

# SYNTHESIS AND CHARACTERIZATION OF POLY(LACTIC-CO-GLYCOLIC-ACID) MICROPARTICLES LOADED WITH FOOT-AND-MOUTH DISEASE VIRUS 40–60 SYNTHETIC PEPTIDE

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The aim of the research was to synthesize and characterize the poly (lactic-co-glycolic-acid) microparticles loaded with foot-and-mouth disease virus 40–60 synthetic peptide. Single emulsion solvent evaporation method was used for the production of microparticles with three different (5; 10 and 15 mg) peptide amounts and obtained microparticles were characterized by using yield reaction, encapsulation efficiency, drug loading, particle size, polydispersity index, and zeta potential.

**Key words:** virus of the foot-and-mouth disease, poly (lactic-co-glycolic-acid) microparticles.

Foot-and-mouth disease (FMD) virus, the pathogen of FMD disease which is a member of the *Picornaviridae* family *Aphthovirus* genus, has a single positively charged RNA chain containing 8500 nucleotides. The un-enveloped capsule of the virus contains VP1, VP2, VP3 and VP4 structural proteins. The synthetic peptide used in this study is defined as the 40–60 viral peptide series of VP1 Foot-and-Mouth disease capsid protein [1–4].

Food-and-mouth disease is an infectious disease that affects the cloven-hoofed animals including cattle, goat, sheep and pigs. This highly infectious disease can be transmitted via infected or contaminated animals, animal products and humans. This disease can be seen all around the globe and can cause enormous economic losses due to production losses and international commerce sanctions. The control and prevention of the disease is extremely difficult. Vaccination is one of the precautions that can be taken against this disease [5–7].

Vaccination with the intention to prevent and control FMD has been used since the 1900 s. Currently, vaccination against this disease depends on cultivation of FMD virus in bio-secure conditions and inactivation via chemical methods. Due to the disadvantages of inactive vaccines such as requirement of safety precautions and cold chain or short shelf life,

new generation more effective vaccines need to be developed. In the last few decades the studies to develop a biotechnological vaccine for FMD is ongoing [8–10].

New vaccine studies involve synthetic peptides, recombinant protein subunits, protein-polysaccharide conjugates and plasmid DNAs [11, 12]. Despite its advantages, these approaches produce weak immune response. For this reason, using adjuvants in order to obtain a more robust immune response is aimed [13, 14].

With the recent studies showing that the size of the particles is crucial for adjuvant activity, using nano- and microparticles as adjuvants has been focused on vaccine studies. Particle carriers are effective delivery systems for antigens and increase the antigen uptake on cellular level. They are effective on controlled antigen release and can protect the antigen integrity from degradation [15].

Carrier polymers, co-polymers, proteins and lipids are widely used in vaccine delivery system production [16–22]. Biocompatibility, biodegradability, rate of degradation, hydrophilic property of the chosen polymer affects the micro-particles formed. Poly (lactic-co-glycolic-acid) (PLGA), which one of the most widely used co-polymer, is a biopolymer approved by FDA and has

several advantages such as inert properties in physical environments, degradability in biological environments, biocompatible properties and being able to be degraded into non-toxic products. PLGA degrades into lactic acid and glycolic acid monomers in body which enter the citric acid cycle where they are metabolized into carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O) [15, 23, 24].

In this study, FMDV 40-60 peptide epitope (Trp-Val-Lys-Ile-Asn-Asn-Thr-Ser-Pro-Thr-His-Val-Ile-Asp-Leu-Met-Gln-Thr-His-Gln-His-Gly), synthesized via solid phase peptide synthesis method, encapsulated PLGA micro-particles were synthesized for using vaccine models. Synthesized microparticles were characterized by methods of ZetaSizer, scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy. Loading and encapsulation yields were calculated, bio-release experimentations were performed.

## Materials and Methods

### Materials

Foot-and-mouth disease 40-60 antigenic peptide (FM) (Trp-Val-Lys-Ile-Asn-Asn-Thr-Ser-Pro-Thr-His-Val-Ile-Asp-Leu-Met-Gln-Thr-His-Gln-His-Gly) was synthesized in our previous study via F-moc chemistry [3]. Poly(lactic-co-glycolic acid) –PLGA – (lactic to glycolic acid ratio in the copolymer is 50:50, inherent viscosity 0.45–0.60 dL/g, Mw — 38–54 kDa P50/50), polyvinyl alcohol (PVA), dichloromethane (DCM) was purchased from Sigma-Aldrich (St. Louis, Mo, USA). All other chemical reagents were of analytical grade. Ultra-pure water was acquired from Millipore MilliQ Gradient system.

### Preparation of Microparticles

In the present study peptide loaded PLGA microparticles were produced according to a previously published single emulsion solvent evaporation methods [25, 26] with brief modifications. Firstly, the synthetic peptide and PLGA, which will form the organic phase, were dissolved in ethanol and DCM, respectively. Peptide solutions were prepared in three different amounts (5 mg, 10 mg, 15 mg) and added to PLGA solutions and mixed. Prepared organic solutions are added on 4 mL of 3% PVA solution, sonicated in ice bath for 2 min with 70 W and a power of 80% (Bandelin Sonopuls, Germany). Obtained uniform emulsion was stirred overnight on a magnetic stirrer for evaporation of the organic

solvents. Subsequently, the particles are collected by centrifugation (Sartorius-Biofuge) for 40 min at 10 000 rpm, washed three times with ultrapure water. The microparticles were lyophilized and stored at –80 °C.

### Characterization of Microparticles

In the study, obtained microparticles were detailed analyzed by following parameters: reaction yield (RY), encapsulation efficiency (EE), drug loading (DL), particle size (Z-ave), polydispersity index (PDI), and zeta potential (ZP).

### Reaction Yield, Encapsulation Efficiency and Drug Loading.

In the present study, the reaction yield was calculated gravimetrically using the formula given below:

$$RY (\%) = \frac{\text{Amount of produced microparticle (mg)}}{\text{Total amount of initial peptide and PLGA (mg)}} \times 100.$$

The drug loading and entrapped peptide was assayed by fluorescence spectroscopy (excitation wavelength 270 nm and emission wavelength 280–600 nm) analysis. EE was determined by using of the supernatants obtained after centrifugation for each of microparticles.

The peptide entrapment efficiency (EE) was determined using the formula given below:

$$EE (\%) = \frac{\text{Encapsulated peptide amount (mg)}}{\text{The amount of peptide initially added (mg)}} \times 100.$$

The drug loading (DL) of peptide was calculated using the formula given below:

$$DL (\%) = \frac{\text{Encapsulated peptide amount (mg)}}{\text{Amount of produced microparticle (mg)}} \times 100.$$

### Particle Size, Polydispersity Index and Zeta Potential Measurements of Microparticles

Particle size, polydispersity index and zeta potential of microparticles were determined by dynamic light scattering (DLS) (Malvern Zetasizer, ZS, Malvern, UK). Measurements were done in triplicate, at 25 °C, using 0.8872 cP viscosity and 1.330 refractive index for the solutions, dielectric constant 79; f(ka) 1.50 (Smoluchowski). Before the measurement all samples were diluted to 1:30 dilution factors with ultra-pure water (Millipore).

### FT-IR Measurements of Microparticles

Fourier transform infrared (FTIR) spectroscopy measurements were performed by IR-Prestige 21 FTIR spectrophotometer

(Shimadzu, Japan) in the range from 4000  $\text{cm}^{-1}$  to 650  $\text{cm}^{-1}$  and a resolution of 4  $\text{cm}^{-1}$  and each sample were scanning 32 times. FTIR spectroscopy was used to characterize structural changes between free peptide and microparticles consisted after encapsulation process.

#### *SEM Measurements of Microparticles*

The surface morphology of microparticles was evaluated by scanning electron microscopy (SEM, Zeiss, EVO-LS 10) as previously described [27]. Lyophilized microparticles were fixed on metallic studs and then coated with gold under vacuum. SEM photomicrographs were taken at an acceleration voltage of 5 kV.

#### *In vitro release*

*In vitro* peptide release was studied at pH 7.4 in phosphate buffer solution (PBS) according to our previous study [27, 28]. 10 mg of peptide loaded microparticles were dissolved in 5 ml (PBS)-0.01% sodium azide and the microparticles suspension was incubated in a shaking incubator (120 rpm) at 37 °C. At specified time intervals (1, 2, 3, 4 h; 1, 2, 3, 7, 10, 16, 24, 30, 33 days), medium was fully removed and fresh medium (PBS) was added. Released peptide amount in the supernatant was measured with fluorescence spectroscopy at 270 nm excitation and 325 nm emission wavelength and calculated by using previously constructed standard calibration curve.

## Results and Discussion

#### *Reaction Yield, Encapsulation Efficiency and Drug Loading of Microparticles*

In the present study, FMDV 40–60 synthetic peptide loaded PLGA microparticles was synthesized via single emulsion solvent evaporation methods by using different peptide amounts (5 mg  $\rightarrow$  FMMP1, 10 mg  $\rightarrow$  FMMP2 and 15 mg  $\rightarrow$  FMMP3). Produced microparticles were detailed characterized for RY, EE, DL using gravimetric method and fluorescence spectrophotometer, respectively. Obtained results were given in Table.

Additionally, the changes in microparticles properties depending on the initial peptide amount are given in Fig. 2. It can be seen that, the DL of the microparticles increases with increasing of initial peptide amount. Conversely, the reaction yield of the microparticles decreases by the increasing of initial peptide amount.

#### *Particle size and Zeta Potential Analysis of Microparticles*

Also, produced microparticles were analyzed Zeta-sizer for particle size, polydispersity index (PDI) and zeta potential. The size distributions of the produced microparticles were shown in Fig. 2 and both zeta potential and PDI values were summarized in Table. It can be concluded that all particles have particle size around micrometers. The size distributions are narrow and there is no agglomeration in the particles.

#### *Fourier transform infrared (FTIR) spectroscopy — FTIR Analysis of Microparticles*

Synthesized microparticles were analyzed by FTIR spectrophotometer in comparison with FMDV 40–60 peptide and PLGA polymer, obtained spectrums were shown in Fig. 3. In the FT-IR spectrums, PLGA molecule showed peaks such as carbonyl  $\text{C}=\text{O}$  stretching (1751  $\text{cm}^{-1}$  strong and narrow),  $\text{C}-\text{O}$  stretching (1087  $\text{cm}^{-1}$ ). Pure FMDV peptide (FM) sample showed amide  $\text{C}=\text{O}$  stretching (1660  $\text{cm}^{-1}$ ). When the obtained spectrums are compared, it is seen that the FT-IR spectrums of microparticles and PLGA have the same chemical characteristics. In the FTIR spectrums of FMMP1, FMMP2 and FMMP3 peptides loaded microparticles, the main peak of peptide at 1 660  $\text{cm}^{-1}$  were dramatically lowered and this situation indicates that FMDV 40–60 peptide was successfully loaded to PLGA microparticles.

**Microparticles properties including RY, EE, DL, particle size, zeta potential and PDI**

Formulation	Particle Size Z-ave ( $\mu\text{m}$ )	Zeta Potential (mV)	PDI	RY (%)	EE (%)	DL (%)
FMMP1	1.82 $\pm$ 0.078	-4.86 $\pm$ 0.13	0.458 $\pm$ 0.066	74.97	40.64	2.58
FMMP2	0.55 $\pm$ 0.012	+11.9 $\pm$ 0.56	0.403 $\pm$ 0.017	73.00	40.66	5.06
FMMP3	1.89 $\pm$ 0.010	+3.37 $\pm$ 0.54	0.505 $\pm$ 0.040	72.59	33.07	5.94

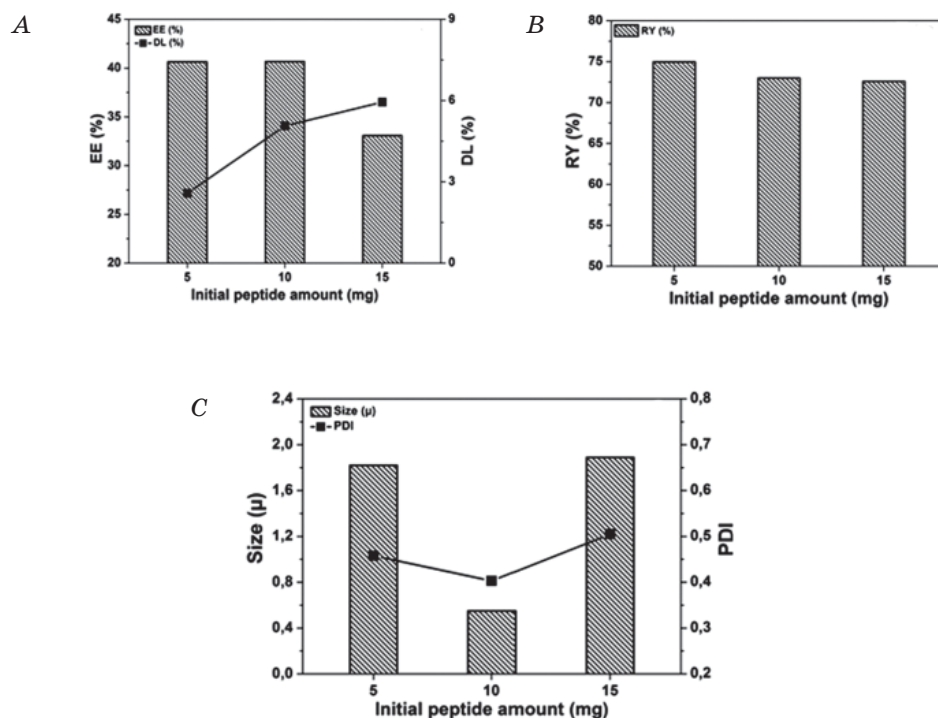


Fig. 1. A — effect of initial peptide amount on EE (bar) and DL (line); B — RY (bar); C — Z-ave of particle size (bar) and PDI (line)

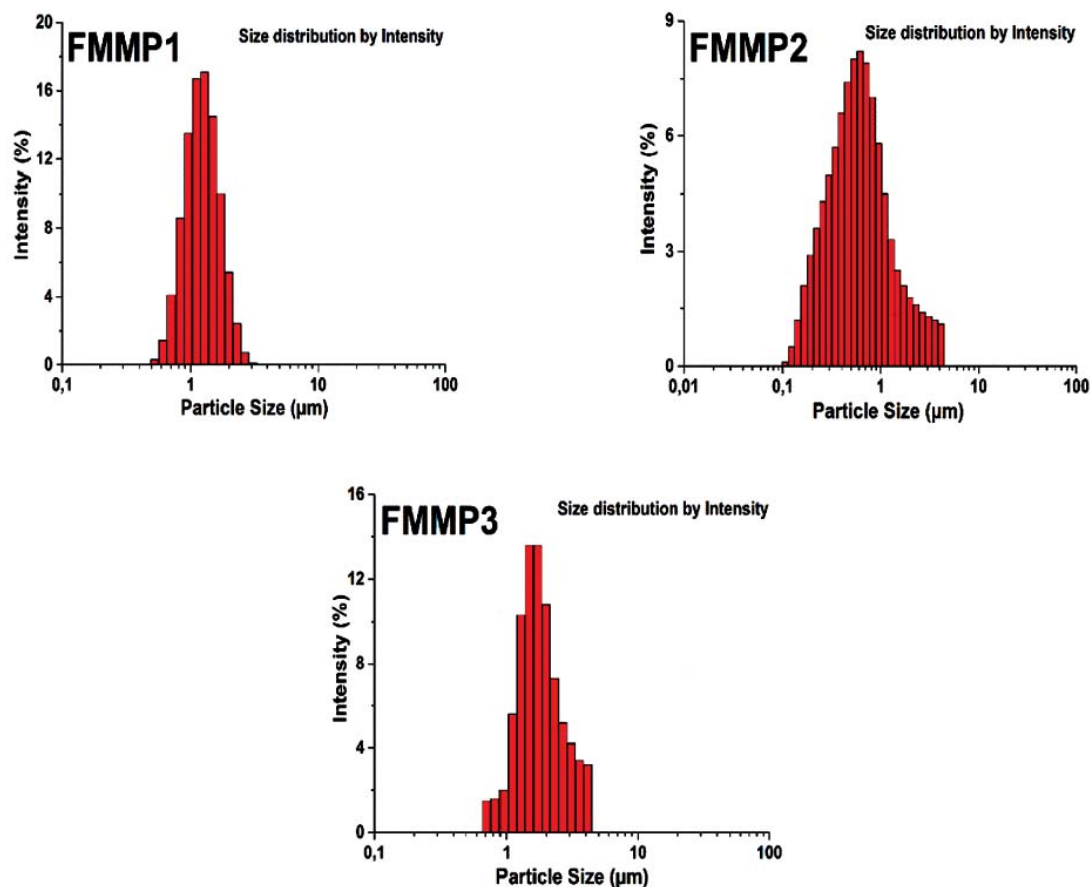


Fig. 2. Particle size distribution of FMMP1, FMMP2 and FMMP3, respectively

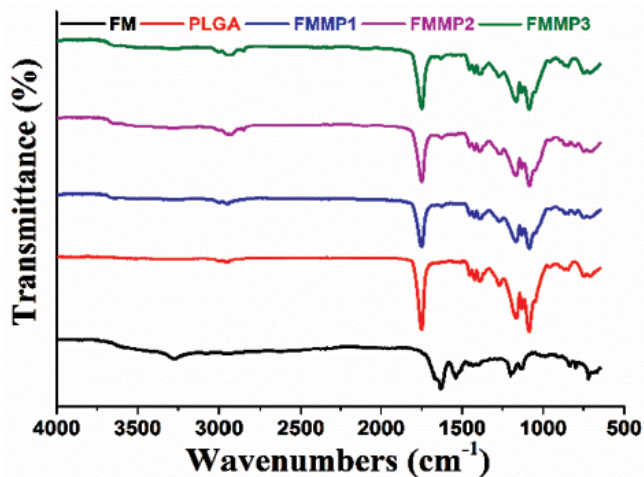


Fig. 3. FT-IR spectrums of FMDV peptide (FM), PLGA, FMMP1, FMMP2 and FMMP3

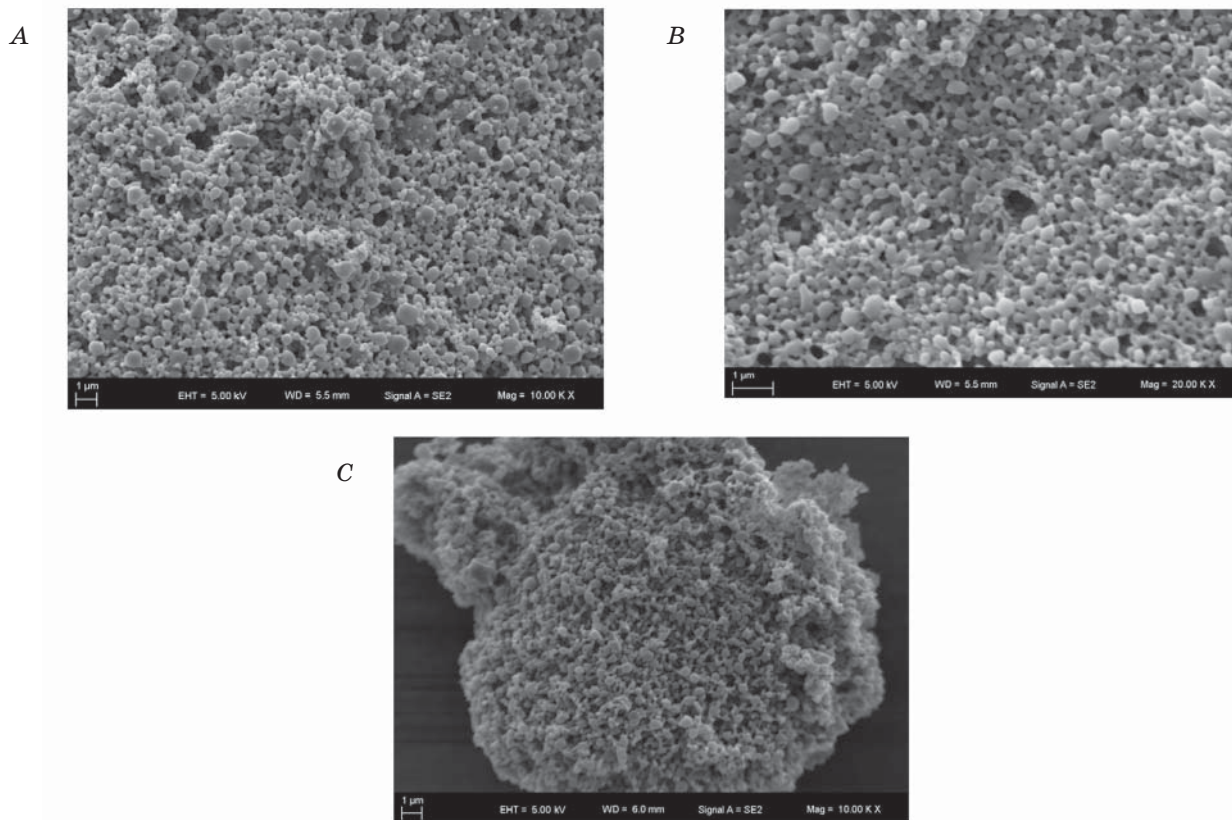


Fig. 4. A — SEM image of FMMP1 microparticles; B — SEM image of FMMP2 microparticles; C — SEM image of FMMP3 microparticles

#### SEM -Analysis of Microparticles

Microparticles synthesized using different amounts of peptides were analyzed morphologically by scanning electron microscopy (SEM). Obtained SEM images were shown in Fig. 4, A, B, C.

When the SEM images of the obtained microparticles are examined, it can be concluded that the microparticles are synthesized smooth/spherically and the size distributions of the particles are in a narrow range. Additionally, obtained SEM results were in agreements with Zeta-Sizer particle results.

*In vitro* Release of Microparticles

Fig. 5 illustrates the *in vitro* release pattern of FMDV 40–60 peptide from microparticles prepared with different initial peptide amounts. Obtained release pattern shows that controlled release of the peptide is observed for 33 days from the microparticles. First two days initial burst release was observed than slow peptide release occurred from microparticles in the subsequent period. The last part of the pattern peptide release continuous release almost close to linear was observed after the 15<sup>th</sup> days.

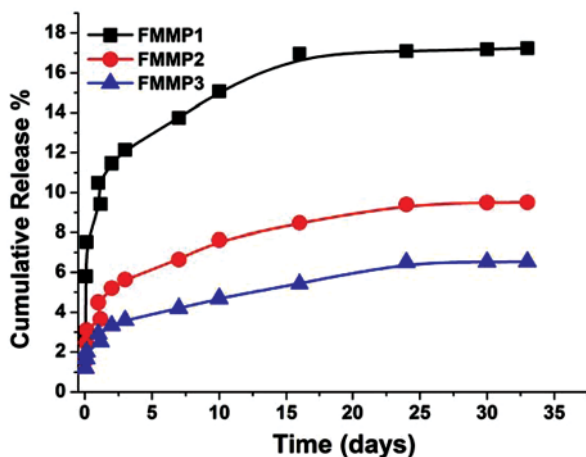


Fig. 5. *In vitro* release profile of FMDV 40–60 peptide from microparticles in phosphate buffer, pH 7.4

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**СИНТЕЗ І ХАРАКТЕРИСТИКА  
МИКРОЧАСТИНОК ПОЛІ(МОЛОЧНОЇ-  
КОГЛІКОЛЕВОЇ) КИСЛОТИ,  
НАВАНТАЖЕНИХ СИНТЕТИЧНИМ  
ПЕПТИДОМ ВІРУСУ ЯЩУРУ 40–60**

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Метою дослідження було синтезувати та охарактеризувати мікрочастинки полі(молочної-когліколевої кислоти), навантажені синтетичним пептидом вірусу ящуру 40–60. Для одержання мікрочастинок з трьома різними кількостями пептиду (5; 10 і 15 мг) було використано метод одноемulsionного випаровування. Отримані мікрочастинки було детально охарактеризовано за допомогою методів визначення виходу реакції, ефективності інкапсулювання, навантаження ліками, розміру частинок, коефіцієнту полідисперсності та зета-потенціалу.

**Ключові слова:** вірус ящуру, мікрочастинки полі(молочної-когліколевої кислоти).

**СИНТЕЗ И ХАРАКТЕРИСТИКА  
МИКРОЧАСТИЦ ПОЛІ(МОЛОЧНОЙ-  
КОГЛІКОЛЕВОЙ) КИСЛОТЫ,  
НАГРУЖЕННЫХ СИНТЕТИЧЕСКИМ  
ПЕПТИДОМ ВИРУСА ЯЩУРА 40-60**

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Цель исследования — синтезировать и охарактеризовать синтетический пептид вируса ящура 40–60, нагруженный микрочастицами поли(молочной-когліколевой кислоты). Для получения микрочастиц с тремя различными количествами пептида (5; 10 и 15 мг) был использован метод одноэмульсионного испарения. Полученные микрочастицы были подробно охарактеризованы с помощью методов определения выхода реакции, эффективности инкапсулирования, загрузки лекарствами, размера частичек, коэффициента полидисперсности и зета-потенциала.

**Ключевые слова:** вирус ящура, микрочастицы поли(молочной-когліколевой кислоты).