# Effects of Ethyl Benzoate on Performance, Morphology, and Erosion of PLGA Implants Formed In Situ

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**ABSTRACT:** An in situ forming implant (ISFI) is a novel drug delivery system used for protein and peptide delivery, especially for cancer treatment. An ISFI based on 33% (w/w) poly(D,L-lactide-*co*-glycolide)(PLGA; 50:50)/3% (w/w) leuprolide acetate (LA)/64% (w/w) *N*-methyl-2-pyrrolidone (NMP) was prepared for this study. After injection of the final formulation, which is a viscous liquid to an aqueous medium, it deforms to become a semisolid or solid matrix. The performance of this matrix was investigated on the basis of peptide release from it. Erosion and morphology of ISFI were also studied. The effects of adding 12.8% (w/w) ethyl benzoate (EB) as a rate-modifying agent on performance, erosion, and morphology of ISFI were assessed. The implant containing EB showed very low burst release (5.53% ± 0.82%) and the morphology turns to closed pore-like structures. After adding EB, the morphology turns to closed pore-like structures. This type of morphology has very close relation to the performance of the implant as well. Finally, the effect of EB on performance, erosion, and morphology is explained by means of solvent–nonsolvent affinity, water permeation, and the rate of phase inversion.  Adv Polym Techn 27: 17–26, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/adv.20114

**KEY WORDS:** Drug release, Erosion, In situ forming implant, Morphology, PLGA

## Introduction

A wide variety of applications have been developed for separation technology, such as purification of liquids, gas separation, biological processes, medical devices, blood purification, and drug delivery.[1–6] One of the common techniques for the preparation of in situ formed implants is the phase separation phenomenon.[7] In situ forming implant (ISFI) can provide controlled release of a drug while offering greater ease of administration than surgical implants. The main parts of an ISFI are nonreactive synthetic biodegradable polymers such as polylactides, polyglycolides, polycaprolactones,[8] additives,[9] and drugs, which are dissolved in a preferably biocompatible and pharmaceutically acceptable solvent such as *N*-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide, or glycoferol.[10] When the solution is injected into the body, the water miscible organic solvent dissipates and water penetrates into the organic phase. This leads to phase separation, which can form an implant at the site of injection.[11] Understanding of polymer properties, membrane morphology, and formation processes is necessary for the improvement of membrane performance in drug delivery.[12] Poly(lactide-*co*-glycolic acid) (PLGA), which has been chosen for this study, is a known polymer that has been used in several branches of medical sciences.

The phase inversion of PLGA solutions is a complicated phenomenon, directly affected by solvent properties.[11] Solvents in the phase separation processes may be categorized as either strong solvents or weak solvents.[13] *N*-methyl-2-pyrrolidone (NMP), which is usually treated as a strong solvent, has been used in our study as well.

A rate-modifying agent that causes a decrease or increase at the release rate of drug and specially for controlling the burst release can be an additive for the formulation of an ISFI. Adding a weak solvent such as ethyl benzoate (EB), which is poorly soluble in aqueous medium or body fluids, is one of the suitable rate-modifying agent.[14]

Furthermore, ISFI morphology has direct effects on ISFI performance and strongly depends on phase inversion conditions such as polymer type, additives, coagulation bath, and polymer solution composition.[15–17]

In the present study, the performance of PLGA/NMP/water systems based on the release of a model peptide, leuprolide acetate (LA), is investigated. LA is used to treat advanced prostate cancer, uterine fibroids, and endometriosis.[18–21] Currently, LA is also being evaluated in Phase II clinical trials for the treatment of Alzheimer's glycofurol.[22,23] Also release, erosion and morphology study of ISFI both in the presence and absence of ethyl benzoate as a rate-modifying agent is reported.

**TABLE I**
**Compositions of the Membrane-Casting Solutions ($n = 3$)**

| Formulation | Polymer (% w/w) | Drug (% w/w) | NMP (% w/w) | Ethyl Benzoate (% w/w) |
|---|---|---|---|---|
| 1 | 33 | 3 | 64 | – |
| 2 | 33 | 3 | 51.2 | 12.8 |

# Experimental

## MATERIAL AND METHODS

The following materials were employed in this study: poly(D,L-lactide-*co*-glycolic acid) (PLGA) 50:50 copolymers, Resomer® RG 504 H ($M_w$ 48 kDa) provided by Boehringer Ingelheim (Dortmund, Ingelheim, Germany) as polymer, leuprolide acetate supplied by Bachem Inc. (Bubendorf, Switzerland) as a model peptide, *N*-methyl-2-pyrrolidone (NMP) was purchased from Merck (Darmstadt, Germany) as a solvent, and ethyl benzoate, sodium azide, Tween® 80, disodium monophosphate as a rate modifying agent were obtained from Merck (Darmstadt, Germany). Other chemicals were obtained commercially, and all were analytical-grade reagents.

## SOLUTION PREPARATION

Homogeneous polymer solutions were prepared from PLGA in NMP without and with 12.8 wt% ethyl benzoate (Table I). Furthermore, drugs were added and dissolved completely in all solutions.

## PREPARATION OF ISFI

The solutions were cast on a homemade holding cell at room temperature. The polymer solutions on the holding cell were immediately immersed in a release medium coagulation bath before any phase inversion in air. To follow the performance and morphology of in situ formed ISFI, the device was stored in a medium for 28 and 3 days for release and morphology studies, respectively. This guarantees complete phase inversion that is needed for morphology results and allows for release of drugs on the other hand.

## PERFORMANCE OF ISFI

The performance of prepared ISFIs was measured on the basis of the drug release. The experiments for release studies were carried out in polypropylene vials for the lowest peptide adsorption. For this purpose, the polymer solutions were placed in a homemade holding cell, which was separated from the receptor phase by a mesh and also solvent–nonsolvent exchange is available.

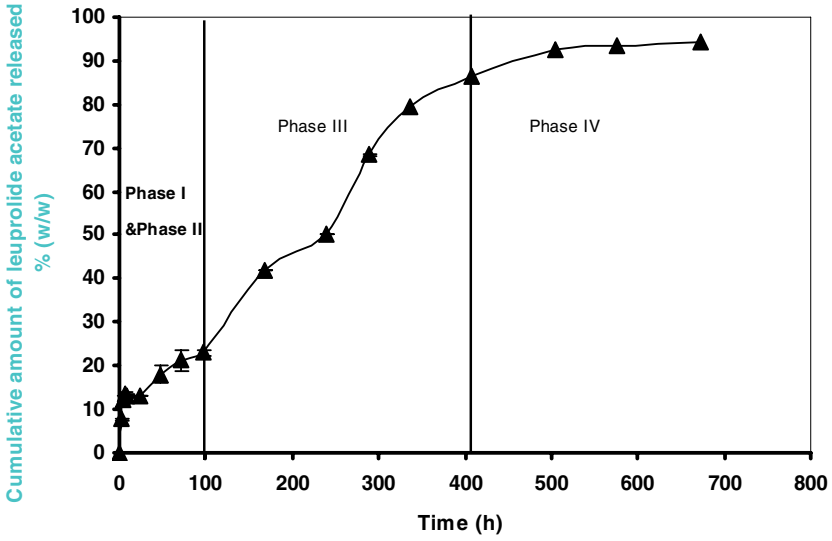


**FIGURE 1.** Drug release profile from an ISFI with PLGA/NMP/LA weight ratio of 33/64/3, respectively. Data are mean ± SD, $n = 3$.

The release medium bath, which consisted of phosphate buffer (0.03 M, pH 7.4) containing sodium azide and Tween® 80,[24] was used at 37°C for all trials. In predetermined time intervals, 1 mL of the receptor phase was withdrawn using a plastic syringe assembly and was replaced with 1 mL of fresh receptor medium. Leuprolide acetate release was analyzed by high performance liquid chromatography (HPLC), operated by the reversed phase C-18 column (Waters Inc., Bedford, MA, USA), isocratic elution of a mobile phase composed of 68:32 volume ratio of deionized water:acetonitrile containing 0.1 % (w/v) trifluoroacetic acid using UV detection at 220 nm.[25]

### SCANNING ELECTRON MICROSCOPY

The surfaces and cross sections of the prepared membranes were studied with a scanning electron microscope (SEM), Cambridge S360. Samples were transferred into the SEM instrument after gold coating. The SEM studies were carried out at room temperature with magnifications of 40, 80, 100, 300, and 800 after 3 and 7 days.[26]

### EROSION OF ISFI

The degradation of ISFIs was characterized by two methods: (a) L-lactic acid detection,[27] which is based on a colorimetric method for enzymatic determination of L-lactate, using a Randox kit and (b) pH change study[28], using a Mettler pHmeter.

### STATISTICAL ANALYSIS

Compiled data were presented as mean ± SD. For comparing the burst release, data were analyzed for statistical significance by the unpaired student *t*-test supported by SPSS 10 for Windows (SPSS Inc., Chicago, USA). For this purpose, the level of significance was set at $P < 0.05$.

## Results and Discussion

### PREPARATION AND PERFORMANCE OF IN SITU FORMED PLGA IMPLANT

The release profile of peptide from PLGA/NMP solution after solidification at a phosphate buffer is

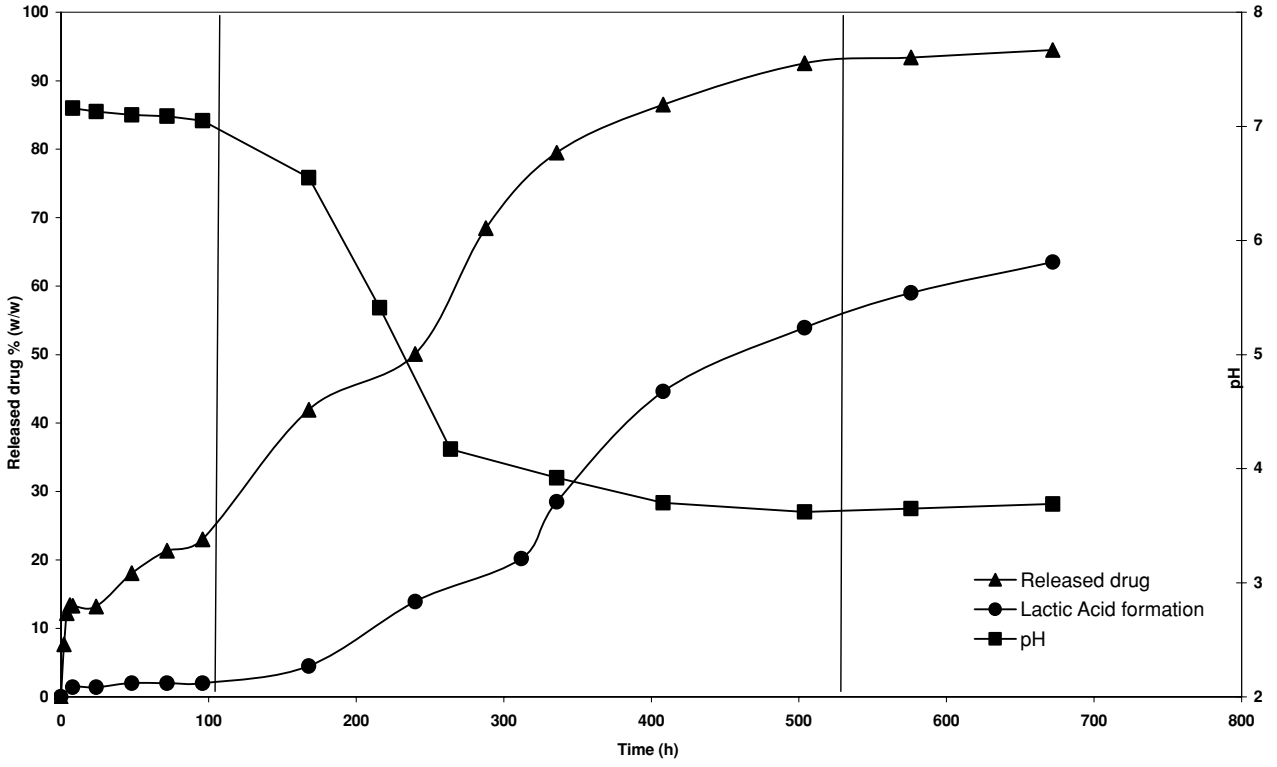


**FIGURE 2.** Lactic acid, pH, and drug release profiles from an ISFI with PLGA/NMP/LA weight ratio of 33/64/3, respectively.

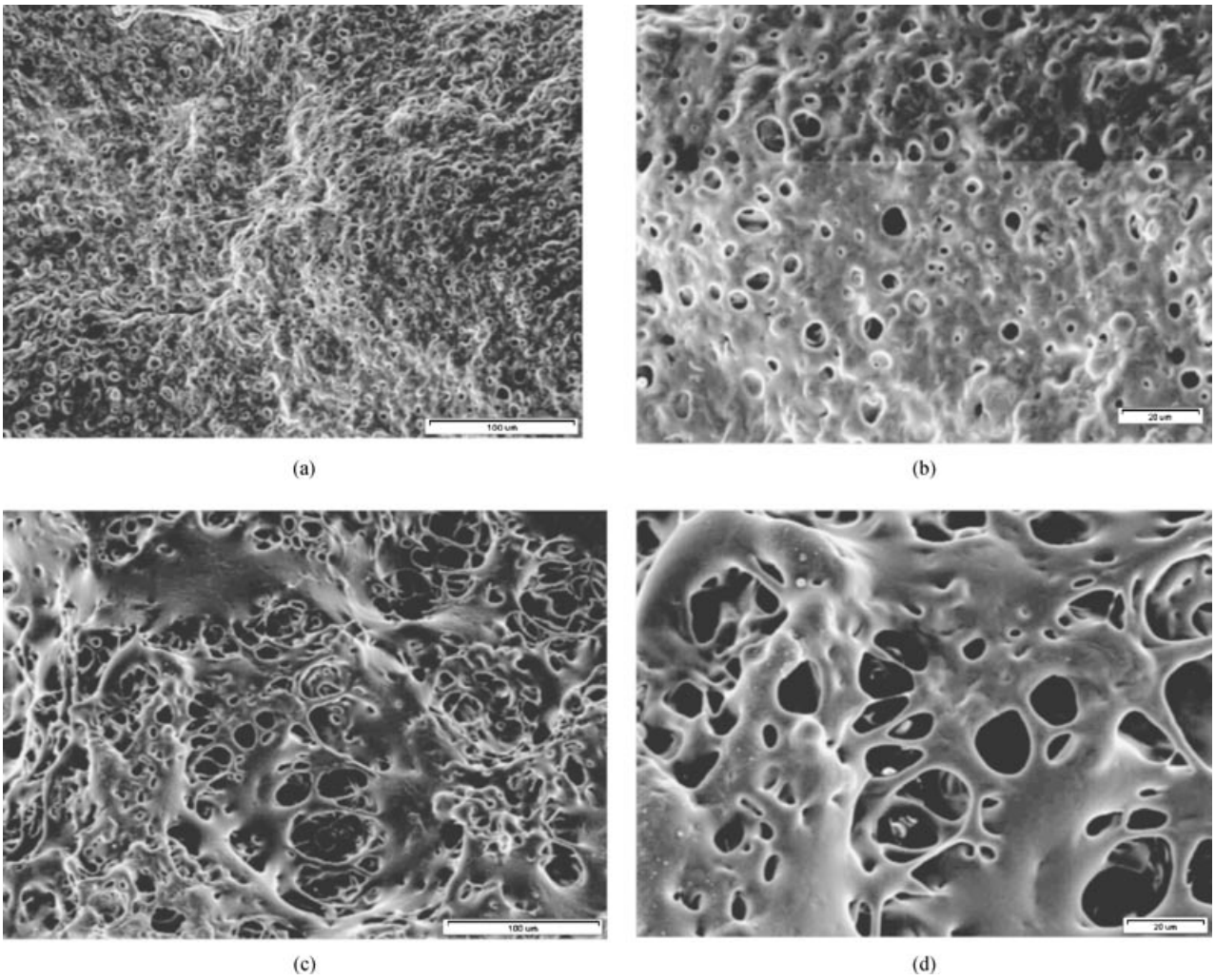


**FIGURE 3.** Surface morphology of an ISFI 3 (a: 300×, b: 800×) and 7 days (c: 300×, d: 800×) after solidification.

shown in Fig. 1. As seen it can be divided into four different phases, with different release rates.[29]

The figure shows an initial burst phase (13% ± 0.078%) over the first 24 h (Phase I) followed by slower drug release pattern (Phase II). At Phase II, the membrane skin layer is formed and initiation of macrovoids takes place in the ISFI that covers approximately 100 h.[26–29]

At Phase III, peptide release is apparently controlled by degradation. In the last phase (Phase IV), the ISFI follows the complete degradation and peptide release shows the constant pattern. For better interpretation, peptide release, pH, and lactic acid profiles of the ISFI were superimposed on the same figure (Fig. 2). Phase II starts with the formation and setting of the polymeric matrix that covers approximately 100 h. At this phase, the skin barrier of ISFI forms. The pH is constant over the first 100 h. It may depend either on the absence of degradation products or on the resistance of buffer against the decrease in pH. As seen, the system shows lag-phases in lactic acid release, which is again related to phase transition and setting of the ISFI. Also release of the peptide from the ISFI in Phase II should be mainly controlled by the passive diffusion. Therefore, this phase can also be called as a diffusion phase.

## MORPHOLOGY OF IN SITU FORMED PLGA IMPLANT

The surface morphology of prepared ISFI solidified after 3 and (Figs. 3a and 3b) 7 days (Figs. 3c and 3d) is shown at two different magnifications. Micrographs show a smooth surface with some pores on it. After 1 week, the size of the pores changes significantly when compared with the ISFI after 3 days.

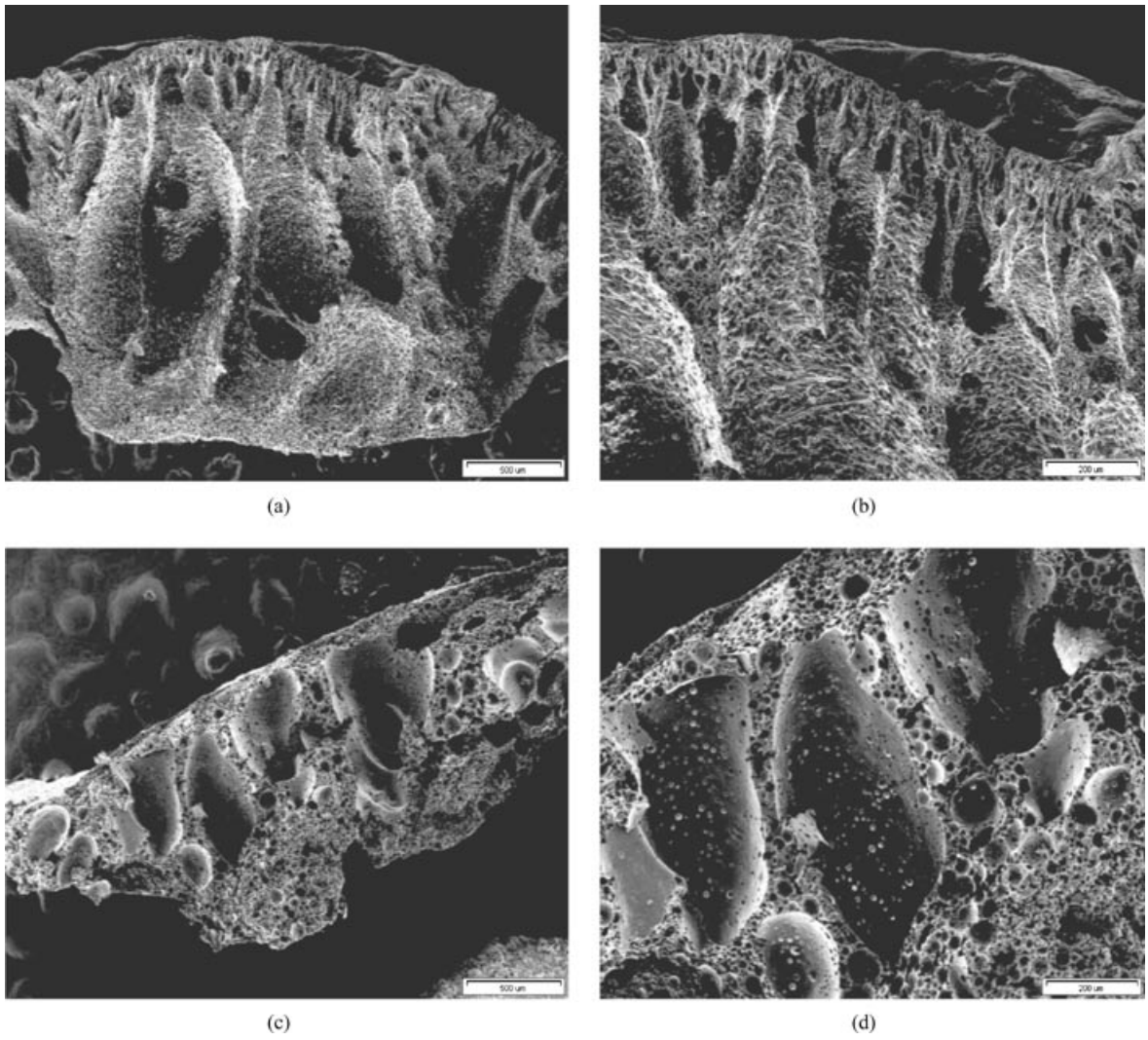


**FIGURE 4.** Cross-section morphology of an ISFI 3 (a: ×300, b: ×800) and 7 days (c: 300×, d: 800×) after solidification.

Figures 4a and 4b show cross-section morphology of the ISFI 3 days after solidification. The micrograph indicates the ISFI cross section including finger- and tear-like pores[30,31] from top to the bottom of it. Figures 4c and 4d show ISFI structure after 1 week, changes appearing in large open pores. This new structure with large volume interconnected pores provides an efficient pathway for release of peptide, also it confirms higher release rate kinetics and bulk erosion of ISFI at phase III as well.

## EFFECT OF ETHYL BENZOATE ON PERFORMANCE OF ISFI

ALZA Corporation has recently introduced more lipophilic solvents such as benzyl benzoate in the field of ISFI.[32] A comparative study using different hydrophilic and hydrophobic solvent systems for an ISFI was recently carried out by Cleland.[33]

EB has been chosen as a more hydrophobic solvent for our study. The release curve of formulations with and without EB has been illustrated in Fig. 5. The release profile of an ISFI without EB displays a large burst release of peptide, whereas the release profile of an ISFI containing EB is seen to be considerably lower. The amount of the peptide released in the initial burst phase was decreased by about 2.8 times at formulation containing EB. The cumulative amount of peptide released (14.50% ± 0.47%) over the first 24 h (burst phase) for the formulation, which was prepared without EB, was significantly ($P < 0.05$) higher than the formulation containing EB (5.53% ± 0.82%). NMP is a good solvent with the Hildebrand solubility parameter of 23.1 $MPa^{1/2}$, so

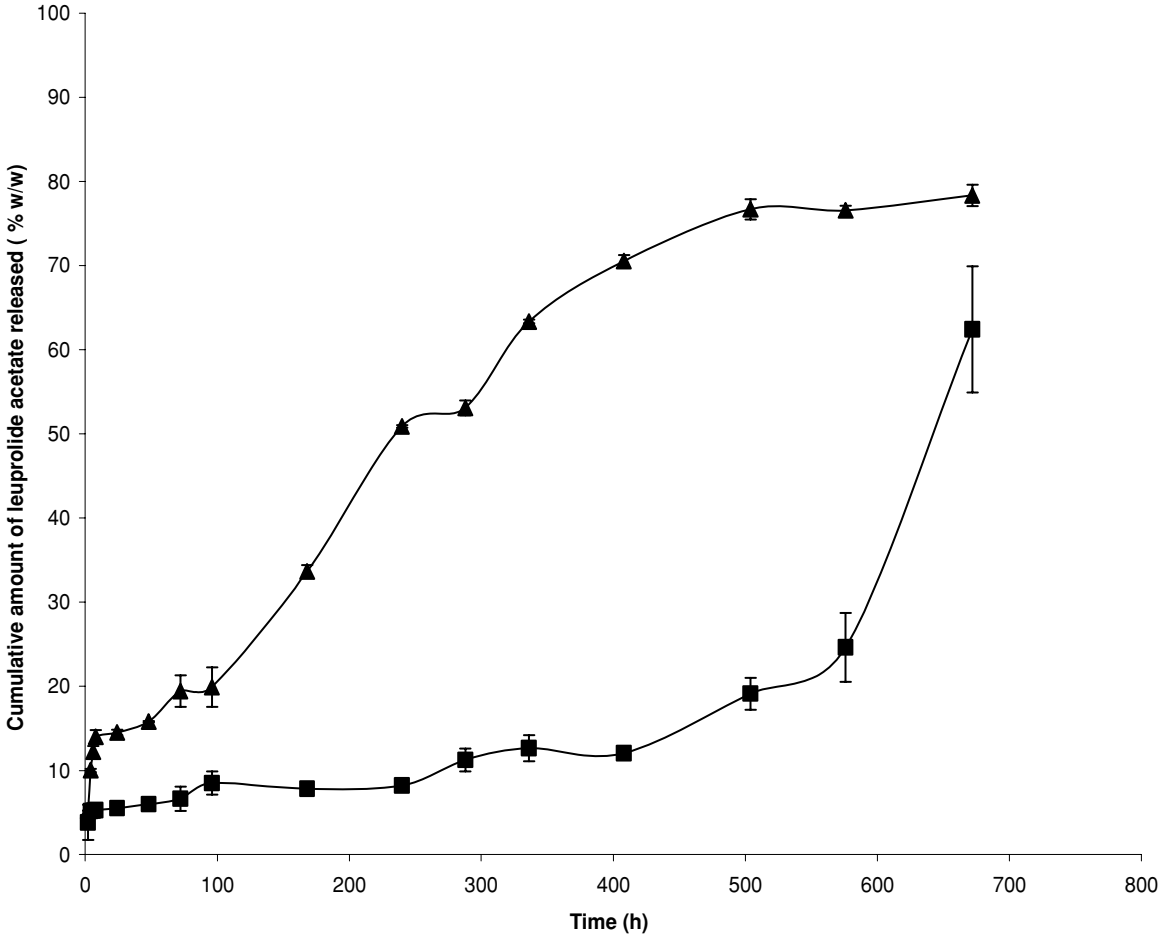


**FIGURE 5.** Leuprolide acetate release profiles from an ISFI without EB (▲) and 12.8 wt% EB (■). Data are mean ± SD, $n = 3$.

the fast phase inversion is characteristic of systems based on strong solvent–nonsolvent (NMP-water) affinity.[26,34]

In contrast to EB with the Hildebrand solubility parameter of 8.2 $MPa^{1/2}$, a slower exchange rate between solvent area (NMP:EB, 80:20) and nonsolvent (water) in release media was seen. The use of EB as a weak solvent additive and rate-modifying agent in the polymer solution can be adapted to cause a decreasing at the release rate of peptide when compared with the ISFI without EB.

Brodbeck et al.[12,26] also demonstrated that protein release kinetics from an ISFI were influenced by solution thermodynamics, for example, solvent strength and water miscibility. They proved that solvent type and polymer concentration are the most critical factors determining the release profile obtained under in vitro and possibly also in vivo conditions.

## EFFECT OF ETHYL BENZOATE ON EROSION OF ISFI

PLGAs as polyester polymers show sigmoidal degradation curves, especially in higher molecular weights [35]. In this study, erosion of PLGA 50:50 is determined with amount of lactic acid formation and pH changes (Figs. 6 and 7). As it is seen in Fig. 6, for both formulations pH remains almost constant over the first 100 h.

The main difference between two formulations is their onset of pH drop, which is shorter for the formulation without EB and could be due to its faster water uptake, degradation, and therefore the higher concentration of degradation products at the same time.

PLGA degradation is based on autocatalyzed hydrolysis of the ester linkages. Accumulation of the degradation products having carboxylic acid end

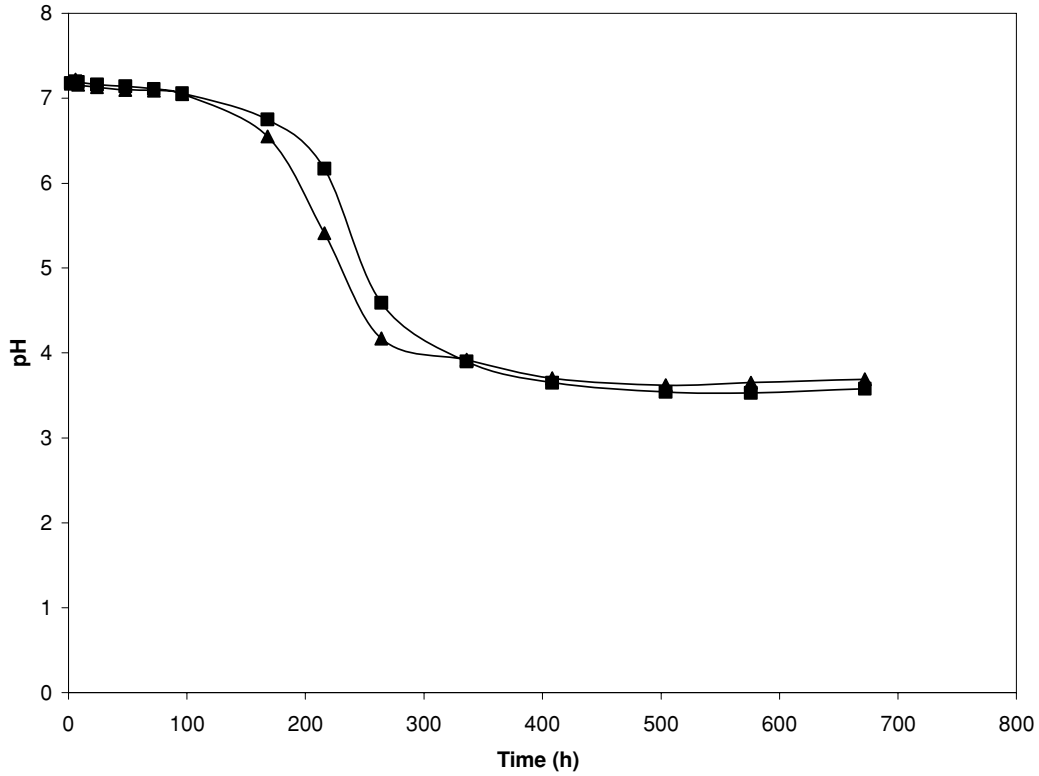


**FIGURE 6.** pH changes profiles from an ISFI without EB (▲) and 12.8 wt% EB (■).

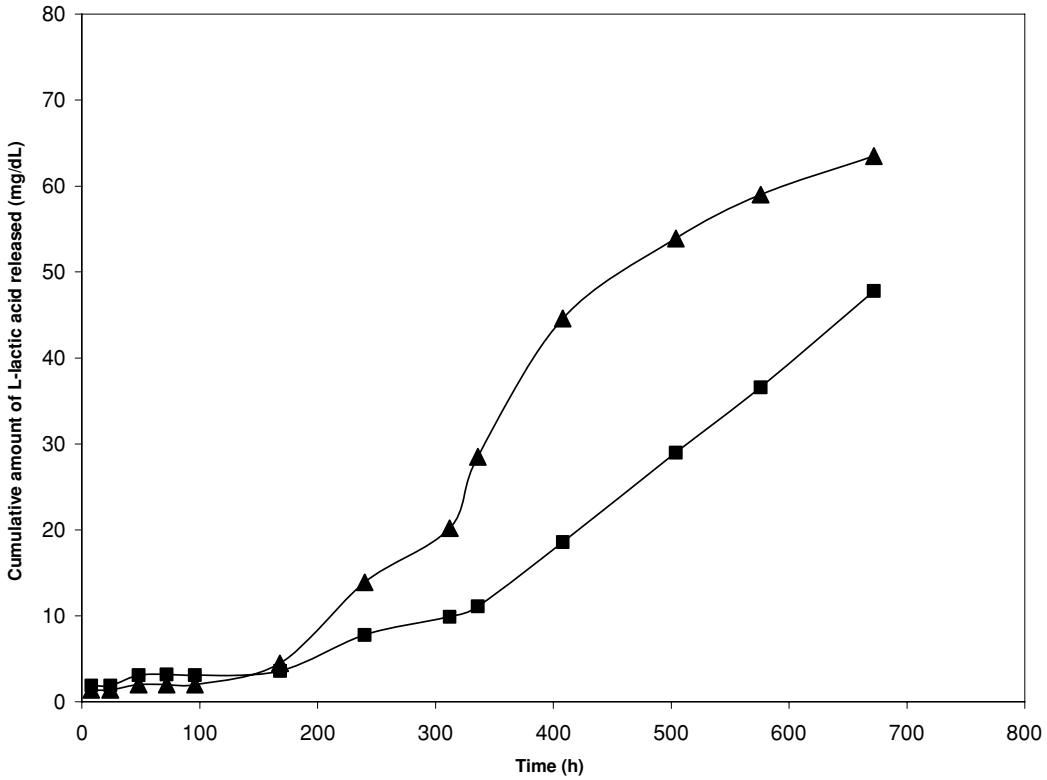


**FIGURE 7.** Lactic acid formation profiles from an ISFI without EB (▲) and 12.8 wt% EB (■).

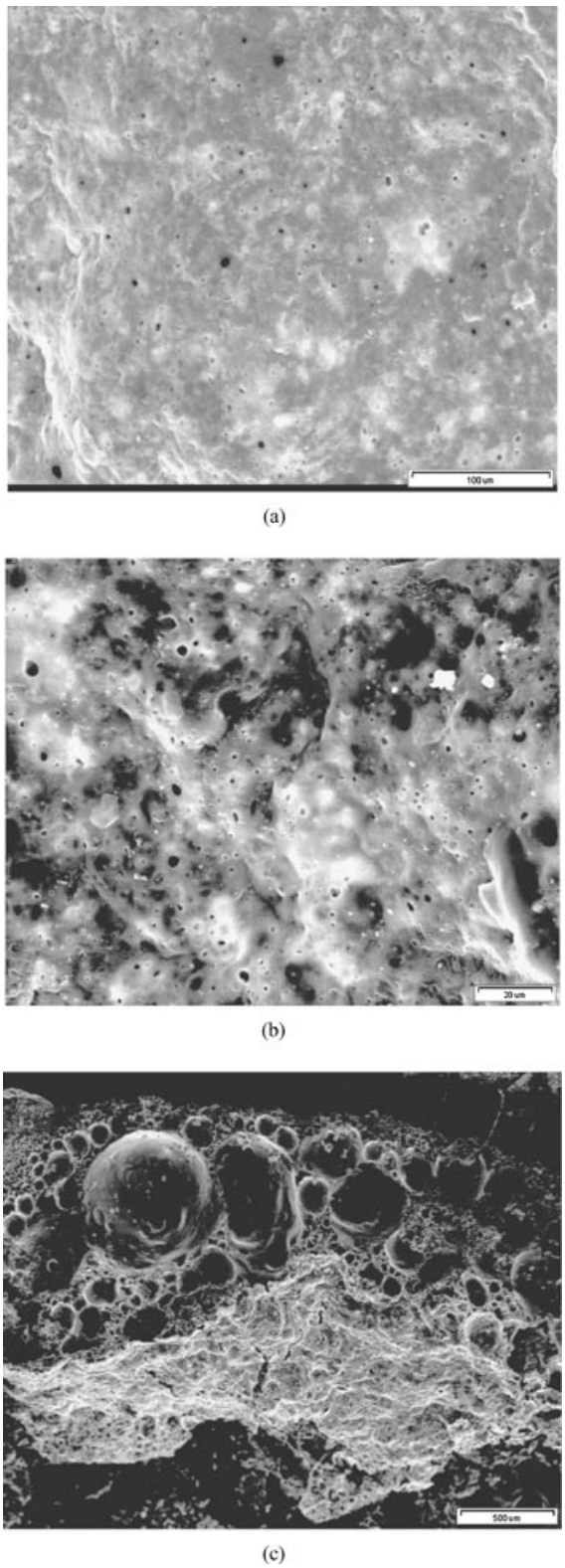

**FIGURE 8.** Surface morphology (a: 300×, b: 800×), cross section (c: 40×) of an ISFI with PLGA/NMP/EB/LA weight ratio of: 33/51.2/12.8/3, respectively.

groups (lactic acid and glycolic acid) induces a decrease in internal pH of the matrix. These alterations further accelerate degradation in the central region.[35,36] For that reason, the degradation profile of PLGA looks like a sigmoidal curve. This is correlatable with lactic acid formation as discussed in the following paragraph.

As it is shown in Fig. 7, formation of lactic acid is also different in the two formulations. Both formulations show lag-phases in lactic acid release, which is again related to phase transition and setting of an ISFI. After this initial lag-phase, both formulations reach a steady-state phase. The slope of the steady-state phase of formulation without EB is dramatically higher than the formulation with EB.

The more hydrophilic formulation without EB induces higher water permeation. Water permeation is one of the main factors that affects the rate of polymer degradation, hence higher amount of lactic acid formation.

### AFFECT OF ETHYL BENZOATE ON MORPHOLOGY OF ISFI

The cross section and surface morphology of ISFI prepared from the formulation containing EB is illustrated in Fig. 8. Figures 8a and 8b show a homogeneous appearance with several small pores on it. A comparison between Fig. 8 (a and b) and Fig. 3 (a and b) clarifies that the rate of phase inversion when adding this additive significantly decreased. So the lower speed of the phase separation resulted in a membrane surface that contains smaller and fewer pores and cross section containing closed pore-like structures instead of finger- and channel-like structures (Fig. 8c).[37,38]

## Conclusions

Formation of ISFI from solutions without EB is predominantly controlled by fast phase inversion dynamics. The surface morphology of these membranes showed smooth surface containing pores on it. Cross-section morphology of ISFI showed the large open pores consisting of a tear-like structure near the surface and finger- or channel-like structures at the body of the matrix.

ISFIs prepared from the solution containing EB showed a lower burst release. Furthermore, the release rate of peptide is lower compared with the

ISFI without EB. Also cross-section morphology showed closed pore-like structures instead of finger-like structures. All of this can be related to the slow phase inversion after adding EB, which is a characteristic of systems based on relatively weaker solvent–nonsolvent affinity.

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