

Release of Naturally-Derived Antimicrobial Agents from LDPE Films

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ABSTRACT: The migration of the natural antimicrobial (AM) agents: linalool, carvacrol and thymol, from low-density polyethylene (LDPE) films containing ethylene vinyl acetate (EVA) into the food simulants: isooctane and various ethanol/water mixtures, was studied with a view towards examining the applicability of a first-order kinetic approach as well as a diffusion model approach for describing these systems. The results suggest the proposed models adequately describe the release of AM agents. The combination of kinetic and diffusion analyses can provide additional information about the release process using the same data set. The analyses suggest that the release of linalool from LDPE/EVA depends on the EVA content in the formulation and that an optimum level of EVA is required to minimize the rate of release. An extension to the existing "idealized diffusion" model is proposed that enables the model to be applied to systems that demonstrate a departure from linearity when subjected to conventional analysis. The applicability of the idealized diffusion model was compared with the "simulant-limited" model and the results suggest that the former model is appropriate for describing most real systems when the simulant (or foodstuff) is favored in the partitioning of the AM agent between the film and the simulant.

Keywords: antimicrobial film, natural antimicrobial agents, migration, diffusion, LDPE, EVA

Introduction

Many food products may be contaminated by undesirable microbes such as fungi, yeast and bacteria (Hotchkiss 1997). In order to prevent or impede such contamination, novel packaging technologies are continually being developed to prolong the shelf life and improve the quality, safety and sensory properties of fresh and processed foods (Ahvenainen 2003). The integration of antimicrobial (AM) agents into packaging materials is aimed at extending the shelf life of packaged food products by eradicating or inhibiting the spoilage and pathogenic microorganisms that cause deterioration of the products (Vartiainen, Skytta et al. 2003). "Active Packaging" (AP) systems, including antimicrobial systems, have been extensively studied and reported in the literature (Rooney 1995).

In recent years there has been an increased emphasis on naturally-derived AM agents (Knowles, Roller et al. 2005; Valverde, Guillén et al. 2005; Zivanovic, Chi et al. 2005; Johnson, Kicklighter et al. 2006; Miltz, Rydlo et al. 2006; Rydlo, Miltz et al. 2006; Yanishlieva, Marinova et al. 2006; Maizura, Fazilah et al. 2007; Neetoo, Ye et al. 2007; Rhim and Ng 2007; Suppakul, Sonneveld et al. 2008) and polymer films containing AM agents derived from basil, for example, exhibit an AM effect against a wide spectrum of microorganisms (EN 1186-1:2002;

Suppakul, Sonneveld et al. 2008). Sweet basil (*Ocimum basilicum* L.) is a popular culinary herb that has been widely used in food products (Dziedzic 1989) and as an ingredient in dental and oral health care products (Guenther 1952). Additionally, basil essential oils have been reported to possess AM activity against some Gram-positive and Gram-negative bacteria and against some important food-borne pathogens (Fyfe, Armstrong et al. 1998), moulds (Arora and Pandey 1977) and yeasts (Conner and Beuchat 1984). Coating of low-density polyethylene (LDPE) films or blending LDPE with basil extracts prior to extrusion are some of the techniques used for creating AM films (Han 2000). The incorporation of basil into LDPE films can result in materials that have effective AM activity against a wide selection of microorganisms including *Staphylococcus aureus*, *Listeria innocua*, *Escherichia coli* and *Saccharomyces cerevisiae* (Suppakul, Sonneveld et al. 2003). Furthermore, the natural product of the essential oil of *Thymus vulgaris*, thymol, is a phenolic monoterpene that has received considerable attention as a possible AM agent (Olasupo, Fitzgerald et al. 2004; Tepe, Daferera et al. 2004) and as a possible food antioxidant (Youdim and Deanes 2000; Shen, Huang et al. 2005). As an AM agent, thymol possesses very high antimicrobial, antifungal and mould inhibitory activity (Thompson 1996; Couladis, Tzakou et al.

2004; Azaz, Kurkcuoglu et al. 2005; Radulovic, Stojanovic et al. 2006).

Antimicrobial activity can be achieved by adding AM agents to a packaging system during manufacturing or by using polymeric AM materials (Hotchkiss 1997). There are three modes in which AM agent activity may be imparted, namely *via* absorption, immobilization and release systems. Absorption systems remove the essential factors of microbial growth from the foodstuff and inhibit the growth of microorganisms. Immobilization systems suppress the growth of microorganisms at the contact surface but they are considered less effective in the case of solid compared to liquid foods because there is generally less contact between the AM package and a solid food product (Han 2000). Release systems allow the migration of the AM agent into the food or the headspace inside the package to inhibit the growth of microorganisms. Active components are usually incorporated into a single-material film, including edible films, or a laminate. Active packaging systems used for wrapped food products involve a direct interaction between the foodstuff and the components in the package (Han 2000). Thus, the layer of the package that is in contact with the foodstuff and is responsible for the activity, is of primary importance (Miltz, Passy et al. 1995; Rooney 1995).

The control of the release rate and the migration of an AM agent from the packages are very important in initiating and maintaining effective AM activity (LaCoste, Schaich et al. 2005; Rardniyon, Miltz et al. 2008). If the concentration of AM agent is at or above the minimum inhibitory concentration (MIC) on the food surface, the system will actively maintain effective AM activity (Suppakul 2004). It is difficult to measure the migration of a given active agent into an actual food product because most foodstuffs are composed of a mixture of substances such as water, carbohydrates, fats, lipids, proteins, vitamins, fibres and minerals. For this reason, migration studies are usually performed using food simulants (Dopico, Lopez-Vilarino et al. 2003). Various food simulants have been identified in current European food-packaging regulations (European 2002). Each of the simulants represents a particular type of food and these include: water; 3% (v/v) acetic acid in water; 8%, 15%, 50% and 95% (v/v) ethanol in water; olive oil; sunflower oil; and synthetic fat simulant HB 307. The compatibility of an AM agent with different types of foods or food simulants is an important factor that must be considered when designing an AM package (Rardniyon, Miltz et al. 2008).

Volatile AM agents that are blended with the substrate can migrate from the packaging material into the food or food simulant *via* diffusion, evaporation or slow release. In some cases, the release of such AM agents can be controlled by incorporating polymer additives such as polyethylene glycol (PEG) or ethylene vinyl acetate (EVA) in the formulation of the packaging system (Cran, Mistry et al. 2007). A number of approaches have been used to characterize the diffusion of active agents and other additives in polymer substrates. An approach that is often convenient is the one proposed by Miltz (1987) that treats the process as one of diffusion.

Theoretical Considerations

The following approximated equations are applied for the time dependence of the mass of additive that migrates from a polymer film into the simulant during short and long terms (Crank 1975; Miltz 1987).

Short-term migration is usually defined as the time for which $m_t/m_\infty < 0.6$ and the relevant equation is:

$$m_t/m_\infty = 4(Dt/\pi l^2)^{1/2} \quad (1)$$

where m_t and m_∞ are the amounts of additive released from the film up to time t and equilibrium ($t = \infty$) respectively, D is the diffusion coefficient and l is the thickness of the film. Thus a plot of m_t/m_∞ versus $t^{1/2}$ should yield a straight line that passes through the origin. The diffusion coefficient can be obtained from the slope of the line.

Long-term migration is defined for $m_t/m_\infty > 0.6$ and the relevant equation is:

$$m_t/m_\infty = 1 - (8/\pi^2)\exp(-\pi^2 Dt/l^2) \quad (2)$$

From equation (2) a plot of $\ln(1 - m_t/m_\infty)$ versus time should yield a straight line that has an intercept with the vertical axis of $\ln(8/\pi^2)$ and a gradient of $k_d = -\pi^2 D/l^2$.

In some cases the analysis of the results can be simplified by considering the entire diffusion process to be a single process that obeys first-order kinetics. This has been pointed out previously (Cran, Mistry et al. 2007) with the merits of such a kinetic treatment being simple and allowing an easy estimation of the initial release rate of the additive (e.g. AM agent), v_0 , from equation (3):

$$v_0 = m_\infty k_1 \quad (3)$$

where k_1 is an overall kinetic rate constant obtained from the gradient of a plot of $\ln(1 - m_t/m_\infty)$ versus time for all data obtained over the entire short-term and long-term time domains.

This approach is clearly an additional approximation over equations (1) and (2). Moreover, the latter equations are approximations based on the assumption that the migrating species diffuse from an infinite "sheet" of a polymer immersed in an infinite volume of food simulant. Nonetheless, these approximations have been found to adequately describe the migration process for many systems and allow a convenient estimation of the diffusion coefficients and kinetic parameters.

In view of the potential benefits that can be obtained by natural AM additives in food packaging films, in the present paper, the diffusion of the natural AM agents linalool, carvacrol and thymol from LDPE films containing EVA as the AM binding agent was investigated. The aim was to determine the applicability of the approximate equations (1) and (2) as well as an overall first-order kinetic approach for the analysis of these systems. The possibility of extending the diffusion modeling by optimizing the position of the short-term/long-term boundary in order to achieve a better fit to the experimental data was also explored.

Materials and Methods

Materials

Polymer films were prepared from low-density polyethylene (LDPE, XJF143/1700 Qenos Ltd., Australia) and ethylene vinyl acetate copolymer (EVA, ELVAX® 3120, Dupont Ltd., Australia). The AM additives used were linalool (L260-2, 97% purity, Aldrich Chemical Company, USA), thymol (AUSTL 21320, 98% purity, Aurora Pty. Ltd., Australia) and carvacrol (W224502, 99% purity, Sigma-Aldrich Pty. Ltd., Australia). The food simulants were prepared using isoctane (Unichrom 2516-2.5L, GL grade, APS Chemicals Ltd., Australia) and ethanol (95 SG, CSR Distilleries Ltd., Australia).

Production of Compression-Molded Films

Films of thickness *ca.* 2 mm consisting of LDPE, EVA and AM agent were prepared by using a compression molding press (Laboratory Press 15T, L0003, IDM Instruments Pty. Ltd., Australia). A hard-chromed steel frame of 2 mm in thickness

was placed between the two platens of the press and set to 120°C. The polymer formulation was placed at the centre of the frame and sandwiched between the two platens. As the polymer melted, a compression force was gradually applied up to 130 kPa. The platens were then quench-cooled to 20°C by water circulation through coils in the platens. The pressure was then released and the films were folded and heated again in the press. This procedure was repeated three times to facilitate uniform mixing. After the pressing operation, a hand-held micrometer (Mitutoyo, Japan) was used for measuring the thickness of the films with an average of five readings taken at different points on the film sample and the films were wrapped in aluminum foil (to minimize the loss of the AM agent) and stored at room temperature.

Production of Extrusion-Blown Films

Films of *ca.* 50 μm in thickness were prepared from a pre-blended LDPE master batch containing EVA and AM agent. A standard single-screw extruder (Telford Smith, Australia) was used with a screw diameter of 50 mm and an operating speed of 40 rpm. The temperature profile was maintained at 150°C from the first barrel zone to the die. The die was a high-density 190 mm centre feed die with a die gap of 1.6 mm. The extruded film was immediately wrapped in aluminum foil to minimize the loss of the AM agent by evaporation and films were stored at room temperature. The thickness of the extrusion-blown films was also measured by a micrometer (Mitutoyo, Japan) using an average of five readings taken at different points on the film sample.

Quantification of AM Agent Release

The release of AM agents from the films into the food simulants was investigated by immersing some weighed film sample *ca.* 0.5 g (4 pieces, 5 × 5 cm) into 100 mL of food simulant. The vessel containing the film samples and simulant was then sealed and placed in a temperature-controlled water bath that was maintained at 25°C. The sample vessels were gently agitated at regular intervals. Accurately known, small-volume aliquots (1.0 μL) of the simulant were taken at different time intervals and the concentration of AM agent in the simulant was determined by gas chromatography (GC). The GC analysis was conducted using a Varian Star 3400-CX GC equipped with a fused silica DB-5 capillary column (30 × 0.25 mm inner diameter, film thickness 0.25 μm , J&W Scientific, USA). The GC was operated using the following conditions: 1.0 μL injection volume; 80°C initial column temperature; 5°C min^{-1} heating rate; 250°C injector temperature; 1:100 split ratio; 300°C FID detector temperature; and nitrogen carrier gas. The concentration of AM agent was calculated from predetermined standard curves.

Results and Discussion

Overall First-Order Kinetics Analysis

In Figure 1, the overall first-order plots for the release of linalool into the non-polar food simulant isooctane (neat) at 25°C from compression-molded LDPE containing 0%, 10% and 50% (w/w) EVA copolymer are shown. The linearity of these plots demonstrates the applicability of an overall first-order kinetic analysis to these systems as a good approximation. This enables a convenient estimation of the initial rate of release of the AM agent from the derived kinetic parameters.

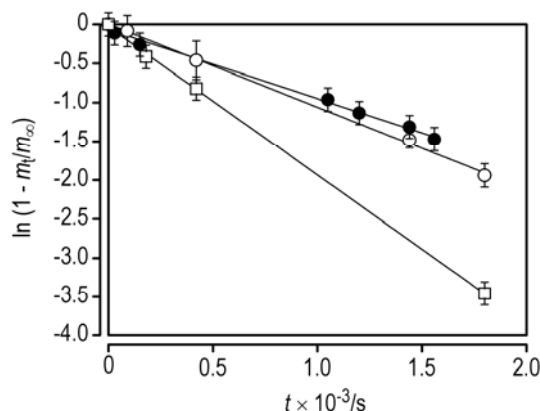


Figure 1 - Plots of $\ln(1 - m_t/m_\infty)$ versus time for linalool released into isooctane at 25°C from LDPE/EVA compression-molded films containing: (O) 0% (w/w) EVA, (●) 10% (w/w) EVA and (□) 50% (w/w).

Table 1 lists the overall first-order rate constants, the initial release rates of linalool and the linear regression coefficients that were derived from the kinetic analyses of the data presented in Figure 1. The results indicate that the values of v_0 and k_1 are lowest in the formulation containing 10% (w/w) EVA which suggests that there is an optimum level of EVA that is necessary to minimize the AM agent release rate in this system (Cran, Mistry et al. 2007). This may arise due to the opposing effects of the hydrogen bonding between the EVA hydrophilic groups with the AM agent. These bonds will tend to retard the release of AM agent in the system and to lower the crystallinity in the compression-molded systems (Dalai and Wenxiu 2002), particularly at the higher EVA levels. These levels would result in an increased amorphous content in the polymer sample that, in turn, may favor a more rapid release of the AM agent.

Table 1 - Results of kinetic analyses for the release of linalool into isooctane at 25°C from compression-molded LDPE films containing EVA.

EVA Content in LDPE/EVA Blend % (w/w)	First-Order Rate Constant $k_1 \times 10^5 / \text{s}^{-1}$	Initial Release Rate $v_0 \times 10^5 / \text{g s}^{-1}$	Linear Regression Coefficient r^2
0	144	30.6	0.959
10	93.4	8.8	0.996
50	193	45.8	0.999

The appropriateness of an overall first-order analysis of the data for each of these systems is confirmed by the linear regression coefficients listed in Table 1. Furthermore, Cran and others (2007) have used the diffusion analysis approach proposed by Miltz (1987) to confirm these results in both short-term and long-term analyses.

Short-Term and Long-Term Diffusion Model Analyses

In view of the results shown in Figure 1 that suggest that the release rate of the AM agent is controlled by the EVA content in the film formulation, extrusion-blown films containing LDPE, 10% (w/w) EVA and the AM agent linalool were prepared. This level of EVA was selected to affect a slow release of the AM agent in the subsequent experiments. Figure 2 shows plots of m_t/m_∞ versus the square root of time (i.e. short-term diffusion analysis where $m_t/m_\infty < 0.6$) and of $\ln(1 - m_t/m_\infty)$ versus time (i.e. long-term diffusion analysis where $m_t/m_\infty > 0.6$) for the release of linalool from the LDPE/EVA films into three different food

simulants, namely 15% (v/v) ethanol/water, 95% (v/v) ethanol/water and neat isooctane at 25°C.

The adequacy of equation (1) for describing the short-term migration and of equation (2) for describing the long-term migration of linalool in these systems is confirmed by the linearity of the plots in Figure 2. Moreover, these results serve to confirm the applicability of the Miltz approach (Miltz 1987) for the analysis of these systems. It is also apparent from the plots that the release of linalool into isooctane occurs faster than in the other simulant studied and that the slowest release was observed in the 15% (v/v) ethanol/water simulant. The fast release of linalool into isooctane may be due to the swelling of LDPE in this solvent as reported by Helmroth and others (2003). The low solubility of linalool in polar aqueous solutions (Sajilata, Savitha et al. 2007) may also explain its faster release into a non-polar simulant such as isooctane.

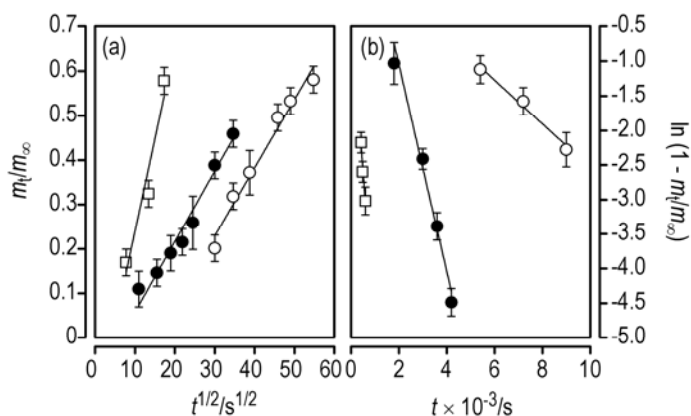


Figure 2. Plots of m_t/m_∞ versus the square root of time and of $\ln(1 - m_t/m_\infty)$ versus time for the release of linalool from LDPE/EVA extrusion-blown films into: (O) 15% (v/v) ethanol/water, (●) 95% (v/v) ethanol/water and (□) neat isooctane at 25°C (Cran, Rupika et al. 2009).

Table 2 lists the diffusion coefficients and rate constants (i.e. k_d values) that were derived from the diffusion analysis. The values of these parameters decrease in the following order of food simulants: isooctane > 95% (v/v) ethanol/water > 15% (v/v) ethanol/water. This reflects the decreasing order of solvent polarity of the simulants and the decreasing order of affinity of the simulant to the polymer substrate (Sajilata, Savitha et al. 2007). From these observations it can also be assumed that the release of linalool into aqueous or acidic foods would be even lower than that observed in the case of ethanol/water mixtures because of the low solubility of linalool in these types of food simulants.

Table 2. Results of the diffusion analyses for the release of linalool from extruded LDPE films containing EVA into three food simulants at 25°C.

Food Simulant	Diffusion Coefficient $D \times 10^{-14}/m^2 s^{-1}$	Diffusion Rate Constant $k_d \times 10^5/s^{-1}$
100% isooctane	41.4	450
95% (v/v) ethanol/water	6.7	142
15% (v/v) ethanol/water	4.5	32.2

The treatment of the vast majority of data obtained from AM migration experiments can be handled by either the overall first-order kinetic approach or the Miltz diffusion approach (Miltz 1987) given above. However, in some cases it has been observed

that the system tends not to be fitted satisfactorily by either of these approaches in the first instance. An example is the analysis of the short-term release of linalool from the LDPE/EVA substrate into 15% (v/v) ethanol/water simulant shown in Figure 2(a) where the fit appears to deviate slightly from linearity for $m_t/m_\infty > 0.5$. Here it appears upon inspection that a better fit to the data may be achieved by moving the short-term/long-term boundary so that the non-conforming data are shifted to the long-term time domain.

Extending the Diffusion Model

The treatment of AM migration data in accordance with the idealized diffusion approach (Miltz 1987) can, in some cases, produce results that deviate from linearity. One explanation for the limited fit of the data relates to the assumptions in the derivation of equation (1). Clearly there exist more complex analytical equations that take into account cases in which the volume of simulant is small in comparison to the surface available for mass transfer of the AM agent from the substrate (Crank 1975; Miltz 1987). Observations of the data obtained for LDPE/EVA blends containing linalool, carvacrol or thymol suggest that an alternative approach which retains the idealized model and adjusts the definition of the short-term/long-term boundary may be more appropriate.

To investigate this possibility further, a computer program was written that calculates the values of m_t/m_∞ for the approximate long-term solution of the AM agent diffusion in an idealized or "infinite" system (equation (2)) and the exact long-term solution given by equation (4) below (Crank 1975; Miltz 1987):

$$m_t/m_\infty = 1 - \sum_{n=0}^{\infty} \{8/[(2n+1)^2 \pi^2] \exp[-(2n+1)2\pi^2 Dt/l^2]\} \quad (4)$$

Figure 3 shows theoretical plots of m_t/m_∞ versus time for both the approximate and the exact solutions to the diffusion equation for an "idealized" system (Crank 1975; Miltz 1987) that have been calculated for the long-term period where the diffusion coefficient is $1 \times 10^{-15} m^2 s^{-1}$ and film thickness of 50 μm (Cran, Rupika et al. 2009).

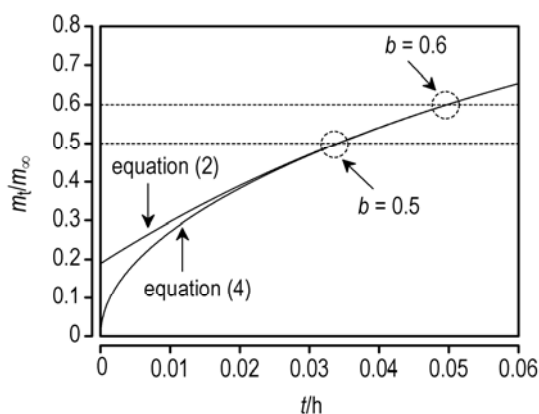


Figure 3 - Theoretical plots of m_t/m_∞ versus time for the exact and approximate solutions to the diffusion equations that pertain to an "idealized" system (Crank 1975; Miltz 1987) where $D = 1 \times 10^{-15} m^2 s^{-1}$ and $l = 50 \mu m$. The summation calculations for the "idealized" solution were performed up to $n = 40$ terms.

It is apparent from Figure 3 that the two functions represented by equations (2) and (4) remain almost convergent for values of m_t/m_∞ down to *ca.* 0.5. Indeed, this suggests that the definition of the short-term/long-term boundary, b , in the analysis can be

shifted from $b = 0.6$ to $b = 0.5$ with little consequence in the theoretical analytical result. Apparently, a shift of the short-term/long-term boundary towards a shorter time period has no adverse effect on the goodness of fit of data that lie in the short-term time domain. The difference between the two functions at $b = 0.6$ has been calculated to be 0.03% and at $b = 0.5$ the difference is 0.23%. This suggests that the error in assuming congruence of the two functions remains acceptably low if the short-term/long-term boundary is shifted downwards from $b = 0.6$ to $b = 0.5$ for the purposes of producing a more convenient data analysis (Cran, Rupika et al. 2009).

Plots of m_t/m_∞ versus the square root of time (short-term diffusion analysis where $m_t/m_\infty < 0.6$ or $m_t/m_\infty < 0.5$) for the release of thymol from extruded LDPE/EVA films into 95% (v/v) ethanol/water at 10°C are shown in Figure 4. Inspection of Figure 4(a) reveals that the inclusion of the data up to the boundary $b = 0.6$ presents an apparent curvature in the plot which should, of course, be linear (Cran, Rupika et al. 2009). A more acceptable fit to the short-term diffusion data is achieved by setting the short-term/long-term boundary to $b = 0.5$ as shown in Figure 4(b).

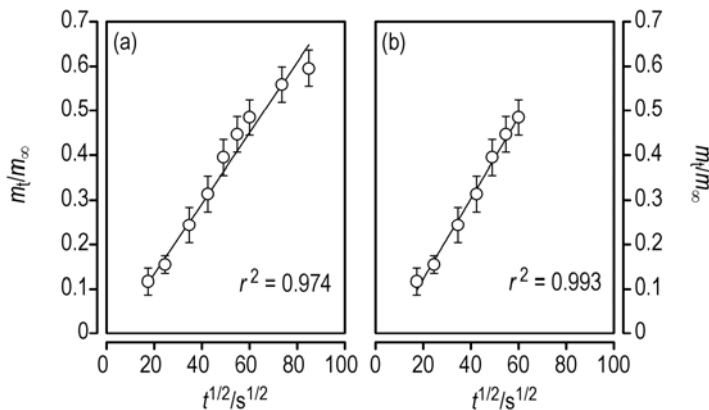


Figure 4 - Plots of m_t/m_∞ versus the square root of time for the short-term release of thymol from LDPE/EVA extrusion-blown films into 95% (v/v) ethanol/water at 10°C where the boundary conditions are: (a) $b = 0.6$ and (b) $b = 0.5$.

Figure 5 shows plots of $\ln(1 - m_t/m_\infty)$ versus time (i.e. long-term diffusion analysis where $m_t/m_\infty > 0.5$ or $m_t/m_\infty > 0.6$) for the release of thymol from extruded LDPE/EVA films into 95% (v/v) ethanol/water at 10°C. These data correspond to the short-term data presented in Figure 4. It is also apparent that in this case a more acceptable fit of the long-term diffusion data is achieved by moving the boundary from $b = 0.6$ to $b = 0.5$. Thus upon considering the data in Figures 4 and 5 simultaneously, it is clear that a better fit to the experimental data is achieved in both the short term and the long term domains by moving the boundary from $b = 0.6$ to $b = 0.5$ (Cran, Rupika et al. 2009).

In order to demonstrate more clearly the potential benefit to the analysis by adopting a flexible definition of the short-term/long-term boundary position, a number of different systems were analyzed using $b = 0.5$ or $b = 0.6$ as the boundary condition. The goodness of fit of the model for both the short-term and long-term analyses was determined in each case by calculating the respective linear regression coefficient, r^2 . Table 3 presents these data for a number of selected systems and demonstrates that in most cases a better fit of the idealized diffusion model is obtained in the case where the short-term/long-term boundary has been moved from $b = 0.6$ to $b = 0.5$.

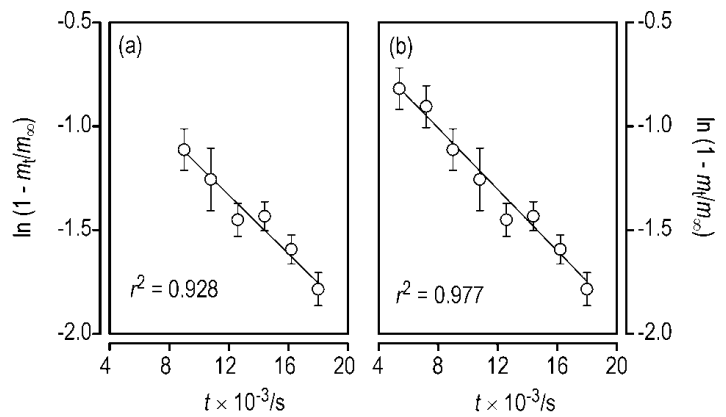


Figure 5 - Plots of $\ln(1 - m_t/m_\infty)$ versus time for the long-term release of thymol from LDPE/EVA extrusion-blown films into 95% (v/v) ethanol/water at 10°C where the boundary conditions are: (a) $b = 0.6$ and (b) $b = 0.5$.

Table 3 - Comparison of linear regression coefficients obtained in the short-term analysis of various AM-containing systems for $b = 0.5$ and $b = 0.6$.

		Linear Regression Coefficient, r^2			
		Short-term analysis		Long-term analysis	
Extruded LDPE/EVA film containing:	Simulant	$b = 0.6$	$b = 0.5$	$b = 0.6$	$b = 0.5$
4% carvacrol	10% ethanol/water, 15°C	0.965	0.984	0.977	0.983
4% carvacrol	95% ethanol/water, 20°C	0.978	0.991	0.932	0.915
4% carvacrol	100% isooctane, 10°C	0.984	0.989	0.949	0.920
2% linalool	100% isooctane, 25°C	0.937	0.996	0.955	0.975
2% linalool	15% ethanol/water, 25°C	0.974	0.987	0.986	0.991
4% thymol	95% ethanol/water, 10°C	0.974	0.993	0.928	0.977

Deviations from an Idealized System

In dealing with all of the above systems, it is important to recall that equations (1) and (2) are approximations that have been derived for the theoretical case of an infinite "sheet" of polymer immersed in an infinite volume of simulant. Nonetheless, provision exists for cases where this assumption is deemed not to be valid and equation (5) has been derived for cases where the volume of simulant is limited (Crank 1975; Miltz 1987).

$$m_t/m_\infty = (1 + \alpha)[1 - \exp(T/\alpha^2)\text{erfc}(T/\alpha^2)^{1/2}] \quad (5)$$

where $\alpha = a/(K_p x)$, $x = l/2$, a is the ratio of simulant volume to surface area for mass transfer, K_p is the partition coefficient (i.e. $K_p = C_{\text{polymer}}/C_{\text{simulant}}$ where C is the concentration of AM agent at equilibrium) and $T = Dt/x^2$. The complementary error function $\text{erfc}(y)$ is given by equation (6):

$$\text{erfc}(y) = 1 - (2/\pi^{1/2}) \int_0^y \exp(-\beta^2) d\beta \quad (6)$$

A computer program was written to investigate the divergence of the "idealized" system as described by equation (4) from the one where the simulant volume is limited, as described by equations (5) and (6). Two extreme cases were investigated for a nominally 50 μm film for which $D = 1.00 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$. The first case involved a system in which transfer of the AM agent to the simulant is highly favored ($K_p = 0.001$) and the second one in which the retention of the AM agent in the polymer substrate is favored ($K_p = 1000$). Theoretical calculations using the computer program were also conducted for a 50 μm film in which $D = 1.00 \times 10^{-15} \text{ m}^2 \text{ s}^{-1}$ and the findings were completely analogous.

Figure 6 shows the results of the computer analysis for a system in which transfer of the AM agent to the simulant is highly favored. The analysis reveals that the "simulant-limited" and "idealized" models remain highly congruent over most of the time domain, almost up to the expected asymptotic approach of the m_t/m_∞ function to unity after "infinite" time. The modeling reveals that the release of the AM agent in the "simulant-limited" model occurs at a faster rate than in the idealized case where an infinite sheet of polymer is in contact with an infinite volume of simulant. An arbitrarily chosen time (e.g. $t = 0.12 \text{ h}$) can be used to assess the extent of the divergence of the two functions (see Figure 6).

The highly contrasting case of a system in which retention of the AM agent in the substrate is favored is presented in Figure 7. The analysis reveals there is little congruence between the two functions, and hence in the corresponding models, under these conditions. This is also reflected at $t = 0.12 \text{ h}$, the arbitrarily chosen time for assessing the extent of divergence.

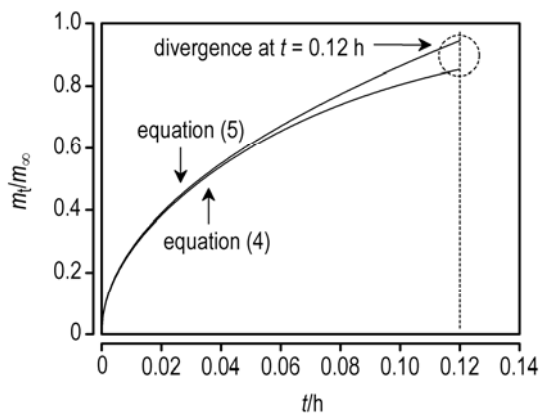


Figure 6 Theoretical plots of m_t/m_∞ versus time for the "simulant-limited" and exact "idealized" solutions to the diffusion equations (Crank 1975; Miltz 1987) for a system where the transfer of AM agent to the simulant is favored. In the analysis $D = 1 \times 10^{-15} \text{ m}^2 \text{ s}^{-1}$, $l = 50 \mu\text{m}$, $K_p = 0.001$ and $a = 1.0 \times 10^{-6} \text{ m}$. The summation calculations for the "idealized" case were performed up to $n = 40$ terms.

A complete analysis of the extreme systems depicted in Figures 6 and 7 is summarized in Table 4 that demonstrates the "simulant-limited" and "idealized" diffusion models are almost congruent over a wide range of conditions as indicated by the magnitude of the divergence of the two functions at the arbitrarily chosen time used for this assessment. Nonetheless, divergence between these functions is observed which depends on the partition coefficient. In the case of systems that favor the retention of the simulant in the polymer the divergence depends critically on the ratio between the simulant volume and the surface area available for mass transfer. The "idealized" diffusion equation can be used as a very good approximation to

the "simulant-limited" equation in systems where the transfer of the AM agent to the simulant is favored. Indeed, the latter is clearly desirable in the formulation of effective AM films where the equilibrium partitioning of the AM agent should lie in favor of the foodstuff albeit attained at a controlled rate of release.

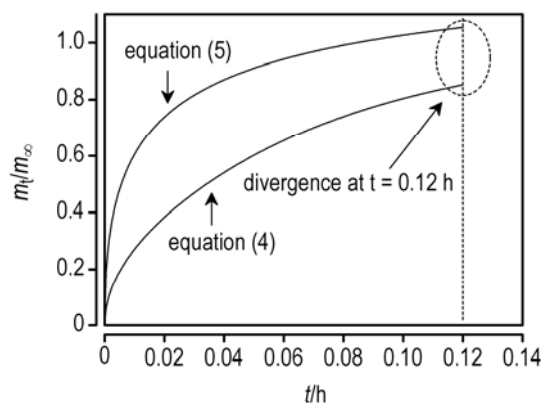


Figure 7 Theoretical plots of m_t/m_∞ versus time for the "simulant-limited" and exact "idealized" solutions to the diffusion equations (Crank 1975; Miltz 1987) for a system where the retention of the AM agent in the substrate is favored. In the analysis $D = 1 \times 10^{-15} \text{ m}^2 \text{ s}^{-1}$, $l = 50 \mu\text{m}$, $K_p = 1.0 \times 10^3$ and $a = 0.01 \text{ m}$. The summation calculations for the "idealized" case were performed up to $n = 40$ terms.

Table 4. Comparison of the "simulant-limited" and exact "idealized" solutions to the diffusion equations (Crank 1975; Miltz 1987) for systems where $D = 1 \times 10^{-15} \text{ m}^2 \text{ s}^{-1}$ and $l = 50 \mu\text{m}$. The summation calculations for the "idealized" case were performed up to $n = 40$ terms.

System	Partition Coefficient	Ratio of Simulant Volume to Mass Transfer Surface Area, a/m	% Divergence of Simulant-Limited Model from Idealized Model at $t = 0.12 \text{ h}$
System favoring transfer of AM agent into simulant	$K_p = 0.001$	1.00×10^{-6}	10.83
		1.00×10^6	9.89
System favoring retention of AM agent in polymer	$K_p = 1000$	1.00×10^{-2}	38.25
		1.00×10^6	10.01

Conclusions

The diffusion of naturally-derived AM agents such as linalool, carvacrol and thymol from film formulations based on LDPE/EVA can be successfully modeled using an overall first-order kinetic analysis. This has the advantage of enabling the initial AM release rate to be conveniently determined. The rate of release of linalool from LDPE/EVA formulations depends on the EVA level and an optimum level of EVA is required to minimize the rate at which the AM agent is released from the substrate. The formulations can also be modeled successfully using an extension of the idealized model involving an infinite "sheet" of polymer immersed in an infinite volume of food simulant. The extension technique involves shifting the short-term/long-term diffusion data boundary to 0.5 instead of 0.6. Such a shift can be made with little consequence to the numerical accuracy of any subsequent diffusion analysis and in some cases can improve the fit of the data.

The extended approach to diffusion analysis enables highly acceptable routine analyses to be performed conveniently without having to resort to more complex equations that require prior knowledge of partition coefficients of the AM agents. Theoretically, there exists general congruency between the "simulant-limited" and "idealized" diffusion models over a wide range of experimental conditions particularly for systems that favor AM agent transfer from the substrate to the foodstuff at equilibrium. As the most practical and effective AM packaging systems will be based necessarily on this premise then the "idealized" diffusion equations, used in conjunction with the extension to that model, should serve as a most reliable means to accurately characterize and quantitatively describe these systems.

Diffusion analyses suggest that the release of linalool from LDPE/EVA formulations is dependent on the polarity of the simulant and the extent to which the simulant may interact with or swell the polymer substrate. This is especially so in the case of non-polar food simulants such as isooctane. It is postulated that AM agents that have poor solubility in aqueous simulants will undergo limited diffusion into aqueous or acidic foodstuffs and may reduce their likelihood of developing off-flavors in the packaged products. Regardless of the food type, however, the relatively high vapor pressure of these agents may result in their extensive release into the package headspace. Thus AM films containing linalool, carvacrol or thymol may be suitable for package/headspace/food systems where the food type could potentially range from high fat content products such as cheese to aqueous foods.

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References

- Ahvenainen, R. (2003). Active and intelligent packaging. Novel Food Packaging Techniques. R. Ahvenainen. Cambridge, England, Woodhead Publishing Limited.
- Arora, R. and G. N. Pandey (1977). "The application of essential oils and their isolates for blue mould decay control in *Citrus reticulata* Blanco." Journal of Food Science and Technology **14**: 137-146.
- Azaz, A. D., M. Kurkcuglu, et al. (2005). "In vitro antimicrobial activity and chemical composition of some *Satureja* essential oils." Flavour and Fragrance Journal **20**(6): 587-591.
- Conner, D. E. and L. R. Beuchat (1984). "Effects of essential oils from plants on growth of food spoilage yeasts." Journal of Food Science **49**: 429-434.
- Couladis, M., O. Tzakou, et al. (2004). "Chemical analysis and antifungal activity of *Thymus striatus*." Phytotherapy Research **18**(1): 40-42.
- Cran, M. J., Y. K. Mistry, et al. (2007). Development of low-density polyethylene films containing natural antimicrobial agents. Proceedings of 23rd IAPRI Symposium on Packaging, Windsor, UK, 3-5 September, Paper FS6 CD ROM.
- Cran, M. J., L. A. S. Rupika, et al. (2009). Migration of Natural Antimicrobial Agents from Low-Density Polyethylene Films. Proceedings of 24th IAPRI Symposium on Packaging, Greenville, South Carolina, USA, 17-20 May, Paper P23 CD ROM.
- Crank, J. (1975). Oxford University Press, Oxford, UK.
- Dalai, S. and C. Wenxiu (2002). "Radiation effects on LDPE/EVA Blends." Journal of Applied Polymer Science **86**(5): 1296-1302.
- Dopico, M. S., J. M. Lopez-Vilarino, et al. (2003). "Determination of antioxidant migration levels from low density polyethylene film into food simulants." Journal of Chromatography **1018**(1): 53-62.
- Dziezak, J. D. (1989). "Spices." Food Technology **43**: 102-115.
- European, S. (2002). Materials and articles in contact with foodstuffs - Plastics - EN 1186-1:2002, June 2002. Part 1: Guide to the selection of conditions and test methods for overall migration.
- Fyfe, L., F. Armstrong, et al. (1998). "Inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* by combinations of plant oils and derivatives of benzoic acid: the development of synergistic antimicrobial combinations." International Journal of Antimicrobial Agents **9**: 130-136.
- Guenther, E. (1952). New York, Van Nostrand Co. Inc.
- Han, J. H. (2000). "Antimicrobial food packaging." Food Technology **54**(3): 56-65.
- Helmroth, I. E., M. Dekker, et al. (2003). "Additive diffusion from LDPE slabs into contacting solvents as a function of solvent absorption." Journal of Applied Polymer Science **90**(6): 1609-1617.
- Hotchkiss, J. H. (1997). "Food packaging interactions influencing quality and safety." Food Additives and Contaminants **14**(6-7): 601-607.
- Johnson, P. M., C. E. Kicklighter, et al. (2006). "Packaging of chemicals in the defensive secretory glands of the sea hare *Aplysia californica*." Journal of Experimental Biology **209**(1): 78-88.
- Knowles, J. R., S. Roller, et al. (2005). "Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium." Applied and Environmental Microbiology **71**(2): 797-803.
- LaCoste, A., K. M. Schaich, et al. (2005). "Advancing controlled release packaging through smart blending." Packaging Technology and Science **18**(2): 77-87.
- Maizura, M., A. Fazilah, et al. (2007). "Antibacterial activity and mechanical properties of partially hydrolyzed sago starch-alginate edible film containing lemongrass oil." Journal of Food Science **72**(6): C324-C330.
- Miltz, J. (1987). Migration of low molecular weight species from packaging materials: theoretical and practical considerations. Food Product-Package Compatibility. J. I. Gray, B. R. Harte and J. Miltz. Lancaster, PA, Technomic Publishing Co.: 30-43.
- Miltz, J., N. Passy, et al. (1995). Trends and applications of active packaging systems. Food and Packaging Materials - Chemical Interaction. P. Ackerman, M. Jagerstad and T. Ohlsson, The Royal Society of Chemistry, Vol 162. **162**: 201-210.
- Miltz, J., T. Rydlo, et al. (2006). "Potency evaluation of a dermaseptin S4 derivative for antimicrobial food packaging applications." Packaging Technology and Science **19**(6): 345-354.
- Neetoo, H., M. Ye, et al. (2007). "Effectiveness and stability of plastic films coated with nisin for inhibition of *Listeria monocytogenes*." Journal of Food Protection **70**(5): 1267-1271.
- Olasupo, N. A., D. J. Fitzgerald, et al. (2004). "Inhibition of *Bacillus subtilis* and *Listeria innocua* by nisin in combination with some naturally occurring organic compounds." Journal of Food Protection **67**(3): 596-600.
- Radulovic, N., G. Stojanovic, et al. (2006). "Composition and antimicrobial activity of *Equisetum arvense* L. essential oil." Phytotherapy Research **20**(1): 85-88.
- Rardniyon, C., J. Miltz, et al. (2008). Parameters affecting the design of antimicrobial films. Proceedings of 16th IAPRI World Conference on Packaging, Bangkok, Thailand, June 8-12.
- Rhim, J.-W. and P. K. W. Ng (2007). "Natural biopolymer-based nanocomposite films for packaging applications." Critical Reviews in Food Science and Nutrition **47**(4): 411-433.
- Rooney, M. L. (1995). Active packaging in polymer films. Active Food Packaging. M. L. Rooney, Blackie Academic & Professional, London, p. 74-110.
- Rydlo, T., J. Miltz, et al. (2006). "Eukaryotic antimicrobial peptides: Promises and premises in food safety." Journal of Food Science **71**(9): R125-R135.

- Sajilata, M. G., K. Savitha, et al. (2007). "Scalping of flavors in packaged foods." Comprehensive Reviews in Food Science and Food Safety **6**(1): 17-35.
- Shen, A.-Y., M.-H. Huang, et al. (2005). "Thymol analogues with antioxidant and L-type calcium current inhibitory activity." Drug Development Research **64**(4): 195-202.
- Suppakul, P. (2004). Study of Antimicrobial Films Containing Basil Extracts. Ph.D. Thesis. Packaging and Polymer Research Unit, Victoria University: 116.
- Suppakul, P., K. Sonneveld, et al. (2008). "Efficacy of polyethylene-based antimicrobial films containing principal constituents of basil." LWT--Food Science and Technology **41**(5): 779-788.
- Suppakul, P., K. Sonneveld, et al. (2003). "Antimicrobial properties of basil and its possible application in food packaging." Journal of Agricultural and Food Chemistry **51**(11): 3197-3207.
- Tepe, B., D. Daferera, et al. (2004). "*In vitro* antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigi*." Journal of Agricultural and Food Chemistry **52**(5): 1132-1137.
- Thompson, D. P. (1996). "Inhibition of growth of mycotoxigenic *Fusarium* species by butylated hydroxyanisole and/or carvacrol." Journal of Food Protection **59**(4): 412-415.
- Valverde, J. M., F. Guillén, et al. (2005). "Improvement of Table Grapes Quality and Safety by the Combination of Modified Atmosphere Packaging (MAP) and Eugenol, Menthol, or Thymol." Journal of Agricultural and Food Chemistry **53**(19): 7458-7464.
- Vartiainen, J., E. Skytta, et al. (2003). "Antimicrobial and barrier properties of LDPE films containing Imazalil and EDTA." Journal of Plastic Film and Sheeting **19**(4): 249-261.
- Yanishlieva, N. V., E. Marinova, et al. (2006). "Natural antioxidants from herbs and spices." European Journal of Lipid Science and Technology **108**: 776-793.
- Youdim, K. A. and S. G. Deanes (2000). "Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain." Journal of Nutrition **83**(1): 87-93.
- Zivanovic, S., S. Chi, et al. (2005). "Antimicrobial activity of chitosan films enriched with essential oils." Journal of Food Science **70**(1): M45-M51.