Release of nisin from polyvinyldichloride lacquer coated on a polyethylene film

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Summary

Polyethylene films coated with commercially available polyvinyldichloride (PVdC) lacquer with the addition of nisin preparation Nisaplin (5.0% to 33.4%, w/w), i.e. with nisin concentration from 16 500 IU·dm^{-2} to 110 200 IU·dm^{-2}, were studied at 25 °C to determine the bacteriocin migration into physiological saline solution. The maximal level of released nisin from films was 5 000 IU·dm^{-2}, which was less than 10% of the total theoretical amount of nisin added to the coating. Equilibrated nisin migration depended on coating thickness. A non-linear relation between the equilibrium nisin migration level and the coating thickness of the nisin preparation was found. A diffusion coefficient of 2.37×10^{-10} cm^2·s^{-1} ± 0.82×10^{-10} cm^2·s^{-1} was determined on the basis of migration data.

Keywords

active food packaging; nisin; bacteriocin; diffusion; polymer film

Active packaging systems, including those with antimicrobial activity, represent one of the most studied topics in food packaging. At present, there is no doubt about their beneficial application potential for prolongation of the shelf-life of many types of foodstuffs, in particular chilled and minimally processed foods. Different types of such systems as well as the possibility of their application in food processing were recently reviewed [1–6].

The bacteriocin nisin, produced by *Lactococcus lactis* ssp. *lactis*, is widely used in dairy industry as a food preserving agent against Gram-positive bacteria [7, 8]. The possibility of nisin application to foodstuffs via packaging has been studied extensively. More than 50 articles have been published on nisin-containing films and/or coatings, both natural and synthetic, after the year 2000. The studies covered the bacteriocin application into edible films formed from peptides [9–14], polysaccharides [15–18], modified cellulose [19–27], bacterial cellulose [28], as well as synthetic polymers, namely, crosslinked polyvinylalcohol [29], polyethylene [30–33], polyvinyl chloride [33], polyamide [33], silicon [34], vinyl acetate-ethylene copolymer [35–37], acrylic polymer [37], vinyl chloride-vinylidene chloride copolymer [38], etc.

In the authors' laboratory, polymer packaging films with linked antimicrobial agents have been studied for nearly ten years [39, 40]. Besides the films treated with the traditional food preservatives, the ionomer films with nisin fixed on the surface using the Ugi reaction were tested [39]. The maximal level of nisin migration into food simulants for this type of the film was about 300 IU·dm^{-2}, which was not enough for practical application. Moreover, there was a little chance of wider use of such films due to their difficult preparation. In order to prepare films suitable for practical application in food industry, we have decided to carry out experiments in which polymers commonly used as a food contact layer in flexible packages were tested, and the antimicrobial agents were incorporated into synthetic lacquers. The application of such lacquers could be carried out

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using equipment utilized by food packaging film producers. Using this strategy, we prepared low-density polyethylene (LDPE) coated with a synthetic lacquer on the basis of polyvinyldichloride. Functionality of this type of packaging materials has already been proved in studies on inhibition of microbial growth on surfaces of packaged cheese and salami [41]. The aim of this article is a more detailed description of nisin migration from a lacquer coated on the LDPE film.

MATERIALS AND METHODS

Packaging material

Polyethylene film (LDPE, thickness 30 μm; Aliachem, Fatra, Napajedla, Czech Republic) with one side treated with corona was used. The corona-treated surface of LDPE film was coated with a lacquer based on polyvinyldichloride (Kombilack L-1917; Rotoflex, Grenchen, Switzerland), containing 5–33.4% (w/w) commercial nisin preparation Nisaplin (Danisco, Copenhagen, Denmark) with activity of 1×10^6 IU·g^{-1}. The preparation Nisaplin contains 2.5% of pure nisin.

Coating of lacquer on LDPE films

The method described below was developed in the authors' laboratory as the optimal procedure under given conditions, i.e concerning final viscosity, lacquer dosage, etc. Before nisin addition, the solid content of the lacquer was determined by drying at 105 °C and adjusted to 30% (w/w) by diluting the lacquer with butan-2-one. Amount of 0.5 g of Nisaplin, i.e. 12.5 mg of nisin with activity of 5×10^5 IU, was dispersed thoroughly in 5 ml of butan-2-one and mixed with 10 g of Kombilack lacquer. A sheet of LDPE film (20 cm × 35 cm) was fixed tightly in the frame with the corona-treated side up, and the lacquer with nisin (5 ml) was poured evenly along the shorter side. The lacquer was manually spread on the film surface using a coating rod (stainless steel rod, 5 mm in diameter, tightly coiled with stainless wire of 0.5 mm in diameter). The film with the coating was let in a fume hood over night at the laboratory temperature, and then it was dried at 40 °C for 1 h. The thickness of the lacquer layer was determined by a micrometer (SE 051, type D2M; Lorentzen and Wettre, Kista, Sweden) and the films with uniform coating with thickness of 13–57 μm were used for migration tests.

One set of LDPE films was coated on an industrial production line (Invos, Svárov, Czech Republic) using the flexography coating machine Soma Flex Midi (Soma Engineering, Lanškroun, Czech Republic). In this case, 10 kg of the PVdC lacquer were prepared and coated on the film using the same ratio of bacteriocin and solvent as mentioned above, i.e. 0.5 kg of Nisaplin and 5 l of butan-2-one.

Nisin migration test

Migration of nisin from packaging films into acidified physiological saline solution was studied under the following conditions: 100 cm^2 of the film was fixed in a glass cell, Helendahl cuvette (Fig. 1), poured over with 70 ml of a sterile acidified physiological saline solution (8.5 g NaCl and 1.7 ml of 36% HCl in 1 l of distilled water) and shaken in a water bath at 25 °C for 48 h at a shaking frequency of 1 Hz. During migration tests, the films were fixed in Helendahl cuvettes in a way that both sides of the tested films were in contact with the simulant. Samples (0.5 ml) were withdrawn with a sterile pipette at various times, maximally twelve times from one cuvette. Nisin released from film into water was determined as described below.

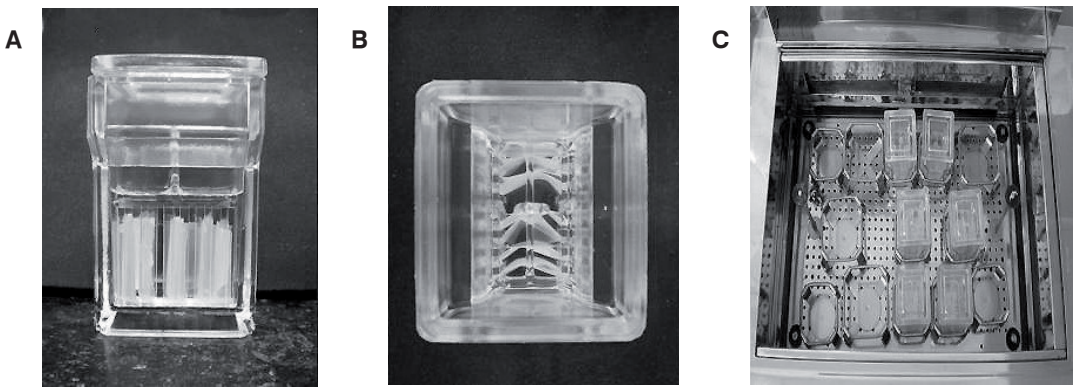

Fig. 1. The testing device used for the nisin migration test - Helendahl cuvettes with tested film (A,B); thermostatic water bath with Helendahl cuvettes (C).

The calculation of the final migration level included the correction for the change in water volume as well as for the amount of nisin withdrawn during previous sampling.

Determination of nisin in the physiological saline solution

A bioassay for the detection of nisin was based on the agar diffusion method [42] and was performed as described previously [43]. The indicator strain *Lactobacillus helveticus* CH-1 was cultivated in MRS broth (Oxoid, Basingstoke, United Kingdom) at 42 °C for 16 h. The diffusion of nisin was carried out in MRS agar (Oxoid) using preincubation of plates at 4 °C for 6 h, followed by incubation at 42 °C for 20 h. Nisaplin was used as a standard for the bioassay. The diameters of inhibitory zones (clear zones) formed around the samples were measured (Fig. 2).

Three parallel determinations by agar diffusion method were done for all of the withdrawn samples and the mean value (x) and the standard deviation (SD) were calculated for each of the following parameters. The results in the following text are in the form $x \pm SD$, SD values are expressed by line segments linked with the plotted points in the figures.

Calculation of diffusion coefficients of nisin in the lacquer layer

The course of migration from the polymer matrix can be described by the following equation [44-46]:

$$\frac{M_{Ft}}{M_{F\infty}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left[-\frac{(2n+1)^2 \pi^2}{4 d_P^2} Dt \right] \quad (1)$$

where M_{Ft} is the amount of the migrant in the food in the particular time t (mg), $M_{F\infty}$ is the amount of the migrant in the food at equilibrium (mg), t is time (s), d_P is the thickness of the polymer layer (cm) and D is the diffusion coefficient of the migrant in the polymer ($cm^2 \cdot s^{-1}$).

The equation (1) is valid on the assumptions that the migrant is distributed uniformly in the packaging film; the migration occurs from one side of the packaging film to a liquid food; the liquid food is well mixed so that there is no migrant concentration gradient in the food; diffusion coefficient and partition coefficient are constant during migration and they are dependent on temperature only; equilibrium exists all the time during the migration at the interface of the packaging film and the food; edge effects and interactions between the packaging film and the food are negligible; the mass transfer is mainly controlled by diffusion taking place in the polymer; and the polymer volume is limited while the food volume is infinite.

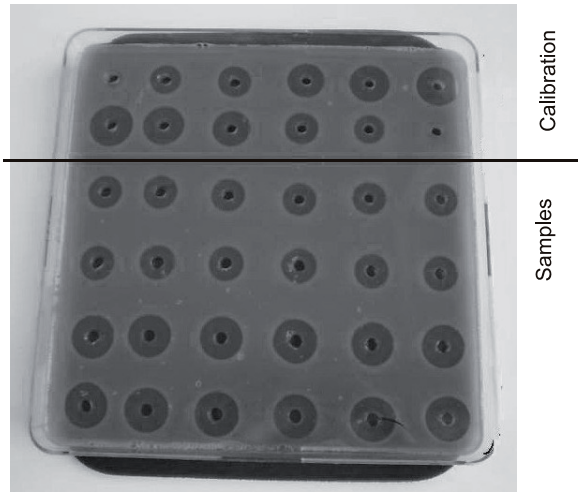

Fig. 2. Illustration of the results of the agar diffusion method with visible inhibitory zones (clear zones).

The experimental data of the course of nisin migration (M_t/M_∞ versus time) were compared with the equation (1). We supposed that the assumptions mentioned above were fulfilled as well as nisin migration from the lacquer layer into the polymer was negligible when compared with that into the physiological saline solution under the test conditions. Diffusion coefficients were calculated from the equation (1) using the following procedure:

1. The dimensionless quantity η representing the relative degree of the establishment of equilibrium between the migrant concentration in the polymer and the food simulant (in our case the physiological saline solution) was introduced as

$$\eta(t) = \frac{M_{Ft}}{M_{F\infty}} \quad (2)$$

   where η is a function of time (t) and its physical meaning is clear. At the beginning of migration, $t = 0$, no migrant was released, $M_{F0} = 0$, and therefore $\eta(0) = 0$. The system was not in the equilibrium state. During the migration, value M_{Ft} grew and value $\eta(t)$ rose. The system was approaching the equilibrium state and, when it was established, $M_{Ft} = M_{F\infty}$, $\eta(\infty) = 1$. It is obvious that values η were in the interval <0, 1>.
2. In order to express the time of migration, the dimensionless quantity Fo (Fourier number) was used:

$$Fo = \frac{Dt}{d_P^2} \quad (3)$$

Tab. 1. Values of the relative degree of equilibrium between migrant concentration in polymer and food η calculated for the specific Fourier numbers *Fo*.

Fo	η(*Fo*)	*Fo*	η(*Fo*)	*Fo*	η(*Fo*)	*Fo*	η(*Fo*)	*Fo*	η(*Fo*)
0.00000	0.00000	0.00500	0.07979	0.25000	0.56223	0.60000	0.81556	1.25000	0.96290
0.00005	0.00798	0.01000	0.11284	0.30000	0.61324	0.65000	0.83697	1.50000	0.97998
0.00010	0.01128	0.02000	0.15958	0.35000	0.65819	0.70000	0.85589	1.75000	0.98920
0.00020	0.01596	0.05000	0.25231	0.40000	0.69788	0.75000	0.87262	2.00000	0.99417
0.00050	0.02523	0.10000	0.35682	0.45000	0.73295	0.80000	0.88740	2.50000	0.99830
0.00100	0.03568	0.15000	0.43695	0.50000	0.76395	0.90000	0.91202	3.00000	0.99951
0.00200	0.05046	0.20000	0.50409	0.55000	0.79135	1.00000	0.93126	–	–

3. Using the expressions (2) and (3), the equation (1) is changed as follows:

$$\eta(Fo) = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left[-\frac{(2n+1)^2\pi^2}{4} \cdot Fo\right] \quad (4)$$

   It can be proven that for $Fo > 0$, i.e. $t > 0$, the sum of infinite series in the equation (4) is in the interval <0, 1). It means that the relevant values η(*Fo*) must be from the interval <0, 1>.
4. The values η calculated for the selected values *Fo* can be found in Tab. 1. It is obvious that values *Fo* from the interval <0, 3> correspond (with precision sufficient for practical applications) to all values η from 0 to 1.
5. Experimental data on the course of nisin migration were couples of time readings and corresponding migration levels, (t_i, M_{Fi}), $I = 1, 2,... N$ at the known equilibrium level of migration $M_{F\infty}$ and thickness of the lacquer layer d_P.

The value η_i was calculated for each experimental value using the equation (2). Fourier numbers *Fo* were assigned to the value η(*Fo*) using interpolation in Tab. 1. Knowing *Fo*, diffusivity *D* was calculated from equation (3) as

$$D_i = \frac{Fo_i \cdot d_P^2}{t} \quad (5)$$

RESULTS AND DISCUSSION

Lacquers permitted for the treatment of food contact films were used. Previous tests had shown that the lacquers diluted with butan-2-one were more suitable for mixing with Nisaplin compared with those diluted with ethanol and/or based on a water dispersion of polymer (PVdC). The application of Kombilack L-1917 provided the best results.

The results on migration from eight samples of a film manually coated with the lacquer layer with a thickness of 23.2 μm ± 8.4 μm containing 16.7% (w/w) of Nisaplin, i.e. nisin concentration 46 500 IU·dm^{-2}, into the physiological saline solution at 25 °C, are given in Fig. 3. It is obvious that the nisin migration was rapid and nearly all of releasable and active nisin was released within less than 10 h. The average level of the total (equilibrium) migration was 1 337 IU·dm^{-2} ± 192 IU·dm^{-2}. When the migration test was done with the sample which had been already extracted in the physiological saline solution for 24 h, no further release of active bacteriocin was observed. Considering the detection limit of the agar diffusion method (about 250 IU·dm^{-2}), more than 90% of releasable active nisin was transferred into the contact medium.

In order to confirm the results mentioned above, three additional series of films were prepared and the experiments were carried out in the same way. In Fig. 4, results for all series are presented with the ovals A, B, C and D represent-

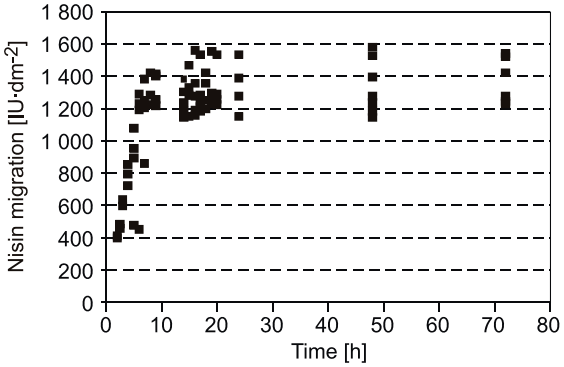

Fig. 3. Course of nisin migration from lacquer coating (thickness 23.2 μm ± 8.4 μm) on LDPE film containing 16.7% (w/w) of Nisaplin, i.e. nisin concentration of 46 500 IU·dm^{-2}, into the physiological saline solution at 25 °C.

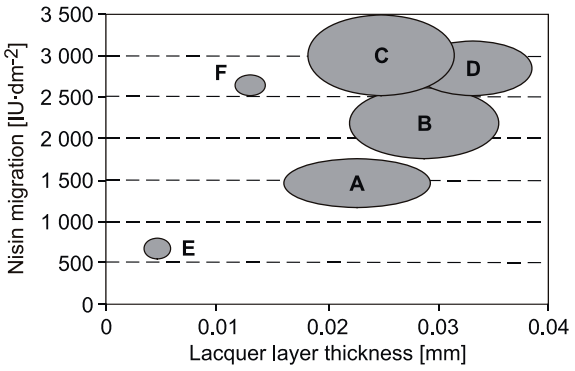

Fig. 4. Relationship between antimicrobial efficiency (corresponding to the ability to release active nisin) of different series of polymer films with nisin, and the range of coating thickness.
Ovals A-D and F represent results for manually coated samples prepared under laboratory conditions, oval E represents results for samples prepared in industrial conditions.

ing the range of coating thickness, and the corresponding level of nisin migration. Due to the manual preparation and impossibility of a precise control of lacquer application, the coating thickness fluctuated from 13 μm to 40 μm. The amount of released nisin ranged from 1 200 IU·dm^{-2} to 3 500 IU·dm^{-2}. On the basis of these data, balance of nisin in the lacquer layer during the migration experiment could be calculated. Considering the thickness range of the prepared coatings, 16.7% (w/w) of Nisaplin in the dry lacquer and a coating density of 1.2 g·cm^{-3}, each square decimeter of prepared films should have contained from 32 000 IU to 71 000 IU of the bacteriocin. It means that from the tested samples, only less than one tenth of the theoretical amount was released in the state of equlibrium. Similar results were published by other authors, reporting that only 15% of the total amount of nisin migrated from gluten and corn zein films at 5 °C during 100 h [9]; 8.6% to 9.3% from the vinyl acetate-ethylene copolymer coating on paper at 10 °C during 8 days [35]; and 7% to 28% from acrylic polymer and vinyl acetate-ethylene copolymer at 10 °C [37].

Under laboratory conditions, we were unable to prepare films with a defined coating thickness different from the samples from series A–D, so we prepared the films in co-operation with the company Invos using their industrial equipment. The prepared film had a coating thickness of 5.0 μm ± ± 0.4 μm (series E in Fig. 2) and the nisin release of 615 IU·dm^{-2} ± 95 IU·dm^{-2}.

Migration is generally considered as a diffusion process and, therefore, the diffusion coefficient of nisin in the lacquer layer could be used for the evaluation of the ability of the substance to be released. The diffusion coefficients of nisin in various polymers have already been calculated from the migration data. Teerakarn et al. [9] determined the diffusion coefficient of nisin in polypeptide films (gluten, corn zein) in the range of 0.77 × 10^{-10} cm^2·s^{-1} to 3.70 × 10^{-10} cm^2·s^{-1} at 25 °C; Buonocore et al. [29] determined D of 0.30 × 10^{-9} cm^2·s^{-1} to 8.60 × 10^{-9} cm^2·s^{-1} in the crosslinked polyvinylalcohol at 25 °C depending on the degree of crosslink; Chan Ho Lee et al. [35] determined D of 0.93 × 10^{-7} cm^2·s^{-1} to 1.13 × 10^{-7} cm^2·s^{-1} in vinyl acetate-ethylene copolymer at 10 °C; Jin Ok Choi et al. [37] determined D of 2.52 × 10^{-8} cm^2·s^{-1} in acrylic polymer and D of 17.5 × 10^{-8} cm^2·s^{-1} in vinyl acetate-ethylene copolymer at 10 °C; and Chollet et al. [26] determined D from 6.5 × 10^{-7} cm^2·s^{-1} to 3.3 × 10^{-6} cm^2·s^{-1} in agarose gel with 5–35% of fat at 25 °C.

As the experiments mentioned above were not suitable for the determination of the diffusion coefficient due to the high variability in the thickness of the lacquer layer (series A–D) and/or the low level of nisin migration (series E), new samples were manually prepared for this purpose (series F in Fig. 4). Probably due to a new batch of Kombilack lacquer, the coating thickness achieved in these experiments was lower in comparison to the previous experiments (series A–D). From about fifty prepared sheets of coated films, seven samples with uniform coating thickness of 14 μm ± 1 μm were selected and used for migration tests. The course of nisin migration from these films is depicted in Fig. 5. It is obvious that the bacteriocin release was quite fast, i.e. nearly all the releasable amount of nisin migrated into the physiological saline solution within 2 h. The final equilibrium effi-

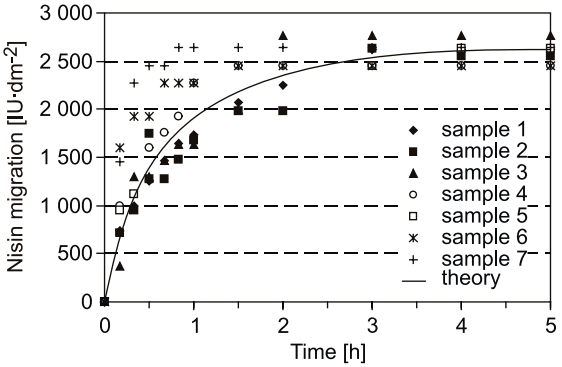

Fig. 5. Course of nisin migration from lacquer coating (thickness 14.0 μm ± 1.0 μm) on LDPE film containing 16.7% (w/w) of Nisaplin, i.e. nisin concentration of 28 000 IU·dm^{-2}, into the physiological saline solution at 25 °C.

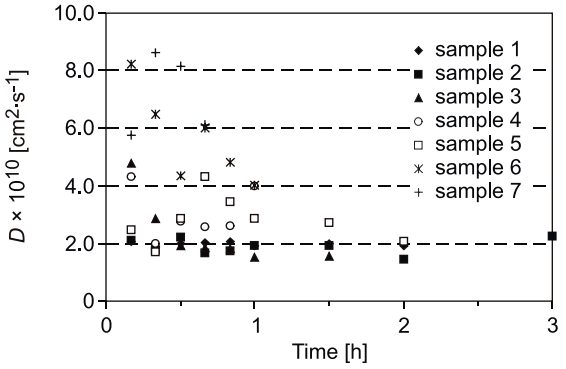

Fig. 6. Estimated values of the diffusion coefficient of nisin in the lacquer layer.

ciency of nisin released into the solution was about 2500 IU·dm^{-2}, which corresponded to the migration level of pure nisin of about 0.625 mg·l^{-1} under the experimental conditions.

Supposing that nisin migration into the physiological saline solution was much faster compared with that into the polymer matrix, and since the experiments were done in good agreement with the theoretical assumptions necessary for the validity of the equation (1), we tried to estimate the diffusion coefficients of nisin in the lacquer layer. The values of diffusion coefficients calculated for all experimental points are plotted against time in Fig. 6. If nisin migration followed Fick's laws, the calculated values should have been close to each other. From Fig. 6 it is obvious that this was true for values obtained for five samples. The differences in samples 6 and 7 could be caused by changes in the lacquer layer structure due to its manual preparation. Considering values calculated for samples 1–5, the value of diffusion coefficient was 2.37×10^{-10} cm^2·s^{-1} ± 0.82×10^{-10} cm^2·s^{-1}. This value was lower compared with the published data mentioned above for nisin diffusion in synthetic polymers.

It has already been mentioned that only less than 10% of the theoretical amount of active nisin added into the lacquer was released into the physiological saline solution. The explanation on the basis of the data mentioned above is not easy, but two reasons may be suggested:

a. a substantial part of nisin is not capable to migrate from the lacquer layer,
b. the activity of bacteriocin in the lacquer is significantly decreased by the film preparation.

Considering the possibility (a), it may also be expected that only the bacteriocin portion close to outer surface can be released into contact media. Under such circumstances, the level of bacteriocin migration should not be related to the thickness of the coating layer as only nisin close to the surface can be released. Fig. 4 indicates that samples with the thinnest coating prepared by Invos provided the lowest nisin migration. However, these samples (series E) were prepared under different conditions compared with the others. To eliminate the influence of conditions under which the lacquer layer was coated, new samples with a controlled thickness of coating layer of 25.0 μm ± 2.8 μm, 26.6 μm ± 3.1 μm, 28.8 μm ± 3.0 μm, 33.9 μm ± ± 3.2 μm, 43.8 μm ± 4.1 μm and 50.7 μm ± 5.5 μm were prepared at a same time. Unfortunately, the preparation of samples out of this range as well as of those with lower thickness variability was out of the authors' experimental possibilities.

The maximum levels of bacteriocin migration from these films are given in Fig. 7. In fact, the results are similar to data given in Fig. 4. The trend of proportionality between the maximal migration and coating thickness is obvious, even though the correlation was not high. Differences between samples covered by thicker and thinner coating were statistically significant ($\alpha = 0.05$). These results indicate that nisin can diffuse through the lacquer layer and the calculation of diffusion quotients is meaningful.

Besides the influence of lacquer layer thickness, the influence of nisin concentration in the lacquer layer on the level of its migration into the physiological saline solution was also determined. Similarly to the previous case, new samples of films with different Nisaplin contents in the lacquer layer (5.0% w/w, 10.0% w/w, 16.7% w/w, 23.4% w/w, 33.4% w/w, corresponding to nisin concentration 16500 IU·dm^{-2}, 33000 IU·dm^{-2}, 55100 IU·dm^{-2}, 77200 IU·dm^{-2}, 110200 IU·dm^{-2}) were prepared together. The samples with coating

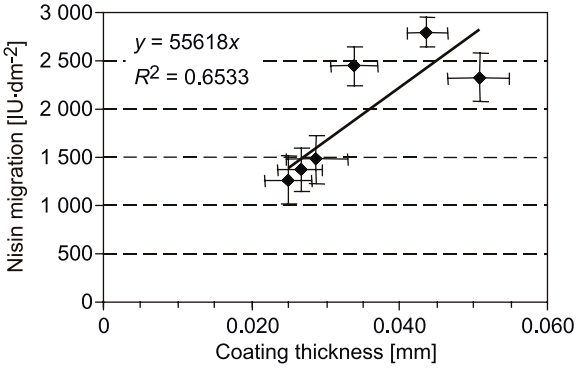

Fig. 7. Relationship between the lacquer layer thickness and the maximal nisin migration at 25 °C.

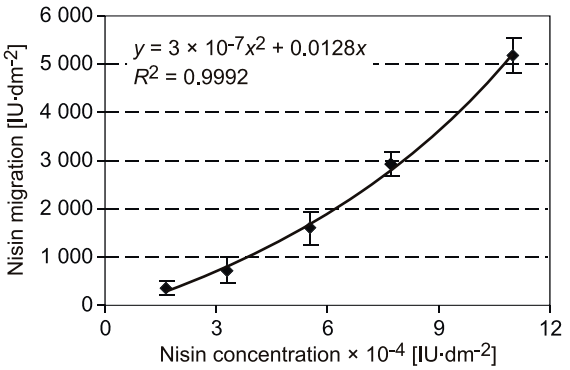

Fig. 8. Relationship between maximal nisin migration and nisin concentration in the lacquer layer (coating thickness 27.5 μm ± 3.0 μm, 25 °C).

thickness of 27.5 μm ± 3.0 μm were selected from the prepared films.

The relationship of nisin concentration in the lacquer layer and the maximal (equilibrium) nisin migration is shown in Fig. 8. The relationship found between the two sets of values was not linear, as it had been theoretically supposed [47]. This could be caused by a large nisin concentration range, as the release of nisin from the lacquer changes the properties of the lacquer and make it more open. Also the bindings between nisin and the lacquer will be different at larger concentrations. At small concentrations, it can be expected that most nisin molecules are uniformly dispersed in the lacquer, whereas nisin molecules will tend to lump together at higher concentrations, while the average binding will decrease with the increase in nisin concentration.

CONCLUSIONS

The aim of this study was to describe in detail the way of the release of nisin from polyethylene films coated with PVdC lacquer with the addition of Nisaplin into the physiological saline solution. The films coated with the lacquer layer (thickness from 13 μm to 57 μm, containing 16.7% of Nisaplin, i.e. nisin concentration from 26 000 IU·dm^{-2} to 114 200 IU·dm^{-2}) released active nisin on the maximal level of about 3 500 IU·dm^{-2} at 25 °C, i.e. less than 10% of the total theoretical amount. Equilibrated nisin migration depended on coating thickness, but the correlation was not high under the used experimental conditions. A non-linear relationship between the maximal nisin migration and nisin concentration in the lacquer layer was found at a coating thickness of 27.5 μm ± 3.0 μm at 25 °C. On the basis of the migration data, the diffusion coefficient of 2.37×10^{-10} cm^2·s^{-1} ± 0.82×10^{-10} cm^2·s^{-1} was determined for the transport of nisin in the lacquer layer at 25 °C.

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