Production and investigation of resorbable micro- and nanoparticles using the solvent evaporation technique and spray-drying method

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СФУ- 22 апреля по 5 мая 2017
* Controlled drug delivery systems

Material + Drugs → Encapsulated drug

Concentrations of drug in the blood plasma:
a - free forms, b - controlled release of the drug

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The minimum therapeutic dose

The maximum tolerated dose

Therapeutic area

Concentrations of drug in the blood plasma:
a - free forms, b - controlled release of the drug

* Polymeric microparticles

Medicinal forms in the form of polymer particles loaded drug

Advantages:

- The biocompatibility of the polymer particles
- The biodegradation of the polymer particles
- Increased therapeutic effect of drug
- Reduce the negative impact of drug on the body

Regulation properties of the particles:

- **Particle size** (from 200 nm up to 10-20 microns) - affects the method of administration (intravenous, intramuscular, subcutaneous), drug release, degradation

- **Zeta potential** (indicator of stability of colloidal dispersions) - affects the distribution in the body

- **The surface of the particles** (porous, smooth) - affects the drug release, degradation
Nano-carriers based on polyesters of hydroxycarboxylic acids

P(LGA) – copolymers of lactic glycolic acid

P(MA) – malic acid polymers

P(HA) – polymers hydroxybutyric acid
Methods of preparation of polymeric microparticles:

**Emulsion method**

- **The average diameter of** 200 nm and above
- **The type and concentration of surfactant** (PVA, Tween, SDS)
- **Speed rate** (300 – 24000 rpm)
- **Chemical composition**
- **The concentration of the polymer solution**
### PHA microparticles of different chemical composition

<table>
<thead>
<tr>
<th>№</th>
<th>Sample</th>
<th>Mw, kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P3HB</td>
<td>1479</td>
</tr>
<tr>
<td>2</td>
<td>P(3HB-co-3HV) 6,5 mol.%</td>
<td>1700</td>
</tr>
<tr>
<td>3</td>
<td>P(3HB-co-3HV) 10,5 mol.%</td>
<td>1500</td>
</tr>
<tr>
<td>4</td>
<td>P(3HB-co-3HV) 20 mol.%</td>
<td>1500</td>
</tr>
<tr>
<td>5</td>
<td>P(3HB-co-3HV) 37 mol.%</td>
<td>2000</td>
</tr>
<tr>
<td>6</td>
<td>P(3HB-co-3HHx) 7 mol.%</td>
<td>440</td>
</tr>
<tr>
<td>7</td>
<td>P(3HB-co-4HB) 6,1 mol.%</td>
<td>410</td>
</tr>
<tr>
<td>8</td>
<td>P(3HB-co-4HB) 16 mol.%</td>
<td>970</td>
</tr>
</tbody>
</table>

SEM images of the microparticles prepared from PHAs of different chemical. The bar 5 µm

Mean diameter zeta-potential of the microparticles prepared from PHAs of different chemical compositions
* Preparation of drug-loaded microparticles

**Antibacterial drugs:**
- Ceftriaxone
- Rifampicin
- Vancomycin

**Antineoplastic drugs:**
- Paclitaxel
- Doxorubicin
- 5 fluorouracil

**Anti-inflammatory drugs:**
- Dexamethasone
- Diclofenac
- Meloxicam
Dynamics of drugs release from PHA microparticles
Methods of preparation of polymeric microparticles:

Mini Spray Dryer B-290, Buchi

Process parameters:
- Aspirator
- Inlet temperature
- Spray gas flow
- Feed rate
- Solid concentration

- High performance
- Automation of the production process

! The average diameter of 2 µm and above
The P3HB microparticles obtained by spray-drying

<table>
<thead>
<tr>
<th>Options</th>
<th>Feed rate, ml/min</th>
<th>Yeld, %</th>
<th>Mean diameter, nm</th>
<th>Zeta potential, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inlet temperature °C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>1,5</td>
<td>10,0</td>
<td>2077±62</td>
<td>-72,8±1,2</td>
</tr>
<tr>
<td></td>
<td>3,2</td>
<td>30,0</td>
<td>3161±59</td>
<td>-83,7±0,7</td>
</tr>
<tr>
<td></td>
<td>5,0</td>
<td>37,5</td>
<td>3129±211</td>
<td>-77,6±0,8</td>
</tr>
<tr>
<td>85</td>
<td>1,5</td>
<td>5,0</td>
<td>6416±616</td>
<td>-69,5±1,4</td>
</tr>
<tr>
<td></td>
<td>3,2</td>
<td>35,0</td>
<td>8604±330</td>
<td>-77,0±2,5</td>
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<tr>
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<td>5,0</td>
<td>42,5</td>
<td>4846±91</td>
<td>-66,6±0,7</td>
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<tr>
<td>95</td>
<td>1,5</td>
<td>60,0</td>
<td>3710±296</td>
<td>-68,7±0,5</td>
</tr>
<tr>
<td></td>
<td>3,2</td>
<td>63,5</td>
<td>6063±742</td>
<td>-81,6±1,6</td>
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<tr>
<td></td>
<td>5,0</td>
<td>63,8</td>
<td>6983±285</td>
<td>-92,7±4,1</td>
</tr>
</tbody>
</table>
## Characteristics of microparticles containing 5-fluorouracil obtained by spray-drying method

<table>
<thead>
<tr>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB)</td>
</tr>
<tr>
<td>P(3HB)-((1%\ 5\text{-fluorouracil}))</td>
</tr>
<tr>
<td>P(3HB)-((5%\ 5\text{-fluorouracil}))</td>
</tr>
<tr>
<td>P(3HB)-((10%\ 5\text{-fluorouracil}))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean diametr, mkm</th>
<th>Zeta-potential, mV</th>
<th>Encapsulation efficiency, %</th>
<th>Yeld, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,7±0,4</td>
<td>-102±2</td>
<td></td>
<td>71±5,6</td>
</tr>
<tr>
<td>2,6±0,1</td>
<td>-106±9,9</td>
<td>47,6±0,5</td>
<td>65±6,8</td>
</tr>
<tr>
<td>2,6±0,2</td>
<td>-83,4±0,8</td>
<td>56±0,6</td>
<td>67±7,6</td>
</tr>
<tr>
<td>2,5±0,1</td>
<td>-86±1,6</td>
<td>57±0,5</td>
<td>78±7,7</td>
</tr>
</tbody>
</table>

SEM images of the microparticles containing 5-fluorouracil obtained by spray-drying method. The bar 5 µm

Dynamics of 5 fluorouracil release from P3HB microparticles

![SEM images of microparticles](image1)

![Dynamics graph](image2)
Evaluation of cytotoxicity of microparticles of PHA of different compositions

Fibroblasts *NIH 3T3*

**[Picture of DAPI staining of fibroblast NIH 3T3 cells on microparticles of different types sterilized with autoclaving (a) and H$_2$O$_2$ plasma (b), 7 days after seeding:](a)**

(a) Autoclaving

(b) Plasma hydrogen peroxide sterilizer Sterad-NX 100
*Penetration of microparticles into L929 cells*

Fibroblasts *L929*

166 nm

426 nm

1.9 mkm

Pictures confocal microscopy and transmission P(3HB-co-3HV) particles with an average diameter of 166 nm, 426 nm and 1.9 microns, containing fluorescent dye Nile Red, in contact with the cells. Cytoskeleton (green, stained with FITC), b) the nanoparticles (containing Nile Red), a) applying (a) and (b), r) cell photomicrograph
The antiproliferative effect of microparticles with cytostatic drugs

Cells were stained using the Live / Dead, 24 h.

Effect of free and deposited in polymer microparticles of paclitaxel on the number of viable cells in culture HeLa: drug concentration - 0.6; 3.2; 6.0 mg / ml
The antiproliferative effect of microparticles with cytostatic drugs

The effect of free DOX concentration on the number of viable cells in HeLa cell (doxorubicin concentration) — 0.6, 3.2, 6 μg/ml

Effect of free and encapsulated 5-fluorouracil on the number of viable cells in culture HeLa: 1 - 0.6 mg P (3HB) - (1% 5-FU), 2 - 1.3 mg P (3HB) - (5% 5-FU), 3 - 6.5 mg P (3HB) - (10% 5-FU), 4 - 0.6 mg of 5-FU, 5 - 1.3 mg of 5-FU, 6 - 6 5 mg 5-FU, 7 - control.

The effect of DOX encapsulated in 0.2 μm (a) and 1.2 μm (b) polymer microparticles on the number of viable cells in HeLa
EXPERIMENTAL FORM ANTI-INFLAMMATORY DRUGS FOR EVALUATION ON ANIMALS

Simulation experimental chemical burns we conducted on laboratory mice (white mice Balb / c, weight 20-23 g)

Model of chemical burns to 8 days from the start of DCC applications: a) macroscopic view, b) microscopic view (hematoxylin-eosin stain, SW. × 100).

n - the zone of necrosis, i - area of inflammatory infiltration

«+» control
Diclofenac + dexamethasone free form (0,08 mg diclofenac и 0,02 mg dexamethasone)
Daily

PHB-Dicl+Dex
(0,08 mg diclofenac и 0,02 mg dexamethasone)
Four times in three days

«-» control
Without drug
The appearance of the skin of mice

Dynamics of changes in the wound area during therapy: traditional and new form of P3HB microparticles (diclofenac and dexamethasone).

Treatment with inflammatory drugs (Diclofenac and Dexamethasone) in the free form and loaded into microparticles of P3HB.

n - zone necrosis; a – acanthosis; ai - area of inflammatory infiltration; cx - greasy zhelezhy.

Dynamics of changes in the wound area during therapy traditional and new form of P3HB microparticles (diclofenac and dexamethasone)
Solid tumors were obtained by injection mouse Erlich solid carcinoma intramuscularly into off hind legs of BALB/c mice. Dox-PHA and a free Dox were injected intratumorally and intravenously on 7 days after injection cancer cells. Duration of experiment – 28 days.

The area of a tumor on a cut