STUDIA UBB PHYSICA, Vol. 63 (LXIII), 1-2, 2018, pp. 61-73
(RECOMMENDED CITATION)
doi:10.24193/subbphys.2018.06

# A COMPARATIVE STUDY FOR NATURAL DEGRADATION OF THREE LOCAL ANESTHETIC DRUGS FOR HUMAN USE BY $^1$H NMR RELAXOMETRY AND FT-IR SPECTROSCOPY

**L. R. DRĂGAN[1], R. CRAINIC[1], R. FECHETE[1,2,*]**

**ABSTRACT.** Three anesthetics, lidocaine (xylene), ropivacaine and propofol, with localized area of action while used in human surgery, were investigated by $^1$H NMR relaxometry and ATR-FT-IR spectroscopy. The injectable liquid anesthetics were then subjected to degradation in natural condition during 36 days by exposure to direct sunlight radiation. The sunlight radiation intensity in the infrared and visible domain as well as UV index was monitored using Adafruit SI1145 breakout board sensor. The temperature and humidity was monitored using a digital DHT11 sensor. The evolution of $^1$H NMR $T_2$-distributions, IR spectra, pH, refraction index, electric conductivity and total dissolved solids (TDS) show a degradation of studied anesthetics but also in some cases a certain recovery.

***Keywords:*** *ATR-FT-IR spectroscopy, $^1$H NMR relaxometry, $T_2$-distribution, lidocaine, ropivacaine, propofol.*

## INTRODUCTION

Modern surgery cannot longer be performed without the use of local anesthetics which decrease the pain feeling into specific area and

[1] *Technical University of Cluj-Napoca, Faculty of Material and Environmental Engineering, B-dul Muncii 103-105, Cluj-Napoca, Romania.*
[2] *Babeş-Bolyai University, Faculty of Physics, 1 Kogălniceanu str., 400084 Cluj-Napoca, Romania*
[*] *Corresponding author: rfechete@phys.utcluj.ro*

relax the muscle power [1]. Therefore, the quality of local anesthetics is a very important factor which can be easily be lost, even in the guaranty period, by improper storage. Modern investigation methods like NMR spectroscopy, GC-FID, GC, GC-MS, HPLC and LC-MS are used for the study forced degradation of some liquid anesthetics in different stress condition such as acidic, basic, and oxidative environment or exposed to sunlight, UV light, elevated temperature and/or humidity [1, 2].

The aim of this study is to evaluate the degradation effect of direct exposal to sunlight radiation during a longer time period up to 36 days from 3 April 2018 to 9 May 2018 of three liquid anesthetics (lidocaine/xylene, ropivacaine and propofol) with local action while are used in human surgery. For that $^{1}$H NMR $T_2$-distributions, ATR-FT-IR spectra, refraction index, pH, electric conductivity and TDS (total dissolved solids) were regularly measured. The natural degradation conditions: relative infrared and visible intensity, UV index, temperature and relative humidity were monitored using Adafruit specialized sensors connected to an Arduino Leonerdo ETH microcontroller and the data were recorded onto SD card.

## EXPERIMENTAL

Three injectable liquid anesthetics with local action such as xylene 1 % (generally known as lidocaine), ropivacaine (10 mg/ml) and propofol (10 mg/ml) were used for the present study. The anesthetics were preserved in dark at room temperature in their original bottle package and extracted using a syringe with a needle for specific measurements of undegraded samples. For degradation in natural conditions the anesthetics were transferred in small volume, cylindrical plastic bottles and exposed at the laboratory window to the direct action of sunlight. Near to the closed bottles two digital sensors: i) Adafruit SI1145 breakout board sensor and ii) DHT11 sensor were placed to monitor the infrared and visible uncalibrated intensity calibrated UV index, temperature and humidity conditions. The data were stored on the onboard microcontroller SD card and recorded if the UV index was larger than 0.6.

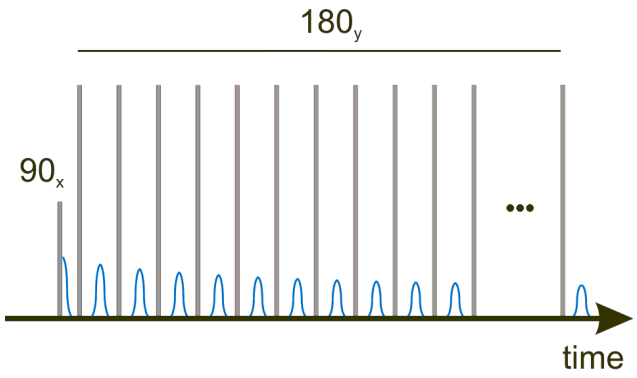


**Fig. 1.** The CPMG pulse sequence used for the $^1$H NMR data acquisition.

The low field Bruker Minispec MQ 20 spectrometer working at 19.69 MHz frequency was used for the $^1$H NMR relaxometry measurement [3-5]. The echo time in the CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence (see Fig. 1) was 5 ms. To cover the full decay of liquid samples a number of 3000 echoes was recorded. The recycle delay (RD) was set at 3 s and a number of 32 scans were accumulated. A Laplace-like inversion algorithm described by [6-8]:

$$M(\tau) = \int_0^\infty f(T_2) e^{-\frac{\tau}{T_2}} dT_2 , \qquad (1)$$

was used for data processing and normalized transverse relaxation time $T_2$-distibutions, $f(T_2)$ were obtained [9, 10].

A Jasco 6200 FT-IR spectrometer was used for the measurements of the FT-IR spectra. The ATR (attenuated total reflection) accessory was used for our liquid samples. For background we use distilled water and we perform the specific ATR correction after each data measurement. The spectrometer set-up was as follow: i) the measurement domain was between 349.053 cm$^{-1}$ up to 4000.6 cm$^{-1}$; ii) due to the noisy data at low wavenumber values the IR spectra were cutoff at 700 cm$^{-1}$; iii) a number of 128 scans were accumulated; iv) the resolution was set at 4 cm$^{-1}$.

The refraction index was measured using a Hanna instrument. This device originally give the refraction index in Brix units. After calibration, finally the refraction index was calculated using:

$$n = 1.13913 + 0.19366 \cdot e^{\frac{Brix}{134.2178}}. \quad (2)$$

For the measurement of pH and electric conductivity we used a IP67 Combo multi-parameter. The TDS (totally dissolved solids) was measured with a pocket TDS&EC instrument with temperature correction.

## RESULTS AND DISCUSSION

In Fig. 2a the normalized $^1$H CPMG decay curves measured for the undegraded anesthetics are compared. At a visual inspection, for the full 15 s decay, small differences can be observed between anesthetics. One can see that the CPMG curve for propofol initially decays faster than the curves measured for xylene and ropivacaine suggesting the existence of a rigid component, then the decay become slower suggesting the existence of a more mobile component. The differences between CPMG decays of xylene and ropivacaine are small even in the initial time regime (see the insert in Fig. 2a).

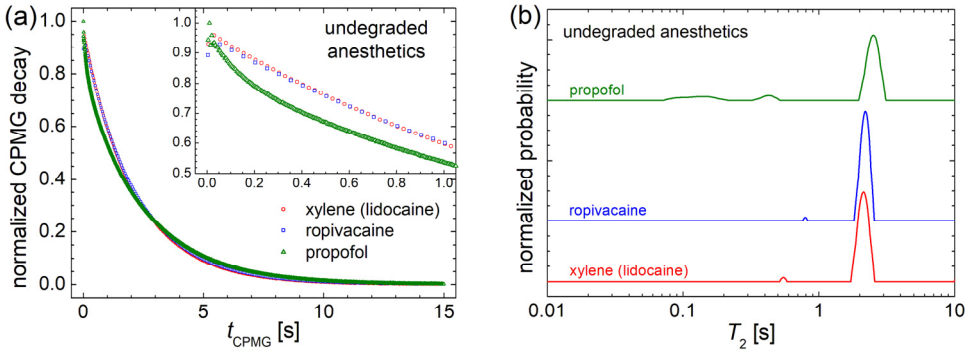


**Fig. 2.** (a) $^1$H NMR CPMG decays and (b) the corresponding $T_2$-distributions measured for undegraded anesthetics: xylene (red), ropivacaine (blue) and propofol (olive).

A better interpretation in terms of components (protons - $^1$H reservoirs) with different mobility can be performed by considering the transverse relaxation time ($T_2$) distributions which are presented in Fig. 2b. The $T_2$-distributions of xylene (lidocaine) and ropivacaine are similar presenting two peaks: i) a large peak located at most probable $T_2$ (maximum of peak) 2.1 s and 2.2 s respectively, which compared to a 2.4 s value measured for distilled water (see ref. [9]) suggests a small amount of dissolved solid and ii) a small peak suggesting a small quantity of undissolved solids. The $T_2$-distributions measured for propofol is different. There it is present one large peak centered at ~ 2.5 s belonging to water and three components with a reduced mobility where two of them (the doublet located at ~ 10 ms) being partially overlapped. These components are most probable responsible with the milky aspect of propofol anesthetic.

Large differences can be observed also in the ATR-FT-IR spectra of our studied liquid anesthetics (see Fig. 3) between propofol spectrum, characterized by well defines absorption lines and xylene and ropivacaine spectra characterized by broad bands. Generally, all spectra can be divided into three regions:

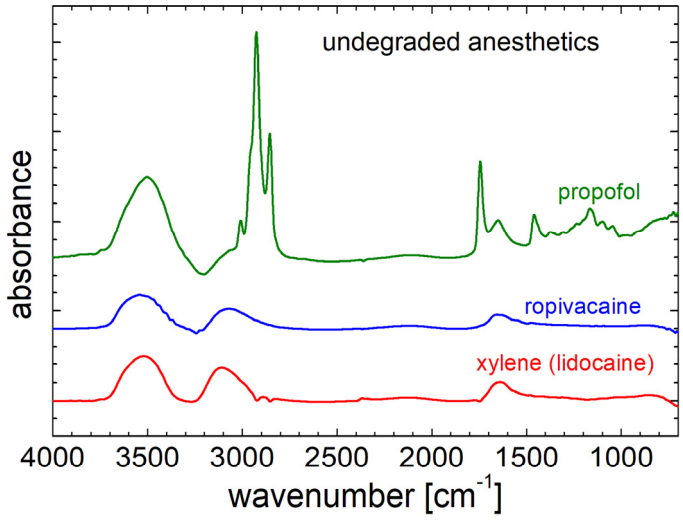


**Fig. 3.** The ATR-FT-IR spectra of undegraded propofol, ropivacaine and xylene(lidocaine).

i) the first one from low wavenumber (here 700 $cm^{-1}$) up to ~ 1900 $cm^{-1}$ present only broad bands for xylene and ropivacaine but for propofol four elevated peaks at ~ 1163, 1462, 1649 and 1747 $cm^{-1}$ can be observed; ii) the middle region between 2690 $cm^{-1}$ up to 3260 $cm^{-1}$ is characterized by broaden absorption peaks for xylene and ropivacaine but again three relatively well resolved peaks belonging to propofol located at ~2857, 2926 and 3012 $cm^{-1}$ are present and iii) the region located between 3260 $cm^{-1}$ and 3780 $cm^{-1}$ which is characterized by broaden peaks for all three samples of liquid anesthetic.

Into an attempt of a specific characterization of the studied anesthetics, a molecular mechanics simulation was performed using Gaussian 09 software. The structure of lidocaine molecule was constructed (see Fig. 4 – top right) and optimized using default settings parameters (Hartree-Fock/ground state method with 3-21G basis set). The vibrational frequencies were also calculated (see Fig. 4 – top left) and the IR spectrum was simulated (Fig. 4 bottom).

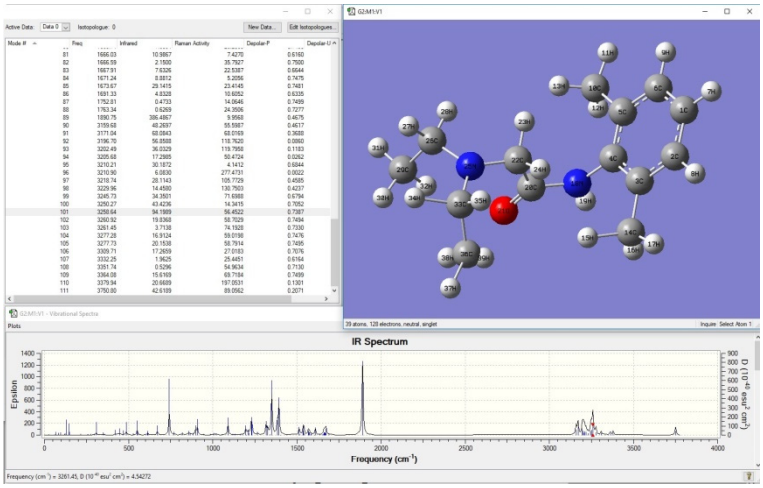


**Fig. 4.** Gaussian 09 simulation of lidocaine (xylene) structure and IR spectrum

The simulated spectra differ from the measured spectrum of lidocaine in to many essential points, then we consider that our broad spectra of small amount of anesthetics in water can be interpreted by numeric simulations

only after a large investigating time which is beyond our present purpose. Therefore, the physico-chemical properties characterization effort of anesthetics was oriented into another direction.

The refraction index, pH, electric conductivity and TDS (total dissolved solids) were measured for our anesthetics and the results are presented in Fig. 5. The larges refraction index was measured for propofol (which present also a milky-like visual aspect) while the refraction index for colorless xylene and ropivacaine (see Fig. 5a) is closed to the distilled water (1.33). Slightly acid character was observed from the pH of our three anesthetics. While xylene 1 % presents an almost neutral character (pH ≅ 6.29) the ropivacaine is the most acid anesthetic (pH ≅ 5.22). Nevertheless, all of these values are into desired limits.

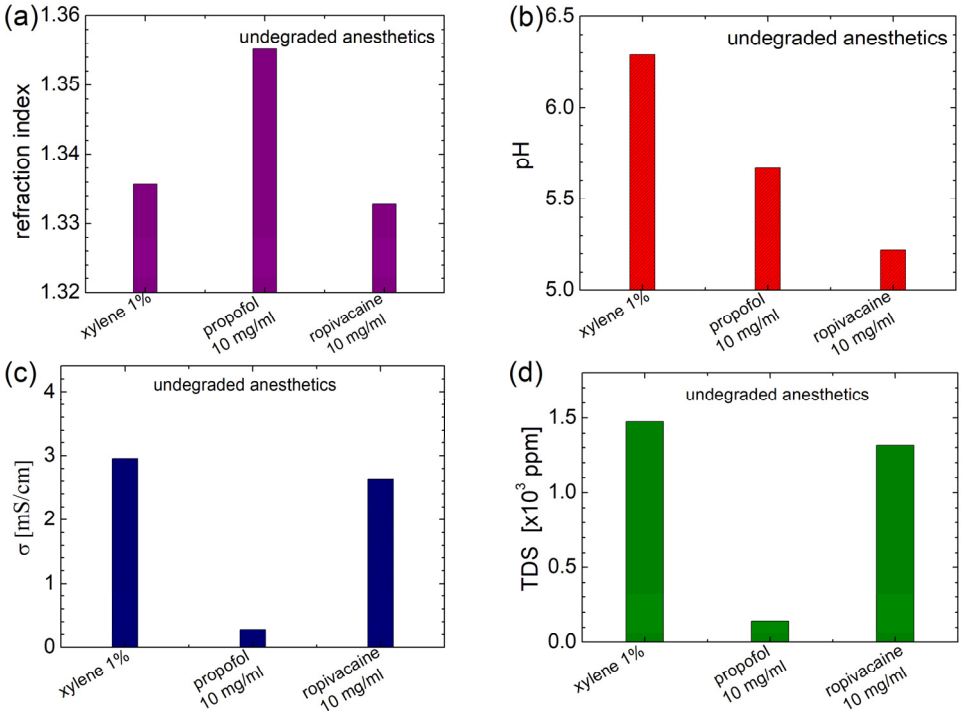


**Fig. 5.** (a) Refraction index; (b) pH; (c) electric conductivity and (d) total dissolved particles measured for undegraded xylene, propofol and ropivacaine.

Elevated values are measured for electric conductivity (Fig. 5c) and TDS (Fig. 5d) parameters of xylene and ropivacaine. These are between 2600 and 3000 µS/cm for electric conductivity (EC) and between 1300 and 1500 ppm

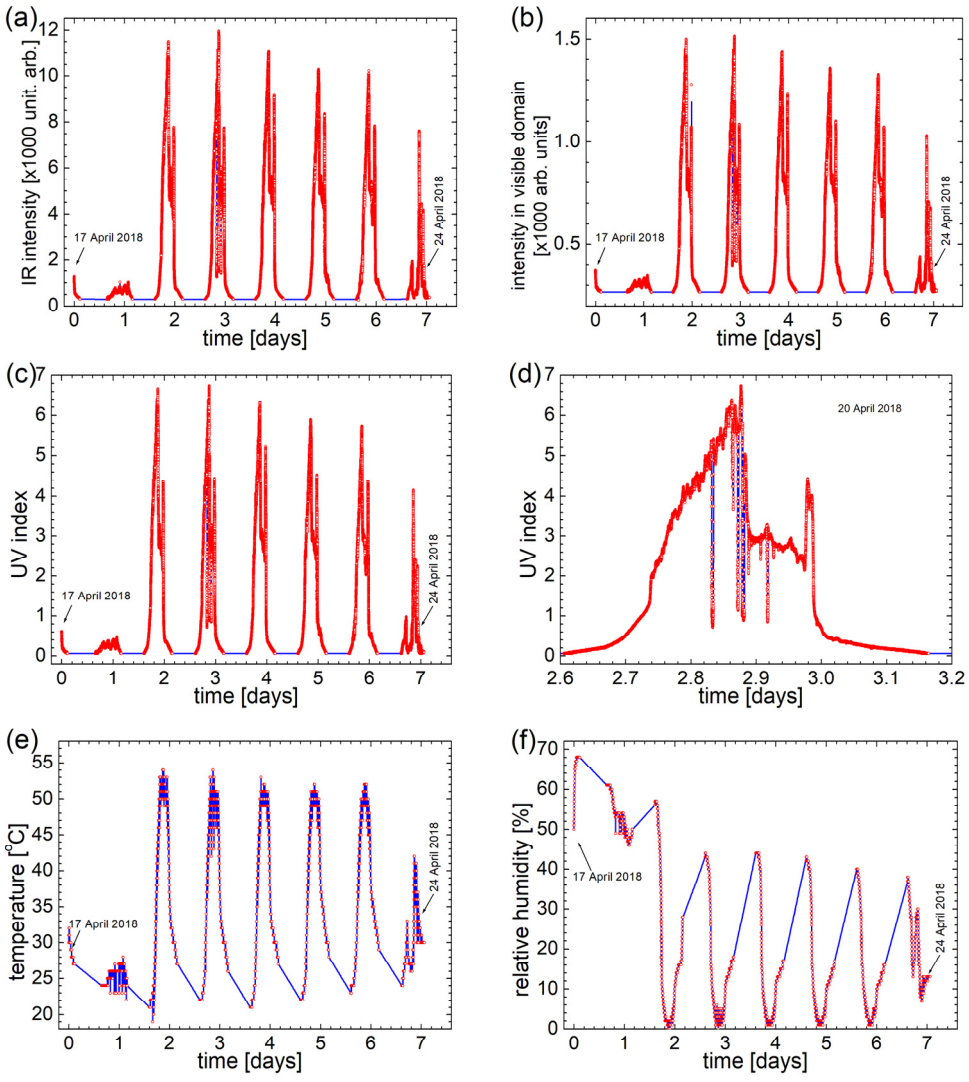


**Fig. 6.** Infrared**,** visible, ultraviolet radiation characteristics, temperature and relative humidity of environmental measured for the natural degradation of anesthetics.

for TDS suggesting the existence of a large number of dissolved solids with electric properties (ions) in anesthetic injectable solution. Much lower values were obtained for propofol: 284 μS/cm for EC and 142 ppm for TDS.

For the study of natural degradation under natural conditions the liquid anesthetics were exposed to the direct sunlight for a period of 36 days in the spring-summer of 2018. Some environmental parameters were measured and presented in Fig. 6 for a period of one week in the middle of monitored period from 17 to 24 April 2018. These parameters are the uncalibrated infrared intensity (Fig. 6a) and light intensity (Fig. 6b), and the calibrated UV index (Fig. 6c), temperature (Fig. 6e) and relative humidity (Fig. 6f). The data were collected from 3 to 3 minutes if the UV index was larger than 0.6. In Fig. 6d the UV index data were represented for a period of approximately one day (20 April 2018). Fine features of UV index variation can be observed during this monitored period. In fact all parameters such as the sun radiation (in IR, visible and UV) as well as the measured temperature and humidity are largely sensitive to the clouding conditions. For example, in 18 April (day 1 as it is presented here) it was mostly clouded reflected into small values of monitored parameters. One can remark also that the relative humidity of air surrounding the anesthetic samples is inversely proportional with the rest of measured parameters.

For the characterization of the degradation effect due to the direct exposure at sunlight of our three anesthetics the samples were periodical measured. In Fig. 7 the $T_2$-distributions measured for the xylene, propofol and ropivacaine degraded for 14 (Fig. 7a) and 36 (Fig. 7b) days in natural conditions are presented. While the $T_2$-distributions measured at 14 days of degradation under direct light exposure are similarly for all samples compared with the corresponding distributions measured for undegraded samples, at 36 day of exposure the degradation effect is visible from the changed in peaks positions, number, and width. From the $T_2$-distributions of xylene the small peak disappeared indicating a dissolution process of solids (see the red curve in Fig. 7b). In the $T_2$-distributions of ropivacaine two peaks appears indicating a dissociation process of undissolved solids into two components with different mobility (hence probably with different size). The doublet located at ~ 10 ms

in the $T_2$-distributions of propofol merges into a single peak indicating (also from the displacement of main peak to smaller $T_2$ values) the dissolution process of less mobile components.

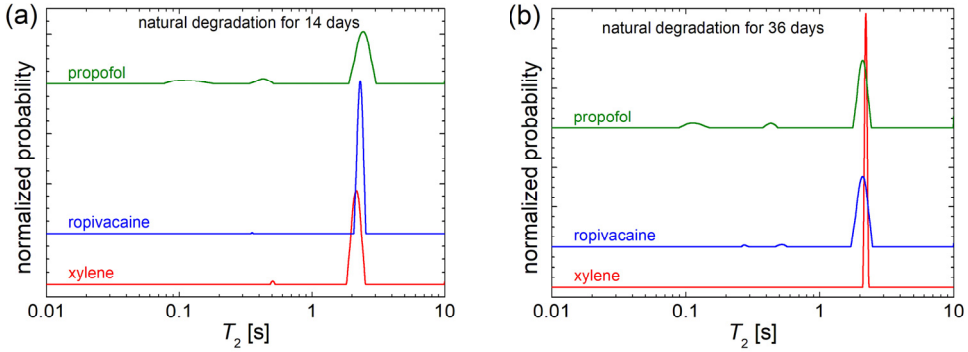


**Fig. 7.** The $T_2$-distributions measured for xylene (lidocaine), ropivacaine and propofol subjected to natural degradation for (a) 14 and (b) 36 days.

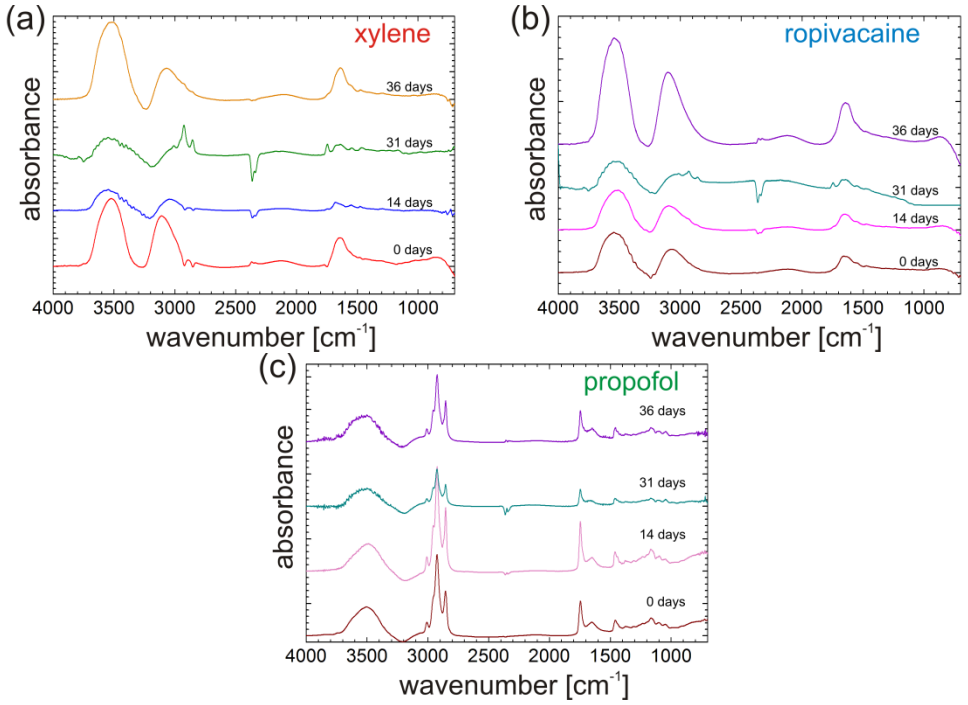


**Fig. 8.** ATR-FT-IR spectra measured for (a) xylene; (b) ropivacaine and (c) propofol undegraded and degraded under natural conditions for 14, 31 and 36 days.

The ATR-FT-IR spectra of degraded anesthetics subjected to direct sunlight are comparatively presented in Fig. 8a for xylene, Fig. 8b for ropivacaine and Fig. 8c for propofol. The samples were measured at 14, 31 and 36 days of natural degradation. As a general remark is the fact that in all cases a degradation effect can be observed from the decay of the overall intensity, in the three spectral regions discussed before, but also a recovery process can be remarked for samples measured in day 36 of degradation in natural conditions. Such recovery process is supported by our other measurements.

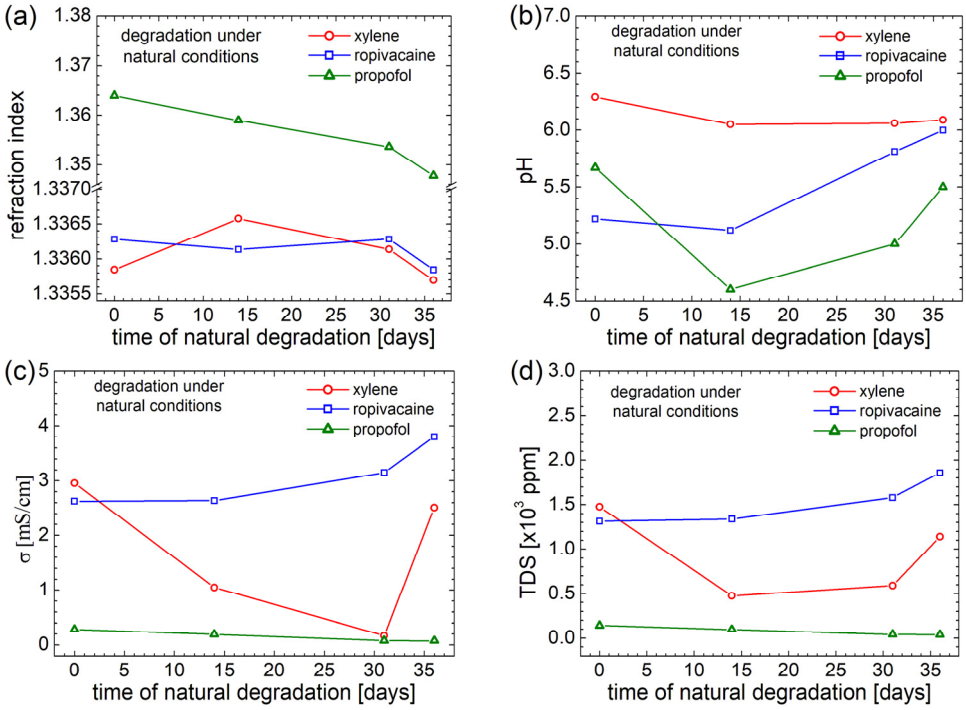


**Fig. 9.** The dependence of (a) refraction index; (b) pH; (c) electric conductivity and (d) TDS function of natural degradation time for human use anesthetics (xylene, ropivacaine and propofol).

In Fig. 9 the refraction index, pH, electric conductivity (EC) and TDS are presented for degraded anesthetics in natural conditions. The continue degradation of propofol (olive triangle) exposed to direct sunlight is observed from the monotone decay of refraction index, EC and TDS. Only the pH of propofol presents a certain recovery after day 14 up to day 36.

The degradation of ropivacaine (blue square) is observed as a monotone increase of pH, EC and TDS parameters. In the case of ropivacaine the refraction index presents a slight recovery. For xylene (red circles) the initial degradation and recovery is most evident for all four measured parameters but especially for electric conductivity (Fig. 9c).

## CONCLUSIONS

The degradation process in natural conditions, by direct exposure to sunlight into a plastic package of three liquid anesthetics, with local action while used in human surgery, was monitored by specific changes of advanced Laplace $^{1}$H NMR $T_2$-distributions, Fourier ATR-FT-IR spectra, but also by global refraction index, pH, electric conductivity and total dissolved solids. Some parameters present changes at a short exposure duration but all measured parameters present significant changes after 36 of exposure to direct sunlight of local xylene, ropivacaine and propofol anesthetics.

## REFERENCES

1. K. M. Patel and A. D. Patel, *Int. J. Curr. Res.*, 9(6) 53036-53043 (2017).
2. Y. Kadioglu, A. Atila, M. S. Gultekin, N. A. Alp, *Iranian J. Pharma. Res.*, 12(4) 659-669 (2013).
3. O. Cozar, N. Cioica, E. M. Nagy, C. Cota, R. Fechete, C. Filip, *Studia UBB Ephemerides*, 62 19-28 (2017).

4. R. I. Chelcea, D. Moldovan, D. E. Demco, E. Culea, R. Fechete, *Studia UBB Chemia*, 62(1), 73-88 (2017).
5. R. Fechete, D. E. Demco, X. Zhu, W. Tillmann, R. Vinokur, M. Möller, *Chem. Phys. Lett.*, 597, 6-15 (2014).
6. L. Venkataramanan, Y. Q. Song, M. D. Hürlimann, *IEEE Trans. Sign. Proc*., 50, 1017–1026 (2002).
7. Y. Q. Song, L. Venkataramanan, M. D. Hürlimann, M. Flaum, P. Frulla, C. Straley, *J. Magn. Reson*., 154, 261–268 (2002).
8. M. D. Hürlimann, M. Flaum, L. Venkataramanan, C. Flaum, R. Freedman, G. J. Hirasaki, *Magn. Reson. Imag.*, 21, 305–310 (2003).
9. E. Jumate, D. Moldovan, D. L. Manea, D. E. Demco, R. Fechete, *App. Magn. Reson*., 47 (12), 1353-1373 (2016).
10. R. S. Sipos, R. Fechete, D. Moldovan, I. Sus, Z. Pávai, D. E. Demco, *App. Magn. Reson.*, 47 (12), 1419-1437 (2016).