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BIOAEROGELS: PROMISING MATERIALS FOR IMPREGNATION OF DRUGS

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BIOAEROGELS: PROMISING MATERIALS FOR IMPREGNATION OF DRUGS

Diploma thesis dissertation

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Abstract

The following work examines the possibility of impregnating chosen model drug into bioaerogels to obtain final formulation with added value. The drug used in this study was esomeprazole, used to treat acid-related diseases.

In the first part of the work, bioaerogels were prepared. Polysaccharide aerogels are lightweight biocompatible and biodegradable materials, suitable for applications in pharmaceutical industry. For this purpose, three different cores were prepared: pectin, alginate and their mixture, followed by coating with chitosan layer. The production of the bioaerogels follows a sol-gel synthesis and supercritical drying technique. All samples were characterised, and optimisation was performed based on examined properties. Aerogels having a pectin core and chitosan coating showed the highest surface area and the highest adsorption capacity.

In the second part, the impregnation of esomeprazole was performed using two different methods: supercritical impregnation and diffusion via sol-gel synthesis. For supercritical impregnation, supercritical carbon dioxide was used as a solvent for impregnation of the drug. In the diffusion method, the model drug was added during sol-gel synthesis using ethanol as solvent. Finally, complete characterisation of prepared formulation followed by drug release studies was performed.

The study showed successful impregnation of esomeprazole using either carbon dioxide or ethanol as a solvent. Bioaerogels proved to be promising as carriers for achieving the optimal release of the chosen drug.

Keywords: Bioaerogels, polysaccharides, supercritical impregnation, diffusion method, esomeprazole.

UDK: 543.635.25:544.774.2(043.2)

Povzetek

V diplomskem delu smo proučevali možnost impregnacije vzorčnega zdravila v bioaerogele z namenom, da dobimo učinkovito končno formulirano obliko. Kot vzorčno zdravilo smo uporabili esomeprazol, ki se uporablja za zdravljenje bolezni povezanih z želodčno kislino.

V prvem delu diplome smo pripravili bioaerogele. Polisaharidni aerogeli so lahki biokompatibilni in biorazgradljivi materiali, ki so primerni za uporabo v farmacevtski industriji. Pektin, alginat in njuno mešanico smo uporabili za pripravo treh različnih jeder, ki smo jih nato prevlekli s plastjo hitozana. Bioaerogele smo sintetizirali s sol-gel sintezo in jih sušili s superkritičnim oglijkovim dioksidom. Opravili smo karakterizacijo in optimizacijo pripravljenih vzorcev. Aerogeli s pektinskim jedrom in obdani s hitozanom imajo največjo površino in največjo adsorpcijsko sposobnost.

V drugem delu smo izvedli impregnacijo esomeprazola po dveh različnih metodah in sicer z metodo superkritične impregnacije in z metodo difuzije s sol-gel sintezo. Pri superkritični impregnaciji smo kot topilo uporabili superkritični ogljikov dioksid. Pri difuzijski metodi pa smo vzorčno zdravilo dodali med sol-gel sintezo, pri čemer smo kot topilo uporabili etanol. Na koncu smo izvedli popolno karakterizacijo pripravljene formulacije in opravili študijo sproščanja zdravila.

Z obema metodama smo uspešno impregnirali esomeprazol ter dosegli optimalno sproščanje izbranega zdravila.

Ključne besede: Bioaerogeli, polisaharidi, superkritična impregnacija, metoda difuzije, esomeprazol.

UDK: 543.635.25:544.774.2(043.2)

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Symbols and Abbreviations

Symbols

pH	numeric scale used to specify the acidity or basicity of a solution
T	temperature (˚C)
p	pressure (bar)
T_c	critical temperature (˚C)
p_c	critical pressure (bar)
A	absorbance
λ	wavelength (nm)
S_{BET}	specific surface area (m^2/g)
I_o	intensity of the initial light
I	intensity of the light that reaches the detector
T_s	transmittance
t	time (h)
SCF	Supercritical Fluid
$scCO_2$	Supercritical carbon dioxide
T_P	Triple point
C_P	Critical point

Abbreviations

FTIR	Fourier transform infrared spectroscopy
ATR	Attenuated total reflectance
SGF	Solution of gastric fluid
SIF	Solution of intestinal fluid
SEM	Scanning electron microscopy
UV	Ultraviolet-visible
TIR	Temperature indicator regulator
PIR	Pressure indicator regulator

Aim of the thesis

The general objective of the thesis was to achieve the successful impregnation of esomeprazole. Bioaerogels were chosen as carriers because of their biocompatibility, biodegradability and low toxicity. The idea was to improve the bioavailability of the chosen model drug by impregnating it into bioaerogels.

The optimisation of the bioaerogels synthesis was performed. Alginate, pectin and their mixture were used for the preparation process. Chitosan was later on used as a coating layer for prepared cores. The effect of coating was studied. The selection of the samples was performed based on characterisation methods. Furthermore, selected bioaerogels were impregnated using two different methods, diffusion and supercritical impregnation. Finally, these two techniques were compared.

1. Introduction

1.1 Polysaccharides

In recent years, natural materials are prevailing over synthetic materials, moving towards sustainable development. This fact is reflected in all kinds of fields, especially in the food and pharmaceutical industry. Moreover, natural materials have many advantages over synthetic polymers [1].

Polysaccharides are polymeric carbohydrate structures which are formed by repeating units joined by glycoside linkages. They are abundant, non-toxic, biocompatible and biodegradable [2]. Due to these properties, polysaccharides are excellent candidates for pharmaceutical and food applications, where their use is increasing. They can be used as excipients, carriers and protecting agents but active substances as well [3].

Polysaccharides are known as thickeners and gelling agents. Their primary use is to either thicken of gel water [4].

Polysaccharides are hydrated in aqueous solution, obtaining a viscous-elastic gel called hydrogel. Peppas et al. define hydrogels. as ‘three-dimensional, hydrophilic, polymeric networks, with chemical or physical crosslinks, capable of imbibing copious amounts of water or biological fluids’ [5]. These networks can be classified according to the type of crosslinking among the macromolecules. These categories can be physical or chemical; however, both can occur in the same network. In physical crosslinking interactions between chains are occurred; meanwhile, chemical crosslinks are covalent bonds [6].

Crosslinked polysaccharides are used for coating active substances. Since their bonds are sensitive to pH, temperature, concentration etc. it is possible to control the phase transition changing the pH of the medium [7].

Polysaccharide systems have been studied as controlled drug release systems because their property of being pH-sensitive, moreover, they can be easily degraded in the human body. However, polysaccharides degrade easily in aqueous medium; therefore, in the human body, they can degrade quickly and release the drug prematurely. However, their properties can be modified to prevent premature drug liberation [8].

1.1.1 Alginate

Alginate is an anionic polysaccharide biopolymer mainly derived from brown algae. Due to its biodegradability, biocompatibility, low toxicity and low cost, can be applied in many different fields [9].

The molecular formula of alginate is $(C_6H_8O_6)_n$, and its molecular weight varies from 10000 to 600000 g/mol. Alginate consists of an α-L-guluronic acid and β-D-mannuronic

acid residues, linearly linked by 1,4-glycosidic linkage. The structural form of alginate is presented in Fig 1.1 [10].

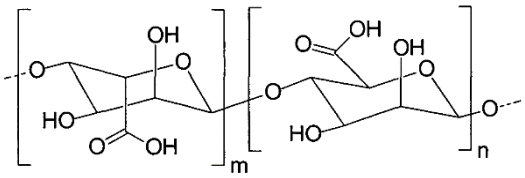

Figure 1.1. The structural formula of alginic acid [10]

Alginate can be extracted from three species of brown algae (*Laminaria hyperborean*, *Ascophyllum nodosum*, and *Macrocystis pyrifera*). Bacterial alginates have also been isolated from *Azotobacter vinelandii* and *Pseudomomas* [10].

Alginate-based materials are pH-sensitive. If Alginate is placed in acidic medium, it shrinks, restricting the release of encapsulated drugs, whereas, in basic medium, alginate dissolves rapidly, therefore, the drug encapsulated release [10].

There are four gelling methods for alginate ionic crosslinking, covalent crosslinking, thermal gelation, cell crosslinking. In ionic crosslinking as the most used, ionic crosslinking agents are used, such as divalent and trivalent cations. These ions connect with the G-block of the polymer chains, resulting in a network of alginate fibres held together by ionic interactions [1].

1.1.2 Pectin

Pectin is a high-molecular-weight carbohydrate biopolymer present mainly in the cell wall of all plant foods, where it contributes to the cell structure [11]. The felling properties of pectin haven been studied for long years, which makes this material one of the most used for gel production. The principal source of pectin are fruits, especially citrus peel and apple pomace [12].

The molecular formula of pectin is $(C_6H_{10}O_7)_{n.}$ If the degree of polymerisation is high, the molecular weight can reach more than 200000 g/mol [12]. Pectin consists of chains of galacturonic acid residues linearly linked by α-1,4-glycosidic linkages, entirely or partly esterified with methanol [11]. The structural form of pectin is shown in Fig. 1.2.

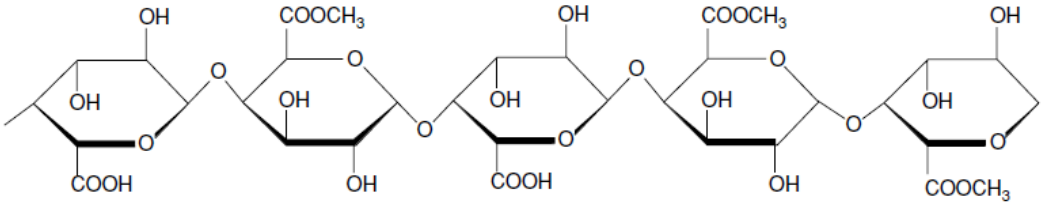

Figure 1.2. The structural formula anhydrous galacturonic acid [12]

Pectin is known because of its facility for gel formation; for this reason, it has many applications in food and pharmaceutical products [12].

The gelation of pectin depends on the type of pectin. High-ester pectins will gel with sugars and acid, while low-ester pectins will gel divalent ions. Cooling of the medium is necessary to decrease molecular movement and permit the formation of intermolecular interactions. Furthermore, a low pH is desirable for the reduction of the distance between the pectin chains. In these conditions, pectins will form gels [12].

1.1.3 Chitosan

Chitosan is a natural amino polysaccharide derived from animals. Chitosan is obtained from chitin which is found in the exoskeleton of crustacean. Most common sources of chitin are crab and shrimp shells [13].

The most common derivate of chitin is chitosan, having improved properties. As shown in Fig 1.3, chitosan is the N-deacetylated form of chitin. It is composed of β-1, 4-linked 2-2amino2-deoxy-α-d-glucopyranose. Being biocompatible, biodegradable, non-toxic, it is appropriate for use in food and pharmaceutical industry, in the same way as alginate and pectin [13].

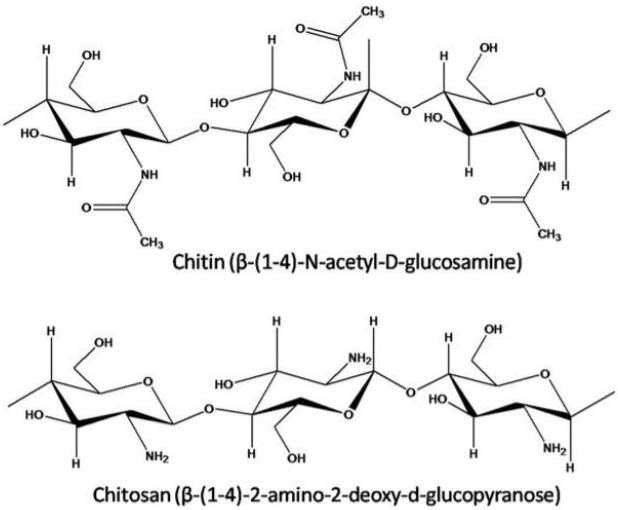

Figure 1.3. The structural formula of Chitin and Chitosan [13]

The molecular formula of chitosan is $(C_{56}H_{103}N_9O_{39})_n$.

Chitosan, as well as most polysaccharides, is pH-dependent. It is soluble in acidic medium at lower pH ranges; however, insoluble in water and higher pH ranger. This property makes it very interesting for application in the biomedical field [13]. There are different methods for chitosan gelation, such as reacetylation of the polymer with acetic anhydride, by using glycerol phosphate with temperature etc. The simplest way of preparing chitosan gels is by precipitation in a high pH medium [14].

1.2 Aerogels

Aerogels are lightweight materials mainly composed of air. Their properties, such as high surface areas, high porosities and extremely low densities, make aerogels great candidates for many and varied applications [1].

The term "aerogel" is usually prescribed to porous materials obtained by removing the solvent, in conditions where the collapse of the structure is avoided. This is achieved by using supercritical fluid technology. The result is a material composed mainly of air (up to 99,8%) [15].

The collapse of the network structure typically occurs during air drying, where the interface between the liquid and the gas is present. It is caused by the capillary forces which create a pressure gradient between the pore walls, leading to the shrinkage of the gel and the collapse of the structure. By using supercritical fluids, the interface between gas and liquid phase is avoided. Since there is no surface distinguishing the liquids and gas, no surface tension or capillary forces are present. Therefore, the structure of the gel is preserved in the final product of aerogel [16].

In 1931, S.S. Kistler introduced the term "aerogels"[17]. First aerogels were made from silica. Nowadays, due to technological progress, it is possible to prepare aerogels from various materials, such as metal oxides, natural materials, like polysaccharides (cellulose, alginate, pectin, chitosan), proteins etc. [15].

The properties and consequently, the application of aerogels, depend on their composition. Aerogels are materials with a wide spectre of applications. To name just a few, they can be used as thermal insulators, for chemical catalysis or catalyst support. Other applications include energy storage, ultrafilters, desalinisation, sensors and fuel cells, among others [18].

1.2.1 Inorganic Aerogels

It is well known that most metals and semimetals can form gels. The most known inorganic aerogel, silica (SiO_2), was the firstly prepared by Kistler. Silica gels have been more studied in chemistry. The formation of silica gels begins with the aqueous solution precursors in organic solvents. Then, the solution is put through sol-gel processing and supercritical drying. The same principles can be applied for non-silicate inorganic gels as titanium [19].

1.2.2 Organic aerogels

Organic aerogels were synthesised in 1987. Simply, organic aerogels were synthesised by polymerisation of organic monomers in solution followed by supercritical drying [20].

Resorcinol/formaldehyde and melamine/formaldehyde were the first mixtures for this organic sol-gel chemistry [19].

Polysaccharide Aerogels

Bioaerogels have gained increasing attention due to their potential applications in different fields, as drug delivery systems, in tissue engineering, regenerative medicine, among others. Polysaccharides and proteins are main components for preparation bio-aerogels due to their stability, renewability, non-toxicity, biodegradability and biocompatibility. [21]

Polysaccharide aerogels are highly porous, lightweight and have high surface areas. [1]

1.2.3 Sol-Gel synthesis

The most common way to prepare aerogels is by sol-gel synthesis.

The process involves four main steps: [22]

1. Sol preparation. A sol is formed by the dispersion of solid particles in an appropriate solvent.
2. Gelation. A gel is formed from the sol by introducing an appropriate crosslinking agent.
3. Ageing of the gel: The gel remains in the solution for a predetermined time to increase the strength of the gel.
4. Drying of the gel. The solvent is removed from the pores of the wet gel usually using supercritical drying technique for avoiding capillary forces.

The production time of polysaccharide aerogels is usually prolonged comparing to other types of aerogels because of the solvent exchange step, which is required before the supercritical drying [23]. Another way to prepare aerogels is by ethanol-induced gelation [24]. The sol-gel principles are the same. However, some steps are reduced. The gelation time is significantly reduced by forming alcogels directly from the sol and introducing them in ethanol. Ethanol triggers the gelation of polysaccharides' solution via hydrogen bonds. By this way, the solvent exchange step is avoided, and the total production time is reduced.

1.3 Supercritical fluids

A supercritical fluid is defined as a substance having pressure and temperature above its critical (Pc, Tc).

The supercritical fluid area is shown in the pressure-temperature diagram in Fig 1.4. As presented, three regions can be distinguished, corresponding to the solid, liquid or gas phase. They are separated by curves, representing the boundary between two phases. All

three stages coexist in the triple point. The liquid-gas line has an endpoint in the critical point (Tc, Pc). Beyond this point, only one phase exists, corresponding to the supercritical fluid (SCF).

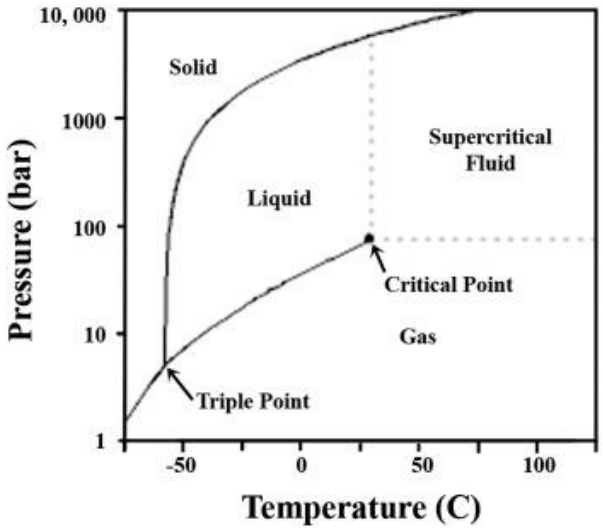

Figure 1.4. Phase diagram for the pure compound (CO2) [25]

SCFs are interesting due to their properties are between liquid and gases ones. The main characteristics are viscosities similar to gases, densities similar to liquids and diffusivities between those of liquids and gases [25]. Characteristic properties are shown in Table 1.1.

Table 1.1 Physical properties of gases, supercritical fluids and liquids [26]

Property	Gas (ambient)	SCF (≥ Tc, Pc)	Liquid (ambient)
Density ρ (kg/m^3)	0.6-2	200-500	600-1600
Dynamic viscosity η (mPa)	0.01-0.3	0.01-0.03	0.2-0.3
Kinematic viscosity v (10^6 m^2 s^{-1})	5-500	0.02-0.1	0.1-5
Diffusion coefficient D (10^6 m^2 s^{-1})	10-40	0.07	0.0002-0.002

SCFs are able to dissolve materials as liquids and are able to penetrate porous materials as gases. Since there are no surface tensions, as explained above, capillary forces are not present during processing [27].

The most common supercritical fluid used is carbon dioxide (CO_2) mainly because it is chemically inert, non-toxic, naturally abundant and recyclable. CO_2 has a critical point at mild conditions (T_c= 31.1 ˚C; P_c=74.3 bar). It allows many processes and chemical reactions to take place at low temperatures, preventing the decomposition of compounds.

This is especially important in food and pharmaceutical industry. By reducing pressure, CO_2 is removed from products, because it is a gas at ambient conditions [28].

As mentioned, the supercritical drying technique is applied for obtaining aerogels. After sol-gel synthesis, the alcogels are placed into an autoclave. The critical conditions of the supercritical fluid (most commonly $scCO_2$) are reached. For this purpose, the temperature and pressure are raised up to the critical values, to ensure the supercritical conditions for avoiding affections of the gels. The supercritical fluid is then introduced into the vessel, replacing the organic solvent. After a determined period, the pressure and temperature are slowly lowed to ambient conditions and aerogels are obtained.

It should be noted that the supercritical drying method is not the only one used today. Besides aerogels, cryogels and xerogels can be formed. Depending on the drying method, there is a clear difference in the structure of the final gel.

In the freeze-drying method, the solvent in the gel network is frozen, followed by the sublimation. The liquid in gel's network is replaced by gas [29]. Obtained gels are named cryogels. The disadvantage of this technique is that crystals of solvent can be formed, destroying the gel structure producing huge pores [18].

Air drying causes capillary forces; therefore, a pressure gradient which provokes the shrinkage of the gel network. The resulting dried gel is called xerogel. Usually, xerogels show the shrinkage up to 90%, while in aerogels, the shrinkage is generally less than 15% [30].

1.4 Impregnation methods

There are two ways to introduce drugs in aerogels.

The first method is by the addition of the drug during the sol-gel process, applying the so-called diffusion method. The method is simple and flexible. The drug has to be dissolved in the given solvent, applied for sol-gel process. However, the most critical step is the drying process because there is a risk to wash out the impregnated.

The second method includes the post-treatment of already dried aerogels, in the process named supercritical impregnation. This method introduces the drug into the aerogel by dissolving it in a supercritical fluid, mostly used $scCO_2$. This technique is presented as a solution when the drug is not soluble or poorly soluble in water and soluble in supercritical fluids [18].

1.5 Model drug

Used model drug for the study was esomeprazole. It is the S-isomer of omeprazole. This drug is orally administered. Principally reduces stomach acid. However, it is used to treat

various diseases, such as gastroesophageal reflux disease, peptic ulcer disease, Zollinger-Ellison syndrome, etc. The stability of esomeprazole depends on the pH of the medium; it rapidly degrades in acidic media while it stable under alkaline conditions.

Esomeprazole is slightly soluble in water and highly soluble in organic solvents and supercritical carbon dioxide [31]. The essential solubilities of Esomeprazole are shown in Table 1.2.

Table 1.2 Solubility of Esomeprazole in different solvents [31]

Compound	Solubility (mg/ml)
Water	0.35
Ethanol	1.00
DMSO	20.0
DMF	25.0

The solubility of esomeprazole in supercritical carbon dioxide varies with temperature and pressure. The solubilities vary from 0.034 to 5.599 g/L, depending on the conditions used (P=120-270 bars and T=35-65 ˚C) [32].

Esomeprazole is solid under ambient conditions. Its skeletal formula is shown in Fig. 1.5. [31].

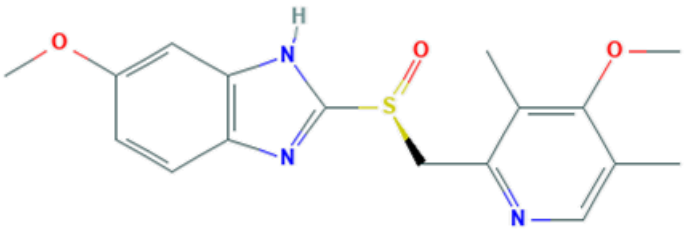

Figure 1.5. Skeletal formula of Esomeprazole [30].

2. Materials and methods

2.1 Materials

2.1.1 Alginic acid sodium

Alginic acid sodium salt used in the experimental part was purchased from Sigma-Aldrich. The source of it is algae. It was in its crystalline form as a yellow powder. The most important properties are given in Table 2.1.

Table 2.1. The most important properties of Alginic acid sodium salt [33]

Synonyms	Alginic acid sodium salt Sodium beta-d-mannuronate
Melting point	99˚
Solubility	Soluble in water. Insoluble in alcohol, chloroform and ether.
Colour	Faint yellow to light brown
Odour	Odourless
Storage	Protect from light

2.1.2 Pectin

Pectin used in the experimental part was purchased from Tokyo Chemical Industry (TCI). The source of the pectin used is citrus. The product was in its crystalline form as a white to light yellow powder. The most important properties, including structural formula, appearance, physical properties and storage conditions, are given in Table 2.2.

Table 2.2. The most important properties of Pectin [34]

Synonyms	Pectin β-D-Galactopyranuroic acid β-D-Galacturonic acid
Melting point	142-144˚C
Solubility	Soluble in water. Insoluble in alcohol, and organic solvents.

Colour	Yellowish white
Odour	Practically odourless
Storage	Protect from light

2.1.3 Chitosan

Chitosan used in the experimental part purchased from Sigma-Aldrich was medium molecular weight. The most important properties, including structural formula, appearance, physical properties and storage conditions, are given in Table 2.3.

Table 2.3. The most important properties of chitosan [35]

Synonyms	Chitosan Poliglusam Deacetylchitin Chicol Flonac C
Melting point	290˚C
Solubility	Insoluble in water or organic solvents. Soluble in a solution of acetic acid.
Colour	Beige
Odour	Odourless
Storage	Protect from light

2.1.4 Esomeprazole

Esomeprazole magnesium trihydrate with purity 99.0% was purchased from Xi'an health biochemical technology co., LTD.

Its most important properties are given in Table 2.4.

Table 2.4. The most important characteristics of esomeprazole [31]

Molecular formula	$C_{17}H_{19}N_3O_3S$

Molecular weight	345.40 g/mol
Synonyms	(S)-Omeprazole (-)-Omeprazole
Melting point	155˚C
Solubility	Slightly soluble in water
Colour	White
Odour	Odourless
Storage	Protect from light

2.1.5 Others

Absolute ethanol with a purity of 99.8% was from Sigma-Aldrich and used to induce gelation replacing the water contained in the hydrogel. Carbon dioxide with a purity of 99.5% was supplied by Messer, Slovenia. It was used for drying the alcogels to obtain aerogels. Also, it has been used as a solvent for impregnating the selected drug into the aerogels. Acetic acid CH_3COOH (Baker, purity ≥ 98%) was used for chitosan dissolution. Hydrochloric acid, HCl (Merck, 37%), potassium phosphate monobasic, KH_2PO_4 (Sigma Aldrich, purity ≥ 98%) and sodium hydroxide, NaOH (Sigma Aldrich, purity ≥ 98%) were employed for the preparation of simulated gastric (SGF) and simulated intestinal fluid (SIF).

2.2 Analytical methods

2.2.1 Nitrogen adsorption-desorption analysis

Gas adsorption is a standard method used for obtaining textural properties of porous materials. Nitrogen is the most commonly used adsorptive; however, other gases can be used depending on the nature of the material.

Nitrogen (N_2) gas adsorption-desorption technique was used to determine the specific surface area (m2/g) of the prepared aerogels using the BET (Brunauer-Emmett-Teller) method [36]. The experiments were performed with liquid nitrogen at -196°C using ASAP 2020MP, a Micrometrics instrument. Before the analysis, the samples were subjected to vacuum at 70°C for 660 min until obtaining a stable 10 µm Hg pressure.

This technique also allows measuring the adsorption and desorption isotherms, which provides additional information. The specific amount of adsorbed gas as a function of the relative pressure at a constant temperature can be represented in the form of an isotherm. These isotherms, presented in Fig. 2.1 can be classified into six groups, microporous adsorbents (type I), nonporous or microporous (types II, III and VI) or mesoporous (types IV and V).

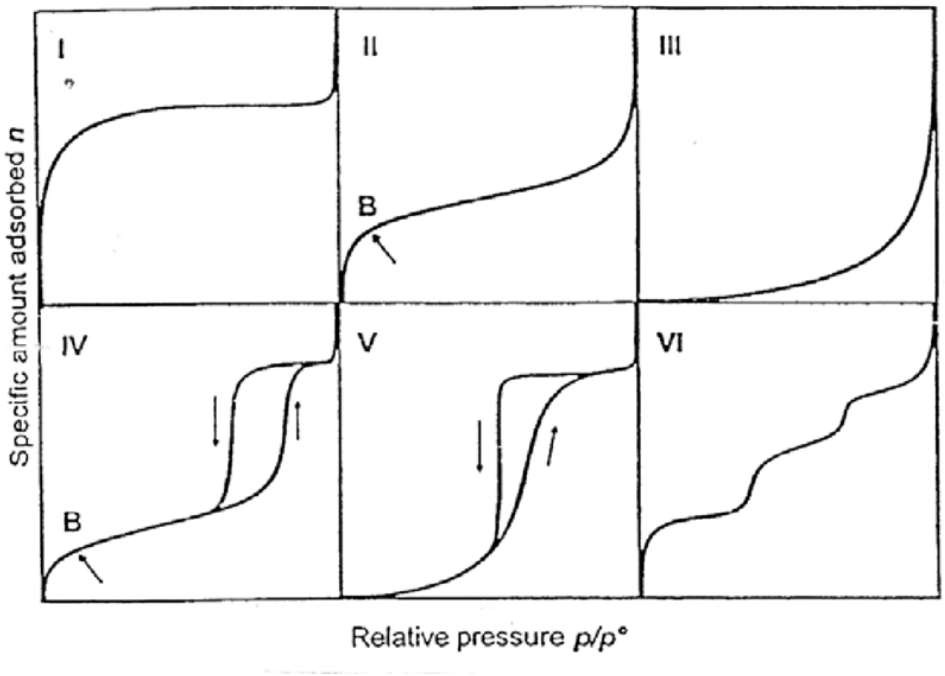

Figure 2.1. IUPAC- Types of adsorption isotherms showing both adsorption and desorption [37]

2.2.2 Scanning Electron Microscopy

The scanning electron microscope (SEM) is a technique that creates images which reveal microscopic-scale information on the shape, size, composition and other physical and chemical properties of organic and inorganic samples. The area to be examined is irradiated with an electron ray, which forms images of the surface of the sample. Besides, this ray can be moved to create a complete image of the surface of the sample or be static to study one zone. This technique is able to provide a really high resolution; therefore, it is the most used technique for examination and analysis of structural characteristics of solid objects [38].

A scanning electron microscope (Sirion 400 NC) was used to determine the surface morphologies of the aerogels. This technique was used to check if some changes occur during the impregnation. Firstly, the samples were covered with gold particles, and then, they were scanned at a voltage of 2-4 kV.

2.2.3 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a technique used for quantitative and qualitative analysis of solids, liquids, gases etc. by measuring their absorbance. Measured absorbances can be used to identify samples. The method consists of grinding the material and mixing it with potassium bromide (KBr) serving as matrix. The other method is attenuated total reflectance (ATR), which may analyse specific samples faster and easier [39].

The apparatus used was a Fourier transform infrared spectroscopy (Shimadzu, IRAffinity-1s) for characterisation of pectin coated aerogels and esomeprazole. The collection of the absorption bands was used to confirm the identity of polysaccharides and esomeprazole and for detection of esomeprazole loaded into aerogels. Aerogels were characterised by ATR-IR method. The samples were cut into pieces and placed on the ATR detector. On the other hand, esomeprazole was grinded into a fine powder and mixed with KBr.

2.2.4 In-vitro dissolution testing

In vitro dissolution tests provide information about the drug release. This technique can be an indicator of the in vivo performance of the drug in the human body. The test is performed in a dissolution medium which simulates the gastrointestinal (SGI) fluids, making in vitro and in vivo performance is close. In vitro studies are therefore necessary prior to in vivo studies, leading to approval of all solid oral drug products [40].

Many apparatus exists for developing this test; however, the most used are basket (apparatus 1) or paddle (apparatus 2). A schematic representation of the main vessels of apparatuses is presented in Fig 2.2.

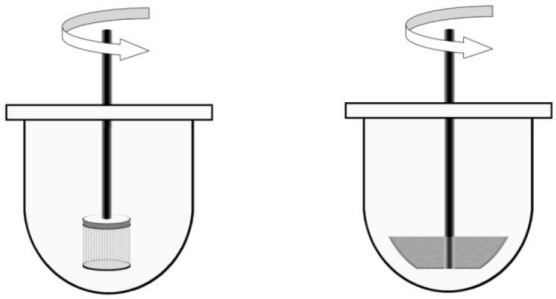

Figure 2.2. Left Apparatus 1; Right Apparatus 2 [40]

Apparatuses consist on a main vessel, a motor, a metallic drive shaft and a cylindrical basket in the case of apparatus 1 and paddle in the case of apparatus 2. The basket is cylindrical in shape with a hemispherical bottom. It is made of a non-reactive material. The temperature of the media inside the vessel is kept constant by a water bath. The volume is 1000 ml. The solution in the vessel is mixed by the rotating stirring element. A

dosage unit is placed in a dry basket at the beginning of each test. Both the vessel and the shaft are generally made of stainless steel or another inert material. The dosage unit can be placed into the piece of the inert material, so the drug does not float. [41].

The apparatus used in this experiments was the apparatus 2. The in vitro test conditions are adjusted to be as much as possible close to physiological conditions. The terms used for the dissolution tests are listed below.

1. The volume used was 900 ml.
2. Agitation was obtained by stirring at 50 rpm.
3. The temperature of the dissolution medium was 37±0.5 ˚C
4. The pH of the test medium was 1.2 and 6.8.

Dissolution medium

The dissolution medium must imitate the human digestive tract pH. This pH varies depending on the part of the tract. Two solutions were prepared. The first one refers to gastric fluid (SGF) and the second one to the intestinal fluids (SIF). SGF (HCl) with pH=1.2 was prepared by diluting 8.3 ml of 37% HCl to 1000 ml with miliQ water. SIF (phosphate buffer solution) with pH=6.8 was prepared by mixing 250 ml of 0.2 M KH_2PO_4 and 112 ml of 0.2 M NaOH and diluted to 1000 ml with miliQ water.

Esomeprazole dissolution testing

Aerogel impregnated with esomeprazole either by diffusion or supercritical impregnation method, were tested for release studies. Firstly, the dissolution testing was performed in SGF for 2 hours and immediately transferred to SIF for the next 22 hours. The overall testing was performed for 24 hours.

In vitro dissolution tests were performed following USP standards [24]. The experiments were carried out on the Farmatester 3, USP II apparatus showed in Fig.2.3. The conditions used for the testing are explained above. Samples of 2 ml were taken at predetermined time periods, and afterwards, 2 ml of fresh dissolution medium was added to maintain a constant volume. The samples were analzide by a Cary 50 Probe UV spectrophotometer at 301 nm. The concentration of esomeprazole was calculated using the calibration curves in SGF and SIF.

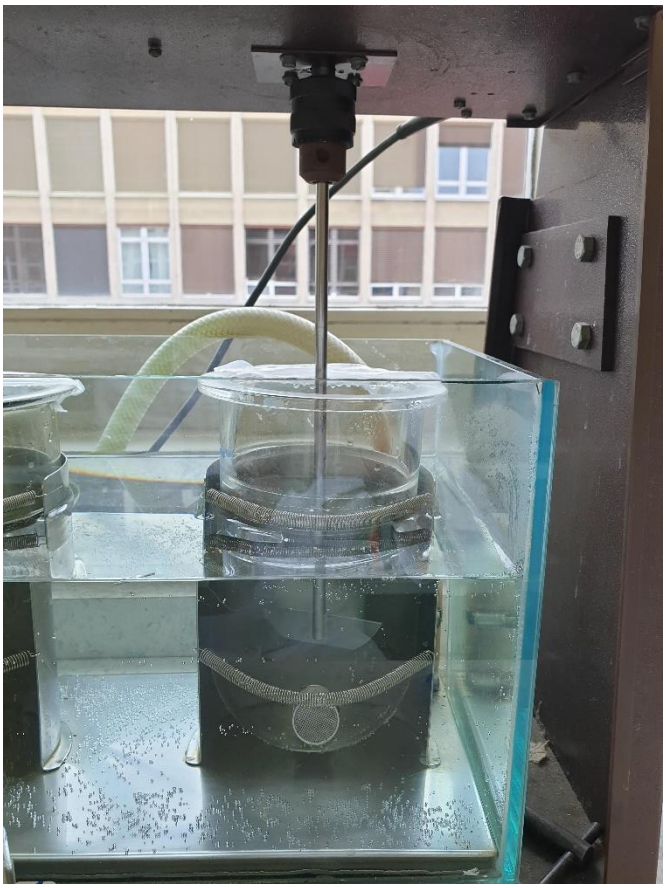

Figure 2.3. Farmatester 3, USP II used during in vitro dissolution test

2.2.5. Ultraviolet-visible spectroscopy

Spectroscopy in the ultraviolet-visible range (UV/VIS) is the most frequently used technique for quantitative and qualitative analysis. The ultraviolet radiation has wavelengths between 400 and 200 nm; visible light between 400 and 200 nm. Therefore, commercial instruments operate between 800 and 200 nm.

Spectrometers measure the amount of light absorbed by the sample. By measuring the intensity of the light that reaches the detector, as well as the initial intensity, it is possible to calculate the transmittance and absorbance of the samples.

When a monochromatic radiation beam of a specific wavelength passes through a solution layer containing an absorbent species, the molecules have the ability to absorb electromagnetic radiation; therefore, the power (energy per unit time and unit area) of the incident beam P is attenuated by decreasing to P_0. These wavelengths are found between 200 to 800 nm corresponding from 200 to 400 nm ultraviolet and from 400 to 800 nm visible light. Spectroscopy in the ultraviolet-visible range (UV/VIS). This technique is the most used for both quantitative analysis and qualitative.

Transmittance (T) is defined as the incident fraction of radiation which can go through the sample. A parameter more interesting is the absorbance. The following equations Eq. (1) and Eq. (2) define these parameters.

$$T = \frac{P}{P_0} \tag{1}$$

$$A = -\log T \tag{2}$$

When there is no absorption $P=P_o$, therefore, the absorbance A=0.

UV spectrometry was applied to determine the final loading of esomeprazole in the aerogels and the released amount esomeprazole during in vitro dissolution tests. The spectrophotometer used was Varian Cary 50 Probe spectrophotometer.

2.3 Processing methods

2.3.1 Synthesis of wet gels

Three different types of gels were produced. The difference lies in the core, prepared from alginate, pectin or their mixture. Cores were covered by chitosan.

Firstly, the 3% (w/w) polysaccharide aqueous solutions were prepared. The polysaccharides were weighed and poured into water at room conditions while mixing at 400 rpm until complete homogenisation. The mixture was prepared in 1:1 ratio. Solutions were transferred to Petri dishes and completely covered with absolute ethanol where it was left until complete the gelation by almost one day.

Secondly, 1.5% (w/w) of chitosan in 0.2M of CH_3COOH was prepared. Once the gelation is completed, tablet shape forms were cut. The cores were dipped into the chitosan solution. To attach chitosan to the cores, tablets were transferred to NaOH solution (0.2 M) in ethanol, which caused the gelation of chitosan. Finally, the gels coated with chitosan were introduced in pure ethanol.

2.3.2 Supercritical drying

To maintain the internal network of aerogels, avoiding the collapse of the structure, supercritical fluids were used. For this porpoise, the supercritical drying technique was applied at the last step of the production.

The supercritical drying system used in the laboratory is shown in Fig. 2.4. The system consists on a CO_2 tank, a pump which introduces $scCO_2$ in the autoclave, a separator, a thermostatic system, pressure and temperature indicator regulators, flow indicator and

inlet and outlet valves. The main container (autoclave) volume is 500 ml and is able to hold up to 500 bars.

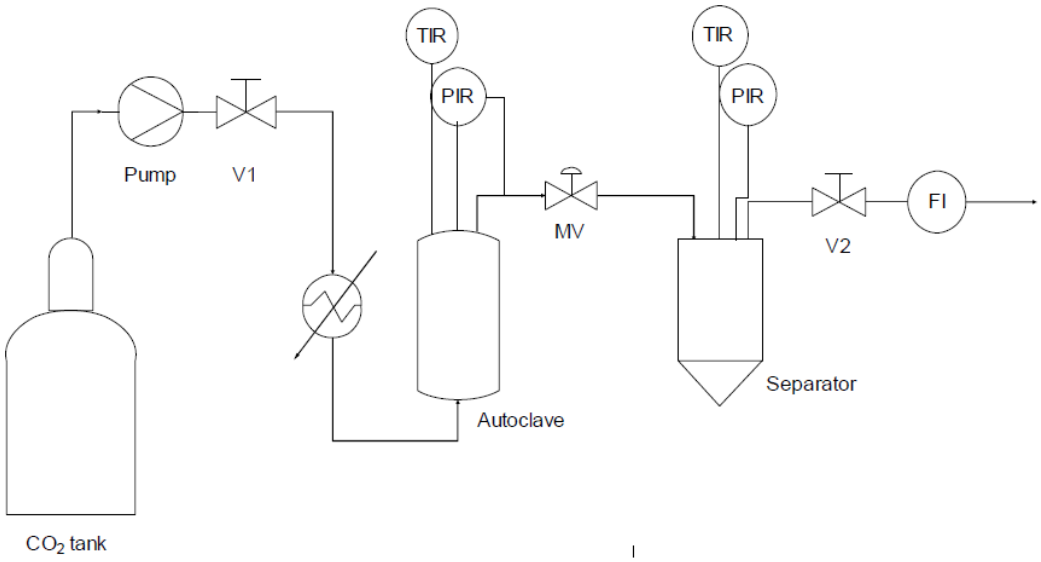

Figure 2.4. Schematic diagram of supercritical drying equipment [1]

Gel samples were placed into the autoclave, which was completely filled with ethanol. The system was heated up to 40ºC. The process of drying started by pumping CO_2 into the autoclave, slowly reaching 120±10 bar (1 bar/min). When the outlet valve was opened, the CO_2 flowed through the autoclave removing the excess of ethanol collecting in the separator. Firstly, the CO_2 flow was set to 200-300 L/h to remove excess ethanol after which was reduced to allow the diffusion of ethanol from the inner part of gels. The drying was performed for approximately 6 hours. Finally, the autoclave pressure was slowly decreased to atmospheric conditions (3 bar/min).

2.3.3 Impregnation during sol-gel synthesis (diffusion method)

Addition of drugs by diffusion method takes place during the sol-gel synthesis. This process is based on loading the drug into the gel by diffusion using a saturated solution in an appropriate solvent.

For this purpose, esomeprazole was dissolved in absolute ethanol to obtain saturated solution. Saturated solution of ethanol was used in all steps of the sol-gel process, allowing the diffusion of esomeprazole into gels. Using this method, the impregnation of drug and synthesis of aerogels occur simultaneously.

Finally, in the supercritical drying, the vessel was poured with the saturated solution instead of only pure ethanol.

2.3.4 Supercritical impregnation

The method consists of impregnation of the esomeprazole by the supercritical CO_2. The experiment was performed in batch mode and without co-solvent.

CO_2 is introduced into the high impregnation cell, where previously has been charged with the aerogels and the drug. The aerogels were placed at the bottom of the cell and the drug above. CO_2 firstly comes into contact with esomeprazole, dissolving it and introducing the drug into the aerogels placed in the bottom of the vessel. The conditions were kept for 24 hours in a static mode at 40˚C and 100±10 bars. At the end of the impregnation process, the cell was slowly depressurised. Slowly depressurisation rate must be maintained because a higher rate could damage the aerogels producing the collapse of the network. When all the pressure was released, the aerogels were removed from the system.

The employed supercritical impregnation apparatus is presented in Fig. 2.5. The device is comprised of a stainless-steel impregnation cell, with an internal volume of approximately 60 ml and maximum operating pressure of 300 bars.

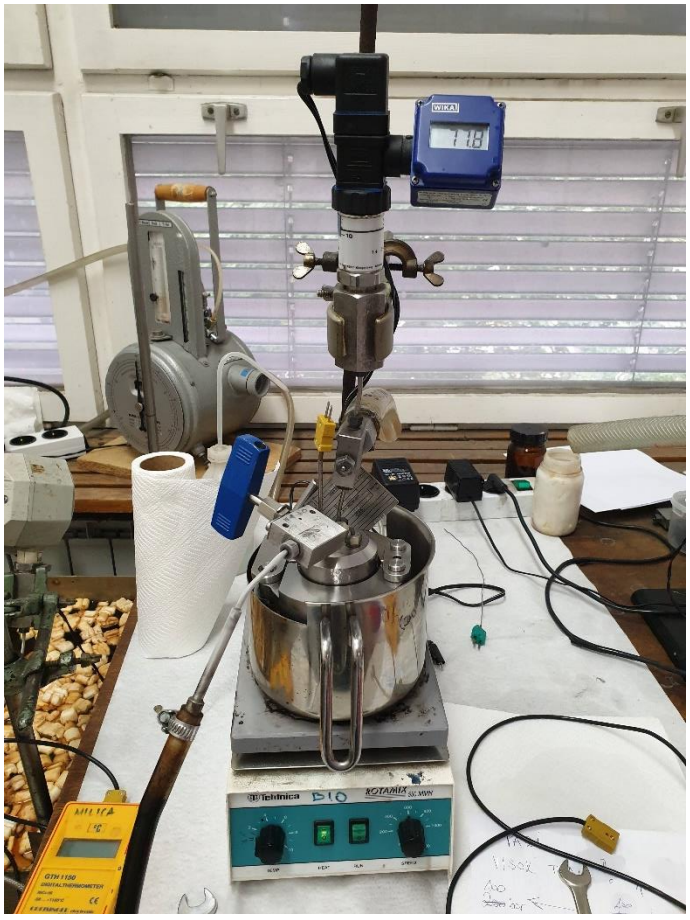

Figure 2.5. Supercritical impregnation apparatus

The temperature inside was measured with a thermocouple. The cell was heated by oil bath. The pressure was measured with a digital manometer. The system was equipped with a micro-metering valve for controlling the pressuring and depressuring. The CO_2 is added to the cell with a pump.

2.3.5 Determination of the drug's impregnation loadings

After the impregnation process, the amount of drug loaded had to be determined. The loaded drug was extracted from the aerogels with absolute ethanol. The aerogels were weighted after and before the loading of the drug. The loaded aerogels were placed in a glass beaker and sealed; then, they were agitated for 30 minutes. Finally, they were sonicated for at least 5 minutes.

The concentration of the drug was measured with UV spectrometry; hence, a calibration curve was made. For this porpoise, different patterns were made in varying concentrations. Besides, the wavelength corresponding to the maximum absorbance peak was scanned, resulting in λ=301 nm.

The absorbance of the drug-loaded was measured and then looked for the concentration in the calibration curve.

The amount of impregnated esomeprazole was recalculated to the loadings following the Eq. (3) where m_e is the mass of impregnated esomeprazole (g) and m_a is the mass of the empty aerogel (g).

$$\text{Esomeprazole loading} = \frac{m_e}{m_a} \tag{3}$$

3. Results and Discussion

3.1 The production of aerogels

Different types of aerogels were prepared including alginate, pectin and their mixture coated with chitosan. Obtained aerogels are presented in Fig 3.1. The average diameter to height ratio was 1.5.

Figure 3.1 Coated aerogels after supercritical drying; a) Pectin b) Alginate c) Alginate and Pectin

Textural properties of prepared samples were determined by gas adsorption using N_2. Table 3.1 shows the values of determined specific areas.

Table 3.1. Specific surface areas, pore volumes and pore diameters of different aerogels dried at 120 bars and 40˚C

Sample	S_{BET} (m^2/g)
Pectin coated with chitosan	314
Alginate coated with chitosan	178
(Pectin+Alginate) coated with chitosan	214

The results indicate that the aerogels made with pectin core coated with chitosan have the highest specific surface area while the once with alginate core have the lowest. As expected, the aerogels with the core made from their mixture have a specific surface area between these two values. Since the aerogels having a pectin core have the highest specific surface area, all further research was conducted with these samples.

N_2 adsorption/desorption isotherms were obtained for all aerogels and are shown in Fig 3.2.

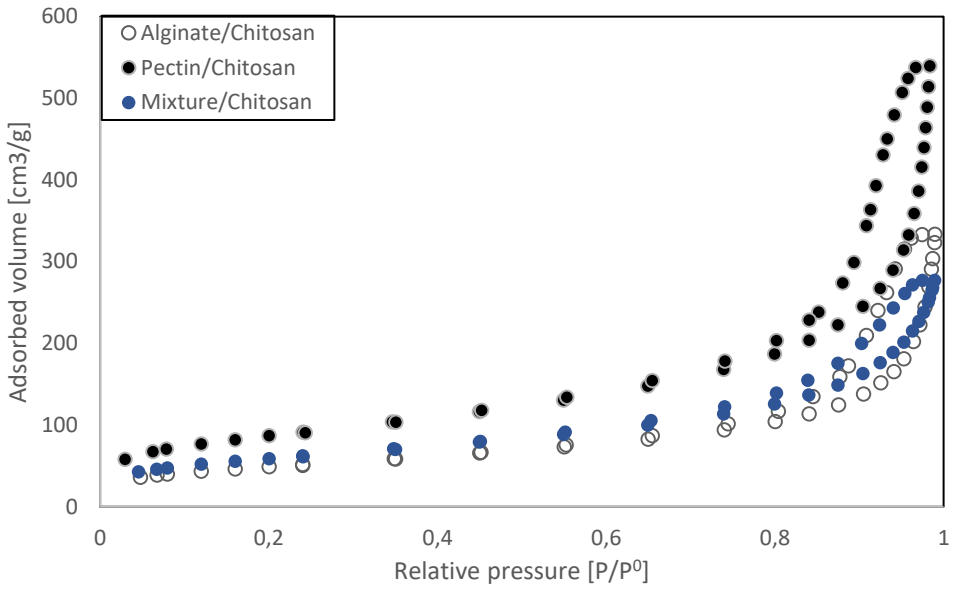

Figure 3.2. N_2 adsorption-desorption isotherms of coated aerogels

From the shape of the curves, it can be seen that all isotherms can be classified as type IV, which are characteristic of mesoporous materials. Moreover, it can be seen that the adsorbed volume of the pectin aerogels is higher than the alginate or the alginate/pectin aerogels, therefore, proving the higher adsorption capacity.

3.2 Drug impregnation loadings

The impregnation experiments were performed with pectin aerogels coated with chitosan since they showed advanced properties compared to alginate or alginate/pectin aerogels coated with chitosan. As a comparison, the impregnation experiments were performed with pectin aerogels without the chitosan coating.

By impregnating esomeprazole into aerogels, their colour change from white to purple as shown in Fig.3.3.

Figure 3.3. Pectin coated aerogels impregnated with Esomeprazole

As explained above, to determinate the loading, the drug impregnated into aerogels is extracted with ethanol and measured using UV spectroscopy. For that purpose, the calibration curve of esomeprazole in ethanol was prepared. Firstly, the solution was

scanned to determine the wavelength corresponding to the maximum absorbance peak. As presented by Fig 3.4, the resulting maximum was obtained at in λ=301 nm.

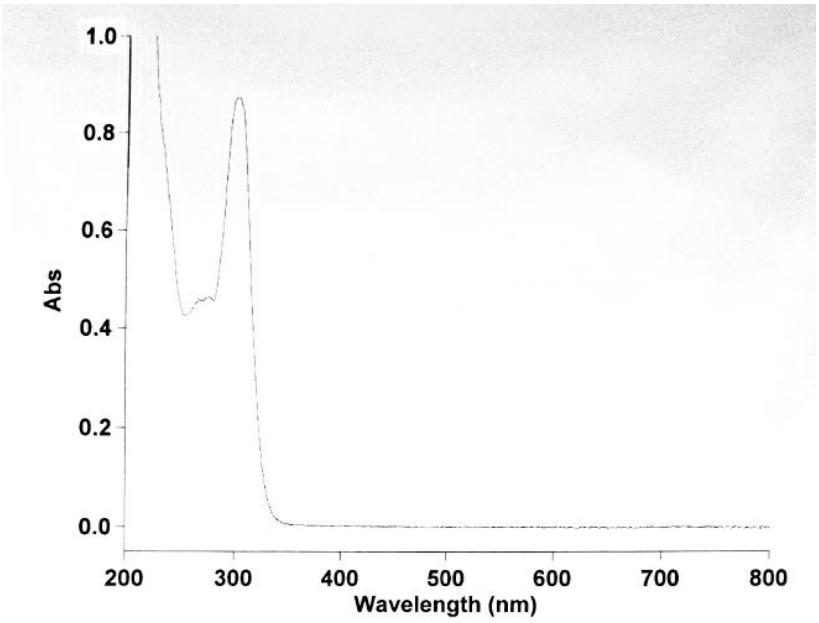

Figure 3.4. Determination of the wavelength where the maximum absorbance occurs

Once the wavelength of the absorbance peak was determined, the calibration curve with known concentrations was measured to obtain a relation between the concentration and the absorbance, as presented by Fig. 3.5. The obtained determination of the fit (R^2) was 0.9985.

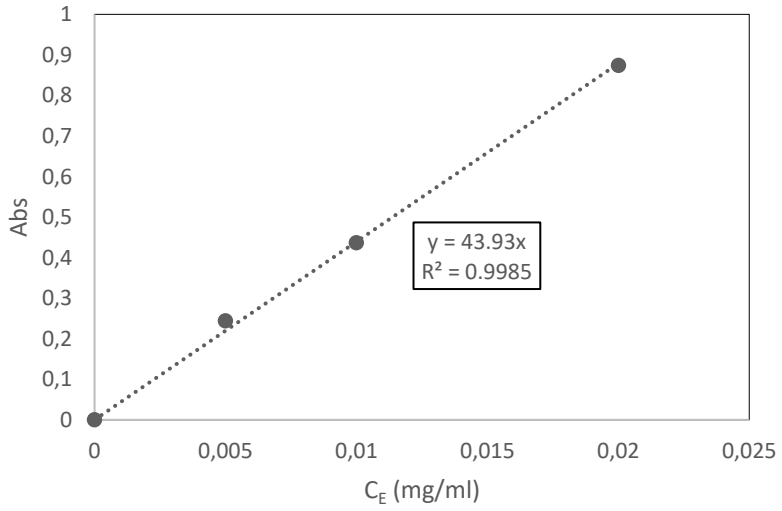

Figure 3.5. Calibration curve for esomeprazole in ethanol

The impregnation of esomeprazole by diffusion was performed during sol-gel synthesis from a saturated solution of the drug in ethanol. Measured loadings are presented in Table 3.2.

In both cases, the impregnation was successfully achieved. However, pectin aerogels show much higher loadings compared to the pectin aerogels coated with chitosan, 20.5 and 4.2 respectively. A possible reason for such behaviour could be slightly damaged structure of pectin aerogels coated with chitosan [42]. The adsorption capacities of coated aerogels are lower and consequently, the final loadings.

The loadings for supercritical impregnation were calculated and are shown in Table 3.2. The experiments were performed at 40 ˚C and the 120 bar to ensure mild impregnation conditions, to avoid possible degradation of the model drug.

Table 3.2. Results of impregnated esomeprazole using diffusion and supercritical impregnation

Method of impregnation	Sample	Loading (%)
Diffusion	Pectin	20.5
	Pectin+Chitosan	4.2
Supercritical impregnation	Pectin	16.2
	Pectin+Chitosan	2.6

Supercritical impregnation showed to be a feasible technique for impregnation of esomeprazole as well. The loadings are slightly lower, compared to the ones obtained by the diffusion method. Here, the same trend is observed, higher loadings are achieved for pectin aerogels, compared with the ones having a chitosan coating, 16.2 and 2.6 respectively.

Loadings for both techniques are comparable. However, the beginning quantity of used model drug is the case of supercritical impregnation is incomparably lower, leading to the conclusion that this technique is more appropriate and economical for the impregnation of esomeprazole than the diffusion method.

3.3 Characterisation of impregnated aerogels

3.3.1 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was applied to identify pure esomeprazole and esomeprazole impregnated into pectin aerogels coated with chitosan. Fig. 3.6 shows the FTIR spectra of the esomeprazole, blank pectin aerogels coated with chitosan and pectin aerogels coated with chitosan impregnated with esomeprazole.

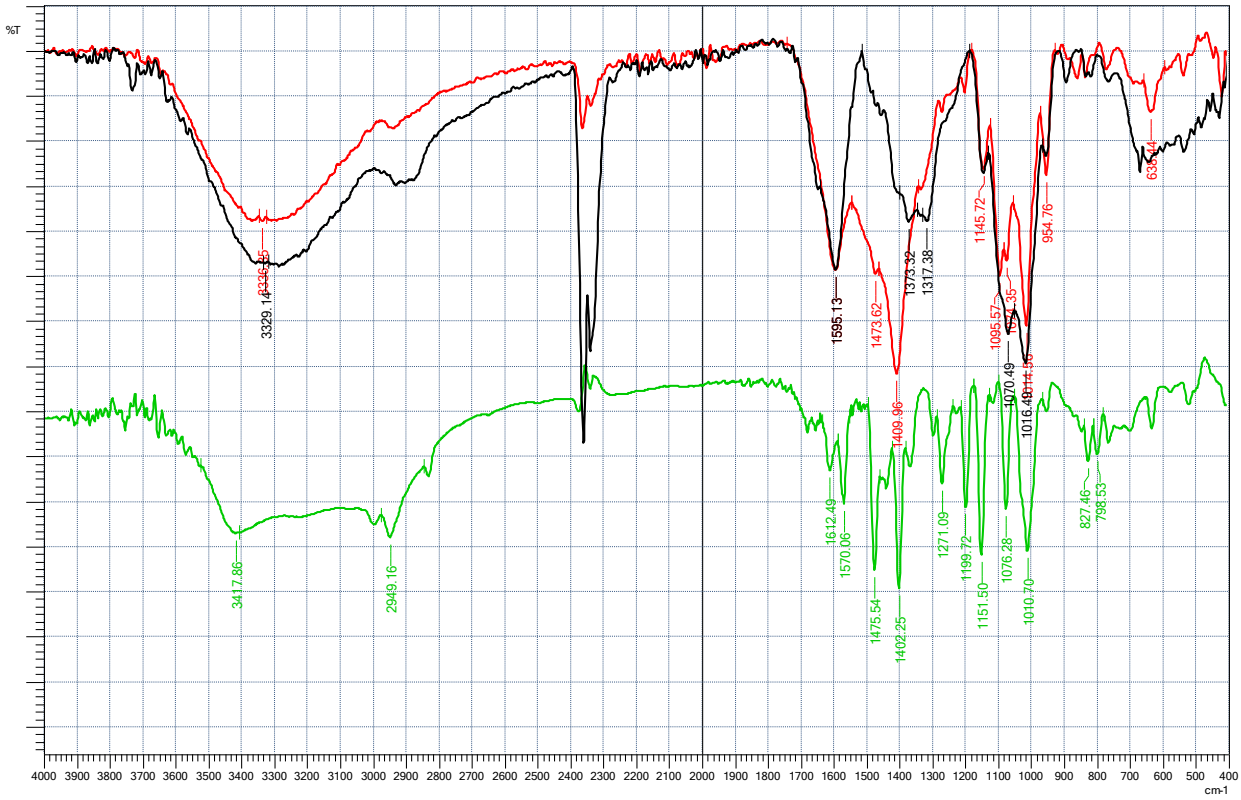

Figure 3.6. FTIR spectra: esomeprazole (green); blank aerogels (black); aerogels impregnated with esomeprazole (red)

The measured spectrum of esomeprazole corresponds to the one found in the literature [43]. The broad peak extended from 3418 to 2949 cm^{-1} corresponds to C=N group. The peaks at 1612 and 1570 cm^{-1} indicate the presence of the carboxyl group. These characteristic peaks are only visible in the spectrum of aerogels impregnated with esomeprazole while they are missing for the blank samples. Furthermore, by comparing spectra of blank and impregnated aerogels, it can be concluded that by impregnating the esomeprazole, the chemical structure of aerogels has not changed.

3.3.2 Scanning electron microscopy

Scanning electron microscopy was used to understand the interior structure of the pectin aerogels coated with chitosan.

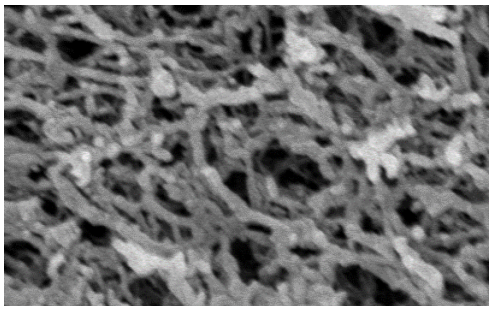

Figure 3.7. SEM image of pectin aerogel coated with chitosan

Fig 3.7. shows a SEM image of pectin aerogel coated with chitosan. In this image, a highly porous structure is visible. Interconnected network of pores is desirable since drugs can be impregnated inside.

3.4 In vitro dissolution tests

Conventional oral drug delivery usually does not provide a target release of the drugs. For better control of the release, bioaerogels are proposed as their carries. Controlling the drug release is an excellent way of focusing on the treatment of disease.

Target release of esomeprazole is the stomach, as it is used to treat various diseases connected with acidic problems. However, it is used to treat syndromes connected with the problems partially in the intestinal part.

To determine the released concentrations of esomeprazole in SGF and SIF, calibration curves were prepared for both solutions (Figs. 3.8 and 3.9). Obtained determination of the fit (R^2) were 0.9996 and 0.9993, respectively.

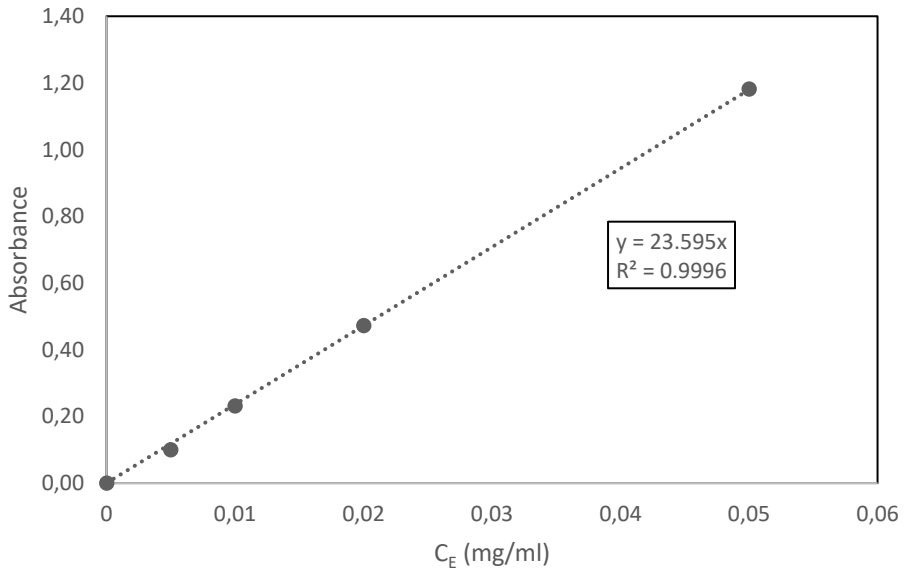

Figure 3.8. Calibration curve for esomeprazole in SGF

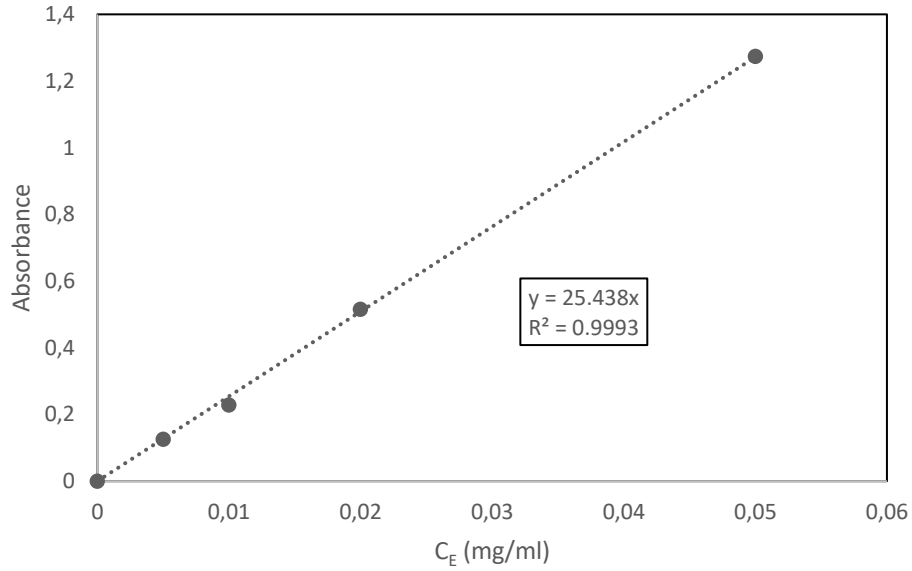

Figure 3.9. Calibration curve for esomeprazole in SIF

In vitro dissolution testing was performed for pectin aerogels coated with chitosan impregnated with esomeprazole using both techniques.

As explained above, the release was simulated in gastric fluid for 2 hours, followed by 22 hours in intestinal fluid. The results obtained are presented in Fig 3.10.

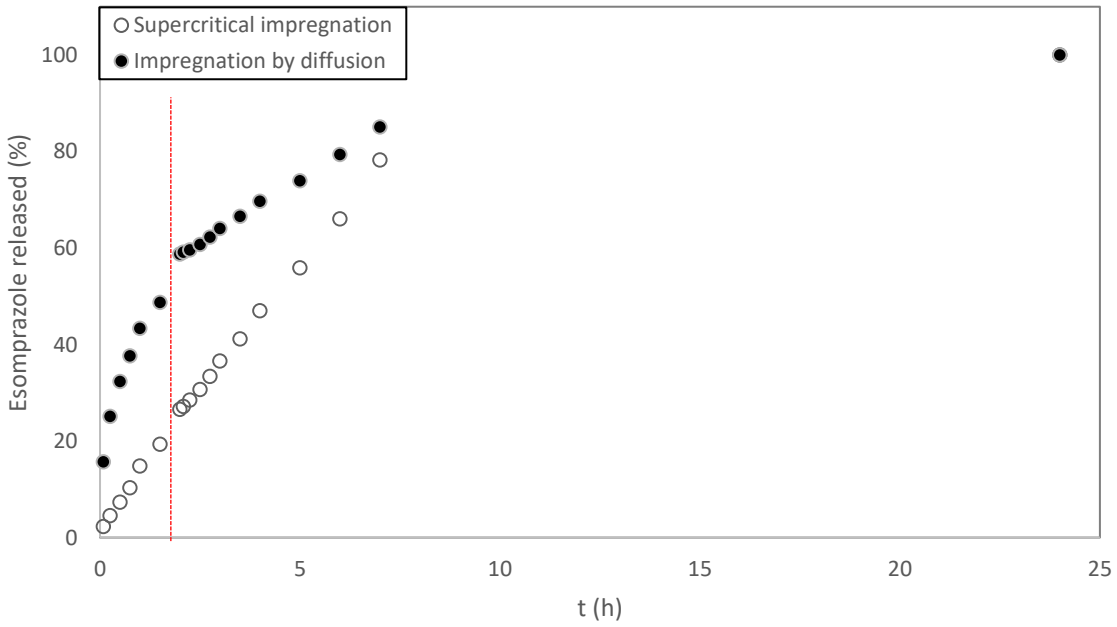

Figure 3.10. Release of esomeprazole in SGF and SIF. The red mark indicates the change of medium.

The red mark indicates the change of medium, from SGF to SIF, representing the transition to the intestinal part. In the case of esomeprazole impregnated by diffusion method, it can be clearly seen that most of the drug is released in SGF (up to 60%). In the

SIF, the release is slower and in a more controllable manner. In the case of esomeprazole impregnated using supercritical impregnation, only 30% was released in SGF, and the rest in SIF.

The reason for this phenomena may lay in the impregnation mechanism of esomeprazole. Apparently, the great amount of esomeprazole loaded by diffusion method via sol-gel synthesis is in the exterior part of prepared aerogels. Using supercritical impregnation method, esomeprazole entered the deepest parts of aerogel, resulting in highly dispersed particles. This led to a slower and more controllable release of esomeprazole. Since the desired effect is the main release of esomeprazole in SGF, a more suitable technique for esomeprazole would be diffusion through sol-gel synthesis.

Pure pectin aerogels impregnated with esomeprazole were not subjected to in vitro dissolution testing. The reason is the nature of the pectin, which in contact with SGF shrinks, enabling the release of the impregnated drug. Since the main release should be achieved in SGF, this formulation, even though having higher loadings, is inappropriate for esomeprazole as model drug.

4. Conclusion

The work covers the development of a complete drug product; synthesis of aerogel carrier, impregnation of chosen model drug and finally, application of the prepared formulation.

Firstly, bioaerogels were prepared. Three different cores were made from alginate, pectin and their mixture. These cores were then coated with chitosan, obtaining a coated aerogels. After characterisation of all prepared aerogels, the one with advanced textural properties was chosen for further investigation. In this case, aerogel having pectin core coated with chitosan layer showed to have the highest specific surface area and the highest adsorption capacity.

Secondly, the chosen model drug esomeprazole was impregnated into pectin aerogel coated with chitosan using two methods: diffusion method via sol-gel synthesis and supercritical impregnation. In any case, by impregnating esomeprazole into aerogels, their colour changes from white to violet, showing successful impregnation. The loadings of esomeprazole into pectin aerogels coated with chitosan are comparable using both techniques. However, there is a huge difference in the amount of used drug required for successful impregnation. In the case of the diffusion method, used esomeprazole is measured in grams, while for supercritical impregnation, in milligrams for achieving similar final loadings.

Finally, in vitro dissolution tests were performed, to simulate the behaviour of prepared formulation in the human body. Esomeprazole impregnated via sol-gel synthesis was mainly released in SGF, while the one impregnated via supercritical conditions was mainly released in SIF. The reason for this phenomenon lies in the penetration of esomeprazole, which in the case of supercritical impregnation is much deeper. Esomeprazole impregnated by diffusion method via sol-gel synthesis could be mainly dispersed in the external part, allowing faster release. In the case of the chosen model drug, this behaviour is more desirable, and this formulation more appropriate.

The future work should be mainly concentrated on improving the formulation in terms of the amount of the drug required for the diffusion method.

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