HYDROGELS WITH PENDANT CYCLODEXTRINS
FOR LOADING AND RELEASE
OF ANTIFUNGAL AGENTS

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First Master in Drug Development

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# TABLE OF CONTENTS

1  INTRODUCTION .................................................................................................................. 1

   1.1  ADVANCED DRUG THERAPY ......................................................................................... 1

   1.2  MICONAZOLE NITRATE ............................................................................................... 2

   1.3  HYDROGELS .................................................................................................................. 3
       1.3.1  Crosslink formation ............................................................................................... 5
       1.3.2  pHEMA hydrogels ................................................................................................. 7

   1.4  CYCLODEXTRINS ......................................................................................................... 8
       1.4.1  Cyclodextrins as drug carriers ............................................................................... 8
       1.4.2  Formation of inclusion complexes ....................................................................... 9
       1.4.3  Recent advances in formulating miconazole with CDs ...................................... 12

   1.5  HYDROGELS AND CYCLODEXTRINS ....................................................................... 14

2  OBJECTIVES ........................................................................................................................ 17

3  MATERIALS AND METHODS ............................................................................................. 18

   3.1  MATERIALS .................................................................................................................... 18

   3.2  HYDROGEL SYNTHESIS ............................................................................................... 18

   3.3  CYCLODEXTRIN IMMOBILIZATION ............................................................................. 19

   3.4  HYDROGEL CHARACTERIZATION ............................................................................. 20
       3.4.1  Degree of swelling ................................................................................................. 20

   3.5  LOADING WITH MICONAZOLE .................................................................................... 20

   3.6  RELEASE STUDY ........................................................................................................... 21

   3.7  UV/VIS SPECTROPHOTOMETRY .................................................................................. 21
       3.7.1  Calibration curve and validation ........................................................................... 23
RESULTS ........................................................................................................................................... 25

4.1 DEGREE OF SWELLING ........................................................................................................... 25
4.2 DRUG LOADING AND RELEASE ............................................................................................ 27

DISCUSSION ..................................................................................................................................... 31

5.1 DEGREE OF SWELLING ........................................................................................................... 31
5.2 DRUG LOADING AND RELEASE ............................................................................................ 32

CONCLUSIONS ................................................................................................................................. 35

REFERENCES .................................................................................................................................... 36
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>Cyclodextrin</td>
</tr>
<tr>
<td>CDs</td>
<td>Cyclodextrins</td>
</tr>
<tr>
<td>EGDMA</td>
<td>Ethylene Glycol Dimethacrylate</td>
</tr>
<tr>
<td>HEMA</td>
<td>Hydroxyethyl Methacrylate</td>
</tr>
<tr>
<td>pHEMA</td>
<td>Poly(Hydroxyethyl Methacrylate)</td>
</tr>
<tr>
<td>UV/VIS</td>
<td>Ultraviolet/Visible</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
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</table>
1 INTRODUCTION

1.1 ADVANCED DRUG THERAPY

Extensive research has been performed over the last years and numerous approaches have been suggested to improve conventional drug delivery systems (Peppas et al., 2000). Trying to overcome the problems of systemic drug administration is one approach to achieve more efficient therapy. Systemic administration has the drawbacks of the even distribution of the drug in the whole body, the lack of drug specific affinity towards a pathological site, the need for a large total dose to achieve high local concentration, non-specific toxicity and other adverse side-effects due to high drug doses used. By targeting the drug and subsequently accumulating the drug in the target zone, independently of the method or route of administration, these problems might be resolved (Torchilin, 2000).

The current options for targeting drugs are: “direct application of a drug into the affected zone, passive drug targeting (spontaneous drug accumulation in the areas with leaky vasculature, or Enhanced Permeability and Retention-EPR-effect), ‘physical’ targeting (based on abnormal pH value and/or temperature in the pathological zone), magnetic targeting (or targeting of a drug immobilized on paramagnetic materials under the action of an external magnetic field), and targeting using a specific ‘vector’ molecules (ligands having an increased affinity toward the area of interest)”. Pharmaceutical carriers such as soluble polymers, microcapsules, microparticles, cells, liposomes and micelles have already been applied as targeted drug delivery devices in vivo (Torchilin, 2000). In addition, hydrogels are assumed to be excellent applicants for controlled release devices, bioadhesive devices or targetable devices of therapeutic agents (Peppas et al., 2000).
1.2 MICONAZOLE NITRATE

Miconazole (C₁₈H₁₄Cl₄N₂O) is an imidazole derivate with a broad-spectrum antifungal activity, used as miconazole base or miconazole nitrate (C₁₈H₁₄Cl₄N₂O·HNO₃). Miconazole nitrate is only slightly soluble in water (1 in 6250 of water or 0.17 ± 0.0002 mg/mL at 25 °C), 1 in 312 of alcohol and 1 in 75 of methanol. The ultraviolet absorption spectrum of miconazole nitrate in methanol shows three maxima at 264, 272 and 280 nm (Al-Badr, 2005; Tenjarla et al., 1998). Miconazole is a weak base with a pKa of 6.7 and its solubility depends on the pH of the medium: the lower the pH (nitrogen becomes protonated), the more miconazole is dissolved (Piel et al., 1998; Kovács et al., 2009).

Its approved indications include superficial candidiasis, dermatophytosis, pytiriasis versicolor and disseminated fungal infections (Al-Badr, 2005). Miconazole can be used as an alternative systemic antifungal agent, in case of either ineffectiveness or contraindication of amphotericin B or ketoconazole (Saag & Dismukes, 1988). Sawyer et al. (1975) found that miconazole can be effective against Candida spp., Trichophyton spp., Epidermophyton spp., Microsporum spp. and Pityrosporon orbiculare (Malassessia furfur); they also observed some activity against Gram-positive bacteria. Though Candida spp. are generally known as benign commensal organisms in the digestive tract of healthy individuals, they can cause a broad range of serious illnesses in compromised hosts (Tenjarla et al., 1998) and should be treated appropriately. Miconazole has a dual mechanism of action: inhibition of ergosterol biosynthesis by the inhibition of 14-alpha-demethylase and direct membrane damage to the fungal cell. By inhibiting the biosynthesis of ergosterol, membrane integrity and fluidity of the fungal cell are lost (Piel et al., 2001b).

Even though there are various antifungal drugs existing, absolute efficacy is hardly reached. This low efficacy in the treatment of systemic mycoses is caused by limited water solubility and poor absorption in the gastrointestinal tract. Parenteral solutions of antifungal agents have been formulated, trying to overcome the slow dissolution and unpredictable bioavailability. These parenteral formulations include various surfactants and Cremophor type vehicles for colloidal stabilization and enhancing solubility. Unfortunately, these adjuvants cause adverse effects such as nausea, vomiting, nephrotoxicity and hepatotoxicity,
limiting their use (Tenjarla et al., 1998). As the overall incidence of systemic fungal infections is increasing and the parenteral formulations have significant limitations (Piel et al., 2001b), there is a strong need for new treatment strategies.

Increasing the water solubility of miconazole is expected to lead to better oral, parenteral or topical formulations, improving the efficacy of antifungal treatment (Tenjarla et al., 1998). To be pharmacological active in the body, a drug should be quite soluble, as well as possess a somewhat lipophilic character to permeate membranes (Arun et al., 2008). For many years, great interest went to the use of cyclodextrins for complexation of miconazole, as cyclodextrins are well known for improving the solubility of lipophilic drugs (Tenjarla et al., 1998). Kovács et al. (2009) tried to enhance the solubility of miconazole by using solubilisers and proved that pH adjusters, co-solvents and surfactants are able to enhance the solubility of miconazole.

Our objective is to improve solubilisation of miconazole by combining hydrogels and cyclodextrins. Hydrogels, as well as cyclodextrins, have the advantage of enhancing solubilisation. In addition, hydrogels are used to ensure drug release during a certain period of time, resulting in sustained release.

1.3 HYDROGELS

Synthetic covalently crosslinked hydrogels were prepared for the first time in 1960 by Wichterle and Lim (Wichterle & Lim, 1960) and since then, many research has been launched to further investigate hydrogels. The terminology ‘hydrogels’ is used when water is the medium for the gel. Hydrogels consist of hydrophilic polymers and form distinct three-dimensional networks, which are capable of absorbing large amounts of water or biological fluids without dissolving. The networks can be composed of homopolymers (i.e., the components are only a single type of repeating unit or monomer), or of copolymers, which are derived from two or more monomers. The characteristics of a gel predominantly rely on the structure of the polymer network and the interaction between the network and solvent.
The crosslinked structure may limit the relative mobility of the polymeric chains in the network (Osada, 2001; Yamauchi, 2001). Thanks to their high water content and softness they are comparable to biological tissue and, thus, biocompatible (Peppas et al., 2000). Biocompatibility means that the material “will not disturb homeostasis, will not cause unwanted rejection reaction, nor cause anomalous growth, absorption or death of organ systems” (Yamauchi, 2001). Being soft and rubbery minimizes irritation to surrounding tissue. Also, because of the low interfacial free energy of their hydrophilic surface, they show a low affinity to proteins and cells, preventing them from sticking together (Hennink & van Nostrum, 2002). Regarding cytotoxicity, hydrogels seem quite safe, although most toxicity is related to unreacted monomers, oligomers and initiators. Thus, it is important to ensure these toxic compounds are washed out very carefully (Peppas et al., 2000).

Hydrogels are versatile and thus can be used in many applications, such as tissue engineering (repairing and regenerating tissues as well as organs), synthetic extracellular matrix, implantable devices, materials controlling the activity of enzymes, phospholipid bilayer destabilizing agents, materials controlling reversible cell attachment, smart microfluidics with responsive hydrogels, and many more (Kopeček, 2007). It has been demonstrated that hydrogels can also be used as contact lenses, drug delivery devices, membranes for biosensors, linings for artificial hearts and material for artificial skin (Peppas et al., 2000).

Hydrogels are able to load and release drugs of various sizes, which makes them useful as drug delivery systems (Satish et al., 2006). Most drugs are released from hydrogels by diffusion, which obviously depends on the mesh size, or the space available in between the macromolecular chains. The mechanisms for drug release can be divided in three groups: diffusion-controlled, swelling-controlled or chemically-controlled. Hydrogels can extend the residence time at the site of delivery and thus improve drug bioavailability. Furthermore, desirable release rates and dissolution profiles can be attained by hydrogels, which may result in a precise release of drugs for a restricted period of time (Peppas et al., 2000; Lin & Metters, 2006). Kim et al. (1992) discovered that the desired kinetics, duration and rate of solute release from hydrogels are all restricted by specific conditions, such as hydrogel properties, amount of incorporated drug, drug solubility, and drug-polymer interactions. Satish et al. (2006) revealed that polymer composition, water content, crosslinking density
and crystallinity are factors determining the release rate and release mechanism from hydrogels.

1.3.1 Crosslink formation

As stated before, hydrogels can form networks because of the occurrence of crosslinks. As a consequence of these crosslinks, hydrogels are insoluble. By adapting the degree of crosslinking, the mechanical properties of the hydrogel can be adjusted. The higher the degree of crosslinking, the stronger and tighter the gel is, but also a more fragile structure is created. The challenge is to achieve the optimal degree of crosslinking, resulting in a strong, but still elastic hydrogel (Peppas et al., 2000). Crosslinked hydrogels can be achieved through either physical interactions or chemical (mainly covalent) bonds (Hennink & van Nostrum, 2002).

Chemical crosslinking involves a crosslinking agent, making the monomers polymerize and forming a strong crosslinked structure (Figure 1.1). Crosslinking by a covalent bond requires the energy input of heat, chemical catalysts, light, radiation, plasma or electric fields. Chemical gels can be obtained by polymerization reactions and crosslinking can occur during or after polymerization. Condensation polymerization and free radical polymerization are two methods where crosslinking takes place during polymerization. Condensation polymerization requires multifunctional monomers, building the network little by little. Free radical polymerization is another technique and involves either thermal polymerization, radiation polymerization, photo polymerization or plasma polymerization. Free radical polymerization involves a vinyl monomer polymerizing with a divinyl compound, acting as crosslinking agent (Okuzaki, 2001; Osada, 2001; Yamauchi, 2001). Low molecular weight monomers are polymerized, promoted by appropriate crosslinking agents, such as ethylene glycol dimethacrylate (EGDMA). Poly(2-hydroxyethyl methacrylate) (pHEMA) is produced according to this technique, by polymerization of hydroxyethyl methacrylate (HEMA) in the presence of EGDMA (Hennink & van Nostrum, 2002). Thermal polymerization requires that monomer, crosslinking agent and initiator are present in a good solvent of the polymer. The most common crosslinking agents include EGDMA and methylene bisacrylamide. Also free
radical initiators are necessary, such as 2,20-azo-bis(isobutyronitrile), which start the polymerization at low temperature. They generate radicals at their optimum temperature, which should match the gel synthesis conditions. Solvent, monomer concentration and polymerization temperature must be chosen carefully, preventing a turbid solution or precipitation, in order to attain a homogenous gel. The following techniques are used to obtain crosslinks after polymerization: radiation crosslinking, photo crosslinking, plasma crosslinking and a method which uses certain functional groups as crosslinker points (Okuzaki, 2001; Osada, 2001). Hennink & van Nostrum (2002) also reported the ability of crosslinking polymers by enzymes. By modifying the mesh size of the network, a precise control of drug release can be attained. These chemical gels are greatly resistant against dilution (Hoare & Kohane, 2008).

![Figure 1.1: Formation of Hydrogel Networks Through Chemical Crosslinking (Jeong et al., 2006).](image)

Physical crosslinking does not require crosslinking agents (Hennink & van Nostrum, 2002). The polymer chains are bonded to each other by non-covalent intermolecular junctions, such as coulombic, dipole-dipole, van der Waals, hydrophobic and hydrogen bonding interactions (Figure 1.2), involving more extended junction areas, instead of occurring at a certain point in the chain like covalent bonds (Kopeček & Yang, 2007). Generally, these secondary forces are readily created, but result in a low stability due to gel-sol transitions caused by changing temperature, ionic strength, pH or by simple addition of more water to the system. Accordingly, physical gels are called reversible gels (Yamauchi, 2001). Drug release from these physical gels is mainly directed by the hindrance effect of viscosity on drug diffusion (Alvarez-Lorenzo et al., 1999).
In summary, hydrogels are very attractive and promising as controlled-release drug devices because of their great tissue compatibility and uncomplicated management of swelling level and of solute permeability (Satish et al., 2006).

1.3.2 pHEMA hydrogels

pHEMA gels are very stable, both chemically and thermally, as they can resist high temperatures, hydrolysis (acid or alkaline) and also have low reactivity (Ferreira et al., 2000). Due to their high water content, they assist diffusion of solutes and oxygen permeability (Alvarez-Lorenzo et al., 2002). Different methods for modifying pHEMA hydrogels have been proposed to improve their poor loading and controlled release qualities. One of the proposed alternatives includes copolymerization with cyclodextrins, enabling in formation of inclusion complexes with drugs. These hydrogels combine physical strength with flexibility, ideal qualities for soft contact lenses, implants and drug delivery platforms (dos Santos et al., 2008).

Miconazole has been incorporated into different kind of hydrogels, e.g. to accomplish swelling controlled release, or local drug delivery by mucoadhesive polymers (Gad et al., 2008; Mandal, 2000).
1.4 CYCLODEXTRINS

Cyclodextrins (CDs) are cyclic oligosaccharides composed of (α-1,4)-linked α-D-glucopyranose monomers. Depending on the number of glucose units (6, 7, 8 or 9), they are named α-, β-, γ-, or δ-CDs, respectively (Figure 1.3). The central cavity is lipophilic and can act as a carrier or ‘molecular cage’, while the outer surface is hydrophilic, carrying primary and secondary hydroxyl groups pointing outward (Arun et al., 2008). Modifications were applied to β-CD to prevent the easy crystallization due to its rigid structure, to augment solubilisation and simultaneously reduce toxicity (Ribeiro et al., 2008).

![Figure 1.3: Structure of β-Cyclodextrin (Arun et al., 2008).](image)

1.4.1 Cyclodextrins as drug carriers

Generally, CDs are incapable of permeating through lipophilic membranes due to their large size and numerous hydrogen donors and acceptors on their surface (Arun et al., 2008). Therefore, only free drug is able to permeate membranes. As the complexed molecules (with CDs) are in equilibrium with the uncomplexed in the aqueous solution (Piel et al., 2001a), the equilibrium is progressively shifted towards decomplexation as the free drug is absorbed. On the other hand, the CDs do increase the amount of drug at the membrane surface, since the drug-CD complexes easily pass aqueous barriers. Figure 1.4 illustrates the process of drug dissolution and absorption facilitated by CDs. CDs can also act as permeation enhancers, as they are able of extracting lipophilic components from the membranes (Frijlink et al., 1990).
1.4.2 Formation of inclusion complexes

CDs are very interesting as components of drug delivery systems as they are able to form inclusion complexes with drugs through non-covalent interactions: the entire drug or lipophilic entities can enter into the cavity. This complexation can enhance solubility, dissolution rate and bioavailability of poorly water-soluble drugs (Arun et al., 2008). Furthermore, other characteristics of molecules can be considerably modified when encapsulated, such as masked or reduced unwanted flavour and improved shelf-life. Through this complexation, sensitive substrates are also protected from oxidation, light-induced decomposition or heat-induced conversions (Wang & Cai, 2008).
Liu & Guo (2002) investigated and proposed the following factors as driving forces for the complex formation: electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding and charge–transfer interaction. Contrary to the findings of Huang et al. (1997) and many others, they stated that the release of conformational strain and the exclusion of cavity-bound high-energy water, do not contribute to the complex formation.

Loftsson et al. (1999) found the complexation efficiency of CDs to be rather low, hence requiring large amounts of CDs to complex a small amount of drug. They also observed an even further decrease in efficiency caused by various pharmaceutical additives. Their aim was to enhance the complexation efficiency by adding polymers (as they increase the stability constant of the drug-CD complex) or by manipulating the solubility of water-insoluble drugs, preparing a more water-soluble drug derivative or a prodrug with higher intrinsic solubility. Hence, less CD can be used, which guarantees an improved effect on toxicology, formulation bulk and production cost.

Higuchi and Connors described a method to investigate inclusion complexation and assessed the solubilisation capability of CDs in a quantitative manner. The phase solubility diagram examines the effect of a ligand or solubiliser (such as CDs) on a substrate, plotting the solubility of the substrate as a function of the concentration of CD (Figure 1.5). This diagram is classified into type A (the solubility of a substrate increases with increasing ligand concentration over the entire concentration range, indicating the formation of soluble inclusion complex) or type B (a plateau occurs in the curve, indicating the formation of a complex with definite solubility). Type A is subdivided into $A_L$ (drug solubility is proportional to CD concentration), $A_P$ (positive deviation) and $A_N$ (negative deviation). Type B is further classified into a $B_S$ (complex with limited solubility) and a $B_I$ curve (insoluble complex) (Arun et al., 2008; Challa et al., 2005; Tenjarla et al., 1998; Uekama et al., 1998). Tenjarla et al. (1998) discovered that complexes of CDs with miconazole nitrate occurred as an $A_L$ type, except those complexes containing $\alpha$- and $\gamma$-CD ($A_N$ type).
FIGURE 1.5: PHASE SOLUBILITY DIAGRAM BY HIGUCHI AND CONNORS, EXPLAINING THE DEPENDENCE OF SUBSTRATE SOLUBILITY ON LIGAND CONCENTRATION (Arun et al., 2008).

The stability constant and stoichiometry of the complexes can be deducted from the phase solubility diagram (Uekama et al., 1998). The association or stability constant \( K \) can be calculated using the following equation, assuming a 1:1 complex (Challa et al., 2005):

\[
K_{1:1} = \frac{\text{slope}}{S_0 (1 - \text{slope})}
\]

where: \( K_{1:1} \): stability or association constant (M\(^{-1}\))

slope: slope of the linear portion of the stability curve

\( S_0 \): intrinsic solubility of the drug under the conditions (M)

The complexation efficiency (CE) for 1:1 complexes is defined by the ratio of drug-CD complex to free CD concentration, and can be calculated by using the slope of the curve (Loftsson et al., 2007).

\[
CE = S_0 \times K_{1:1} = \frac{\text{slope}}{(1 - \text{slope})}
\]
1.4.3 Recent advances in formulating miconazole with CDs

Various different types of CDs have been investigated, trying to improve the solubility of miconazole. Piel et al. (2001b) found no significant difference in the minimum inhibiting concentration between an intravenous solution of miconazole (which was withdrawn from the Belgian market due to the assumed toxicity of polyoxyl 35 castor oil) and a formulation containing miconazole in combination with hydroxypropyl-β-CD and lactic acid. Piel et al. (2001a) compared β-CD and γ-CD, and found β-CD being a good vehicle to solubilise miconazole in aqueous medium. They proposed a highly probable and favourable inclusion complex of miconazole-β-CD incorporating the dichlorobenzene–CH–O– group of the enantiomeric S-form of miconazole (Figure 1.6). Wang & Cai (2008) confirmed the inclusion complex formation between β-CD and miconazole nitrate using IR spectrophotometry, differential scanning calorimetry (DSC) and X-ray diffraction. They found that the benzene ring entered the cavity and that the reaction was spontaneous, gaining the highest yields when prepared at lower pH and lower temperature.


Piel et al. (1998) noticed a synergistic effect between acids and CDs when an acid ternary compound was used; this combination again showed enhanced solubility. Redenti et al. (2000) enhanced solubility by adding a low molecular weight organic acid, which stabilizes the drug-CD complex and, at the same time, increases the solubility by salt formation of the
basic drug. Barillaro et al. (2004) detected improved inclusion turnover when combining hydroxypropylated CD with an acid. This effect was much weaker with β-CD and γ-CD.

Ribeiro et al. (2008) also studied various methods for the preparation of inclusion complexes and found solid miconazole-methyl-β-CD inclusion complexes formed by coevaporation, spray-drying and lyophilisation; the latter being the most effective, economic and easy method and delivering the highest yield. Table 1.1 summarizes the capability of some CDs to form inclusion complexes with miconazole, under different experimental conditions.

### TABLE 1.1: CAPABILITY OF CDs TO FORM INCLUSION COMPLEXES WITH MICONAZOLE.

<table>
<thead>
<tr>
<th>CD type</th>
<th>Conditions experiment</th>
<th>Affinity constant $K_{1:1}$ $(M^{-1})$</th>
<th>Complexation Efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CD</td>
<td>25 ± 1°C</td>
<td>333 ± 18.5</td>
<td></td>
<td>Tenjarla et al., 1998</td>
</tr>
<tr>
<td></td>
<td>0.05 M phosphate buffer pH 7.1, at 23°C</td>
<td>&gt;2.23 x 10⁶</td>
<td></td>
<td>Jacobsen et al., 1999</td>
</tr>
<tr>
<td>β-CD</td>
<td>25 ± 1°C</td>
<td>293 ± 17.6</td>
<td></td>
<td>Tenjarla et al., 1998</td>
</tr>
<tr>
<td></td>
<td>0.05 M phosphate buffer pH 7.1, at 23°C</td>
<td>&gt;2.20 x 10⁵</td>
<td></td>
<td>Jacobsen et al., 1999</td>
</tr>
<tr>
<td></td>
<td>pH 7.0, 25°C</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 8.0, 25°C</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 9.0, 25°C</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20°C, pH 6</td>
<td>117</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25°C, pH 6</td>
<td>96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30°C, pH 6</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37°C, pH 6</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45°C, pH 6</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>methyl-β-CD</td>
<td>25 ± 2°C</td>
<td>145.69 ± 4.1 $(K_{1:1})$</td>
<td>11.11 ± 0.5 $(K_{1:2})$</td>
<td>Ribeiro et al., 2008</td>
</tr>
</tbody>
</table>
TABLE 1.1: CAPABILITY OF CDs TO FORM INCLUSION COMPLEXES WITH MICONAZOLE (CONTINUED).

<table>
<thead>
<tr>
<th>CD type</th>
<th>Conditions experiment</th>
<th>Affinity constant K1:1 (M⁻¹)</th>
<th>Complexation Efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-hydroxy-propyl-β-CD</td>
<td>25 ± 2 °C</td>
<td>126.94 ± 4.4 (K-1:1)</td>
<td></td>
<td>Ribeiro et al., 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.20 ± 0.4 (K-1:2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ambient temperature</td>
<td>260 (using S₀)</td>
<td>0.055</td>
<td>Loftsson et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55 (using Sₘ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 ± 1 °C</td>
<td>363 ± 34.1</td>
<td></td>
<td>Tenjarla et al., 1998</td>
</tr>
<tr>
<td>Hydroxyl-ethyl-β-CD</td>
<td>25 ± 1 °C</td>
<td>312 ± 31.0</td>
<td></td>
<td>Tenjarla et al., 1998</td>
</tr>
<tr>
<td>γ-CD</td>
<td>25 ± 1 °C</td>
<td>695 ± 39.6</td>
<td></td>
<td>Tenjarla et al., 1998</td>
</tr>
<tr>
<td></td>
<td>0.05 M phosphate</td>
<td>&gt;4.30 x 10⁴</td>
<td></td>
<td>Jacobsen et al., 1999</td>
</tr>
<tr>
<td></td>
<td>buffer pH 7.1, at 23°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyl-propyl-γ-CD</td>
<td>25 ± 1 °C</td>
<td>305 ± 27.6</td>
<td></td>
<td>Tenjarla et al., 1998</td>
</tr>
</tbody>
</table>

Thus, CDs are able to enhance solubility, stability, safety and bioavailability (Arun et al., 2008), all outstanding characteristics that could improve the efficacy of the therapy with miconazole. Nevertheless, the release of drugs from CD solutions is generally instantaneous after dilution of the complexes in aqueous fluids (Stella et al., 1999).

1.5 HYDROGELS AND CYCLODEXTRINS

Loading hydrogels with drugs has several limitations. For example, hydrogels should be extensively washed after synthesis to remove impurities and unreacted monomers. If the drug was integrated into the gel during synthesis, this washing step could imply rinsing the drug from the gel. Also, side reactions between drug and reactants should be restricted, as this could affect the biological activity, as well as the high temperatures needed during
synthesis (Kanjickal et al., 2005). Moreover, poorly water-soluble drugs have a low affinity for the hydrophilic hydrogels, while sustained release is hardly attained with hydrophilic drugs (Rodriguez-Tenreiro et al., 2006).

Bibby et al. (2000) described the mechanisms by which CDs can adjust drug release from physically crosslinked polymeric drug delivery systems and emphasized the profit of integrating CDs in gels concerning drug entrapment and release control.

Not only solubility of the drug can be altered by incorporation of CDs into hydrogels, also diffusivity can be changed, enhanced hydration of the polymer matrix can occur or erosion of the polymer can be encouraged. Hydrophobic drugs are sheltered in the cavities of the CDs within the aqueous environment of the hydrogel network. But this combination implicates some shortcomings as well: stable complex formation between drug and CD might be constrained in case of a physical mixture between CDs and hydrogel polymers. When more CD is incorporated, restricted hydrogel swelling and release rate of drug is observed, caused by increased crosslinking and a subsequent decrease in mesh size of the network (Bibby et al., 2000). Kanjickal et al. (2005) were able to load PEG hydrogels with the poorly water soluble drug cyclosporine by forming inclusion complexes with CDs and proved that CDs can adjust the drug release from polymers.

Networks made of chemically crosslinked hydrogels and covalently bonded CDs are able to reduce dilution, as CDs are linked by covalent bonds, and water entrance into the network is restricted by the chemically crosslinked polymer chains. Therefore, the gel can still swell, but chains will not dissolve or spread out. Consequently, drug delivery will be mainly controlled by the drug-CD affinity (Rodriguez-Tenreiro et al., 2006). As earlier released drug molecules can be attracted again into the network, drug will not be released immediately; again a favourable contributing factor to sustained release (Gazpio et al., 2008; Layre et al., 2002).

Attaching CDs to hydrogels can be accomplished using different methods: direct crosslinking of CDs, copolymerization of CDs with vinyl- or acrylic-comonomers, or by connecting the CDs to a previously prepared network. Direct crosslinking is established by a
condensation reaction between a crosslinker (e.g. epichlorhydrine, EPI) and CDs, resulting in an EPI-CD hydrogel (Kobayashi et al., 1989). Rodriguez-Tenreiro et al. (2007) attached hydroxypropyl-β-CD to ethyleneglycol diglycidylether in carbopol dispersions, revealing good affinity for hydrophobic drugs. The second method involves a modified CD (containing a monomer), able to copolymerize with the monomers building up the hydrogel (Harada et al., 1976). This technique was used by dos Santos et al. (2008), copolymerizing pHEMA hydrogels with methacrylated-β-CD and loading them with acetazolamide or hydrocortisone.

For the present investigation the latter method was used, copolymerizing poly(HEMA) hydrogels with GMA and subsequently grafting CDs to the hydrogel throughout the glycidyl groups. Consequently, the CDs are hanging on two or three ether bonds through their hydroxyl groups, not being part of the crosslinked network (Figure 1.7). These hydrogels with pendant CDs were designed and patented by dos Santos et al. (2009), who discovered enhanced diclofenac loading, drug affinity and sustained delivery.

FIGURE 1.7: HYDROGEL NETWORK BUILT UP OF HEMA AND GMA MONOMERS, CONTAINING PENDANT CDs ATTACHED TO THE GLYCIDYL GROUPS OF GMA (dos Santos et al., 2009).
2 OBJECTIVES

The aim of this work was to design and characterize hydrogels able to uptake the antifungal drug miconazole nitrate and to release it at an appropriate rate for being useful preventing fungal infections. To carry out the work, pHEMA hydrogels were prepared with several GMA proportions and, after synthesis, various CDs were attached to the preformed networks.

We examined drug loading and release of miconazole nitrate from the hydrogels with pendant CDs measuring the swelling degree and performing a release study, using the spectrophotometer.

Adjusted loading and release of miconazole from hydrogel networks containing CDs was extensively explored.
3 MATERIALS AND METHODS

3.1 MATERIALS

Ophthalmic grade 2-hydroxyethyl methacrylate (HEMA) (Figure 3.1) and dimethylformamide (DMF) were provided by Merck (Germany). Ethylene glycol dimethacrylate (EGDMA) (Figure 3.1), glycidyl methacrylate (GMA) (Figure 3.1) and 2,2-azobis(isobutyronitrile) (AIBN) were supplied by Sigma-Aldrich (Spain). β-cyclodextrin (β-CD) was provided by Roquette-Laisa (Spain), α- and γ-cyclodextrin (α-CD and γ-CD) by Wacker (Germany). Miconazole nitrate was distributed by Fagron (Spain), Lot No. 0708072. Ultrapure water (resistivity > 18.2 MΩ cm) was obtained by reverse osmosis (MilliQ®, Millipore Spain). All other reagents were of analytical grade.

![Figure 3.1: Chemical structure of HEMA (left) and GMA (middle), monomers used for the synthesis of hydrogels, and EGDMA (right), crosslinking agent (Satish et al., 2006; http://www.sigmaaldrich.com/)](image)

3.2 HYDROGEL SYNTHESIS

We synthesized poly(2-hydroxyethyl methacrylate) hydrogels (pHEMA hydrogels) from the monomer HEMA and the comonomer GMA, with EGDMA as crosslinking agent. First, we dissolved 0.0714mL EGDMA (8mM) and increasing amounts of GMA (0mM, 100mM or 0.164mL, 200mM or 0.328mL, 300mM or 0.490mL and 400mM or 0.654mL) in HEMA (6mL). Later, GMA will be used to couple CDs (through their hydroxyl groups) onto the hydrogel, as GMA contains epoxide groups. Then, 0.074g of AIBN (10mM), the initiator, was added and stirred for several minutes in order to make the mixture homogenous. We used a syringe to
inject the solution carefully into a mold, which consisted of two glass plates pressed together. In between the glass plates we placed a polypropylene sheet and a 0.9mm wide silicone frame. Polypropylene is used to make sure the gel does not stick to the glass. Next, the molds were placed in an oven at 50°C and after 12 hours they were heated for 24 hours at 70°C. Once polymerization occurred, unreacted monomers were removed by boiling each gel sheet in distilled water for 15 minutes. This facilitated the cutting of disks out of the sheet. Disks with a diameter of 10mm were made and immersed in water for 24 hours and another 24 hours in NaCl (0.9%). This ensured that all unreacted monomers are removed. Finally, they were kept in water (dos Santos et al., 2008). The gels which were not for immediate use were washed twice a week to prevent growth of microorganisms.

3.3 CYCLODEXTRIN IMMOBILIZATION

Three types of CDs (α, β and γ) were attached to each hydrogel. First, we weighed NaOH (39g) and NaCl (37.96g). Next, we added 650mL of water and 650mL of DMF. This solution was profoundly mixed until no particles could be detected. In different beakers we placed α-CD (7.56g), β-CD (8.8g) or γ-CD (10g), another beaker served as blank (no CDs added). We added 400mL of the solution to the different CDs and stirred them for a while. For 1g of disks (± 10 disks) we used 100mL of the solution. In each bottle we carefully chose 5 disks and added 50mL of the solution, a duplicate was made. Next, the bottles were placed for 24 hours in a hot water bath at 80°C. Then, the gels were washed for several times to make sure DMF was washed out. Next, the gels were placed in the oven to dry. Finally, the dried gels were placed in ethanol 100% until the solution evaporated completely, to remove the remnants of DMF that might not have been removed yet (dos Santos et al., 2009). This was three times repeated.
3.4 HYDROGEL CHARACTERIZATION

3.4.1 Degree of swelling

The swelling ratio $Q$ of the hydrogels was gravimetrically determined as follows. First, the dry weight $W_0$ of a disk was measured (before swelling). Next, the disks were immersed in 10-15 mL of distilled water at 25°C, allowing them to swell. After soft removal of the excess water with a tissue, the weight of the swollen disk $W_t$ was determined with ten minutes interval. After measurement, the disk was placed in the medium again. After 40 minutes, the weight was measured every half an hour (at 60, 90, 120, 150, 180 minutes). The last measurements were performed after 24 hours. The measurements were carried out in triplicate for each attached CD (α, β and γ) and a blank. The swelling degree was measured by using the following equation:

$$Q_t = 100 \times \frac{W_t - W_0}{W_0}$$  \hspace{1cm} (3.1)

where:

- $Q_t$: swelling degree
- $W_t$: weight of disk at time $t$
- $W_0$: weight of dried disk

3.5 LOADING WITH MICONAZOLE

First, a saturated suspension of miconazole nitrate (Figure 3.2) in water was prepared (2-3g/500mL). Six disks were placed in a bottle and each bottle was filled with 60mL of the suspension. Next, the bottles were placed in the Raypa AES-12 autoclave (Raypa Espina, S. L., Barcelona, Spain) for 20 minutes at 121°C. Immediately after heating, the solution was removed and the disks were washed in distilled water to make sure free miconazole was removed from the surface, as this would lead to over-estimated results and instantaneous release. Next, the disks were dried again, to prevent from leaching miconazole.
MATERIALS AND METHODS

3.6 RELEASE STUDY

To check if the disks loaded miconazole, a release study was carried out as follows. Each disk was weighed and placed in a small flask and 5.00mL of sodium laurilsulphate (0.3% w/v) was added. The surfactant solution was used as blank and release medium. We prepared the 0.3% w/v solution of sodium laurilsulphate by weighing 6.0g sodium laurilsulphate and adding distilled water until the final volume was 2L. Samples of the medium were taken (± 1mL) at preestablished time intervals and the absorbance at 272 nm was measured using a UV spectrophotometer Agilent 8453 (Agilent Technologies Deutschland Gmbh, Waldbronn, Germany). The samples were returned to the flask immediately after measurement. When the absorbance increased above 0.7, we added more sodium laurilsulphate solution (1.00 or 5.00 mL). The experiment was performed in triplicate.

3.7 UV/VIS SPECTROPHOTOMETRY

In ultraviolet/visible (UV/VIS) spectrophotometry, light within the UV/VIS range is sent through a sample. Absorption of light by a compound can trigger a transition from an energetic ground state to a specific excited state. These excited states depend on the energy of light and chemical character of the compound. If a photon contains an energy identical to the difference in energy between the excited and non-excited state, an electron will move from a lower to a higher energy level causing absorption of energy. The accessible electronic, vibrational and rotational energy levels of the compound determine at which
energies absorption can occur. In the range of UV/VIS, electronic and vibrational excitations occur after absorption of light. The UV spectral region reaches from 190 to 400 nm, while the VIS region starts at 400 nm until 780 nm. The apparatus contains a light source, a monochromator, a compartment for the sample and a detector (Figure 3.3). The light source supplies illumination of the appropriate range and the monochromator selects the desired wavelength. The detector quantifies the amount of light transmitted by the sample, also known as the intensity. Spectrophotometry is a frequently used technique to measure the transitions in molecules, providing qualitative (characterisation) and quantitative information. It has been found an easy method for measuring concentrations in solution (Gauglitz & Vo-Dinh, 2003; Harris & Bashford, 1987).

\[ A = \log_{10} \left( \frac{I_0}{I_t} \right) \]  

where:  
- \( A \): absorption  
- \( I_0 \): incident intensity  
- \( I_t \): intensity of transmitted light

**FIGURE 3.3: ILLUSTRATION OF A UV/VIS SPECTROPHOTOMETER: INCIDENT LIGHT \( I_0 \) PASSES THROUGH A CUVE, WITH LENGTH \( \ell \) AND CONTAINING A SAMPLE WITH CONCENTRATION \( C \), AND FINALLY THE INTENSITY OF TRANSMITTED LIGHT \( I_t \) IS MEASURED BY THE DETECTOR.**
MATERIALS AND METHODS

The transmittance $T$ of a sample is given by the following formula:

$$T = \frac{l_i}{l_o}$$  \hspace{1cm} (3.3)

Quantifying the concentration in a solution has been facilitated by Lambert and Beer. The law of Lambert-Beer states that the intensity of light absorbed (absorbance, $A$) is related to the concentration $C$ of a component in the solution. There is a linear relationship between absorbance and concentration at sufficiently low concentrations of the measured substance \textit{(Harris & Bashford, 1987)}.

$$A = \varepsilon \cdot C \cdot \ell$$  \hspace{1cm} (3.4)

where: $\varepsilon$: molar absorptivity or extinction coefficient (L mol$^{-1}$ cm$^{-1}$)
$C$: concentration (mol L$^{-1}$)
$\ell$: length of light path through sample (usually 1 cm) (cm)

3.7.1 Calibration curve and validation

To determine the concentration of a compound in a solution with UV/VIS spectrophotometry, we need to plot a calibration curve first. We prepared the solutions with increasing concentrations varying from 10 up to 200 mg/L (15 different concentrations). In a beaker, an amount of distilled water was added to 200 mg of miconazole nitrate. This mixture was set in an ultrasound bath to make the particles smaller. Then, in a volumetric flask, distilled water was added to the mixture until 1L. Further dilutions were precisely made. The absorbance of each sample was determined at 272 nm with a Agilent 8453 spectrophotometer (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The experiments were conducted in triplicate. A calibration curve was plotted with concentration as x-coordinate and absorbance as y-coordinate, as demonstrated in Figure 3.4.
MATERIALS AND METHODS

The data were analyzed with SigmaPlot for Windows Version 11.0 and linear regression analysis was performed. The equation of the calibration curve was: $y = 0.0016x + 0.0047$. Linearity was observed within the range of 10 - 200 mg/L at 272 nm, which means that in this range, Lambert-Beer’s law is obeyed. The mean correlation coefficient was 0.9995, being greater than 0.999. All performed statistical tests (Durbin-Watson Statistic, Normality Test and Constant Variance Test) proved that the calibration curve was precise and accurate.

FIGURE 3.4: CALIBRATION CURVE OF MICONAZOLE NITRATE, PERFORMED IN TRIPlicate.
4 RESULTS

4.1 DEGREE OF SWELLING

After immersing the dried disks in water, all of them reached equilibrium within approximately 3 hours. In Figures 4.1, 4.2 and 4.3 the degree of swelling is plotted versus time. The error bars show the standard deviation of the experiment that was carried out in triplicate. All three graphs show a quite similar degree of swelling. The blank showed a significant lower swelling profile. The hydrogels with higher amount of GMA confirmed a somewhat lower degree of swelling. At equilibrium, all gels with pendant CDs showed a swelling degree more or less between 66% and 72%, the blank had a swelling degree in the order of 61%.

FIGURE 4.1: SWELLING PROFILE OF pHEMA HYDROGELS WITH PENDANT α-CD AND BLANK IN WATER. DIFFERENT AMOUNTS OF ADDED GMA (mM) WERE COMPARED: 400 ( ), 300 ( ), 200 ( ), 100 ( ) and 0 ( ).
FIGURE 4.2: SWELLING PROFILE OF pHEMA HYDROGELS WITH PENDANT β-CD AND BLANK IN WATER. DIFFERENT AMOUNTS OF ADDED GMA (mM) WERE COMPARED: 400 (●), 300 (▲), 200 (◆), 100 (■) and 0 (●).

FIGURE 4.3: SWELLING PROFILE OF pHEMA HYDROGELS WITH PENDANT γ-CD AND BLANK IN WATER. DIFFERENT AMOUNTS OF ADDED GMA (mM) WERE COMPARED: 400 (●), 300 (▲), 200 (◆), 100 (■) and 0 (●).
4.2 DRUG LOADING AND RELEASE

After loading the disks by autoclaving with miconazole nitrate, a release study was performed, measuring spectrophotometrically the amount of miconazole released in the medium. The amount of miconazole was calculated in mg per gram of dried hydrogel (mg/g). A graph was plotted showing the amount of miconazole released versus time. The results of the release study are presented in Table 4.1 and Figures 4.4, 4.5 and 4.6. The table shows the amount of miconazole released for blank and hydrogels with α-, β- and γ-CDs, with different amounts of GMA (400, 300, 200 or 100).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Blank (mg/g)</th>
<th>400 α (mg/g)</th>
<th>300 α (mg/g)</th>
<th>200 α (mg/g)</th>
<th>100 α (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>2.64 (0.51)</td>
<td>1.37 (0.24)</td>
<td>2.21 (0.08)</td>
<td>2.83 (0.15)</td>
<td>2.32 (0.19)</td>
</tr>
<tr>
<td>1</td>
<td>3.29 (0.71)</td>
<td>2.44 (0.49)</td>
<td>3.32 (0.73)</td>
<td>2.91 (0.16)</td>
<td>2.89 (0.32)</td>
</tr>
<tr>
<td>1.5</td>
<td>6.14 (0.39)</td>
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<td>4.18 (0.41)</td>
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<td>4.54 (0.29)</td>
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<tr>
<td>2</td>
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<td>7.27 (0.84)</td>
<td>4.98 (0.55)</td>
<td>5.08 (0.08)</td>
<td>5.00 (0.68)</td>
</tr>
<tr>
<td>3</td>
<td>7.63 (1.12)</td>
<td>6.77 (1.22)</td>
<td>6.20 (0.49)</td>
<td>6.21 (0.51)</td>
<td>5.90 (0.003)</td>
</tr>
<tr>
<td>4</td>
<td>8.53 (1.30)</td>
<td>7.77 (0.86)</td>
<td>6.71 (1.26)</td>
<td>4.54 (0.12)</td>
<td>6.58 (1.10)</td>
</tr>
<tr>
<td>5</td>
<td>9.45 (1.95)</td>
<td>9.14 (0.51)</td>
<td>6.80 (1.07)</td>
<td>5.31 (0.07)</td>
<td>6.49 (1.86)</td>
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<tr>
<td>6</td>
<td>11.82 (2.95)</td>
<td>10.50 (0.005)</td>
<td>7.58 (1.31)</td>
<td>5.31 (0.47)</td>
<td>7.31 (1.55)</td>
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<tr>
<td>8</td>
<td>11.62 (2.66)</td>
<td>12.53 (0.53)</td>
<td>8.69 (1.45)</td>
<td>6.49 (0.06)</td>
<td>7.89 (1.55)</td>
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<tr>
<td>24</td>
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<td>12.15 (2.49)</td>
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<td>14.23 (3.97)</td>
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<td>12.78 (0.38)</td>
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<td>72</td>
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<td>30.89 (2.09)</td>
<td>27.96 (0.77)</td>
<td>21.56 (1.49)</td>
</tr>
<tr>
<td>96</td>
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<td>70.99 (1.74)</td>
<td>63.34 (2.25)</td>
<td>58.53 (3.88)</td>
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<tr>
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<td>89.89 (12.68)</td>
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<tr>
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<td>94.75 (6.76)</td>
<td>88.56 (14.11)</td>
<td>83.49 (17.08)</td>
</tr>
</tbody>
</table>
RESULTS

TABLE 4.1: RESULTS RELEASE STUDY, SHOWING THE AMOUNT OF MICONAZOLE RELEASED WITH STANDARD DEVIATION IN TIME FOR BLANK, ALFA-, BETA- AND GAMMA-CD (CONTINUED).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Blank (mg/g)</th>
<th>400 β (mg/g)</th>
<th>300 β (mg/g)</th>
<th>200 β (mg/g)</th>
<th>100 β (mg/g)</th>
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</tr>
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<td>102.40 (11.76)</td>
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</table>

<table>
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<tr>
<th>Time (h)</th>
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<th>300 γ (mg/g)</th>
<th>200 γ (mg/g)</th>
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<td>12.12 (6.98)</td>
<td>6.10 (1.28)</td>
<td>9.77 (2.61)</td>
</tr>
<tr>
<td>3</td>
<td>7.63 (1.12)</td>
<td>8.77 (1.56)</td>
<td>13.82 (6.46)</td>
<td>7.14 (1.27)</td>
<td>11.99 (2.74)</td>
</tr>
<tr>
<td>4</td>
<td>8.53 (1.30)</td>
<td>9.28 (1.59)</td>
<td>16.75 (6.94)</td>
<td>7.03 (1.52)</td>
<td>12.46 (2.59)</td>
</tr>
<tr>
<td>5</td>
<td>9.45 (1.95)</td>
<td>10.02 (1.36)</td>
<td>17.96 (6.15)</td>
<td>8.06 (1.13)</td>
<td>13.69 (2.76)</td>
</tr>
<tr>
<td>6</td>
<td>11.82 (2.95)</td>
<td>12.05 (1.29)</td>
<td>19.29 (5.89)</td>
<td>9.06 (2.20)</td>
<td>16.22 (3.93)</td>
</tr>
<tr>
<td>8</td>
<td>11.62 (2.66)</td>
<td>11.92 (1.28)</td>
<td>21.53 (6.36)</td>
<td>9.62 (1.37)</td>
<td>16.64 (3.54)</td>
</tr>
<tr>
<td>24</td>
<td>15.59 (4.72)</td>
<td>16.43 (0.72)</td>
<td>28.94 (4.69)</td>
<td>12.47 (1.67)</td>
<td>23.46 (7.23)</td>
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<tr>
<td>48</td>
<td>17.67 (4.30)</td>
<td>22.53 (4.19)</td>
<td>31.93 (3.07)</td>
<td>18.86 (3.83)</td>
<td>30.62 (8.87)</td>
</tr>
<tr>
<td>72</td>
<td>34.27 (2.40)</td>
<td>40.85 (2.81)</td>
<td>42.51 (0.34)</td>
<td>31.34 (2.30)</td>
<td>58.54 (16.93)</td>
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<td>96</td>
<td>50.60 (7.05)</td>
<td>81.61 (5.23)</td>
<td>70.13 (0.42)</td>
<td>59.41 (8.93)</td>
<td>87.49 (14.51)</td>
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<td>168</td>
<td>73.99 (6.73)</td>
<td>133.63 (24.55)</td>
<td>138.61 (24.17)</td>
<td>85.48 (5.10)</td>
<td>135.67 (16.55)</td>
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<tr>
<td>192</td>
<td>82.27 (5.70)</td>
<td>143.09 (18.24)</td>
<td>140.06 (15.99)</td>
<td>96.65 (4.25)</td>
<td>145.90 (12.51)</td>
</tr>
<tr>
<td>216</td>
<td>85.07 (13.50)</td>
<td>160.14 (28.38)</td>
<td>141.43 (4.96)</td>
<td>102.04 (0.70)</td>
<td>169.93 (6.01)</td>
</tr>
<tr>
<td>336</td>
<td>84.78 (11.78)</td>
<td>150.76 (38.66)</td>
<td>122.52 (12.64)</td>
<td>98.78 (6.83)</td>
<td>169.36 (6.41)</td>
</tr>
</tbody>
</table>
As shown in Figures 4.4, 4.5 and 4.6 the CD-hydrogel network was able to sustain the delivery of miconazole nitrate during at least 14 days. Initially, the graph exhibits a quite fast increase in drug release, resulting in a sustained release starting around day 7 (168 h). The sustained release suggests interaction between miconazole and CD. We found that the gels with pendant γ-CDs (100, 300 and 400 GMA) released the highest amount of miconazole. After 168 hours, the release of γ-CD (300 GMA) drops. Also, β-CD (400 and 300 GMA) and α-CD (400 GMA) released quite a high amount of miconazole, compared to the other disks. Error bars are shown in the graphs, indicating the standard deviation of the experiment, conducted in triplicate.

FIGURE 4.4: RESULTS OF RELEASE STUDY, SHOWING THE AMOUNT OF MICONAZOLE RELEASED VERSUS TIME, FOR HYDROGELS WITHOUT CDs (BLANK) AND HYDROGELS WITH α-CD. DIFFERENT AMOUNTS OF GMA (mM): 400 ( ), 300 ( ), 200 ( ), 100 ( ) and 0 ( ).
FIGURE 4.5: RESULTS OF RELEASE STUDY, SHOWING THE AMOUNT OF MICONAZOLE RELEASED VERSUS TIME, FOR HYDROGELS WITHOUT CDs (BLANK) AND HYDROGELS WITH β-CD. DIFFERENT AMOUNTS OF GMA (mM): 400 ( ), 300 ( ), 200 ( ), 100 ( ) and 0 ( ).

FIGURE 4.6: RESULTS OF RELEASE STUDY, SHOWING THE AMOUNT OF MICONAZOLE RELEASED VERSUS TIME, FOR HYDROGELS WITHOUT CDs (BLANK) AND HYDROGELS WITH γ-CD. DIFFERENT AMOUNTS OF GMA (mM): 400 ( ), 300 ( ), 200 ( ), 100 ( ) and 0 ( ).
5 DISCUSSION

5.1 DEGREE OF SWELLING

Hydrogels can be characterized in many ways, for example by infrared analysis, by differential scanning calorimetry (DSC), by measurement of light transmission, by its degree of swelling or by viscoelasticity (dos Santos et al., 2008). We opted to study the swelling kinetics.

Hydrogels have the capability to swell in aqueous solutions. Instead of dissolving the network of polymers, the solvent is incorporated into the crosslinked structure, forming a swollen gel phase. The degree of swelling is limited and reaches an equilibrium, as seen after 3 hours. The degree of swelling depends on several factors: degree of crosslinking of the network, interaction between solvent and polymer, conditions of polymerization and presence of drug. A higher degree of crosslinking implies a lower degree of swelling. This can be explained by looking in more detail at the mechanism of swelling. The solvent entering the network will push the network junctions further away, causing a strain on the polymer chains attached to the junctions. This strain will be counterbalanced by the natural effect of the polymer, trying to return to its original state. Thus, a higher crosslinked gel will have smaller chains in between the junctions and will not extend as much as a less crosslinked gel. At a certain point, the equilibrium will be reached and no more solvent will enter the hydrogel. This equilibrium depends on the length of the chains between the network junctions and the affinity between solvent and network (Lin & Metters, 2006).

Drug loading can be estimated by studying the degree of swelling. Drug release is also influenced by swelling (Kim et al., 1992).
5.2 DRUG LOADING AND RELEASE

The amount of drug loaded on the CDs depends on two factors: the concentration of drug in solution and the affinity of the drug for this network. Heating may boost the complexation of drugs with CDs: as the probability of drug-CD interaction might be augmented by autoclaving, advanced distribution of the drug into the network could be achieved (dos Santos et al., 2008).

An important factor concerning drug diffusion is the mesh size of the network. The mesh size depends on the grade of crosslinking, the chemical structure of the monomers of the network and external stimuli (e.g. temperature, pH and ionic strength). The mesh size determines the physical properties of a gel such as its mechanical strength, its ability to degrade but also, the diffusivity of the incorporated drug (Lin & Metters, 2006).

When drugs are small enough to circulate through the pores of the network and form inclusion complexes with the CDs, loading may be achieved in an efficient way and their release may be sustained during several days. Drugs dissolving in the aqueous phase of the hydrogel or weakly interacting with the network are generally easily released (dos Santos et al., 2008).

The amount loaded by just a simple equilibrium between the aqueous phase of the network and the loading solution, which leads the drug concentration within the hydrogel to be equal to that of the loading solution, can be estimated using the following equation proposed by Kim et al. (1992):

\[
\text{Loading (aqueous phase)} = \left(\frac{V_s}{W_p}\right) \times C_0
\]  

(5.1)

where:  
- \(V_s\): volume of water sorbed by hydrogel (mL)  
- \(W_p\): dried hydrogel weight (g)  
- \(C_0\): concentration of drug in the loading solution (mg/mL)
DISCUSSION

Since the loading was carried out inside a miconazole suspension, $C_0$ is the solubility of miconazole in water, i.e., 0.17 mg/mL (Tenjarla et al., 1998). Table 5.1 summarizes the amounts of miconazole loaded by each hydrogel in its aqueous phase. The values are remarkably smaller than the total amount loaded, as estimated from the release tests. This means that the drug could establish hydrophobic interactions with the network and form inclusion complexes with CD cavities through its aromatic rings. To gain an insight into the role of the interaction of miconazole with the CD units, the partition coefficient, $K$, between the polymer network and the drug loading solution was estimated from the following expression (Kim et al., 1992):

\[
\text{Loading (total)} = C_0 \times \frac{V_s + KV_p}{W_p} 
\]

(5.2)

where:  
$K$: partition coefficient  
$V_p$: volume of dried polymer (mL)  
and the other symbols maintain the meaning of equation (5.1).

A density of 1g/mL was assumed for $V_p$.

The high $K$ values obtained indicate that the affinity of miconazole for pHEMA networks (blank) is quite relevant and that such a high affinity can be even enhanced by attaching CDs to the network structure.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Degree of swelling (ml water/g dried gel)</th>
<th>Miconazole loaded in aqueous phase (mg miconazole/g dried gel)</th>
<th>Total miconazole loaded (mg miconazole/g dried gel)</th>
<th>Partition coefficient $K$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.6077 (0.0395)</td>
<td>0.1033 (0.0067)</td>
<td>84.78 (11.78)</td>
<td>498</td>
</tr>
<tr>
<td>400 α</td>
<td>0.6819 (0.0205)</td>
<td>0.1159 (0.0035)</td>
<td>120.50 (2.94)</td>
<td>708</td>
</tr>
<tr>
<td>300 α</td>
<td>0.6892 (0.0097)</td>
<td>0.1172 (0.0017)</td>
<td>94.75 (6.76)</td>
<td>557</td>
</tr>
<tr>
<td>200 α</td>
<td>0.7041 (0.0131)</td>
<td>0.1197 (0.0022)</td>
<td>88.56 (14.11)</td>
<td>520</td>
</tr>
<tr>
<td>100 α</td>
<td>0.7047 (0.0159)</td>
<td>0.1198 (0.0027)</td>
<td>83.49 (17.08)</td>
<td>490</td>
</tr>
<tr>
<td>400 β</td>
<td>0.6708 (0.0052)</td>
<td>0.1140 (0.0009)</td>
<td>124.32 (2.56)</td>
<td>731</td>
</tr>
<tr>
<td>300 β</td>
<td>0.6995 (0.0036)</td>
<td>0.1189 (0.0006)</td>
<td>118.35 (10.12)</td>
<td>696</td>
</tr>
<tr>
<td>200 β</td>
<td>0.7174 (0.0103)</td>
<td>0.1220 (0.0017)</td>
<td>102.40 (11.76)</td>
<td>602</td>
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<tr>
<td>100 β</td>
<td>0.7009 (0.0219)</td>
<td>0.1191 (0.0037)</td>
<td>82.40 (16.31)</td>
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</tr>
<tr>
<td>400 γ</td>
<td>0.6631 (0.0114)</td>
<td>0.1127 (0.0019)</td>
<td>150.76 (38.66)</td>
<td>886</td>
</tr>
<tr>
<td>300 γ</td>
<td>0.6857 (0.0294)</td>
<td>0.1166 (0.0050)</td>
<td>122.52 (12.64)</td>
<td>720</td>
</tr>
<tr>
<td>200 γ</td>
<td>0.7027 (0.0053)</td>
<td>0.1195 (0.0009)</td>
<td>98.78 (6.83)</td>
<td>580</td>
</tr>
<tr>
<td>100 γ</td>
<td>0.7037 (0.0212)</td>
<td>0.1196 (0.0036)</td>
<td>169.36 (6.41)</td>
<td>996</td>
</tr>
</tbody>
</table>
6 CONCLUSIONS

pHEMA hydrogels with pendant cyclodextrins showed a slightly greater degree of swelling than unmodified pHEMA networks, but a remarkably greater capability to load miconazole and to control its release.

Preliminary microbiological tests with Candida albicans, carried out by Prof. T. Coenye and co-workers at the Laboratory of Pharmaceutical Microbiology at Ghent University, evidenced that the amount of miconazole loaded by the hydrogels with the highest content in pendant CDs as well as the delivery rate are suitable to prevent the growth of this microorganism. These findings open great perspectives for the hydrogels developed in the present work.
7 REFERENCES


REFERENCES


REFERENCES


