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Research paper

# Monolithic glyceryl trimyristate matrices for parenteral drug release applications

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**Abstract**

Monolithic lipid matrices were developed that allow parenteral drug release for days, weeks or even months. The cylindrical matrices consist of triglycerides or triglyceride/cholesterol mixtures and allow, due to their small dimensions, an application via injection. Pure triglyceride matrices showed less than 3%, triglyceride matrices containing 70% and more cholesterol less than 10% water uptake over 30 weeks. This swelling behavior would allow the use of such matrices even for sophisticated applications such as interstitial drug delivery to the brain where excessive swelling is highly undesirable. The drug release kinetics were found to depend strongly on the fatty acid chain length of the triglyceride and the cholesterol content of the matrices. Increasing the chain length from $C_{12}$ to $C_{18}$ allowed an increase in the release of pyranine, a low molecular weight model compound, from approx. 60 days to more than 120 days. Adding cholesterol to glyceryl trimyristate matrices made it possible to adjust the release within a time span varying from days to weeks. While matrices containing 50% cholesterol released pyranine within 8 days, cholesterol contents of 90% allowed a release of the dye for more than 3 weeks.


*Keywords*: Lipid matrices; Triglyceride; Cholesterol; Drug release; Implants

## 1. Introduction

The design of controlled release systems for peptide or protein drugs is hampered by a number of obstacles related to their chemical and physical properties. Due to their susceptibility to chemical and physical instability, their bioavailability after oral administration is usually low [1]. Although the parenteral application of peptides and proteins is a potent alternative, it requires, however, frequent injections or continuous intravenous infusions due to low plasma half-lives that are often in the order of only a few minutes [2]. An attractive way to overcome these problems are delivery systems that release proteins continuously for extended periods of time to maintain the plasma level at therapeutic concentrations and concomitantly protect the drug reservoir from inactivation [3]. The incorporation of drugs into biodegradable biocompatible polymers such as poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) is a well known and a widely accepted concept to achieve this goal [3]. However, the use of synthetic polymer matrix materials for protein release applications often entails detrimental effects on incorporated compounds. Peptide and protein drugs are susceptible to various sources of inactivation during polymer erosion which is related to the acidic microclimate and the accumulation of degradation products inside PLA and PLGA matrices [4,5].

In an attempt to overcome these limitations, we focused our attention on the manufacture of miniaturized monolithic triglyceride matrices. The size of these matrices had to be small enough to be injectable via a trochar but large enough to allow for the release of drugs over a period of months. The question of how the release rate of a substance could be modulated in such a system was of key interest. To this end we investigated different types of triglycerides and triglyceride mixtures with cholesterol. To assess if these materials would be suitable for interstitial drug delivery to the brain, we investigated if they undergo excessive swelling that could cause brain damage. Pyranine served as a model compound for release experiments, as it is highly water soluble and, therefore, a good indicator for the ability of the matrices to control release [6]. The overall aim of this study was to evaluate the potential of triglycerides as a substitute for biodegradable polymers as a carrier for drug release applications.

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## 2. Materials and methods

### 2.1. Materials

Glyceryl trilaurinate, -trimyristate, -tripalmitate and -tristearate (Dynasan® 112, 114, 116, 118) were obtained from Sasol (Witten, Germany). Cholesterol was bought from Acros Organics (NJ, USA). Pyranine was purchased from Molecular Probes (Eugene, OR, USA). Sodium hydroxide, potassium hydrogen phosphate, sodium azide and methylene chloride were purchased in analytical grade from Merck (Darmstadt, Germany). Water used in this study was always of double-distilled quality.

### 2.2. Methods

#### 2.2.1. Lipid matrix manufacturing

Triglycerides or mixtures of cholesterol with glyceryl trimyristate (10–50% (w/w) cholesterol content) were loaded with 3% (w/w) pyranine using an emulsion technique. In a typical experiment 6 mg pyranine were dissolved in 200 μl water and dispersed in a solution of 194 mg lipid in 5 ml methylene chloride under vigorous vortex mixing. The resulting mixture was sonicated for 30 s at a frequency of 20 kHz and an intensity of 120 Watts using a B12 sonifier made by Branson (Sonic Power Company, Danbury, CT, USA). Water and solvent were removed from the resulting W/O emulsion by vacuum drying. After drying, the lipid was ground in a mortar to obtain a free flowing powder with a particle size of less than 400 μm.

For the manufacture of lipid matrix cylinders, a set of 2 mm diameter cylindrical punches and a die were machined from hardened steel and V4A steel, respectively. Matrices of 2 mm height were obtained by manual compression of 7 mg powder applying a force of approximately 250 N for 10 s.

#### 2.2.2. Differential scanning calorimetry (DSC) analysis

For recording DSC thermograms, matrices were sealed into hermetic AutoDSC aluminum sample pans (TA Instruments, Alzenau, Germany). Thermograms were recorded on a 2920 differential scanning calorimeter made by TA Instruments (Alzenau, Germany) using an empty pan as a reference. Scans were recorded with a rate of 5°C/min after equilibration at 0°C for 5 min between 0 and 90°C for the triglycerides and between 0 and 160°C for cholesterol and cholesterol/triglyceride mixtures.

#### 2.2.3. Matrix swelling and erosion

Matrices ($n = 3$) were eroded at 37°C in 10 ml 0.08 M phosphate buffer solution (pH 7.4) containing 0.02% sodium azide to suppress the growth of bacteria and fungi. The weight increase was taken as a measure of water uptake and consequently for matrix swelling. In order to determine the degree of erosion the samples were dried under vacuum. The mass loss of a matrix in relationship to its original value was then taken as a measure of erosion.

#### 2.2.4. In vitro release studies

To investigate the release of pyranine from lipid matrices in vitro, pyranine-loaded matrices ($n = 5$) were eroded under the same conditions as for the erosion tests. The buffer was analyzed for released dye using a CS-9301PC microplate reader (Shimadzu, Kyoto, Japan). Pyranine was excited at $\lambda_{ex}$ 407 nm and analyzed for fluorescence emission above 436 nm.

## 3. Results and discussion

### 3.1. Lipid matrix manufacturing and DSC analysis

The aim of this study was to develop lipid matrices for the parenteral application of drugs that allow the release period to be adjusted from days to weeks and even months. As these delivery systems were intended to be an alternative for biodegradable polymers, a number of additional requirements such as minute geometry to allow injection and a low degree of swelling to allow drug delivery to the brain had to be met.

The matrices were prepared by simple compression which has the advantage that the system can be manufactured by means of mass production such as compression on tablet machines or by means of extrusion in the future. The investigation of matrix cylinders by light microscopy revealed that the matrices had a well defined geometry that would meet the needs for application via injection using a trocar (Fig. 1).

As it was known that polymorphic transformations may occur during the processing of lipids [7], it deemed necessary to investigate if the manufacturing protocol or the blending with cholesterol affected the modification of the triglyceride components. Therefore, DSC thermograms were recorded for glyceryl trimyristate and cholesterol prior to and after compression (Fig. 2). Glyceryl trimyristate bulk material (Fig. 2a) showed a single endothermic transition at 60°C that stemmed from the melting of the crystalline

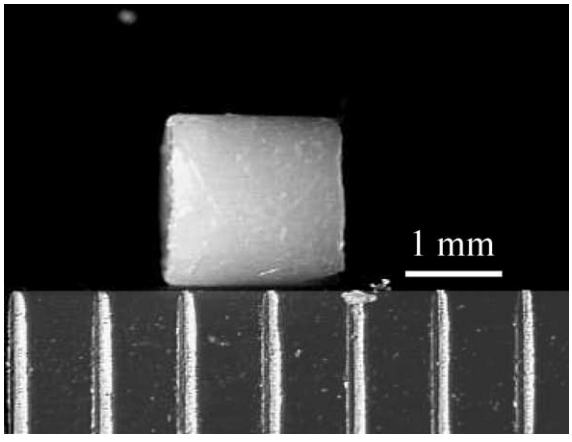


Fig. 1. Light microscopy picture of a pyranine loaded glyceryl trimyristate matrix cylinder.

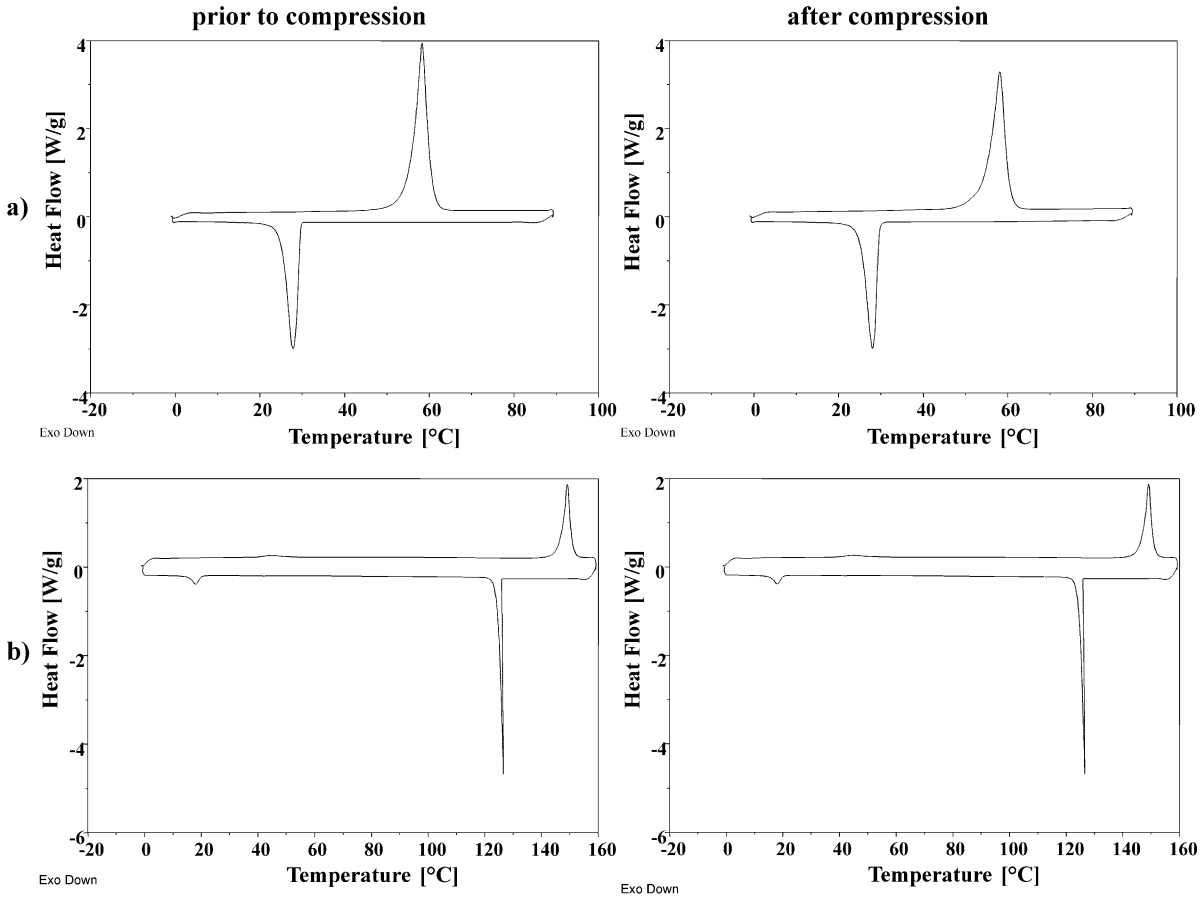


Fig. 2. DSC thermograms obtained for bulk lipid material (prior to compression) and lipid matrices (after compression): (a) glyceryl trimyristate; and (b) cholesterol.

β-modification [8]. Scans obtained for glyceryl trimyristate matrices were identical with those obtained for the bulk material. This showed that no polymorphic transformations occurred during compression and that the stable crystalline β-modification was largely maintained [8]. In the case of cholesterol, the melting endotherm was constant at 150°C before and after compression, which indicates that the crystalline state of the material was not affected when processed into cylinders (Fig. 2b).

Thermograms of glyceryl trimyristate/cholesterol mixtures (Fig. 3) showed two endothermic peaks corresponding to the melting endotherm of the two substances. While the location of the triglyceride melting endotherm remained constant at 60°C, the peak area increased continuously with rising cholesterol content. From these observations we conclude that glyceryl trimyristate crystallized again in the stable β-modification [8]. Furthermore, one can assume that cholesterol and triglyceride seem to coexist in separate phases. The intensity and shape of the cholesterol endotherm, however, changed significantly. With decreasing cholesterol content, the signal shifted from 150°C for pure cholesterol to 125°C for a mixture containing equal amounts of triglyceride and cholesterol. Concomitantly, the area under the melting endotherm decreased significantly. This result may be interpreted as a melting point depression and is consistent with the findings of Ekman et al., who also found depressed cholesterol melting points at high triglycerides concentration [9]. The results are indicative of the dissolution of small amounts of triglyceride inside the cholesterol phase. An additional small endotherm persisted at 45°C for all triglyceride/cholesterol mixtures. Due to the purity of cholesterol (95% purity) we hypothesized that it might be attributed to impurities since this peak was also observed in the thermograms obtained for the bulk cholesterol sample and cholesterol matrices (Fig. 2b).

### 3.2. Matrix swelling and erosion

Prior to release studies, the swelling and an eventual erosion of triglyceride matrices was investigated in vitro. Another parameter of interest was the swelling and erosion behavior of glyceryl trimyristate matrices as a function of the cholesterol content.

Matrices made of triglycerides alone showed no significant water uptake for more than 6 months. The glyceryl trimyristate matrices took up less than 3% water (data not shown). The constant mass of the matrices after drying indicates that there was no erosion throughout the entire observa-

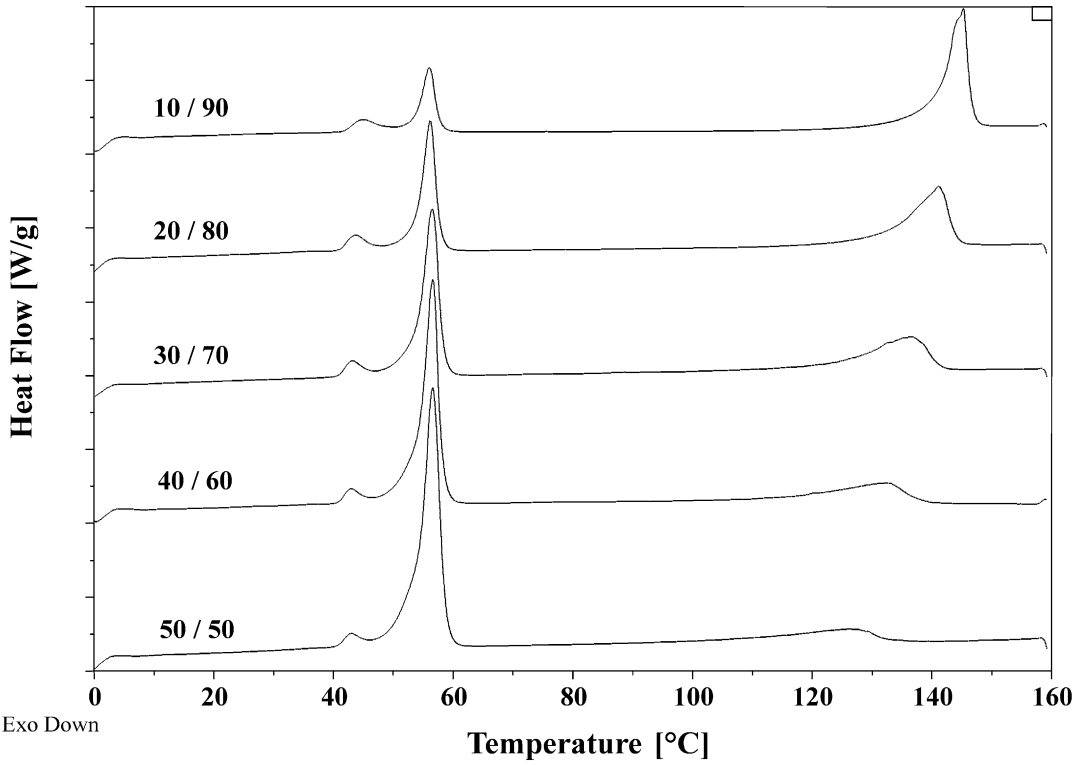


Fig. 3. DSC thermograms obtained for glyceryl trimyristate cholesterol mixtures (figures next to a thermogram reflect the triglyceride/cholesterol ratio (w/w)).

tion period of 32 weeks. This is not surprising as triglycerides are essentially insoluble in water and the erosion medium contains no lipases that would allow cleavage of the fatty acids into more water soluble degradation products.

When different glyceryl trimyristate/cholesterol mixtures were investigated (Figs. 4a–f), there was again no significant mass loss noticeable. When such matrices were implanted subcutaneously into nude mice, they exhibited a similar stability (data not shown) which makes them an ideal candidate for long term drug delivery. When the same matrices were investigated for their swelling behavior, water uptake was moderate at less than 10% in matrices containing 80–100% cholesterol (Figs. 4a–c). However, lipid matrices with a content of 50–70% cholesterol (Figs. 4d–f) showed a marked mass increase over the research period of 32 weeks. The biggest mass gain of approx. 30% was recorded for matrices with equal amounts of triglyceride and cholesterol (Fig. 4f). One explanation for this phenomenon could be that equal amounts of triglyceride and cholesterol lead to a melting point depression in the cholesterol phase of the matrix that softens the matrix and leads to a more flexible orientation of molecules with an increased possibility of water diffusing into the matrices. This is supported by the DSC thermograms that indicate a shift of the cholesterol melting endotherm in combination with a decreasing melting enthalpy indicating the presence of less crystalline phases [9]. The new phases that are created in this way may explain the change of the system's swelling properties.

The moderate swelling of less than 3% water uptake for glyceryl trimyristate matrices and glyceryl trimyristate matrices containing 70% and more cholesterol make them suitable candidates for interstitial drug delivery to the brain. Their degree of swelling is of the same magnitude as for polyanhydrides, a class of polymer currently in use for the treatment of brain tumors in humans [10].

### *3.3. In vitro release studies*

For the investigation of in vitro release, lipid matrices were loaded with pyranine as a model compound. Fig. 5 shows the release profile from glyceryl trimyristate matrix cylinders in comparison to other triglycerides. After an initial burst release of approx. 15–20% within the first 24 h, pyranine was released according to more or less concave release profiles. The release from glyceryl trilaurinate cylinders was most rapid and was complete after approx. 80 days. A total of 50% of the pyranine was released from these matrices within the first 20 days, and the remainder of the dose over a period of approx. 60 days. In contrast, cylinders made of glyceryl tristearate were able to release pyranine for more than 120 days. After approx. 20% of the dye had been released within the 1st day, the profile was almost linear. A total of 30% of the incorporated model compound was still inside the matrices after 120 days. Lipid matrices made of glyceryl trimyristate or glyceryl tripalmitate released pyranine for approx. 120 days. Both triglycerides showed almost identical kinetics with a moderate burst effect. After 50% of

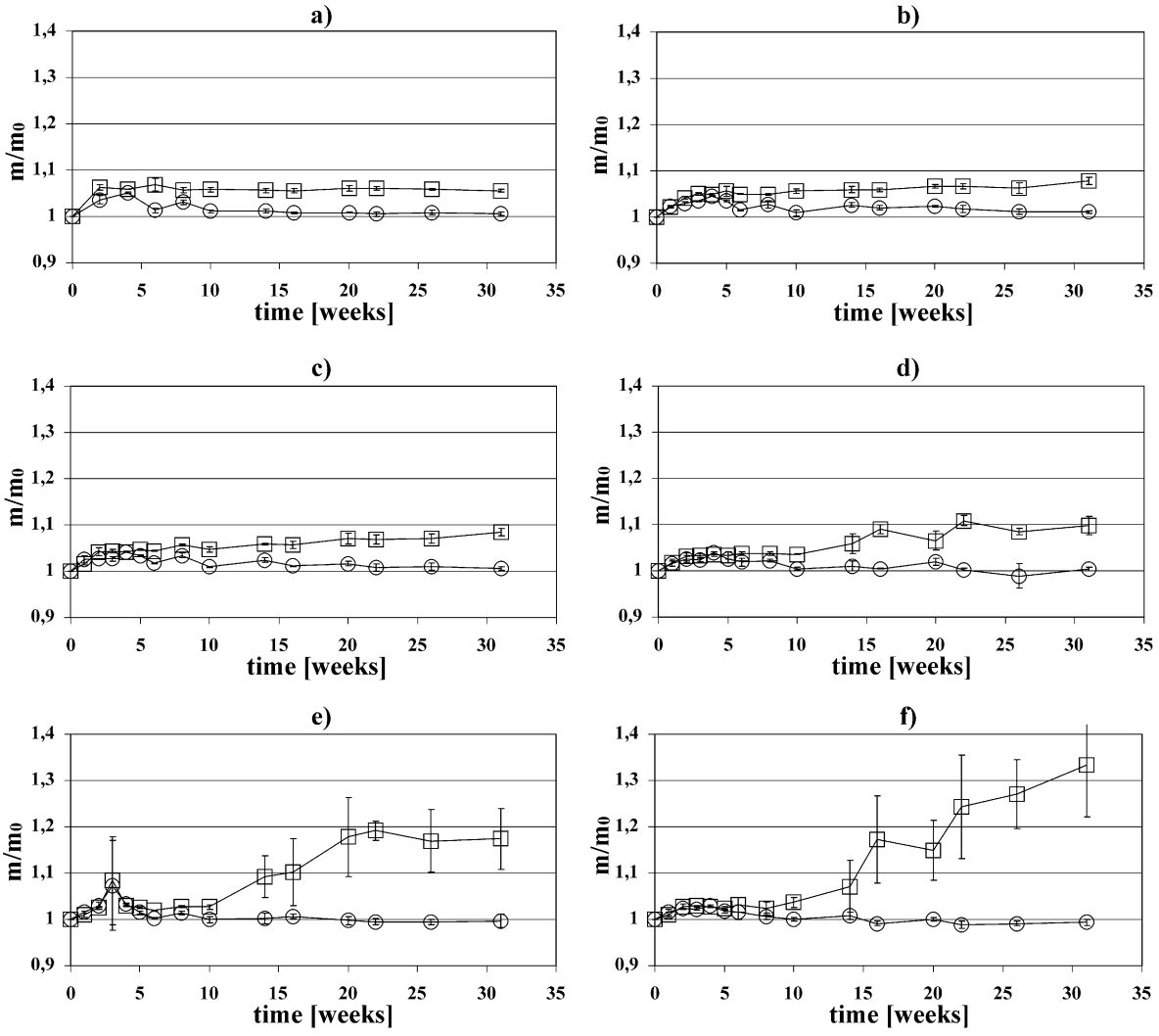


Fig. 4. Swelling (□) and erosion (○) profiles of lipid matrices made of: (a) cholesterol; (b) glyceryl trimyristate:cholesterol 10:90 (w/w); (c) glyceryl trimyristate:cholesterol 20:80 (w/w); (d) glyceryl trimyristate:cholesterol 30:70 (w/w); (e) glyceryl trimyristate:cholesterol 40:60 (w/w); and (f) glyceryl trimyristate:cholesterol 50:50 (w/w).

the model compound had been released by day 40 the rest of the dose was set free at an almost constant velocity over a period of 80 days.

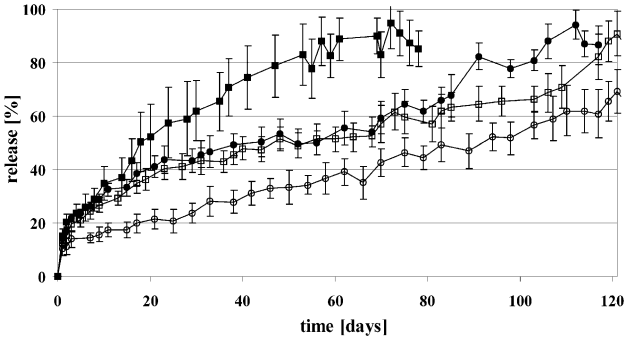


Fig. 5. In-vitro release of pyranine from different triclyceride matrices: (■) glyceryl trilaurinate; (●) glyceryl trimyristate; (□) glyceryl tripalmitate; and (○) glyceryl tristearate.

Matrices made of different triglyceride/cholesterol mixtures showed almost linear release profiles (Fig. 6). A general trend which could be observed was that increasing cholesterol content delayed the release of pyranine substantially. For example, increasing the cholesterol content of gyceryl trimyristate matrices from 50% (w/w) to 90% (w/w) increased the release period of pyranine from 8 to more than 25 days.

The swelling behavior of the lipid matrices correlates well with the release of pyranine from triglycerides (Fig. 5) and glyceryl trimyristate/cholesterol mixtures (Fig. 6). The release from the pure triglyceride cylinders was slow and can, depending of the type of lipid, last for more than 120 days. After a burst release of less than 20% of the dose, pyranine was released from the matrices in a sustained and controlled way. The experiment demonstrates that the release from triglyceride matrices was strongly affected by the chain length of the fatty acids. The shorter the carbohy-

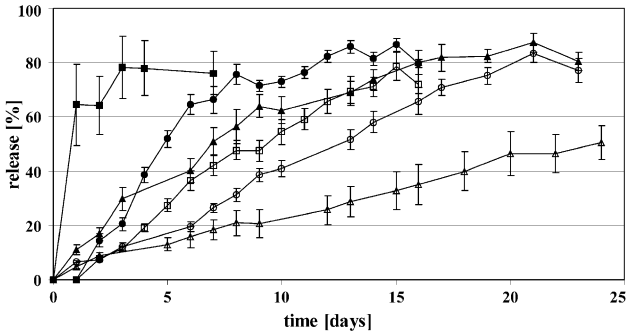


Fig. 6. Effect of the cholesterol content on the release of pyranine from glyceryl trimyristate in vitro: (■) pure cholesterol; (△) glyceryl trimyristate:cholesterol 10:90 (w/w); (○) glyceryl trimyristate:cholesterol 20:80 (w/w); (□) glyceryl trimyristate:cholesterol 30:70 (w/w); (▲) glyceryl trimyristate:cholesterol 40:60 (w/w); and (●) glyceryl trimyristate:cholesterol 50:50 (w/w).

drate chain, the faster the release. This makes the type of triglyceride a valuable tool for modifying the release kinetics. However even glyceryl trilaurinate which released the dye fastest did not allow complete release of pyranine before 60 days. This problem can be overcome by blending glyceryl trimyristate with cholesterol. Depending on the amount of cholesterol, the release periods for pyranine could be reduced to weeks or days (Fig. 6).

Although the release studies showed promising release properties, there are a number of parameters that will have to be investigated in the future such as the impact of lipid particle size on release. Another question is that for the stability of proteins and peptides compared to biodegradable polymers.

## 4. Conclusions

Monolithic triglyceride matrices are a promising controlled release system for parenteral drug release that permits adjustment of drug release periods from days to months. Their release, erosion and swelling behavior make them promising candidates for parenteral protein and peptides release systems as well as for interstitial drug delivery to the brain.

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