Incorporating Opioids into Micro - to Nano - Structurally Optimized Silica Xerogel Controlled Release Delivery Systems Prevents Abuse

George K. Toworfe, PhD
School of Foundation, Bahrain Polytechnic University, Isa Town, Kingdom of Bahrain, Center for Bioactive Materials and Tissue Engineering, Department of Bioengineering, School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, USA

Doi:10.19044/esj.2019.v15n24p1 URL:http://dx.doi.org/10.19044/esj.2019.v15n24p1

Abstract

Analgesics is a multibillion dollar worldwide industry. Oxycodone, an opioid, is currently administered using controlled release tablets; although this has resulted in considerable control over pain management, the controlled-release tablets have been abused by drug users. Measures to curb illicit use of the drug have not been successful, to date. This study proposes that by incorporating the medicine into a micro- to nano-structurally optimized delivery material, release concentrations that cannot exceed therapeutic target levels will be achieved and therefore, curbing its misuse, even when the carrier material is crushed. Using Dextromethorphan (Dextro) as a model drug, silica xerogels (sol-gels) were synthesized by mixing tetramethoxysilane, Deionized water and HCl, in addition to Dextro-methanol solution. Different drug loads and water-to-alkoxide ratios were observed to alter the micro- and nano-structurural properties. Different particle sizes were produced by crushing the xerogel discs, after which they were sieved and immersed in 5mg/ml PBS solution. Concentration of released Dextro was measured spectrophotometrically at 280 nm. The effect of particle size on *in vitro* release of Dextro from xerogels, as a function of immersion time, demonstrated that both micro- to nano-sized particles exhibited time-dependent release of Dextro. Although the release from nano-sized particles was noticeably faster than from the micro-sized ones, they did not show any burst release. Data obtained demonstrate that synthesizing a delivery system capable of achieving the controlled-release of therapeutically relevant doses and misuse resistance are attainable.

Keywords: Silica xerogel (sol-gel), Dextromethorphan (Dextro), microstructure, nanostructure, controlled-release, opioids, abuse

Introduction

Analgesics represent hundreds of billions of dollars per year industry worldwide, and especially in the United States**.** OxyContin was introduced in 1996 and sales grew from $48 Million to $1.1 Billion in 2000 (Art, 2009). The market consists of drugs used for the alleviation of pain due to injury, surgery, illness and chronic diseases. In previous years, morphine and many opioids derivatives have been widely used for the treatment of pain resulting from trauma, cancer or surgery. OxyContin is a semisynthetic opioid analgesic prescribed for acute, chronic or long-lasting pain. It contains between 10-160 mg of oxycodone HCl (active ingredient) in a time-release tablet (CSAT Advisory, 2001). The sales of OxyContin in the United States generated $1.2 billion in 2003 (Oxycontin, 2004); this rose up to $2.6 billion in 2012, although sales have declined in recent years, sales went down to $1.74 in 2017. In addition, opioids abuse has been linked to more than 42,000 overdose deaths in the United States in 2016, according to a report by the U.S. Centers for Disease Control and Prevention (Rudd et al, 2016). Studies have proven OxyContin to be effective in the treatment of various types of pain. It is most often prescribed for pain associated with arthritis, lower back conditions, injuries, and cancer. In 1996, controlled-release OxyContin became available in 12-hour time release tablets in doses ranging from 10 mg to 160 mg per tablet (Oxycontin, 2004). The higher doses of 80 mg and 160 mg are administered to opioid (narcotic) tolerant individuals. Unlike other analgesics, like aspirin or acetaminophen, with threshold to their effectiveness, OxyContin can potentially provide up to four times the relief of a non-opioid analgesic, such that the most severe degree of pain can be managed (Kaplan et al, 1998; Hale et al, 1999; Staumbaugh et al, 2001; Art, 2009). The development of these controlled-release tablets was a marked improvement over the conventional tablets that only lasted for 3 to 4 hours and required patients to swallow more tablets each day in order to control their intense pain.

Although controlled-release tablets have enabled patients to gain considerable control over their pain, OxyContin has become a significant source of drug abuse. It has been documented that emergency rooms in the US treated an estimated 5,261 people from January to June 2000 for abuse of OxyContin (Pleurvy, 2004, CBHSQ, 2016) and these figures have risen astronomically since. In 2015 alone, drug overdose was reported to be the leading cause of accidental death in the US, with 52,404 lethal drug overdoses. Opioid addiction has been identified to be driving this epidemic, with 20,101 overdose deaths related to prescription pain relievers, and 12,990 overdose deaths related to heroin (Rudd et al, 2016) It is estimated that 20.5 million people in the United States are dependent on or abuse opioid painkillers and that 23% of the population develop opioid addiction (NIDA, 2014). Drug

overdose is the leading cause of accidental death in the US and opioid addiction is driving this epidermic (NIDA, 2014; Rudd et al, 2016).

OxyContin is abused by crushing or dissolving the tablets and swallowing, snorting or injecting the drug contents released. When abused, OxyContin can be dangerously addictive. In addition to addiction due to drug abuse, psychological well-being of individuals can be inhibited as well (Olufunke, 2017). Measures taken by manufacturers and traditional pharmaceutical companies to curb the illicit use of the drug have not been successful yet. Thus, there is a considerable interest in the development of alternative controlled release methods (Kota et al, 2005; Wang et al, 2009, Tahereh et al, 2013; Purdue Pharma, 2019) that are abuse-proof. This study proposes that by incorporating the medicine in a nanostructurally optimized delivery material, release concentrations will be achieved that cannot exceed therapeutic target levels even when the carrier material is crushed. This preliminary data represents an outstanding basis for achieving controlled-release of Oxycontin for pain relief over a long period of time, as well as, inhibiting its use for recreation.

Using silica precursors such as tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS), silica sol gels can be synthesized via hydrolysis and condensation reactions (Kota et al, 2005, Yoshio et al, 2005, Beibei et al, 2011). The hydrolysis reaction, which could be either acid or base catalyzed, could replace alkoxide groups with hydroxyl groups. Siloxane bonds (Si-O-Si) may be formed during subsequent condensation. Alcohol and water which are byproducts of the condensation reaction evaporate while drying. The step by step chemical reactions within the process starts with the Stöber Process (Radin and Ducheyne, 2004; Tian-Song et al, 2009, Li and Jianjun, 2013) hydrolysis, and ends with condensation.

Theoretically, the chemical reactions that occur in the process are modelled according to the Stöber Process (Stöber, 1968; Wang et al, 2009; Tahereh et al, 2013) which involved hydrolysis and condensation. The overall reaction may be summed up as:

$$n\mathrm{Si(OR)_4} + 2n\mathrm{H_2O} \rightarrow n\mathrm{SiO_2} + 4n\mathrm{ROH}$$

However, the completion of the reaction and the chemical composition of the resulting products depend on the excess of water above the stoichiometric H2O/Si ratio of 2. Many other sol-gel processing parameters (such as pH of the sol, type and concentration of solvents, temperature, aging and drying schedules) can also affect the composition, structure, and properties of the resulting products (Kota et al, 2005, Yoshio et al, 2005, Beibei et al, 2011).

Drug molecules are then incorporated into the nano-sized pore channels of the micro-to-nano-sized particles and are released by diffusion through the aqueous phase that penetrates these pores. In our laboratory, we

have developed a deep understanding regarding nano-structural control of the release properties of sol-gels (Oxycontin, 2004; Rudd et al, 2016). The variation of processing parameters leads to variations of pore sizes and porosity, which in turn affect the release rates of the drug molecules (Tian-Song et al, 2009; Li and Jianjun, 2013). These findings were summarized in a review paper titled "Nanostructural control of implantable xerogels for the controlled release of biomolecules" (Falaize et al, 1999; Radin and Ducheyne, 2004).

The goals of this research were (i) to develop a sol-gel system for the release of an opioid compound with the potential to provide sustained relief from post-operative pain over a period of time (from one to several weeks); (ii) formulate a drug carrying system that will be impervious to recreational use/abuse, either by crushing, or by dissolving in solution to obtain a burst release of the active ingredients. By using a model opioid such as dextromethorphan, it is possible to determine the feasibility of loading high concentrations (up to 80 mg/g) of the drug into the xerogels; demonstrate the principle of preventing abuse of drug by determining the effects of drug load (high versus low) and particle size (fine versus large) on the release profile of dextromethorphan from silica xerogels.

Materials and Methods:

In this first series of experiments, we used a model molecule, Dextromethorphan, which is an inactive opioid. The experimental design was based on the assumptions that: the use of low water/alkoxide ratio would enable the prevention of burst release from finely crushed particles (the water-to-alkoxide ratio is a parameter associated with the synthesis of the nanostructure sol-gel controlled release carriers); since solubility of dextromethorphan is low in water (15 mg/ml) and high in alcohols (up to 200 mg/ml in methanol), the use of alcohol solutions for the synthesis of drug-loaded xerogels would result in obtaining higher loads (up to 80 mg/g) of this drug.

Table 1 shows comparison between the characteristic properties of Oxycontin and the model opioid, Dextromethorphan, used in the study. Silica xerogels with water/alkoxide ratio of 4 and nominal dextromethorphan concentrations (W, %) of either 20 mg/g or 80 mg/g, were synthesized by using acid-catalyzed hydrolysis of Tetramethyl Orthosilicate (TMOS-98%, Aldrich). After mixing TMOS, DI water and 1N HCl a sol was formed and corresponding amounts of Dextromethorphan - Methanol solution were added.

The sols were cast into polystyrene vials (1 ml in each vial), sealed, allowed to gel and age for 3 days. The vials were unsealed, and the gels were dried at room temperature till constant weights were attained. Weight loss for both 20mg/g and 80 mg/g gels was about 70%. The resulting xerogel disks

were crushed and particles of three different size ranges, 200-500 □m, 40-70 □m, and 20-40 □m, were produced by sieving, although the smallest micro- to nano-sizes were exceedingly difficult to produce. The various stages in the chemical processes including hydrolysis and condensation are outlined below:

Chemical Reactions:

Hydrolysis: $\mathbf{Si\text{-}OR + H_2O \longrightarrow Si\text{-}OH + ROH}$

Alcohol: $\mathbf{Si\text{-}OH + RO\text{-}Si \longrightarrow Si\text{-}O\text{-}Si + ROH}$

Water Condensation: $\mathbf{Si\text{-}OH + HO\text{-}Si \longrightarrow Si\text{-}O\text{-}Si + H_2O}$

Overall Reaction: $\boldsymbol{n\mathrm{Si(OR)_4 + 2}n\mathrm{H_2O \longrightarrow }n\mathrm{SiO_2 + 4}n\mathrm{ROH}}$

- **Stöber Process** (Stöber, 1968; Wang et al, 2009; Tahereh et al, 2013)

  Hydrolysis:

  $$\mathbf{Si(OR)_4 + 4H_2O \xrightarrow[ROH]{NH_3} Si(OH)_4 + 4ROH}$$

  $$\mathbf{Si(OH)_4 \longrightarrow SiO_2 + 2H_2O}$$

  R = Alkyl, preferred $\boldsymbol{C_2H_5}$

NB: Using TEOS alkoxide and ammonia catalyst, spherical nanoparticles with well-defined sizes were produced.

The drug release procedure was carried out in phosphate buffered saline solution (PBS) environment at pH of 7.4 and at a physiological temperature of 37 °C. The setup was placed on a continual vibrating and rotating platform at 100 rpm.

The particles which were obtained as a result of the crushed xerogels were immersed in PBS at a ratio between weight and solution volume of 5 mg/ml. Meanwhile, the solutions in which the particles were immersed were exchanged daily. The concentration of released dextromethorphan (into the surrounding PBS solution) was measured spectrophotometrically at 280 nm.

Results:

Figure 1 shows a schematic and spatial orientation of the Dextro molecule indicating the positions of the Oxygen and Nitrogen in the molecule, showing the spatial orientation of Carbon, Nitrogen and Hydrogen atoms. The Nitrogen atom is very prominent in the structure (Falaize et al, 1999) and the chemical formula of Dextro: C18H28BrNO2 (Table 1).

In vitro release of dextromethorphan as a function of immersion time, for 20mg/g and 80 mg/g, xerogels is shown in Figures 2 and 3 respectively. The figures show the mean cumulative release of Dextromethorphan from 20 mg/g-xerogels (Fig. 2) and from 80 mg/g-xerogels (Fig. 3) as a function of immersion time in PBS. The percentage release at various time points is also indicated (n = 3, error bars represent standard deviation – by their small sizes). The data demonstrates that both large (200-500 μm) and fine (20-40 and 40-70 μm) particles showed time-dependent and load-dependent release of Dextro. Although the release from smaller micro- to nano-sized particles was noticeably faster than from larger ones, the smaller particles did not show any burst release. Specifically, after one day of immersing particles in PBS, only 2% to 4% of the original load were released from the micro- to nano- sized particles obtained from the 20mg/g- and 80 mg/g-xerogels, respectively.
The controlled burst release continued throughout the period in which particles were immersed in PBS. Over the seven days duration, only 17% of the original drug load was released from the micro- to nano-sized particles obtained from both the 20 mg/g- and 80 mg/g-xerogels.

Table 1: *Table shows a comparison between the characteristic properties of Oxycontin and the model opioid, Dextromethorphan Hydrobrmide (NCBI, 2019a; NCBI, 2019b)*

Property	OxyContin	Dextromerthophan HBr
1. Molecular weight	351.83	370.331
2. Chemical Formula	$C_{18}H_{21}NO_4.HCl$	$C_{18}H_{28}BrNO_2$
3. Physical properties:	White, odorless, crystalline	White, odorless, crystalline
4. Solubility	16g/100mL	1.5g/100mL
a) In water	Slightly soluble in alcohol	Soluble 1 in 10 of ethanol (10g/100mL)
b) In alcohol		Freely soluble in chloroform
5. pH		Of 1 % aqueous solution is 5.2 – 6.5
6. Chemical name	4, 5α-epoxy-14-hydroxyl-3-methyl-17-methylmorphinan-6-one hydrochloride	3 methoxy-17-methylmorphinan monohydrate
7. Type	Opioid analgesic (oxycodone is pure agonist opioid)	Synthetic compound; d-isomer of levophenol, a codein analogue and opioid analgesic
8. Drug name	Oxycodone hydrochloride	Dextromethorphan hydrobromide Dextromethorphan hydrobromium Demorphan hydrobromide
9. Trade name	OxyContin	Polistirex Exended Release Suspension
10. Therapeutic dosages	10, 20, 40 80 & 160 mg	10, 20, 30 and 60 mg

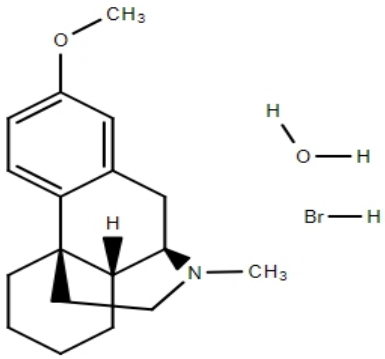

Figure 1. *A-2D schematic of the Molecular structure of Dextromethorphan Hydrobromide (Dextro), with formula C18H28BrNO2, similar to OxyContin. (NCBI, 2019c)*

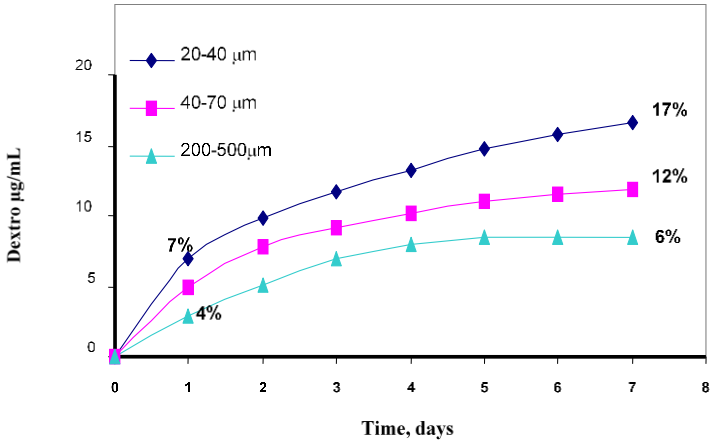

Figure 2. *Mean cumulative release of Dextromethorphan from 20 mg/g-xerogels.*

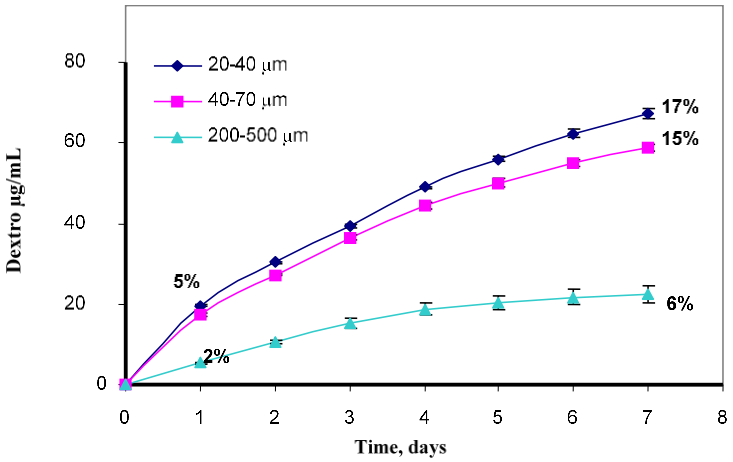

Figure 3*. Mean cumulative release of Dextromethorphan from 80 mg/g-xerogels.*

Discussion and Conclusion:

The formulation of the research question was based on the premise that a novel sol-gel controlled-release technology will offer a fundamental solution to the issue of abuse associated with controlled-release opioids. The data obtained in this study, so far, demonstrates that a delivery material can be synthesized to achieve controlled release of therapeutically relevant doses. The experiment was carried out over a seven-day period, for a start. The researcher intends to extend the duration of the experiments for longer periods in order to further monitor the controlled-release mechanism of opioids.

The data obtained so far, affirms that the principle of misuse resistance can be accomplished, since no burst release associated with the controlled-release sol-gel materials (Santos et al, 1999; Radin et al, 2001; Cicero et al, 2012), was observed. These finding were valid and independent of the size of the controlled release particles. It is obvious, therefore, that it may not be feasible to dissolve the drug and obtain large quantities sufficient for recreational use in any reasonable time frame. In addition, the controlled-release carrier cannot be cast into much smaller particle sizes, less than the micro-to nano-sized particle sizes obtained, which may be useful for injection (abuse). That is, reducing the particle sizes, further, to ranges below what was realized, in this study, may be very challenging, unrealistic and may not lead to any significant increase in the release or burst effect. Therefore, any attempt to formulate the products to obtain below the nano-sized formulations, may be highly impracticable and may only lead to strengthening the misuse resistance concept.

In 2009, an abuse-deterrent formulation of the prescription opioid OxyContin was introduced with the intention of making OxyContin more difficult to solubilize or crushed, thereby discouraging its abuse through injection and inhalation. Subsequently, a study was done (between 2009 and 2012) to examine the effect of the abuse-deterrent formulation on its use in relation to other opioids (Ciero et al, 2012). The study showed that users unanimously preferred older versions of drugs since those were more prone to abuse. There was, however, no evidence to support the assertion that abusers of OxyContin terminated their drug misuse because of the abuse-deterrent formulation. Instead, it appeared that they simply switched their attention to alternative drug choices. The data, nevertheless, shows that an abuse-deterrent formulation can successfully reduce abuse of an opioid to a very large extent. However, an unanticipated outcome that may be generated, which is, replacing the abuse-deterrent formulation with an alternative opioid medication may pose a greater overall risk to public health than OxyContin. The study therefore concluded that abuse-deterrent formulations is not the "magic bullet" that may hopefully solve the growing problem of opioid abuse (Ciero et al, 2012). In view of this and many other findings, it is imperative that an

alternative and viable solutions are sought to address the abuse problem. The solution lies, therefore, in engineering an abuse-free drug carrier to alleviate the problem, which this study addresses.

The objective of this study was to control opioid release, independent of the size of the pellet or powder particles in which the drug was incorporated. This was achieved by encasing the active substance in pore spaces within the fabricated sol-gels, such that they could not be breached mechanically since this class of controlled-release materials were made as monoliths (Aughenbaugh et al, 2002, Radin et al, 2004, Taylor et al, 2016) and could not be crushed to smaller particle sizes. These sol-gel particle sizes are three orders of magnitude larger than the pore channels (about 2 nm) that were created to contain the opioid. Consequently, the data obtained in this study, therefore, is proof of the principle that synthesizing a micro- to nano-sized dimensional drug delivery carrier that may be highly resistant to misuse and abuse is feasible and achievable.

Acknowledgements

The author would like to acknowledge the immense guidance and technical support received from Paul Ducheyne, Shula Radin both of the Centre for Bioactive Materials and Tissue Engineering, the University of Pennsylvania and Alan Zeiger of the Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University Hospital, Philadelphia. This work was supported by Nanotechnology Institute Grant (NTI - USA).

References:

1. Art, V.Z. (2009). The Promotion and Marketing of OxyContin: Commercial Triumph, Public Tragedy. *Am J Public Health*. 99(2): 221 -227
2. Aughenbaugh, W., Radin, S., Ducheyne, P. (2002). Silica sol-gel for the controlled release of antibiotics. II. The effect of synthesis parameters on in vitro release kinetics of vancomycin. *J Biomed Mater Res*, 57: 321-326.
3. Beibei, Z., Caihua, T., Yan, Z., Tao, T., Fengyun, W. (2011). Size control of monodisperse nonporous silica particles by seed particle growth. *Particuology*, 9(3): 314-317.
4. Center for Behavioral Health Statistics and Quality (CBHSQ). (2016). Key substance use and mental health indicators in the United States: Results from the 2015 *National Survey on Drug Use and Health* (HHS Publication No. SMA 16-4984, NSDUH Series H-51). Retrieved from http://www.samhsa.gov/data

5. Cicero, T.J., Ellis, M.S., Surratt, H.L. (2012). Effect of Abuse-Deterrent Formulation of OxyContin. *N Engl J Med,* 2012(367): 187-189. DOI: 10.1056/NEJMc1204141
6. CSAT Advisory (2001). *Breaking News for the Treatment Field,* April 2001, Vol 1, Issue 1.
7. Falaize, S., Radin, S., Ducheyne, P. (1999). In Vitro behavior of Silica-Based intended of controlled release carriers. *J Am Ceram Soc,* 1999(82): 969-976.
8. Hale, M.E., Fleischmann, R., Salzman, R. (1999). Efficacy and safety of controlled-release versus immediate-release oxycodone: randomized, double-blind evaluation in chronic back pain. *Clin J Pain,* 1999(15): 179–183.
9. Kaplan, R., Parris, W.C., Citron, M.I. (1998). Comparison of controlled-release and immediate-release oxycodone in cancer pain. *J Clin Oncol*, 1998(16): 3230–3237.
10. Kota, S.R., Khalil, E., Tsutomu, K., Kazumi, M., Keisuke, M. (2005). A novel method for synthesis of silica nanoparticles. *Journal of Colloid and Interface Science,* 289(1): 125-131.
11. Li, T., Jianjun, C. (2013). Nonporous silica nanoparticles for nanomedicine application. *Nano Today,* 8(3): 290-312
12. National Institute on Drug Abuse (NIDA). (2014). Drug Facts: Heroin.: *National Institute on Drug Abuse.* Bethesda, MD, USA.
13. NCBI, 2019a; National Center for Biotechnology Information. PubChem Database. Oxycodone, CID=5284603, https://pubchem.ncbi.nlm.nih.gov/compound/Oxycodone.
14. NCBI, 2019b; National Center for Biotechnology Information. *PubChem Database*. *Dextro methorphan,* CID=5360696, https://pubchem.ncbi.nlm.nih.gov/compound/ Dextromethorphan.
15. NCBI, 2019c: National Center for Biotechnology Information. PubChem Database. Dextromethorphan hydrobromide, CID=5462351, https://pubchem.ncbi.nlm.nih.gov/compound/ Dextromethorphan-hydrobromide
16. Olufunke, B.R. (2017). Academic stress and drug abuse as factors inhibiting psychological well-being among undergraduates: its counseling implications. *European Scientific Journal,* 13(8): 60-74.
17. Oxycontin: Facts and Statistics. (2014). *Greater Dallas Council on Alcohol and Drug Abuse*. November 2004.
18. Pleurvy, A. (2004). Analgesics on the World Market. *The Virtual Consulting Group.* December 2004. http://www.v-c-g.co.uk/.
19. Purdue Pharma, 2019. Considerations in Opioid Comparator Selection at the College on Problems of Drug Dependence. *81st Annual Scientific Meeting*, San Antonio, Texas, USA)

20. Radin, S., Ducheyne, P., Kamplain, T., Tan, B.H. (2001). Silica sol-gel for the controlled release of antibiotics. I. Synthesis, characterization, and in vitro release. *J Biomed Mater Res*, 2001(57): 313-320.
21. Radin, S., Ducheyne, P. (2004). Nanostructural control of implantable xerogels for the controlled release of biomolecules. In R. Reis, S. Wiener (eds). *Learning from nature how to design new implantable biomaterials*. Kluwer, The Netherlands, 2004.
22. Radin, S., El-Bassyouni, G., Vresilovic, E., Schepers, E., Ducheyne, P. (2004). In vivo tissue response to resorbable silica xerogels as controlled release materials, *Biomaterials*. 26 (9): 1043-1052.
23. Rudd, R.A., Seth, P., David, F., Scholl, L. (2016). Increases in Drug and Opioid-Involved Overdose Deaths - United States, 2010–2015. *Morb Mortal Wkly Rep*, 2016(65):1445–1452. DOI: http://dx.doi.org/10.15585/mmwr.mm655051e1
24. Santos, E., Radin, S., Ducheyne, P. (1999). Sol-Gel derived carrier for the controlled release of proteins. *Biomater*, 20: 1695-1700.
25. Staumbaugh, J.E., Reder, R.F., Stambaugh, M.D. (2001). Double-blind, randomized comparison of the analgesic and pharmacokinetic profiles of controlled- and immediate-release oral oxycodone in cancer pain patients. *J Clin Pharmacol*, 41: 500–506.
26. Stöber, W., Fink, A. (1968). Controlled growth of monodispersed silica spheres in the micron size range. *Journal of Colloid and Interface Science*, 26(1): 62 – 69. https://doi.org/10.1016/0021-9797(68)90272-5.
27. Tahereh, G., Masoud, S.N., Mehdi, B., Elham, N. (2013). Synthesis and characterization of spherical silica nanoparticles by modified Stöber process assisted by organic ligand. *Superlattices and Microstructures*, 61: 33-41.
28. Taylor, C.P., Traynelis, S.F., Siffert, J., Pope, L.E., Matsumoto, R.R. (2016). Pharmacology of dextromethorphan: Relevance to dextromethorphan/quinidine (Nuedexta®) clinical use. *Pharmacol. Ther*, 164: 170-82. doi:10.1016/j.pharmthera.2016.04.010.
29. Tian-Song, D., Qi-Feng, Z., Jun-Yan, Z., Xin, S., Kong-Tao, Z. (2009). One-step synthesis of highly monodisperse hybrid silica spheres in aqueous solution. *Journal of Colloid and Interface Science*, 329(2): 292-299.
30. Wang, X. D., Shen, Z.X., Sang, T. (2010). Preparation of spherical silica particles by Stöber process with high concentration of tetra-ethyl-orthosilicate. *Journal of Colloid and Interface Science*, 341(1): 23-29. https://doi.org/10.1016/j.jcis.2009.09.018

31. Yoshio, K., Hironori, K., Eiichi, M., Daisuke, N., Mikio, K. (2005). Silica coating of silver nanoparticles using a modified Stöber method. *Journal of Colloid and Interface Science*, 283(2): 392-396.