

Low Molecular Weight Poly(lactic acid) Microparticles for Controlled Release of the Herbicide Metazachlor: Preparation, Morphology, and Release Kinetics

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ABSTRACT: The preemergence chloroacetamide herbicide metazachlor was encapsulated in biodegradable low molecular weight poly(lactic acid) micro- and submicroparticles, and its release to the water environment was investigated. Three series of particles, S, M, and L, varying in their size (from 0.6 to 8 μm) and with various initial amounts of the active agent (5%, 10%, 20%, 30% w/w) were prepared by the oil-in-water solvent evaporation technique with gelatin as biodegradable surfactant. The encapsulation efficiencies reached were about 60% and appeared to be lower for smaller particles. Generally, it was found that the rate of herbicide release decreased with increasing size of particles. After 30 days the portions of the herbicide released for its highest loading (30% w/w) were 92%, 56%, and 34% for about 0.6, 0.8, and 8 μm particles, respectively. The release rates were also lower for lower herbicide loadings. Metazachlor release from larger particles tended to be a diffusion-controlled process, while for smaller particles the kinetics was strongly influenced by an initial burst release.

KEYWORDS: poly(lactic acid), controlled release, metazachlor, oil-in-water solvent evaporation technique, microparticles, kinetics

INTRODUCTION

In the last three decades, bioactive compound delivery systems have attracted increasing interest in the pharmaceutical industry^{1,2} and agriculture and environmental engineering^{3,4} because they are able to reduce undesirable effects of some of these bioactive molecules considerably. In agriculture, such controlled release formulations, including particles loaded with various agrochemicals, could prevent the unwanted phenomena associated with conventional applications of agrochemicals such as leaching through the soil, volatility, and degradation (photolytic, hydrolytic, and microbial),⁵ and simultaneously they could extend their activity in soil, improve their stability, and reduce unwanted toxicity and thus safety for the environment and for operators along with easier handling and application.

Suitable materials meeting the demands for low impact on the environment could particularly be found among biodegradable polymers capable of gradual decomposition either during or after the release of an active agent.⁶ In the area of microparticle formulations, one of the materials used regularly appears to be poly(lactic acid) (PLA), belonging to the family of biodegradable polyesters. The utilization of PLA as a matrix in microparticle release systems has been widely investigated through various techniques of encapsulation including emulsification–diffusion method,⁷ nanoprecipitation,⁸ supercritical antisolvent coprecipitation,⁹ etc. Among these, the oil-in-water solvent evaporation technique, which has been already described in detail in a number of research papers,^{10–14} proved to be the most preferred encapsulation method due to its relative simplicity, with no need for specialized equipment. In addition, this method is frequently used for encapsulation of

lipophilic drugs with low water solubility and high efficiencies of encapsulation are achieved.¹⁵

PLA particles as a matrix were tested with a wide spectrum of drugs with various targeting in the field of pharmaceutical industry^{16,17} and with some agrochemicals.^{18,19} Almost uniformly, researchers used relatively high molecular weight (MW) PLA, with the exception of Takei et al.,¹⁸ where low MW PLA was used in a granulate formulation prepared through melting and blending of the polymer and the bioactive compound at rather high temperature. In our laboratory we ran a series of preliminary experiments with high MW PLA (MW about 100 000) and low MW PLA (MW about 30 000) and blends of both materials, and we found that high MW PLA and the blends of both materials tended to form rather large aggregates in the relatively broad interval of processing conditions, while low MW PLA produced fine and homogeneous suspensions of particles tunable by processing properties. Utilization of low MW PLA can bring some further advantages over high MW PLA because of its faster biodegradation in the environment and possibly some other advantageous features that could arise during particle preparation and a bioactive agent encapsulation. We decided to investigate this eventuality.

Metazachlor (MTZ) is a common preemergence chloroacetamide herbicide frequently used to control grass and some dicotyledonous weeds in rape, soybean, potatoes, and other crops.²⁰ The agent is usually used in water suspension formulations, where one of the drawbacks is that the crystals

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(triclinic form) tend to form agglomerates, which can lead to clogging of the spray nozzles during application.²¹

The aim here was to study the encapsulation of herbicide metazachlor in micro- and submicroparticles made from low molecular PLA. The oil-in-water solvent evaporation technique was selected as the preferred method of particle preparation. Particle characteristics and active agent release were examined for various particle formulations. Fully biodegradable gelatin proved to be a suitable emulsion stabilizer,^{21,22} and was applied through this work as the principal surface-active agent. Compared with common commercial surfactants used in most research works,^{23–25} gelatin is remarkable for its low price and biodegradability, and hence could be considered as a potentially good emulsion stabilizer for environmental applications.

MATERIALS AND METHODS

Materials. The polymer used throughout the study was poly(lactic acid) (PLA, $M_w = 34\,000\text{ g}\cdot\text{mol}^{-1}$, $M_w/M_n = 2$) prepared in our laboratory. PLA was synthesized through direct melt polycondensation of lactic acid. At the beginning, 50 g of 80% L-lactic acid (optical purity 99.9%, Penta) was placed into a two-neck flask equipped with a condenser. The contents were stirred and heated for 4 h at 160 °C under reduced pressure of 20 kPa. After 4 h 0.5% (w/w) of catalyst tin(II) octoate was added, the pressure was reduced to 0.1 kPa, and reaction continued for a further 24 h. Finally, the product was cooled, dissolved in acetone, precipitated from water/methanol (1:1) solution, filtered, and washed with distilled water several times. The obtained powder was dried at 50 °C under vacuum for 24 h and subsequently analyzed by GPC.²⁶

The bioactive agent used for the encapsulation was the herbicide metazachlor [2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl)acetamide], purchased from Chemos (Czech Republic), in the monoclinic and triclinic form, off-white powder with molecular weight 277.75, water solubility 430 mg·L⁻¹, purity >98%, melting point 74–78 °C, and density 1.19 g·cm⁻³.

Surfactants used in the oil-in-water evaporation technique were gelatin from porcine skin type A (Sigma Aldrich) and Tween20 (Sigma Aldrich). Gelatin was in form of yellowish powder, with water solubility 67 mg·mL⁻¹ at 50 °C. Tween20 (polyoxyethylenesorbitan monolaurate) was in the form of clear, yellow to yellow-green viscous liquid, with $M_w = 1225\text{ Da}$ and water solubility 100 g·L⁻¹.

Preparation of PLA Micro- and Submicroparticles. PLA (0.4 g) was dissolved in 4 mL of chloroform. The exact amount of MTZ solution was then used to reach the required final mass fraction in a formulation (from 5% to 40%) and emulsified in water solution of selected surfactant system. Gelatin A (0.5%) and Tween20 (0.5%) were used in combination to produce larger size particles marked L, or gelatin A alone (0.5%) was used for smaller or medium particles marked S or M, respectively. The mixture was dispersed under continuous stirring at speed from 11 500 to 18 000 rpm for 5 min (homogenizer DI 18 basic, Yellow Line by IKA, Belgium), while the mixture was cooled on ice. The ratio between water and oil phases was kept the same in all experiments (10:1). To obtain smaller and medium size particles marked S or M, the emulsion was further sonicated with an ultrasonic probe (Hielscher UP 400S, Germany) for 5 min with amplitude 20% or 35%, respectively. In the last step, the prepared emulsion was placed into an Erlenmeyer flask, the organic solvent was evaporated under reduced pressure with stirring, and the suspension of microparticles was formed. The blank samples for all series were prepared in the same way without MTZ.

Characterization of Particles. Metazachlor Encapsulation. Efficiency of the encapsulation process was characterized by two parameters: encapsulation efficiency (EE) and herbicide loading (HL). The EE (%) was defined as a ratio between the weight of MTZ encapsulated and its total weight at the beginning of the process:

$$EE (\%) = \frac{\text{weight of encapsulated metazachlor}}{\text{initial weight of metazachlor}} \times 100$$

One milliliter of the obtained suspension was centrifuged (Hettich Universal 320) at 10 000 rpm for 10 min to separate water phase from particles, then each of them was handled in a different way. Water phase was decanted and analyzed later. Distilled water (40 mL) was then added and the mixture was stirred to resuspend particles. The total time of contact of particles with fresh water phase was exactly 60 s. It should represent a sufficient time to dissolve nonencapsulated MTZ on particle surface but short time for the release of encapsulated MTZ from the particles.¹⁵ The mixture was then immediately filtered through a 0.22 μm poly(tetrafluoroethylene) (PTFE) syringe filter.

MTZ concentrations in both separated water phases were analyzed by HPLC on a Reprosil 5 μm C₁₈ end-capped reversed-phase column (Watrex, Czech Republic), isocratic acetonitrile/water (60:40) mobile phase, 0.6 mL/min flow and dual UV detection (Waters 2487) at 220 and 266 nm.

Weight of encapsulated MTZ was calculated as the difference between its initial amount and combined amounts of MTZ in the initial suspension water phase and the readily soluble fraction on the surface of particles.

Herbicide loading (HL, %) was defined as the amount of MTZ encapsulated divided by the final weight of particles with encapsulated MTZ at the end of the process:

$$HL (\%) = \frac{\text{weight of encapsulated metazachlor}}{\text{weight of particles}} \times 100$$

Size of Particles. Size and morphology of large particles were evaluated by optical microscopy on a glass slide with a calibrated grid. Evaluation of particle size distribution (PSD) and z-average (Z_D) was performed with the help of ImageJ software. For particles smaller than 5 μm, PSD and Z_D were measured with Zetasizer (Zetasizer Nano ZS, Malvern Instruments) following manufacturer instructions. In both cases, PSD was expressed in the form of polydispersity (Pd), also called the coefficient of variation, calculated as the quotient of standard deviation σ and Z_D :

$$Pd (\%) = \frac{\sigma}{Z_D} \times 100$$

Particles Morphology by Scanning Electron Microscopy. Size and morphology of particles were also investigated by scanning electron microscopy (SEM; VEGA-II, Tescan). Particles were washed with distilled water three times to remove soluble substances from their surface, dried at laboratory temperature, and coated with a gold-platinum layer. Electron accelerating voltage was 10 kV.

Thermal Properties. Determination of thermal properties of particles were measured by differential scanning calorimetry (DSC) on Mettler Toledo DSC1 STAR system under nitrogen atmosphere (20 cm³·min⁻¹) according to the following program: (i) first heating scan 0–170 °C (10 °C·min⁻¹); (ii) annealing at 170 °C for 1 min; (iii) cooling scan 170–0 °C (10 °C·min⁻¹); (iv) annealing at 0 °C for 1 min; (v) second heating scan 0–170 °C (10 °C·min⁻¹). Melting point temperature (T_m) as well as the exothermal response relating to cold crystallization temperature (T_c) was obtained from the first heating cycle. From the second heating scan, the region of glass transition temperature (T_g) was determined. Both onset and midpoint were recorded. The degree of crystallinity χ_c was calculated from the measured heat of fusion (ΔH_m) and crystallization (ΔH_c) according to the following equation:

$$\chi_c = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0} \times 100$$

where ΔH_m^0 is the enthalpy of fusion for 100% crystalline PLA (93.1 J·g⁻¹).²⁷

Fourier Transform Infrared Spectroscopy. Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectra were recorded on a Nicolet iS10 instrument equipped with a diamond crystal (Thermo Fisher Scientific, Waltham, MA). Total light reflection

Table 1. Conditions Used in the Preparation of Particles: Size, Surfactant Incorporation, and EE and HL Values

sample	MTZ ^a (%)	St ^b (rpm)	Amp ^c (%)	Z _D ^d (μm)	Pd ^e (%)	surf ^f (%)	EE ^g (%)	HL ^h (%)
S0	0	18 000	35	0.603	48.8	5.5	0	0
S5	5	18 000	35	0.592	52.1	4.4	35.1	1.8
S10	10	18 000	35	0.626	51.7	4.2	51.4	5.3
S20	20	18 000	35	0.587	56.2	5.3	59.1	11.1
S30	30	18 000	35	0.607	51.6	3.8	64.7	18.0
M0	0	11 500	20	0.752	50.7	3.2	0	0
M5	5	11 500	20	0.737	48.2	2.2	64.2	3.7
M10	10	11 500	20	0.760	51.5	2.6	62.5	6.8
M20	20	11 500	20	0.778	55.9	2.2	57.7	11.1
M30	30	11 500	20	0.803	49.5	3.0	61.9	17.8
L0	0	18 000	no	5.25	63.9	0.5/0.9	0	0
L5	5	18 000	no	6.72	36.4	0.6/1.8	73.0	3.7
L10	10	18 000	no	5.66	35.3	1.0/0.8	70.2	7.9
L20	20	18 000	no	4.94	27.6	0.4/1.4	64.8	13.5
L30	30	18 000	no	8.09	30.7	1.0/0.3	65.2	22.9

^aPercentage of MTZ in a formulation at the beginning of the process. ^bStirring speed. ^cAmplitude of sonication. ^dZ-average diameter. ^ePolydispersity. ^fSurfactant incorporation in particles (gelatin for S and M series, gelatin/Tween20 for L series). ^gEncapsulation efficiency. ^hHerbicide loading.

from surface was measured in the wavenumber range 500–4000 cm⁻¹. The collected spectra represented the average of 32 scans at a spectral resolution of 2 cm⁻¹. Spectra of formulations before and after herbicide release tests were recorded.

Surfactant Incorporation. The amount of surface-active agents encapsulated in particles was determined by elemental analysis in Thermo Electron Corp. Flash EA 1112 series analyzer in a configuration CHNS/O. Particles were washed with distilled water three times to remove soluble substances from their surface. Contents of individual compounds (PLA, MTZ, and surfactants) in formulations were calculated from the system of linear equations, which was based on known percentages of measured elements (C, H, N, S, and O) in pure compounds and in a given sample.

Release of Herbicide. Aliquots of particle suspensions containing exactly 5 mg of MTZ were centrifuged. Separated particles were resuspended in 100 mL of phosphate buffer (20 mmol·L⁻¹, pH 7) containing 0.2% sodium azide to prevent undesirable microbial degradation. All was done in triplicate. Suspensions were shaken (120 rpm) at 25 °C. Subsamples of 1.5 mL were taken in time intervals, centrifuged at 14 000 rpm for 10 min, and filtered through a 0.22 μm syringe PTFE filter to remove remaining particles. MTZ in samples was determined by the HPLC method described above.

Hydrolysis of PLA. Three sets of experiments at different pH were performed to determine the extent of PLA hydrolysis in the aquatic environment. Purified PLA powder (1.6 g) was suspended in 80 mL of phosphate buffer (0.1 mol·L⁻¹, pH 3, 7, and 9), in triplicate for each pH and kept at 25 °C. Samples (1.5 mL) were taken at intervals and centrifuged, and supernatants were analyzed for dissolved organic carbon (TOC 5000A analyzer, Shimadzu).

RESULTS AND DISCUSSION

Particle Preparation and Herbicide Encapsulation. PLA particles containing MTZ were prepared by the above-described oil-in-water solvent evaporation technique. The size of particles was influenced by some of the procedure parameters. These process parameters and characteristics of the resulting particles are listed in Table 1. The study approach included preparation of three series of particles marked S, M, and L, which were intended to differ mainly in size. In order to achieve the maximal amount of encapsulated MTZ, its initial ratio in a formulation was increased up to 40% limit, where PLA particles tended to precipitate. Similar phenomenon has

been already observed with a lipophilic drug encapsulated in PLA matrix.¹⁸

Particle diameters were controlled by ultrasonication parameters, stirring speed, and use of auxiliary surfactant Tween20 in the case of L series. Finally, S series of particles differed from M series by only about 200 nm, whereas L series particles were about 10 times larger. In contrast to the expectations derived from literature data, no significant increase in particle size with herbicide loading was detected in comparison with empty particles (S0, M0, L0);^{16,17} in S series the values fluctuated close to 600 nm, in M series a slightly growing trend could be detected, and in L series a significantly higher z-average was achieved for 30% MTZ loading only. No significant differences in PSD represented by polydispersity (Pd) could be observed, either between various formulations in S and M series or between the individual series. Pd values seem to be more variable in L series, with the broadest PSD found for blank sample and PSD of loaded formulations considerably narrower in comparison with other formulations. It could be concluded that in the described case the process conditions, mainly the use of ultrasonication, have a crucial impact on particle size and PSD, unlike MTZ loading, which appeared to have almost no effect.

The amount of surfactants enclosed in particles was determined to evaluate its transfer with particles and potential influence on the herbicide release process. In the case of L series, the appropriate amount of Tween20 had to be added along with gelatin, used as the main stabilizer, in the process of submicroparticle preparation, because PLA precipitation occurred when gelatin alone was used. The decrease of surfactant incorporation with increasing size of particles can be observed in Table 1. Since a surfactant is expected to be present near the particle surface, the trend can be attributed to the reduction of surface area related to particle volume with increasing diameter of particles. There is no clear trend related to MTZ loads.

The encapsulation efficiencies obtained were about 60%, which are lower values in comparison with the study describing encapsulation of another herbicide, tubeconazole,¹⁴ where EE

values over 95% were achieved. An explanation could be found in about 10 times lower water solubility of tubeconazole ($0.032 \text{ g}\cdot\text{L}^{-1}$) in comparison with MTZ ($0.45 \text{ g}\cdot\text{L}^{-1}$). Higher EE are easily reached with less water-soluble agents. EE values were significantly lower for smaller particles (S and M series). Again this could be explained by higher specific surface area of smaller particles.

As expected according to a number of research papers,^{5,17} HL values increased with increasing initial amounts of MTZ in all series. The highest HL values with respect to initial MTZ amounts were achieved in the L series, which is in agreement with the highest obtained EE values for this series.

Morphology of Particles. SEM micrographs representing a comparison between blank particles without MTZ and maximally loaded particles containing 30% MTZ are assembled in Figures 1 and 2 for S, M, and L series of particles. No marked

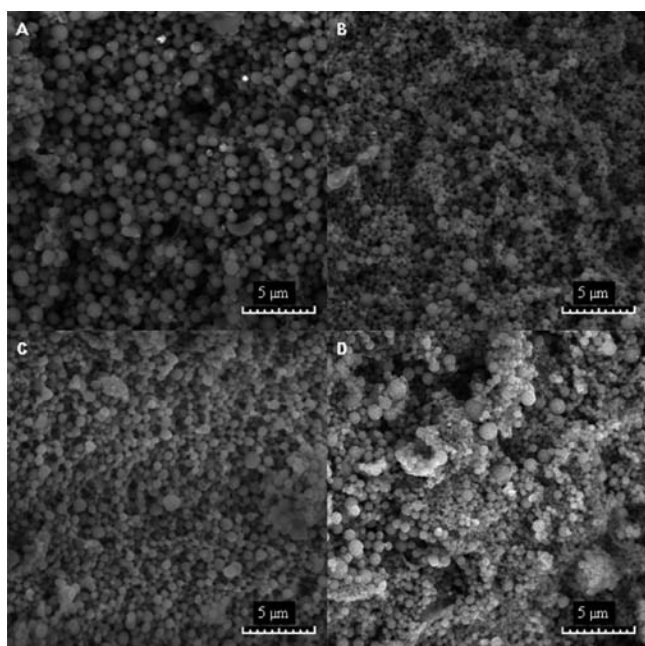


Figure 1. Scanning electron microscopy of polymer submicroparticles (10000 \times): (A) S0, (B) S30, (C) M0, and (D) M30 formulations.

differences in the size and the surface morphology of particles were observed between empty (Figures 1A,C and 2A) and maximally filled particles (Figures 1B,D and 2B) for S, M, and L series. All types of particles from S and M series appears to be spherical (Figure 1); however, in L series, significant deviations

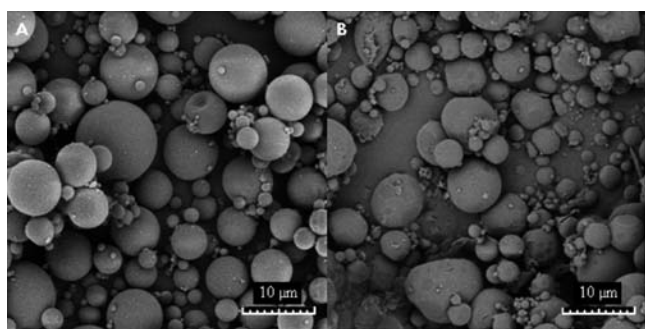


Figure 2. Scanning electron microscopy of polymer submicroparticles (5000 \times): (A) L0 and (B) L30 formulations.

from spherical shape of 30% MTZ loaded particles (Figure 2A) in comparison with empty particles (Figure 2B) are quite evident.

Differential Scanning Calorimetry. Thermal analysis of particles was performed to clarify the effect of polymer properties, especially crystallinity, on MTZ release. Thermal properties of loaded and unloaded microparticles investigated are summarized in Table 2. It can be seen that both T_g and T_m

Table 2. Thermal Properties of Microparticle Formulations

sample	T_m^a ($^{\circ}\text{C}$)	T_c^b ($^{\circ}\text{C}$)	T_g , midpoint ^c ($^{\circ}\text{C}$)	T_g , onset ^d ($^{\circ}\text{C}$)	χ_c^e (%)
S0	149.8	85.2	46.6	42.9	58.9
S5	147.0	80.0	43.8	39.7	50.1
S10	143.5	80.7	39.4	36.2	49.9
S20	141.3	79.7	35.2	31.2	44.4
S30	139.8	79.7	34.6	31.1	40.3
M0	148.7	85.7	46.3	44.2	64.9
M5	147.7	88.1	45.2	42.3	63.9
M10	145.5	86.5	42.0	39.3	61.0
M20	142.7	85.0	39.2	36.1	55.9
M30	140.0	83.3	35.3	32.3	55.0
L0	149.3	90.4	42.4	37.8	58.7
L5	148.5	90.6	36.5	32.8	64.3
L10	146.6	86.4	40.1	36.3	59.6
L20	142.5	85.0	36.5	32.8	51.1
L30	140.0	81.7	32.3	28.1	43.9

^aMelting temperature. ^bCrystallization temperature. ^cGlass-transition temperature at midpoint. ^dGlass-transition temperature at onset. ^eDegree of crystallinity.

decreased with increasing particle loading. For S and M particles, the glass transition temperature dropped by nearly 12°C , while for L particles the decrease was 9.7°C . This could be explained by an assumption that MTZ acted as a plasticizer in PLA matrix and increased free volume of polymer chains. Similarly, melting temperature declined, probably because MTZ limited crystal development in PLA matrix and decrease of crystallinity χ_c is a logical consequence.

Herbicide Release Study. The influence of herbicide loadings in micro- (L, Figure 5) and submicroparticles (S, Figure 3; M, Figure 4) on the cumulative release of herbicide

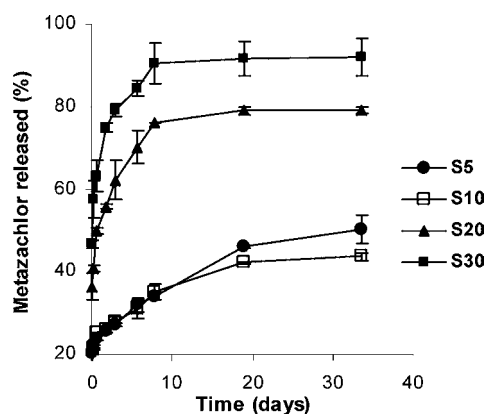


Figure 3. MTZ release profiles from PLA particles for S formulations. Error bars correspond to twice the standard deviation ($n = 3$).

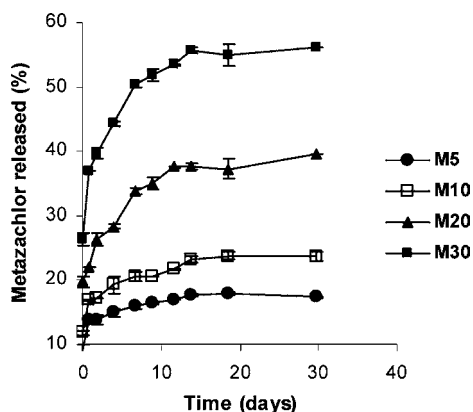


Figure 4. MTZ release profiles from PLA particles for M formulations. Error bars correspond to twice the standard deviation ($n = 3$).

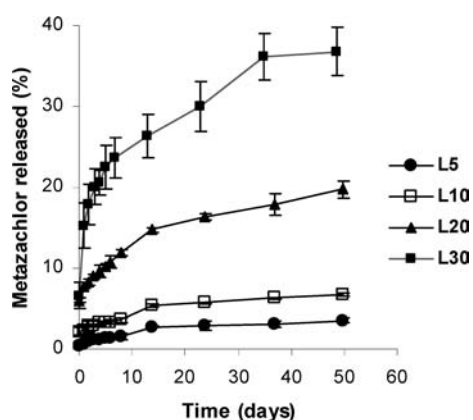


Figure 5. MTZ release profiles from PLA particles for L formulations. Error bars correspond to twice the standard deviation ($n = 3$).

MTZ for various particle sizes was investigated. As can be seen, for all formulations the release of MTZ could be characterized by a nonlinear biphasic profile, where the initial rapid release was followed by a considerably slower phase. The initial fast phase might be interpreted as the release of MTZ situated close to the surface.⁵ Although a certain portion of MTZ was lost during a relatively fast initial period, the principal part of the agent was being released gradually during days and weeks as required for a controlled release formulation.

Results in Figures 3–5 show that the percentage of released MTZ in the initial phase increased significantly with increasing particle loadings. Such behavior corresponds well with previously published observations made with another polymer matrix and herbicide^{5,15} and might primarily be caused by the increased percentage of the herbicide close to the surface and the consequent shortening of the diffusion pathway from particles for this portion of the active agent. Described trends, however, could also be potentiated by changes in thermal properties of particles. It is well-known that the release of agents from polymer matrix changes dramatically with the glass transition of the polymer matrix, because of the pronounced shift of diffusion coefficients and other parameters. Generally, above T_g the release is expected to be faster than below T_g .^{16,28,29} In our experiments the glass transition region of highly loaded formulations (Table 2) was relatively close to the temperature in release experiments (25 °C), which can, at least partially, explain higher MTZ release rates from highly loaded particles.

By comparison of formulations with identical MTZ loadings, it is apparent that the size of particles affects drug release significantly. The diminution of herbicide cumulative release with increasing z -averages of particles can be observed for all formulations. For instance, after 30 days the percentage of released herbicide for the highest loading was 92% for S30 (Figure 3), 56% for M30 (Figure 4), and 34% for L30 (Figure 5) formulations. Similar behavior was already observed for the same technique of particle preparation^{25,30} and can be explained by the prolongation of an active agent diffusion pathway in larger particles. However, as can be detected from Figures 3–5, this trend is also strongly influenced by the magnitude of the release in the initial burst phase. As an illustration of this fact, the decrease of herbicide released from the above-mentioned formulations (47% from S30, 27% from M30, and 7% from L30) at the beginning of tests can be used. Since the initial burst relates to the release of the agent situated close to the surface of particles, the effect can probably be attributed to the reduction of specific surface area with increasing particle diameters.

For largest and maximally loaded particles (L30), their morphology before and after releasing test can be compared in Figure 6 as an example. The SEM microphotographs suggest

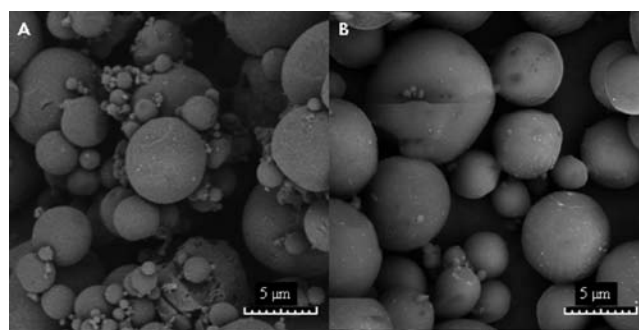


Figure 6. Scanning electron microscopy of polymer submicroparticles (10000 \times): L30 formulation (A) before and (B) after release tests.

that before testing the particles have somewhat rougher surface, in comparison with a rather smooth surface after the release test. The roughness could be a consequence of some portion of MTZ deposited on the surface. However, despite the release of a considerable amount of MTZ, surprisingly, no surface porosity was detected after the test.

As supporting evidence of MTZ incorporation and its subsequent release, FTIR-ATR spectra of pure MTZ and PLA particles without MTZ, as well as loaded particles before and after release tests, were recorded (Figure 7). The spectrum of pure PLA can be characterized by signals at 1751 cm^{-1} (attributed to C=O bond), 1452 cm^{-1} (typical for CH_3 bending), 1366 cm^{-1} (asymmetric $-\text{CH}-$ bending), and the region between 1300 and 1040 cm^{-1} with four intensive absorption peaks at 1185, 1126, 1088 (C–O–C stretching), and 1046 cm^{-1} ($-\text{OH}$ bending).^{25,30,31} The spectrum of MTZ is more complex²⁰ but could be characterized by a distinctive signal at 1685 cm^{-1} of C=O stretching in the amide group, clearly visible also in the background of PLA spectra.³² As can be seen for loaded particles, signals at 1685 cm^{-1} were clearly reduced after release tests, witnessing a drop in MTZ concentrations and its release from particles. The fact that the signal did not disappear completely also confirmed above-presented data, obtained by HPLC, that some herbicide

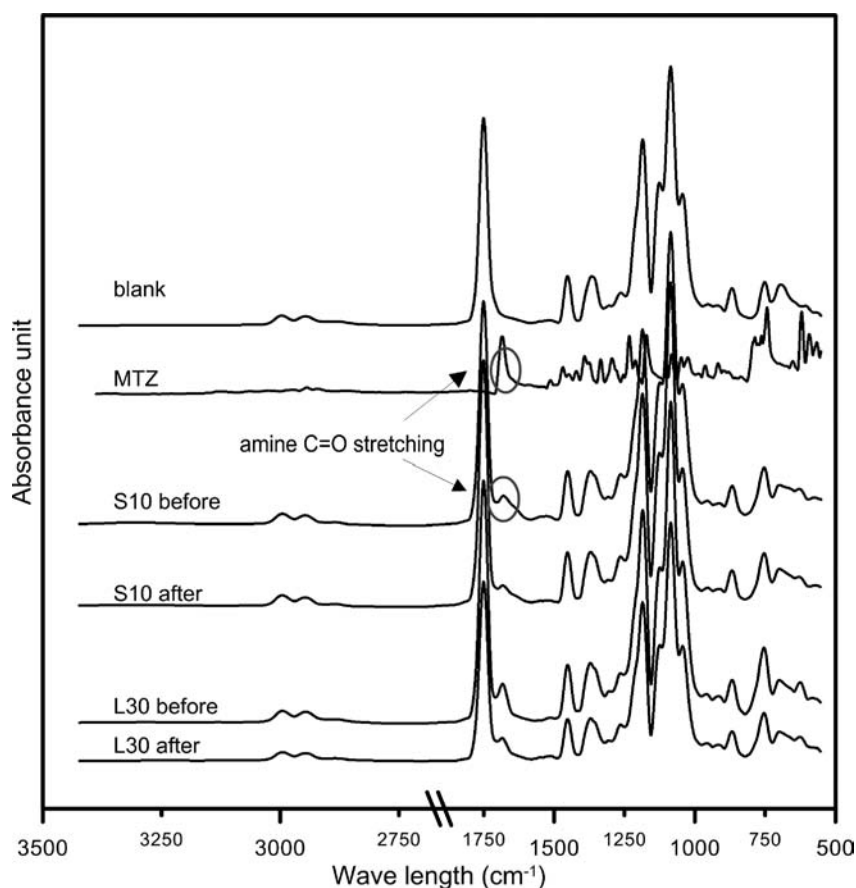


Figure 7. FTIR-ATR spectra of MTZ, unloaded particles, and MTZ-loaded particles.

remained in particles at the end of the release test period. This residual MTZ probably would be released during a longer time period and/or along with the degradation of particles.

PLA Hydrolysis. PLA hydrolysis, if taking place to a significant extent, could have an important influence on the kinetics of MTZ release from PLA particles. Cumulative amount of hydrolyzed PLA was calculated from the measured release of dissolved organic carbon. Three pH values (3, 7, and 9) corresponding to the theoretical interval of common soil pH were investigated (Figure 8). Results showed that the rate of PLA hydrolysis increased with increasing pH value. However, during the recorded period, corresponding with the period of MTZ release tests, percentages of the released carbon

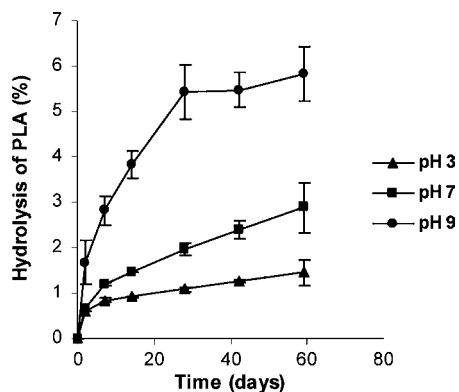


Figure 8. PLA hydrolysis at various pH values. Error bars correspond to twice the standard deviation ($n = 3$).

hydrolyzed from the polymer were rather low for all pH values and especially for pH 7 used in MTZ release experiments. It could be concluded then that PLA hydrolysis should not influence considerably MTZ release from PLA particles under given conditions.

Metazachlor Release Kinetics. Obtained data from MTZ release experiments were analyzed by applying the common semiempirical exponential equation to describe and assess the influence of size and herbicide loading on the release kinetics:¹⁵

$$\frac{M_t}{M_\infty} = Kt^n + C$$

where M_t is the amount of active agent released at time t , M_∞ is the total amount of agent in particles. K is a release constant, C is a constant that takes into account the initial burst effect, and n is a diffusional exponent describing the mechanism of release depending on the geometry of the system.³³ For a system described by Fickian diffusion, where the concentration gradient of a released compound is the dominant driving force, the diffusion parameter is equal to 0.50; for a degradation controlled-release system, where the erosion of particles is the major force, the diffusion parameter is close to 1.0. In the case of spheres, n should be corrected to 0.43 and 0.85, respectively.^{33,34}

Parameters of the equation along with the correlation coefficient R and the time needed for 50% release of MTZ were obtained by nonlinear regression and are listed in Table 3. High R values indicate good fitting of exponential equations on measured MTZ release profiles from all particle formulations.

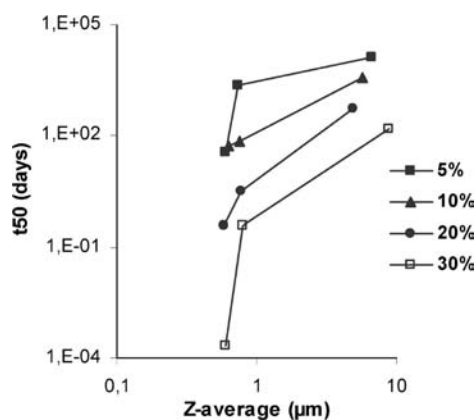
Table 3. Parameters Characterizing Fitting of the Model Equation on MTZ Release Data

sample	K^a (10^{-2})	n^b	C^c (10^{-2})	R^d	t_{50}^e (days)
S5	1.374	0.459	19.360	0.986	36.35
S10	2.820	0.347	16.750	0.993	50.82
S20	9.183	0.255	33.910	0.971	0.38
S30	13.660	0.196	45.170	0.968	0.000 21
M5	4.612	0.199	9.188	0.986	2362.09
M10	5.125	0.271	11.820	0.989	69.44
M20	6.554	0.361	19.060	0.980	3.08
M30	13.330	0.269	25.880	0.988	0.38
L5	0.112	0.483	0.262	0.974	12 634.83
L10	0.126	0.521	1.951	0.977	3730.47
L20	0.510	0.471	5.684	0.994	541.04
L30	3.557	0.306	6.619	0.996	149.33

^aRelease constant. ^bInitial burst constant. ^cDiffusional exponent. ^dCorrelation coefficient. ^eTime corresponding to 50% agent release.

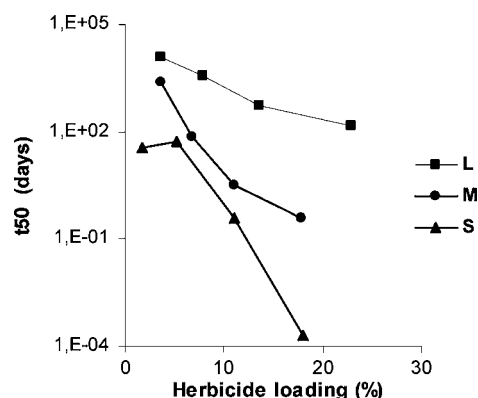
As can be seen, the release exponent $n < 0.52$ obtained in all experiments indicate that particle degradation was not the crucial driving force of the release. This observation is in agreement with the data from PLA hydrolysis at pH 7, when negligible hydrolysis was detected. In formulations with n close to 0.43, the release was probably controlled by diffusion; however, n values lower than 0.43 indicate another mechanism governing the herbicide release. There the high C values suggested that the release kinetics was probably influenced by "burst release",¹⁵ which could be regarded as an undesirable effect.³⁵

The resulting relationships of MTZ release half-times (t_{50}) to z -averages and herbicide loadings in three series of preparations are shown in Figures 9 and 10.

**Figure 9.** Dependence of t_{50} on particle z -averages for various initial percentages of MTZ.

These trends calculated from nonlinear regression analysis confirmed our previous observations that generally the rate of MTZ release decreased with increasing z -average of particles and increased with herbicide loading. Steep initial phases of t_{50} versus z -average dependences are probably a consequence of the fact that for the smallest particles the release kinetics is most strongly influenced by the initial burst release.

Three series of polymer micro- and submicroparticles varying in size were prepared from low molecular PLA by oil-in-water

**Figure 10.** Dependence of t_{50} on herbicide loading for various formulations.

solvent evaporation technique and loaded with various initial amounts of the herbicide metazachlor. Particle loadings up to 30% of the theoretical herbicide content were investigated: for higher loadings, polymer precipitation occurred during solvent evaporation. Encapsulation efficiency reached about 60% and was lower for smaller particles (S and M series). Consequently, the highest actual herbicide loading values (HL) were found in L series, which was in agreement with encapsulation efficiency (EE) results. Ultrasonication was found to have a crucial impact on particle size and particle size distribution (PSD), unlike MTZ loading, which had almost no effect. All formulations appeared to be spherical and not affected by the amount of MTZ incorporated, with the exception of the highest loaded formulations in L series, which exhibited deviations from spherical shape. MTZ release was characterized by the typical nonlinear biphasic profile, where the release from formulations with diffusional exponent n close to 0.43 was found to be diffusion-controlled, while for formulations with n lower than 0.43 it was significantly influenced by the initial burst release. Low degree of PLA hydrolysis means that particle erosion has not considerably influenced MTZ release from PLA particles under given conditions. Generally, it was found that the rate of release depends inversely on the z -average and directly on herbicide loading. Since MTZ acted as a plasticizer in PLA matrix and decreased T_g , MTZ release rate could have been enhanced by this effect in highly loaded formulations, whose T_g was relatively close to the temperature employed in release experiments. Our study proved that preparation of a controlled-release formulation of the herbicide metazachlor with relatively high water solubility based on biodegradable PLA microparticles is possible in principle. By combination of different formulations, a particular release profile could be tuned to fit desired parameters.

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Notes

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ABBREVIATIONS

DSC, differential scanning calorimetry; EE, encapsulation efficiency; GPC, FTIR-ATR, Fourier transform infrared attenuated total reflectance; GPC, gel-permeation chromatography; HL, herbicide loading; HPLC, high-performance liquid chromatography; M_n , number-average molecular mass; MTZ, metazachlor; MW, molecular weight; M_w , weight-average molecular mass; Pd, polydispersity; PLA, poly(lactic acid); PSD, particle size distribution; SEM, scanning electron microscopy; T_c , cold crystallization temperature; T_g , glass-transition temperature; T_m , melting point temperature; Z_D , z-average; ΔH_c , heat of crystallization; ΔH_m , heat of fusion; σ , standard deviation; χ_c , degree of crystallinity

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