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# INFLUENCE OF MICROEMULSION COMPONENTS ON TRANSDERMAL DELIVERY OF IMMUNOMODULATOR GLUCOSAMINYLMURAMYL DIPEPTIDE

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This paper demonstrates a chemical way of enhancing transdermal delivery using immunomodulator glucosaminylmuramyl dipeptide (GMDP) as an example. **Objective:** to study *in vitro* the effect of various components of the microemulsion composition on GMDP diffusion through the skin from a transdermal therapeutic system (TTS). **Materials and methods.** Medicinal substance – glucosaminylmuramyl dipeptide (Peptek, Russia). Excipients and raw materials: sodium chloride, purified water, sodium dodecyl sulfate, docusate sodium, oak bark, apricot kernel oil, alpha-tocopheryl acetate and Decaglyn PR-20 emulsifier. Equipment: Heidolph DIAX 900 mechanical disperser (Germany) and Hielscher UIS250V ultrasonic homogenizer (Germany). GMDP diffusion from TTS through unpreserved rabbit skin was studied on diffusion tester Copley (UK). GMDP in aqueous solutions was determined by reversed-phase high-performance liquid chromatography (RP-HPLC) on an Agilent 1200 chromatography system (Agilent Technologies, USA). **Results.** A microemulsion system composed of 20% docusate sodium in an oil phase and an oak bark decoction as an aqueous phase was developed. This made it possible to increase GMDP transdermal delivery by ~70% in comparison with the basic composition. **Conclusion.** The characteristic parameters of microemulsion components of GMDP contained in TTS, influencing GMDP diffusion through unpreserved rabbit skin *in vitro*, were determined. Introducing relative indicators would be advisable in order to correctly evaluate the results of different series of *in vitro* experiments with biological objects.

*Keywords: transdermal therapeutic system, microemulsion, immunomodulator, drug diffusion.*

## INTRODUCTION

Currently, 74% of all drugs are administered orally, but such a dosage form often does not have sufficient efficacy due to low bioavailability. To increase it, intact skin can be used as a route of administration, which requires the creation of an appropriate dosage form, namely the transdermal therapeutic system (TTS) [1].

The major problem in the development of transdermal delivery systems is overcoming the medicinal substance (MS) of the skin barrier, and mainly the stratum corneum. Researchers use various chemical, physical, or combined approaches to increase cutaneous absorption and percutaneous diffusion, the choice of which is determined by the characteristic properties of MS [1, 2].

Physical methods always imply the use of a device, which makes this method of enhancing skin permeability expensive and not always convenient for the patient to use [2].

The essence of the chemical method is either modifying the MS molecules or introducing transfer activators into the TTS, which can directly affect the skin structure. They are often administered as part of complex formulations, such as micro- and nanoemulsions, biphasic vesicles, spheroid particles, or liposomes [3].

In our opinion, the most effective of the above chemical methods for enhancing transdermal MS delivery include nano- and microemulsions due to the possibility of a wide selection of transfer activators for a specific drug substance, ease of manufacture, and low cost. In addition, the submicron size of the dispersed phase and the high sorption capacity of microemulsions make it possible to achieve a noticeable increase in the diffusion of some MS through the skin. When MS is introduced into emulsions, in some cases it becomes possible to avoid hydrolysis, decomposition and oxidation of the introduced substances [4]. Also, when using them, it is possible to prevent skin irritation, which is sometimes observed upon contact with an active substance [4–7]. We have proved the prospects of using microemulsion compositions when creating TTS with such medicinal

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substances as insulin, bromocaine, caffeine, and others [8–10]. The choice of components and their ratio in the microemulsion composition depends on the nature of the drug substance [5, 6, 11].

The range of drugs that can be used in the form of TTS using microemulsions is quite wide. The greatest interest in recent years is represented by drugs from the pharmacological group of immunomodulating agents. Delivery of an immunomodulator (MI) in the form of TTS, if it is necessary to maintain its constant concentration in the blood, can be relevant for a number of patients: children, people with difficulty chewing and swallowing, bedridden patients, including those in serious condition with infectious diseases.

Glucosaminylmuramyl dipeptide (GMDP), a synthetic analogue of the structural fragment of the membrane (peptidoglycan) of bacterial cells, is an activator of innate and acquired immunity, enhances the body's defense against viral, bacterial and fungal infections, and has an adjuvant effect in the development of immunological reactions [12].

In Russia, the drug with GMDP is produced only in the form of tablets under the trade name Likopid® (registration certificate No. LS-001438) [13]. The high efficiency and safety of GMDP use, confirmed by the results of clinical trials [14], determine the prospects for the development of its new dosage forms, including the transdermal therapeutic system.

When developing the composition of multicomponent microemulsions, a necessary stage is a comparative analysis of the contribution of the main components of the emulsion composition to the percutaneous transfer of a particular MS.

The **purpose** of this work is to study in vitro the effect of various components of the microemulsion composition on the diffusion of GMDP through the skin from a transdermal therapeutic system.

## MATERIALS AND METHODS

When developing various formulations of emulsion compositions for TTS, excipients were used that were approved for medical use and that meet the requirements of the current regulatory documentation.

Glucosaminylmuramyl dipeptide ($C_{25}H_{43}N_5O_{15}$) produced by JSC Peptek, Russia was taken as a drug substance.

For the *production of microemulsion* purified water (FS 42-2620-97, distiller DE-10 and filter "MILLIPORE SIMPAKOR 1"); 0.9% sodium chloride solution (solution for infusion of JSC NPK ESCOM); sodium dodecyl sulfate (CAS 151-21-3, Appli Chem Panreac, Spain); oak bark (Krasnogorskleksredstva JSC, Russia); apricot kernel oil (CAS 72869-69-3, Desert Whale Jojoba Company Ltd., USA); sodium docusate (DNS) (D4422-50G, Sigma, USA); alpha-tocopherol acetate (Customer Product # 4904352421, BASF SE, Germany); emulsifier NIKKOL Decaglyn PR-20 (CAS 29894-35-7, Nikko Chemicals Co., Ltd, Japan) were used.

The sorbing layer of the dressing (PALV-01, Palma Group, Russia) served as a depot of the microemulsion composition in the transdermal therapeutic system, and the Skotchpak film (97303M, USA) served as a waterproof protective base.

The following reagents were used to determine *GMDP by the HELC* method: acetonitrile (for UHPLC, AppliChem GmbH – AnITWCompany, Germany); potassium hydrogen phosphate (extra pure, Scharlab S.L., Spain); potassium dihydrogen phosphate (extra pure, Scharlab S.L., Spain).

*Equipment used*: analytical balance (GH-200 AND, Japan); magnetic stirrer with heating (IKA, Germany); mechanical submersible disperser (T18 basic Ultra-Turrax IKA-WERKE Gmbh & Co. Kg, Germany); ultrasonic homogenizer (UIS 250V Heilscher, Germany); drug diffusion analyzer (HDT 1000 Copley Scientific Ltd., UK); dispersion analyzer (LUMi Sizer, Germany); spectrophotometer (UV-2600 Shimadzu, Japan); moisture analyzer (MX-50 AND, Japan); chromatographic system (1200 Agilent Technologies, USA).

### Production of laboratory GMDP TTS samples

The manufacture of laboratory samples of microemulsion TTS GMDP was carried out according to the previously developed method [8]. Each 1 $cm^2$ TTS contained 0.1 g of microemulsion composition.

### Preparation of oak bark decoction

5 g sample of oak bark was placed in a 200 ml conical flask and 100 ml of purified water was poured, then heated to 90 °C in a water bath for 30 minutes. Thereafter, it was cooled to room temperature over 1 hour. Then the broth was poured into a 100 ml volumetric flask through filter paper and the volume was brought up to the mark with purified water. In a volumetric flask with a volume of 100 ml was placed 10 ml of a decoction of oak bark and the volume was brought up to the mark with purified water.

### Determination of moisture content in oak bark

Moisture content of oak bark was determined in accordance with the requirements of GPM 1.5.3.0007.15 "Determination of the moisture content of medicinal plants and herbal medicinal products" with a moisture analyzer. The moisture content $W$ of raw materials in percent was calculated by the formula:

$$W = \frac{(m - m_1) \times 100}{m},$$

where $m$ – mass before drying, g; $m_1$ – mass after drying, g.

The moisture content of raw was (8.2 ± 0.8)%, n = 5.

## Determination of tannins content in oak bark concoction

The content of tannins in plant raw was determined by spectrophotometry at 277 nm wavelength (Guidelines for quality control methods and safety of biologically active food additives. Guidelines. R 4.1.1672-03, 2004). An aliquot of the decoction of oak bark, equal to 1 ml, was placed in a 50 ml volumetric flask and made up to the mark with purified water. Purified water was used as a reference solution. The total content of tannins in the bark of oak X,% in terms of gallic acid was calculated by the formula:

$$X = \frac{D_1 \times V_1 \times V_2 \times 100\%}{D_2 \times V_3 \times m \times (100\% - W) \times 1000},$$

where $D_1$ – optical density of the test solution; $D_2$ – optical density of a solution of gallic acid with 1 mg/ml (0.508) concentration; $m$ – weight of raw sample, g; $V_1$ – total volume of aqueous extract, 100 ml; $V_2$ – flask volume at dilution, 50 ml; $V_3$ – aliquot volume, 1 ml; $W$ – raw moisture, %.

## Research methods for emulsion compositions

The particle size of the microemulsion composition with GMDP by the time of its separation were determined with a dispersion analyzer in infrared light (865 nm) at 40 °C. Light transmission profiles were recorded every 600 seconds at a rotor speed of 4000 rpm, the light factor was chosen equal to 3.

## Laboratory animals

In *in vitro* experiments to study the diffusion of the immunomodulator from TTS, the skin of male New Zealand White rabbits was used, weighing 2–2.5 kg, obtained from the laboratory animal nursery of KrolInfo LLC. All manipulations with animals were carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123) Strasbourg, 1986).

## *In vitro* study of medical substance diffusion through skin

After euthanasia of an animal, the skin flap was taken from the abdomen with previously removed hair cover.

The dynamics of the release of GMDP from TTS was studied in Franz glass diffusion cells according to the standard technique [9]. The duration of the experiments was 24 hours. Samples of GMDP aqueous solutions taken during the experiment from the receiving chambers of the diffusion cells were studied by the HELC method.

The amount of MS passing through unconserved rabbit skin from the TTS contact area (ω) was calculated as follows:

$$\omega = \frac{C \times V}{M} \times 100\%,$$

where $C$ – GMDP concentration in the receiving chamber of the Franz diffusion cell, mg/ml; $V$ – diffusion cell receiving chamber volume, ml; $M$ – GMDP content in TTS in the contact area, mg.

The rate of transdermal MS diffusion can be very different in laboratory animals, even in the same litter, due to the different thickness and density of the skin flap. In this regard, a comparative analysis of the effect of different compositions of the microemulsion composition for TTS on the percutaneous transfer of MS is expedient to be carried out in relative units. The relative amount $\omega_{rel}(i, j)$ was calculated using the following formula:

$$\omega_{rel(i\,j)} = \frac{\omega_j}{\omega_i},$$

where $i$, $j$ – microemulsion composition numbers (Table 2).

## HELC method for study of aqueous GMDP solutions

Earlier, the authors developed a method for the quantitative determination of glucosaminylmuramyldipeptide in aqueous solutions by the method of reverse phase HELC [12].

Chromatographic separation was carried out in an isocratic mode on a MediterraneaSea18 column 15 × 0.4 cm, 5 μm (Teknokroma Analitica SA, Spain), with an 8 × 4 mm guard column filled with the same sorbent. The temperature of the column thermostat is –25 °C. The volume of the injected sample is 10 μl. The mobile phase is a mixture of acetonitrile: 25 mM phosphate buffer solution (3:97), pH 7.3. A phosphate buffer solution was prepared by mixing 25 mM $K_2HPO_4$ solution with 25 mM $KH_2PO_4$ solution in a ratio of 80:20. The eluent was prefiltered and degassed on a vacuum filtration device. The flow rate of the mobile phase is 0.7 ml/min. Detection was carried out at a wavelength of 200 nm, corresponding to the absorption maximum of GMDP. The chromatography time was 10 min, the retention time of the GMDP anomers was about 3.3 and 4.9 min.

Registration and processing of chromatographic data were performed with ChemStation software (Agilent Technologies, USA). Statistical processing of the results was performed in accordance with GMP.1.1.0013.15 "Statistical processing of the results of a chemical experiment" with Microsoft Office Excel 2010.

## RESULTS AND DISCUSSION

As a microemulsion composition for TTS GMDP, the basic composition was chosen (Table 1), which we developed earlier for the transfer of drugs of various pharmacological groups and molecular weights through

Table 1

**The base composition № 1 of the microemulsion composition**

| | Substance | TTS indication | Characteristics |
|---|---|---|---|
| Aqueous phase | Purified water | Aqueous phase base | GMP 42-2620-97 |
| | GMDP | Medical substance | White hydrophilic powder |
| Oil phase | Apricot kernel oil | Base of oil phase | Contains linoleic (30–45%) and oleic (55–70%) acids |
| | α-tocopherol acetate | Skin disintegrant and antioxidant | Lipophilic liquid |
| | Sodium dokuzat | MS carrier | Amphiphilic anionic detergent |
| | Decaglyn PR-20 | Emulsifier | Lipophilic surfactant with hydrophilic-lipophilic balance of 3.2 |

Table 2

**Changes in microemulsion compositions with GMDP compared with the base composition**

| Composition # | Aqueous phase | Oil phase |
|---|---|---|
| 2 | Decrease in volume of purified water by 1.9 times | Volume increase by 1.7 times |
| 3 | Sodium dodecyl sulfate 0.5% solution | Similar to base composition |
| 4 | 0.9% sodium chloride solution | Similar to base composition |
| 5 | Similar to base composition | Increase in sodium docusate content from 15 to 20% |
| 6 | Decoction of oak bark (concentration of gallic acid 2.60 ± 0.18%, n = 5) | Increase in sodium docusate content from 15 to 20% |

the skin, such as insulin [10], anilocaine [9], sodium aminodihydrophthalazinedione [8].

At choosing vegetable oils for application dosage forms, the fatty acid composition is of particular importance [15, 16]. The basis of the oil phase of the microemulsion composition was the oil of apricot kernels (acid number 0.04), which contains a large amount of oleic acid (from 55 to 70%), due to which it is well absorbed into the deep layers of the skin and enhances the penetration of active components into the stratum corneum. In addition, apricot kernel oil has a low viscosity, which makes it possible to use it as a solvent for lipophilic drug transfer activators, in our case, vitamin E and sodium docusate.

The selected microemulsion composition containing GMDP had a particle size of 1 to 15 μm and a very high stability: it only partially separated when centrifuged and heated to 40 °C for 12 hours. This stability of the microemulsion can be explained by the fact that medicinal substances of a protein nature can act as a stabilizer. The authors observed a similar effect when developing emulsion insulin TTS [10].

The investigated medical substance, glucosaminylmuramyldipeptide, has a small molecular weight (695.67 g/mol) in terms of transdermal transfer in comparison, e. g., with insulin (5500 g/mol). However, the amount of GMDP (ω) diffused from TTS from the contact area through unconserved skin in vitro in 24 hours was only (14.4 ± 3.0)% (n = 10) of that contained in the sample.

To clarify the role of one or another component of the microemulsion composition in the transdermal diffusion of MS (at its constant concentration in TTS of 46 mg/g), 5 microemulsion compositions were prepared (Table 2), which differ from the pre-selected (base) composition No. 1 in the following parameters: change in the amount of the oil phase; increasing the concentration of the sodium docusate transfer activator in the oil phase; the introduction of hydrophilic activators for the transdermal transfer of sodium dodecyl sulfate or sodium chloride; the use of oak bark decoction as an aqueous phase of a microemulsion, a well-known source of biologically active substances used in medical practice in the treatment of skin diseases [17].

Table 3 shows the results of percutaneous diffusion of GMDP from TTS with different compositions of the microemulsion composition for 24 hours *in vitro* in relative values.

Changes to the base microemulsion composition in the case of formulations # 2, 3 and 4 did not lead to an

Table 3

**Relative amount of GMDP (%) passed through unreserved rabbit skin, for TTS of various composition**

| Microemulsion composition # | Relative amount $\omega_{rel(i,j)}$ |
|---|---|
| 2 | $\omega_{rel(1,2)} = 0.62 \pm 0.24$ |
| 3 | $\omega_{rel(1,3)} = 0.85 \pm 0.14$ |
| 4 | $\omega_{rel(1,4)} = 0.68 \pm 0.05$ |
| 5 | $\omega_{rel(1,5)} = 1.44 \pm 0.30$ |
| 6 | $\omega_{rel(5,6)} = 1.16 \pm 0.19$ |
| | $\omega_{rel(1,6)} = 1.67 \pm 0.26$ |

increase in the yield of GMDP from TTS through unconserved rabbit skin.

For TTS with microemulsion formulation # 5, in which the concentration of docusate sodium salt transporter in the oil phase was 20%, an increase in transdermal transfer of GMDP was observed by 44% compared to base formulation # 1 containing 15% of the same carrier. The use of an aqueous extract of oak bark (composition No. 6) as a dispersed phase of a microemulsion made it possible to further increase the percutaneous diffusion of GMDP in comparison with composition # 5 by another 16%.

Thus, the simultaneous increase in the sodium docusate content in the oil phase to 20% and the use of an aqueous extract of oak bark (composition No. 6) led to an increase in the transdermal transfer of GMDP by 67% compared to the base composition.

## CONCLUSION

The effect of changes in the composition and phase ratio of microemulsion compositions on the diffusion of GMDP from TTS through unconserved rabbit skin *in vitro* was studied.

The developed composition of the microemulsion composition containing 20% sodium docusate in the oil phase and a decoction of oak bark as the aqueous phase made it possible to increase the transdermal transfer of GMDP by about 70% compared to the basic composition used by us in the development of the TTS series [8–10].

For a correct assessment of the results of different series of in vitro experiments with biological objects, it is advisable to introduce relative indicators.

*The authors declare no conflict of interest.*

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