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Reduction of the "burst release" of copper ions from copper-based intrauterine devices by organic inhibitors

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Abstract

Background: The copper intrauterine device is a contraceptive method that is based on the release of copper ions from a copper wire. Immediately after insertion, the dissolution of copper in the uterine fluid is markedly higher ("burst release") than that necessary for contraception action, leading to a variety of harmful effects.

Study Design: Pretreatments with organic compounds [thiourea (TU) and purine (PU), 10^{-4}–10^{-2} M concentration range, 1- and 3-h immersion times] were tested. The dissolution of copper with and without pretreatments in TU and PU solutions was analyzed by conventional electrochemical techniques and surface analysis.

Results: Pretreatments in PU solutions reduced the initial corrosion rate of copper in simulated uterine solutions, with inhibitory efficiencies that depend on the PU concentration and on the immersion time assayed. Inhibitory efficiency values higher than 98% for pretreatments with $\geq 10^{-3}$ M PU were found. Conversely, after TU pretreatments, a high copper release was measured.

Conclusions: It was concluded that 10^{-3} M PU pretreatment is a promising strategy able to reduce the "burst release" of copper and to ensure contraceptive action.

Keywords: IUD; Metal ion release; Corrosion; Adsorption; Metal surface treatment; Copper

1. Introduction

The intrauterine device (IUD) is used by approximately 162 million women worldwide [1], being the most common form of reversible contraception [2–9] that is frequently provided free in developing countries. Its contraceptive action is mainly based on the release of copper ions.

Most of Cu-IUDs have a plastic T-shaped frame that is wound around with pure electrolytic copper wire (Fig. 1) and/or has copper collars (sleeves). Copper ions released from an IUD enhance the inflammatory response and reach concentrations in the luminal fluids of the genital tract that are toxic for spermatozoa [9,10].

The local effect of IUDs on the endometrium depends on the rate of release of copper ions and on the area of contact between the device and the endometrium. The tissues around the IUD undergo changes due to sterile inflammation or by the presence of a foreign body. A local effect of copper ions is also found in serum, cervical mucus and endometrium. Overexposure to copper ions may produce a variety of effects on the surrounding cells, including pyknotic changes in endometrial cells [11–15].

The copper corrosion rate depends on several factors like amino acids concentration, oxygen level, and quality, shape and size of the copper surface, among others [16]. Both, in vivo and in vitro studies of the liberation of copper ions from IUD revealed that copper dissolution decreases exponentially over time. However, dissimilar amounts of released copper in vivo were reported by different authors since several areas of copper and different exposure periods were assayed. Patai et al. [12] reported the average values of copper ions release in utero, during

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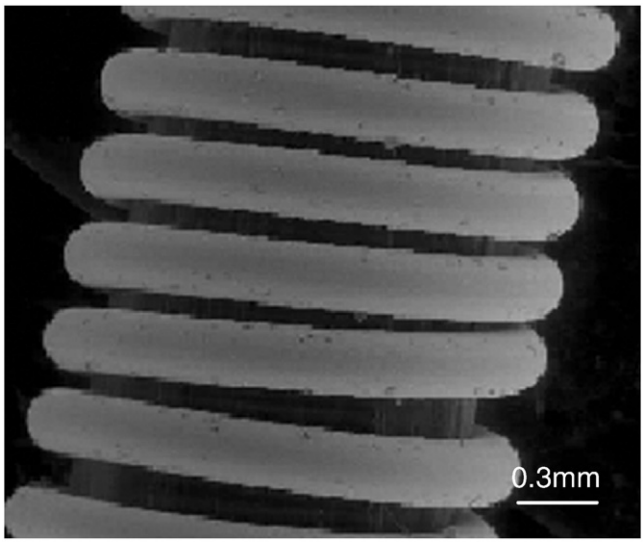


Fig. 1. SEM image of the Cu wire of the UID.

the first month after insertion, between 100.8 and 296.1 mcg/day decreasing drastically after 3 to 5 months (10.08–39.69 mcg/day). However, the area of the copper surface was not stated in their studies. Other authors [17,18] measured 44.4 and 73.6 mcg/day in utero for 200- and 400-mm^2 IUD samples after the first month. Kjær et al. [4] reported for a TCu200 IUD an average value of copper ions release of 82.6 ±6.4 mcg/day and observed the reduction of copper release with time. Thiery et al. [19] analyzed 118 Copper T22OC devices removed from users and reported average values of copper release in utero between 7.3 and 62.1 mcg/day for periods in the range 12 to 72 months. Unfortunately, data of copper released in utero after the first week following the IUD insertion could not be found in the literature.

In vitro studies of Gao et al. [20] with TCu 380A IUD showed a high amount of copper liberated into simulated uterine fluid (SUF) during the first 3 days after dipping the metal into the solution (75 mcg). Cao et al. [21] also reported that the highest corrosion rates of the TCu380A IUD and TCu220C IUD in SUF were obtained after the first and third days of immersion in this solution, respectively. However, a dramatic reduction in the copper dissolution was observed after 9 days, indicating that the "burst release" effect mainly occurs during the first week after insertion. On the other hand, it was reported that in the stable phase, the average levels of copper ion release of TCu 380A and MCu 375 in SUF were low (4.25±2.71–7.62±6.42 and 4.92±1.23–8.62±3.08 mcg/day, respectively). Nevertheless, copper amounts as low as 2 mcg/day (measured for IUD with indomethacin [22]) have proved to be enough to guarantee the contraception action.

The in vivo situation is even more complex than in vitro, since the concentration of copper in the uterus also varies with the distance from the device (the higher values are close to the IUD surface) [23]. Previous in vitro studies from our laboratory showed cytotoxic and genotoxic effects of copper ions at concentrations close to 11 mcg/mL [14,15]. Consequently, it may be speculated that cytotoxic and genotoxic effects could be found in vivo during the first week after insertion.

Based on these findings, study of the biodegradation of copper and the reduction of the corrosion rate together with the initial "burst release" effect in the uterine fluid is crucial since it is related not only with the IUD effectiveness but also to its harmful effects. Complaints reported during the follow-up period were mostly related to uterine bleeding, and it has been stated as the most frequent side effects of IUD [1,9,24–26]. Abdominal pain, particularly during menstruation, was also described [23]. Statistical data reported by Yu et al. [26] showed that pelvic pain, intermenstrual spotting and excessive bleeding decrease markedly over time mainly after the first month following the insertion. Copper burst release during this initial period has been hypothesized to account for pain and bleeding symptoms during the early moths after insertion [1,27].

Considering that the decrease of copper release may be a way of reducing side effects, the use of organic corrosion inhibitors is proposed here as strategy for controlling the copper dissolution within the initial period after the insertion of the IUD. In a previous work [28], purine (PU) (Fig. 2) has been proposed for industrial use as a friendly corrosion inhibitor for the environment since it is biodegradable. Two of the four ribonucleotides and two of the four deoxyribonucleotides, which are the respective building blocks of RNA and DNA, are PUs. Thus, PU is probably nontoxic to the body and, importantly, can be easily obtained at high purity (greater than 99%). It was demonstrated that PU is a good inhibitor of copper corrosion in 1 M NaCl solutions at pH 6.8 acting as a cathode and anode inhibitor [28]. In acidic $NaNO_3$ media, PU also proved to be effective [29].

Thiourea (TU) is an organosulfur compound (Fig. 2) that is effective to reduce corrosion in acidic solutions [30–32]. The inhibitory capacity in these media would be related to the formation of a protective layer. Although less biocompatible than PU, TU has the advantage of protecting against protein oxidation mediated by copper through the formation of complexes with the metal [33].

The aim of this research work is to evaluate the efficacy of TU and PU to inhibit the initial corrosion of IUD copper wires in SUF. The pretreatment should be able to reduce the initial high release of copper from the IUD without affecting

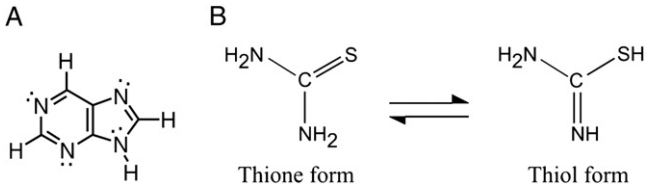


Fig. 2. Molecular representation of PU (A) and TU (B).

the contraceptive action. In addition, it should be easily handled and inexpensive, due to the widespread use of IUDs.

2. Materials and methods

2.1. Simulated uterine fluid

The degradation of copper with PU and TU as corrosion inhibitors has been previously investigated in electrolyte media completely different from the uterine fluid. Thus, it is necessary to test the effect of this pretreatment, consisting of immersion in PU and TU solutions, on the dissolution of copper in SUF. This SUF has been widely used and consists mainly of chlorides, phosphates and bicarbonates (Table 1) [16,34,35].

Considering that the pH of the uterine fluid is in the 6.0–8.0 range, tests were conducted in SUF at pH 6.0, which represents the condition with the higher copper release [36]. It was prepared with analytical grade chemicals and bidistilled water. This solution, formulated by Zhang et al. [36], is widely accepted as a suitable medium to simulate the uterine fluid [16,20,34,35]. All measurements were made at 37.0±0.1°C.

Copper ions released from copper in SUF after different exposure times were evaluated by two different methods: electrochemical tests and measurements of copper ions concentration in SUF by colorimetric method and by flame atomic absorption spectrometry.

2.2. Electrochemical tests and microscopic observations

Electrochemical tests widely used to investigate the degradation of copper [32,34,35] were used. The electrochemical experimental setup and the mechanical pretreatment of the copper electrodes are similar to that used in a previous work [16]. Copper electrodes (99.99% electrolytic metal copper; Merck) were used as disks (0.785 cm^2). The potential values in the text are referred to a saturated calomel electrode (SCE). The polarization curves were carried out in triplicate, and the results were repetitive. Solutions of NaCl 5 g/L containing either TU or PU in the 1×10^{-2}–1×10^{-4} M concentration range were used to pretreat the electrodes by dipping into the solution for 1 and 3 h. Inspections of the electrode surface before and after each experiment were made by optical microscopy (Olympus BX51; Olympus Corp., Tokyo, Japan).

Polarization curves were performed at 1 mV s^{-1} from the open-circuit potential to 0.0 V, and potentiostatic current transients were measured at –0.1 V. In all cases, a Radiometer potentiostat PGP201 was used.

To quantify the effectiveness of the corrosion inhibition, the dissolution rate of copper with and without the inhibitor was evaluated, and the inhibition efficiency (IE) was calculated according to Grillo et al. [37]

$$\mathrm{IE\%} = \frac{J_c - J_{inh}}{J_c} \times 100$$

where J_c is the current measured in the voltammogram at −0.1 V (close to the open-circuit potential) and corresponds to the control, and J_{inh} is the current measured at the same conditions for electrodes with the inhibition pretreatment. The lower values of J_{inh} at −0.1 V with respect to the control and, consequently, the high values of IE%, are related to low copper dissolution (inhibition of the burst release).

2.3. Copper concentration measurements and surface analysis

Copper wires of 0.3-mm diameter and 200-mm length (area 188 mm^2), similar to those used in IUD devices, were used for these experiments. New identical wires were used in each assay. In order to obtain reproducible and free of copper oxide surfaces, the wires were cleaned by immersion in H_2SO_4 5% for 1 min before each measurement [38]. Subsequently, samples were pretreated with PU or TU as described above and immersed in SUF during exposure periods between 1 and 14 days.

After the selected exposure time, 0.75-mL aliquots of the solutions were mixed with identical volumes of NH_3 to measure copper ions concentration by spectrophotometric analysis. Then, the UV-vis spectra were obtained, and absorbance at 640 nm was measured (Perkin-Elmer, Cambridge Technology, Inc., Cambridge, MA, USA; spectrophotometer model UV-160 UV-Vis, equipped with a 1-cm quartz cell) [39].

On the other hand, flame atomic absorption spectrometer (Shimadzu AA-6650, Kyoto, Japan) was also used for the determination of copper released from the wires with and without treatment with PU. Hallow cathode lamps were used as radiation sources. Samples were filtered by membrane (0.45-μm pore size). The concentration of the remaining soluble copper was determined. The solids on the membranes were dissolved with concentrated nitric acid, and the insoluble copper concentration was measured. Copper wires of identical size and cleaned in the same way as the others used for colorimetric analysis were used.

Fourier transform infrared (FTIR) spectra were performed in a Varian 660 spectrometer equipped with an attenuated total reflection (ATR) accessory (MIRacle ATR, Pike Technologies) with a ZnSe prism. Control spectra of PU

Table 1
Composition of the SUF (g/L)

Composition (g/L)	$NaHCO_3$	$NaH_2PO_4.2H_2O$	$CaCl_2$	KCl	NaCl	Glucose	Urea
	0.25	0.072	0.167	0.224	4.97	0.50	0.48

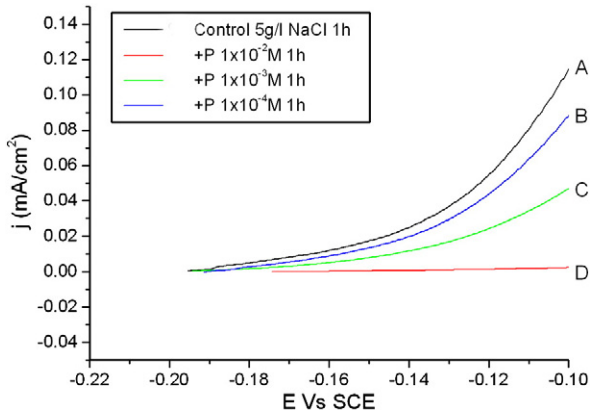


Fig. 3. Polarization curves at 1 mV s^{-1} recorded with pretreated copper electrodes in simulated uterine fluid. Pretreatment (1 h of immersion time) in solutions: (A) control: 5 g/L NaCl; (B) control + 1×10^{-4} M PU; (C) control + 1×10^{-3} M PU; (D) control + 1×10^{-2} M PU.

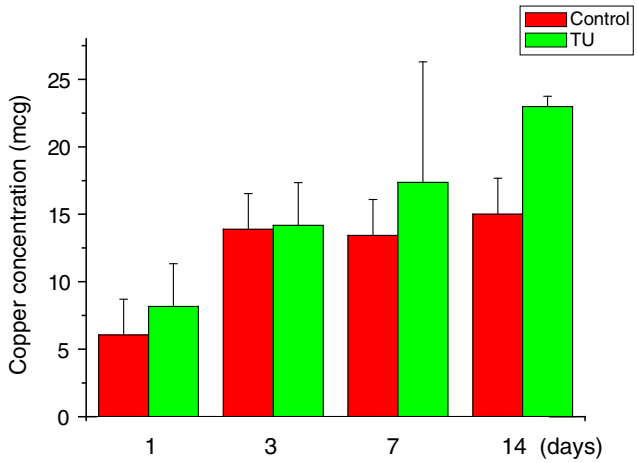


Fig. 4. Dissolution of copper wires with and without pretreatment with 10^{-2} M TU (1-h immersion period).

were carried out in transmittance mode by using KBr pellets. Samples for FTIR–ATR measurements were prepared by immersing a copper disk in a 1×10^{-3} M dissolution of PU for 24 h. In all cases, each spectrum results from 256 scans, taken with a resolution of 4 cm^{-1}.

3. Results

3.1. Electrochemical tests

The electrochemical measurements were performed with electrodes pretreated by immersion in 5 g/L NaCl solution with PU concentrations between 1×10^{-2} and 1×10^{-4} M. Copper samples pretreated in 5 g/L NaCl in the absence of the organic inhibitor were used as controls. Voltammograms were subsequently recorded in the potential range between the open-circuit potential (close to −0.2 V) and 0.0 V in SUF (Fig. 3). In Fig. 3 the vertical line at −0.1 V indicates the current values used for the IE calculation. IE data are reported in Table 2. This table shows that for 1-h exposure period, the most efficient behavior corresponds to 1×10^{-2} M solutions, since an IE=98.10%, at −0.1 V, was detected (Fig. 3 and Table 2). Markedly higher current values were recorded for solutions 1×10^{-3} and 1×10^{-4} M (IE: 61.20% and 23.27%, respectively).

The effectiveness of treatments with PU was also examined by potentiostatic techniques monitoring the change of current with time at −0.1 V (not shown). In agreement with the polarization curves (Fig. 3), potentiostatic results show that pretreatments with PU 1×10^{-2} and 1×10^{-3} M are more effective inhibition strategies than that with lower concentrations. It can also be noticed that after 1-h exposure, the current increases very slowly with time indicating that the IE is slowly lost.

In addition, experiments with copper electrodes pretreated in PU solutions during 3 h were made, in order to test the influence of the dipping time. In this case, IE=99.18% and IE=98.61% were obtained for 1×10^{-2} and 1×10^{-3}M solutions, respectively, indicating a better performance than that obtained for 1-h pretreatment (Table 2). Again, potentiostatic measurements at −0.1 V were in agreement with voltamperometric measurements.

Table 2
Current density measured at −0.1 V and IE (%) for each pretreatment

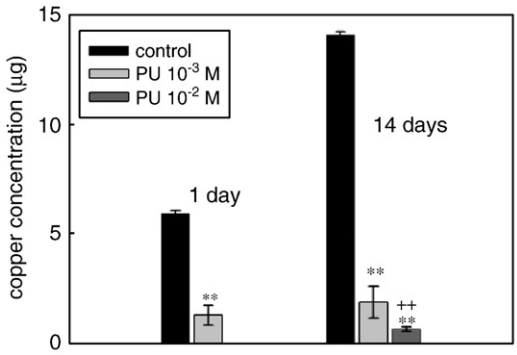
Inhibitor	Concentration	Immersion period of the pretreatment			
		1 h		3 h	
		j (mA/cm^2)	Efficiency IE (%)	j (mA/cm^2)	Efficiency IE (%)
Control without inhibitor	–	0.116		0.123	
PU	1×10^{-2}M	0.0022	98.10	0.0010	99.18
	1×10^{-3}M	0.045	61.20	0.0017	98.61
	1×10^{-4}M	0.089	23.27	0.0989	19.59
TU	1×10^{-2}M	0.059	49.13	0.054	53.44
	1×10^{-3}M	0.0198	82.93	–	–
	1×10^{-4}M	0.080	31.03	–	–

As a second alternative, we evaluated the effect of TU as a weak inhibitor of copper corrosion. Once more, we evaluated both the concentration and time of exposure to inhibitor. Voltammograms recorded after different pretreatments show that the immersion of copper wires in 1×10^{-3} M TU solutions for 1 h is the most effective treatment, since an IE=82.93% (Table 2) was calculated from the current value measured at −0.1 V. Surprisingly, lower efficiencies were found for 1×10^{-2} M after 3-h immersion time.

3.2. Copper concentration measurements and microscopic observations

Fig. 4 shows the amount of copper ions released by copper wires (similar to those used in IUDs) after 1-h immersion time in 10^{-2} M TU solution. It can be seen that the concentration of copper ions released from TU-pretreated wires is similar or higher than that obtained for the control. Pretreatments with TU concentrations lower than 10^{-2} M for 3 h also show that the amount of dissolved copper is close to that measured for the control (i.e., 5.56±4.01, mcg/mL, 10^{-3} M TU, 1 day).

Electrochemical tests showed that the inhibitory action of 10^{-2} M PU is very effective with IE close to 99.18%. This inhibition may be too high to guarantee the minimal copper concentration necessary for good contraceptive action. In fact, a cumulative concentration of dissolved copper of only 0.634 mcg was found after 14 days using this pretreatment. This amount of copper is lower than the minimum necessary to ensure contraception action. Consequently, the most adequate treatment seems to be 10^{-3} M PU because lower IE was measured. Fig. 5 shows the copper amounts found when the PU-pretreated wires were exposed to the SUF. It is noted that copper releasing was reduced from 5.922±0.14 mcg (control wire) to 1.269±0.45 (10^{-3} M PU-pretreated wire, 3-h immersion time) after 1 day of exposure to SUF. The cumulative dissolution of the PU-pretreated wires slightly increased with time in the 14-day periods assayed (1.86±0.72 mcg), while a marked



** Significant difference at p<.01 in relation to the control
++ Significant difference at p<.01 (treatments with PU 10^{-2} M vs. treatments with PU 10^{-3} M)

Fig. 5. Dissolution of copper wire with and without PU pretreatment.

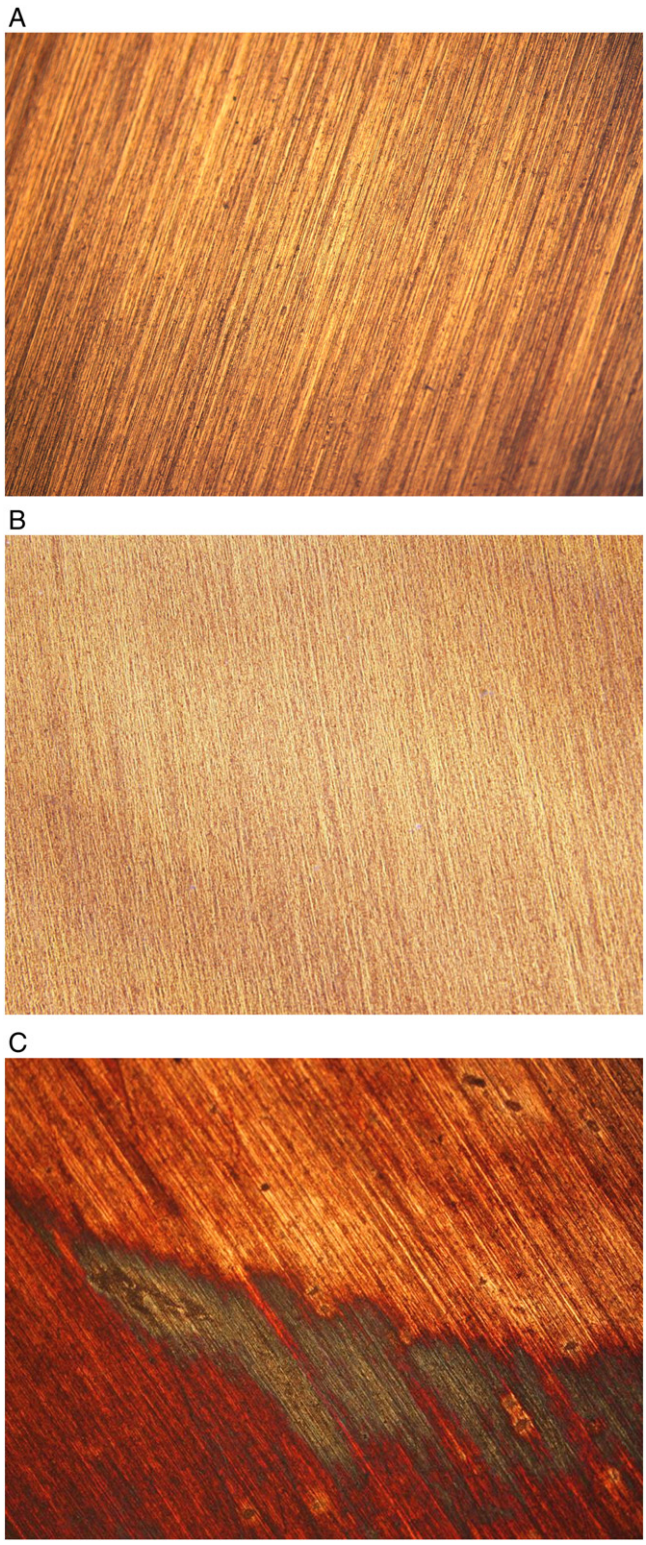


Fig. 6. Microphotographs of the copper surface obtained with an optical microscope before (A) and after 3-hour immersion in 5 g/L NaCl + 1×10^{-2}M PU (B) and 5 g/L NaCl + 1×10^{-2}M TU (C) (100×).

increase of the copper released by the control was observed (15.09±0.14 mcg). It can also be observed that the amount of copper released after 10^{-2} M PU treatment is only half

of that measured for 10^{-3} M PU (Fig. 5). It is worth noting that the area of the wire was close to 186 mm^2, and consequently, a larger area (220 mm^2) could provide higher copper ions release to reach the minimum value (2.0 mcg/day).

Microscopic observations of the samples revealed that a bright homogeneous film was formed on the PU-pretreated electrodes, while the TU-pretreated samples were covered by a nonuniform layer (Fig. 6). The different properties of the adsorbed coatings probably account for the different dissolution behavior of the samples.

4. Discussion

Common side effects of Cu-IUDs like bleeding, spotting, pain and dysmenorrhea are a cause of great concern during the first days after insertion into the uterus [1,9,23–27]. Some authors have hypothesized that these clinical effects might be correlated to the initial "burst release" of copper ions [2,26,27]. The use of weak corrosion inhibitors is proposed here as a strategy to reduce the burst release effect.

4.1. Reduction of the initial copper release

Electrochemical tests have been widely used to evaluate the corrosion of copper in uterine fluids [16,34–36] as well as to study the behavior of corrosion inhibitors like TU [32,37]. The effect of PU and TU pretreatments on copper dissolution was firstly assessed by electrochemical tests. Electrochemical results and microscopic observations revealed that adsorbed PU is more protective because it probably forms a more stable and uniform layer on copper surface than TU. In fact, IE calculated for TU pretreatment was lower than that of PU pretreatment, which is in agreement with the nonuniform layer detected by microscopic observations. Besides, the colorimetric measurements of copper release confirmed that after the first day of exposure, the dissolution rate of copper treated with PU was reduced to values fivefold lower than that corresponding to the control. It is worth mentioning that the surface area of the wires used in these assays was 188 mm^2, while TCu 380A and Multiload Cu375 have areas close to 400 mm^2. Consequently, the amounts of copper released from the pretreated wires should be twice if their area is double (376 mm^2). Thus, the minimal copper concentration value necessary for the contraceptive action can be attained with PU pretreatment.

Electrochemical tests (transient currents) showed that the PU inhibitory action was progressively lost. It was reported [35] that a gradual calcification of the copper wires occurs and leads to lower copper dissolution. Consequently, for periods longer than 14 days, it is expected that the dissolution rate in vivo will be enough to ensure contraception and will not increase significantly with time. Again, it is worth noting that the amount of copper ions released in the stationary state could be regulated by selecting the adequate copper area.

4.2. Mechanism of the dissolution of copper in the absence and presence of PU and TU

Several authors have reported the mechanisms to interpret the copper corrosion in chloride-containing solutions like SUF. They include the formation of Cu_2O, $Cu(OH)_{ad}$, $Cu(Cl)_{ad}$ and CuCl on the electrode and soluble species ($CuCl_2^-$) and probably oxygen-reactive species [16,35–46].

Remarkably, the pH of the uterine media changes during the menstrual cycle between 6 and 8.0 [35,36]. Zhang et al. [36] showed that higher copper dissolution is found when the pH is lower. If copper is in contact with acidic solutions (pH 6), a larger amount of soluble products is formed. In this work, SUF pH 6 was used to simulate the worse clinical condition due to the higher copper release.

According to previous reports [46], under the open-circuit conditions, Cu(I)-TU adsorbed complexes, which partially cover the electrode surface, can be formed. Our results show that corrosion is enhanced by the presence of the nonuniform layer that partially covered the electrode after TU pretreatment. Occasionally, micropits can be found on the TU-pretreated wires after immersion in the uterine fluid.

Pretreatments with PU have shown a better IE related to the presence of PU adsorbed derivatives ($Cu(PU)^+$) on the copper surface. Fig. 7 shows FTIR spectra of PU in a KBr pellet (Fig. 7a) with expected bands for this compound [47]. These typical bands were also found in the FTIR–ATR spectra corresponding to copper pretreated in 10^{-2}M PU (1-h immersion time) confirming that PU was adsorbed on the surface (Fig. 7b). According to microscopic observations, the coating formed on PU-treated electrodes was more uniform and shielding than that of TU-pretreated samples. This accounts for the better protection of the PU pretreatment.

Overall, results of electrochemical tests, copper dissolution measurements and microscopic observations show that

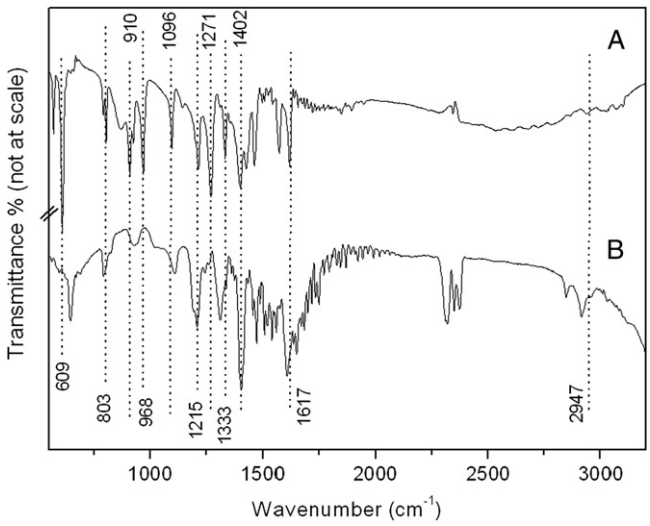


Fig. 7. FTIR spectra of PU (A) KBr pellet (B) PU adsorbed on copper. Dashed lines point out the typical bands expected for PU.

it is possible to reduce the early copper releasing from IUD by treating the IUD copper wires with 10^{-3} or 10^{-2} M PU solutions. However, to reach the adequate copper release and to ensure the contraception, the use of pretreatments with 10^{-3} M PU is the more suitable strategy. The advantage of PU-pretreated Cu-IUD is that the amount of the contraception agent (copper ions) could be controlled initially by adjusting the concentration of PU in the solution used as inhibition treatment. For longer exposures, when the PU effect has been lost, the area of the copper wire used (between 220 and 400 mm^2), which is related to the amount of copper ions released, should be adequately selected.

Pretreatments with PU may be made by the manufactures under sterile conditions before packing the IUD. Another alternative is to provide sterile PU solutions in glass ampoules to the gynecologists to immerse or moisten the nonpretreated IUD under sterile conditions for 3 h. On this respect, different possibilities are actually assayed in the laboratory to reduce the immersion time. In addition, cytotoxic and genotoxicity evaluation under different conditions will be performed in the near future followed by test in an animal model.

5. Conclusions

The control of biodegradation of copper in uterine fluids is crucial to avoid initial toxic effects due to high ions release ("burst release"). Results of this work demonstrate that the IE depends not only on the type and concentration of organic inhibitor but also on the duration of the pretreatment. PU is more appropriate for IUD treatments because its IE is higher and its action remains for a longer period than TU. After pretreatments, adsorbed PU or its derivatives remained on the copper surface inhibiting copper dissolution reaction in SUFs.

It was concluded that the pretreatment of IUD with 10^{-3} M PU solutions (immersion period=3 h) is promissory because it is easily handled and inexpensive and ensures both a sufficient concentration of copper ions to guarantee contraceptive action of the device and a controlled release of copper ions to avoid harmful effects in the body related to the initial "burst release" effect.

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