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# STUDY OF FACTORS AFFECTING THE IN VITRO RELEASE OF KETOPROFEN FROM CARBOMERS-BASED GELS

**Olena Bezugla, Anna Liapunova, Igor Zinchenko, Oleksii Liapunov, Nikolay Lyapunov, Yurij Stolper**

***The aim.*** *To identify some factors affecting the in vitro release of ketoprofen from carbomer-based gels.*
***Materials and methods.*** *Carbomer-based gels containing ketoprofen as well as a Newtonian liquid without carbomer, which was the dispersion medium of the gel, were studied by rotational viscometry and spin probe method. The flow behaviour and rheological parameters were determined using the rheograms, and the rotational correlation times of the two dissolved spin probes, the molecules of which contain a carboxyl group or an amino group, were determined by EPR spectra. In vitro release tests were performed using vertical diffusion chambers according to a validated method. The quantitative determination of ketoprofen in gels, liquid and receptor medium was performed by liquid chromatography, and ethanol was quantified by gas chromatography according to validated procedures. Gels with different brands of carbomers, neutralised with trolamine or trometamol, with different contents of ketoprofen and ethanol, and with pH from 6.0 to 7.0 were studied.*
***Results.*** *The sol→gel transition due to the neutralisation of the carbomer did not affect the shape and parameters of the EPR spectrum of the spin probe containing a carboxyl group in the molecule (like a carbomer and ketoprofen) in contrast to the probe with an amino group. If the substance dissolved in the gels does not interact with the carbomer, then its molecules/ions rotate rapidly in the liquid medium. This facilitates the release of a such substance from carbomer-based gels. The medicinal product Nobi Gel® gel 2.5 % and Newtonian liquid were equivalent in relation to the in vitro release parameters of ketoprofen from these objects. Carbomer-based gels, which differed significantly in terms of rheological parameters, were also found to be equivalent in terms of ketoprofen release parameters. The in vitro release of ketoprofen was affected by its concentration and ethanol content in the gel. A change in pH from 6.0 to 7.0 practically did not affect the parameters of in vitro release of ketoprofen from gels.*
***Conclusions.*** *The formation of a carbomer-based gel did not affect the rotational correlation time of the probe, which did not interact with the carbomer. Parameters of in vitro release of ketoprofen from the gel and Newtonian liquid differed little; these parameters were also little affected by the difference in apparent viscosity of the gels. The in vitro release of ketoprofen depended on its concentration and ethanol content*
***Keywords:*** *carbomer, gel, liquid, ketoprofen, ethanol, viscosity, rotational correlation time, in vitro release test (IVRT)*

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## 1. Introduction

Gels consist of liquids gelled using suitable gelling agents [1, 2]. Gels can be divided into several categories: organogels, hydrogels, emulgels (including nanoemulgels), bigels etc. [3]. Medicinal products in a such dosage form as gels are intended for topical administration for local or transdermal delivery of active substances with the aim of topical or systemic effects. At present, there are many publications devoted to newly developed gel formulations. For example, the study of the gels with liposomes was performed by Dragicevic N. et al. [4]. Ambala R. et al. consider that emulgels are promising dosage form due to the possibility of combining hydrophilic and hydrophobic active substances in a medicinal product [5]. The semi-solid preparation (SSP) containing nanoparticles of ketoprofen (KETnano gel) was developed by Nagai N. et al. According to the authors, KETnano gel has an advantage over other SSP with ketoprofen (KET) in terms of effectiveness due to the accumulation of KET in skin tissues and its low concentration in blood plasma [6].

Effective transdermal delivery of ketoprofen to the joints is optimal for treating osteoarthritis, as it can provide the maximum therapeutic effect while minimising side effects [7]. The advantages of transdermal delivery of non-steroidal anti-inflammatory drugs (NSAIDs) are connected to a significant decrease in the risk of side effects compared to systemic drugs and avoiding the pre-systemic metabolism in the liver. However, for the effective action of topical preparations, it is necessary to overcome the skin barrier [8].

For an appropriate effect and prolonged action, a semi-solid preparation should contain substances to improve or assist skin penetration to deliver an active substance to the target tissues or organs [9]. In the case of transdermal delivery to tissues and joints, NSAIDs initially penetrate through the stratum corneum and then

pass through the deeper epidermis and dermis [10, 11]. Penetration enhancers facilitate drug permeation across the skin, they include hydrophilic non-aqueous solvents (alcohols, dimethyl sulfoxide, *N*-methyl pyrrolidone), solubilisers for poorly water-soluble substances etc. [9].

In order to penetrate the skin, NSAIDs must be released from a gel or other dosage form for cutaneous application. In the case of topical semi-solid preparations, the determination of *in vitro* release parameters is a regulatory requirement for the demonstration of the extended pharmaceutical equivalence of the new topical medicinal product (hybrid product) and comparator medicinal product (i.e. existing medicinal product) [12, 13]. In addition, there are requirements for the equivalence of other properties such as appearance, spreadability, microstructure/physical properties, evaporation of volatile excipients etc. According to the recommendations of the EMA draft guideline [12] for quantitative quality characteristics, the 90 % confidence interval for the difference of means of the test product and comparator drug should be contained within the acceptance criteria of ±10 % of the mean value for the comparator product, assuming a normal distribution of data. Qualitative quality characteristics should be essentially the same [12]. The flow behaviour and the values of some rheological parameters can potentially affect the pharmaceutical and therapeutic equivalence of hybrid products and comparator drugs in the form of gels and creams [14]. But some researchers found that the *in vitro* release of active substances was the same even if the difference in rheological parameters was more than 10 % [15]. In some scientific publications, the acceptance criterion of ±10 % in regard to the certain physical properties (rheological, for instance) was recognised as incorrect and discriminatory [16].

Different polymers are used to form gels, for example, carbomers, cellulose derivatives, poloxamers, etc. [17]. The rheological properties of gels depend on the relevant quality attributes of these excipients [1, 2], the accepted range for which could be beyond ±10 %.

Carbomers are the most widely used polymers for the production of gels [17, 18]. Numerous studies were conducted with carbomer-based gels. Back in 1996, Gürol Z. et al., using the model of carrageenan-induced paw oedema in rats, found that carbomer 940-based gels with ketoprofen were more effective than ointments on a hydrophilic basis, creams, as well as ointments with soft white paraffin as a basevehicle [19]. Kolman M. et al. [20] studied the influence of the composition of the dispersion medium containing water, ethyl alcohol, and isopropyl alcohol on the rheological properties of gels. These solvents could be important factors regarding the «pharmaceutical equivalence» of semi-solid preparation [16]. Toderescu C. D. et al. [21] found that *in vitro* release of ketoprofen from carbomer-based gels was influenced by the type of alcohol and the gel production process; the authors recommended ketoprofen gel containing ethanol and glycerin. Salamanca C. H. et al. [22] demonstrated that *in vitro* release of ketoprofen from the gel depended on the dispersion state of this active substance. Release from the gel with dissolved ketoprofen was significantly more intense and complete than in the case of suspension. In addition, the type of membrane also affected the *in vitro* release of ketoprofen [22, 23].

It was previously shown that the formation of carbomer-based gels with a plastic flow behaviour and high apparent viscosity did not lead to an increase in the microviscosity of the dispersion medium of the gels. The spin probes dissolved in the gel base remained in a state of rapid isotropic rotation, which could be a prerequisite for the rapid release of dissolved active substances from these gels [24]. But during that work, the influence of this factor on the release of any medicinal substance was not studied.

It is of interest to study the *in vitro* release of ketoprofen from carbomer-based gels, which differ in the type of carbomer and organic base, pH, and ethanol content, as well as from a water-alcohol solution that does not contain a carbomer. The mentioned factors could potentially affect the rheological parameters of the gels and the *in vitro* release of ketoprofen. These studies are reasonable, taking into account the existing assortment of semi-solid preparations with ketoprofen [25, 26] and scientific research currently being conducted in this direction.

**The aim** of this study was to identify some factors affecting the *in vitro* release of ketoprofen from carbomer-based gels.

## 2. Planning (methodology) of the research

The carbomer-based gels available on the market in Ukraine: Ketonal® gel 2.5 % and Nobi Gel® gel 2.5 %, differing only in the type of carbomer [25], were the objects of research. Gels that differed quantitatively and/or qualitatively from Nobi Gel® gel 2.5 % in ketoprofen content or ethanol content, or pH, or the organic base were studied. In addition, a liquid with the same composition and pH as the dispersion medium of Nobi Gel® gel 2.5 %, but without carbomer, was under study. Ethanol can affect the passive diffusion of ketoprofen, so gels with different ethanol contents were studied [27].

Regarding the studied gels and liquid, the flow behaviour as well as the rheological parameters: apparent or dynamic viscosity (η), and in the case of a plastic flow, the yield stress ($\tau_0$) should be determined [1, 2]. Since the preparations under study should be stored at 25 °C [25], and the *in vitro* release tests should be carried out at 32 °C [13], it is necessary to study rheological properties at these two temperatures.

The parameters of the EPR spectra of two spin probes should be determined, namely: rotational correlation time ($\tau_{+1}$, $\tau_{-1}$, $\tau_{\pm1}$) anisotropy parameter (ε) and hyperfine splitting constant ($A_N$) [28, 29]. The effect of the carbomer on the apparent viscosity of gels and the parameters of the EPR spectra spin probes should be compared. In addition, it is necessary to examine the parameters of the EPR spectra in gel and liquid in the case of two spin probes containing different functional groups. There is a carboxyl group in the molecule of one of the probes, as in the keto-

profen molecule, and there is an amino group in the molecule of another probe, which can react with carboxyl groups of carbomer [24]. The difference between the parameters of the EPR spectra in liquid and gel could indicate the interaction between the spin probe and the carbomer. Without such a difference, the interaction between the probe and the carbomer does not occur. This, in turn, might suggest that carbomer would not influence the *in vitro* release of ketoprofen from the gel.

The actual content of ketoprofen and ethanol (96 %), as well as pH in the studied preparations, should be determined.

*In vitro* release of ketoprofen from medicinal products and experimental preparations should be studied [13]. For this purpose, the analytical procedure for the determination of ketoprofen concentration in the receptor medium by liquid chromatography (HPLC) should be developed and validated in the appropriate range [2, 30]. In addition, certain studies regarding the validation of *in vitro* release method should be conducted according to the methodology described in the literature [16, 31].

The results of comparative studies of *in vitro* release of ketoprofen from gels and liquids should be evaluated according to the acceptance criteria established in the EMA draft guideline [12] and the USP General Chapter <1724> [13]. The release parameters should be compared with the rheological parameters of dispersed systems and the results of studies by the spin probe method. According to the research results, it is necessary to identify significant factors affecting the *in vitro* release of ketoprofen from gels, in particular, the presence of carbomer and organic base in the dispersion system. It is possible that factors will be identified that should be taken into account during pharmaceutical development, postapproval changes in the composition of excipients and standardisation of ketoprofen gels to reduce the risks of manufacturing batches that are not equivalent in terms of *in vitro* release.

### 3. Materials and methods

The medicinal products Ketonal® gel 2.5 % (No. UA/8325/05/01; batch LE8721) and Nobi Gel®-gel 2.5 % (No. UA/15144/01/01; batch 71021) [25] were under study.

Ketoprofen – (2*RS*)-2-(3-benzoylphenyl)propionic acid (Societa Italiana Medicinali Scandicci s.r.l., Italy) [1] as well as such excipients as ethanol (96 %) [32], Carbopol® Ultrez 21 Polymer (Lubrizol, USA) [33], trolamine (triethanolamine) [1], trometamol [32], lavender oil and purified water (hereinafter referred to as water) [32] were used in order to produce the experimental preparations in forms of gel and liquid.

For both studied medicinal products, the nominal content of ketoprofen is 25.0 mg/g (±5 %), and ethanol (96 %) is 285.0 mg/g (±10 %); the acceptable range for pH is 6.0 to 7.0. Carbomer 980 NF is used in Ketonal® gel 2.5 %, and Carbopol® Ultrez 21 Polymer is used in Nobi Gel® gel 2.5 %. In both preparations, trolamine is used to neutralise carbomer and ketoprofen; their salts with trolamine are dissolved in the mixed solvent consisting of ethanol and water. The nominal values for the all research objects, including mentioned medicinal products and the experimental laboratory preparations, are given in Table 1.

Table 1

Characteristics of research objects

| Research object | Nominal content, mg/g | | pH |
|---|---|---|---|
| | Ketoprofen | Ethanol (96 %) | |
| 1. Ketonal® gel 2.5 % | 25.0 | 285.0 | ~6.5 |
| 2. Nobi Gel® gel 2.5 % | 25.0 | 285.0 | ~6.5 |
| 3. Ketoprofen gel 2.5 %* | 25.0 | 285.0 | ~6.5 |
| 4. Ketoprofen gel 2.0 % | 20.0 | 285.0 | ~6.5 |
| 5. Ketoprofen gel 3.0 % | 30.0 | 285.0 | ~6.5 |
| 6. Ketoprofen gel 2.5 % | 25.0 | 228.0 | ~6.5 |
| 7. Ketoprofen gel 2.5 % | 25.0 | 342.0 | ~6.5 |
| 8. Ketoprofen gel 2.5 % | 25.0 | 285.0 | ~6.0 |
| 9. Ketoprofen gel 2.5 % | 25.0 | 285.0 | ~7.0 |
| 10. Ketoprofen trolamine solution 2.5 %** | 25.0 | 285.0 | ~6.5 |

*Note: * – Trometamol was used in gel No. 3 and trolamine was used in the other gels; ** – 2.5 % calculated with reference to ketoprofen*

Nobi Gel® gel 2.5 % was considered a reference product and all studied objects were compared with this drug. In the laboratory gels made with Carbopol® Ultrez 21 Polymer and trolamine the following factors were varied: ketoprofen content was 2.0 % and 3.0 % (gels No. 4 and No. 5), ethanol content (96 %) – 22.8 % and 34.2 % (gels No. 6 and No. 7), pH – 6.0 and 7.0 (gels No. 8 and No. 9). Liquid No. 10 did not contain carbomer. Gel No. 3 contained trometamol as an organic base instead trolamine.

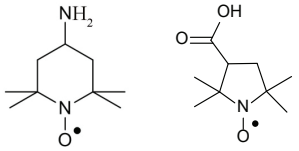
In order to obtain a gel, a dispersion of carbomer in water was prepared and neutralised by trolamine (or trometamol in the case of gel No. 3) (up to pH≈6.5); the resulting gel base was mixed with a portion of ethanol. Ketoprofen and lavender oil were dissolved in another part of ethanol (96 %), and an equimolar amount of trolamine (or trometamol in the case of gel No. 3) was added. This alcohol solution was mixed with the gel base and homogenised. Dissolution, mixing and homogenisation were carried out under vacuum (–0.05 MPa to –0.07 MPa).

In addition to the gels listed in Table 1, gels with a ketoprofen content of 1.25 %, 2.50 %, and 3.75 % were prepared to validate the IVRT method.

Rheograms (plots of the shear stress ($\tau_r$) *vs* the shear rate ($D_r$)) were obtained at 25 °C and 32 °C by rotational viscometry (*2.2.10*) [1, 2] using a rotating viscometer «Rheolab QC» with coaxial cylinders CC-27 (for gels) and DG42 (for liquids) («Anton Paar GmbH»; software RHEOPLUS, 2.66 version). A circulating thermostat Julabo F12-ED (Julabo Labortechnik GmbH, Germany) was used to maintain a necessary temperature (with an accuracy of ±0.1 °C). Rheograms were used to characterise the flow behaviour as well as to determine the yield stress ($\tau_0$) and the apparent viscosity of gels or the dynamic viscosity of liquid (η) [1, 2].

Potentiometric determinations of pH (*2.2.3*) [1, 2] were conducted directly in the gels and liquid using a pH meter Metrohm 827 lab with an electrode «Porotrode» (Metrohm, Switzerland).

Electron paramagnetic resonance (EPR) spectroscopy was used for the research [28, 29]. For this study, 2 hydrophilic spin probes with different functional groups were used: 4-amino-TEMPO ($C_9H_{19}N_2O$; $M_r$ 171.26; CAS: [14691-88-4]) and 3carboxy-2,2,5,5-tetramethylpyrrolidine 1-oxyl (PCA) ($C_9H_{16}NO_3$; $M_r$ 186.23; CAS: [2154-68-9]):




4-amino-TEMPO PCA

Each of the spin probes was added into the Nobi Gel® gel 2.5 % and Ketoprofen trolamine solution 2.5 % at the concentration of $10^{-4}$ mol/l. EPR spectra were obtained using an EPR Spectrometer CMS8400 («Adani», software EPRCMD) at (25±1) °C. Using the EPR spectra, which were triplets, the height of the low-field, central and high-field peaks ($h_{+1}$, $h_0$ та $h_{-1}$, respectively) as well as the width (*Gs*) of the low-field component ($\Delta H_{+1}$) and central component ($\Delta H_0$) were determined. Rotational correlation times of spin probes ($\tau_{+1}$, $\tau_{-1}$, $\tau_{\pm1}$) and anisotropy parameter (ε) were calculated by the following equations [28, 29]:

$$\tau_{+1} = \left(\sqrt{\frac{h_0}{h_{+1}}} - 1\right) \cdot \Delta H_0 / 2 \cdot 10^8, \quad (1)$$

$$\tau_{-1} = \left(\sqrt{\frac{h_0}{h_{-1}}} - 1\right) \cdot \Delta H_0 / 3.6 \cdot 10^9, \quad (2)$$

$$\tau_{\pm1} = \left(\sqrt{\frac{h_{+1}}{h_{-1}}} - 1\right) \cdot \Delta H_{+1} \cdot 6.65 \cdot 10^{-10}, \quad (3)$$

$$\varepsilon = \frac{\sqrt{h_0 / h_{+1}} - 1}{\sqrt{h_0 / h_{-1}} - 1}. \quad (4)$$

The rotational correlation time of the spin probe (τ) is directly proportional to the effective radius of the molecule (*R*) and to the microviscosity of its local surrounding (η) and inversely proportional to the absolute temperature (*T*) [28, 29]:

$$\tau = \left(4 \cdot \pi \cdot R^3 \cdot \eta\right) / 3 \cdot k \cdot T. \quad (5)$$

The hyperfine splitting constant ($A_N$) was determined as the distance (*mT*) between the central and high-field components by the EPR spectra, which were triplets; the $A_N$ characterises the micropolarity of the environment in the vicinity of the nitroxyl radical [28].

In order to study the release of ketoprofen from gels or liquids, the IVRT method was used. The IVRT experiments were performed using vertical diffusion cells (capacity of receptor chamber 6.3 ml; orifice area 1 $cm^2$; Copley Scientific Ltd., UK) and cellulose membranes (GOST 7730-89); the membranes were pre-soaked in the receptor medium (phosphate buffer solution pH 6.8) for 24 hours. The tests were performed at 32 °C; in order to evaluate the robustness of the IVRT method to minor perturbations in temperature, two additional IVRT runs were conducted at temperatures 30 °C and 34 °C. The medium in the receptor chamber was stirred by a magnetic stirrer with a mixing rate 600 rpm; in order to evaluate the robustness of the IVRT method to minor perturbations in mixing rate, two additional IVRT runs were conducted at 540 rpm and 660 rpm. Samples (0.3 ml) were collected from the receptor chamber at 0.5, 1, 2, 3, 4, 5, and 6 h after application of the tested product, and the volume withdrawn was replaced with stock receptor medium (phosphate buffer solution pH 6.8). The results were assessed according to the requirements of EMA draft guidelines [12] and USP General Chapter <1724> [13].

The IVRT method was validated by assessing membrane inertness, the solubility of ketoprofen (ketoprofen salts) in the receptor medium, and the linearity, precision, reproducibility, sensitivity, specificity, selectivity, and robustness of the method. The recovery of ketoprofen was also calculated [16, 31].

Quantitative determination of ketoprofen in the gels, liquid and samples of receptor medium was performed by HPLC (*2.2.29*) [1, 2] according to developed analytical procedures using Shimadzu Prominence-i LC-2030C 3D liquid chromatograph with a diode-array detector (Shimadzu; software: LabSolutions Lite version 5.82). During the analytical studies, Ketoprofen BP CRS (cat. No. 668; content 99.9 %) was used.

Quantitative determination of ethanol (96 %) in the gels and liquid was performed by gas chromatography (*2.2.28*) [1, 2] using Shimadzu GC-2030 gas chromatograph with FID detector and IOC-20 autosampler (Shimadzu; software: LabSolutions version 5.99). Propanol (Merck, cat. No. 100997) was used as an internal standard.

Validation studies in regards to the procedures for the quantitative determination of ketoprofen in gels, liquid and samples of receptor medium, as well as the procedure for the quantitative determination of ethanol (96 %), were carried out according to the accepted methodology [2, 30]. Acceptance criteria for validation characteristics were calculated in accordance with the requirements of State Pharmacopoeia of Ukraine [2, 30].

### 3. 1. Analytical procedure for the quantitative determination of ketoprofen in the gels and liquid

Ketoprofen solutions should be protected from daylight!

*Test solution*. Dissolve 0.5 g of gel or liquid in the mobile phase and dilute to 100 ml with the same solvent.

Dilute 10.0 ml of this solution to 250 ml with the mobile phase.

*Reference solution*. Dissolve 125 mg of Ketoprofen BP CRS in the mobile phase and dilute to 250 ml with the same solvent.

Dilute 5.0 ml of this solution to 500 ml with the mobile phase.

Chromatographic conditions:

– mobile phase: acetonitrile for chromatography R – methanol R2 – phosphate buffer solution pH 4.0 (solution of potassium dihydrogen phosphate R (13.6 g) in water for chromatography R (2000 ml) adjusted to pH (4.0±0,05) with phosphoric acid R) (315:135:550);

– column: stainless-steel chromatographic column, 250×4.6 mm, packed with octadecylsilyl silica gel for chromatography R (5 μm) Spherisorb® ODS1 («Waters»);

– flow rate: 1.5 ml/min;

– detection: at 254 nm;

– injection: 10 μl;

– temperature: 40 °C;

*System suitability* (reference solution): column performance calculated by the peak due to ketoprofen should be at least 1000 theoretical plates; symmetry factor for ketoprofen peak should be from 0.8 to 1.5, and relative standard deviation (RSD) for areas of ketoprofen peaks should meet the requirements of State Pharmacopoeia of Ukraine (2.2.46(N)) [2].

**3. 2. Analytical procedure for the quantitative determination of ethanol (96 %) in the gels and liquid**

*Test solution*. Add 5.0 ml of the solution of internal standard to 0.25 g of gel and dilute to 50 ml with the water R. Mix, centrifuge at 8000 rpm for 15 min and filter (membrane filter with pore size≤0.45 μm).

*Reference solution.* Add 5.0 ml of the solution of internal standard to 72 mg of ethanol (96 %) R and dilute to 50 ml with water R.

*Solution of internal standard*. Dissolve 1.4 ml of propanol R in water R and dilute to 100 ml with water R.

Chromatographic conditions:

– column: fused silica, 30 m×0.53 mm, packed with stationary phase polydimethyldiphenylsiloxane R (film thickness 5.0 μm) (DB-5);

– carrier gas: nitrogen for chromatography R;

– linear velocity: 50 cm/min;

– split ratio: 1:25;

– temperature: thermostat – 90 °C; injection port – 260 °C; detector – 250 °C;

– detection: flame ionisation;

– injection: 1 μl.

*System suitability* (reference solution): column performance calculated by the peak due to ethanol should be at least 4000 theoretical plates; resolution should be at least 3.0 between the peaks due to ethanol and propanol and relative standard deviation for the ratio of areas of ethanol peaks to areas of propanol peaks should meet the requirements of State Pharmacopoeia of Ukraine (*2.2.46*(*N*)) [2].

**3. 3. Analytical procedure for the quantitative determination of ketoprofen in the receptor medium**

*Test solution*. Filtered sample (receptor medium with released ketoprofen).

*Reference solution*. Dissolve 50 mg of Ketoprofen BP CRS in 40 ml of acetonitrile for chromatography R and dilute to 50 ml with the same solvent (ketoprofen concentration is 1.0 mg/ml).

Dilute 5,0 ml of this solution to 20 ml with phosphate buffer solution pH 2.5 (solution of potassium dihydrogen phosphate R (2.38 g) in water R (1000 ml) adjusted to pH (2.50±0,05) with phosphoric acid R) and filter (ketoprofen concentration is 0.25 mg/ml).

Chromatographic conditions:

– mobile phase: acetonitrile for chromatography R – buffer solution pH 2.5 (55:45);

– column: stainless-steel chromatographic column, 250×4.6 mm, packed with end-capped octadecylsilyl silica gel for chromatography R (5 μm) Nucleosil® 1005 $C_{18}$ («Macherey-Nagel»);

– flow rate: 1.5 ml/min;

– detection: at 254 nm;

– injection: 5 μl;

– temperature: 40 °C;

*System suitability* (reference solution): column performance calculated by the peak due to ketoprofen should be at least 5000 theoretical plates; symmetry factor for the ketoprofen peak should be from 0.8 to 1.5, and relative standard deviation (RSD) for areas of ketoprofen peaks should meet the requirements of State Pharmacopoeia of Ukraine (*2.2.46*(*N*)) [2].

**4. Research results**

**4. 1. Research by rotating viscometer method and spin probes method**

The rheograms of Ketonal® gel 2.5 % and Nobi Gel® gel 2.5 % at 25 °C and 32 °C are shown in Fig. 1. The rheological parameters of these gels, as well as Ketoprofen gel 2.5 % containing trometamol instead of trolamine are presented in Table 2.

Table 2

Rheological parameters of Ketonal® gel 2.5 %, Nobi Gel® gel 2.5 % and Ketoprofen gel 2.5 % at 25 °C and 32 °C

| Препарат | *t*, °C | $\tau_0$, Pa | η, Pa·s, at $D_r$ of: | | |
|---|---|---|---|---|---|
| | | | 14.56 $s^{-1}$ | 41.63 $s^{-1}$ | 82.28 $s^{-1}$ |
| Ketonal® gel 2.5 % | 25 | 93.06 | 9.38 | 4.25 | 2.59 |
| | 32 | 100.90 | 11.01 | 4.88 | 3.01 |
| Nobi Gel® gel 2.5 % | 25 | 85.20 | 8.60 | 4.03 | 2.54 |
| | 32 | 70.20 | 6.65 | 3.17 | 2.05 |
| Ketoprofen gel 2.5 % (with trometamol) | 25 | 52.43 | 5.24 | 2.50 | 1.60 |
| | 32 | 48.25 | 5.05 | 2.36 | 1.49 |

All gels with ketoprofen were characterised by a plastic flow behaviour and lower yield point ($\tau_0$), as well as certain values of apparent viscosity (η) at different shear rates ($D_r$) (Fig. 1, Table 2). At 25 °C, the differences between the rheoparameters of Nobi Gel® gel 2.5 % and Ketonal® gel 2.5 % did not exceed 10 %. Ketoprofen gel 2.5 % containing Carbopol® Ultrez 21 Polymer in the same concentration as Nobi Gel® gel 2.5 % but neutralised by trometamol, had significantly lower values of rheological parameters. For instance, the value of $\tau_0$ was lower by approximately 33 % compared to the value of $\tau_0$

for Nobi Gel® gel 2.5 % and the apparent viscosity (at $D_r$=14.56 $s^{-1}$) was lower by 39 %.

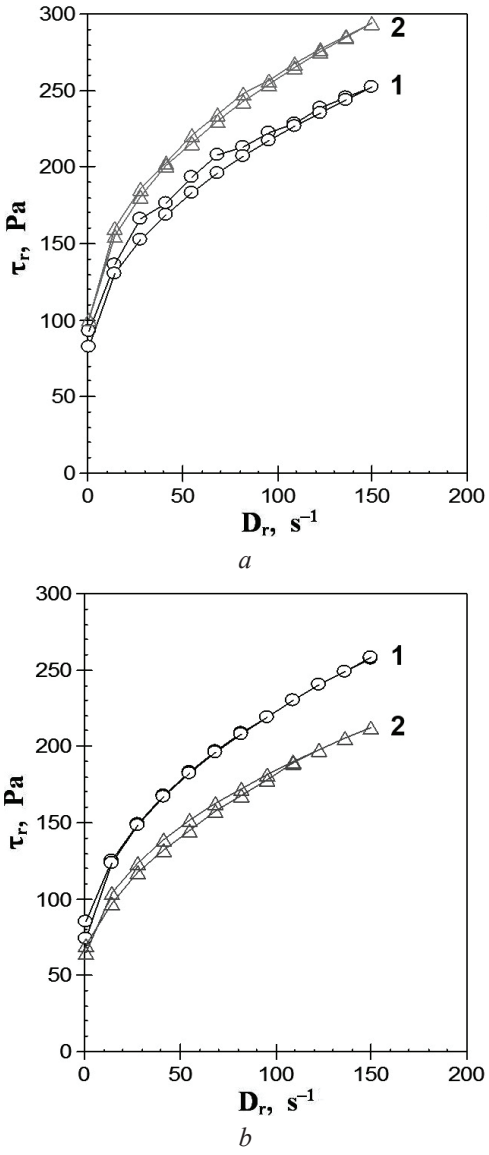



Fig. 1. Rheograms of: *a* – Ketonal® gel 2.5 %; *b* – Nobi Gel® gel 2.5 % at 25 °C (1) and 32 °C (2)

At a higher temperature of 32 °C, the rheological parameters of the gels with Carbopol® Ultrez 21 Polymer decreased, and the rheological parameters of medicinal product Ketonal® gel 2.5 % increased – the value of $\tau_0$ was greater by 30.4 %, and the apparent viscosity (at $D_r$=14.56 $s^{-1}$) was greater by 39.6 % compared to the corresponding parameters of Nobi Gel® gel 2.5 %. This was probably due to a change in the equilibrium between trolamine salts with carbomer and ketoprofen. Therefore, during IVRT, the rheological parameters of the test sample of Nobi Gel® gel 2.5 % were significantly lower than those of Ketonal® gel 2.5 %.

Ketoprofen trolamine solution 2.5 % was Newtonian liquid with a dynamic viscosity of 2.04 mPa·s at 25 °C and 1.42 mPa·s at 32 °C, which was less than 3 orders of magnitude of the apparent viscosity of gels at different shear rates (Table 2).

The EPR spectra of both spin probes in Nobi Gel® gel 2.5 % and in Ketoprofen trolamine solution 2.5 % were triplets (Fig. 2). The structural transition Newtonian liquid → gel did not affect the shape of the EPR spectra of spin probes dissolved in the dispersion medium of the gel, in contrast to the rheograms, which in the case of gels became characteristic for the systems with plastic flow behaviour (Fig. 1).

The parameters of the EPR spectra of both probes indicated their fast isotropic rotation regardless of the consistency of the dispersed system (Fig. 2, Table 3). The spin probes rotated in a liquid medium, and the polarity of the local environment of the radicals was identical in the corresponding solutions and gels ($A_N$=1.61 mT and 1.69 mT).

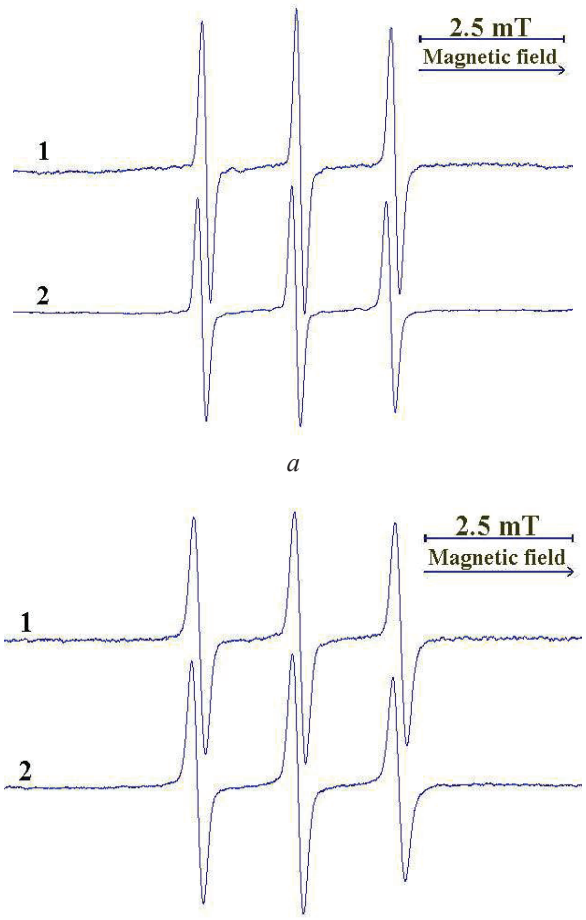



Fig. 2. EPR spectra of: *a* – PCA probe; *b* – 4-amino-TEMPO probe in Ketoprofen trolamine solution 2.5 % (**1**) and Nobi Gel® gel 2.5 % (**2**) at 25 °C

Table 3

EPR spectra of the probes PCA and 4-amino-TEMPO in ketoprofen trolamine solution 2.5 % and Nobi Gel® gel 2.5 % at 25 °C

| Spin probe | Disperse system | Parameters of EPR spectra | | | | |
|---|---|---|---|---|---|---|
| | | $A_N$, mT | $\tau_{+1}$, ps | $\tau_{-1}$, ps | $\tau_{\pm1}$, ps | ε |
| PCA | Solution | 1.61 | 308.8 | 27.6 | 23.9 | 0.62 |
| | Gel | 1.61 | 287.6 | 26.1 | 24.7 | 0.61 |
| | **Δ, %** | **0** | **6.9** | **5.4** | **3.3** | **–1.6** |
| 4-amino-TEMPO | Solution | 1.69 | 294.7 | 31.9 | 37.0 | 0.51 |
| | Gel | 1.69 | 329.2 | 67.0 | 118.1 | 0.27 |
| | Δ, % | **0** | **11.7** | 110.0 | 219.2 | –47.1 |

The parameters of the rotational diffusion of spin probes in liquid and gel differed depending on the functional groups in their molecules. In the case of the spin probe PCA, which contained a carboxyl group in the molecule, like ketoprofen, the rotational correlation times and the anisotropy parameter have remained almost unchanged (Table 3). A small relative increase (Δ) in values of $\tau_{+1}$ by 6.9 %, $\tau_{-1}$ – by 5.4 %, and $\tau_{\pm1}$ – by 3.3 %, and a decrease in value ε by 1.6 % can be attributed to the formation of the salt of PCA with trolamine.

In the case of the spin probe 4-amino-TEMPO, which contained an amino group in the molecule, the value of $\tau_{+1}$ increased by only 11.7 %, but values of $\tau_{-1}$ and $\tau_{\pm1}$ increased by 110.0 % and 219.2 %, respectively. At the same time, the anisotropy parameter (ε) decreased by 47.1 % (Table 3), which indicated a significant decrease in the frequency and increase in the ordering of the rotational diffusion of the probe 4-amino-TEMPO. These changes were not as substantial as changes in rheological parameters during the liquid→gel transition, but they were considerable and probably indicated the interaction of the probe 4-amino-TEMPO with the carboxyl groups of the carbomer.

According to the research results, it can be assumed that the gel structure formed by the carbomer will have a slight effect on *in vitro* the release of ketoprofen.

Taking into account the obtained results, it was reasonable to study *in vitro* release of ketoprofen from liquid and gels, as well as factors that could affect the release of ketoprofen from gel [34], in particular, ethanol content.

The analytical procedures for the quantitative determination of ketoprofen and ethanol in liquid and gels, as well as for quantification of ketoprofen in the receptor medium (dialysate), are outlined previously (see section 3), and the results of the validation of these procedures are presented below.

**4. 2. Validation of the analytical procedure for the quantitative determination of ketoprofen in gels and liquid**

Validation of the analytical procedure was carried out in the concentration range of model solutions from 3.20 μg/ml to 7.20 μg/ml (from 64 % to 144 % of the nominal concentration of 5 μg/ml of ketoprofen in the reference solution). This range was chosen due to the study of gels with ketoprofen content of 1.25 % and 3.75 % for the validation of the IVRT method.

The specificity of the analytical procedure was confirmed by the fact that on the chromatograms obtained with the solvent (blank) and solution of a placebo, there was no peak with a retention time, which would coincide with the retention time of the ketoprofen peak on the chromatograms obtained with the reference solution and test solution (Fig. 3). There was no difference in the retention times of the ketoprofen peaks on the chromatograms obtained with the test solution (*Rt*=6.647 min) and reference solution (*Rt*=6.645 min) (difference was 0.03 %, and acceptance criterion ≤1.85 %) (Fig. 3). Ketoprofen peaks on the chromatograms obtained with the reference and test solutions were spectrally pure (peak purity index was 1.000000).

Ketoprofen model solutions were stable during the entire period of analysis: the difference between the obtained values of $Z_i$ for the first and last analysis was $\Delta Z_i$=0.33 % and did not exceed the critical value: 0.33 %<√2×1.6 %=2.26 % (Table 4).

Table 4

Stability data for model solutions

| Substance | $Z_{first}$, % | $Z_{last}$, % | $\lvert\Delta Z_i\rvert$, % | $\leq\sqrt{2}\times\max\Delta_{As}$, % | Conclusion |
|---|---|---|---|---|---|
| Ketoprofen | 99.51 | 99.84 | 0.33 | 2.26 | Pass |

According to the results of validation studies, the procedure for the quantitative determination of ketoprofen in gels and liquid in the established range of application met the acceptance criteria for linearity, repeatability and accuracy (Table 5).

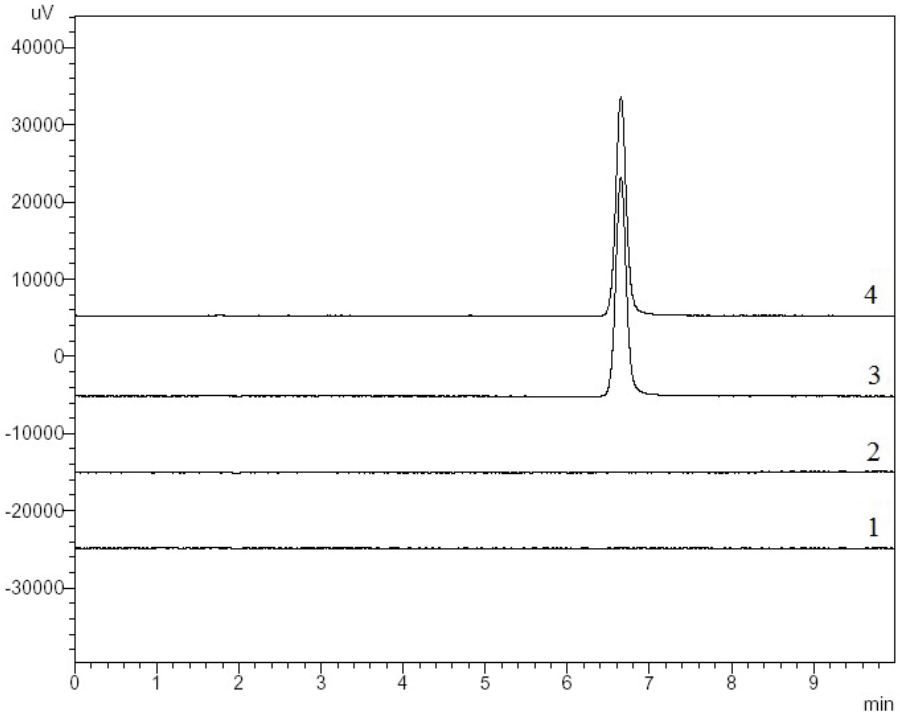



Fig. 3. Chromatograms obtained with solvent («blank») (1), solution of a placebo, reference solution (3) (*Rt*=6.645 min) and test solution (4) (*Rt*=6.647 min) (peaks with *Rt*≈6.5 min are due to ketoprofen)

Table 5

Validation characteristics of the analytical procedure for the ketoprofen assay in the gels and solution and their evaluation against the acceptance criteria [2]

| Parameter | Value | Criterion ($n$=9) | Conclusion |
|---|---|---|---|
| Linearity | | | |
| $b$ | 0.99043 | – | – |
| $S_b$ | 0.01531 | – | – |
| α | 1.10333 | $\|1.10\|<\|S_\alpha \times 1.8946\|=\|2.97\|$<br>$\|1.10\|<\|1.42\|$ | Pass |
| $S_\alpha$ | 1.56498 | | |
| $S_0$ | 0.60113 | | |
| $S_0/b \leq \Delta_{As}$:$t$(95 %, $n$–2)=0.845 % | 0.60693 | 0.607<\|0.845\| | Pass |
| $r$ | 0.99950 | 0.99950>\|0.99943\| | Pass |
| Repeatability | | | |
| Relative standard deviation $RSD_z$, % | 0.5878 | – | – |
| Relative confidence interval: $\Delta_z = t(95\,\%, n-1) \times RSD_z$ | 1.0930 | 1.09 % < 1.60 % | Pass |
| Accuracy | | | |
| Systematic uncertainty (δ), % | 0.14 | – | – |
| 1) statistical insignificance: | δ=0.14 %<$\Delta_z$:√9=0.364 % | | Pass |
| 2) practical insignificance: | δ=0.14 %<1.6 %·0.32=0.51 % | | |

**4. 3. Validation of the analytical procedure for ethanol assay (96 %) by GC in gels and liquid**

Validation of the procedure for the quantitative determination of ethanol (96 %) was carried out in the concentration range of model solutions from 64 % to 144 % of the nominal concentration of ethanol (96 %) in the reference solution. This range was chosen due to the study of gels with ethanol (96 %) content of 22.8 % and 34.2 % (Table 1).

The specificity of the analytical procedure was confirmed by the fact that on the chromatograms of the solution of placebo, there was no peak with a retention time, which would coincide with the retention times of the ethanol peak and propanol peak (Fig. 4). The resolution between peaks due to ethanol and propanol was appropriate – 7.124 (the acceptance criterion is ≥3). RRT of ethanol with reference to propanol on the chromatograms obtained with the test solution and reference solution were practically the same (Table 6).

Table 6

Retention times (*Rt*) of ethanol and propanol (internal standard) on the chromatograms of the reference solution and the test solution, as well as the relative retention times (*RRt*) of ethanol with reference to propanol

| Solution | Substance | *Rt*, min | *RRt* |
|---|---|---|---|
| Reference solution | Ethanol | 1.616 | 0.7520 |
| | 1-propanol | 2.149 | 1 |
| Test solution | Ethanol | 1.618 | 0.7519 |
| | 1-propanol | 2.152 | 1 |

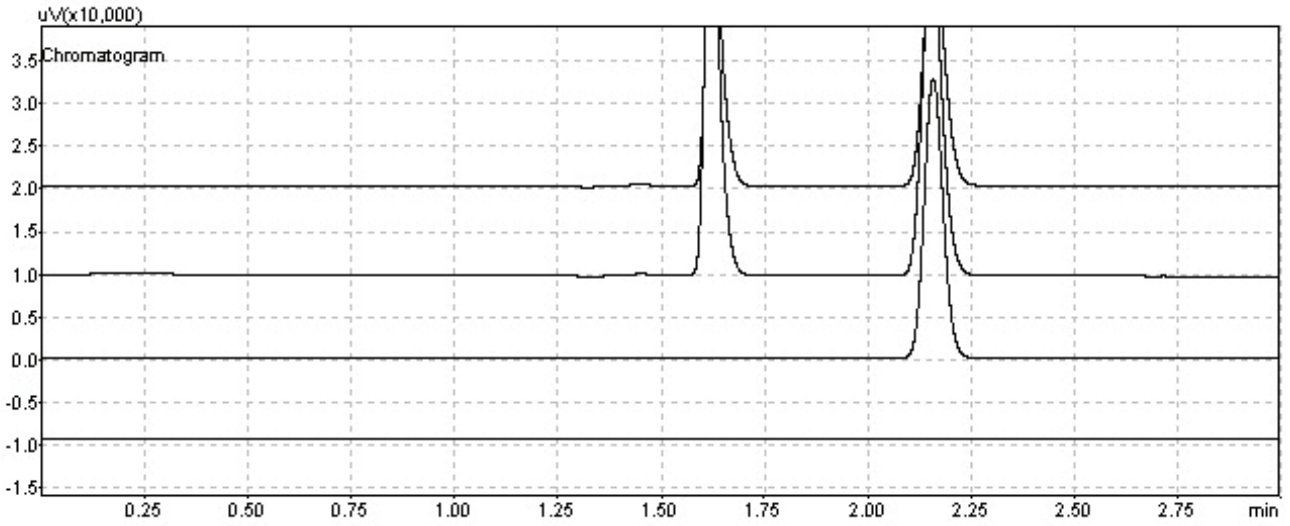



Fig. 4. Chromatograms (from top to bottom) obtained with the test solution, reference solution, solution of internal standard, solution of placebo

Ethanol (96 %) model solutions were stable during the entire period of analysis: the difference between the obtained values of $Z_i$ for the first and last analysis was $\Delta Z_i$=0.52 % and did not exceed the critical value: 0.52 %<√2×3.2 %=4.53 % (Table 7).

Table 7

Stability data for model solutions

| Substance | $Z_{first}$, % | $Z_{last}$, % | $\lvert\Delta Z_i\rvert$, % | $\leq\sqrt{2}\times\max\Delta_{As}$, % | Conclusion |
|---|---|---|---|---|---|
| Ethanol (96 %) | 99.84 | 100.36 | 0.52 | 4.53 | Pass |

According to the results of validation studies, the procedure for the quantitative determination of ethanol (96 %) in gels and liquid in the established range of application met the acceptance criteria for linearity, repeatability and accuracy (Table 8).

The pH values of the studied objects, as well as the results of their analyses using validated procedures, are shown in Table 9.

**4. 4. Validation of the analytical procedure for the ketoprofen assay in the receptor medium**

Validation of the analytical procedure for the quantitative determination of ketoprofen in receptor medium was carried out in the concentration range of model solutions from 5 % to 300 % of the nominal ketoprofen concentration of 0.25 mg/ml in the reference solution.

The specificity of the analytical procedure was confirmed by the fact that on the chromatograms obtained with the solvent (blank) and solution of placebo there was no peak with a retention time, which would coincide with the retention time of the ketoprofen peak on the chromatograms obtained with the reference solution and test solution (Fig. 5). There was no difference in the retention times of the ketoprofen peaks on the chromatograms obtained with the test solution (*Rt*=4.887 min) and reference solution (*Rt*=4.880 min) (difference was 0.14 % and acceptance criterion ≤1.85 %). Ketoprofen peaks on the chromatograms obtained with the reference and test solutions were spectrally pure.

Table 8

Validation characteristics of the analytical procedure for the ethanol (96 %) assay in the gels and solution and their evaluation against the acceptance criteria [2]

| Parameter | Value | Criterion ($n$=9) | Conclusion |
|---|---|---|---|
| Linearity | | | |
| $b$ | 1.00112 | – | – |
| $S_b$ | 0.01137 | – | – |
| α | –0.25844 | $\lvert 0.26\rvert<\lvert S_\alpha\times 1.8946\rvert=\lvert 2.20\rvert$ $\lvert 0.26\rvert<\lvert 2{,}84\rvert$ | Pass |
| $S_\alpha$ | 1.16005 | – | – |
| $S_0$ | 0.44566 | – | – |
| $S_0/b\leq\Delta_{As}$:$t(95\ \%, n-2)$=1.690 % | 0.44566 | 0.446<\|1.690\| | Pass |
| $r$ | 0.99955 | 0.99955>\|0.99770\| | Pass |
| Repeatability | | | |
| relative standard deviation $RSD_z$, % | 0.4104 | – | – |
| relative confidence interval: $\Delta_z = t(95\ \%, n-1)\times RSD_z$ | 0.7631 | 0.76 %<3.20 % | Pass |
| Accuracy | | | |
| systematic uncertainty (δ), % | 0.14 | – | – |
| 1) statistical insignificance: | δ=0.14 %<$\Delta_z$:√9=0.25 % | | Pass |
| 2) practical insignificance: | δ=0.14 %<3.2 %·0.32=1.02 % | | |

Table 9

Results of analyses of research objects

| Research object | Content, mg/g | | pH |
|---|---|---|---|
| | Ketoprofen | Ethanol (96 %) | |
| 1. Ketonal® gel 2.5 % | 25.37 | 287.15 | 6.49 |
| 2. Nobi Gel® gel 2.5 % | 25.28 | 284.12 | 6.57 |
| 3. Ketoprofen gel 2.5 %* | 25.80 | 283.10 | 6.68 |
| 4. Ketoprofen gel 2.0 % | 20.08 | 285.63 | 6.61 |
| 5. Ketoprofen gel 3.0 % | 29.97 | 284.95 | 6.67 |
| 6. Ketoprofen gel 2.5 % | 25.17 | 228.05 | 6.50 |
| 7 Ketoprofen gel 2.5 % | 25.21 | 342.30 | 6.57 |
| 8. Ketoprofen gel 2.5 % | 25.25 | 284.77 | 6.00 |
| 9. Ketoprofen gel 2.5 % | 25.15 | 285.05 | 7.02 |
| 10. Ketoprofen trolamine solution 2.5 %** | 24.90 | 283.02 | 6.58 |

*Note: * – Trometamol was used in gel No. 3 and trolamine was used in the other gels; ** – 2.5 % calculated with reference to ketoprofen*

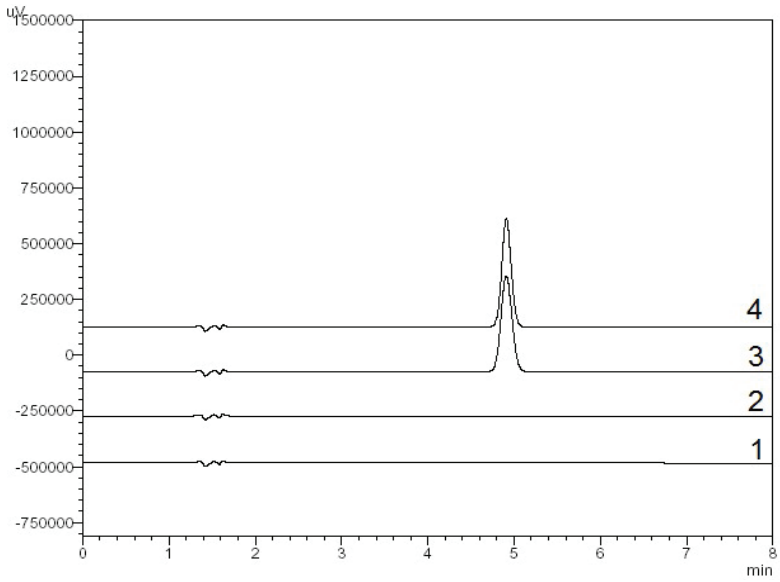



Fig. 5. Chromatograms obtained with solvent («blank») (1), solution of placebo (2), reference solution (3) (*Rt*=4.880 min) and test solution (4) (*Rt*=4.887 min) (peaks with *Rt*≈4.9 min are due to ketoprofen)

According to the results of validation studies, the procedure for the quantitative determination of ketoprofen in the established range from 5 % to 300 % of its nominal concentration in the reference solution (0.25 mg/ml) met the acceptance criteria for linearity, repeatability and accuracy (Table 10).

Ketoprofen model solutions were stable during the entire period of analysis: the difference between the obtained values of $Z_i$ for the first and last analysis was $\Delta Z_i$=0.24 % and did not exceed the critical value: 0.24 %<√2×3 %=4.24 % (Table 11).

According to the results of the linearity study (Table 10), the limit of quantification (LOQ) of ketoprofen in normalised coordinates was [30]:

$$LOQ=10\times S_\alpha : b=$$
$$=10\times 0.22468 : 0.9995=2.25\ \%.$$

LOQ, which was 2.25 % of nominal concentration of ketoprofen in the reference solution, corresponded to its concentration of 5.63 μg/ml in the receptor medium.

**4. 5. Validation of the IVRT method**

*Membrane inertness.* The inertness of cellulose membrane was studied by immersing each of three membranes into 6.3 ml of identical test solutions (ketoprofen trolamine solution 650 μg/ml in phosphate buffer solution pH 6.8). Three test solutions and three control solutions (the same solutions without immersed membranes) were kept at (32±0.5) °C for 6 hours. Then, the ketoprofen concentration was determined and recovery was calculated by dividing the mean value of ketoprofen concentration in the test solutions by the mean value of ketoprofen concentration in the control solutions. The recovery was 99.87 % (SD: 0.14 %) (the acceptance criterion was ≥95 %). Thus, cellulose membrane did not interact with ketoprofen and did not present a rate limiting barrier for the diffusion of this active substance.

*Solubility of ketoprofen salts in the receptor medium* (*phosphate buffer solution pH 6.8).* In order to determine the solubility of ketoprofen in phosphate buffer solution pH 6.8 these solutions were prepared:

1. The equimolar amount (41.1 mg) of trolamine was added to 70 mg of ketoprofen, then, mixture was diluted to 10.0 ml with a phosphate buffer solution pH 6.8 and mixed.

2. The equimolar amount (33.3 mg) of trometamol was added to 70 mg of ketoprofen, then, mixture was diluted to 10.0 ml with a phosphate buffer solution pH 6.8 and mixed.

These solutions were kept at (32±0.5) °C for 24 h. No precipitate was formed in any of the solutions. Content of the active substance (in terms of ketoprofen) in these solutions was 7 mg/ml, which was 10.8 times higher than the highest concentration (0.65 mg/ml) in the samples obtained during IVRT validation (acceptance criterion:

Table 10

Validation characteristics of the analytical procedure for the ketoprofen assay in the receptor medium and their evaluation against the acceptance criteria [24]

| Parameter | Value | Criterion ($n$=9) | Conclusion |
|---|---|---|---|
| Linearity | | | |
| $b$ | 0.9995 | – | – |
| $S_b$ | 0.00151 | – | – |
| α | –0.06187 | 1) ≤\|$S_\alpha$×1.8946\|=\|0.43\|; 2) if it does not meet criterion (1), then ≤\|1.01\| | Pass |
| $S_\alpha$ | 0.22468 | – | – |
| $SD_{rest}$ | 0.41211 | ≤\|1.58\| | Pass |
| $r$ | 0.99999 | ≥\|0.99999\| | Pass |
| Repeatability | | | |
| standard deviation $SD_{\Delta Zi}$, % | 0.39 | – | – |
| confidence interval: $\Delta_{\Delta Zi}=t$ (95 %, 9–1)×$SD_{\Delta Zi}$ | 0.72 | ≤3.0 % | Pass |
| Accuracy | | | |
| mean value Δ$Z$, % | –0.12 | – | – |
| 1) statistical insignificance \|Δ$Z$\|: | 0.12 | $\lvert\Delta Z\rvert \le \frac{t(95\ \%, 9-1)}{\sqrt{9}} \times SD_{\Delta Zi} = 0.24\ \%$ | Pass |
| 2) practical insignificance \|Δ$Z$\|: | | \|Δ$Z$\|≤0.32×3.0 %=0.96 % | |

Table 11

Stability data for model solutions

| Substance | $Z_{first}$, % | $Z_{last}$, % | \|Δ$Z_i$\|, % | ≤√2×max$\Delta_{4s}$, % | Conclusion |
|---|---|---|---|---|---|
| Ketoprofen | 99.60 | 99.84 | 0.24 | 4.24 | Pass |

solubility should be more 10 times higher than the maximum concentration of analyte in the receptor medium).

*Linearity, precision and reproducibility.* Fig. 6 shows the mean release rates (the released amount of ketoprofen per unit area of the membrane versus the square root of time) for three IVRT runs using experimental gel No. 1 with ketoprofen content of 2.5 %. The relevant release parameters are given in Table 12.

Table 12

Parameters of KET release from the 2,5 % gel No. 1 for three IVRT runs

| Parameter | Results | | |
|---|---|---|---|
| | Run 1 | Run 2 | Run 3 |
| Release rate ($R$), mg/cm$^2$/h$^{-1/2}$ | 1.43±0.05 SD: 0.02 | 1.44±0.073 SD: 0.04 | 1.46±0.05 SD: 0.02 |
| Cumulative amount ($A$) (at time the point 6 h), mg/cm$^2$ | 3.14±0.09 SD: 0.05 | 3.14±0.07 SD: 0.04 | 3.13±0.10 SD: 0.05 |
| Content ($C$) in the receptor medium (at the time point 6 h), mg/ml | 0.50±0.01 SD:0.01 | 0.50±0.01 SD: 0.01 | 0.50±0.02 SD: 0.01 |
| Correlation coefficient $r$ | 0.999 | 0.998 | 0.999 |
| Coefficient of determination $R^2$ | 0.999 | 0.997 | 0.999 |
| Recovery (at the time point 6 h), % | 8.36±0.25 SD: 0.12 | 8.36±0.19 SD: 0.10 | 8.35±0.26 SD: 0.13 |

According to the presented plots and values of correlation coefficients (Fig. 6, Table 12), the relationship between ketoprofen amount released per unit area of the membrane versus the square root of time was linear for all three IVRT runs. The coefficients of determination were greater than 0.99 (acceptance criterion $R^2$>0.90).

The maximum relative standard deviation for the release rate did not exceed 1.91 % in the individual IVRT run ($RSD_{intra\text{-}run}$) and did not exceed 0.75 % between IVRT runs ($RSD_{inter\text{-}run}$). These results met the acceptance criteria of USP (RSD<15 %) and EMA (RSD<10 %) and confirmed the precision and reproducibility of the IVRT method.

*Sensitivity, specificity and selectivity.* The mean KET release rates for three IVRT runs using gels with different content of ketoprofen: 1.25 %, 2.50 % and 3.75 % are shown in Fig. 7. The relevant release parameters are given in Table 13.

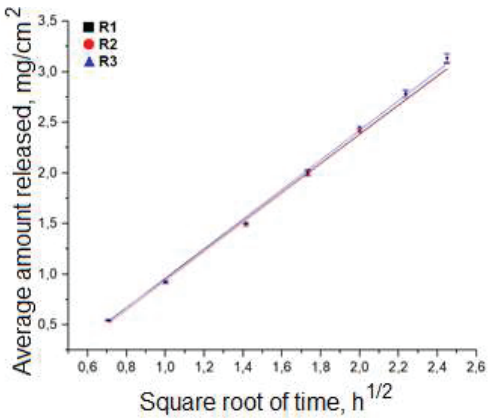



Fig. 6. Release rate plots obtained from the three IVRT runs using 2.5 % gel

The mean KET release rate was lower in the case of 1.25 % gel compared to 5.0 % gel, and the mean KET release rate was higher in the case of 3.55 % gel compared to 5.0 % gel (0.71 mg/cm$^2$/h$^{-1/2}$<1.44 mg/cm$^2$/h$^{-1/2}$< <2.17 mg/cm$^2$/h$^{-1/2}$) (Table 13), so the IVRT method was considered to be sensitive.

Fig. 8 shows the evidence of a linear, proportional relationship between the ketoprofen concentration in the gels and respective release rates; the coefficient of determination $R^2$ was 0.99998 (acceptance criterion >0.90). Thus, the specificity of the IVRT procedure was confirmed.

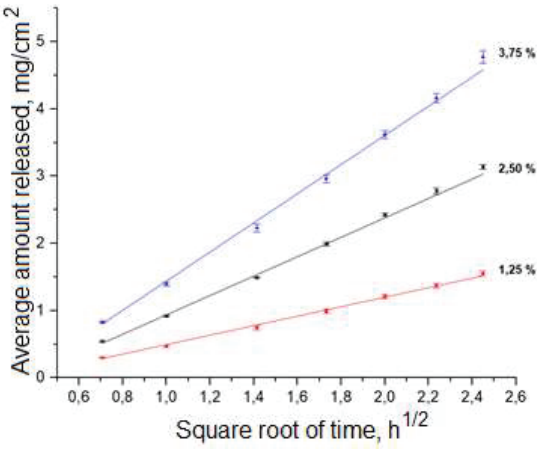



Fig. 7. Release rate plots obtained from the IVRT runs using gels with different content of ketoprofen: 1.25 %, 2.50 % and 3.75 %

Table 13

Parameters of KET release from the gels with different content of the active substance: 1.25 %, 2.50 % i 3.75 %

| Parameter | Results | | |
|---|---|---|---|
| | 2.50 % gel | 1.25 % gel | 3.75 % gel |
| Release rate ($R$), mg/cm$^2$/h$^{-1/2}$ | 1.44±0.07 SD: 0.04 | 0.71±0.03 SD: 0.01 | 2.17±0.09 SD: 0.04 |
| $R_{1.25\ \%/3.755\ \%}/R_{2.50\ \%}$, % | – | 45.61–50.00 | 144.30–156.18 |
| Cumulative amount (A) (at the time point 6 h), mg/cm$^2$ | 3.14±0.07 SD: 0.04 | 1.55±0.07 SD: 0.03 | 4.77±0.18 SD: 0.09 |
| $A_{1.25\ \%/3.75\ \%}/A_{2.50\ \%}$, % | – | 47.03–51.42 | 145.99–157.79 |
| Content ($C$) in the receptor medium (at the time point 6 h), mg/ml | 0.50±0.01 SD: 0.01 | 0.25±0.01 SD: 0.01 | 0.76±0.03 SD: 0.01 |
| Correlation coefficient $r$ | 0.998 | 0.999 | 0.999 |
| Coefficient of determination $R^2$ | 0.997 | 0.998 | 0.998 |
| Recovery (at the time point 6 h), % | 8.36±0.19 SD: 0.10 | 8.27±0.37 SD: 0.19 | 8.48±0.33 SD: 0.16 |

The selectivity of the IVRT method (the ability to discriminate the nonequivalent characteristics in the case of a product with different KET content: 1.25 %, 2.50 % and 3.75 %) was evaluated by assessing the ratio of KET release rates from 1.25 % and 3.75 % gels and KET release rate from 2.50 % gel. The values of the ratio in case of comparison of the KET release rates for 1.25 % gel and 2.50 % gel were in the range of 45.61–50.00 %, and if the release rates

for 3.75 % gel have been compared with the same parameter for 2.50 % gel, the ratios were from 145.99 % to 157.79 %. In both cases, the ratios $R_{1.25\,\%}/R_{2.50\,\%}$ and $R_{3.55\,\%}/R_{2.50\,\%}$, converted to percent, were outside the limits of 75.00 % and 133.33 % (USP criterion). So, the IVRT procedure was considered to be selective in regard to its ability to accurately discriminate the different release rates.

To prove the ability of the IVRT procedure to accurately identify equivalent product performance, the pairwise comparison were performed using the results of three IVRT runs with 2.50 % gel (Table 12). The results indicate that the computed limits for all pairwise comparisons were within the range of 75.00–133.33 % (USP criterion) and even in the range of 90–111 % (EMA criterion): run 1 vs run 2 – 96.37–102.45 %; run 1 vs run 3 – 96.43–102.54 % and run 2 vs run 3 – 96.70–103.56 %. The results confirmed the ability of IVRT procedure to accurately detect the equivalent product performance.

*Robustness in regards to minor changes in the temperature.* The results obtained in three IVRT runs at different temperatures (30 °C, 32 °C and 34 °C) using 2.50 % gel are presented in Fig. 9; the relevant release parameters are given in Table 14. According to the presented data, the mean release rates obtained in IVRT runs at different temperatures (i.e. 32 °C and 34 °C) did not deviate by more than 15 % (acceptance criterion) from the mean release rates obtained in IVRT runs at nominal temperature – 32 °C. These results confirmed that the IVRT procedure was robust in regard to minor temperature changes.

Table 14

Parameters of KET release at different temperatures

| Parameter | Results at a temperature of: | | |
|---|---|---|---|
| | 32 °C | 30 °C | 34 °C |
| Release rate ($R$), mg/cm$^2$/h$^{-1/2}$ | 1.44±0.07 SD: 0.04 | 1.40±0.06 SD: 0.02 | 1.56±0.054 SD: 0.02 |
| $R_{30/34}/R_{32}$, % | – | 91.56–97.37 | 100.68–105.85 |
| Cumulative amount ($A$) (at the time point 6 h), mg/cm$^2$ | 3.14±0.07 SD: 0.04 | 2.98±0.06 SD: 0.02 | 3.25±0.06 SD: 0.02 |
| $A_{30/34}/A_{32}$, % | – | 93.15–97.09 | 101.63–105.84 |
| Coefficient of determination $R^2$ | 0.997 | 0.998 | 0.999 |

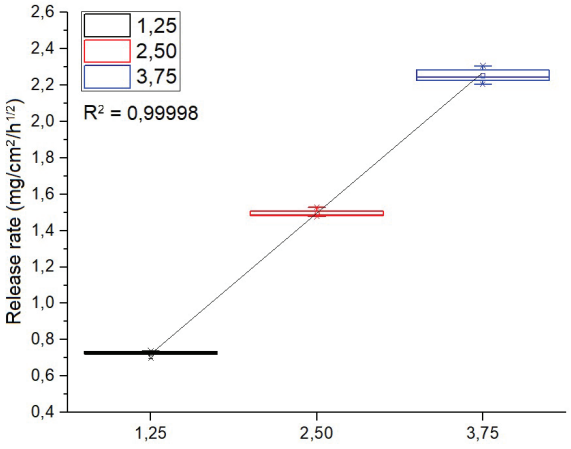



Fig. 8. Box and whiskers plot of the KET released rates for three gels with different content of the active substance

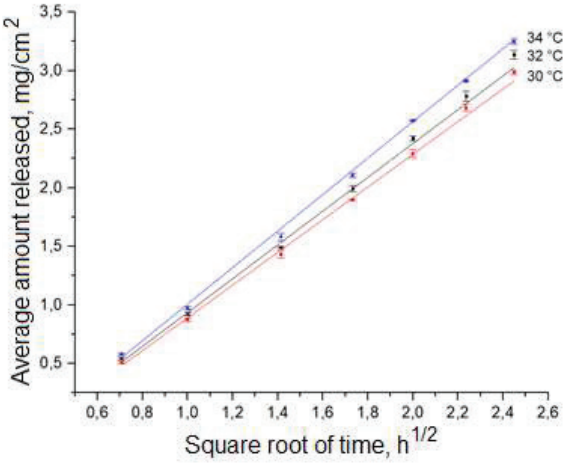



Fig. 9. KET release rate plots obtained from the three IVRT runs performed at different temperatures

*Robustness in regards to minor changes in the mixing rate.* The results obtained in three IVRT runs at different mixing rates (540 rpm, 600 rpm and 660 rpm) using 2.50 % gel are presented in Fig. 10; the relevant release parameters are given in Table 15. According to the results, the mean release rates obtained in IVRT runs at different mixing rates (i.e. 540 rpm and 660 rpm) did not deviate by more than 15 % (acceptance criterion) from the mean release rates obtained in IVRT runs at nominal mixing rate – 600 rpm. These results confirmed that the IVRT procedure was robust in regard to minor changes in mixing rate.

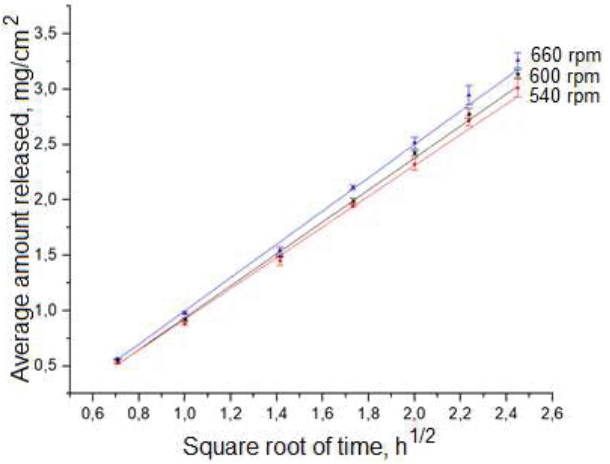



Fig. 10. KET release rate plots obtained from the three IVRT runs performed at a different mixing rate

Table 15

Parameters of KET release at different mixing rates

| Parameter | Results at mixing rate of: | | |
|---|---|---|---|
| | 600 rpm | 540 rpm | 660 rpm |
| Release rate ($R$), mg/cm$^2$/h$^{-1/2}$ | 1.44±0.07 SD: 0.04 | 1.39±0.06 SD: 0.02 | 1.50±0.07 SD: 0.02 |
| $R_{30/34}/R_{32}$, % | – | 92.19–100.20 | 101.03–107.66 |
| Cumulative amount ($A$) (at the time point 6 h), mg/cm$^2$ | 3.14±0.07 SD: 0.04 | 3.01±0.23 SD: 0.08 | 3.26±0.20 SD: 0.07 |
| $A_{30/34}/A_{32}$, % | – | 92.55–100.08 | 100.40–107.72 |
| Coefficient of determination $R^2$ | 0.997 | 0.999 | 0.999 |

*Recovery.* This parameter characterises the extent of dose depletion during the IVRT. Regarding 2.50 % gel, the recovery was 8.36 % after a 6-hour experiment which did not exceed USP acceptance criterion (30 %). Thus the extent of dose depletion was considered to be acceptable.

**4. 6. Study of ketoprofen release from gels and Newtonian liquid**

Fig. 11 shows the KET release rate in the case of medicinal products Nobi Gel® gel 2.5 % (*R*) and Ketonal® gel 2.5 % ($T_1$) as well as experimental preparations Ketoprofen gel 2.5 % with trometamol instead trolamine ($T_T$) and Ketoprofen solution 2.5 % ($T_S$) without Carbopol® Ultrez 21 Polymer (this solution was Newtonian liquid with low dynamic viscosity). The relevant release parameters are presented in Table 16.

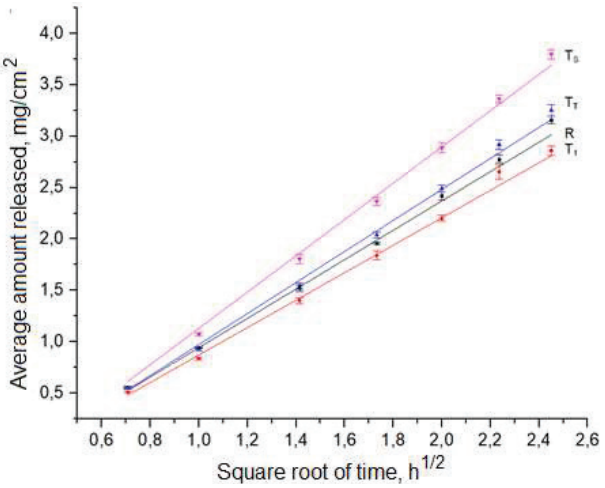



Fig. 11. KET release rate plots in the case of medicinal products Nobi Gel® gel 2.5 % (*R*) and Ketonal® gel 2.5 % ($T_1$), experimental preparations Ketoprofen gel 2.5 % with trometamol ($T_T$) and Ketoprofen solution 2.5 % ($T_S$)

As evidenced by the presented data (Fig. 11, Table 16), the relationship between the released amount of ketoprofen per unit area of the membrane and the square root of time was linear for both gels and liquid ($R^2$>0.9). Among the studied gels, the lowest values of KET release parameters were in the case of Ketonal® gel 2.5 % ($T_1$), for which the greatest values of rheological parameters were observed (Table 2). Ketoprofen gel 2.5 % containing trometamol instead of trolamine ($T_T$) had the greatest values of KET release parameters (Fig. 11, Table 16). This experimental preparation was characterised by the lowest values of rheological parameters (Table 2). But despite the significant difference in the rheological parameters, all three studied gels were equivalent in terms of the release rate of ketoprofen according to EMA acceptance criteria (90–111 %) and furthermore according to USP criteria (75–133.33 %) [12, 13].

The greatest values of KET release parameters were observed in the case of the experimental preparation of Ketoprofen solution 2.5 % ($T_S$). The viscosity of this liquid was more than 3 orders of magnitude lower than the viscosity of Nobi Gel® gel 2.5 % (*R*). But in terms of release parameters of ketoprofen, Ketoprofen solution 2.5 % ($T_S$) was equivalent to Nobi Gel® gel 2.5 % (*R*) as well as two other gels, taking into account USP acceptance criteria (Table 16). That is, the presence of carbomer in the gel composition and the resulting high apparent viscosity of the gels had little effect on the KET release; the functional purpose of carbomers was limited mainly to the modification of the flow behaviour and viscosity. The apparent viscosity of Nobi Gel® gel 2.5 % (*R*) at 32 °C was greater than the dynamic viscosity of Ketoprofen solution 2.5 % ($T_S$) by several thousand times (depending on the shear rate), but the parameters of ketoprofen release from the solution ($T_S$) were only about 1.2 times larger compared to the gel (Tables 2, 16).

The results of the study of three gels with ketoprofen content of 2.0 %, 2.5 % and 3.0 % are presented in Fig. 12 and Table 17.

The release parameters for the preparations containing 2.0 % and 3.0 % ketoprofen differed almost proportionally from the release parameters for the gel with the nominal content of ketoprofen (2.5 %). The release rate differed by –20.1 % and +22.0 %, respectively, the cumulative content, the content in the dialysate after 6 h and the recovery differed by –23.0 % and +20.6 %. Both samples $T_{2.0}$ and $T_{3.0}$ did not meet the specification regarding the ketoprofen content in Nobi Gel® gel 2.5 %, which should be 2.5 g/100 g±5 %. But with regard to the release rate of ketoprofen, these preparations could be considered equivalent to the Nobi Gel® gel 2.5 % according to USP criteria.

The results of the study of gels with different ethanol (96 %) content are presented in Fig. 13 and in Table 18.

Table 16

Parameters of KET release from Nobi Gel® gel 2.5 % (*R*), Ketonal® gel 2.5 % ($T_1$), Ketoprofen gel 2.5 % ($T_T$) and Ketoprofen solution 2.5 % ($T_S$)

| Parameter | *R* | $T_1$ | $T_T$ | $T_S$ |
|---|---|---|---|---|
| Release rate (*R*), mg/cm²/h$^{-1/2}$ | 1.43±0.06 SD: 0.03 | 1.34±0.07 SD: 0.03 | 1.51±0.06 SD: 0.03 | 1.76±0.09 SD: 0.04 |
| $R_T/R_R$, % | – | 89.61–98.23 | 102.0–109.13 | 116.77–125.81 |
| Cumulative amount (*A*) (at the time point 6 h), mg/cm² | 3.16±0.07 SD: 0.04 | 2.86±0.09 SD: 0.05 | 3.25±0.12 SD: 0.06 | 3.81±0.09 SD: 0.05 |
| $A_T/A_R$, % | – | 87.69–94.32 | 99.16–106.27 | 116.56–123.50 |
| Content (*C*) in the receptor medium (at the time point 6 h), mg/ml | 0.50±0.01 SD: 0.01 | 0.45±0.01 SD: 0.01 | 0.52±0.02 SD: 0.01 | 0.60±0.01 SD: 0.01 |
| Correlation coefficient *r* | 0.999 | 0.996 | 0.999 | 0.998 |
| Coefficient of determination $R^2$ | 0.998 | 0.993 | 0.998 | 0.997 |
| Recovery (at the time point 6 h), % | 8.43±0.20 SD: 0.10 | 7.63±0.25 SD: 0.12 | 8.67±0.43 SD: 0.15 | 10.13±0.37 SD: 0.12 |

Table 17

Parameters of KET release from Nobi Gel® gel 2.5 % (*R*), Ketoprofen gel 2.0 % ($T_{2.0}$) and Ketoprofen gel 3.0 % ($T_{3.0}$)

| Parameter | *R* | $T_{2.0}$ | $T_{3.0}$ |
|---|---|---|---|
| Release rate (*R*), mg/cm²/h$^{-1/2}$ | 1.43±0.06 SD: 0.03 | 1.14±0.03 SD: 0.01 | 1.74±0.05 SD: 0.03 |
| $R_T/R_R$, % | – | 75.76–80.17 | 117.07–126.25 |
| Cumulative amount (*A*) (at the time the point 6 h), mg/cm² | 3.16±0.07 SD: 0.04 | 2.43±0.04 SD: 0.02 | 3.81±0.16 SD: 0.08 |
| $A_T/A_R$, % | – | 75.22–79.12 | 116.14–124.63 |
| Content (*C*) in the receptor medium (at the time point 6 h), mg/ml | 0.50±0.01 SD: 0.01 | 0.397±0.01 SD: 0.004 | 0.61±0.026 SD: 0.01 |
| Correlation coefficient *r* | 0.999 | 0.9997 | 0.999 |
| Coefficient of determination $R^2$ | 0.998 | 0.999 | 0.999 |
| Recovery (at the time point 6 h), % | 8.43±0.20 SD: 0.10 | 6.49±0.12 SD: 0.06 | 10.17±0.42 SD: 0.21 |

Table 18

Parameters of KET release from Nobi Gel® gel 2.5 % (*R*), Ketoprofen gel 2.5 % with ethanol content 22.8 % ($T_{E80}$) and Ketoprofen gel 2.5 % with ethanol content 34.2 % ($T_{E120}$)

| Parameter | *R* | $T_{E80}$ | $T_{E120}$ |
|---|---|---|---|
| Release rate (*R*), mg/cm²/h$^{-1/2}$ | 1.43±0.06 SD: 0.03 | 1.63±0.06 SD: 0.03 | 1.28±0.05 SD: 0.02 |
| $R_T/R_R$, % | – | 108.31–117.85 | 86.52–91.44 |
| Cumulative amount (*A*) (at the time the point 6 h), mg/cm² | 3.16±0.07 SD: 0.04 | 3.56±0.11 SD: 0.06 | 2.76±0.08 SD: 0.04 |
| $A_T/A_R$, % | – | 109.52–117.72 | 84.97–90.65 |
| Content (C) in the receptor medium (at the time point 6 h), mg/ml | 0.50±0.01 SD: 0.01 | 0.57±0.02 SD: 0.01 | 0.44±0.01 SD: 0.01 |
| Correlation coefficient r | 0.999 | 0.999 | 0.999 |
| Coefficient of determination $R^2$ | 0.998 | 0.998 | 0.998 |
| Recovery (at the time point 6 h), % | 8.43±0.20 SD: 0.10 | 9.49±0.30 SD: 0.15 | 7.35±0.22 SD: 0.11 |

Table 19

Parameters of KET release from Nobi Gel® gel 2.5 % (pH 6.57) (*R*), Ketoprofen gel 2.5 % with pH 6.0 ($T_{pH6}$) and ketoprofen gel 2.5 % with pH 7.0 ($T_{pH7}$)

| Parameter | *R* | $T_{pH6}$ | $T_{pH7}$ |
|---|---|---|---|
| Release rate (*R*), mg/cm²/h$^{-1/2}$ | 1.43±0.06 SD: 0.03 | 1.44±0.06 SD: 0.03 | 1.44±0.09 SD: 0.05 |
| $R_T/R_R$, % | – | 94.41–103.76 | 94.33–103.64 |
| Cumulative amount (*A*) (at time the point 6 h), mg/cm² | 3.16±0.07 SD: 0.04 | 3.08±0.10 SD: 0.05 | 3.12±0.14 SD: 0.07 |
| $A_T/A_R$, % | – | 94.22–101.52 | 94.12–102.69 |
| Content (*C*) in the receptor medium (at the time point 6 h), mg/ml | 0.50±0.01 SD: 0.01 | 0.49±0.02 SD: 0.01 | 0.50±0.02 SD: 0.01 |
| Correlation coefficient r | 0.999 | 0.999 | 0.997 |
| Coefficient of determination $R^2$ | 0.998 | 0.998 | 0.995 |
| Recovery (at the time point 6 h), % | 8.43±0.20 SD: 0.10 | 8.21±0.24 SD: 0.12 | 8.33±0.36 SD: 0.18 |

In the case of ethanol content in the preparation of 22.8 %, which was 20 % less than its nominal content, the values of the KET release parameters were greater, and with an ethanol content of 34.2 %, which was 20 % more than its nominal content, the values of these parameters, on the contrary, were less than KET release parameters at nominal ethanol content of 28.5 % (Fig. 13, Table 18). The KET release rate in the case of preparation $T_{E80}$ increased by approximately 14.5 %, and other parameters were greater by 12.6 %. The KET release rate in the case of the preparation $T_{E120}$ decreased by approximately 10.5 %, and other parameters were lower by 12.8 %.

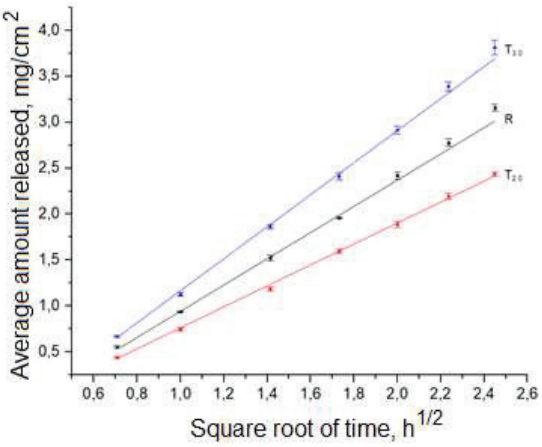



Fig. 12. KET release rate plots in the case of Nobi Gel® gel 2.5 % (*R*), Ketoprofen gel 2.0 % ($T_{2.0}$) and Ketoprofen gel 3.0 % ($T_{3.0}$)

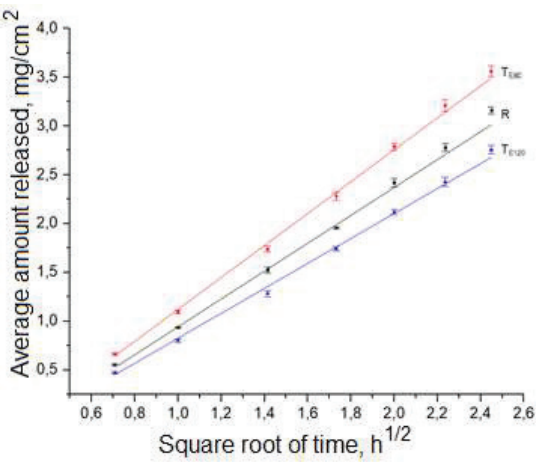



Fig. 13. KET release rate plots in the case of Nobi Gel® gel 2.5 % (*R*), preparations Ketoprofen gel 2.5 % with ethanol content 22.8 % ($T_{E80}$) and Ketoprofen gel 2.5 % with ethanol content 34.2 % ($T_{E120}$)

The results of the study of ketoprofen release from Nobi Gel® gel 2.5 % (pH 6.57) (*R*), Ketoprofen gel 2.5 % with pH 6.0 ($T_{pH6}$) and Ketoprofen gel 2.5 % with pH 7.0 ($T_{pH7}$) are presented in Fig. 14 and Table 19. It was shown that a change in the pH in the range from 6.0 to 7.0 practically did not affect the parameters of KET release from gels, that is, pH is not a significant factor for the *in vitro* release of ketoprofen [35].

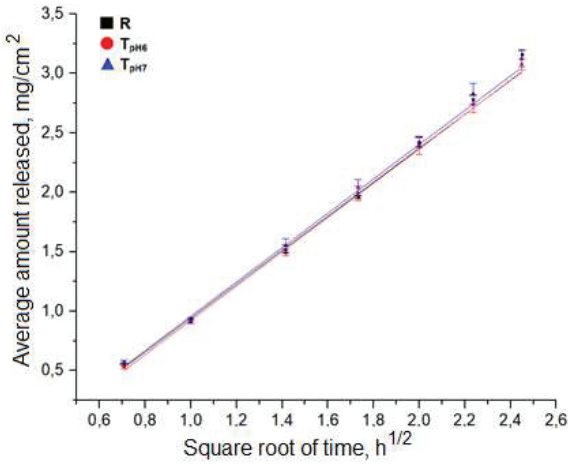



Fig. 14. KET release rate plots for Nobi Gel® gel 2.5 % (pH 6.57) (*R*), Ketoprofen gel 2.5 % with pH 6.0 ($T_{pH6}$) and ketoprofen gel 2.5 % with pH 7.0 ($T_{pH7}$)

**5. Discussion of research results**

Carbomers in gels perform the function of gelling agents. The sol→gel transition, which occurs when the carbomer is dissolved in a Newtonian liquid and then neutralised, leads to a change of the Newtonian flow to a plastic flow characterised by yield stress and apparent viscosity. But the formation of a carbomer-based gel (in particular, with Carbopol® Ultrez 21 Polymer) practically did not affect the type and parameters of the EPR spectrum of the dissolved spin probe with carboxyl group in its molecules like the molecules of carbomer and KET (Fig. 2, Table 3). An increase in rotational correlation times and a decrease in the anisotropy parameter were observed in the case of a spin probe with an amino group that interacted with the carboxyl groups of the carbomer. That is, if in gels the active substance does not interact with the carbomer, then its molecules/ions rapidly rotate in the liquid dispersion medium. This creates prerequisites for the rapid and complete release of a such medicinal substance from carbomer-based gels.

The conclusions based on the results of studies by the spin probe method are confirmed by data regarding the *in vitro* release of ketoprofen. According to the KET release parameters in the case of Nobi Gel® gel 2.5 % and the liquid that was its dispersion medium, they were equivalent, taking onto account the USP acceptance criteria (Fig. 11, Table 16). Moreover, gels with carbomers that differed significantly in terms of apparent viscosity and yield stress were equivalent to Nobi Gel® gel 2.5 % according to USP acceptance criteria as well as taking into account the requirements of the EMA draft guideline [12, 13].

Ketoprofen content and ethanol content were the factors which affected the KET release from the gel. When the KET content increased from 2.0 % to 2.5 % and up to 3.0 %, the release parameters increased proportionally (Table 17, Fig. 12). Content of 2.0 % and 3.0 % of ketoprofen was out of the specification for 2.5 % gel [36]. But these preparations can be considered equivalent to Nobi Gel® gel 2.5 % in terms of the release rate of ketoprofen according to USP requirements [13].

The ethanol content is an important factor for the release of ketoprofen from the gel. When the ethanol content in the gel was reduced by 20 %, the KET release parameters increased, and conversely, when the ethanol content was increased by 20 % relative to the nominal content, the KET release rate decreased (Table 18, Fig. 13). According to the specification for Nobi Gel® gel 2.5 % ethanol content (96 %) should be 28.5 g/100 g±10 %. Both preparations $T_{E80}$ and $T_{E120}$ did not meet the specification for Nobi Gel® gel 2.5 % regarding the ethanol content (96 %) [37]. But these studied products can be considered equivalent to Nobi Gel® gel 2.5 % in terms of the KET release rate according to the requirements of the USP [13].

Within the established limits, the change in pH from 6.0 to 7.0 pH practically did not affect the parameters of KET release from gels (Table 19, Fig. 14).

**Study limitations.** The experiments only with regard to one active substance (ketoprofen) could be considered as a limitation of the study. In addition, some other types of carbomer could be used, and the release of ethanol (which is a significant factor for the release of ketoprofen) could be studied depending on its content in the gel and the Newtonian fluid.

**Prospects for further research.** The possibility of interaction between substances containing an amino group in the molecule with carbomers has been shown based on the results of research by the spin probe method. Such interaction of active substances or excipients can lead to a decrease or loss of their activity/functionality or to problems during the production process. Such issues require further research and novel technological solutions.

Some contradictions between the pharmacopoeial and regulatory standards regarding the content of the active substance or excipients [36, 37] and the pharmacopoeial acceptance criteria regarding the *in vitro* release of an active substance [12, 13] need some coordination. The same acceptance criteria for the release of active substances from carbomer-based gels, creams with o/w emulsion bases, and hydrophilic and hydrophobic ointments should not be applied. This might be a particular challenge in the case of suspension preparations. The results of this work should attract the attention of the regulatory authorities that individual guidelines should be developed based on the results of certain research in regards to *in vitro* release testing for preparations that differ in the type of bases and dispersed state. In order to correctly confirm an extended pharmaceutical equivalence using *in vitro* release testing, it is necessary during pharmaceutical development to identify those factors that are significant for the release of the active substance from a particular medicinal product.

Additional scientific rationale for the acceptance criteria regarding *in vitro* release of substances is necessary for semi-solid preparations with different types of bases and different dispersed states of active substances.

**6. Conclusions**

The formation of a carbomer-based gel did not affect the rotational correlation time of the probe, which did not interact with the carbomer. Parameters of *in vitro* release of ketoprofen from the gel and Newtonian liquid differed little; these parameters were also little affected by the difference

in apparent viscosity of the gels. The *in vitro* release of ketoprofen depended on its concentration and ethanol content.

**Conflict of interests**

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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