear time on changes in viscosity of formulation containing 15% Pluronic F-127 was investigated at shear rate ($\dot{\gamma}$) 10 and 100 s⁻¹ at constant temperature of 20 and 30°C. Moreover, the effect of methotrexate concentration and different shear rates on sol-gel transition temperature of systems containing 14.8% Pluronic F-127 was investigated.

Determination of sol-gel transition temperature of the prepared systems on the basis of viscosity changes

Sol-gel transition temperature was measured by means of a Brookfield rheometer type DV-III. The measurements were three times repeated for every formulation at temperature increasing by 0.2°C/5 s in ranges from 20°C to 40°C, constant shear rate of 1, 10 and 100 s⁻¹. The determination of changes in viscosity with increasing temperature for increased-viscosity preparation for intramuscular injections, i.e. oil solution of estradiol benzoate, were performed in the same conditions at 100 s⁻¹.

Methotrexate release from selected formulations to water in flow chambers

Methotrexate, used in therapy of many cancers, was used as a model substance for the investigation of release kinetics. Semi-permeable membrane Membra-Cel[®] Dialysis Tubing (Serva, Heidelberg) with pore size of 3500 Da was immersed in bi-distilled water for 30 minutes prior to the investigation. Weighed exact to 0.001 g portions of formulations containing 0.01% methotrexate were introduced with a syringe to active substance release chamber constructed by Z. Olszewski and A. Kubis, separated by a semi-permeable membrane, and immersed in water bath at 37°C. Dialysis fluid was collected in 1ml aliquots after 30, 60, 90, 120, 150, 180, 210 and 240 minute. The obtained sample was supplemented with bi-distilled water to 3 ml. The amount of released active substance was determined spectrophotometrically using a UV/VIS spectrophotometer manufactured by Cecil Instruments-Chemist-Handel (M. B. H. Austria), in quartz dishes with 1 cm thick layers in relation to bi-distilled water. The measurements were six times repeated and mean values were calculated.

The data were analyzed on STATISTICA Version 5,97' software, using the method of least squares at confidence level of $p=0.05$. Release rate constant (K) and semi-release time ($t_{0.5}$) were determined on the basis of pharmacokinetic equations. The significance of relations between the amount of released methotrexate from individual formulations was evaluated statistically using uni-factorial analysis ANOVA/MANOVA post hoc NIR test.

RESULTS

Altogether, 11 formulations prepared on the base of Pluronic F-127 with various additives were evaluated. The obtained formulations were transparent, colourless, of liquid consistency at room temperature. Bringing a parenterally administered drug to the state of isohydria is essential in order to avoid the possibility of adverse effects when the drug is injected, i.e. pain or tissue inflammation. According to FP VI, pH of the drug forms for parenteral administration should approach physiological pH of body fluids from 7.2–7.6 [17].

Deviation of pH from 6.5 to 7.8 is acceptable on short-term administration of the drug. Table II presents pH for the prepared formulations. Prepared formulations had pH values approaching physiological reaction. The pH of systems containing 1% addition of glucose (A, A1), lactose (B, B1), glycerol (C, C1) and propylene glycol (D, D1) was within the physiological limits for systemic fluids. The highest pH deviations, from 8.255 to 8.891 were revealed in systems prepared on the base of 14.8% Pluronic F-127 with addition of 0.2% sodium diphosphate (E, E1). On parenteral administration, these formulations can cause tissue irritation after application.

The measurements of density were six times repeated for every formulation in a picnometer at 20°C. Mean density values in g/ml are given in Table 2. The prepared formulations revealed various density values depending on composition. The highest density was revealed by formulations obtained with the addition of 0.2% sodium phosphate solution and 1% lactose. Formulations prepared on 14.8% Pluronic F-127 with the addition of 1% solution of propylene glycol were characterized by the lowest density ranging from 1.0179 g/ml for systems free from methotrexate (D) and 1.0174 g/ml for formulations containing methotrexate (D1). The density of the investigated formulations was higher than that of oil solution of estradiol benzoate for intramuscular injections, which at 20°C was 0.9625 g/ml.

According to FP VI, if tissue irritation has to be avoided after administration, the osmotic pressure of drug forms for injections should range from 280-320 mOsm/l. The results for systems 0, A, B, C, D, E, not containing methotrexate, are presented in Fig.1. Osmotic pressure of the obtained formulations was within the minimal limits recommended for parenteral application. The addition of adjuvant substances to the formulation based on 14.8% Pluronic with the addition of 0.01% benzalkonium chloride enabled to obtain isotonic or low-hypertonic formulations which should not cause tissue irritation following administration.

The measurements of transmittance determine clarity, and indirectly, also of dispersion of the prepared systems. The results of transmittance measurements for the investigated formulations are presented in Table 2. Obtained transmittance results ranging from 95-100% point to a high clarity of the formulations and indirectly to a significant dispersion of the additives in the prepared system.

Investigation of the relationship between changes in viscosity in relation to temperature in the studied formulations has revealed that all the systems reveal sol-gel transition occurring at increasing temperature. It was demonstrated that the transition for the system based on 14.8% Pluronic F-127 with the use of certain shear rates, 10 and 100 s⁻¹, occurred at the same temperatures. Viscosity changes for the formulation containing 14.8% Pluronic F-127 at 10 and 100 s⁻¹ and temperature increasing by 0.2°C/5 s are presented in Fig. 2. In order to eliminate the effect of shear time in the transition processes at constant temperatures of 20 and 30°C and constant shear rate 10 and 100 s⁻¹, the changes in viscosity in time were registered for the system based on 15% Pluronic F-127. The results presented in Fig 3 demonstrate that the time of measurements at constant temperature was not associated with changes in viscosity of the formulations.

The effect of various concentrations of methotrexate on sol-gel transition temperature was investigated further in the study. No changes in viscosity were observed with increase of temperature between formulations containing 14.8% Pluronic F-127 and 0.01% and 0.1% methotrexate. In comparison to the above systems, formulation containing 0.5% methotrexate investigated in the same conditions was characterized by sol-gel transition temperature higher by 1-2°C, revealing at the same time lower viscosity values. The results are presented in Fig. 4.

Formulations not containing active substances revealed similar transition in similar temperature ranges, i.e. about 33-35°C. Introduction of methotrexate to the systems resulted in the decrease of their viscosity, while the transition temperature was increased by 2-3°C towards physiological temperature of the body. Changes in viscosity at increasing temperature are presented in Fig. 5-7.

The highest viscosity was revealed by formulations based on 14.8% Pluronic F-127 and 0.01% benzalconium chloride with 1% addition of lactose and glucose. Sol-gel transition temperature of the systems after addition of methotrexate was within physiological ranges of 36-37°C.

In order to evaluate fully the pharmaceutical usefulness of obtained formulations, we have investigated changes in viscosity of a commercially available oil solution of estradiol benzoate with increased viscosity for intramuscular injections. The dynamic viscosity of the product at 20°C and 100 s⁻¹ shear rate was determined at 65 mPa.s, while at 25°C it was 55 mPa.s. The dynamic viscosity of the investigated formulations at 20°C was in the range of 17.9-27.8 mPa.s, depending on their composition, while at 25°C it ranged from 39.8 to 67.6 mPa.s. Obtained results demonstrate that the viscosity of the systems does not deviate from values determined for a preparation with increased viscosity. Thus there administration of liquid formulations in direct injection to the tumour should not be difficult at room temperature. The results of viscosity changes at increasing temperature and 100 s⁻¹ shear rate for a reference formulation are presented in Fig. 3.

The release of methotrexate from the obtained vehicles characterized by sol-gel transition under the effect of increasing temperature was performed for 4 hours 37°C. Consecutive fractions were collected every 30 minutes. The measurements were six times repeated for every formulation and mean percentage of released substance was calculated. The course of methotrexate release process is presented in Fig. 8. Analysis of the results included comparison of R correlation coefficients for empirical data in the linear and semi-logarithmic scales. Obtained values of the coefficients for selected systems at the significance level $p < 0.05$ evidence high matching of the experimental data to the linear

function of changes in the residual (100-% - released methotrexate) methotrexate in the investigated systems in relation to duration of the release process. A thorough analysis of the functional relationship between the remaining percentage of released methotrexate and time in linear and semi-logarithmic systems on the basis of R^2 coefficients demonstrated that the process of methotrexate release from formulations D1 and E1 may be described on the basis of first order kinetics model. The release of methotrexate from formulations A1, B1, C1 containing the additives, 1% glucose, 1% lactose and 1% glycerol respectively, approached zero order kinetics. Release rate constants and semi-release times were calculated on the basis of respective pharmacokinetic equations. The results are presented in Table 3.

Interpretation of the obtained findings demonstrated that the system based on 14.8% Pluronic with the addition of 0.2% sodium diphosphate revealed the highest pharmaceutical availability. The significance of relations between the total percentage of released methotrexate from individual formulations evaluated statistically by means of a uni-factorial ANOVA/MANOVA analysis, post hoc NIR test, confirmed statistically significant differences in the percentage of released methotrexate from various formulations. Table 4. presents the significance levels of obtained results for post hoc NIR test. The longest semi-release time ($t_{0.5}$) 22.04 h was observed in case of formulation prepared with the addition of 1% lactose. The investigations have shown that the use of 14.8% concentration of Pluronic F-127 in preparation of vehicles for drug delivery made it possible to obtain such a form which, depending on applied additives, enables active substance release at various times.

CONCLUSIONS

1. The results of *in vitro* studies demonstrate that the prepared systems characterized by sol-gel transition under the effect of temperature increase may be used as vehicles for active substance administered as direct injection into the neoplastic tumour. Transition of the prepared formulations at physiological temperature ranges of the body makes possible their injection in the liquid state and subsequent gelification *in situ* providing a prolonged release of the active substance at the application site. The viscosity of the prepared formulations did not deviate from values determined for a selected commercially available product with increased viscosity for intramuscular administration and should not cause any problems on application.
2. The investigations have demonstrated that the use of 14.8% concentration of Pluronic F-127 with addition of certain additives as a thermosensitive drug carrier makes it possible to obtain systems characterized by various active substance semi-release times.
3. Obtained findings indicate that formulation based on Pluronic F-127 and benzalconium chloride with 1% addition of lactose revealed the best physico-chemical properties. Methotrexate release from this system approached zero order kinetics and active substance semi-release time ($t_{0.5}$) determined for this formulation supports its usefulness in constructing a prolonged release drug from.

LITERATURE

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TABLE 1. Composition of the investigated formulations

TABELA 1. Skład badanych formułacji. Oznaczenie formułacji

Content/ Zawartość (%)	Formulation version/ Oznaczenie formułacji										
	0	A	B	C	D	E	A1	B1	C1	D1	E1
Pluronic F-127	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8
Glucose/ Glukoza	-	1.0	-	-	-	-	1.0	-	-	-	-
Lactose/ Laktoza	-	-	1.0	-	-	-	-	1.0	-	-	-
Glycerol 85%/ Glicerol 85%	-	-	-	1.0	-	-	-	-	1.0	-	-
Propylene glycol-1.2/ Glikol 1,2-propylenowy	-	-	-	-	0.5	-	-	-	-	0.5	-
Di-sodium hydrogen phosphate/ Difosforan sodowy	-	-	-	-	-	0.2	-	-	-	-	0.2
Benzalconium chloride/Chlorek benzalkoniowy	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Methotrexate/ Metotreksat	-	-	-	-	-	-	0.01	0.01	0.01	0.01	0.01

TABLE 2. pH, density in g/ml and transmittation (T) values for prepared formulations

TABELA 2. Wyznaczona wartość pH, gęstości w g/ml i transmitacji (T) dla sporządzonych formułacji

Formulation version/ Oznaczenie formułacji	pH X ± S. D.	Density/ Gęstość (g/ml) X ± S. D.	T (%)
0	7.077 ± 0.032	1.0187 ± 0.000644	99.6
A	7.039 ± 0.026	1.0199 ± 0.000539	94.5
B	7.046 ± 0.008	1.0200 ± 0.000397	99.8
C	7.075 ± 0.016	1.0194 ± 0.001169	96.4
D	7.051 ± 0.021	1.0179 ± 0.001136	96.1
E	8.256 ± 0.025	1.0216 ± 0.000836	97.9
A1	7.076 ± 0.015	1.0200 ± 0.001432	95.5
B1	7.071 ± 0.019	1.0220 ± 0.001300	99.5
C1	7.141 ± 0.215	1.0177 ± 0.001113	99.6
D1	7.030 ± 0.050	1.0174 ± 0.000207	97.8
E1	8.890 ± 0.034	1.0187 ± 0.001198	99.6

TABLE 3. Interpretation of the release process on the basis of zero order and first order mathematical orders.

Release rate constants (K) and semi-release times ($t_{0.5}$)

TABELA 3. Interpretacja procesu uwalniania w oparciu o modele matematyczne zerowego rzędu i pierwszego rzędu. Wartości stałych szybkości procesu uwalniania (K) oraz czasy półuwalniania ($t_{0.5}$). Równanie opisujące kinetykę uwalniania w układzie liniowym i R^2 . Równanie opisujące kinetykę uwalniania w układzie półlogarytmicznym

Formulation /Formulacja	Equation describing the release kinetics in linear system and R^2 / Równanie opisujące kinetykę uwalniania w układzie liniowym	Equation describing the release kinetics in semi-logarithmic system and R^2 / Równanie opisujące kinetykę uwalniania w układzie półlogarytmicznym	K (min^{-1}) $X \pm S. D.$		$t_{0.5}$ [h]
A1	$y = 100.00 - 0.0656 x$ $R^2 = 0.9931$	$y = 4.605 - 0.0007 x$ $R^2 = 0.9870$	K dla kinetyki zerowego rzędu	0.0656 ± 0.00874	12.70
B1	$y = 100.00 - 0.0378 x$ $R = 0.9970$	$y = 4.605 - 0.0004 x$ $R^2 = 0.9949$		0.0378 ± 0.00652	22.04
C1	$y = 100.00 - 0.0680 x$ $R = 0.9910$	$y = 4.605 - 0.0007 x$ $R^2 = 0.9841$		0.0680 ± 0.00437	12.25
D1	$y = 100.00 - 0.0586 x$ $R = 0.9948$	$y = 4.605 - 0.0006 x$ $R^2 = 0.9965$	K dla kinetyki pierwszego rzędu	0.0006 ± 0.000013	19.25
E1	$y = 100.00 - 0.0764 x$ $R = 0.9993$	$y = 4.605 - 0.0008 x$ $R^2 = 0.9995$		0.0008 ± 0.000022	14.44

TABLE 4. Significance levels for post hoc NIR test

TABELA 4. Poziomy istotności dla testu post hoc NIR

	A1 X =16,41875	B1 X =9,181375	C1 X =16,72000	D1 X =14,04175	E1 X =17,6254
A1	-	0,000001	n. s.	0,015385	n. s.
B1	0,000001	-	0,000000	0,000052	0,000000
C1	n. s.	0,000000	-	0,007622	n. s.
D1	0,015385	0,000052	0,007622	-	0,000905
E1	n. s.	0,000000	n. s.	0,000905	-

n. s. - statistically insignificant/ nieistotne statystycznie.

List of figure:

FIG. 1. Mean osmotic pressure in mOsm/l for formulations not containing methotrexate

Ryc. 1. Średnia wartość ciśnienia osmotycznego w mOsm/kg dla formulacji nie zawierających metotreksatu

FIG. 2. The relationship between viscosity and temperature for formulation containing 14.8 % Pluronic F-127 at 10 and 100 s⁻¹ shear rate

Ryc. 2. Zależność lepkości od temperatury dla formulacji zawierającej 14,8 % Pluronic F-127 przy szybkości ścinania 10 i 100 s⁻¹

FIG. 3. Changes in viscosity of formulation containing 14.8% Pluronic F-127 at shear rate of 100 s⁻¹ and increasing temperature in relation to methotrexate concentration. The graph also presents changes in viscosity of Oestradiolum benzoicum with increased viscosity for intramuscular administration

Ryc. 3. Zmiany lepkości formulacji zawierającej 14,8% Pluronic F-127 przy szybkości ścinania 100 s^{-1} i wzrastającej temperaturze w zależności od stężenia metotreksatu. Na wykresie przedstawiono również zmiany lepkości dla preparatu Oestradiolum benzoicum o zwiększonej lepkości podawanego domięśniowo.

FIG. 4. The effect of temperature on viscosity at 1 s^{-1} shear rate for formulations not containing methotrexate

Ryc. 4. Zależność lepkości od temperatury przy szybkości ścinania 1 s^{-1} dla formulacji nie zawierających metotreksatu

FIG. 5. The effect of temperature on viscosity at 10 s^{-1} shear rate for formulations not containing methotrexate

Ryc. 5. Zależność lepkości od temperatury przy szybkości ścinania 10 s^{-1} dla formulacji nie zawierających metotreksatu.

FIG. 6. The effect of temperature on viscosity at 1 s^{-1} shear rate for formulations containing methotrexate

Ryc. 6. Zależność lepkości od temperatury przy szybkości ścinania 1 s^{-1} dla formulacji zawierających metotreksat

FIG. 7. The effect of temperature on viscosity 10 s^{-1} shear rate for formulations containing methotrexate

Ryc. 7. Zależność lepkości od temperatury przy szybkości ścinania 10 s^{-1} dla formulacji zawierających metotreksat.

FIG. 8. The course of methotrexate release from formulations A1, B1, C1, D1, E1

Ryc. 8. Przebieg procesu uwalniania metotreksatu z formułacji A1, B1, C1, D1, E1.