

SOL-GEL DERIVED SILICA GEL MONOLITHS AND MICROPARTICLES AS CARRIER IN CONTROLLED DRUG DELIVERY IN TISSUE ADMINISTRATION

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Division of Biopharmaceutics and Pharmacokinetics

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Pirjo Kortesus

Academic Dissertation

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ABSTRACT

The synthesis of inorganic oxide material by a sol-gel technique through the formation of colloidal suspension (sol) and gelation of the sol into the gel enables incorporation of heat-sensitive active substances into the material during processing. Sol can be further processed into various forms, such as monoliths, fibers, coatings or microparticles. This versatile technique has recently been investigated in diagnostics as well as in pharmaceutical applications.

The general objective of this study was to evaluate sol-gel derived silica gel as an implantable (monoliths) or injectable (microparticles) biodegradable carrier for controlled drug delivery. Sol-gel derived silica gel monoliths were prepared by casting while microparticles were prepared by spray drying. The effect of various synthesis parameters on the release rate of two model drugs, toremifene citrate and dexmedetomidine hydrochloride from monoliths and microparticles, was studied *in vitro*. In addition, the *in vivo* release of toremifene from subcutaneously administered monoliths, the degradation of monoliths and the tissue effects of silica gel were estimated in mice. Pharmacokinetic parameters of dexmedetomidine were evaluated in dogs after subcutaneous administration of dexmedetomidine hydrochloride in monoliths and microparticles.

In vitro release of dexmedetomidine from silica gel and degradation of the silica gel was controlled by modifying processing parameters, e.g. water/tetraethoxysilane (TEOS) ratio, pH, by alkyl-substitution or by using spray drying instead of casting as the manufacturing method. The release of dexmedetomidine was slowest from silica xerogel monoliths and microparticles prepared at the isoelectric point (IEP) of silica. Decreasing the water/TEOS ratio either increased (monoliths) or decreased (microparticles) the release rate of dexmedetomidine. Since alkyl-substituted silica gel was more hydrophobic than 100% TEOS based silica, the release rate of dexmedetomidine decreased. The release of model drugs typically corresponded either to the square root of time kinetics or zero-order kinetics with simultaneous degradation of the silica gel matrix.

The silica xerogel materials used were biodegradable and did not cause any adverse effects in the surrounding tissue or various organs in the preliminary study with mice. Sustained release was achieved with toremifene in mice and with dexmedetomidine in dogs. Silica gel formulation giving typical toremifene-related changes in the mouse uterus for more than six weeks and a formulation that produced an effective serum level of dexmedetomidine in a dog for at least 24 hours were developed. Silica xerogel was shown to be suitable for controlled delivery of drug substances both as an implantable and an injectable drug delivery system.

ABBREVIATIONS

AUC	area under the concentration vs. time curve
C_0	total amount of drug in polymer
C_s	solubility of drug in polymer
C_{max}	maximum concentration of drug substance in serum
CH_3COOH	acetic acid
D	diffusional coefficient of the drug in the polymer
DMDES	dimethyl(diethoxy)silane
ε	porosity factor
ETES	ethyl(triethoxy)silane
FDA	U.S. Food and Drug Administration
3H	tritium
HCl	hydrochloric acid
IEP	isoelectric point
LD_{50}	lethal dose, 50%
$\log P_{app}$	logarithm of apparent partition coefficient (octanol/water)
METES	methyl(triethoxy)silane
M_t/M_∞	fractional released amount of drug
NaOH	sodiumhydroxide
NH_4OH	ammoniumhydroxide
NIH	National Institute of Health
PEG	polyethyleneglycol
PGA	poly(glycolic acid)
pKa	ionisation constant/protonisation constant
PLA	poly(lactic acid)
PLGA	poly(lactic acid-glycolic acid)copolymer
r	water/alkoxide ratio
SBF	simulated body fluid
SDS	sodium dodecyl sulphate
SEM	scanning electron microscopy
Si-O ⁻	deprotonated silanol group
Si-OH	silanol group
$Si(OH)_4$	silicic acid
SiO_2	silicon dioxide (silica)
Si-O-Si	siloxane group
SSA	specific surface area
τ	tortuosity factor
TEOS	tetraethoxysilane
Tmax	time of maximum concentration
TMOS	tetramethoxysilane

LIST OF ORIGINAL PUBLICATIONS

I Kortesuo P., Ahola M., Karlsson S., Kangasniemi I., Yli-Urpo A., Kiesvaara J. 2000. Silica xerogel as an implantable carrier for controlled drug delivery – evaluation of drug distribution and tissue effects after implantation. *Biomaterials* 21, 193-198.

II Kortesuo P., Ahola M., Kangas M., Kangasniemi I., Yli-Urpo A., Kiesvaara, J. 2000. In vitro evaluation of sol-gel processed spray dried silica gel microspheres as carrier in controlled drug delivery. *Int. J. Pharm.* 200, 223-229.

III Kortesuo P., Ahola M., Kangas M., Yli-Urpo A., Kiesvaara J., Marvola M. 2001. In vitro release of dexmedetomidine from silica xerogel monoliths: Effect of sol-gel synthesis parameters. *Int. J. Pharm.* 221, 107-114.

IV Kortesuo P., Ahola M., Kangas M., Leino T., Laakso S., Vuorilehto L., Yli-Urpo A., Kiesvaara J., Marvola M. 2001. Alkyl-substituted silica gel as a carrier in controlled release of dexmedetomidine. *J. Control. Release* 76, 227-238.

V Kortesuo P., Ahola M., Kangas M., Jokinen M., Leino T., Vuorilehto L., Laakso S., Kiesvaara J., Yli-Urpo A., Marvola M. Effect of synthesis parameters of the sol-gel processed spray dried silica gel microparticles on the release rate of dexmedetomidine. Submitted to *Biomaterials*

1. INTRODUCTION

Several drugs that are used for treatment of diseases can not be administered through the gastrointestinal tract due to their poor physicochemical properties or due to a high first-pass metabolism in the liver or degradation in the acidic atmosphere of the stomach. Digestive enzymes in the intestine or enzymes in the gut wall are responsible for the presystemic degradation of many drugs. Conventional administration of such drugs by repetitive injections is inconvenient and causes fluctuation of the blood drug level. The administration of drug by implantation or injection as a polymeric drug delivery system provides advantages over conventional drug therapies. The entire drug dose needed for a desired period of time is administered at one time and released in a controlled manner. Other potential advantages include drug targeting, improved compliance and comfort. Polymeric systems also protect biologically sensitive active agents, e.g. proteins and peptides from degradation in the human body. On the other hand, incorporating highly potential drugs, like doxorubicin or nitrosoureas in a polymer matrix, decrease toxicity to several organs (Langer and Peppas, 1981, Langer, 1990). The development of modern drug delivery technology has led to sophisticated systems that allow drug targeting and a sustained or controlled release of drug substances (Langer, 1998, Santini et al., 1999).

The availability of various types of polymers generally referred to as biomaterials that can be introduced into the body without being rejected due to the inflammation process at the implantation site, make such an approach possible. These polymers control the rate of drug release by remaining intact while the drug diffuses out from the matrix or by releasing the drug by simultaneous degradation of the matrix. Drug release mechanisms depend on the degradation behaviour of the polymer. A diffusion controlled release of drug substance is achieved if the drug substance is released prior to degradation of the polymer undergoing homogenous degradation throughout the polymer matrix. Zero order release of the drug substance is attained if the drug substance is released simultaneously with polymers eroding from the surface (heterogeneously) (Heller, 1980, Langer, 1980, Baker, 1987, Langer, 1993).

In recent years, sol-gel method has attracted many researchers (Böttcher et al., 1998, Jokinen et al., 1998, Peltola et al., 1998, Falaize et al., 1999, Ahola et al., 2001). The name refers to a low-temperature method using chemical precursors that can produce homogenous and pure ceramics and glasses. Recently, biotechnology applications, where biomolecules (such as proteins, enzymes, antibodies etc.) are incorporated into sol-gel matrix, have been extensively studied (Braun et al., 1990, Lev, 1992). Applications include biochemical process monitoring, environmental testing, food processing and drug delivery for medicine. Sol-gel glasses have been used as matrix for catalysts, in hybrid materials, such as ceramic-polymer, ceramic-metal composites or biosensors in diagnostic applications (Braun et al., 1990, Lev et al., 1995,). In 1983 Unger and co-workers introduced the idea using sol-gel derived silica gel in drug delivery applications (Unger et al., 1983). In addition, sol-gel based materials are bioactive i.e. they bond to bone and have osteoconductive properties and can be used

in biomedical and dental applications (Klein et al., 1995, Wilson et al., 1995, Suominen and Kinnunen, 1996, Stoor et al., 1999).

The aim of the present thesis was to develop an implantable or injectable sol-gel derived silica xerogel formulation for the controlled release of drug substances using dexmedetomidine hydrochloride and toremifene citrate as model drugs. The aim was to attain formulations giving a few days effect for dexmedetomidine conventionally used as intramuscular bolus injection in premedication of anaesthesia and an effect of several months for toremifene used ordinarily as oral medication for long term treatment of metastatic breast cancer.

2. REVIEW OF LITERATURE

2.1. BIOMATERIALS

Regarding their use in medicine, biomaterials can be divided mainly into three classes: scaffolds for tissue engineering, load bearing applications for surgery and drug delivery systems (Ikada, 1999, Langer, 1999). Biomaterials should be biocompatible without any toxic, inflammatory, carcinogenic and immunogenic response. Accordingly, biomaterials can be divided into biotolerant, bioinert, biodegradable and bioactive materials (Heimke and Griss, 1983). They include polymers, metals, ceramics and composite materials.

Tissue engineering involves tissue regeneration and tissue substitution, such as artificial organs (Ikada, 1999, Langer, 1999). Scaffolds for tissue engineering should provide space and substrate for cell differentiation and proliferation in the body. Transplanted cells encapsulated in an artificial scaffold might avoid destruction by the immune system eliminating the need for lifelong immunosuppression.

Several surgical applications exist, such as sutures, membranes to cover bone defects, adhesives as well as osteosynthesis pins, screws and plates in maxillofacial surgery. Most of the classical materials, such as steel, titanium and aluminium are intended to remain chemically inert in contact with human tissue. In contrast, bioactive materials, such as bioactive glass and sol-gel derived silica gel, form strong bonds with the surrounding tissue, e.g. bone and even promote growth of new tissue. Professor Hench launched in his pioneering work in the early 1970s a bioactive glass called Bioglass® (Hench et al., 1971), which is later used to replace bone in the middle ear or to fill defects in the jaw (Wilson et al., 1995). Bioactive glass has also been used in the treatment of facial injuries to replace the bone supporting the eye (Suominen and Kinnunen, 1996). Bioactive glass granules seem to provide promising option in odontology, where it can be used to fill defects associated with periodontal disease. It can also be used in frontal sinusitis and in special type of rhinitis (Peltola et al., 1998, Stoor et al., 1999). Biodegradable polymers, such as poly(lactic acid) (PLA) used for self-dissolving sutures and for fracture fixation have the advantage that they do not have to be surgically removed afterwards (Törmälä et al., 1998).

The concept of biodegradation is also used in polymeric drug delivery systems, which should release a therapeutic agent in a controlled manner during a predetermined period (Langer, 1980, Baker, 1987, Nitsch and Banakar, 1994). Implant systems are used to provide sustained drug release for long-term systemic therapy, such as hormone replacement therapy or cancer therapy (Okada et al., 1988, Brem et al., 1991, Okada et al., 1994, Johnson et al., 1997). Although biodegradable polymers are widely studied only a few implantable or injectable drug products are commercially available. They are based mainly on PLA and poly(glycolic acid) (PGA) or their copolymers (Pitt et al., 1979, Okada et al., 1988, Okada et al., 1994, Johnson et al., 1997, Lemmouchi and Schacht, 1997, Kumar and Kumar, 2001). Implantable products based

on poly(anhydride) for treatment of glioma and osteomyelitis are also commercially available (Brem et al., 1991, Domb et al., 1997).

2.2. DRUG RELEASE MECHANISMS FROM POLYMERIC DEVICES

Drug release from a controlled release system can be achieved by several mechanisms, such as diffusion through a rate-controlling membrane, by osmosis, ion exchange or by degradation of the matrix (Langer, 1990, Park et al., 1993, Nitsch and Banakar, 1994, Göpferich and Langer, 1995).

2.2.1. Diffusion controlled release from non-degradable systems

Diffusion controlled systems can be divided into non-degradable reservoirs and monolithic devices (Baker and Lonsdale, 1974, Park et al., 1993). Reservoir systems provide constant release of a drug over a substantial period. In this system, a core of drug is surrounded by a polymer film which serves as diffusion barrier and the release of the drug occurs through this polymer membrane. As long as the drug concentration inside the film stays constant, the drug release obeys zero order kinetics. Ocusert® (pilocarpine) and Progestasert® (progesterone), for instance, are membrane-enclosed reservoir systems in commercial use.

In monolithic devices of various shapes, the active agent is either dispersed or dissolved in the polymer matrix. In both cases drug diffusion through the polymer matrix is the rate limiting step and the release rates are determined by the choice of the polymer and its consequent effect on the diffusion and partition coefficient of the drug (Baker, 1987).

Mathematical treatment of diffusion depends on whether the drug is dissolved or dispersed in the polymer. In a matrix system, where the drug is dissolved in the polymer matrix, the release follows Fick's law. The following equation describes drug release from a slab-shaped device:

$$\frac{M_t}{M_0} = 4 \left[\frac{Dt}{\pi h^2} \right]^{1/2} \quad (1)$$

M_t/M_0 is the fractional released amount of drug, D is the diffusion coefficient of a drug in the matrix, h is the thickness of the slab device and t is time. This equation is valid for the release of the first 60 percent of the drug total (early time approximation). Thereafter the release kinetics follow first order kinetics (late time approximation). The reason for a decrease in the release rate is an increase in the diffusional path length (Baker and Lonsdale, 1974, Langer, 1980, Park et al., 1993). This problem can be avoided by using special geometry that provide increasing surface areas over time

(Langer, 1980). Mathematical expressions describing the release from other geometries can be found in literature (Baker, 1987).

Higuchi developed an equation for the release of solid drugs dispersed in matrix dosage systems, from which the drug substance diffuses through non-porous polymer (Baker, 1987). Drug release is affected, apart from the geometry of the device, also by the concentration of drug. Drug release follows square root of time kinetics until the concentration in the matrix falls below the saturation value ($C_0 \gg C_s$).

$$\frac{dM_t}{dt} = \frac{A}{2} \left(\frac{2DC_s C_0}{t} \right)^{1/2} \quad (2)$$

In this equation dM/dt is the release rate of drug, A is the total area of the slab (both sides), D is the diffusion coefficient of the drug in the matrix, C_s is the solubility of the drug in a polymeric matrix, C_0 is the total amount of drug (dissolved plus dispersed) in unit volume of the matrix and t is time. In monolithic systems where excess drug is dispersed, the drug release rate increases with increasing drug concentration. The Higuchi expression is a good predictor of release rates from systems containing 5 to 10 vol% of active agent.

At higher drug concentrations, drug particles are in contact with each other and the drug is released by diffusion through the water filled pores. (Baker and Lonsdale, 1974, Baker, 1987). The diffusion of drug from a porous matrix is described by a second form of the Higuchi equation.

$$Q = \left[\frac{D\varepsilon}{\tau} (2C_0 - \varepsilon C_s) C_s t \right]^{1/2} \quad (3)$$

In this case the amount of drug released per unit area of the matrix, Q , depends on the diffusion coefficient of the drug in the matrix (D), its solubility in the polymeric matrix (C_s), the total amount of the drug (dissolved plus dispersed) in unit volume of the matrix (C_0), the porosity factor (ε) and the tortuosity factor (τ) of the matrix and time (t) (Langer, 1980, Baker, 1987).

2.2.2. Solvent activation controlled systems

In a solvent activation controlled system, the active agent is dissolved or dispersed within a polymeric matrix or is surrounded by a polymer and is generally not able to diffuse through the matrix. Permeation of the moving dissolution medium through the polymer controls the release behaviour of drugs from these systems (Langer and Peppas, 1981, Baker, 1987, Leong and Langer, 1987). Solvent controlled systems can be divided in two types; osmotic systems and swelling controlled systems. Drug release from the swelling polymer follows Fick's law, when penetration of water into

the polymer is rapid as compared to drug diffusion, whereas non-Fickian diffusion is achieved when drug diffusion and the solvent induced relaxations in the polymer are within the same range. When drug diffusion is rapid compared to the constant rate of solvent induced relaxation and swelling in the polymer, zero-order drug release is achieved and drug release is referred to as Case II transport (Baker, 1987). An osmotic system is constructed by enclosing a drug in a semipermeable membrane equipped with an orifice. The release rate of drug is governed by the nature of the membrane and the osmotic activity of the drug core. A constant release is maintained as long as the drug core remains saturated (Leong and Langer, 1987).

2.2.3. Biodegradable systems

The mechanism of biodegradation and drug release from biodegradable controlled release systems can be described in terms of three basic parameters. To begin with, the type of the hydrolytically unstable linkage in the polymer, affects the design of the system and next, the position of the labile group in the polymer is important. Secondly the way the biodegradable polymer degrades, either at the surface or uniformly throughout the matrix, affects device performance substantially. The third significant factor is the device design. The active agent may be covalently attached to the polymer backbone and is released as the bond between drug and polymer cleaves. The active agent may also be dispersed or dissolved into a biodegradable polymer matrix in the same way it is in a monolithic system made from non-biodegradable polymer and the release is controlled by diffusion, by a combination of diffusion and erosion or solely by biodegradation of the matrix (Baker, 1987, Siepmann and Göpferich, 2001).

Biodegradable polymers are divided in homogenous (bulk) and heterogeneous (surface eroding) degrading polymers. These mechanisms are the extreme cases and most biodegradable polymer systems constitute a combination of the two types of mechanisms (Baker, 1987, Siepmann and Göpferich, 2001). Degradation is the process of polymer scission by the cleavage of bonds in the polymer backbone. Degradation leads to size reduction of the polymer chains. Erosion is the mass loss of the polymer matrix (Göpferich, 1997, Siepmann and Göpferich, 2001).

Homogenous (bulk) degradation appears to be the most common polymer degradation mechanism, where the polymer degrades homogeneously throughout the matrix. The hydrolysis of bulk degrading polymers usually proceeds by losing molecular weight at first, followed by loss of mass in the second stage when molecular weight has decreased to 15 000 g/mol or less. (Pitt et al., 1981). The biodegradation rate can be changed by changing the composition of the polymer but not by changing the size or shape of it (Tamada and Langer, 1993, Grizzi et al., 1995).

Drug release from a matrix undergoing homogenous degradation may be governed by the equations derived from simple diffusion-controlled systems if the drug diffuses rapidly from the device before degradation of the matrix begins (figure 1). However, bulk degradation causes difficulties in the control of drug release, because the release

rate may change as the polymer degrades. As the polymer begins to lose mass, the release rate accelerates because it is determined by a combination of diffusion and simultaneous polymer erosion (figure 1) (Heller, 1997, Ahola et al., 1999b, Rich et al., 2000). The bulk degrading polymers most extensively studied are poly(esters), such as copolymers of PLA and PGA.

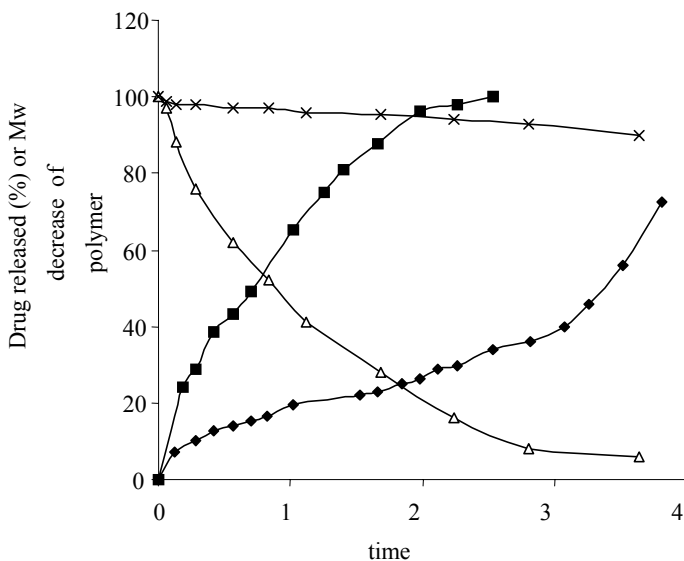


Figure 1. Drug release and biodegradation of aliphatic poly(ester). Drug released by diffusion (■), a burst in drug release as the mass loss begins (◆); decrease of molecular weight (Δ) and mass loss of the polymer (x).

Surface eroding systems (heterogeneous erosion) lose material from the surface and the erosion rate is dependent on the surface area and the geometry of the device, i.e. the radius to thickness ratio controls the matrix erosion time, rather than the volume of the polymer matrix (Tamada and Langer, 1993, Katzhendler et al., 1997, Akbari et al., 1998). The molecular weight of the polymer generally does not change significantly as a function of time (Baker, 1987). Achieving surface erosion, however, requires that the degradation rate of the polymer matrix surface be much faster than the rate of water penetration into the matrix (Langer, 1990).

Zero order drug release is obtained with surface erosion controlled systems such as poly(anhydrides) or poly(orthoesters). The surface eroding system device design is made easier due to the fact that release rates can be controlled by changes in system thickness and total drug content. Hopfenberg et al (1976) developed a general mathematical equation for drug release from surface degrading slabs, spheres and infinite cylinders. This model described in equation 4 assumes that the actual erosion process is the rate-limiting step and that the drug release occurs from the primary surface area of the device without seepage from the matrix.

$$\frac{M_t}{M_\infty} = 1 - \left(1 - \frac{k_0 t}{C_0 a}\right)^n \quad (4)$$

M_t/M_∞ is the fractional released amount of drug, C_0 is the initial concentration of the drug in the matrix, a is the initial radius for a sphere or cylinder, k_0 is the zero order rate constant for surface erosion and n is the shape factor. A shape factor that was defined in the equation by Hopfenberg, has in subsequent studies been applied to other geometrical forms, such as squares and half-spheres (Karasulu et al., 2000). According to Katzhendler, the erosion rates are different in the radial and axial directions (Katzhendler et al., 1997). Drug release from a surface eroding polymer may be controlled solely by erosion of the polymer matrix and the release of drug is constant provided that the surface area of the matrix and the drug concentration remain constant during the drug release period (Langer, 1980). However, the surface area decreases as the implant is eroded, with a consequent decrease in the release of drug. Consequently, a geometry that does not change its surface area as a function of time is required to attain more uniform and zero order release (Park et al., 1993).

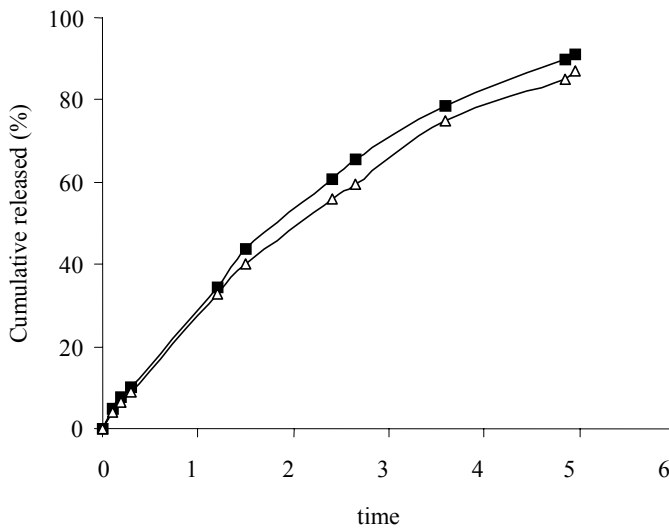


Figure 2. Drug release and mass loss of polymer from heterogeneously degrading polymer (surface erosion). Drug released (■), mass loss of the polymer (Δ).

True surface erosion where matrix mass loss is equal to the drug release rate (figure 2) is difficult to achieve and often diffusion of the drug molecules may still be rate limiting. For highly water-soluble drugs especially, the release rate is controlled mainly by diffusion through the matrix, whereas the erosion process controls the release rate of low water-soluble drugs. Thus, the release rate may be a combination of

erosion control (zero-order) and diffusion control (square root of time kinetics) (Urtti, 1985).

2.2.4. Empirical equation for describing drug release

To simplify the analysis of controlled release data from polymeric devices of varying geometry, an empirical, exponential expression was developed to relate the fractional release of drug to the release time, (Ritger and Peppas, 1987b, Ritger and Peppas, 1987a):

$$\frac{M_t}{M_\infty} = kt^n \quad (5)$$

where M_t/M_∞ is the fractional solute release, t is the release time, k is a constant and n is the exponent characteristic of the release mechanism (Ritger and Peppas, 1987a, Ritger and Peppas, 1987b). This equation applies until 60% of the total amount of drug is released. It predicts that the fractional release of drug is exponentially related to the release time and it adequately describes the release of drug from slabs, spheres, cylinders and discs from both swellable and non-swellable matrices (Table 1). The slope (n) of the $\log(\text{drug released})$ vs. $\log(\text{time})$ plot is 0.5 for pure Fickian diffusion. An anomalous non-Fickian diffusion pattern ($n = 0.5-1$ or $n = 0.45-0.89$) is observed when the rates of the solvent penetration and drug release are in the same range. This deviation is due to increasing drug diffusivity in the matrix by the solvent induced relaxation of the polymers. Zero order drug release ($n = 0.89$ or $n = 1$) can be achieved when drug diffusion is rapid compared to the constant rate of solvent induced relaxation and swelling in the polymer (Case II transport for swellable polymers). Use of this equation to analyse data of drug release from a porous system will probably lead to $n < 0.5$, since the combined mechanisms (diffusion through the matrix and partially through water-filled pores) will shift the release exponent toward smaller values (Peppas, 1985). Figure 3 describes the effect of exponent n on the release profile from controlled release systems.

Table 1. Diffusional exponent and mechanism of diffusional release from cylindrical and spherical non-swallowable and swallowable controlled release systems (Peppas, 1985, Ritger and Peppas, 1987b, Ritger and Peppas, 1987a).

Controlled release system	Diffusional exponent n	Drug release mechanism
Non-swallowable	< 0.5	Release from porous material
	0.5	Fickian diffusion
	0.5-1.0	Anomalous (non-Fickian) transport
	1.0	Zero-order release
Swallowable	0.45	Fickian diffusion
	0.45-0.89	Anomalous (non-Fickian) transport
	0.89	Case-II transport
	>1	Super-Case II transport

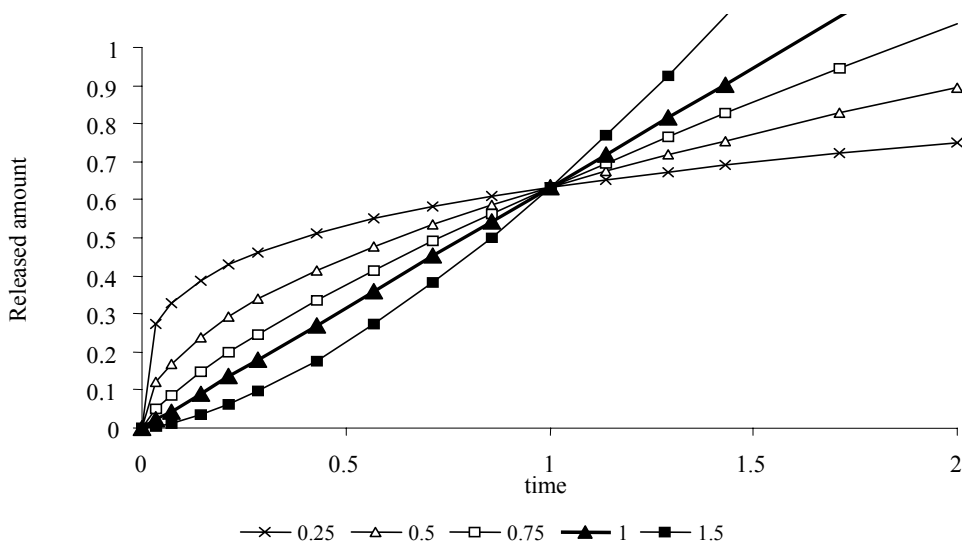


Figure 3. Fractional drug release versus time curves with different values of exponent n (0.25-1.5) when the constant (k) in the equation (5) is 0.6.

2.3. SOL-GEL TECHNOLOGY

Sol-gel produced oxides are used in numerous applications, such as coatings and thin films in electronic or optical components and devices. In addition, sol-gel monoliths and coatings have been investigated as matrices for catalysts, optical filters or as biosensors in diagnostic applications (Braun et al., 1990, Lev, 1992, Lev et al., 1995). The process has been used to produce amorphous materials in various forms,

including powders, fibers, coatings and thin films, monoliths and porous membranes (Böttcher et al., 1999, Ahola et al., 2000, Peltola et al., 2001). Sol-gel processing offers advantages such as low processing temperature and high homogeneity and purity of the material enabling preparation of hybrid materials and incorporation of drug substances or even cells (Pope, 1995, Baker et al. 1999). A large number of proteins (trypsin, glucose oxidase and hydrogen peroxidase), that retain their activity and stay in the matrix, have been incorporated into silica xerogel matrix through moulding.

The sol-gel process involves the manufacture of inorganic matrices through the formation of a colloidal suspension (sol) and the gelation of the sol to form a wet gel, which, after spontaneous drying, forms dry gel called xerogel. Most sol-gel techniques use water and low molecular weight alkoxy silanes, such as tetraethoxysilane (TEOS) or tetramethoxysilane (TMOS), as silica precursors. The alkoxy silanes hydrolyse with water forming silanols, with either acid or base as catalyst (Brinker and Scherer, 1990). The reactions of alkoxy silanes can be summarised in terms of three steps: hydrolysis of the alkoxy, silanol-silanol condensation, and silanol-ester condensation (Figure 4). Hydrolysis occurs by the nucleophilic attack of the oxygen contained in water in the silicon atom. Subsequent condensation reactions take place producing siloxane bonds. The polymerisation stages may be described as, i) polymerisation of monomers to polymers, ii) condensation of polymers to primary crystals, iii) growth or agglomeration of primary crystals to particles and iv) linking of particles into chains and to three dimensional network (figure 4) (Iler, 1979, Brinker and Scherer, 1990). Networks of the chains extend throughout the liquid medium, thickening the network into a gel. In the last stage water and alcohol are evacuated from the network structure causing gradual shrinkage and even cracking of the monolithic gel.

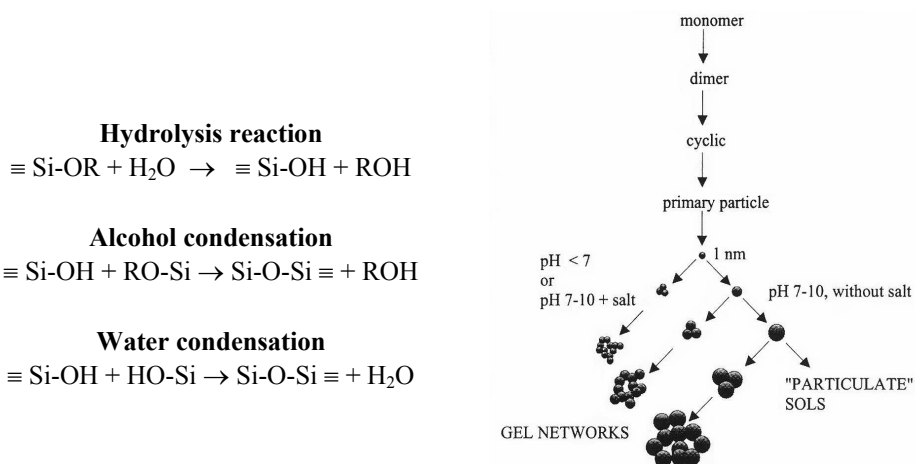


Figure 4. Reactions of alkoxy silanes (Brinker and Scherer, 1990) and polymerisation behaviour of silica. After Iler by Jokinen (Iler, 1979, Jokinen et al., 1998)

2.3.1. Factors affecting the structure of sol-gel processed silica xerogel

A porous and amorphous structure is one of the most characteristic features of sol-gel derived silica xerogel. Reactivity of the matrix, due to free hydroxyl groups, is the other typical property of silica xerogel. The microstructure of silica xerogel can be controlled by changing the water/alkoxide molar ratio, the catalyst type or concentration, by consolidating /sintering the silica xerogel by heat treatment or by using alkyl-substituted alkoxides or other additives.

2.3.1.1. Water/alkoxide molar ratio

The water/alkoxide molar ratio (r) has significant effect on the silica xerogel microstructure (Brinker and Scherer, 1990). When the water/alkoxide molar ratio is low, alcohol condensation is dominating and gelation time is longer, leading to more microporous materials. Gels made from higher water content sol ($r > 4$) have shown more coarse microstructure than gels made from lower water content sols ($r < 4$) (Ro and Chung, 1991). However, when the water/alkoxide ratio is more than 10, the microstructure was only slightly dependent on water content. The gels made from lower water content sols have more unreacted alkoxy ligands than those from higher water content sol and therefore form more linear chain-like structures (Kusakabe et al., 1999). At higher water concentration, more branched polymers are formed. Fiber drawing is possible from sols made at low water/TEOS ratio and low pH (Lev et al., 1995).

2.3.1.2. Catalyst

The effect of pH on the pore structure and morphology has been extensively studied (Brinker and Scherer, 1990). Changes in solution pH alter the relative rates of hydrolysis and condensation, yielding products ranging from weakly branched to particulate silica sols (Brinker and Scherer, 1990). Iler divides the polymerisation process into three approximate pH domains: $\text{pH} < 2$, $\text{pH} 2\text{-}7$ and $\text{pH} > 7$ (Iler, 1979). $\text{pH} = 7$ appears as a boundary because the silica solubility and dissolution rates are maximised at or above $\text{pH} = 7$. The kinetics and growth mechanisms of the reaction depend on the pH value of the solution. With acidic pH, particle growth stops once the size of 2 to 4 nm is reached. Above $\text{pH} = 7$ particle growth is mainly dependent on the temperature and particles of more than 100 nm in diameter can be formed (particulate sols). Above $\text{pH} = 7$ particles are negatively charged and they repel each other and no aggregation of particles occurs (particulate sols). At low pH, near the IEP, repulsive forces between particles are low and particles collide and form continuous networks leading to gels (gel networks)(figure 4).

The relative rates of hydrolysis and condensation effectively determine the morphology of the final xerogel. Generally, silica particles are positively charged at low pH and negatively charged at high pH. At the isoelectric point of silica (IEP, between $\text{pH}=1$ and $\text{pH}=3$), where the electrophoretic mobility of particles is zero, or at the point of the zero charge, where the surface charge is zero the condensation rate is slowest (Iler, 1979, Brinker and Scherer, 1990). Weakly basic to moderate acidic sols

have significant amounts of deprotonated silanol groups (SiO^-) which increase the condensation rate causing a formation of highly branched silica species. Gelation of these branched species results in the formation of mesoporous regions with a pore size between 2 nm and 50 nm. As the pH is lowered to the isoelectric point (between 1 to 3), the gelation times are increased and this leads to linear or randomly branched silica gel having highly microporous structure with the pore diameter < 2 nm (Meixner and Dyer, 1999). At very high acid concentrations, below isoelectric point (below 1) the dried xerogels become more mesoporous (Curran and Stiegman, 1999). This is due to the protonation of silanols to produce (SiOH^{2+}) groups, which are good leaving groups and act to increase the rate of condensation (Brinker and Scherer, 1990).

2.3.1.3. Organic-inorganic composite materials

Organically modified silicates represent hybrid systems in which several precursor types are combined. They are intermediate between glasses and polymers. Silica xerogels where organic chains are covalently attached to the silica atom of the network are called type II compounds (Baker et al., 1999). Organic groups linked to the oxide network by stable chemical bonds change the inner structure by reducing the degree of cross-linking. The amount of surface silanol groups decreases and leads to modified chemical reactivity and enhanced hydrophobicity (Schmidt, 1989, Lev et al., 1995, Mah and Chung, 1995). By adjusting the ratio of tetra alkoxy silane and alkyl substituted alkoxide, the structure and hydrophobicity of the silica gel can be controlled. The pore size and the specific surface area of the silica xerogel matrix increases with increasing length of the alkyl-group (Kusakabe et al., 1999). The presence of mesopores is more significant in silica gels with longer alkyl-chains. These changes in the structure of silica gel also influence the release rate of drug substances (Unger et al., 1983, Böttcher et al., 1998, Ahola et al., 2001).

Type I compounds have organic agents, like surfactants or inorganic salts, proteins or charged polymers attached with van der Waals or hydrophobic interactions within the silica xerogel network (Sanchez and Ribot, 1994). The presence of these agents controls the pore size and the specific surface area of the silica gel (Sato et al., 1990, Murakata et al., 1992, Vong et al., 1997). Polyethylene glycol affects the formation of silica particles at the colloidal level during polycondensation by forming hydrogen bonds with residual silanol groups in the silica network (Jokinen et al., 1998). It only affects the amount of micropores and the mean pore size of calcined silica xerogel increases (Sato et al., 1990). Organic compounds may also serve as templates to control the pore size and are added to the initial sol, as in the production of mesoporous silica (Kresge et al., 1992, Lindén et al., 1998, Vallet-Regi et al., 2001).

2.3.2. Sol-gel derived silica xerogels in drug delivery

Sol-gel derived silica xerogel has also been studied as a carrier material for various drugs, peptides and proteins (Sieminska and Zerda, 1996, Nicoll et al., 1997, Böttcher et al., 1998, Falaize et al., 1999, Santos et al., 1999, Ahola et al., 2000, Ahola et al., 2001). Bioactive agents can be incorporated into silica xerogel either by adsorbing

drug onto the surface of the heat-treated silica xerogel (Sieminska et al., 1997, Ahola et al., 1999a, Otsuka et al., 2000) or by adding the drug during the sol-gel manufacturing process (Unger et al., 1983, Nicoll et al., 1997 Böttcher et al., 1998, Ahola et al., 2000, Ahola et al., 2001). The solubility of drugs may limit the amount of drug added during the sol-gel phase (Sieminska and Zerda, 1996).

Incorporation of drug substances into sol-gel derived silica sol was introduced as early as 1983 (Unger et al., 1983). Unger found that the physicochemical characteristics of the drug molecule significantly control the release rate. Basic drugs are released in a controlled manner, whereas neutral or acidic drugs are released very quickly. This is due to the ionic properties of silica xerogel structure. Drug molecules with reactive functional groups, such as alcoholic or phenolic OH-groups can be chemically linked to silica surface by $\equiv\text{Si-O-C}\equiv$ bonds (Eckert-Lill et al., 1987) or by hydrogen bonds (Sieminska and Zerda, 1996). The amount of hydroxyl and carbonyl groups in a drug molecule affects the bonding and release rate of the drug. Stronger hydrogen bonds are formed with weak nitrogen bases, like pyridine, than with oxygen atoms of the ether or keto groups (Iler, 1979). Hydrogen bonds are formed between pH 1.5 to 3.0. Compounds that contain cationic groups combine with silica at all pH values and the bonds may involve both ionic bonding and hydrogen bonding. However, at a low pH below 3 to 4 interactions involve only hydrogen bonding. In addition large hydrophobic moieties intensify the adsorption to the silica surface (Iler, 1979). In some cases hydrophobic drug molecules, like hydrocortisone, may be permanently trapped within silica xerogel (Sieminska and Zerda, 1996, Otsuka et al., 2000). They are probably trapped to inter-particle volume (Otsuka et al., 2000).

The drug release rate from a silica xerogel matrix can be controlled by adding water soluble polymers such as polyethylene glycol or sorbitol or by substituting alkoxide with covalently linked organomodified alkoxide during synthesis (Unger et al., 1983, Böttcher et al., 1997, Böttcher et al., 1998, Ahola et al., 2001). The surface of silica gel can also be modified by using silane-coupling agents to improve the surface affinity and drug release rate of very hydrophobic drugs (Otsuka et al., 2000). In addition, the drug release rate can be controlled by adjusting the degree of drying or the particle size (Nicoll et al., 1997, Ahola et al., 2001). The effect of pore size, which can be modified by water/alkoxide ratio, on the release rate of drugs is also obvious (Sieminska and Zerda, 1996, Aughenbaugh et al., 2001). Crushed silica xerogel containing incorporated drug molecules have also been mixed with polyester copolymers in order to control the release of the drug (Ahola et al., 1999b, Rich et al., 2000).

The release mechanisms of drugs from a silica xerogel matrix are not extensively evaluated. The release of nifedipine from crushed silica xerogel particles is assumed to occur through solvent filled capillary channels and to obey square root of time kinetics (Böttcher et al., 1998). In addition, progesterone diffused from the porous silica xerogel obeys Fick's second law equation (Sieminska and Zerda, 1996). The release rate is governed by the rates of dissolution and diffusion. The release of toremifene is

also diffusion controlled from crushed particles but conforms to zero order kinetics from monoliths (Ahola et al., 1999a, 2000). In addition, the release of heparin from silica xerogel rods obeys zero order release kinetics (Ahola et al., 2001). The release mechanism of oily drugs, such as phytonadione, proceeds in two phases, a rapid release phase, not due to interaction with the silica surface and a slower drug release due to interaction (Otsuka et al., 2000).

2.3.3. Biocompatibility of silica based glasses

Biomaterials are expected to perform without any adverse effects, such as toxic, carcinogenic immunogenic and inflammatory responses. Biocompatibility of the material is defined as the appropriate effects, either local or systemic, of a biomaterial on a host (Williams, 1988). The biocompatibility of biomaterials is often described in terms of the acute and chronic inflammatory response and as the fibrous capsule seen at various time intervals after implantation of the material. Events following implantation of foreign material are injury, acute inflammation, chronic inflammation, granulation tissue, foreign body formation and fibrosis. Neutrophils predominate during the first several days and are replaced by monocytes. The acute inflammation lasts up to a few days, depending on the extent of the injury. Chronic inflammation is characterised by the presence of macrophages, monocytes and lymphocytes with the proliferation of blood vessels and connective tissue. The end stage of the healing response to biomaterials is generally fibrous encapsulation and has been considered as a sign of biocompatibility of the material. However, the fibrous capsule surrounding the implant may affect the drug release and absorption into the circulation. (Anderson et al., 1981, Anderson, 1994, Tang and Eaton, 1995, Park and Park, 1996, Andersson and Langone, 1999).

Silicon has been recognised as an essential trace element in the body and to participate in connective tissue, especially cartilage and bone formation (Carlisle, 1986). Crystalline silicon dioxide is known as a cytotoxic agent in macrophages and is known to cause enhanced formation of granulation tissue (Allison et al., 1966, Absher et al., 1989). Sol-gel derived glasses, on the other hand, are biocompatible and also bioactive, i.e. they form a chemical bond with living tissue and promote bone formation (Hench and Wilson, 1986, Li et al., 1992, Klein et al., 1995). Subcutaneously implanted pure silica xerogel as well as bioactive glass are surrounded by collagen fibres containing only few inflammatory cells after 4 weeks (Hench and Wilson, 1986, Radin et al., 1998, Kortessuo et al., 1999). Wilson and co-workers conducted a series of both in vitro and in vivo tests to evaluate the toxicity of glasses containing silica (Wilson et al., 1981). All tests showed silica-containing glass to be non-toxic and biocompatible. The proliferation of cultured murine fibroblasts and the activation of human polymorphonuclear leukocytes by sol-gel derived glasses has also been studied. Sol-gel glass neither caused the inhibition of fibroblast growth nor induced a significant inflammatory response by polymorphonuclear leukocytes (Wilson et al., 1981, Palumbo et al., 1997). Therefore sol-gel-derived glass is

considered a non-toxic material, due to its ability to induce normal growth of various cells.

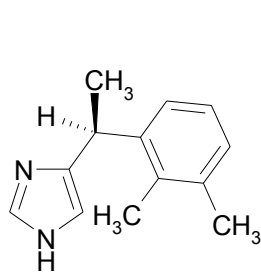
2.3.4. Elimination of silica based material

When silica xerogel is exposed to water it starts to degrade through hydrolysis of the siloxane bonds to $\text{Si}(\text{OH})_4$ (Brinker and Scherer, 1990), which diffuses into the local tissue around the implant, enters the bloodstream or lymphic circulation and is excreted in the urine through the kidneys or is actively phagocytised by the macrophages (Lai et al., 1998). During the resorption the Si concentration remains within the physiological range and no accumulation of silica was found in peripheral organs. In other words silica is harmlessly excreted in a soluble form through the kidneys.

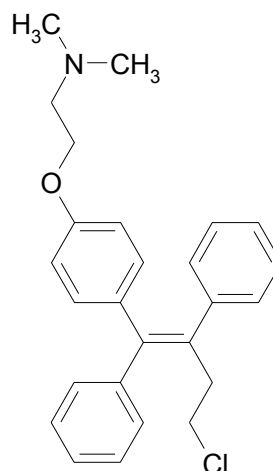
2.4. CHARACTERISATION OF DEXMEDETOMIDINE AND TOREMIFENE

2.4.1. Physico-chemical characteristics of the drug

Dexmedetomidine, d-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole (figure 5), is a very specific, even full agonist at several alpha 2-adrenergic receptor sites (Aantaa et al., 1993). It is the highly receptor selective dextro enantiomer of medetomidine (Savola and Virtanen, 1991). The molecular weight of hydrochloride salt is 236.7 (Rajala et al., 1994). It is weakly basic with a pKa value of about 7.1 and lipophilic with the logP about 2.8 at pH=7.4 with octanol as an organic solvent (Savola et al., 1986).



Dexmedetomidine



Toremfene

Figure 5. Structure of dexmedetomidine and toremifene

Toremifene, (Z)-4-chloro-1,2-diphenyl-1-[4-(2-(N,N-dimethylamino)ethoxy) phenyl]-1-butene (figure 5), is a selective estrogen receptor modulator for treatment of metastatic breast cancer. Toremifene is weakly basic with the pKa 8.0 and lipophilic having the logP value about 3.4 at pH 7.4 with octanol as an organic solvent. The molecular weight of citrate salt is 598.

2.4.2. Basic pharmacokinetics of dexmedetomidine and toremifene

Dexmedetomidine is used as intramuscular injection or as a slow intravenous infusion, because rapid intravenous injection due to transient peak concentration values during the distribution phase, causes vasoconstriction (Scheinin et al., 1989). The pharmacokinetics of dexmedetomidine in men is best described by a three-compartment model after intravenous administration (Dyck et al., 1993a, Dyck et al., 1993b). The effect of racemic medetomidine is dose dependent at the dose range 10-80 µg/kg for dogs. At higher doses the strength of sedation does not increase, only the duration of the effect (Vainio, 1989). The estimated LD₅₀ value for dexmedetomidine in dogs is 2000 µg/kg.

Dexmedetomidine is rapidly absorbed after i.m. injection. Time to maximum concentration after i.m. injection in men was 1.6 to 1.7 hours (Scheinin et al., 1992). Peak concentration was achieved within 0.5 h after i.m. injection in dogs (Salonen, 1989). Dexmedetomidine distributes into tissue including the brain. The mean distribution half-life is less than 10 minutes in dogs after i.v. injection (Salonen, 1989). The apparent volume of distribution is about 2.8 l/kg in dogs (i.v.) (Salonen, 1989) and 2.1 to 2.6 l/kg in men (Scheinin et al., 1992). The elimination half-life is 1.6 to 2.4 h after i.m. administration in men (Scheinin et al., 1992). Elimination from serum occurs with a half-life of 0.97 to 1.6 h in dogs (Kuusela et al., 2000). Most (85%) of the racemic medetomidine is present in protein bound form (Salonen, 1989).

Racemic medetomidine is metabolised in the liver and the metabolites are excreted in the urine. Unchanged medetomidine represented about 1-10 % of the urinary excretion products (Salonen and Eloranta, 1990). The primary hepatic metabolic route of medetomidine is hydroxylation of the methyl substituent at the aromatic ring. Hydroxymedetomidine is further metabolised either into O-glucuronide or to carboxylic acid conjugate (Salonen and Eloranta, 1990). The first pass metabolism in the liver is extensive and pharmacological effects are achieved at very high doses (Vainio, 1989). The metabolisms of medetomidine and dexmedetomidine differ in their respective rates of hydroxylation. The P450 isozyme has a higher affinity to dexmedetomidine, causing higher rates of oxidation (Salonen and Eloranta, 1990).

Toremifene is completely absorbed from the gastrointestinal tract without a first-pass metabolism. More than 99% is bound to plasma proteins (Sipilä et al., 1988). The kinetics are linear in the range of 10-680 mg (Anttila et al., 1990). Peak concentration in the serum after oral administration is achieved after 4 hours (Anttila et al., 1990). The apparent volume of distribution is 11.8 l/kg after oral administration (Anttila et

al., 1995). The mean half-life of distribution after oral administration is 4 hours. The elimination is slow with a mean half-life of 5 days (Anttila et al., 1990). The consequence of the long elimination half-life, is that a significant accumulation of the drug occurs during repeated administration. The steady state level is achieved within 6 weeks after the start of peroral therapy (Anttila et al., 1990).

Toremifene is metabolised in the liver by cytochrome P450 (Berthou et al., 1994). More than ten metabolites have been identified, the main metabolite being N-demethyltoremifene, that show antitumor activity (Anttila et al., 1990, Coradoni et al., 1991).

2.4.3. Clinical applications of dexmedetomidine and toremifene

Alpha 2-agonists are widely used as a sedative, analgesic and anaesthetic premedication. Dexmedetomidine (Precedex®) is commercially available for sedation of patients in intensive care. Dexmedetomidine is well tolerated and the most frequently observed adverse effects are hypotension and bradycardia, which, however, are quite easily controlled. The effect is achieved with im, iv and sc modes of injection. The racemic mixture, medetomidine (Domitor®), which also includes the pharmacologically inactive *l*-isomer, has been approved for veterinary use in several countries.

Toremifene (Fareston ®) is a triphenylethylene antiestrogen, which acts as competitive antagonist at the estrogen receptor (Kangas, 1990) and is used in hormonal therapy as an anticancer drug for the treatment of metastatic breast cancer (Valavaara, 1990).

3. AIMS OF THE STUDY

The main objective of this study was to evaluate sol-gel based silica gel as an implantable or injectable biodegradable carrier for controlled drug delivery. The effect of various synthesis parameters and manufacturing methods, casting and spray drying on the release rate of model drugs was studied in vitro. Two model drugs (toremifene citrate and dexmedetomidine hydrochloride), both basic drugs but with different physicochemical and pharmacokinetic properties, were incorporated into silica gel during the synthesis phase. In vivo release of toremifene from a silica xerogel monolith and degradation of the matrix was studied in mice. Bioavailability of dexmedetomidine from implanted silica xerogel monoliths or injected silica gel microparticles was estimated from the pharmacokinetic data in dogs.

The specific aims of this study were:

1. To confirm biodegradation and evaluate tissue effects of the sol-gel derived silica xerogel carrier material at the implantation site and in the specific organs in mice
2. To study the effects of manufacturing methods (spray drying, casting method) on the release behaviour of dexmedetomidine and toremifene and degradation of the matrix in vitro
3. To study effects of sol-gel synthesis parameters (pH of the sol, water/TEOS ratio and partial substitution of silica precursor with alkyl-substituted alkoxide) on the release behaviour of dexmedetomidine and degradation of the matrix in vitro
4. To investigate in vivo whether an implantable or injectable controlled release formulation for toremifene citrate and dexmedetomidine hydrochloride could be developed.

4. MATERIALS AND METHODS

4.1. MODEL DRUGS

Dexmedetomidine hydrochloride (II-V) and toremifene citrate (I-II) were synthesised at Orion Corporation (Espoo, Finland). Physicochemical characteristics of drugs are described in section 2.4.1. ³H-toremifene was synthesised as described earlier (Kangas et al., 1989). The specific activity of the material was 16 Ci/mmol (I).

4.2. PREPARATION OF SOL-GEL PROCESSED SILICA GEL MONOLITHS AND MICROPARTICLES

Silica sols were prepared at room temperature by acid hydrolysis of TEOS or TEOS co-hydrolysed with alkyl-substituted alkoxides and deionised water (I – V). Toremifene citrate or dexmedetomidine HCl were dissolved into hydrolysed sol. Silica xerogel monoliths containing ³H-toremifene/toremifene citrate for implantation study in mice were prepared by dissolving 33 mg/g of toremifene citrate in the silica sol (I). ³H-toremifene was added to the silica sol to produce a final radioactivity of 16 μ Ci/g.

The drug concentration in monoliths and microparticles was calculated as a theoretical concentration by assuming that all of the drug dissolved in the silica sol was incorporated into the silica gel structure (I-V). The actual concentration of drug could not be determined because silica gel dissolves at reasonable speed only at a very high pH (> 10) (Brinker and Scherer, 1990).

4.2.1. Monoliths

Hydrolysed silica sol was cast into Teflon moulds (rod or disc shaped) that were tightly closed and kept at 40°C and 40% relative humidity for polycondensation and gelation. After gelation the silica gel monoliths were dried to a constant weight at 40°C and 40% relative humidity (I, III, IV, V).

4.2.2. Microparticles

Hydrolysed silica sol was spray dried with a mini spray dryer (B-191, Büchi Labortechnik AG; Switzerland). Spray drying process parameters were: inlet air temperature 135°C, pump 16 (dialling setting), aspirator 95 (dialling setting) and spray-flow 600 Nl/h. A 0.77 mm nozzle was used throughout the experiments (II, IV, V).

Volumetric particle size and size distribution of the microparticles was obtained by laser diffractometry using Sympatec/Helos laser diffraction equipment (Sympatec GmbH, Germany). The samples were dispersed in water and treated with ultrasound equipment for 5 seconds to ensure a homogenous dispersion. The measurements were

carried out using a 50-mm or 100-mm lens. The results are the average of five withdrawals (II, IV, V).

The shape and surface morphology of the microparticles was determined from micrographs taken with scanning electron microscope (SEM-EDX Stereoscan 360, Cambridge Instruments, UK) (II, IV, V).

The yield of microparticles was calculated from the theoretical amount silica gel (SiO_2). The yield varied between 42.8 and 77.7 % for silica gel microparticles containing toremifene citrate and between 20.6 and 49.2 % for microparticles containing dexmedetomidine HCl (II).

4.3. THE SOL-GEL SYNTHESIS PARAMETERS STUDIED IN THE PROCESSING OF SILICA GEL MONOLITHS AND MICROPARTICLES

4.3.1. pH of the sol

The hydrolysis and condensation reactions were catalysed in two different ways, one-step acid catalysis with various concentrations of HCl and CH_3COOH or two-step catalysis with HCl and NH_3 in order to get silica gel monoliths or microparticles manufactured at pH 1; 2.3; 3 and 5 at water/TEOS ratio 14 (III, table 1; V, table 1).

4.3.2. Water/TEOS ratio

Silica monoliths were manufactured at water/TEOS ratios 6, 14 and 28 (III, table 1) and microparticles at ratios 6, 10, 14, 28 and 35 at pH = 2.3 (V, table 1).

4.3.3. Alkyl-substituted silica xerogel

Alkyl-substituted silica gels were prepared at pH = 2.3 with a water/TEOS ratio of 14 using HCl as catalyst. Partial substitution of silica sol was carried out by co-hydrolysis of TEOS with 5, 10 or 25 mol-% of DMDES, METES or ETES (IV).

4.3.4. Size and shape of the monoliths

The influence of silica xerogel monolith size on the release rate of dexmedetomidine was studied with rod-shaped monoliths with a length of 11.5 mm and diameters of 1.9 mm, 1.4 mm and 0.95 mm and with disc-shaped monoliths with a diameter of 4.6 mm and a thickness of 1.59 mm prepared at pH = 3 with a water/TEOS ratio of 14 having 1 wt-% of dexmedetomidine HCl in the sol (III).

4.3.5. Drug concentration

Toremifene citrate was added to silica sol with a concentration of 0.5, 1.0, 2.0, 2.5 and 3.0 wt-% and the sol was spray dried to microparticles (II). The concentration of dexmedetomidine HCl varied between 0.5 and 2 wt-% in silica sol (II, III, IV).

4.4. DRUG RELEASE AND MATRIX DEGRADATION STUDIES IN VITRO

The release of toremifene and dexmedetomidine from silica gel monoliths and microparticles was performed in a dissolution apparatus II (U.S.P 24) at 37°C with a rotation speed of 50 rpm using 250 ml of either 0.5% SDS/SBF- buffer (pH 7.4) (I-IV) or 0.9% NaCl as dissolution medium (V). Toremifene and dexmedetomidine were analysed with an UV-spectrophotometer at the maximum absorbance of the drug.

Simultaneously with the drug release studies, the degradation of the silica gel monoliths and microparticles was determined. Dissolved silicon was determined as $\text{Si}(\text{OH})_4$ as a molybdenum blue complex at 820 nm (Koch and Koch-Dedic, 1974) (I-V).

4.5. IN VIVO STUDIES

All animal experiments were approved by the animal care committee of Orion Corporation.

4.5.1. Tissue effects and release of toremifene from silica xerogel implants in mice

Thirty female mice (C57Bl, Denmark) with an average weight of 19.6 g (SD 1.2) received implants subcutaneously on the back on either side of the backbone with ^3H -toremifene loaded silica implants (a total of 2 implants were placed in each mouse) and thirty mice with untreated silica implants. The ^3H -toremifene dose was about 80 $\mu\text{Ci}/\text{kg}$ (0.8 $\mu\text{Ci}/\text{implant}$), toremifene citrate 350 mg/kg (app. 3.4 mg/implant) and silica xerogel about 1.53 g/kg body weight. The silica xerogel implants were round with a diameter of approximately 4.7 mm and a thickness of 0.9 mm (I).

The animals were sacrificed at 7, 14, 21, 28, 35 and 42 days (5 mice per time period) after implantation. The silica xerogel implants on the left side of the backbone were removed together with surrounding tissue and fixed in 70% ethanol and embedded in PMMA (Technovit). Sections of 20 μm were stained with toluidine blue. Samples of liver, kidney, mesenterial lymph node and uterus were fixed in buffered formaldehyde and embedded in paraffin. Sections of 6 μm were stained with hematoxylin eosin. The samples were evaluated using light microscopy. The samples of the implantation site

were studied from all interim kills, but liver, kidney, lymph node and uterus only from the animals treated 7, 28 and 42 days (I).

The percentage of implant remaining at each time point was calculated. To determine the amount of toremifene remaining in the implants, the dried implants were dissolved in 0.1 N NaOH solution and the activity was measured in liquid scintillation counter (I).

4.5.2. Bioavailability of dexmedetomidine in dogs

Formulations. Silica gel formulations administered to dogs are described in table 1. As a reference dexmedetomidine HCl dissolved in 0.9% NaCl (10 and 20 µg/ml) was administered subcutaneously (5 ml) with a dose of 0.05 or 0.1 mg. The test formulations were rod-shaped silica gel monoliths prepared by the casting method or microparticles prepared by spray drying. The monoliths were prepared from a sol, where 25 mol-% of TEOS was substituted by alkyl-substituted alkoxide, dimethyl(diethoxy)silane (DMDDES) at pH = 2.3 with a water/TEOS ratio of 14. The amount of dexmedetomidine HCl dissolved in the sol was either 1 wt-% or 2 wt-% corresponding respectively to 7.7 wt-% and 15.4 wt-% in dry gel (IV). The spray-dried microparticles were processed from a sol synthesised at pH = 2.3 with a water/TEOS ratio of 14 containing 1 wt-% dexmedetomidine HCl in the sol (V).

Table 1. Dexmedetomidine HCl containing silica gel microparticles (prepared at pH = 2.3, water/TEOS ratio 14), monoliths (25 mol-% DMDDES/75 mol-% TEOS, pH = 2.3, water/TEOS 14) and reference doses used in bioavailability studies in dogs.

Treatment	Dexmedetomidine HCl dose range, µg/kg	Dogs (n), weight of dogs
0.05 mg of dexmedetomidine HCl in 5 ml of 0.9% NaCl (IV)	4.8 – 6.1	n = 4 8.2 – 10.4 kg
0.1 mg of dexmedetomidine HCl in 5 ml of 0.9% NaCl (V)	9.5 – 12.0	n = 4 8.3 – 10.5 kg
60 mg of silica gel microparticles containing 4.6 mg of dexmedetomidine HCl in 5 ml of 0.9% NaCl (V)	460– 540	n = 3 8.6 - 10.1 kg
Two silica gel monoliths (53.9 mg ± 0.8) containing 4.15 mg (± 0.007) of dexmedetomidine HCl (IV)	420 – 440	n = 3 9.1 – 9.8 kg*
Two silica gel monoliths (57.8 mg ± 0.3) containing 8.9 mg (± 0.04) of dexmedetomidine HCl (IV)	910 - 980	n = 4 9.5 – 9.9 kg*

* Typing mistake in original paper IV.

Bioavailability study. Three beagle dogs, two males and one female were subcutaneously given 60 mg of silica gel microparticles containing 4.6 mg of dexmedetomidine HCl suspended in 5 ml of 0.9 % NaCl solution per dog (V). The

amount of dexmedetomidine given varied between 0.46 to 0.54 mg/kg (table 1). Four dogs, two males and two females were subcutaneously given two silica gel monoliths (total weight 53.9 mg) containing a total of 4.15 mg of dexmedetomidine HCl per dog corresponding to 0.42 – 0.44 mg/kg (IV). Three dogs, two male and one female, were given two silica gel monoliths (57.8 mg) containing 8.9 mg of dexmedetomidine HCl per dog corresponding to 0.91 – 0.98 mg/kg (IV). As a reference, either 0.05 mg (IV) or 0.1 mg (V) of dexmedetomidine HCl (in 5 ml of 0.9% NaCl) was given subcutaneously each to four dogs, three males and one female in each group. The amount of dexmedetomidine given varied between 4.8 – 6.1 and 9.5 – 12.0 µg/kg respectively (table 1).

The blood samples for dexmedetomidine serum concentration determination were taken from the jugular vein at the predose and 0.5, 1, 2, 4, 6, 8, 24 and 48 hour time points.

Assay methods. The dexmedetomidine concentrations in serum were determined by a specific and sensitive gas chromatographic mass spectrometric (GC-MS) method, a modification of the published method (Vuorilehto et al., 1989). The method is linear from 50 pg/ml to 2500 pg/ml for dexmedetomidine (IV, V).

Determination of bioavailability. The pharmacokinetic parameters were calculated by non-compartmental analysis using the commercially available software package WinNonlin Professional, version 3.0 (Pharsight Corporation, Mountain View, CA, USA). The kinetic parameters were derived from the individual dexmedetomidine concentration time curves and included: C_{\max} (the maximum observed dexmedetomidine concentration in serum), t_{\max} (the time of occurrence of C_{\max}) and AUC_{0-48h} or AUC_{0-8h} (the area under the concentration versus time curves from time zero to either 48 hours or 8 hours using the linear trapezoidal rule) (Gibaldi and Perrier, 1982). After the s.c. dosing of reference compound, the last quantifiable concentration was reached before eight hours and the area beyond that point was approximated using a triangular area up to the next sampling point, the concentration of which was deemed to be zero. The individual AUC values were averaged and the mean dose normalised values were used to calculate the relative bioavailability of dexmedetomidine from silica xerogel. The relative bioavailability of the silica gel test formulations was calculated by comparing the AUC values with the AUC values of the reference dose used in the study (IV, V).

5. RESULTS

5.1. THE EFFECT OF SYNTHESIS PARAMETERS AND MANUFACTURING METHOD ON THE RELEASE RATE OF DRUGS AND DEGRADATION OF THE SILICA GEL IN VITRO

5.1.1. Size and shape of silica gel monolith

The effect of device size on the release rate of dexmedetomidine was studied in rod-shaped monoliths with various diameters or in disc-shaped monoliths (III, fig 4a). The burst release of dexmedetomidine was about 40% from rod-shaped monoliths with a diameter of 0.95 mm, whereas it was less than 20% from disc-shaped monoliths with a diameter of 4.6 mm and rod-shaped monoliths with diameter of 1.4 mm and 1.9 mm. After 30-hours of dissolution, more or less 100 % was released from 0.9-mm rod-shaped monoliths, 80% from 1.4-mm monoliths and 70% from 1.9-mm monoliths. From disc-shaped monoliths dexmedetomidine release was initially as fast as from the rods for the first six hours. Later the release rate slowed down and about 54% was released after 30 hours.

The amount of silica xerogel degraded after 30-hours of dissolution was at least 15% for a disc-shaped monolith (diameter 4.6 mm) and at most 28% for a rod-shaped monolith with a 0.95 mm diameter (III, fig 4 b). Rod-shaped monoliths with diameters of 1.9 mm and 1.4 mm as well as disc-shaped monoliths had a lag phase before the degradation of the silica xerogel matrix began.

5.1.2. pH and water/TEOS ratio

Monoliths. The sols with pH = 2.3 (hydrochloric acid as catalyst) and pH = 3 (acetic acid as catalyst) were synthesised near the isoelectric point of silica, which generally is dependent on the acid used as a catalyst (Brinker and Scherer, 1990). The release of dexmedetomidine was faster from acetic acid catalysed silica gel (pH = 3) than from hydrochloric acid catalysed silica gel prepared at pH = 2.3 with a water/TEOS ratio of 14 (III, fig 1). The amount of dexmedetomidine released varied between 44 % (pH = 2.3) and 92 % (pH = 5) during the 30-hour dissolution period (table 2). The initial burst was highest, 34%, from monoliths prepared at pH = 1 and lowest, about 6% from monoliths prepared at pH = 2.3. The diffusional exponent (n) characteristic for the release mechanism varied between 0.23 (pH = 1) to 0.61 (pH = 2.3) (III, table 2).

Decreasing the water/TEOS ratio of the silica sol from 28 to 6 decreased the released amount of dexmedetomidine from about 64% to 30% during a 30-hour dissolution period (table 2, III, fig 2). Drug release could be regarded as diffusion controlled from silica xerogel monoliths synthesised at pH = 2.3 having $r = 14$ ($n = 0.61$) and $r = 28$ ($n = 0.56$). From $r = 6$ silica xerogel the exponential coefficient clearly deviated from diffusional release mechanism ($n = 0.71$) (III, table 2).

At the end of the dissolution test (30 h) about 83 % (pH = 2.3) to 75% (pH = 1 and pH = 3) of the matrix remained when the water/TEOS ratio was 14 (table 2, III, fig 1). When the water/TEOS ratio was changed from $r = 6$ to $r = 28$ the amount of matrix remaining after 30 hour varied between about 82 % ($r = 6$) and 80 % ($r = 28$) at pH = 2.3 (table 2, III, fig 2).

Microparticles. Scanning electron microscopy analysis showed that microparticles prepared at pH = 2.3 ($r = 14$) containing either dexmedetomidine HCl or toremifene citrate were spherical and had a smooth surface without visible pores on the surface (II, fig 1). Microparticles containing dexmedetomidine HCl spray-dried from a sol synthesised at pH = 1 with a water/TEOS ratio of 14 had, however, a rough surface (V, figure 1c). The size of microparticles containing toremifene citrate or dexmedetomidine HCl and prepared at different pH had a very similar, a quite narrow particle size distribution between 1.2 μm (D 10%) and 42 μm (D 90%) (II, table 2, V, table 2). The specific surface area of the microparticles was less than 10 m^2/g (II, table 2).

Microparticles containing dexmedetomidine HCl were synthesised at same pH values as monoliths (pH = 1, pH = 2.3, pH = 3 and pH = 5) at a water/TEOS ratio of 14 (V). The release rate of dexmedetomidine was slowest from microparticles prepared at pH = 2.3 (HCl as catalyst) and pH = 3 (CH_3COOH as catalyst) near the IEP of silica. The burst effect increased from microparticles prepared above or below the isoelectric point (table 2, V, fig 2). The amount of released dexmedetomidine varied between about 10 % (pH = 2.3 and pH = 3) and 40 % (pH = 1) during a 30-hour dissolution period (table 2). When the synthesis pH was at IEP, the released amount during a 30-hour dissolution period was so low that drug release kinetics could not be evaluated. Below or above IEP the slopes (n) of $\log Q$ vs. $\log t$ plots deviated from diffusion controlled release kinetics ($n < 0.5$) (V, table 3).

The rate of dexmedetomidine release was significantly decreased from silica xerogel microparticles with increasing dilution of the sol before spray drying (V, fig 4). Decreasing the mole ratio of water/TEOS from 35 to 6 increased the amount of dexmedetomidine released from about 0.5% to 71% at 30 hours from microparticles prepared at pH = 2.3 (table 2, V, fig 4). Dexmedetomidine release obeyed zero order kinetics from microparticles prepared at water/TEOS ratios 6 as determined from the slope (n) of $\log Q$ versus $\log t$ plots (V, table 3). However the slope of the $\log Q$ vs. $\log t$ plot could not be reliably calculated for release profiles of water/TEOS ratios between 10 and 35, because the released amount during a 30-hour dissolution period was too low.

The synthesis pH as well as the water/TEOS ratio clearly affected the degradation rate of silica gel microparticles (V). After a 30-hour dissolution period about 99 % (pH = 3 and pH = 2.3) to 83 % (pH = 5) of the matrix remained (table 2, V, fig 3). The amount of matrix degraded during a 30-hour dissolution period varied between 20 % to 0.3%,

when the water/TEOS ratio (pH = 2.3) varied between 6 and 35 respectively (table 2, V, figure 5).

5.1.3. Alkyl-substituted silica gel

Monoliths. One object of study was the partial substitution of TEOS with alkyl-substituted alkoxides (5, 10 or 25 mol-%) with covalently bound methyl (METES and DMDES) or ethyl groups (ETES) on the release rate of dexmedetomidine. The addition of METES did not have any effect on the release rate of dexmedetomidine (table 2, IV, fig1). Partial substitution with 10 or 25 mol-% of ETES decreased the released amount of dexmedetomidine from 44% (100% TEOS, pH = 2.3, water/TEOS 14) to 37 and 22% respectively (table 2, IV, fig 1). An almost 3-fold decrease in released amount of dexmedetomidine during 30-hour dissolution was obtained, when the TEOS was substituted with 25 mol-% of DMDES having two covalently bound methyl groups attached to silicon (table 2, IV, fig 1). A long-term study showed that 80 % of the drug was released from the matrix substituted with 25 mol-% of DMDES during four-month release test (IV, fig 2). The release obeyed diffusion controlled kinetics from monoliths containing 25 mol-% of METES, ETES or 100% TEOS ($0.5 < n < 0.63$, IV, table 1). The release of dexmedetomidine deviated from diffusion controlled kinetics ($n > 0.6$) from rods containing 25 mol-% DMDES or 10 or 5 mol-% of METES or ETES as determined from the slope (n) of the log Q vs. log t plots. The release conformed to zero order release from silica gels substituted with 5 or 10 mol-% DMDES (IV, table 1).

The amount of silica xerogel left after a 30-hour dissolution period varied between about 98% (25 mol% DMDES or ETES) and 87 to 85% (5 mol% of alkyl-substituted alkoxide) (table 2, IV, fig 3).

Microparticles. Alkyl-substituted silica xerogel microparticles were spherical with aggregated clusters, the size distribution ranging from 1.36 to 35.02 μm (IV, table 2, fig 4)

As compared to 100% TEOS, microparticles with a lag time in drug release, partial substitution with alkyl-substituted alkoxides DMDES or METES (5 mol-% and 25 mol-%) increased the amount of released dexmedetomidine after one hour to about 3 to 15% (table 2, IV, fig 5a). After the burst the drug was released nearly at the same rate as from 100% TEOS microparticles.

The degradation of the silica microparticles, co-hydrolysed with 5 mol-% of alkyl-substituted alkoxide (METES or DMDES) or with 25 mol-% DMDES, was faster than that of the 100% TEOS matrix synthesised at pH = 2.3 at water/TEOS ratio 14 (table 2, IV, figure 5b). The amount of matrix remaining after dissolution was between 99% (25 mol-% METES) and 95 % (5 mol-% DMDES or METES) (table 2).

Table 2. In vitro release data for dexmedetomidine from silica gel monoliths and microparticles and degradation of silica gel (III – V).

Formulation	Dexmedetomidine released after one hour, %	Dexmedetomidine released after 30 h, %	Silica gel degraded after 30 h, %
pH (water/TEOS 14)			
Monoliths (III)			
pH = 1	34.1	71.1	25.5
pH = 2.3	6.4	43.8	16.9
pH = 3	13.6	77.7	25.7
pH = 5	21.9	92.2	21.7
Microparticles (V)			
pH = 1	21.5	41.4	13.6
pH = 2.3	0	11.5	1.2
pH = 3	0	7.3	0.7
pH = 5	10.3	34.0	16.7
Water/TEOS (r) (pH = 2.3)			
Monoliths (III)			
r = 6	2.5	30.2	17.8
r = 14	6.4	43.8	16.9
r = 28	8.9	63.6	20.4
Microparticles (V)			
r = 6	0.9	71.1	20.0
r = 10	0	26.7	2.8
r = 14	0	11.5	1.2
r = 28	0	3.0	0.4
r = 35	0	0.5	0.3
Alkyl substitution			
Monoliths (IV)			
25 mol-% DMDDES	1.7	15.7	1.3
10 mol-%	0.6	33.5	5.7
5 mol-%	0.04	34.8	14.9
25 mol-% METES	7.8 (2h)	48.7	5.8
10 mol-%	2.8	43.3	11.5
5 mol-%	1.8	41.5	14.3
25 mol-% ETES	4.1	21.7	2.1
10 mol-%	3.8	36.7	7.8
5 mol-%	1.8	46.0	13.2
Microparticles (IV)			
25 mol-% DMDDES	8.1	29.4	3.5
5 mol-%	15.0	40.2	5.4
25 mol-% METES	3.3	15.5	1.3
5 mol-%	4.0	17.9	5.8

5.1.4. Drug concentration

The release rate of dexmedetomidine from silica gel monoliths was proportional to the drug concentration between 0.5 and 1 wt-% in the sol, corresponding to 3.9 and 7.7 wt-% in the dry gel in monoliths, whereas the released amount seemed to be slightly lower from monoliths containing 15.4 wt-% of dexmedetomidine HCl (corresponding to 2 wt-% in the silica sol)(III, fig 3). From alkyl-substituted matrix (25 mol-% DMDES/75 mol-% TEOS), however, the release of dexmedetomidine was faster from monoliths containing 2 wt-% of dexmedetomidine HCl in the sol (15.4 wt-% in dry gel) than from monoliths containing 1 wt-% of drug in the sol (7.7 wt-% in dry gel) (IV, fig 6b).

The release of toremifene from silica gel microparticles was linear with respect to the square root of time up to 15.4 wt-% of drug in silica gel (II, fig 2), whereas release of dexmedetomidine was linear with respect to the square root of time when the drug concentration was 9.6 wt-% or lower (II, fig 3). The release rate of toremifene remained about the same when the concentration of drug was below 15 wt-%. The burst of toremifene increased appreciably when the drug concentration was 19.3 wt-% or above. For dexmedetomidine the release rate was the same when the drug concentration was 3.9 wt-% or 5.8 wt-% (II, fig 3). Above 5.8 wt-% of dexmedetomidine HCl, the drug release rate had a tendency to increase with increasing drug concentration. In addition, release of dexmedetomidine was slower than that of toremifene when the drug concentration was below 11.6 wt-% (II, fig 2-4).

5.2. TISSUE EFFECTS OF SUBCUTANEOUSLY ADMINISTERED SILICA XEROGEL IN MICE

The local effect of the silica xerogel implant was restricted to the immediate vicinity of the implant. A well-organised fibrous capsule was formed around the implant from 14 days time point onward (I, fig 4a). The implant did not cause necrosis and the inflammatory response observed was mainly due to a minor infiltration of macrophages at time points 14 days and later. Acute inflammation was observed only at the 7-day point. Phagocytic macrophages were present close to the implant at 14 days or later (I, fig 4b). No accumulation of dissolved material occurred. No extensive silica xerogel related histological changes could be observed in the liver, lymph nodes or kidney at the 1.5 g/kg dose.

5.3. DRUG RELEASE IN VIVO

5.3.1. Toremifene release in mice

Toremifene was released in a sustained manner from silica xerogel monoliths in mice. The amount of ^3H -toremifene remaining in the monolith as determined by dissolving the excised monolith in NaOH and measuring the radioactivity, was about 16% after 42 days from implantation (I, fig 2). About 40% of the loaded amount eluted during the first week. Weight loss of the matrix was about 75 % during the test period, 42 days (I, fig 2). The release rate of toremifene in mice was lower than in vitro where about 90% of the loaded amount was released after 99 hours (I, fig 1). The amount of silica released is equivalent to about 65% weight loss of the total silica xerogel monolith weight (I, fig 1).

The radioactivity of tritiated toremifene was detected up to 42 days in the liver, kidneys, and lung. The radioactivity reached a maximum value at 14 days and then remained relatively constant until the end of the implantation test. The original data showed that radioactivity was not found in the blood or uterus (I, fig 3). Toremifene induced uterine glandular hyperplasia, the characteristic hormonal change seen in mice treated with this drug, was observed at all studied time points.

5.3.2. Bioavailability of dexmedetomidine in dogs

Table 3 presents a summary of mean pharmacokinetic parameters resulting from a subcutaneous administration of alkyl-substituted silica gel monoliths (IV) containing dexmedetomidine HCl at two dose levels (4.15 mg dexmedetomidine HCl and 8.9 mg dexmedetomidine HCl), microparticle suspension (containing 4.6 mg of dexmedetomidine HCl) (V) and a reference dose at two dose levels (0.05 mg and 0.1 mg dexmedetomidine HCl in 0.9 % NaCl solution). The relative bioavailability was calculated using a dose of 0.05 mg as reference for alkyl-substituted silica gel monoliths and a dose of 0.1 mg as a reference for microparticles.

The C_{max} value after the lower dose of the alkyl-substituted silica gel monoliths was 0.6 ng/ml and that of the higher dose was 3.8 ng/ml (table 3, IV, fig 6). The C_{max} after microparticle formulation containing approximately the same amount of dexmedetomidine as the lower dose of monoliths was about 1.6 ng/ml (table 3, V fig 6). The mean maximum concentration was achieved at about 5 hours after administration of silica gel formulations whereas it was reached at about one hour after administration of the references. After the reference, the dexmedetomidine concentration declined rapidly, decreasing below the quantitation limit after six hours. In contrast, measurable serum concentrations were still seen in serum samples taken 48 hours after the dosing of alkyl-substituted silica gel monoliths or silica gel microparticles (IV, fig 6; V, fig 6). The concentration of dexmedetomidine in serum after 48 hours was 0.09 ng/ml for 4.15 mg and 0.23 ng/ml for 8.9 mg of dexmedetomidine HCl in alkyl-substituted silica gel. After the administration of 4.6

mg of dexmedetomidine HCl in the microparticle formulation, the serum concentration was 0.13 ng/ml after 48 hours.

Table 3. The pharmacokinetic parameters after subcutaneous administration of 0.1 mg or 0.05 mg of dexmedetomidine HCl as reference, 4.6 mg of dexmedetomidine HCl incorporated into silica gel microparticles (100 mol-% TEOS, pH = 2.3, r = 14) and 4.15 mg or 8.9 mg incorporated into alkyl-substituted (25 mol-% DMDES) silica xerogel monoliths in dogs. Means \pm SD .

Parameter	Reference 0.05 mg (n = 4)	Reference 0.1 mg (n = 4)	Microparticles 4.6 mg (n = 3)	Alkyl- substituted 4.15 mg (n = 4)	Alkyl- substituted 8.9 mg (n = 3)
C_{max} (ng/ml)	0.5 (0.3)	1.3 (0.5)	1.6 (0.6)	0.6 (0.3)	3.8 (0.5)
T_{max} (h)	1.1 (0.6)	1.5 (0.6)	4.7 (1.2)	5.0 (1.2)	5.3 (2.3)
AUC_{0-48h} (ng*h/ml)	0.98 (0.24)*	3.31 (0.95)*	24.8 (7.7)	9.8 (2.5)	67.9 (12.9)
Relative bio- availability	1.0	1.0	0.16	0.12	0.39

C_{max} = peak concentration of dexmedetomidine, t_{max} = time to reach C_{max} ,

AUC_{0-48h} = area under the concentration vs. time curve

* AUC_{0-8h}

6. DISCUSSION

6.1. CONTROLLING THE RELEASE RATE OF MODEL DRUGS AND DEGRADATION OF SILICA GEL BY SYNTHESIS PARAMETERS AND MANUFACTURING METHOD

Silica sol is mostly manufactured to monoliths by casting. Spray drying was evaluated as an alternative method for the manufacturing of silica gel and as a means to control the release rate of drug substances. During spray drying silica sol is forced to condense very quickly at an increased temperature, whereas monoliths are allowed to polymerise spontaneously during a longer time period. The difference in the polymerisation process leads to changes in the inner structure of the silica gel. Microparticles have a condensed structure with a specific surface area $< 10 \text{ m}^2/\text{g}$ (II, table 2), which is significantly lower than that of typical porous monoliths ($\approx 400 \text{ m}^2/\text{g}$) (Ahola et al., 2000). The structure of silica gel and the release rate of dexmedetomidine from monoliths and microparticles were controlled by synthesis factors. In general the porous structure of cast silica xerogel monoliths resulted in a faster release of drug and degradation of the matrix as compared to spray-dried microparticles. The release of dexmedetomidine from monoliths prepared at various pH levels and water/TEOS ratios varied from days to a few weeks, whereas with microparticles it was from a few days to several months. Drug release was faster than

the degradation of the silica matrix, which varied from days to months with monoliths and from days to more than a year with microparticles as calculated on the basis of the 30-hour dissolution period results in table 2 (Table 4). The major differences between monoliths and microparticles are highlighted in table 4 and discussed in detail below.

Table 4. Comparison of main properties of sol-gel processed silica gel monoliths and microparticles

Property	Monolith	Microparticles
Drug concentration	< 35 wt-% (Ahola et al., 2000)	< 15 wt-%
Specific surface area	$\approx 400 \text{ m}^2$ (Ahola et al., 2000)	< 10 m^2
Optimum synthesis pH	Between pH = 2-3	Between pH =2-3
Release kinetics of drug	Diffusion	Zero order (at IEP)
Release time of dexmedetomidine*	From days to weeks	From days to months
Release kinetics of SiO ₂	Zero order	Zero order
Appr. degradation time of silica gel*	From days to months*	From days to more than a year*

*Release time of dexmedetomidine and degradation time of silica gel are calculated on the basis of a 30 hour dissolution period results described in table 2

6.1.1. Sol-gel synthesis parameters

6.1.1.1. pH and water/TEOS ratio

The optimum release profile for dexmedetomidine was obtained from silica gels (monoliths and microparticles) prepared at the isoelectric point of silica (pH = 2.3 and pH = 3) where the structure of silica gel is most condensed (Brinker and Scherer, 1990) (III, V). The amount of dexmedetomidine released after the dissolution period (30 h) was greater with a decreasing diameter of the rod (II, fig 4). Similar results were obtained with bulk degrading polymers (Tamada and Langer, 1993, Lemmouchi and Schacht, 1997). Changing the water /TEOS ratio of the starting materials from 6 to 35 with microparticles and from 6 to 28 with monoliths at the pH = 2.3 had an opposite effect on the release of dexmedetomidine from microparticles and from monoliths. The amount of released dexmedetomidine from microparticles decreased 140-fold when silica sol was diluted with water during a 30-hour dissolution period (table 2, V, fig 4). Contrary to microparticles, the amount of released dexmedetomidine increased by appr. 50% from monoliths with an increasing water/TEOS ratio (table 2, III, fig 2). Previously Aughenbaugh and co-workers (Aughenbaugh et al., 2001) have observed that an increasing water/TEOS ratio increased the release rate of vancomycin from silica xerogel monoliths. It is well known that the water/TEOS ratio affects the structure of silica gel monoliths and a more condensed matrix is formed at low water/TEOS ratios (Meixner and Dyer, 1999). However, during spray drying, silica particles are forced to form quickly from

the sol at elevated temperatures. Dilution of the sol influences several factors and the main effect is that the volume fraction of the solids and viscosity of sols are decreased. In addition, some attraction is needed to form dense particles at a colloidal level. It is also known that due to exceedingly strong attraction, the particles stick together tightly where they first make contact and more open structures are easily formed. However, dilution decreases the attractive forces between colloidal silica particles causing a more dense packing at high water/TEOS ratios. Thus the sol diluted with an increasing amount of water produces slower degrading silica particles and slower drug release.

Modification of the matrix by changing the processing method from casting to spray drying changed the drug release mechanism. The release of dexmedetomidine was mainly diffusion controlled from 100% TEOS monoliths (III, table 2), whereas zero order release was obtained from certain microparticle formulations (V, table 3). The diffusional coefficient calculated from the slope of the log (released drug) versus log (time) plot and describing the release mechanism of dexmedetomidine varied between $0.2 < n < 0.7$ for monoliths prepared at various pH and water/TEOS ratios, whereas it was between 0.2 (for microparticles prepared at pH = 1 and pH = 5 and about 1.0 (for microparticles prepared at pH = 2.3 with water/TEOS ratio 6). Values below 0.5, however, indicate porous structure (Peppas, 1985). The release of dexmedetomidine gave values below 0.5 from monoliths and microparticles prepared at pH = 1 and pH = 5, where the structure of the silica xerogel matrix is more porous than at the isoelectric point causing an increased burst and a faster drug release rate (Curran and Stiegman, 1999). More mesoporous structure is formed due to higher condensation rate with monoliths when the pH deviates from IEP. Microparticles prepared at pH = 1 or pH = 5 are probably more porous than microparticles prepared at IEP because of repulsion preventing formation of dense particles at colloidal level. This difference in structure is also showed in SEM pictures where the surface of microparticles prepared at pH = 1 is more rough (V, fig 1).

6.1.1.2. Alkyl-substituted silica gel

The structure of the silica xerogel matrix can be modified by adjusting the amount of alkyl-substituted siloxane. Organic groups linked to the oxide network by stable chemical bonds change the porosity by lowering the degree of cross-linking and provide hydrophobicity and ion exchange capacity due to the decreased amount of surface silanol groups (Schmidt, 1989, Mah and Chung, 1995, Lev et al., 1995). The pore size of the silica gel matrix increases with increasing length of the alkyl group (Kusakabe et al., 1999). It is known from previous studies that the release rate of active agents can be modified by chemical modification of the silica xerogel matrix (Unger et al., 1983, Böttcher et al., 1998, Ahola et al., 2001). In the present study, a decrease in the release rate of drug and in initial bursts was dependent on the amount of the alkyl-substituted alkoxide as well as on the length and number of the functional groups attached covalently to silicon (table 2, IV, fig 1). The observed decrease in release rate may be due to hydrophobic nature of ethyl and methyl groups or due to changes in the porous structure of silica gel depending on the amount and

type of alkoxide. Interactions of dexmedetomidine with the silica gel also influence the release rate. Dexmedetomidine probably binds to silica gel with hydrogen bonds and with hydrophobic interactions (Iler, 1979).

Value of the diffusional coefficient varied between $0.52 < n < 1.06$ for alkyl-substituted silica gel monoliths. With 25 mol-% of alkyl-substitution the release was diffusion controlled ($n = 0.52-0.71$) but changed with 5 or 10 mol-% of alkyl-substitution towards non-Fickian or zero order release ($n = 0.72-1.06$) (IV, table 1). By adjusting the alkyl-substituted alkoxide/TEOS ratio upward, a decreased ion exchange capacity, an increased hydrophobicity and porosity is gained (Kusakabe et al., 1999). The modifications in the silica gel structure cause also changes in the release mechanism of dexmedetomidine.

The effect of alkyl-substitution on the release profile of dexmedetomidine from microparticles was that the burst increased from DMDDES and METES substituted microparticles compared to 100% TEOS microparticles and the effect was more pronounced with DMDDES (IV, fig 5a). This may indicate that alkyl-substituted microparticles have a more open structure than 100% TEOS microparticles due to covalently bound alkyl groups that prevent formation of a dense structure during spray drying. They may also be too hydrophobic to keep dexmedetomidine HCl in the silica gel structure during spray drying.

6.1.2. Drug concentration

Dexmedetomidine is apparently in dissolved form in silica gel monoliths. Thus the release of the drug substance should be proportional to the drug concentration (Baker, 1987). However, the release of dexmedetomidine from monoliths was proportional to the dexmedetomidine concentration when the drug concentration was 0.5 or 1 wt-% in the sol corresponding to 3.9 or 7.7 wt-% in silica xerogel, whereas the release rate decreased from monoliths containing 15.4 wt-% of drug (III, fig 3). In an earlier study the release of toremifene from a silica gel monolith was proportional to the drug concentration between 1.9 and 5.5 wt-% in the sol corresponding to concentration between 11.4 wt-% and 34.4 wt-% in dry silica gel (Ahola et al., 2000). However, from alkyl-substituted silica gel monoliths dexmedetomidine released at higher rate from rods containing higher amount of dexmedetomidine. This is possibly due to modified ion exchange capacity and polarity of the silica xerogel meaning that the loading capacity of organomodified silica xerogel could be lower than that of unmodified silica xerogel

Drug release from microparticles was studied both with toremifene citrate and dexmedetomidine HCl. The release rate of dexmedetomidine was slower than that of toremifene when the drug concentration was below 11.6 wt-% (II, fig 2 - 4). This could be due to the difference in physicochemical properties of drugs. Unger and co-workers have earlier reported that the basic drugs act as external catalysts, increasing the release rate of the drug in the concentration and in a pKa dependent manner

(Unger et al., 1983). Toremifene citrate is more hydrophobic, a stronger base, less soluble and has probably less hydrogen bonding capacity than dexmedetomidine. In a forced spray drying process toremifene does not stay in dissolved form and precipitates more easily than dexmedetomidine on the air/sol interface of silica microparticles. This precipitation tendency may cause an increasing release trend for both drugs in the amount of drug released during the first hour from microparticles when the concentration of drug increases (II, fig 2 and 3). The release profile of toremifene deviated from the diffusion controlled release at drug concentration above 19 wt-% and the burst was increased to more than 20 % (II, fig 2). The drug loading capacity of microparticles was also shown to be lower than that of monoliths, where the release rate of toremifene was proportional to the drug concentration up to 34.4 wt-% (Ahola et al., 2000).

6.1.3. Degradation of the silica gel

According to the literature on the subject, the degradation of a silica matrix is linear with time and generally slower than the release rate of drugs (Ahola et al., 2000, Ahola et al., 2001). In studies described in this thesis the degradation of silica gel appeared to have a lag phase before degradation begun (III, fig 1, 2 and 4; IV, fig 3). For monoliths prepared by co-hydrolysis of TEOS with alkyl-substituted alkoxides, the lag time might be due to the hydrophobic structure of the silica gel.

The lifetime of the silica gel microparticles varied between about 6 days ($r = 6$, pH = 2.3) and a year and half ($r = 35$, pH = 2.3) as calculated from the in vitro dissolution results showed in table 2. Microparticles prepared at IEP have a dense structure and diluting the sol from water/TEOS ratio 6 to 35 leads to even denser particles. The degradation time of the monoliths prepared with different water/TEOS ratio, at different pH or in different size varies between 4 and 8 days. Larger silica gel monoliths degraded faster than smaller ones, giving approximately equal erosion times. The degradation rate of the monolithic matrix seemed to be dependent on the total amount of material, suggesting that the SA/V ratio (surface area to volume ratio of the device) defines the erosion rate (Tamada and Langer, 1993, Grizzi et al., 1995). Monoliths substituted with 25 mol-% of DMDES and showing the slowest degradation rate among alkyl-substituted silica xerogels degraded in about 4 months (table 2). The degradation rate of alkyl-substituted silica gels was dependent on the amount and type of the alkyl-substituted alkoxide (table 2). These results show that the degradation rate of the silica xerogel can be modified by varying the composition of starting materials and subsequently the structure of the silica gel matrix or by varying the manufacturing method from casting to spray drying.

The in vivo degradation time of a monolith ($r = 14$, pH = 2.3) weighing appr. 15 mg is about 2 months (I, fig 2). This means that the degradation time in vivo is longer than in vitro, which was about 8 days as calculated from the in vitro dissolution profile (I, fig 1). This difference between degradation rates is partially due to serum proteins that

bind onto the surface of silica gel preventing the dissolution of the material in vivo (Falaize et al., 1999).

6.2. IN VIVO STUDIES

6.2.1. Tissue effects

The plain silica xerogel implant (without drug substance) did not cause irritation of the surrounding tissue during 42-day treatment in mice (I, fig 4a). Effects at the implantation site were considered part of a normal wound healing process. The final stage of the healing was the development of a fibrous capsule around the implant at 14-d point. The end-stage healing response to biomaterials is generally fibrous encapsulation, which is typically considered a sign of biocompatibility (Anderson et al., 1981, Anderson, 1994).

Subcutaneously implanted untreated silica xerogel as well as bioactive glass are surrounded by of a thin to medium thick fibrous capsule containing only few inflammatory cells at the tissue implant interface after 4 weeks (Hench and Wilson, 1986, Radin et al., 1998, Gerritsen, 2000). The proliferation of cultured murine fibroblasts and activation of human polymorphonuclear leukocytes by sol-gel derived glasses has also been studied. Sol-gel glass neither caused the inhibition of fibroblast growth nor induced a significant inflammatory response by polymorphonuclear leukocytes (Wilson et al., 1981, Palumbo et al., 1997).

No silica related histological changes in liver, kidney, lymph nodes and uterus were detected during the test period even though the amount applied was high (1.5 g/kg body weight) (I). Lai and co-workers have measured the release of silica from glass implants and the distribution of dissolved silica to various organs in the body. They found that silica is excreted in the urine through kidneys or is actively phagocytised by the macrophages (Lai et al., 1998).

6.2.2. Toremifene release in mice

About 40 % of toremifene was released during the first seven days and resulted in the peak radioactivity at 7 to14 days and after that radioactivity in various organs was quite steady for the rest of the implantation time (42 days) (I, fig 3). Sustained release of toremifene was obtained for more than six weeks (I, fig 2). The results were similar to earlier studies where progesterone incorporated during sol-gel synthesis or toremifene citrate impregnated into heat-treated silica xerogel was administered subcutaneously in rats or mice (Sieminska and Zerda, 1996, Kortusuo et al., 1999). After an initial burst effect the release rate was nearly constant for more than 4 weeks.

The fibrous capsule around the implant may function as a permeability barrier and decrease the drug release from the implantation site to the systemic circulation

(Anderson, 1994). Toremifene related changes in the uterus were detected at all time points. However, the amount of toremifene in the implants was high, 350 mg/kg. It is possible that only part of the drug diffused through the fibrous capsule, which is, however, enough to cause characteristic changes in the uterus.

6.2.3. Bioavailability of dexmedetomidine in dogs

The rate and amount of bioavailability of dexmedetomidine was clearly reduced from silica gel microparticles or alkyl-substituted silica gel monoliths as compared to the reference dose (table 3). The relative bioavailability was slightly higher with microparticles containing 4.6 mg of dexmedetomidine (0.16) than with alkyl-substituted monoliths containing 4.15 mg of dexmedetomidine (0.12) (table 3). The C_{\max} value was appreciably higher with microparticle formulation than with alkyl-substituted silica gel monoliths containing appr. the same amount of dexmedetomidine HCl (table 3). However the duration of effect was similar. Alkyl-substituted silica gel is hydrophobic and thus diffusion of tissue fluid in to the matrix and the subsequent release of drug may be prevented. On the other hand, microparticles are widely spread in the subdermal tissue after the hypodermal injection and this probably facilitates drug release. The comparison of C_{\max} and AUC values shows that a two-fold increase in dexmedetomidine HCl concentration in alkyl-substituted silica xerogel formulations resulted in a 7-fold increase in C_{\max} and AUC values in dogs (table 3, IV, fig 6a). This supra-proportional increase might be due to faster release of dexmedetomidine from formulations with a greater amount of dexmedetomidine HCl (IV, fig 6b).

The reduced bioavailability of dexmedetomidine from silica gel formulations as compared to reference, may be due to the condensed non-porous structure of silica microparticles or the hydrophobicity of the alkyl-substituted matrix of monoliths. Dexmedetomidine that was absorbed into the body during the test period was possibly released from the surface of the silica xerogel formulation. Most of the drug, however, is incorporated inside the silica structure and is released as the matrix degrades. In order to keep the serum concentration on the desired level, the release rate should be faster, because the elimination half-life of dexmedetomidine is short, about 1h and therefore it eliminates faster than more drug substance is released from the formulation to the circulation (Li et al., 1988, Salonen, 1989).

6.3 SOL-GEL DERIVED SILICA MONOLITHS AND MICROPARTICLES AS A POTENTIAL DRUG DELIVERY MATRIX IN TISSUE ADMINISTRATION

The applicability of sol-gel derived silica gel was studied as an implantable or injectable matrix for controlled drug delivery. Various means to control the structure and the release rate of drugs *in vitro* and *in vivo* were studied. An ideal matrix used in tissue administration should possess the following characteristics. 1) It should have an accurate and precise release profile and 2) be biocompatible *in vivo* and have a predictable *in vivo* degradation rate. 3) It should be comfortable to the user with the relevant indication and 4) should be easily administered. The system should possess 5) an adequate reservoir capacity and 6) be safe, free from leaks and dose dumping and also adaptable for drug substances with different physicochemical properties. In addition, the production method should be capable, robust and preferably cost-effective.

Sol-gel technology is a gentle room temperature process. Different active agents including proteins and other macromolecules retain their biological activity in the sol-gel processed silica gel matrix (Nicoll et al., 1997, Ahola et al., 2001). Physicochemical properties, such as solubility, hydrophobicity and pKa of the releasing drug seem to affect the drug release behaviour *in vitro*. The solubility of drug into silica sol is a factor that has to be taken into account with each new drug candidate. If the solubility is low, the drug may crystallise during the gelation and drying steps causing an inhomogeneous distribution and non-constant release of drug. Release rates of the model drugs toremifene citrate and dexmedetomidine HCl were controlled by varying the sol-gel synthesis parameters or by the manufacturing method. The drug release was prolonged, constant and conformed to zero order release from certain spray-dried microparticles and alkyl-substituted silica gel monoliths. From 100% TEOS monoliths the release was quite rapid and occurred mainly by diffusion. The release rate of dexmedetomidine could be controlled over periods lasting from days to more than a year *in vitro*. Silica gel was shown to be biodegradable and did not cause adverse effects in the surrounding tissue or organs studied, although the amount of silica gel was quite significant. Silica xerogel, either cast or spray dried, did not cause dose dumping and was easily administered with a hypodermic needle into the desired area.

Nowadays silica based material is used in biomedical applications. Consequently silica xerogel, especially silica gel microparticles and alkyl-substituted silica gel monoliths seem to be promising material for a matrix in controlled drug delivery systems in tissue administration. However, there are open questions that could be solved in further studies *in vitro* as well as *in vivo*. These include the applicability of sol-gel techniques for drugs with different physicochemical properties, long-term stability studies and safety tests. The preparation of silica xerogel in laboratory scale was quite trouble-free but in industrial scale it is more demanding and especially the production of sterile silica gel products requires special equipment and facilities.

7. CONCLUSIONS

The following conclusions can be drawn on the basis of the results of the studies.

1. The preliminary implantation study showed that silica xerogel degraded in the body and did not cause adverse reactions in various organs or at the implantation site in mice.
2. Various injectable and implantable silica gel formulations for controlled drug delivery were developed. The release rate of model drugs, toremifene and dexmedetomidine and degradation of the silica gel matrix was controlled by synthesis parameters or by choice of manufacturing method. Release mechanism of dexmedetomidine was governed by diffusion from monoliths and obeyed zero order release in certain microparticle and alkyl-substituted formulations in vitro.
3. Sustained release was achieved with toremifene in mice and with dexmedetomidine in dogs. A sustained release silica gel formulation for toremifene citrate with an effect duration in excess of six weeks and a formulation for dexmedetomidine with an effect duration of 24 hours were developed. Toremifene and dexmedetomidine were released with simultaneous degradation of silica matrix. The degradation rate of silica gel, however, was slower than the release rate of drugs.

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