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EXTENT OF DIFFUSION OF ORGANIC MOLECULES IN THE ENAMEL TISSUE OF DOGS

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The objective of this study was to evaluate the extent of diffusion of organic molecules in the enamel tissue, of permanent teeth. The experimental study was performed in vivo on dogs.

To the experimental dogs the molars on the right side of the lower jaw underwent enamel tissue reduction without additionally placing any tooth protection. Teeth on the left side of the lower jaw were left intact thus representing the control.

After 12 months animals were sacrificed, experimental and control teeth extracted and the study of diffusion in vitro carried on. For that purpose an organic spin marker 4-amino – 2,2,6,6-tetramethyl-piperdiny 1-oxi (4-Amino-Tempo) was selected. The property of this marker is that it contains a nitroxide radical which is stable in a neutral and base environment, and due to the unbound spin of the free radical it is highly suitable for the electronic paramagnetic resonance method (EPR).

Studies on the diffusion of organic spin markers in the enamel tissue are performed by submerging the prepared tooth in a water solution containing the marker substance. From the intensity of the EPR spectrum filmed after tooth withdrawal from the water solution, distribution, diffusion time and penetration depth of marker molecules were established. Coefficients of organic spin marker diffusion and saturation concentrations of marker molecules in the enamel were determined. The observed variations in the areal and time for distribution of diffused molecules were explained by the binding possibility of the N-O-radical from the marker molecule to the enamel tissue. The results of the experiment contained in this work contribute to a better understanding of the spin marker organic molecule movement through the enamel tissue which is controlled by the mechanism of diffusion and chemical bonding of the marker molecule to the enamel structure.

The results of the experimental research also indicate the harmful effects of leaving the enamel tissue without protection.

Key words: diffusion, enamel tissue, diffusion coefficient, EPR.

INTRODUCTION

The enamel tissue is continuously exposed to the effect of agents which can be augmented by the effects of preparations and materials applied locally on the tissue.

We meet every day with a great number of new materials introduced in the dentistry practice. The mechanism of their effect on the enamel tissue is in the greatest number of cases, investigated only within clinical studies. The basis for testing the effect of a new dentistry material on the dental tissue is the chemical composition of the material and the histological structure of the dental tissues, whereas there are still not valid data on the dynamics of diffusion processes.

Reduction of the enamel tissue in the daily dental practice is performed for the purpose of:

- occlusion adaptation by selective drilling and
- preparation of teeth for receiving the elements of a partial skeletal prosthesis,

Reduced enamel is exposed to the effect of the external harmful agents which result in irreversible changes. In order to make possible the protection of enamel it is necessary to know the dynamics of diffusion processes of organic structures through the enamel tissue.

The mechanisms of the effect of all these diverse chemical structures depend primarily on:

- chemical composition of the material,
- properties of the bilateral diffusion process through the enamel tissue,
- manner of binding to the enamel tissue and
- enamel tissue structure.

Diffusion is a process of mutual amalgamation of gases, liquids and solids. Good knowledge of the dynamics of diffusion processes through the oral tissues contributes to:

- I. clarification of the mechanism of penetration of harmful agents from the environment,
- II. research of biological effects of dental material under *in vivo* and *in vitro* conditions,
- III. protection of functional and anatomic integrity of oral tissues.

Vrbošek and Ravnik *et al.* (1973), Zipkin (1964) and Weatherell *et al.* (1965;1977) state that the process of diffusion through crystal structures takes place as several different mechanisms of movement of atoms and atomic groups within a crystal grid.

The structures of the crystal grid are not always ideal and contain certain imperfections which may be expressed as:

- I. dot defects stemming from evacuated atomic places in the crystal grid (Gwinnett, 1966;1967),
- II. line defects or defects within the plane, which represent a dislocation or layered atom vacancies (Little *et al.*; 1966) and
- III. complex of defects which are a result of interaction or fusing of primary defects. (Ripa, 1966).

The direction of the diffusion process through the enamel tissues has two diverse direction, Tihaček Šojić (1996):

- I. *CENTRIPETAL DIFFUSION* which takes place from the surface of the enamel tissues towards its interior and
- II. *CENTRIFUGAL DIFFUSION* whose direction is from the enamel-dentine border to the surface of the enamel tissue.

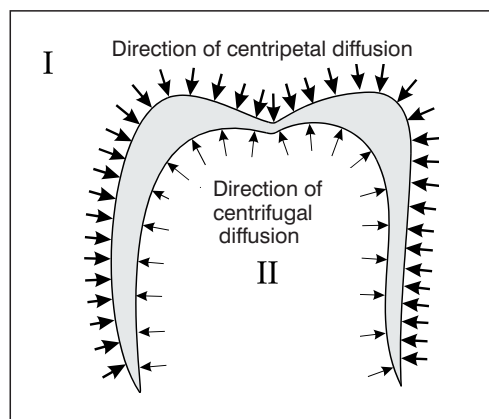


Figure 1. Directions of diffusion processes through the enamel tissue:
a) centripetal direction,
b) centrifugal direction

Beloica *et al.* (1980), Borowsky (1978), Cevs *et al.* (1986), Skalerić *et al.* (1974;1986) and Tihaček Šojić (1992;1993). The surface of the enamel tissue is exposed to the effect of a great quantity of diverse organic and inorganic substances originating from the saliva, food, dental plaque and dental material due to which the diffusion from the centripetal direction, namely from the surface of the enamel tissue towards the interior, is very intensive. At the same time the centrifugal direction of diffusion cannot be neglected in spite of the smaller quantity of particles whose diffusion is possible, in view of:

- I. *density of the enamel tissue*
- II. *size of the interprism space* and
- III. *dentine structure*

The density value of the enamel tissue is greatest on the enamel surface and has a tendency of dropping towards the enamel-dentine border Silverstone (1986), Stearns (1971), Stern *et al.* (1985) and Toth *et al.* (1986).

The size of the interprism space, contrary to the enamel, grows from the enamel surface towards the enamel-dentine border. Young (1974) and Schwancar (1970) state that dentine at the level of the enamel-dentine border has openings of dentine tubules whose mean diameter is 1.74 μm so that an undisturbed centrifugal direction of diffusion is possible.

The diffusion of particles is carried out through the enamel mostly in one direction, but in both senses and is called the Bilateral diffusion. It represents a physiological diffusion.

The research performed up to now on the diffusion processes (Borggreven *et al.* 1980, Funduk *et al.*, 1986 and Kambara, 1977) through the dental tissues relates only to the unilateral diffusion of inorganic molecules, namely to the determination of the intensity of the centripetal direction of diffusion, which moves from the enamel tissue surface to the enamel-dentine border. Other tissue surfaces were considered hermetically closed, Johnson (1971), Borowsky (1978), Burke (1975), Doi (1986) and Spektrova (1967).

On analysis of unilateral water diffusion processes through the enamel tissue (Dibdin, 1972 and Chick, 1963) a diffusion coefficient was obtained:

$$D_{H_2O} = (1.16 + 0.08) \times 10^{-8} \text{ cm}^2/\text{s}, \quad t = 20^\circ\text{C} \quad (1)$$

Coefficients of the unilateral diffusion through the enamel tissue for sodium and manganese ions, according to Arwill (1965), Kambara (1977), and Funduk (1975), amount to:

$$D_{Na^+} = 2.0 \times 10^{-10} \text{ cm}^2/\text{s}, \quad t = 20^\circ\text{C} \quad (2)$$

$$D_{Mn^{2+}} = 1.4 \times 10^{-10} \text{ cm}^2/\text{s}, \quad t = 20^\circ\text{C} \quad (3)$$

Data on the diffusion of organic molecules through the enamel tissue has been found in only a few published articles Waters (1971) and Tihaček Šojić (1996).

The aim of the research was to determine properties of transport and distribution of organic molecules in the enamel tissue.

MATERIAL AND METHODS

For the research of the properties of the bilateral organic molecule diffusion in the enamel a water solution of an *organic 4-Amino-Tempo spin marker* was used. The concentration of the marker was $C=0.02335 \text{ M}$ (250 ml $H_2O+1 \text{ gr}$ Marker) and the number of spins $M_s=7 \times 10^{16}/0.0005 \text{ cm}^3$. According to its structure the marker is similar to the toxins (or parts of toxins) of oral bacteria, as well as the detritus that can be found in the saliva and dental plaque. Its empirical formula is $C_9H_{19}N_2O$, molecular weight 171.3, and the structural formula is presented on Figure 2.

The oxygen within the nitrogen oxide radical has an unpaired electron -0^\bullet , and the electronic configuration $1s^22s^2ps^2$ has a spin $S = 1/2$ and orbital moment $L = 1$.

Molecules that have an unpaired electron with S spin are paramagnetic molecules which make paramagnetic centers in the diamagnetic matrix.

Organic spins marker 4-Amino-Tempo with a stable nitrogen oxide radical represents a paramagnetic molecule whose EPR spectrum has a characteristic

triplet structure, which is presented at Figure 3. The value of the first integral of the first derivative of the EPR spectrum is presented in Figure 4.

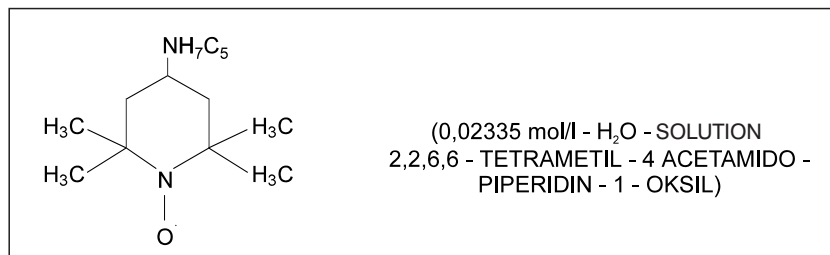


Figure 2. Structural formula of organic 4-Amino-Tempo spin marker

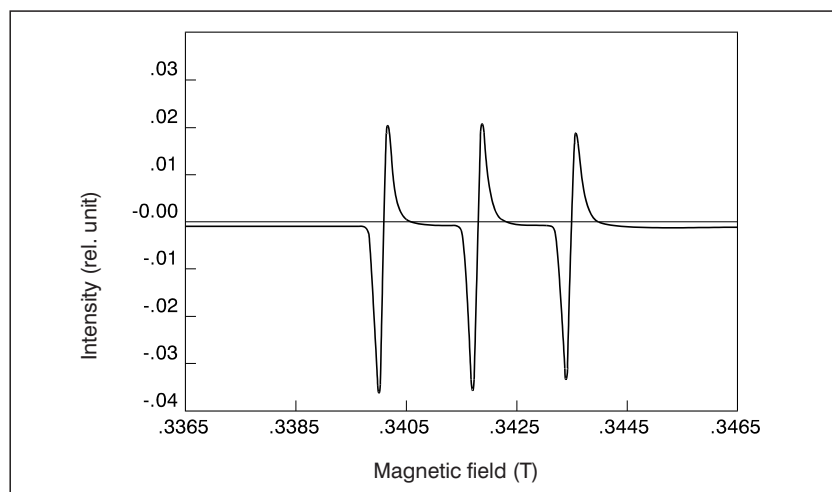


Figure 3. First derivation of the ERP spectrum obtained on the water solution of the 4-Amino-Tempo marker concentration $C = 0.02335 \text{ M}$

Three groups of enamel tissue preparations, prepared in different ways, have been used as a test material.

The experimental study *in vivo* was performed on three dogs.

Molars on right side of the lower jaw, in all experimental animals had enamel tissue reduction, without putting protection, while teeth on left side were intact and they represent the control group. After 12 months animals were sacrificed, experimental and control teeth extracted and the studying of diffusion carried on *in vitro*.

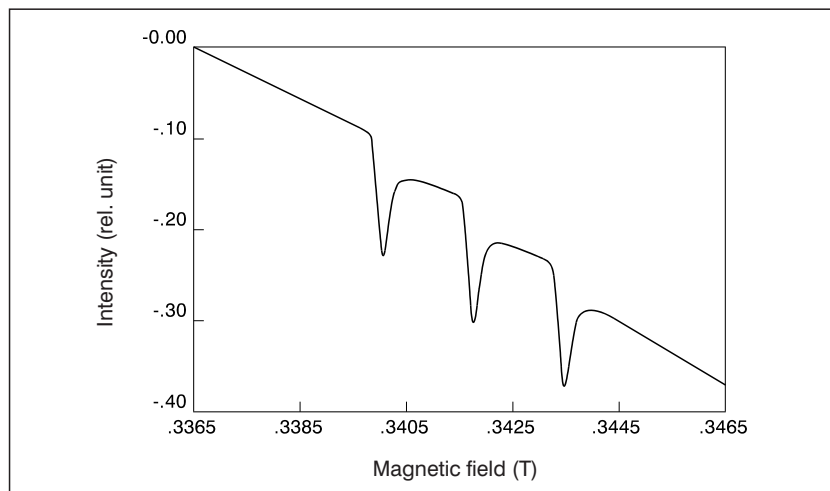


Figure 4. Integral of the first derivation of EPR spectrum of water solution of 4 Amino-Tempo marker concentration $C = 0,02335 \text{ M}$

The size of the prepared enamel preparations was approximately $0.4 \text{ cm} \times 0.3 \text{ cm} \times 0.25 \text{ cm}$, where the $h = 0.4 \text{ cm}$ is the cervico-occlusion orientation, $s = 0.3 \text{ cm}$ vestibulo-oral orientation and $l = 0.25 \text{ cm}$ thickness of the enamel.

Based on the mode of sample and tissue preparation the experimental groups were formed in the following way:

I GROUP – Intact enamel – samples of the intact enamel tissue obtained from extracted intact teeth of experimental dogs.

II GROUP – Young non-intact enamel – enamel tissue was reduced after tooth extraction.

III GROUP – Old non-intact enamel – Reduction of enamel tissue was performed one year prior to the extraction of the tooth. Protection of the reduced enamel tissue has not been performed.

Immediately after tooth extraction in a dog, enamel tissue preparation was cut out by a high velocity machine with a diamond grinding plate. Checking for the presence of dentine was made with a UV light. For each group 20 preparations of enamel tissue were made.

Observation on EPR Spectrometer was performed prior to the immersion in the organic spin marker solution. None of the samples showed the presence of resonant signals which would be a consequence of paramagnetic centers within. The prepared preparations could thus be used in further experiments with marker diffusion.

All samples were marked and immersed in the water solution of organic spin marker 4-Amino-Tempo. The time of monitoring the bilateral diffusion process was for the 1st and 2nd group 842.5 hours (35 days), and for the 3rd group 1010.5 hours (42 days).

The method of electronic paramagnetic (*spin*) resonance, representing a spectroscopy method, was used for the test. The experiment was carried out by an EPR Spectrometer Varian E-9 at the Institute for Nuclear Sciences in Vinča.

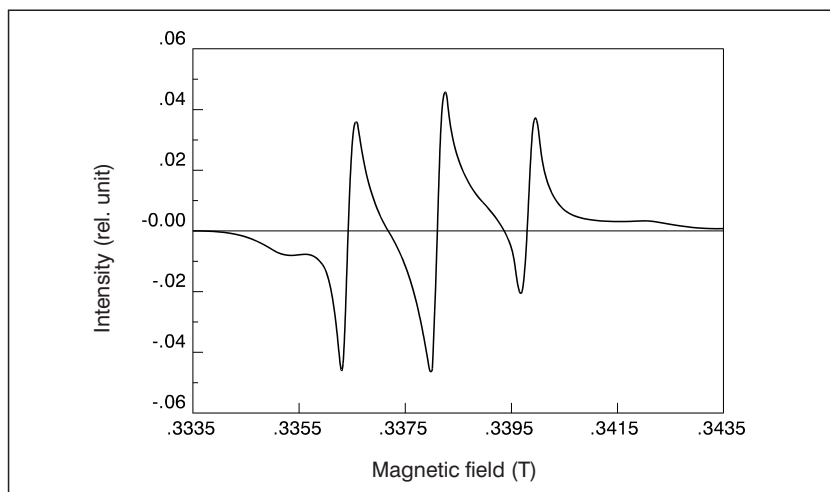


Figure 5. First derivative of EPR spectrum observed on the preparation of intact enamel tissue (G_5) held in a water solution of 4-Amino-Tempo marker for 46 hours at a temperature of $t = 17^\circ\text{C}$

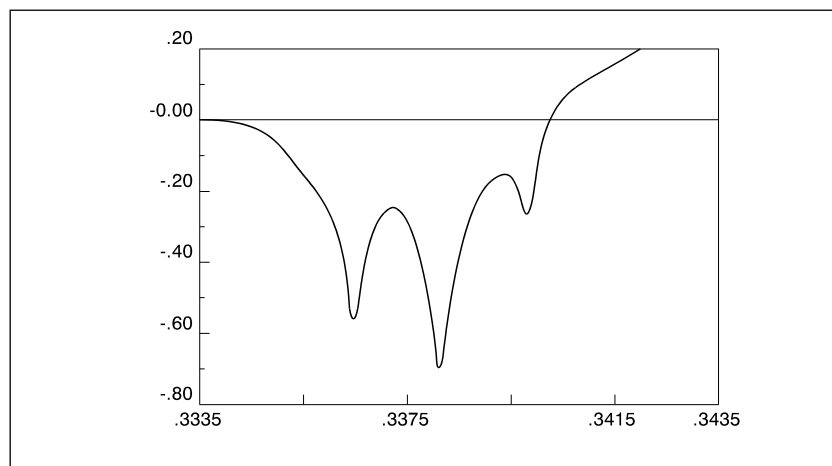


Figure 6. Integral of the first derivative of ERP spectrum of preparation of intact enamel tissue (G_5) observed after 46 hours of diffusion of 4-Amino-Tempo at a temperature of $t = 17^\circ\text{C}$

The quantity of diffused paramagnetic marker molecules was determined by measurements of the EPR enamel spectrum at different time intervals. The enamel samples (rinsed with distilled water and dried) were placed within a test tube in the EPR Spectrograph resonant cuvette. Within the range of the magnetic field of the resonant cuvette there was an interaction of the magnetic effects and paramagnetic molecules was detected by the appearance of a triplet spectrum.

The spectrums are detected by the EPR Spectrometer (Figure 5) and the integrals of the first derivative of the EPR spectrum have been calculated by a special computer program (Figure 6).

RESULTS

Analysis of the bilateral process of organic spin 4-Amin-Tempo marker diffusion in dogs, enamel tissue was made by measurements of the first derivative of the EPR spectrum, of all three groups in defined time intervals. Determination of the values of the integrals below the central absorption line was observed for all spectrums.

I GROUP – INTACT ENAMEL

Table 1. Samples of the 1st Group - Intact enamel: Values of integrals, number of molecules in the entire volume of the sample and number of molecules that have entered a sample per unit of surface

Sec	Hours	Days	Integrals	Q_{mer}	$Q=Q_{mer}/sh$ ($1/cm^2$)
1.818×10^5	50.5	2	–	–	–
3.546×10^5	98.5	4	–	–	–
6.138×10^5	170.5	7	2.43	4.2×10^{14}	42×10^{14}
8.730×10^5	242.5	10	3.74	5.6×10^{14}	56×10^{14}
2.186×10^5	338.5	14	3.66	5.6×10^{14}	56×10^{14}
13.914×10^5	386.5	16	4.05	6.1×10^{14}	61×10^{14}
15.642×10^5	434.5	18	4.59	6.8×10^{14}	68×10^{14}
18.234×10^5	506.5	21	4.17	6.2×10^{14}	62×10^{14}
19.962×10^5	554.5	23	3.82	5.8×10^{14}	58×10^{14}
21.690×10^5	602.5	25	4.53	6.6×10^{14}	66×10^{14}
24.282×10^5	674.5	28	4.13	6.2×10^{14}	62×10^{14}
26.010×10^5	722.5	30	4.95	7.2×10^{14}	72×10^{14}
27.738×10^5	770.5	32	4.67	6.8×10^{14}	68×10^{14}
30.330×10^5	842.5	35	4.87	7.2×10^{14}	72×10^{14}
33.786×10^5	890.5	37	–	–	–
37.242×10^5	938.5	39	–	–	–
38.970×10^5	1010.5	42	–	–	–

The Table 1 presents the values of integral I, the number of unbound molecules of markers that have diffused through the intact enamel over the entire volume of the sample Q_{mer} , and the number of unbound molecules that have entered the sample with thickness l per surface

$$Q = Q_{mer} / sh \quad \text{for different diffusion times.}$$

The number of diffused molecules of Q marker in a sample of intact enamel is presented in Figure 7.

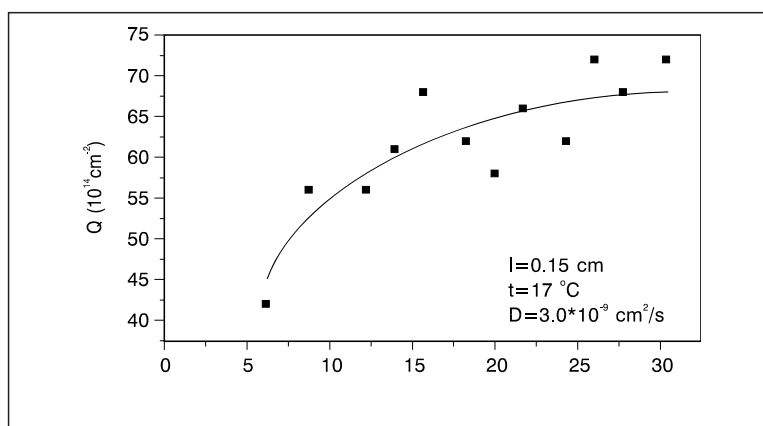


Figure 7. Number or unbound marker molecules that have entered the intact enamel, thickness $l = 0.15 \text{ cm}$, per unit of sample surface subject to diffusion time

II GROUP – YOUNG NON-INTACT ENAMEL

Parameters of molecule marker diffusion in the young non-intact enamel tissue determined from the EPR spectrum of these samples are presented in Table 2 .

The dependence on the of the diffusion of Q marker molecules in the young non-intact enamel is presented in Figure 8.

Table 2. Samples of the 2nd Group – Young non-intact enamel: Values of integrals, number of molecules in the entire volume of the sample and number of molecules that have entered a sample per unit of surface

Sec	Hours	Days	Integrals	Q_{mer}	$Q=Q_{mer}/sh$ ($1/cm^2$)
1.818×10^5	50.5	2	0.56	2.2×10^{14}	24.4×10^{14}
3.546×10^5	98.5	4	1.08	2.8×10^{14}	31.1×10^{14}
6.138×10^5	170.5	7	1.18	2.9×10^{14}	32.2×10^{14}
8.730×10^5	242.5	10	1.39	3.1×10^{14}	34.4×10^{14}
12.186×10^5	338.5	14	–	–	–
13.914×10^5	386.5	16	2.81	4.6×10^{14}	51.1×10^{14}
15.642×10^5	434.5	18	3.20	5.1×10^{14}	56.7×10^{14}
18.234×10^5	506.5	21	2.80	4.6×10^{14}	51.1×10^{14}
19.962×10^5	554.5	23	3.22	5.1×10^{14}	56.7×10^{14}
21.690×10^5	602.5	25	3.17	5.0×10^{14}	55.6×10^{14}
24.282×10^5	674.5	28	3.61	5.6×10^{14}	62.2×10^{14}
26.010×10^5	722.5	30	3.45	5.4×10^{14}	60.0×10^{14}
27.738×10^5	770.5	32	–	–	–
30.330×10^5	842.5	35	3.19	5.1×10^{14}	56.7×10^{14}
33.786×10^5	890.5	37	–	–	–
37.242×10^5	938.5	39	–	–	–
38.970×10^5	1010.5	42	–	–	–

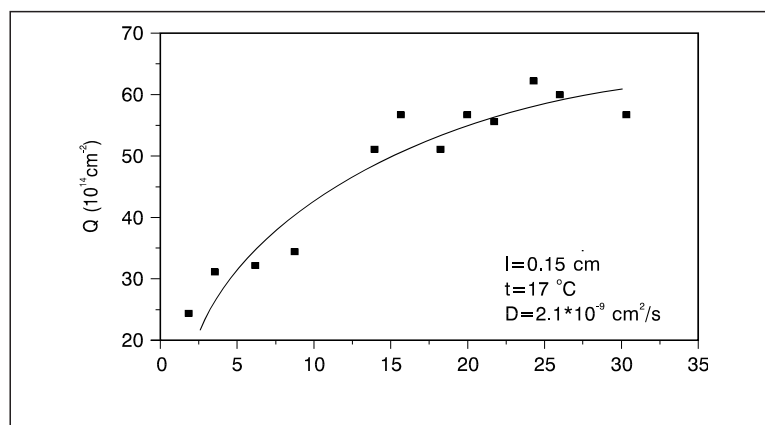


Figure 8. Number of unbound marker molecules that have entered the young non-intact enamel, thickness $l=0.15$ cm, per unit of sample surface subject to diffusion time

III GROUP – OLD NON-INTACT ENAMEL

Table 3. Samples of the 3rd Group – Old non-intact enamel: Values of integrals, number of molecules in the entire volume of the sample and number of molecules that have entered a sample per unit of surface

Sec	Hours	Days	Integrals	Q_{mer}	$Q=Q_{mer}/sh$ ($1/cm^2$)
1.818×10^5	50.5	2	1.07	5×10^{14}	45.5×10^{14}
3.546×10^5	98.5	4	1.66	5.6×10^{14}	50.9×10^{14}
6.138×10^5	170.5	7	2.01	6.3×10^{14}	57.3×10^{14}
8.730×10^5	242.5	10	–	–	–
12.186×10^5	338.5	14	2.43	6.6×10^{14}	60.0×10^{14}
13.914×10^5	386.5	16	2.37	6.5×10^{14}	59.1×10^{14}
15.642×10^5	434.5	18	3.17	7.5×10^{14}	68.2×10^{14}
18.234×10^5	506.5	21	2.95	7.0×10^{14}	63.6×10^{14}
19.962×10^5	554.5	23	2.91	7.0×10^{14}	63.6×10^{14}
21.690×10^5	602.5	25	2.64	6.7×10^{14}	60.9×10^{14}
24.282×10^5	674.5	28	2.84	6.9×10^{14}	62.7×10^{14}
26.010×10^5	722.5	30	2.74	6.8×10^{14}	61.8×10^{14}
27.738×10^5	770.5	32	2.84	6.9×10^{14}	62.7×10^{14}
30.330×10^5	842.5	35	2.76	6.8×10^{14}	61.8×10^{14}
33.786×10^5	890.5	37	2.93	7.0×10^{14}	63.6×10^{14}
37.242×10^5	938.5	39	–	–	–
38.970×10^5	1010.5	42	–	–	–

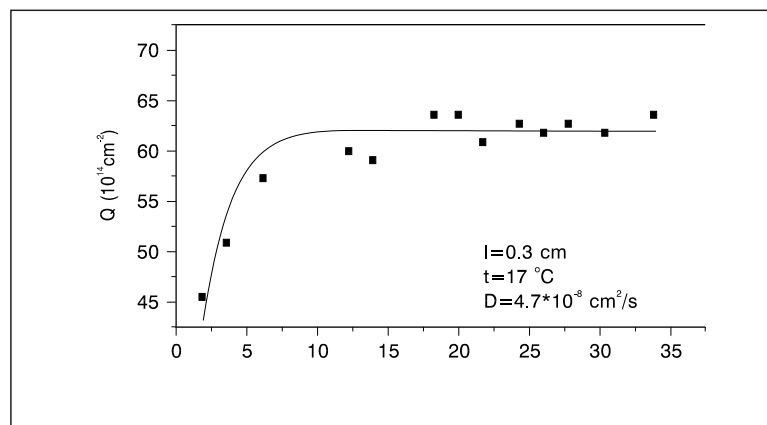


Figure 9. Number of unbound marker molecules that have entered the old non-intact enamel, thickness $l=0.3 \text{ cm}$, per unit of sample surface subject to diffusion time

Interprismatic spaces of experimental teeth were accessible to imbibition of different agents from the environment, before the beginning of the experiment while still in the dog's mouth. The market diffusion parameters in the old non-intact enamel are presented in Table 3, and the dependence of the number of diffused molecules on time of diffusion is presented on Figure 9.

The experimentally determined dependence of the number of diffused marker molecules in the enamel tissue for a sufficient diffusion time may be approximated by the formula:

$$Q = N_0 \left(1 - \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} e^{-\pi^2 D n^2 t / l^2} \right) \quad (4)$$

where l = sample thickness, N_0 – number of particles and D – diffusion coefficient. Full lines on figures 7, 8 and 9 represent the dependence (4) adjusted to the experimental value.

The saturation value of the number of diffused molecules and the diffusion parameters at a temperature $t = 17^\circ\text{C}$ are presented on Table 4.

Table 4. Saturation value of the number of diffused marker molecules and diffusion coefficient at a temperature of $t = 17^\circ\text{C}$

Preparations	l (cm)	s (cm)	h (cm)	N_0 (cm ⁻²)	D (cm ² s ⁻¹)
(1)	0.15	0.20	0.50	69.3×10^{14}	3.0×10^{-9}
(2)	0.15	0.20	0.45	65.0×10^{14}	2.1×10^{-9}
(3)	0.30	0.20	0.55	61.7×10^{14}	4.7×10^{-8}

DISCUSSION

Organic molecules of the 4-Amino-Tempo spin marker organic molecules diffused through the enamel tissue thanks to its molecular structure and high reactivity of the nitrogen oxide radical.

The bilateral process of marker diffusion going on in the enamel tissue is characterized by concentration of diffused molecules and diffusion coefficient.

It was observed that the intact enamel and the freshly reduced enamel tissue show similar behavior with respect to diffusion. Saturation of diffused marker molecule occurs after 4-5 weeks, which is also corroborated by the same saturation concentration of the diffused marker molecules:

$$\begin{aligned} N_0^{\text{IG}} &= 0.46 \times 10^{17} \text{ cm}^{-3} && \text{INTACT ENAMEL} \\ N_0^{\text{RG}} &= 0.43 \times 10^{17} \text{ cm}^{-3} && \text{YOUNG NON-INTACT ENAMEL} \end{aligned}$$

And approximately the same diffusion coefficient:

$$\begin{aligned} D^{\text{IG}} &= 3.0 \times 10^9 \text{ cm}^2 \text{ S}^{-1} \\ D^{\text{RG}} &= 2.1 \times 10^9 \text{ cm}^2 \text{ S}^{-1} \end{aligned} \quad t = 17^\circ\text{C}$$

Contrary to these tissues the reduced enamel tissues which had been in the dog's mouth for a long time (period of one year) without protection, had a smaller saturation value of concentration of diffused marker molecules.

$$N_0^{\text{SIG}} = 0.21 \times 10^{17} \text{ cm}^{-3} \quad (\text{OLD NON-INTACT ENAMEL})$$

Marker molecules appear after 10 days showing a significantly higher diffusion coefficient:

$$D^{\text{SRG}} = 4.7 \times 10^8 \text{ cm}^2 \text{ s}^{-1}, \quad t = 17^\circ\text{C}$$

Faster diffusion and penetration of organic molecules into the old non-intact enamel tissue indicates the harmful effects of leaving reduced enamel without protection.

In monitoring the diffusion process oscillations of the number of diffused marker molecules around the monotonous dependence of their concentration on the time and thickness of the preparation, which indicates the process of binding the marker molecules in the enamel tissue. One of such processes could be the substitution of ions from the crystal grid of the apatite by an N-O ion from the marker molecule.

The available reference data on the properties of diffusion in the enamel (Borggreven, 1980; Dibdin, 1972; Chick, 1963; Kambera, 1977 and Funduk, 1975) can only be roughly compared. Analyses of diffusion process of water, sodium and manganese in the enamel tissue represent unilateral diffusion processes, whereas diffusion of the organic spin marker 4-Amino-Tempo is the result of a bilateral diffusion process (Tihaček-Šojić, 2002; 2004).

CONCLUSIONS

From the results the following may be concluded:

I. organic molecules of the 4-Amino-Tempo spin marker diffused through enamel tissue

II. diffusion of the organic molecules is a bilateral process which is characterized by the concentration of diffused molecules and the diffusion coefficient.

III. In the intact and fresh non-intact enamel tissue during diffusion a maximum quantity of diffused organic molecules is observed after several weeks. Their diffusion coefficients have almost the same values

IV. With the old non-intact enamel tissues, harmful consequences of leaving the reduced enamel tissue without any protection are observed

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DUBINA DIFUZIJE ORGANSKIH MOLEKULA U GLEDNOM TKIVU KOD PASA

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SADRŽAJ

U ovom radu prikazani su rezultati dubine difuzije organskih molekula u glednom tkivu stalnih zuba. Eksperimentalna istraživanja *in vivo* vršena su na psima. Na molarima desne strane donje vilice, kod svih životinja eksperimentalno je izvršena redukcija glednog tkiva, bez postavljanja zaštite, dok su zubi leve strane mandibule ostali intaktni i predstavljali su kontrolnu grupu. Period *in vivo* je trajao 12 meseci, posle čega su životinje žrtvovane, eksperimentalni i kontrolni zubi izolovani i nastavljeno ispitivanje procesa difuzije *in vitro*. U te svrhe izabran je organski spinski marker 4-amino-2,2,6,6-teramentil-piperidinil-1-oksi (4-Amino Tempo). Osobina ovog markera je da ima u svom sastavu nitroksidni radikal koji je stabilan u neutralnoj i baznoj sredini, a zbog nesporenog spina slobodnog radikala veoma je pogodan za istraživanja metodom elektronske paramagnetne resonance.

Ispitivanja difuzije organskog spinskog markera u gleđnom i dentinskom tkivu su izvršena potapanjem zubnih preparata u vodeni rastvor markera. Iz inteziteta EPR spektara, snimljenih na preparatima posle njihovih vađenja iz vodenog rastvora, ustanovljena je raspodela broja difundovanih molekula markera u preparatima u zavisnosti od vremena difuzije i dubine prodiranja. Određeni su koeficijenti difuzije organskog spinskog markera za gleđ i dentin i saturaciona koncentracija molekula markera u gleđnim preparatima. Uočene varijacije u prostornoj i vremenskoj raspodeli roja difundovanih molekula objašnjene su sa mogućnošću vezivanja N-O – radikala iz molekula markera za gleđno tkivo.

Rezultati eksperimentalnih istraživanja sadržani u ovom radu doprinose boljem razumevanju procesa kretanja molekula organskog spinskog markera kroz gleđno tkivo koji je kontrolisan mehanizmom difuzije i hemijskim vezivanjem molekula markera za strukturu gleđi. Rezultati eksperimentalnih istraživanja takođe ukazuju na moguće štetne posledice ostavljanja redukovano gleđnog tkiva bez zaštite.