# DEVELOPMENT OF THE TECHNOLOGY OF POLYMER FILMS CONTAINING IMMOBILIZED PROTEASE AND DIOXIDINE

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A water-soluble polymeric film (Prodioxilong pellicles) containing the proteolytic enzyme procelan and the broad-spectrum antimicrobial drug dioxidine has been created. This medicinal form ensures full bioaccessibility and exact dosage of the drugs, retains activity for a prolonged time, and is convenient to use for the treatment of deep suppurative necrotized wounds and purulent inoculations, in particular, in stomatology. Based on the results of biological evaluation of the duration of action and physicochemical properties of the new preparation, the shape and dimensions of the pellicles were selected, the composition and technology were optimized, and the performance characteristics were established. The pellicles were characterized by the appearance, dimensions, pH of the pellicle suspension, the size of procelan and dioxidine particles, the weight loss on drying, the average weight, proteolytic activity, dioxidine content, and the level of microbiological contamination. The polymeric pellicles are stable for twelve months when stored under normal conditions.

Immobilized protease (protosubtilin) preparation, called procelan, was created and the corresponding technology was developed at the Institute of Applied Enzymology (Ferment Corporation, Vilnius). This preparation was intended for the enzymatic purification of purulent wounds [1, 2] and initially produced for veterinary purposes in the form of a 20% suspension in isotonic sodium chloride solution. Preliminary results allowed procelan to be recommended for clinical testing [3]. Investigation of the therapeutic action of procelan showed that the drug possesses a pronounced enzymatic activity and is practically nontoxic. Such properties allow this compound to be used for the development of new medicinal forms [4]. Previously, it was reported that stable and convenient forms of procelan were created, including a lyophilized preparation [5], an ointment [6], and a combined preparation prodioxin [7]. The latter ointment contains procelan and dioxidine, an antibacterial compound with a broad spectrum of action [8].

This study was aimed at the development of water-soluble polymer films (pellicles), also containing procelan in combination with dioxidine, which would provide full bioaccessibility, ensure exact dosage of the drugs, and retain their activity for a prolonged time. Such ready-to-use medicinal form is convenient for the treatment of deep suppurative necrotized wounds and purulent inoculations, in particular, in stomatology.

In order to evaluate the effect of activity prolongation and estimate the physicochemical properties of polymeric pellicles, we have biologically tested polymeric bases of three types (I – III) prepared from the components allowed for use in medicine. Type I was based on a biodegradable copolymer of acrylamide, N-vinylpyrrolidone, and ethylacrylate (AVPE polymer) [9]. Previously, this polymer base was used for the development of films for anginal and ophthalmology purposes [10]. Type II was based on hydroxypropylmethyl cellulose (HPMC) [11], and type III, on the sodium salt of carboxymethylcellulose (Na-CMC). The compositions of type I – III polymeric bases are given in Table 1.

The biological tests were performed on rabbits with model purulent wounds induced by inoculating a virulent *Staphylococcus aureus* culture into a dentogingival recess cryogenically pretreated with a chloroethane stream. The dissolution of pure polymeric pellicles prepared using compositions I – III was studied on the animals with model wounds, which were divided into three groups (each of five rabbits). The samples of pellicles were introduced into the wounds and covered with sterile pads. Then, the state of each sample was examined every 30 min until complete dissolution. It was established that the pellicles made from a polymer composition I completely dissolved within 6 h, while the pellicles

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**TABLE 1.** Polymer Film Base Compositions

| Component | Base composition | | |
|---|---|---|---|
| | I | II | III |
| AVPE polymer | 10.25 | — | — |
| Cacao oil | 1.26 | — | — |
| OPMC | — | 5.00 | — |
| Macrogol-400 | — | 0.90 | — |
| Shellac | — | 0.16 | — |
| Na-CMC | — | — | 5.00 |
| Glycerol | — | — | 10.00 |
| Ethanol | 58.49 | 87.00 | — |
| Water | 30.00 | 6.94 | 85.00 |

**TABLE 2.** Comparative Therapeutic Efficacy of Polymeric Pellicles with Different Drug Compositions

| Preparation | Drug composition (g/pellicle) | | Healing stages (in days) * | | |
|---|---|---|---|---|---|
| | procelan content, g | dioxidine content, g | granulation | onset of epithelization | full epithelization |
| Formula 1 | 0.006 | 0.002 | 1.0 | 2.3 | 3.4 |
| Formula 2 | 0.003 | 0.001 | 1.2 | 2.7 | 3.8 |

* Average for $n = 5$.

of types II and III dissolved within 3.5 and 1.5 h, respectively. Thus, the slowest dissolution and, hence, the most prolonged release of potential drugs into the environment could be expected for polymeric pellicles based on composition I.

Other important parameters of the medicinal form under consideration are the shape and dimensions of the pellicles. These characteristics are related to the field of application and, in turn, determine the average polymer weight and, hence, the dose of drugs immobilized in the polymer matrix. Based on the results of preliminary investigations, involving pellicles of various shapes and dimensions, and the expert estimates of dentists, it was established that optimum pellicles must have an oval shape (without of acute angles), a length of 6 – 9 mm, a width of 3 – 5 mm, a thickness of 0.2 – 0.6 mm, and a weight of 10 – 30 mg.

The films were prepared from polymer solutions spread over a moving ribbon substrate of a casting machine. The introduction of ethanol into cast solutions increases the rate of solvent evaporation and, accordingly, decreases the time of film drying. The formation of a polymer film of homogeneous thickness is ensured by the output effectors, which provide uniform spreading of the solution flowing out of the injectors. The flow regime is determined by the plastic viscosity of each solution. The results of preliminary experiments showed that polymer solutions most convenient for the formation and subsequent processing are prepared using a 40% aqueous ethanol and must possess the optimum dynamic viscosity in the range 100 – 300 P, which corresponds to a polymer solution concentration of 7 – 13% [11].

In order to select the optimum concentrations of active components, we have prepared three series of polymeric pellicles weighing 0.025 g with the following contents: (1) 0.006 g of lyophilized procelan and 0.002 dioxidine; (2) 0.003 g of lyophilized procelan and 0.001 dioxidine; and (3) drug-free (blank) pellicles. The biological tests were performed on rabbits with model purulent wounds induced in the dentogingival recess as described above. The animals were divided into three groups (each containing five rabbits) and their wounds were treated daily by application of polymeric pellicles with a certain content of drugs covered with pads. The efficacy of the treatment was evaluated in terms of objective parameters characterizing the healing process, including a change in the wound area, the volume of purulent discharge, the intensity and character of inflammatory response in the wound and surrounding tissues, the time of the onset of granulation and epithelization, and the time to complete healing.

As can be seen from the experimental results summarized in Table 2, the use of polymeric pellicles with the given drug compositions significantly reduces the time required for healing the model purulent wounds. Indeed, the period of purification of the initially purulent wounds decreases on the average by two days, which favors rapid decay of the inflammation and leads to complete healing 5 days before that in the control group of spontaneous healing. The therapeutic efficacy of polymeric pellicles with both drug compositions is virtually identical. For this reason, proceeding from economic efficiency and therapeutic efficacy, formulation (2) was selected as the optimum.

Thus, we have developed polymeric pellicles containing immobilized drugs with the following composition: procelan lyophilized, 0.03 g; dioxidine, 0.001 g; cacao oil, 0.03; biodegradable AVPE polymer base, at an amount sufficient to obtain pellicles with an average weight of 0.025 g. In order to ensure more pronounced and prolonged action of the proposed medicinal form, the pellicles were fabricated using a four-layer technology. The mixture compositions of two types for a 100-g batch of polymeric pellicles are presented in Table 3.

Mixture No. 1 was prepared in a vessel equipped with a stirrer (operating at 86 rpm), which was charged with the required amount of the polymer base and a 96% ethanol and allowed to stand for 2 h to provide for polymer swelling. Then, lyophilized procelan (comminuted on a hammer mill to a particle size of 25 – 150 μm) and dioxidine (comminuted in a homogenizer to a particle size of 5 – 10 μm) were added with the necessary amount of distilled water. This mixture was stirred at room temperature until obtaining a homogeneous mass. This mass was filtered through a sieve with mesh size 0.2 – 0.5 mm under air pressure of 100 kPa, and then al-

**TABLE 3.** Bulk Compositions of Polymeric Pellicles (per 100 g)

| Component | Mixture 1 | Mixture 2 |
|---|---|---|
| Procelan lyophilized | 0.96 | 1.04 |
| Dioxidine | 0.32 | 0.34 |
| AVPE Polymer | 6.29 | 5.47 |
| Ethanol 96% | 26.0 | 29.8 |
| Cacao oil | — | 1.82 |
| Water | 14.5 | 13.44 |

lowed to stand for 15 h in order to provide for the complete removal of air bubbles.

Mixture No. 2 was prepared in a jacketed vessel equipped with a stirrer. The procedure was analogous to that used for preparing mixture No. 1, except that cacao oil in the form of an ethanol solution was added and the mixture was stirred at 35 ± 5°C.

The four-layer polymer plates were manufactured using a casting machine (Sanitas Company) with a maximum permissible temperature of 30°C in the drying chamber. Preliminarily, the machine was heated with a stream of warm air from a ventilator, and the gap between the working die edge and the ribbon was adjusted using calibration plates so as to obtain the desired polymer film thickness. Then, four layers were continuously applied by casting polymer solutions, comprising two layers of mixture No. 2 sandwiched between layers of mixture No. 1. The polymer films were dried with a stream of warm air from a heater set at 30°C. The aqueous-ethanol vapor was removed via exhaust ventilation system connected to the casting machine. The dry polymer film was separated from the substrate and cut into plates and stripes, from which the pellicles were made. The pellicles were packed into blisters (a package of 10 sticks) or into bottles (50 sticks) closed with polyethylene caps.

The polymeric pellicles appeared as thin, odorless light-yellow oval plates of homogeneous composition without mechanical inclusions. The plates had rounded ends and were 9.0 ± 0.5 mm long, 4.5 ± 0.3 mm wide, and 0.6 ± 0.1 mm thick. The dimensions were determined with the aid of a micrometer in an average sample set taken according to the sampling rules. A 5% aqueous polymer suspension was characterized by pH determined using a potentiometric technique [12]. The size of procelan and dioxidine particles was determined with the aid of a microscope [12]. We have also determined the water content (in a set of 40 films), proteolytic activity [5], content of dioxidine [7], average weight [12], and microbiological purity of the films [12].

For determining the maximum storage time, Prodioxilong pellicles were kept under standard natural conditions (temperature, 25 ± 2°C; relative humidity, 60 ± 5%). The samples were taken for investigation after storage for 3, 6, 9, and 12 months (the test is still continuing). The results of these preliminary investigations showed that polymeric pellicles with immobilized drugs retain their activity after one-year storage, since the proteolytic activity was unchanged and remained within the interval from 0.016 to 0.026 units and the quantitative content of dioxidine was constant (about 1 mg). The appearance of polymeric pellicles in the course of storage also remained unchanged.

Thus, the results of our investigation show that polymeric films (Prodioxilong pellicles) containing immobilized procelan and dioxidine can be used for the treatment of purulent wounds and are worthy of further medico-biological and pharmacological characterization.

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