Task 1A

KINETICS OF DICLOFENAC SODIUM DESORPTION FROM MCM-41 AND Si 60

I. Aim of the task

The purpose of this task is estimation of the diclofenac sodium desorption kinetics from porous silicas to phosphate buffer using a spectrophotometric method.

II. Introduction

1. Characterization of silica adsorbents
2. Diclofenac sodium
3. UV-VIS spectrophotometry. The Beer-Lambert law.
4. Kinetics of reactions. Integrated rate laws.

References:

1. P. Atkins, *'Physical Chemistry'*, Oxford University Press, 1998.
2. P. Atkins, J. de Paula, *'Physical Chemistry for the Life Sciences'*, Oxford University Press, 2006.
3. K. Iler, *'The colloid chemistry of silica and silicates'*, Cornell University Press, Ithaca, New York, 1955.
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III. Theory

III. 1. Characterisation of silica adsorbents

Silicates are the oxides of silica, where the Si atom shows tetrahedral coordination, with four oxygen atoms surrounding the central Si atom – see Fig. 1.

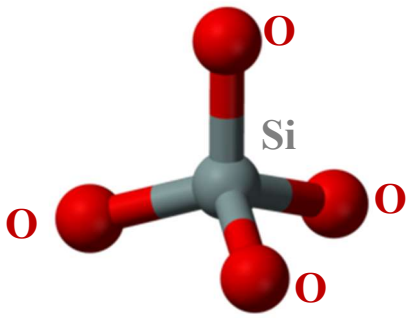


Fig. 1. Tetrahedral structural unit of silica [1].

In each of the thermodynamically stable forms of silica all oxygen atoms of the SiO_4 tetrahedra are shared with others, yielding the net chemical formula: SiO_2. Silicon dioxide has a number of distinct crystalline forms (polymorphs, e.g. quartz, tridimite, cristobalite, faujasite, coesite, stishovite, moganite) in addition to the amorphous forms. Silica gels belong to amorphous silicates possessing non-recurrent network of tetrahedra, where all the oxygen corners connect two neighbouring tetrahedra. The amorphous structure of silica is shown in Fig. 2, where for clarity some oxygen atoms behind the plane of the picture or in front of it are omitted.

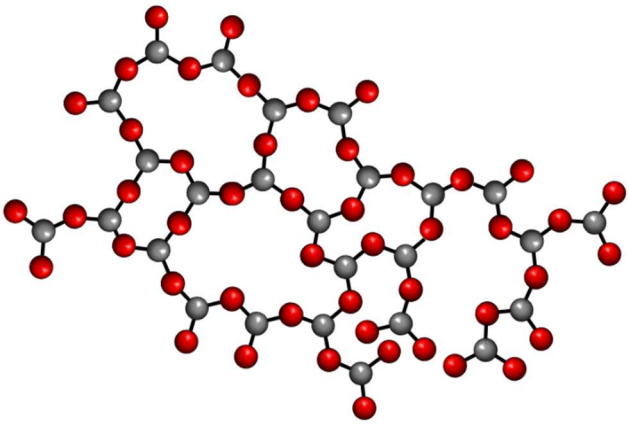


Fig. 2. The amorphous structure of silica gel (red spheres – oxygen, grey spheres – silicon).

Silica gel, a porous solid amorphous form of hydrous silicon dioxide, has the nominal chemical formula of $SiO_2 \cdot xH_2O$. It is constituted by randomly linked spheroidal polymerized silicate particles – the primary particles. The properties of silica gels are a result of the state of aggregation of the primary particles and the chemistry of their surfaces. The surface area, porosity and surface chemistry can be controlled during the production process. Silica gels are fabricated in the sol-gel process as a result of polycondensation of orthosilicic acid:

$$(OH)_2SiOH + HOSi(OH)_3 \rightarrow (OH)_3Si - O - Si(OH)_3 + H_2O$$

This reaction leads to obtaining enlarged silicic acid macromolecules – by elongation, branching and cyclization of oxy-silicone chains. As a result, the silica particles covered by SiOH groups are obtained. The forms of created hydroxyl groups are collected in Fig. 3

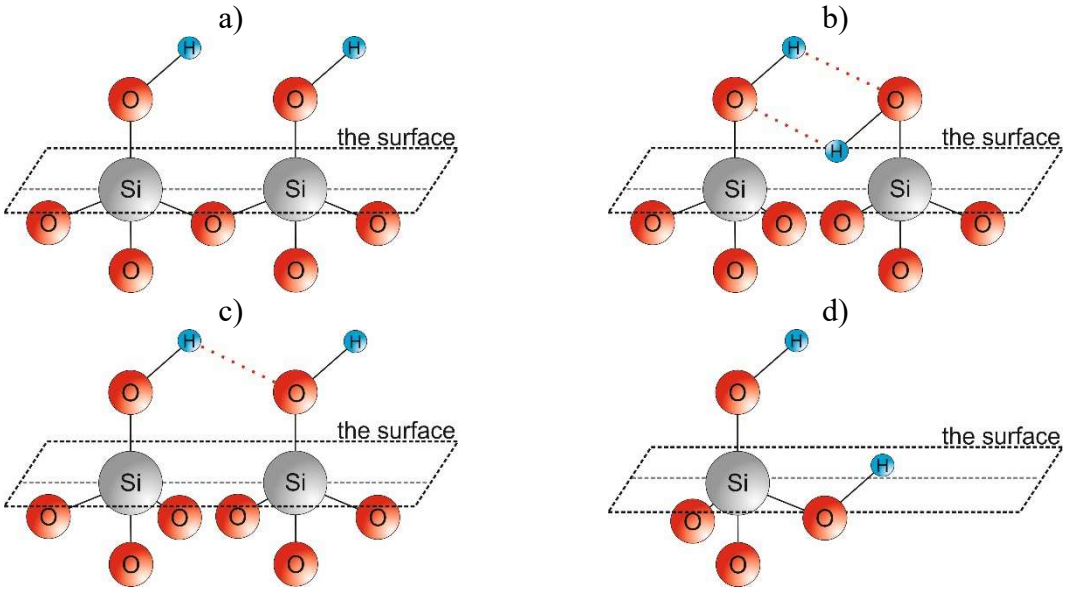


Fig. 3. Forms of hydroxyl groups on the surface of silica gel: a) single (isolated); b) hydrogen bonded; c) active; d) double (germinal) (based on [2]).

Structure characterization of amorphous silica gel Si-100 obtained using the low temperature nitrogen adsorption/desorption method is presented in Fig. 4 (a) and (b).

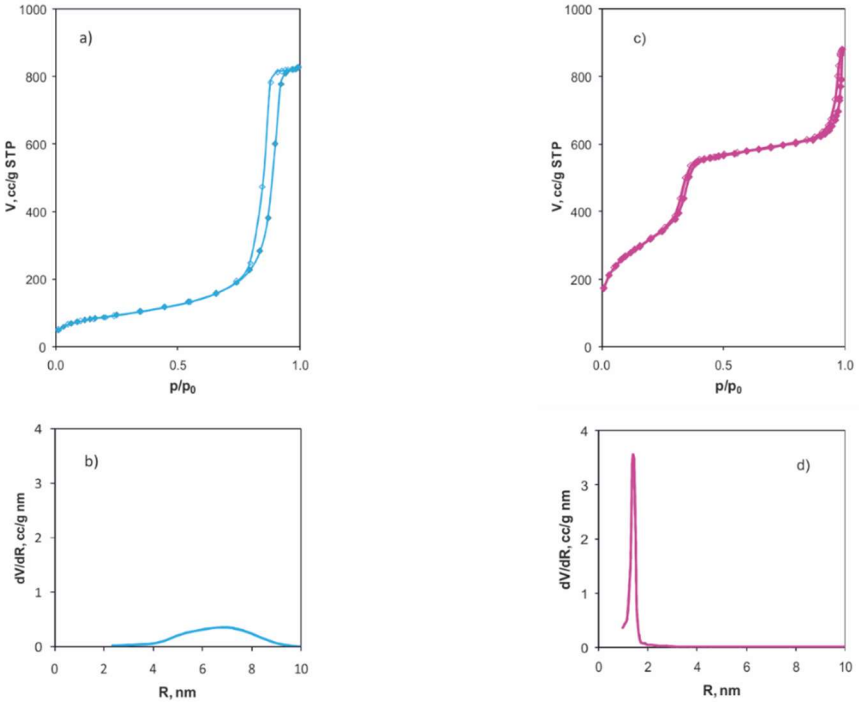


Fig. 4. Nitrogen adsorption/desorption isotherms (a, c) and pore size distributions (b, d) for Si-100 (a, b) and MCM-41 (c, d) samples.

Ordered mesoporous silica (OMS) materials are synthesized using a surfactant templating method (see Fig. 5). They present long-range ordered porous structures with narrow pore size distributions and large surface areas as well as pore volumes. Mobil Corporation research and development scientists for the first time reported the synthesis of novel ordered mesostructured materials known as MCM-41 family in 1992. These materials are characterized by regular arrays of uniform pores whose dimensions can be tailored through the choice of surfactant, additives and synthesis conditions. One of preparation theories is based on the formation of liquid crystals in mixtures of polar solvents and surfactants with non-polar tail group as follows: an increasing amount of surfactant molecules is dissolved in aqueous solution, and when the surfactant concentration reaches the critical micellar concentration (CMC), the surfactant molecules cluster together as micelles. These micelles are formed because the hydrophobic tails of the surfactant tend to agglomerate while their hydrophilic heads procure protection from water.

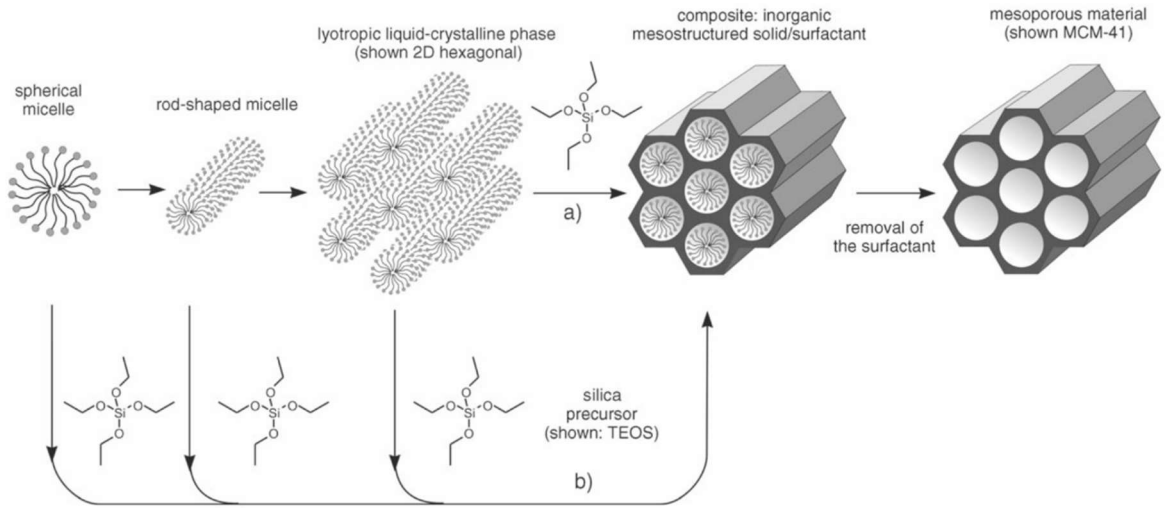
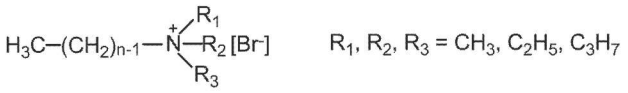
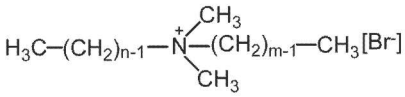
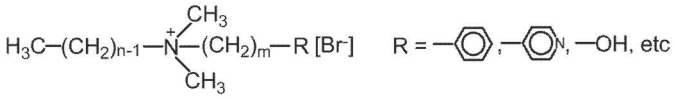
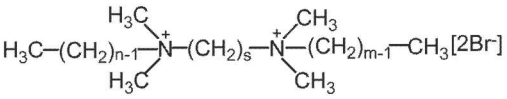
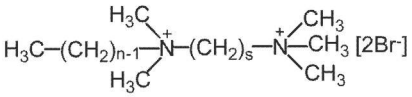
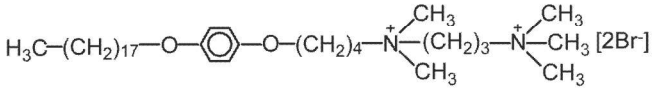
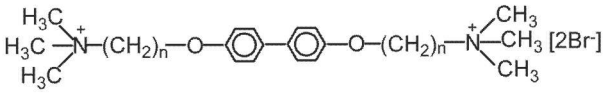
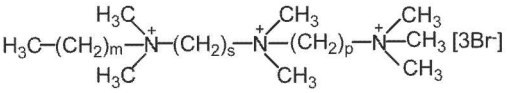
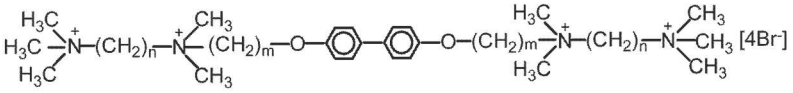


Fig. 5. Scheme of synthesis of ordered silica materials [3].

The final mesostructure of the material will depend on the organization of the surfactant molecules into the micellar liquid crystals which act as templates for the formation of the mesoporous materials. These liquid crystal structures depend on the composition and chemical nature of the surfactant and on the solution mixture conditions such as pH, temperature, surfactant concentration etc. The silica source has condensed around the micelles and the surfactant is removed by calcination (thermal degradation) or solvent extraction. The molecular formulae of cationic surfactants used in preparation of ordered mesoporous materials are collected in Table 1.

Table 1. Molecular formulae of cationic surfactants [4].

Alkyltrimethyl quaternary ammonium surfactant	 $R_1, R_2, R_3 = CH_3, C_2H_5, C_3H_7$ $n = 8 – 22$  $n = 8 – 22$; $m = 2 – 22$  $n = 8 – 22$; $m = 0 – 3$
Gemini surfactant ($C_{n\text{-}s\text{-}m}$)	 $n = 8 – 22$; $s = 2 – 6$; $m = 1 – 22$
($C_{n\text{-}s\text{-}1}$)	 $n = 8 – 22$; $s = 2 – 6$
($18B_{4\text{-}3\text{-}1}$)	
Bolaform surfactant (R_n)	 $n = 4, 6, 8, 10, 12$
Tri-headgroup cationic surfactant ($C_{m\text{-}s\text{-}p\text{-}1}$)	 $m = 14, 16, 18$; $s = 2$; $p = 3$
Tetra-headgroup rigid bolaform surfactant ($C_{n\text{-}m\text{-}m\text{-}n}$)	 $n = 2, 3, 4$; $m = 8, 10, 12$

This way prepared silica materials display exceptional properties: long-range ordering of the structure (see Figs 6 and 7), large surface area (usually over 1000 m^2/g), large pore volume (ca 1 cm^2/g) and uniform pore sizes (2-50 nm) – see Fig. 4 (c) and (d). The conditions of reaction (pH, concentration of reagents) affect kind of the obtained materials (e.g. MCM-41, MCM-48, SBA-15) – see Fig. 8.

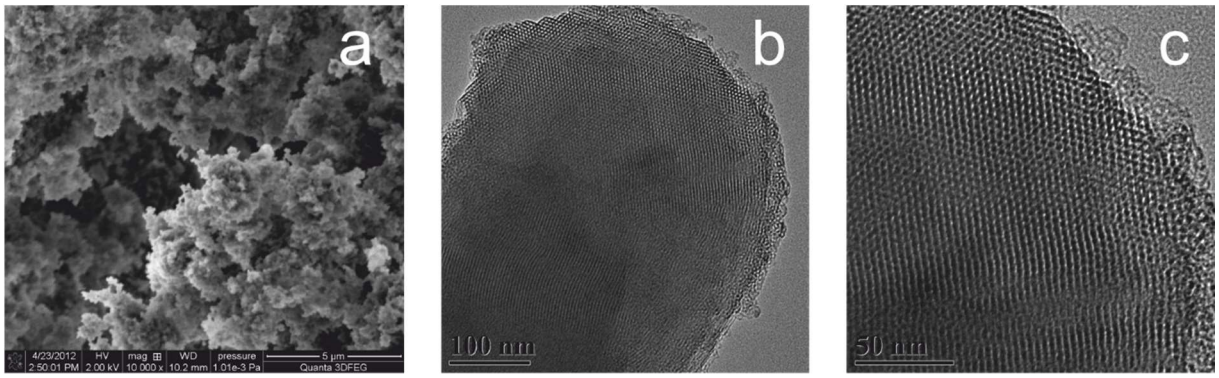


Fig. 6. Images of MCM-41 materials made using: a) scanning electron microscopy (SEM); c and b) transmission electron microscopy (TEM) methods [5].

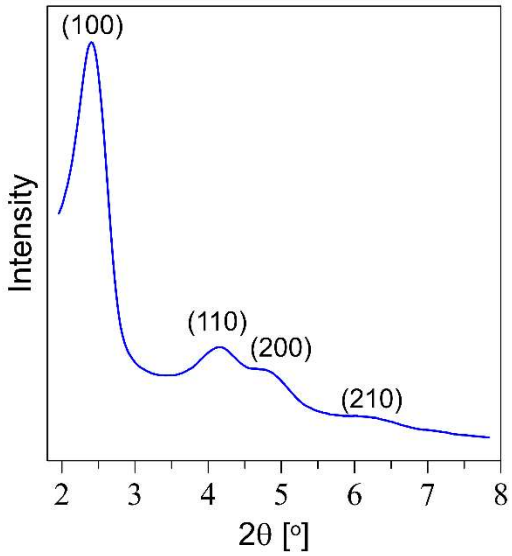


Fig. 7. X-ray diffraction patterns of MCM-41 material.

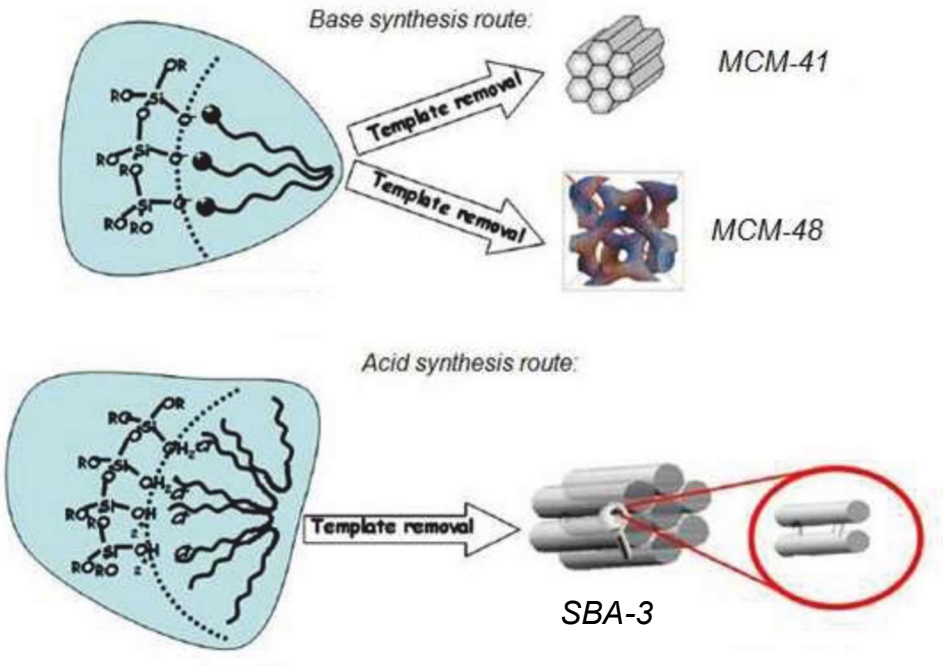


Fig. 8. Preparation routes of some mesoporous silica materials.

The ordered mesoporous silica materials offer the possibility of achieving desired chemical surface properties. All surface features such as the nanostructure or the degree of atoms/chemical groups organization depend on both the chemical nature and synergy between the components. The hybrid materials has been divided into two main classes. The first class corresponds to all systems where **no covalent or ion-covalent bonds are present** between the organic and inorganic components. All interactions in such materials are based only on weak interactions i.e. hydrogen bonding, van der Waals contacts, electrostatic forces. In second class materials a fraction of the organic and inorganic component is linked through strong chemical bonds (covalent, ion-covalent, or Lewis acid-base bonds).

The next important feature in tailoring hybrid networks concerns the chemical pathways that are used to design a given hybrid material. These chemical routes are schematically shown in Fig. 9. Path A corresponds to the conventional sol-gel chemistry. The hybrid network is obtained through the hydrolysis of organically modified metal alkoxides condensed with or without simple metallic alkoxides. This strategy yields amorphous hybrid materials, generally polydispersed in size and locally heterogeneous in chemical composition. For achieving the structure control at the nanoscopic level three main approaches can be conceived: self-assembled procedures (route B in Fig. 9), assembly of well-defined nanobuilding blocks (NBBs) (route C) and combination of the two abovementioned (route D).

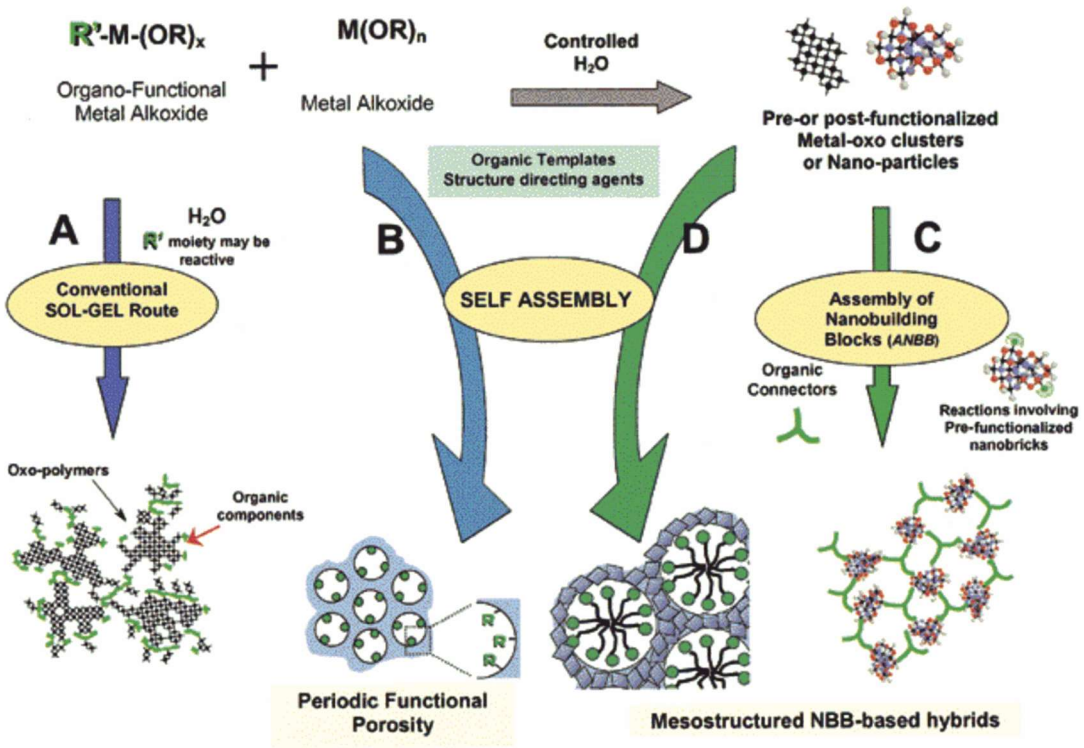


Fig. 9. Main paths for obtaining hybrid materials [6].

Routes B and D involve the use of organic structure-directing agents capable of self-assembly, giving rise to meso-organized phases. The organic phase is spread throughout inorganic matrix and can be relatively easily controlled. Route D represents strategy combining the nanobuilding block approach with the use of organic templates that self-assemble and allow controlling the

association step. The NBBs units exhibit a large variety of interfaces between the organic and inorganic components (e.g. covalent bonding, electrostatic interactions). Depending on the set of chosen experimental conditions they will keep or lose their integrity. Therefore, they can be used as true building blocks that can be connected through organic spacers or surface-driven condensation reactions or as a reservoir of inorganic matter that can be delivered at the hybrid interface to build an extended inorganic network. The functional groups which are usually introduced into the silica matrix are shown in Fig. 10. These groups can be incorporated during precipitation as is described above or by post-synthesis treatment and binding to silanol groups.

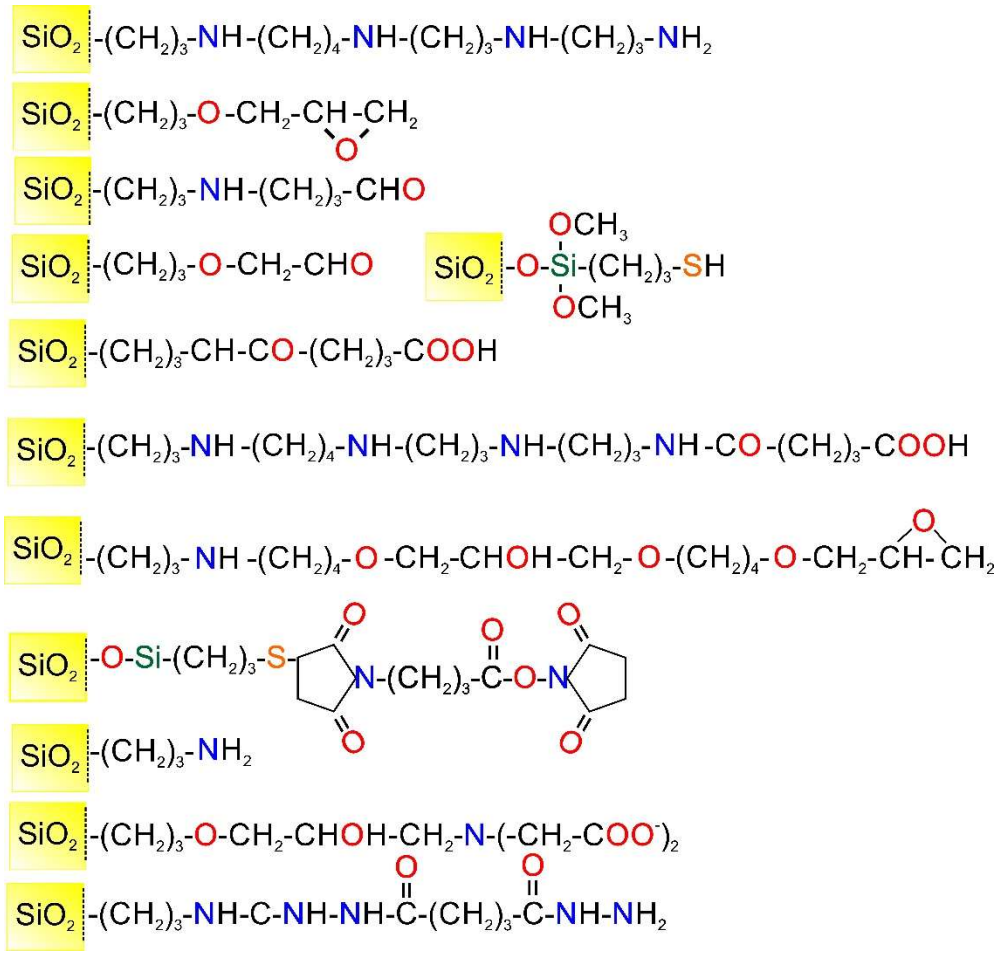


Fig. 10. Functional groups usually incorporated into the mesoporous silica matrix.

III. 2. Diclofenac sodium

Diclofenac (i.e. 2-(2,6-dichloranilino) phenylacetic acid) and its salts (Fig. 11) belong to the class of medicinal products known as NonSteroidal Anti-Inflammatory Drugs (NSAIDs). The members of NSAIDs also are e.g. aspirin, ibuprofen and its salts as well as naproxen, all are well known and available over-the-counter (OTC) in most countries.

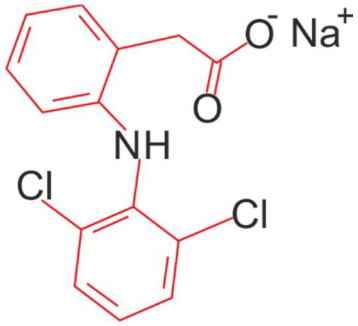


Fig. 11. The structural formula for diclofenac sodium.

NSAIDs are medications widely used to relieve pain, reduce inflammation, and bring down a high temperature (fever). Thus, they are used to relieve symptoms of headache, painful periods, cold and flu. NSAIDs are also indicated for the treatment of the long term therapy of chronic musculoskeletal pain, chronic inflammatory conditions, and for treatment of degenerative joint diseases such as rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis, diclofenac in particular [7]. Unfortunately, the frequent and long-term using of NSAIDs are associated with a significant number of side effects and complications. Side effects include an increased risk of having a range of gastrointestinal (GI) adverse events, e.g. nausea, stomach ulcers or bleeding. They also may interfere with kidney function and increase the risk of heart attacks [8].

Moreover, NSAIDs have a short half-life (<2 h), which necessitates multiple dosing for maintaining therapeutic effect throughout the day [9]. In the case of diclofenac sodium which is used for the long-term treatment an effective controlled release formulation would be preferred. An appropriate formulation should provide an initial burst of drug to facilitate rapid onset of action and then maintain a constant plasma level for a prolonged period of time. The development of such a formulation will not only avoid systemic accumulation of drug and related side effects, but will also decrease dosing frequency.

Therefore, a large number of studies demonstrate the development of controlled release administration of NSAIDs, diclofenac salt in particular. Among them, porous silicas emerge as a very attractive drug carriers due to the possibility to synthesize them of the desired chemical character with controlled size, morphology, porosity or/and mechanical rigidity. Moreover, it has been reported that silica gel produced by sol-gel method may be successfully used as a solid carrier for various biologically active molecules. It is possible to produce the silica gel immobilized with drugs without any loss in its biological activity enzymes, proteins and others sensitive molecules [10].

III. 3. UV-VIS spectrophotometry. The Beer-Lambert law.

Spectrophotometry is the basis for a number of techniques used in chemical investigations. The key result for using the intensity of absorption at a particular wavelength to determine the concentration, *c,* of the absorbing species is the empirical *Beer-Lambert law*:

$$A = \varepsilon\, c\, l \qquad (1)$$

where *l* is the length of the path that light is traveling through sample solution (in practice it is the depth/width of spectrometric cuvette) .
The dimensionless absorbance, *A*, of the sample can be expressed by the light intensities:

$$A = log\left(\frac{I_0}{I}\right) \qquad (2)$$

Where I_0 and I are the incident and transmitted light intensities, respectively – see Fig. 11.

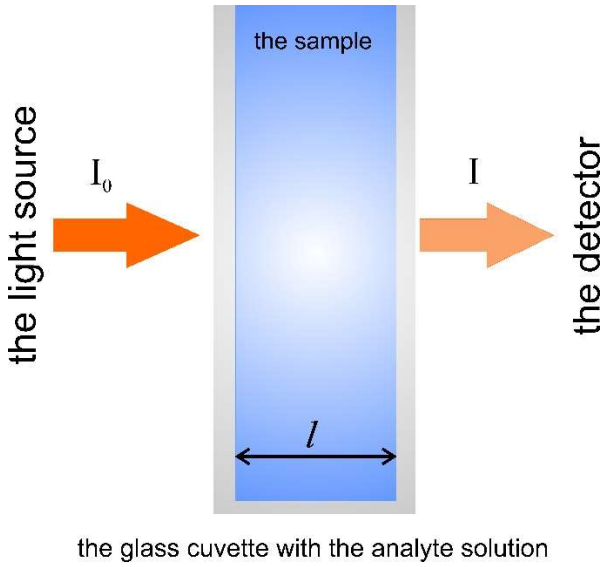


Fig. 11. Schematic representation of spectrophotometric measurements.

The quantity ε is called the molar absorption coefficient. It depends on the wavelength of the incident radiation and is greater when the absorption gets more intense. Typical values of ε for strong transitions are of the order 10^4-10^5 L $mol^{-1}cm^{-1}$. It follows from the Beer-Lambert law that we can observe the appearance or depletion of a species during a reaction by monitoring changes in the absorbance of the reaction mixture.
The UV-Vis spectrophotometers can emit and detect electromagnetic wave (light) in the range from the ultraviolet to near infrared. The electromagnetic spectrum is shown in the figure below in Fig. 12. The range of wavelength used in the spectrometric measurement should be experimentally adjusted to ensure that the light absorbed by the analyte molecules/ions is within this range and so that no other substances present in the solution have ability to absorb the light in chosen range. Usually to get precise information about the wavelength range several

solutions of known concentration are prepared and whole spectrum from 200-800 nm is collected for each of them. Spotted diminishing tendency in absorbance along with decreasing analyte concentration indicates that this region can be used for the analyte concentration monitoring.

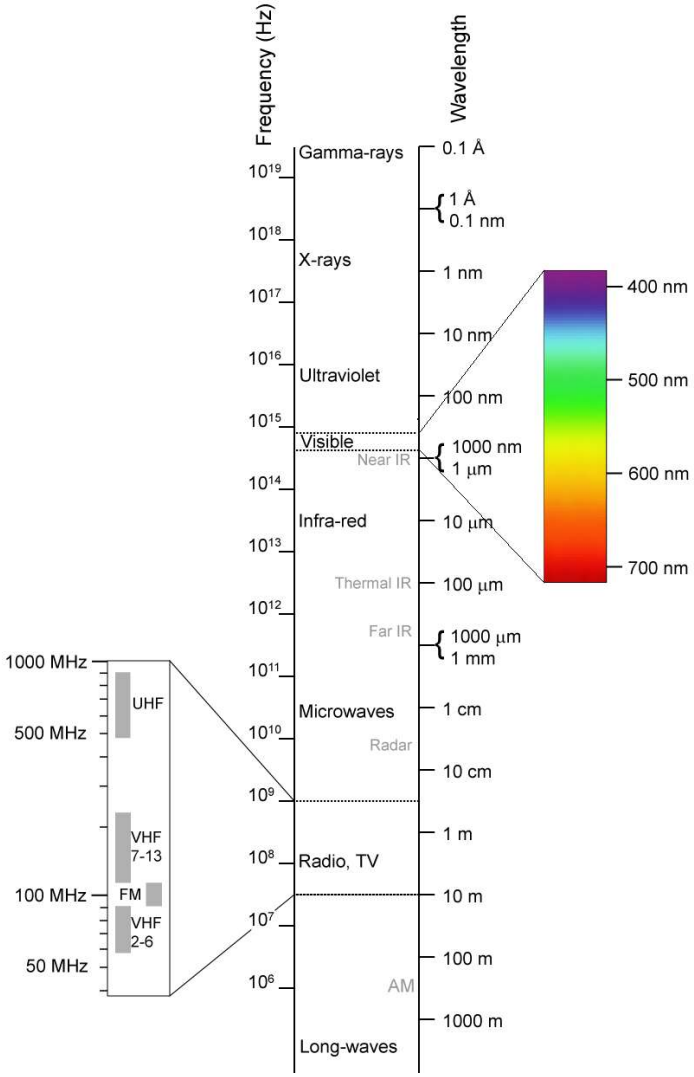


Fig. 12. Electromagnetic spectrum [11].

From pratical point of view if the analyte solution is transparent in the visible light its maximum absorbance will be present somewhere in the ultraviolet region (200-350 nm).

III. 4 Kinetics of reactions. Integrated rate laws.

The rate of reaction is defined as concentration change, Δc, in given period of time, Δt,:

$$v = \pm\frac{\Delta c}{\Delta t} \quad (3)$$

Usually Δc is the change in the molar concentration of the investigated species. If Δc is the substrate loss the minus sign is applied, if otherwise plus sign is present (eq. 3). An empirical observation is that the rate of reactions is often found to be proportional to the molar concentrations of the reactants raised to a simple power. The rate of reaction can be expressed as:

$$v = k[A]^m[B]^n \quad (4)$$

[A] and [B] are the molar concentrations of the reactants A and B. The coefficient *k*, which is characteristic for the reaction being studied, is called the rate constant. It is independent of the concentrations of the species taking part in the reaction but depends on the temperature. The *m* and *n* powers have nothing in common with respective stoichiometric coefficients of the balanced equation; both parameters should be determined experimentally. Equation 4 is called the rate law of the reaction.

Reactions can be classified on the basis of their order, the power to which the concentration of a species is raised in the rate law. A reaction with the rate law in equation 4 is *m*-order for A and *n*- order for B. The overall order of a reaction is the sum of the orders of all the components.

Table 2. Kinetic equations for zero-, first- and second-order reactions.

	Zero-order	**First-order**	**Second-order**
Rate law	$-\frac{d[A]}{dt} = k$	$-\frac{d[A]}{dt} = k[A]$	$-\frac{d[A]}{dt} = k[A]^2$
Integrated rate law	$[A] = [A]_0 - kt$	$[A] = [A]_0 e^{-kt}$	$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$
Linear plot	$[A]\ vs.\ t$	$\ln([A]) vs.\ t$	$\frac{1}{[A]}\ vs.\ t$

To obtain by graphical method the order of the reaction it crucial to find linear relation between concentarion and the time (as shown in last row of Table 2). Sometimes it is impossible to estimate order of the reaction for both substrates. In that case one of the substrates is used in excess so that it can be assumed that its concentration during the experiment does not change. Obtained order is called *pseudo order* e.g. if B is the reactant whose concentration is constant, then the equation $V = k\,[A][B]$ can be written as $V = k'[A]$. The second order rate equation has

been reduced to a *pseudo first order* rate equation. This simplifies acquiring the integrated rate equation [12].

References to the *Theory*

[1] https://en.wikipedia.org/wiki/Silicon%E2%80%93oxygen_tetrahedron
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[4] Y. Wan, D. Zhao, Chemical Reviews, 107 (2006) 2821-2860.
[5] Fig 6. b & c b& c https://www.acsmaterial.com/mcm-232.html
[6] C. Sanchez, G.J. de A.A. Soler-Illia, F. Ribot, T. Lalot, C.R. Mayer, V. Cabuil, Chemistry of Materials, 13 (2001) 3061-3083.
[7] M. Tuncay, S. Calis, H. S. Kas, M. T. Ercan, I. Peksoy, A. A. Hincal, Journal of Microencapsulation 17 (2000) 145-155.
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[9] M. Tunçay, S. Caliş,H.S. Kaş, M.T. Ercan, I. Peksoy, A.A. Hincal, Journal of Microencapsulation 17 (2000) 145-155.
[10] M. Prokopowicz, J. Lukasiak, A. Przyjazny, Journal of Biomaterials Science, Polymer Edition, 15 (2004) 343–356.
[11] http://en.wikipedia .org/viki/File:Electromagnetic-Spectrum.png
[12] P. Atkins, J. de Paula – "Physical Chemistry for the Life Sciences', Oxford University Press, 2006.

IV. Experimental

A. Equipment and reagents

1. Instrumentation:

· UV-Vis Spectrometer Varian Cary 100 BIO.
· Automatic pipette (5 ml) 1 pcs.
· 2 quartz cuvettes for the spectrophotometer (4 ml).

2. Equipment:

· Calibrated glass vessels (100 ml) 5 pcs.

3. Reagents:

· Diclofenac sodium , M = 318,129 g/mol.
· Phosphate buffer pH=7.4.4
· Diclofenac sodium stock solution (0.5% w/w)
· Distilled water.

Safety instructions:
Diclofenac sodium is an anti-inflammatory drug. Safety gloves should be on during its solution handling. Wastes with diclofenac should not be disposed into the sink.

B. Preparation of the spectrometer for the measurements

1. Turn on the computer and the spectrometer (the ON/OFF buttons are on the front panels of both devices).
2. For calibration curve:
    a. Click: *Start (Windows)* → *Scan*.
    b. Click: *Setup*. In *Cary* set *Start* and *Stop* according to the C. 2. Change *Ymax* to 3.00.
    c. Click: *Baseline* and check *Baseline correction*. Click *OK*.
3. On the main panel click *Baseline*. Put blank sample (for which A = 0) into the cuvette holder and confirm OK. Baseline measurement needs to be done only once.
4. Put sample in the cuvette into the spectrometer and click *Start,* indicate file directory and confirm OK. Name the sample and press OK.
5. After the last calibration curve measurement click *File*→*Save data as*. Introduce the name of the data and save as .CSV file.
6. For release measurements repeat 2a-2c procedure. In *Cary* check *Cyclic mode*, set *cycle count* for 60 and *cycle time* to 1.00. The device will measure UV-Vis spectrum every minute for 60 minutes.
7. To turn off the spectrometer simply press OFF button at the front of the device.

C. The execution of exercise

1. By diluting diclofenac sodium stock solution prepare four calibration solutions (5.27×10^{-6} mol/dm^3, 1.54×10^{-5} mol/dm^3, 7.71×10^{-5} mol/dm^3, 1.58×10^{-4} mol/dm^3) in 100 ml calibrated glass vessels.
2. Measure absorbance for each solution (3 ml sample) in range of 200-350 nm. Set baseline (A = 0) for buffer solution. Choose maximum absorbance value at which spectrophotometric analysis will be conducted.
3. Weight MCM 41 and Si 60 with adsorbed drug materials. (Previously to the class, the teacher will absorb on approx. 50 mg of vacuum dried material the diclofenac sodium from 0,5 % w/w ethanolic solution).
4. Add MCM 41 into the quartz cuvette with phosphate buffer and start measuring UV spectra every minute, up to 40 min. Desorption starts when solid drug carrier has contact with aqueous solution.
5. Monitor concentration changes for at least 60 min.
6. Repeat procedure in point 4 and 5 with Si 60 material.

D. Analysis of the results

1. Present in one graph changes in time for diclofenac sodium release spectra.
2. On the basis of calibration solutions measurements, plot $A = f(c_{diclo})$ graph and estimate quantitative relation between absorbance and concentration.
3. Using graphic method estimate order of the desorption kinetics.
4. Calculate rate constant for the process.
5. Try to explain why drug release curves have at least two regions of the substance release.
6. Estimate whether or not whole adsorbed drug was released and which solid material is better for potential medical application.