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EPR studies of defects in pure sol-gel matrices and their influence on cytotoxicity of the material

AGNIESZKA ULATOWSKA-JARŻA[1*],
MAŁGORZATA KOMOROWSKA[2], HALINA PODBIELSKA[2]

[1]Bio-Optics Group, Institute of Physics, Wrocław University of Technology,
Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

[2]Biophysics Group, Institute of Physics, Wrocław University of Technology,
Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

Sol-gel based biomaterials may be used for various applications, including biomedical ones. In this respect it is important to investigate the influence of sol-gel matrices on biological systems in order to establish their cytotoxic activity. The results of EPR studies of sol-gels are described in this work. They demonstrate that various defects are present in sol-gel matrices: surface defects, peroxy- centres or hydrogen-related centres. These defects arise spontaneously during the gelation, being responsible for cytotoxicity of sol-gels. It was found that the ratio of the number of solvent moles to the number of precursor moles (the molar ratio *R*) is one of the factors determining the level of cytotoxicity. More defects were present in samples prepared with a lower molar ratio *R*. A higher concentration of defects was detected in freshly prepared samples, as compared to samples aged for a longer time.

Key words: sol-gel; xerogel; electron paramagnetic resonance (EPR); cytotoxicity

1. Introduction

A major advantage of the sol-gel technique is the possibility of preparing pure or doped silica xerogels, single- or multicomponent systems at room temperature. This feature can be exploited for the construction of various sensing devices, as well as carriers of biomedical components, e.g. drugs, steroids or cells.

For medical applications, the sol-gel matrix should not be cytotoxic. The absence of cytotoxicity can be confirmed by various biological laboratory tests. Recently, we have described several routes allowing us to obtain non-cytotoxic sol-gels [1]. Our recent studies

[*]Corresponding author, e-mail: Agnieszka.Ulatowska-Jarza@pwr.wroc.pl.

have demonstrated that some of xerogels can cause haemolytic reaction [2–4]. The experiments were done on human erythrocytes obtained from citrated blood 0Rh+, diluted with 150 mmol/l NaCl to the concentration of 10%. Some results are presented in Fig. 1.

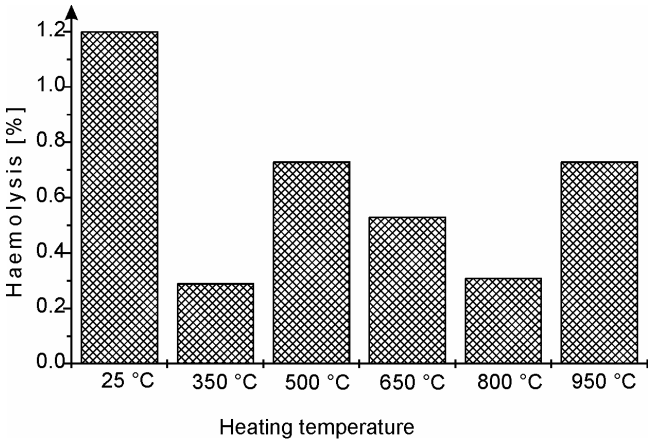

Fig. 1. Percentage of haemolysis depending on the temperature of thermal treatment of a xerogel matrix

The laboratory tests, as well the examination of the haemolytic reactions proved that freshly prepared sol-gels are cytotoxic due to their chemical instability. In order to rule out the problem of biological impurity of the investigated samples, the freshly prepared sol-gel bulks were subjected to the hot steam sterilization process. Interesting conclusions have been drawn from the analysis of the results obtained. The temperature of sterilization does positively influence the haemolytic reactions. This can be the result of a higher chemical stability due to the thermal process. Therefore, further examinations were performed on the xerogels exposed to higher temperatures after the condensation, as well as on materials aged for 6 months at room temperature (25 °C). For long-aged and heated materials the haemolysis is below the toxic values (< 1%).

A promising technique that can be used to describe and explain the material properties is the electron paramagnetic resonance (EPR). Some applications of the EPR technique to doped sol-gel materials have been reported [5–7]. The results include analysis of EPR spectra of pure sol-gel matrices (no dopants added). These materials, prepared by the sol-gel route, have been γ-ray irradiated and vacuum dried [8], heated at 700 °C and irradiated with X-rays [9] or dried at 920 °C and X-ray irradiated [10].

In the present work, we report the studies of paramagnetic defect centres in silica -based sol-gel materials that were produced at room temperature during the gelation process with various solvent amounts (different *R* values). Endogenous defects in the materials were examined. No exogenous defects caused by irradiation or additives were introduced.

2. Materials and methods

The samples were prepared from TEOS (Tetraethoxysilane, $Si(OC_2H_5)_4$, Aldrich), mixed with suitable amount of solvent (96% ethyl alcohol) for 4 h. The following molar ratios *R* were chosen: 5, 15, 32, and 50. The mixtures were acidified with 36% $HCl_{aq.}$ to obtain pH = 2 during the hydrolysis. The sol-gels were prepared in the form of bulks in plastic containers and left for drying after gelation stimulated by addition of ammonia solution. One-week-old samples were examined by means of UV-VIS spectroscopy. After one-month storage at room temperature the samples were powdered and weighed. Then, 9.44 GHz electron spin resonance spectra of the sol-gel materials were recorded by means of an EPR spectrometer made at the Wrocław University of Technology (EPR spectra were obtained with a standard SE/X-28 spectrometer operating in the X band). The MX-20/R microwave unit with a frequency counter was applied. The magnetic field was modulated at 100 kHz and the spectra were displayed as the first derivative of the absorption curves. All spectra were recorded at room temperature.

3. Results and discussion

Defects in amorphous SiO_2 with and without OH^- ions has been reviewed by Weil [11], Halliburton [12] and Griscom [13]. The description of these centres according to notation given by Ikeya [14] will be used in our further considerations (Table 1). The EPR spectra of the examined sol-gel matrices are presented in Fig. 2.

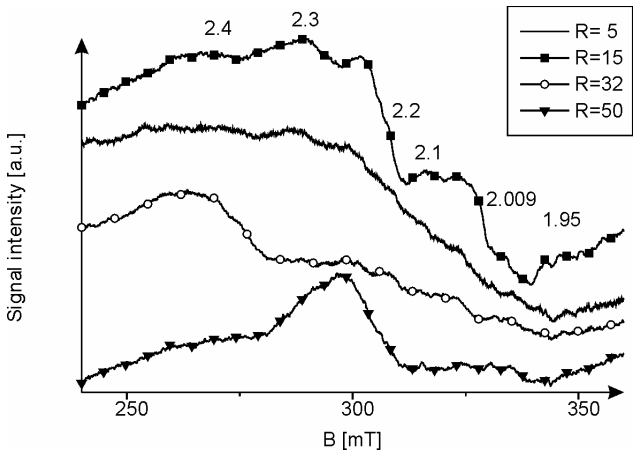

Fig. 2. EPR spectra of the sol-gel matrices dependent on the molar ratio *R*, recorded in the range of 240–360 mT

Figures 2 and 3 demonstrate that EPR signals of the sol-gel materials are mainly the broad structured bands with the average factor $g = 2.06$ (width of about 70 mT).

Table 1. Possible defects observed in silica materials by EPR and UV spectroscopy

Normal glassy structure	≡Si–O–Si≡	Each Si atom has 4 oxygen atoms tetrahedrally positioned, two different bond lengths: 1.62 Å and 1.60 Å. Si–O–Si angle 144°
g parameters		
E_1' centre	≡Si • Si≡ 2.00179 2.00053 2.00030	The centre is an electron trapped in the oxygen vacancy, produced at room temperature by γ-irradiation, annealed out above 373 K. The EPR signal is easily saturated at microwave power of 10^{-2} mW. Optical absorption at 212 nm.
E_2' centre E_4' centre	2.0022, 2.0006 2.00154, 2.00065, 2.00060	Absorption band at 225 nm. H^0 in an O^{2-} vacancy with a different relaxation.
Surface *E*′-like centre CO^{3-}- type centre	2.0017, 2.0003 centre: 2.0007 2.0048, 2.0063, 2.0248	Not stable when the sample contacts with water due to formation of gaseous H_2. Adsorption of CO_2 on the surface of *E*′-like centre.
Peroxy centre: two types OHC (dry OHC) NBOHC (non bonding oxygen hole centre). wet OHC	 ≡Si–O–(–O •)–Si≡ 2.0014, 2.0074, 2.067 (av.*) ≡Si–O • 2.0010, 2.0095, 2.078 (av.)	Oxygen-associated trapped hole centres. Peroxy radical, oxygen interstitial. Model: a complimentary centre of E_1' in a Frenkel pair (interstitial oxygen ion O^- which bonds with O^{2-}). Optical absorption at 325 nm. Usually observed as a shoulder of the OHC. Model: proton is released from Si–OH and a hole is trapped at the remaining oxygen in nonbonding 2p orbital. Optical absorption at 260 nm and 630 nm.
O_2^-	2.318, 1.992, 1.959	
Hydrogen centres	$[(OH)_4]^+$ 2.0911, 2.0103, 2.0002 $O(HO)_3$ 2.1351, 2.0047, 1.9962	Trapping a hole in p orbital of oxygen associated with one of four hydrogen atoms replacing a Si site $[H_4O_4]$ one proton is released

*Av. – average value of the *g* parameter.

With the increase of the solvent amount (increasing *R*), the fine structure of the EPR signal is more visible. For $R = 15$ three dominating groups of signals are observed: a low-field group with 2 signals $g_1 = 2.3–2.4$ and a more intensive $g_2 = 2.2$; mid-field with signals $g_3 = 2.1$ and more intensive $g_4 = 2.06$; high-field with narrow signals g_5 and g_6 between 2.009–1.95. For higher *R* factors, the dominating characteristics are low-field signals, although other signals are present as well (Fig. 2). The amount of

defects is strongly dependent on *R*. For small values of *R*, the number of defects is larger, what is demonstrated by wide bands with slightly marked fine structure of the spectrum.

With the increase of *R*, the amount of defects clearly decreases, particularly in the mid-field and high-field areas. In Figure 3, the EPR spectra are shown of the sol-gel matrices produced with $R = 15$ and 50 recorded in the range of 310–350 mT.

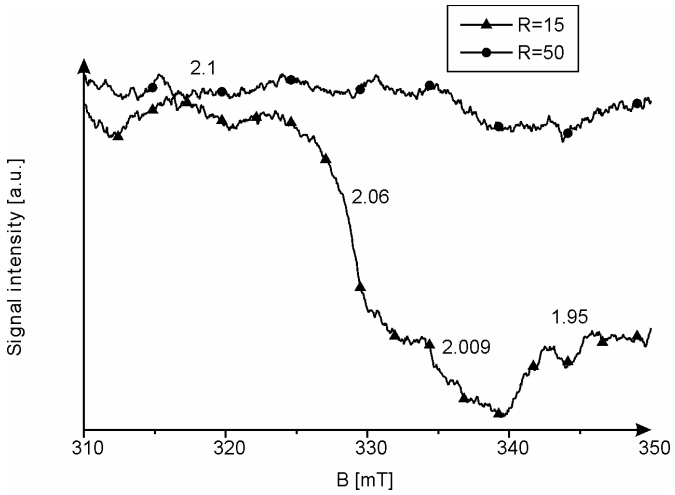

Fig. 3. EPR spectra in the range of 310–350 mT of the sol-gel matrices prepared with the molar ratios $R = 15$ and $R = 50$

Drying time at room temperature influences the amount of defects as well. For the shorter-dried sample (A), the highest concentration of centres in the high-field area is observed. The defects disappear with the increase of the ageing time (sample B). The centres with signals in the low-field part of the spectrum are prevalent (Fig. 4).

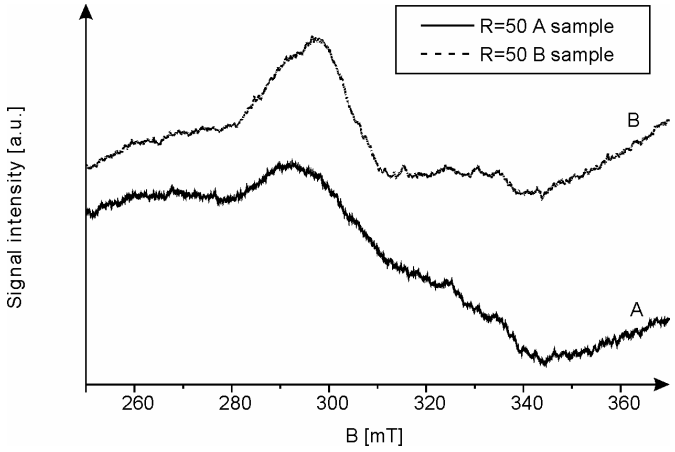

Fig. 4. The comparison of the EPR spectra depending on the ageing time of sol-gel matrices

Since some defects (for example E' – easily saturated) are better observed by the UV spectroscopy, the absorption spectra of matrices with R = 15 and 50 were measured (Fig. 5). For R = 15, two absorption bands with the maxima at 225 (E_2' centre) and 260 nm (peroxy centre) are observed. For R = 50 a third band with maximum at 212 nm appears (E_1' centre). The number of centres which absorb at 260 nm is constant and does not depend on the R factor. However, the 212 nm and 225 nm bands occur for the larger R factors.

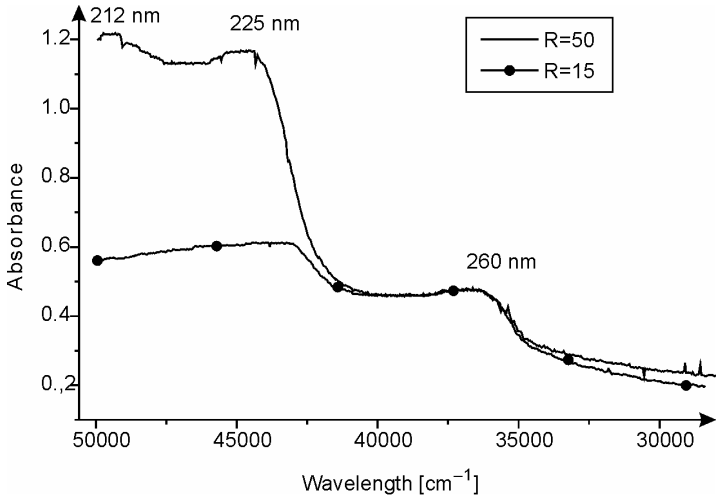

Fig. 5. UV-VIS spectra of sol-gels prepared with R = 15 and R = 50

Table 2. Absorption bands (UV spectra)

Parameter R	Absorption band at [nm]	Absorbance	I_{225}/I_{260}
15	225	0.9	1.1
	260	0.8	
50	212	1.2	2.4
	225	1.2	
	260	0.5	

Regarding the g factor, the presence of peroxy-centres: OHC (g_1 = 2.0014, g_2 = 2.0074, g_3 = 2.067 (av.)) and NBOHC (2.0010, 2.0095, 2.078 (av.)) can be assumed. E_1' centres cannot be detected by EPR because of saturation, even at low-power microwaves (50 mW used in experiment). Surface centres E' are unstable in contact with water, therefore they are present only in areas with the smallest amount of solvent. These centres are responsible for signals in the mid-field and high-field areas, therefore the defect concentration for these signals is much higher at the low R factors than

at the higher R values. Signals observed in the low-field and high-field areas (g about 1.9) may be related to the presence of the O^{2-} centres. The value of $g = 2.13$ may indicate the presence of hydrogen-related centres. Table 3 contains the summary of the above considerations.

Table 3. Proposed assignments of defects in the samples studied

Defect in a sample	g values	Absorption bands [nm]	Remarks
E_1'	not available	212	Yes. Invisible in EPR spectra because of saturation, stated by UV spectrometry.
E_2' E_4'	high magnetic field	225	Yes. The concentration is higher with the increasing parameter R
Surface like E_1'	high magnetic field	No UV absorption observed	Probably. Unstable in the presence of water. Dominating for lower R
O^{2-}	low and high magnetic field	No UV absorption observed	Yes The most stable. Dominating for high R values
Peroxy centres OHC NBOHC	mid and high magnetic field	260	Yes Dominating for lower values of the R parameter
Hydrogen -related defects $[(HO)_4]^+$, $[O(HO)_3]^+$	mid and high magnetic field	No UV absorption observed	Yes

4. Possible influence of the defects observed on cytotoxicity

Macroporous silica gels can be exploited as carriers for cells, bacterias or other microorganisms, provided they are biocompatible. Gels may be cytotoxic since the hydrolysis of alcohol-based reactants leads to creation of non-biocompatible materials. In addition, our studies have demonstrated that all types of E' defects can be present. Thus when the samples contact with water, free electrons or hydrogen atoms are produced. Both products could act as redox reagents.

According to the results obtained, the toxicity is expected to be higher when samples are freshly prepared or prepared with a lower R ratio. Increased R value accelerates the reaction of hydrolysis and causes more complete hydrolysis of monomers before condensation occurs. As a consequence, the gelation time increases. Longer gelation times reduce the amount of defects formed during gelation. For lower R values the alcohol-producing condensation mechanism is favoured, whereas the water-forming condensation is favoured at higher values of the R ratio.

5. Conclusions

The number of defects in sol-gel matrices, which can be responsible for cytotoxicity, depends on the *R* ratio and the ageing time. This confirms the results of laboratory and biological testing of aqueous extracts of sol-gels presented in our previous papers [2, 3]. We have proved that it was possible to produce the sol-gel derived materials that are non-haemolytic. This can be achieved by heating the materials at elevated temperatures or by ageing them for a suitably long time (minimum 6 months). Examination of the haemolytic reaction showed that freshly prepared xerogels are cytotoxic due to their chemical instability. EPR can be exploited as a suitable method for searching for defects responsible for cytotoxicity in the materials tested.

Acknowledgements

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References

[1] CONROY J.F.T., POWER M.E., MARTIN J., EARP B., HOSTICKA B., DAITCH C.E., NORRIS P.M., J. Sol-Gel Sci. Technol., 18 (2000), 296.
[2] ULATOWSKA-JARŻA A., PODBIELSKA H., SZYMONOWICZ M., STANISZEWSKA-KUŚ J., PALUCH D., Polym. Med., 30 (2000), 45.
[3] ULATOWSKA-JARŻA A., PODBIELSKA H., HOŁOWACZ I., LECHNA-MARCZYŃSKA M., SZYMONOWICZ M., STANISZEWSKA-KUŚ J., PALUCH D., SPIE Proc. vol. 4597 (2001), 81.
[4] ULATOWSKA-JARŻA A., *Elaboration of project for enzymatic optode based on the own results of examination of the sol-gel matrices properties*, Ph.D. Thesis, Wrocław University of Technology, Wrocław, 2001.
[5] KŁONKOWSKI A., KLEDZIK K., OSSOWSKI T., JANKOWSKA-FRYDEL A., J. Mater. Chem., 8 (1998), 1245.
[6] KŁONKOWSKI A., KOEHLER K., WIDERNIK T., GROBELNA B., J. Mater. Chem., 6 (1996), 579.
[7] KŁONKOWSKI A., WIDERNIK T., GROBELNA B., MOZGAWA W., JANKOWSKA-FRYDEL A., Microporous Mesoporous Mater., 31 (1999), 175.
[8] KORDAS G., WEEKS R.A., KLEIN L.C., J. Non-Cryst. Solids, 71 (1985), 327.
[9] WOLF A.A., FRIEBELE E.J., TRAN D.C., J. Non-Cryst. Solids, 71 (1985), 345.
[10] GRISCOM D.L., BRINKER C.J., ASHLEY C.S., J. Non-Cryst. Solids, 92 (1987), 295.
[11] WEIL J.A.,Phys. Chem. Miner., 10 (1984), 149.
[12] HALLIBURTON L.E., Appl. Radiat. Isot., 40 (1989), 859.
[13] GRISCOM D.L., Rev. Solid State Sci., 4 (1990), 565.
[14] IKEYA M., *New Applications of Electron Spin Resonance: Dating, Dosimetry and Microscopy*, World Scientific Publ., London, 1993.

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