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Tracking of the micro-structural changes of levonorgestrel-releasing intrauterine system by positron annihilation lifetime spectroscopy

Kálmán Patai[a], Virág Szente[b], Károly Süvegh[c], Romána Zelkó[b,*]

[a] Second Department of Obstetrics and Gynecology, Semmelweis University, Üllői Str. 78/a, H-1082 Budapest, Hungary
[b] University Pharmacy Department of Pharmacy Administration, Semmelweis University, Hőgyes Endre Str. 7-9, H-1092 Budapest, Hungary
[c] Laboratory of Nuclear Chemistry, Eötvös Loránd University, P.O. Box 32, H-1518 Budapest 112, Hungary

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ABSTRACT

The morphology and the micro-structural changes of levonorgestrel-releasing intrauterine systems (IUSs) were studied in relation to the duration of their application. The morphology of the removed IUSs was examined without pre-treatment by scanning electron microscopy. The micro-structural changes of the different layers of IUSs were tracked by positron annihilation lifetime spectroscopy. Besides the previously found incrustation formation, the free volume of the hormone containing reservoir was remarkably increased after 3 years of application, thus increasing the real volume of the core of the systems. Although the free volume of the membrane encasing the core was not significantly changed in the course of the application, as a result of the core expansion, microcracks could be formed on the membrane surface. Along these cracks, deposits of different compositions can be formed, causing inflammatory complications and influencing the drug release of IUSs.

Stability tests in combination with micro-structural screening of such IUSs could be required during their development phase to avoid the undesired side effects.

1. Introduction

The levonorgestrel-releasing intrauterine systems (LNG-IUSs) are small plastic T-shaped systems with a hormone containing reservoir which sits inside the womb and releases tiny doses of the contraceptive agent directly into the womb lining, thus decreasing the systemic hormone exposition [1,2].

When choosing a controlled release system, the important aspects include the ease of manufacturing, the reliability and the stability of the release rate during long storage periods. Among the excipients of these systems, the amorphous polymers are frequently used in different dosage forms. Most of these applications require long-term stability, but as amorphous polymers are not in equilibrium below their glass transition temperature, these polymers usually undergo spontaneous, however slow, transformations towards low-energy equilibrium states. This so-called physical ageing is usually manifested also in volume and enthalpy relaxation [3,4]. Poly(dimethylsiloxane) (PDMS) is one of the most valuable polymers for use in biomedical devices. PDMS has flexibility, high gas permeability, processability, flexible surface chemistry, and optical transparency. However, due to the hydrophobicity of the surface, the interface of the PDMS surface with the biological environment is inadequate and non-specific biofouling, i.e., protein adsorption and cell adhesion frequently occurs on the surface. Biofouling induces contamination, inflammation, infection, and the reduction of material function [5,6].

A specific, *in utero* calcification process – incrustation on the surface of intrauterine devices – was discovered in the early 1980s. Since then, several aspects of incrustation, like scanning electron microscopic morphological characterization of incrustments, the time-dependence of incrustation on the surface of devices and the related composition and medical consequences, have been extensively studied. Incrustation formation significantly increases the chance of side effects (abdominal complaints, bacterial and fungous infections, inflammatory complications) and is related to the shape and chemical composition of the calcified deposits [7,8]. Since the most important feature of a drug releasing system is its continuous, near zero-order release property, the long-term stability of the free volume plays a very significant role determining the use of the device. Thus, controlling free volume changes in such systems is an important point. Positron annihilation lifetime spectroscopy, used routinely for characterizing the free volume in polymers, may be an adequate tool for this purpose.

The purpose of the present work was to illustrate the morphological and micro-structural changes of different parts of IUSs as a

* Corresponding author. Tel.: +36 1 2170927; fax: +36 1 2170927.
E-mail address: zelrom@gytk.sote.hu (R. Zelkó).

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function of the duration of application and their consequences on the safe and effective applicability of the system.

2. Materials and methods

T-LNG-IUS, Mirena® (Schering AG, Berlin, Germany), T-type IUSs containing levonorgestrel were examined after having been in place for up to 5 years. It has a 32 mm long T-shaped plastic frame with a reservoir on the vertical stem of the intrauterine system containing 52 mg of levonorgestrel mixed with polydimethylsiloxane. This allows a steady, local release of 20 μg levonorgestrel per day through the rate-limiting surface membrane with a near zero-order release rate [9] over a period of 5 years [10]. The device comprises a core containing levonorgestrel, and a membrane encasing the core wherein the membrane is made of an elastomer. The elastomer is a siloxane-based polymer comprising 3,3,3-trifluoropropyl groups attached to the Si-atoms of the siloxa units, and the release rate of levonorgestrel of the delivery device is regulated by the amount of 3,3,3-trifluoropropyl groups. IUSs were removed because of abdominal complaints and suspected inflammatory complications to the patients after 3 and 5 years of application. The morphology and the related free volume changes and composition of these samples were examined without pretreatment.

2.1. Scanning electron microscopy

The surface characteristics of sterile IUS and those applied for 3 and 5 years were examined, without mounting, by means of a scanning electron microscope (Philips XL 30, The Netherlands). Examination was carried out at 12 kV and 20 kV accelerating voltage and 25–1500 times magnifications were used. Magnifications of scanning electron microscopic images are measured by the micrometer-line on the pictures; the accuracy of these magnifications was ±2%.

2.2. Positron annihilation lifetime spectroscopy

Positron annihilation lifetime spectroscopy (PALS) injects positrons into materials and study their faith to gain information on the structure of the sample [11]. In polymers, a large fraction of positrons form positronium (Ps), a hydrogen-like exotic atom with the electrons of the substance. These Ps-atoms live several nanoseconds longer than free positrons. Their lifetime reacts to the changes of the free volume very sensitively. A Ps-atom, as any other atom, sits into the "empty" holes in the material. In polymers, these places trapping Ps are the spaces between polymeric chains. As these free volume holes are "empty" the trapped Ps can only annihilate with electrons at the "walls" of the hole. The larger a free volume hole in the material, the longer the lifetime. There is a monotonous correlation between the hole radius and the lifetime of Ps-atoms [11].

The void size a positronium is able to scan is several angströms in diameter, i.e., it reacts to changes occurring at the molecular scale. Since Ps scans the free volume and not the atoms, it is a unique method.

To study the free volume changes in LNG-IUS samples, a conventional fast–fast coincidence system was used. It consisted of BaF_2 based detectors, ORTEC electronics and a Nucleus multichannel analyzer card. The time resolution of the system was around 250 ps. Spectra were recorded in 4096 channels, each channel representing 9.6 ps.

The layers of LNG-IUSs were separated for the measurements. Three independent measurements were performed on every layer. The data given below are the averages of these three independent measurements. Also, the error bars given in Fig. 1 are the standard deviations calculated from these parallel measurements.

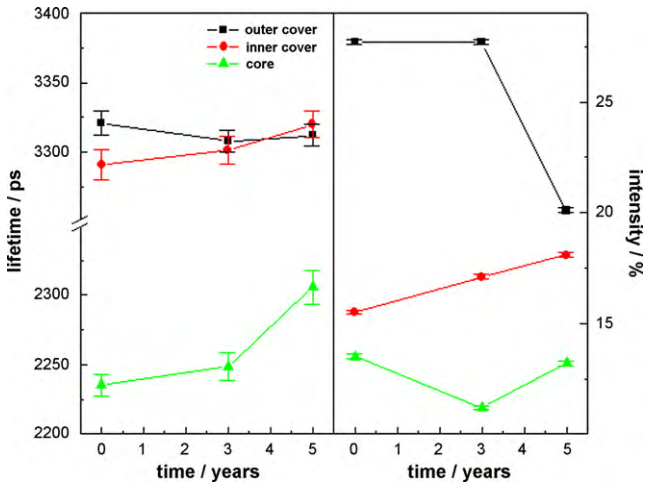

Fig. 1. o-Ps lifetime values of different parts of IUS as a function of the duration of application. The lines are only drawn to guide the eye.

Each spectrum was evaluated assuming that positrons form three different states after they have been injected into the material: free positrons and the two ground states of positronium. From the two latter ones the longest living, the ortho-positronium (o-Ps) is used to study the free volume. Although the two shorter lifetimes varied similarly to the o-Ps lifetime discussed below, the interpretation of these lifetime components is very hard. Thus, we give only their ranges here: the shortest lifetime varied between 175 and 205 ps and the medium one between 400 and 510 ps in the three layers of the studied LNG-IUSs.

3. Results and discussion

The electromicrograph of the cross-section of an IUS indicates the different layers of the system (Fig. 2). The PDMS core containing levonorgestrel and the two layers of the siloxane-based membrane are shown clearly. The core is separated from the membrane by a well-expressed crack. On the other hand, the two layers of the membrane, having very similar constitutions, look to be two different regions of the same material showing a 'continuous' interface between the two layers. This is confirmed by physical examinations of the IUS. The core can be separated from the membrane easily, while the two layers of the membrane remain

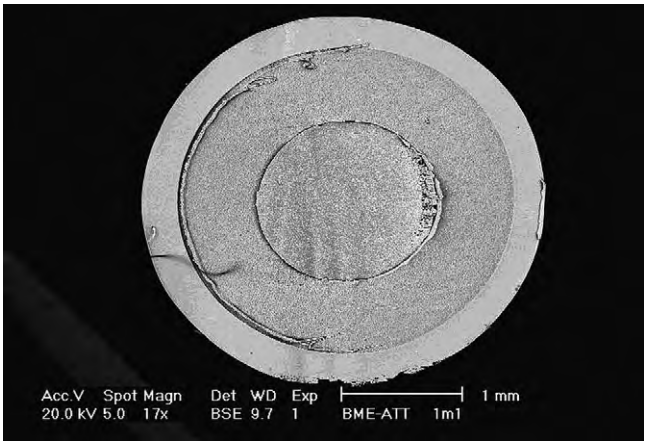

Fig. 2. The cross-section of a sterile IUS, magnification: 17×.

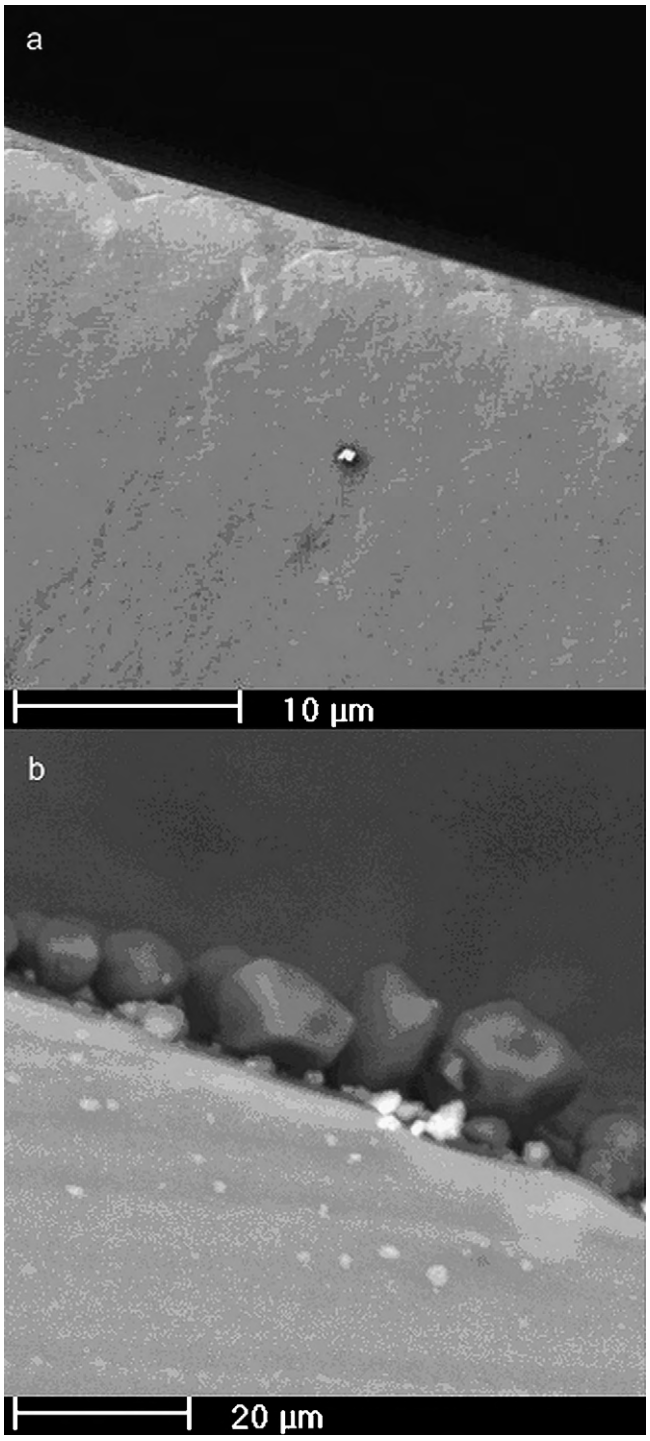

Fig. 3. The surface of a sterile IUS (a) and the characteristic incrustment layer on the surface of the membrane after 3 years (b) of application. Magnification: 1500×.

inseparable even after a 5-year use. The electromicrographs of the membrane surface controlling the drug release (Fig. 3) illustrate the changes of the surface of the IUS during application. As a negative control, the image of an IUS prior to insertion presents a clear surface (Fig. 3a). It is revealed by these photos that, after 3 years of application, the device becomes covered by crystalline deposits (Fig. 3b).

The observed incrustation might not cause sudden dose dumping but the deposit could inhibit further levonorgestrel diffusion (0.8 μg/h) through the rate-controlling membrane. This might reduce the hormonal effect of the IUS. Not only the possible incrustation of levonorgestrel, but also the decrease of the concentration gradient in the course of application could result ineffective hormone release, thus risking the safe application.

According to PALS spectra, the incrustment affects even the positronium formation in the membrane. Although the size of free volume holes has been surprisingly stable for 5 years (Fig. 1), positronium formation intensity dropped significantly after 3 years. The stable, very long positronium lifetime indicates large (about 11 Å in diameter) free volume holes in both the inner and the outer layers of the membrane. Moreover, even if the two layers look different even by the naked eye, o-Ps lifetimes indicate very similar structures. At least, the average sizes of free volume holes are the same for the whole period in the two layers (Fig. 1). On the other hand, the outer layer of the membrane, on which the incrustment is formed, loses its stability after 3 years of use. Something on (or in) this layer inhibits positronium formation in the 5-year old sample. This 'inhibitor' might well be the formed incrustment.

Another indication of instability can be found if we consider o-Ps lifetime in the core of the IUS. The o-Ps lifetime indicates much smaller holes than for the membrane (9.6 Å in diameter) and this is stable only for 3 years. In the sample having been applied for 5 years, the lifetime is increased significantly indicating a larger space for o-Ps to live in.

There are two possibilities to explain such an increase in the lifetime. The first is that the core loses its levonorgestrel content after 3 years and the free volumes in the core are emptied. Considering the results of former measurements on the drug releasing rate of IUSs, this is almost impossible. As it was shown, the IUS still have enough levonorgestrel to maintain a steady release even after 5 years. (Note that the releasing rate is controlled by the membrane which is stable. However, an almost constant levonorgestrel concentration in the core is equally significant to form the steady release.) The other possibility is the increase of the size of free volume holes, i.e., the physical ageing of the core material.

The increase of the free volume might have serious consequences on the applicability of IUSs. Since the free volume of the rate-controlling membrane did not change (Fig. 1), the increase of the free volume, and consequently the expansion of the inner core, could induce microcracks in the rate-controlling membrane [12]. Along these cracks, deposits of different compositions (depending on the biologic variability of the patients) can be formed, which then cause inflammatory complications because of the hollow, uneven coating serving as a hiding place for pathogens. The deposits might also influence the drug release of IUSs.

To sum up our results, sensitive monitoring of the free volumes of different parts of IUSs in the course of their real-time stability studies is of great impact from the point of their safe and effective long-term application. Positron annihilation lifetime method can be recommended as sensitive means for the stability tests of IUSs during the preformulation.

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