UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

“DISEÑO DE COMPOSITOS FUNCIONALES DE PSi/QUITOSANO: SÍNTESIS, CARACTERIZACIÓN Y EVALUACIÓN EN TERAPÉUTICOS”

TESIS PARA OBTENER EL GRADO DE:

DOCTOR EN CIENCIAS EN INGENIERÍA QUÍMICA

P R E S E N T A

M.C. CÁNDIDA ANAHY CISNEROS COVARRUBIAS

DIRECTORA DE TESIS

DRA. ALMA GABRIELA PALESTINO ESCOBEDO

SAN LUIS POTOSÍ, S.L.P. MAYO DE 2021

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

"DISEÑO DE COMPOSITOS FUNCIONALES DE PSi/QUITOSANO: SÍNTESIS, CARACTERIZACIÓN Y EVALUACIÓN EN TERAPÉUTICOS"

TESIS PARA OBTENER EL GRADO DE:

DOCTOR EN CIENCIAS EN INGENIERÍA QUÍMICA

P R E S E N T A:

M.C. CÁNDIDA ANAHY CISNEROS COVARRUBIAS

DIRECTORA DE TESIS

DRA. ALMA GABRIELA PALESTINO ESCOBEDO

SINODALES:

Dra. Alma Gabriela Palestino Escobedo

Dr. Sergio Rosales Mendoza | **Dr. Omar González Ortega**

Dr. Raúl Ocampo Pérez | **Dr. Raúl González García**

SAN LUIS POTOSÍ, S.L.P. MAYO 2021

El programa de Doctorado de la Universidad Autónoma de San Luis Potosí pertenece al Programa Nacional de Posgrados de Calidad (PNPC) del CONACYT, registro 000897.

Número de registro de la beca otorgada por CONACYT: 335248

Este trabajo fue apoyado por donaciones del CONACYT: CB 2017/2018 Project, No. A1-S-31287.

AGRADECIMIENTOS

PERSONALES

Principalmente agradezco a Dios por ser mi fortaleza en los momentos difíciles, por permitirme obtener un logro más en mi vida profesional y por concederme vida, salud y amor.

A mi padre, el Sr. José Santos Cisneros Medrano por ser mi fortaleza y mi pilar, por apoyarme en cada una de mis decisiones a pesar de la distancia, por comprenderme, por el esfuerzo que hace en todo momento para darme lo mejor y por el amor incondicional que me ha brindado.

A mi hermano Juan Alberto por estar siempre a mi lado, por los momentos especiales que hemos vivido juntos, por llenar mi vida de alegría y amor, y por apoyarme durante esta etapa.

Al destino, por poner a mi lado a mi esposo Hugo René. Gracias por toda la felicidad y el amor que me das, por ser mi compañero de vida, por caminar a mi lado durante estos 4 años, por comprenderme en mis momentos de estrés, por darme ánimos cuando quiero rendirme, por el tiempo que me dedicas, por tu paciencia, por motivarme a ser mejor, por apoyarme a cumplir mis metas, por los momentos bonitos y, sobre todo, por aguantar mi carácter en momentos complicados. Te amo!!!.

A la Dra. Gaby Palestino por aceptarme como su estudiante para realizar este trabajo de investigación, por darme la oportunidad de ser parte de su equipo de trabajo, por las palabras de apoyo brindadas en momentos difíciles, por todos los conocimientos brindados, por sus consejos tanto personales como académicos, por escuchar mis inquietudes, mis tristezas y alegrías, y por ser además de mi asesora, mi amiga. Mi admiración y enorme cariño siempre para usted. Este logro no hubiera sido posible sin su apoyo, mil gracias.

A mi tío Javier Cisneros, por ser mi segundo papá, por guiarme y apoyarme siempre, por escucharme, por quererme y por estar siempre al pendiente de mi avance personal y profesional, sin duda alguna es una persona esencial en mi vida.

A mi tía Angélica Cisneros por su cariño y amor incondicional, por estar siempre para mí, por apoyarme en todos los sentidos y por el esfuerzo que hace día a día para ayudarme a concluir esta etapa.

A mi segunda mamá, Elena, gracias por ser quién eres conmigo, por educarme, por los valores que me diste, por tu inmenso amor y por cuidarme siempre a pesar de la distancia.

A mis compañeros y amigos de laboratorio por sus comentarios constructivos y positivos, por los momentos agradables, por la convivencia, por las pláticas en momentos de estrés, agradezco a todos su tiempo y cariño.

A la Dra. Lourdes Betancourt por su tiempo, por compartir conmigo sus conocimientos, por su apoyo incondicional en la escritura y discusión del artículo científico, por su compañerismo y sobre todo por su bonita amistad.

Al Dr. Azael Gómez Durán por estar siempre al pendiente de mis avances, por su apoyo en cada duda, por compartir conmigo sus conocimientos y por brindarme momentos de diversión y distracción en días pesados.

Al Dr. Omar González Ortega por los comentarios constructivos en cada avance de tesis, por el apoyo en la revisión del artículo científico y por las asesorías brindadas durante mis estudios de doctorado.

Al Dr. Raúl González García por las asesorías brindadas y conocimientos compartidos en la discusión del diseño de experimentos y por su tiempo.

A mi amiga Diana Moreno por el bonito lazo de amistad que hemos formado, por hacer mi vida más feliz, por brindarme siempre palabras de motivación, por su cariño, por apoyarme en las mejores etapas de mi vida y por su amistad incondicional.

A mi amiga Fide Báez por apoyarme en la realización de mis experimentos, por la bonita amistad que formamos, por tomar café conmigo para distraernos un poco mientras realizamos los experimentos de liberación, por su confianza y disponibilidad siempre, por las risas y pláticas agradables.

A mi amiga Janeth Moreno por su amistad, confianza y apoyo en algunos experimentos de mi tesis y por los momentos de convivencia en días pesados de

experimentos (fines de semana).

A mi amigo César Del Ángel por su linda amistad y por las pláticas por whatsapp en momentos de estrés y desesperación.

A Elioth y Jeny por su apoyo durante su servicio social.

INSTITUCIONALES

A la Universidad Autónoma de San Luis Potosí, que a través del Doctorado en Ciencias en Ingeniería Química me dio la oportunidad de realizar mis estudios de doctorado y de desarrollar el presente trabajo de investigación.

Al laboratorio de Biopolímeros y Nanoestructuras de la Facultad de Ciencias Químicas, por brindarme el espacio, material, reactivos y equipos necesarios para desarrollar mi proyecto de tesis.

Al laboratorio de Bionanomateriales del Centro de Investigación en Ciencias de la Salud y Biomedicina por el espacio otorgado para desarrollar parte experimental del proyecto de tesis.

Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por la beca No. 335248 otorgada durante los estudios de doctorado, y la beca otorgada por el proyecto A1-S-31287 para concluir la tesis doctoral y la escritura de un artículo científico. Un agradecimiento adicional por los fondos brindados realizar esta investigación a través del proyecto CB 2017/2018, No. A1-S-31287.

A mi Comité Tutorial: Dr. Omar González Ortega, Dr. Raúl Ocampo Pérez, Dr. Raúl González García y Dr. Sergio Rosales Mendoza por las aportaciones realizadas durante las revisiones de avance, por sus comentarios constructivos y por el apoyo que sin duda alguna enriqueció este trabajo de tesis.

Al Dr. Jaime Reyes Hernández por el apoyo brindado, permitiendo el uso de sus instalaciones y equipo para la realización de mis experimentos de TGA.

A la Dra. Patricia Aguirre Bañuelos y a la M.C. Rosa Alejandra Hernández Esquivel del Laboratorio de Farmacología por su aportación a este proyecto mediante la realización de los experimentos in vivo.

DEDICATORIA

Al gran amor de mi vida: Mi madre

Profra. Ma. De la Paz Covarrubias Bernal

1969 - 2013

No solo te agradezco mi vida, te dedico cada paso que doy

"TU CORAZÓN VIVE EN EL MÍO, PORQUE NO IMPORTA DONDE TE ENCUENTRES, YO SIEMPRE TE LLEVO CONMIGO"

A mi hija

Ana Victoria Carrizalez Cisneros

El mejor regalo de la vida, mi felicidad de cada día

Mi motivo para crecer personal y profesionalmente siempre

"La vida no es fácil, para ninguno de nosotros. Pero…. ¡qué importa! Hay que perseverar y, sobre todo, tener confianza en uno mismo. Hay que sentirse dotado para realizar alguna cosa y esa cosa hay que alcanzarla, cueste lo que cueste".

Marie Curie

PRODUCTOS OBTENIDOS

ARTÍCULOS CIENTÍFICOS

- Cisneros-Covarrubias C. A., Palestino G., Gómez-Durán C. F. A. Rosales-Mendoza S. and Betancourt-Mendiola M. L. Optimized microwave-assisted functionalization and quantification of superficial amino groups on porous silicon nanostructured microparticles. Anal. Methods, 2021, 13, 516. DOI: 10.1039/d0ay02083d.
- Cisneros-Covarrubiasa C. A., Gómez-Durán César F. A., Aguirre Bañuelos P., Hernández-Esquivel R. A. and Palestino G. Porous silicon microparticles as nanovehicles for the sustained release of tramadol: Kinetic, physicochemical and biological evaluation (**En correcciones**).

CONGRESOS

- 4° Simposio Potosino de Investigación en Ciencia de Materiales. IPICYT. San Luis Potosí, S.L.P. 11 – 13 de Abril de 2018. “Estudio comparativo de los parámetros de síntesis de micro/nanopartículas de silicio poroso obtenidas por ataque electroquímico”. Autores: Cándida Anahy Cisneros Covarrubias a, César Fernando Azael Gómez Durán a, Alma Gabriela Palestino Escobedo.
- 1° Simposio Interno de Investigación. CICSAB. UASLP. San Luis Potosí, S.L.P. 3 de Diciembre de 2018. Ponencia. “Síntesis, caracterización y evaluación de propiedades de vehículos nanoestructurados de silicio poroso para la liberación sostenida de fármacos.
- XXVIII International Materials Research Congress. Cancún, Quintana Roo, México. 18 – 23 de Agosto de 2019. “Porous silicon microparticles as nanovehicles for the sustained release of tramadol: kinetic, physicochemical and biological evaluation”. Autores: Cándida A. Cisneros-Covarrubias, César F. A. Gómez-Durán, Gabriela Palestino.

- Segundo Encuentro de Ingeniería Química. Facultad de Ciencias Químicas. UASLP. 24 de Septiembre de 2019. "Micropartículas de silicio poroso como nanovehículos para la liberación sostenida de tramadol: evaluación fisicoquímica y cinética". Autores: Cándida Anahy Cisneros-Covarrubias, César F. A. Gómez-Durán, Gabriela Palestino.
- Segundo Curso-Taller: Eliminación de compuestos tóxicos del agua: Caracterización de materiales y sus aplicaciones. Facultad de Ciencias Químicas. UASLP. San Luis Potosí, S.L.P. "Micropartículas de silicio poroso como nanovehículos para la liberación sostenida de tramadol: evaluación fisicoquímica y cinética". Autores: Cándida Anahy Cisneros-Covarrubias, César F. A. Gómez-Durán, Gabriela Palestino.
- Concurso de Exhibición de Carteles de Proyectos de Investigación de Estudiantes de Posgrado. Centro Cultural Universitario Bicentenario, UASLP. San Luis Potosí, S.L.P. 11 de Octubre de 2019. "Micropartículas de silicio poroso como nanovehículos para la liberación sostenida de tramadol: evaluación fisicoquímica y cinética". Autores: Cándida Anahy Cisneros Covarrubias, Alma Gabriela Palestino Escobedo, César F. A. Gómez Durán, Lourdes Betancourt Mendiola.
- Simposio Virtual: Nanotecnología y sus aplicaciones en las áreas de la Química. San Luis Potosí, S.L.P. 14 – 16 de Octubre de 2020. "Micropartículas de silicio poroso como nanovehículos para la liberación sostenida de tramadol: evaluación fisicoquímica, cinética y biológica". Autores: Cándida A. Cisneros-Covarrubias, César F. A. Gómez-Durán, Gabriela Palestino, Aguirre Patricia, Hernández Alejandra.

PREMIOS

- 24 de Septiembre de 2019. Mejor cartel de la LGAC Materiales avanzados y nanoestructuras: polímeros, sensores y adsorbentes, con el Póster: "Micropartículas de silicio poroso como nanovehículos para la liberación

sostenida de tramadol: evaluación fisicoquímica y cinética". Autores: Cándida Anahy Cisneros-Covarrubias, César F. A. Gómez-Durán, Gabriela Palestino. Segundo Encuentro de Ingeniería Química. Facultad de Ciencias Químicas. UASLP. San Luis Potosí, S.L.P.

- 14 – 16 de Octubre de 2020. Segundo lugar en el concurso de carteles con el Póster: "Micropartículas de silicio poroso como nanovehículos para la liberación sostenida de tramadol: evaluación fisicoquímica, cinética y biológica". Autores: Cándida A. Cisneros-Covarrubias, César F. A. Gómez-Durán, Gabriela Palestino, Aguirre Patricia, Hernández Alejandra. Simposio Virtual: Nanotecnología y sus aplicaciones en las áreas de la Química. UASLP. San Luis Potosí, S.L.P.

RESUMEN

La investigación relacionada con los sistemas de liberación controlada de medicamentos ha crecido rápidamente en los últimos años, ya que estos sistemas ofrecen propiedades ventajosas tales como eficiencia mejorada, toxicidad mínima y administración amigable en comparación con los procedimientos tradicionales de administración de medicamentos. La mayoría de estos sistemas se han sintetizado utilizando biopolímeros biocompatibles y biodegradables con partículas nanoestructuradas con propiedades biocompatibles, no tóxicas y biodegradables. La combinación de estos materiales permite mejorar las propiedades del sistema de liberación. Principalmente mejorando la presentación temporal y espacial de los fármacos en el organismo y protegiéndolos de la degradación o eliminación fisiológica. En este trabajo se diseñaron, sintetizaron, caracterizaron y evaluaron las propiedades fisicoquímicas y morfológicas de partículas de silicio poroso térmicamente oxidadas (TOPSip) y de compositos funcionales a base de partículas de silicio poroso (PSip) y quitosano (CH). Se utilizó clorhidrato de tramadol (TR) como fármaco modelo para evaluar los perfiles de liberación acumulativa in vitro de TOPSip y de los compositos. Se obtuvieron los porcentajes de capacidad de carga de TR en TOPSip y compositos, y se demostró que la adsorción de TR se rige por fuerzas intermoleculares (fuerzas electrostáticas ion-ion y enlaces de hidrógeno) y por la dimensión de los poros. Se obtuvieron los perfiles de liberación acumulada de TR in vitro en fluidos simulados (gástrico e intestinal) para todos los sistemas de liberación diseñados, obteniendo un tiempo de liberación de 24 h para TOPSip desnudas y tiempos de 30 h para los compositos de PSip-CH con bajo efecto de estallido. Finalmente, la evaluación in vivo utilizando microportadores TOPSip mostró evidencia de efectos antinociceptivos y antiinflamatorios mejores y sostenibles cuando se usan los compositos TOPSip-OH/TR en comparación con TR solo.

ABSTRACT

Studies related to the controlled release system have developed rapidly in recent years to provide beneficial properties such as increased efficiency, reduced toxicity, and helpful administration compared to conventional treatment regimens. Many of these systems are synthesized by combining biopolymers and nanostructured particles, both materials with biodegradable and biocompatible properties and low toxicity. The combination of these materials makes it possible to improve the properties of the delivery system. Mainly improving the spatial and temporal presentation of drugs in the body and protecting them from degradation or physiological elimination. In this work, the physicochemical and morphological properties of thermally oxidized porous silicon particles (TOPSip) and functional composites based on porous silicon particles (PSip) and chitosan (CH) were designed, synthesized, characterized, and evaluated. Tramadol hydrochloride (TR) was used as a model drug to evaluate the in vitro cumulative release profiles of the TOPSip and composites. The percentages of TR loading capacity in TOPSip and composites were obtained, showing that TR adsorption was governed by intermolecular forces (ion-ion electrostatic forces and hydrogen bonding) and by pore dimension. The in vitro TR cumulative release profiles in simulated fluids (gastric and intestinal) were obtained for all the designed delivery systems, obtaining a release time of 24 h for bare TOPSip and for the PSip-CH composites times of 30 h with low burst effect. Finally, the in vivo evaluation using TOPSip microcarriers showed evidence of better and sustainable anti-nociceptive and anti-inflammatory effects when using TOPSip-OH/TR composites compared with TR alone.

INDEX

INDEX OF FIGURES

CHAPTER 1. Porous silicon microparticles as nanovehicles for the sustained release of tramadol: Kinetic, physicochemical, and biological evaluation

CHAPTER 2. Optimized Microwave-Assisted Functionalization and Quantification of Superficial Amino Groups on Porous Silicon Nanostructured Microparticles

CHAPTER 3. Design of functional PSi/CH composites: synthesis, characterization, and evaluation in therapeutics

INDEX OF TABLES

CHAPTER 1. Porous silicon microparticles as nanovehicles for the sustained release of tramadol: Kinetic, physicochemical and biological evaluation

CHAPTER 2. Optimized Microwave-Assisted Functionalization and Quantification of Superficial Amino Groups on Porous Silicon Nanostructured Microparticles

CHAPTER 3. Design of functional PSi/CH composites: synthesis, characterization, and evaluation in therapeutics

1. INTRODUCTION

For many years, pharmaceutical companies have focused on the synthesis of chemical compounds for the treatment of various diseases. For their administration, these therapeutic systems are traditionally obtained in different presentations, including tablets, capsules, suppositories, creams, aerosols, and injections, among some others. The typical formulation of these systems entails certain disadvantages, among them, an instantaneous release of the drug, which makes it difficult to maintain drug concentration levels within adequate therapeutic limits and a low availability of the drug. These disadvantages lead to a multiple dose regimen, which results in significant fluctuations of drug levels in the blood plasma. This effect can decrease the effectiveness of the therapeutic and / or cause a certain degree of toxicity that leads to side effects for patients [1,2]. One option to solve this problem is to use the new emerging technologies offered by nanotechnology, an area in which great progress has been made in the manipulation of supramolecular molecules and structures, which has made it possible to produce controlled drug delivery systems with programmed functions, that drive the drug to a specific site, with the optimal concentration, in a suitable time profile [3].

1.1 Controlled drug delivery systems

Studies related to the controlled release system have developed rapidly in recent years to provide beneficial properties such as increased efficiency, reduced toxicity, and helpful administration compared to conventional treatment regimens [4]. Controlled drug release systems are designed primarily with the objectives of i) obtaining greater control during exposure of the therapeutic substance over time and protecting them from premature elimination, ii) guiding the desired site of action, iii) reducing minimizing their exposure to other parts of the body and iv) helping to cross physiological barriers [5]. Nowadays, the most relevant in this area of study is the design of hybrid systems in which biodegradable and biocompatible biopolymers are combined with various nanostructured materials, producing a new generation of composites with special

properties. This better control of properties shown by hybrid systems has generated great scientific interest to design nanostructured materials with controlled morphologies and pore size that can be used for the development of controlled drug release systems.

In recent years, nanostructured materials have been considered as emerging vehicles for their application as controlled release systems. They have a great variety of advantages, one of the main ones being their great stability, in addition, they have physicochemical and structural properties that make them ideal for the transport of drugs. They can be designed with morphological and chemical properties that increase their biocompatibility and at the same time induce a controllable degradation rate in low toxicity degradation products, which can be eliminated via the kidneys [6]. Some of the most widely used emerging nanomaterials are clays [7], silica nanoparticles [8], graphene [9], carbon nanotubes [10] and porous silicon (PSi) [11].

1.2 Nanostructured porous silicon (PSi)

In recent years, nanostructured porous silicon (PSi) has been extensively used as an attractive and flexible material for biomedical applications mainly related to the development of platforms for drug delivery [12]. PSi is prepared by electrochemical anodization of highly doped crystalline silicon wafers, in aqueous and organic solutions containing hydrofluoric acid (HF) and a surfactant (ethanol). This type of synthesis makes it possible to modulate various characteristics of PSi to control the load and release kinetics of therapeutic agents according to the required applications. Porous silicon particles (PSip) are characterized by having a large surface area (~ 500m^2/cm^3) and a highly reactive surface that can be chemically modified, through functionalization or chemical conjugation with other biomolecules of interest [13]. The pore size and porosity of the PSip can be adjusted by manipulating the synthesis parameters (current density and attack time) to use them as reservoirs of therapeutic agents of different sizes [14]. In addition, it is important to highlight that PSip have high biocompatibility and biodegradability since they degrade, in a physiological conditions, it is converted

to monomeric silicic acid ($Si(OH)_4$), which is the most natural form of silicon that exists in the environment [15].

It has been shown that PSip have resistance to chemical degradation and changes in pH, due to this PSip can be considered as transporting and releasing vehicles for drugs [16]. Some studies that have demonstrated the use of PSip as vehicles are those carried out by Salonen et al, they studied the releases of 5 drugs: furosemide, griseofulvin, antipyrine, ranitidine and ibuprofen and showed that release kinetics can be obtained in periods of 50 - 350 minutes, depending on interaction of the PSip with the drugs, the pH of the medium and the solubility of the drugs [17]. Another example is that of Maniya et al, they studied the release of an antiviral Acyclovir and demonstrated that release kinetics can be obtained in periods of 3-8 hours, which depends on the surface chemistry of the PSip [18].

Although PSip can control drug release behavior, increase oral bioavailability, and drug efficacy, and reduce adverse side effects, they have the disadvantage of the rapid rate of drug release and/or therapeutic agent. One strategy to control the rate of drug release is to coverage the PSip with a biopolymer. The most used biopolymers in this application are natural polymers such as agarose, collagen, polylysine, dextran, hyaluronic acid, chitosan, pectin, carboxymethylchitin, gelatin; in addition, synthetic polymers, and a combination of natural and synthetic polymers [19].

1.3 Chitosan (CH)

Chitosan is a cationic polysaccharide that is commercially available with different degrees of deacetylation (DD) and molecular weights (MW). This polysaccharide is produced from chitin through a deacetylation process that involves alkaline hydrolysis. It is interesting highlight that chitin is the second most abundant biopolymer in the world, is a renewable and sustainable product, and it has a low-cost. Chitin is obtained mainly from the seafood industry as a waste product, this product is used as a pharmaceutical

raw material. Other sources of obtaining chitin are bacteria, fungi, and crustacean shells [20,21]. The properties of CH come from the combination of DD and MW [20].

CH is a hydrophilic copolymer of glucosamine and N-acetylglucosamine. It is considered a reliable excipient due to its advantageous properties. CH has a pKa value of 6.3, that is, at pH values > 6 CH has low solubility, its improve effect is conditioned at the sites of absorption and loses its positive charge, forming aggregates and precipitating from solution [22]. However, a contrary behavior is shown at pH values < 6. CH has high solubility and positive charge, this characteristic provides good properties such as the ability to open tight junctions improving absorption, better interaction with cell membranes and excellent mucoadhesive properties [23]. This attractive property has been used to design drug delivery systems for different vias of administration. The positive charge of CH is the main reason for the mucoadhesion properties. Electrostatic interactions between positively charged CH and negatively charged mucin, presents in the mucus layer, are considered the reason for its good adhesion on mucosal surfaces [21]. Mucoadhesive properties of CH are mainly a function of MW and DD. It has been shown that the mucoadhesive properties of CH increase with high MW and DD, however, high MW and DD decrease the biodegradability of CH, thus preventing the release of therapeutic agents [24]. In vitro studies to determine the mucoadhesion of chitosan have been carried out indirectly, in which the adhesion of mucin on chitosan was evaluated, obtaining mucoadhesion forces ranging from 61.9 to 72% [25].

Based on this information, the idea is to produce a vehicle that allows the drug to be protected and transported until the target site is reached. Once there, fix it in the physiological membrane of interest, so that it can act as a metering valve for the therapeutic agent. Finally, the SiP/CH composite is a promising material for both controlled release and permeability of drugs through intestinal cells.

1.4 Tramadol hydrochloride (TR)

In this work, TR is used as a model drug to evaluate the release profiles of TOPSip and designed composites. TR is an analgesic used for the treatment, management, and relief of moderate to severe pain. TR exhibits opioid and non-opioid activity primarily in the central nervous system (CNS) and it is associated with morphine and codeine, which is 1/10 less powerful than codeine and 1/6000 than morphine. TR was approved as a synthetic analgesic in 1995 by the Food and Drug Administration (FDA) [26]. TR is a reuptake inhibitor of norepinephrine and serotonin. It is suggested for patients who do not respond to certain therapies, or who have contraindications to cyclooxygenase-2 (COX-2) inhibitors and non-selective anti-inflammatory drugs (NSAIDs) [27]. There is a wide variety of pharmaceutical formulations available for TR, which have been administered by different vias including oral, intramuscular, subcutaneous, intravenous, and sublingual [28]. After TR oral administration (main route of administration), TR shows a very fast and almost complete absorption, showing a maximum mean plasma concentration after 2 hours of being administered. TR undergoes first-pass metabolism in the liver and has a bioavailability of approximately 70%. Approximately 20% of the drug binds to plasma proteins and has a half-life of 6 hours. The extraction process involves the kidneys almost entirely. TR is excreted in the urine in different forms, approximately 30% as unchanged drug, 60% as metabolites, and 10% is excreted in the bile. Regarding its solubility, TR is not soluble in organic compounds, it is slightly soluble in acetone and very soluble in methanol and water (more than 20 mg/ml in the pH range of 1.2 to 7.5) [26]. TR is administered every 4 to 6 hours in doses of 50 mg to 100 mg, depending on the needs of the patient, without exceeding a maximum daily dose of 400 mg. The average effective daily dose of TR is between 100 mg and 300 mg [27].

REFERENCES

1. Kost J., Lapidot S. A., Kumar N., Chaubal M., Domb A. J., Kumar R. and Majeti N. V. Drug delivery systems. Enc. of Pol. Sc. Tech. 2004, 5, 697-721, John Wiley & Sons, Inc.
2. Hnawate, R. M. and Deore P. Nanoparticle - novel drug delivery system: A Review. PharmaTutor, 2017, 5, 9-23.
3. Kumar, D. S., Banji D., Madhavi B., Bodanapu V., Dondapati S. and Sri A. P. Nanostructured porous silicon a novel biomaterial for drug delivery. International Journal of Pharmacy and Pharmaceutical Sciences. 2009, 1, 8-16.
4. Hamidi M., Azadi A. and Rafiei P. Hydrogel nanoparticles in drug delivery. Adv. Drug Deliv. Rev., 2008, 60, 1638–1649.
5. Savage D., Liu X. and Curley S. Porous silicon advances in drug delivery and immunotherapy, Curr. Opin. Pharm. 2013, 5, 834-841.
6. Bae K. H., Wang L. S., and Kurisawa M. Injectable biodegradable hydrogels: progress and challenges. J. Mater. Chem. B., 2013, 1, 5371-5388.
7. Viseras C., Aguzzi C., Cerezo P. and Bedmar M. C. Biopolymer–clay nanocomposites for controlled drug delivery. Mater. Sci. Tech., 2008, 24, 1020-1026.
8. Song B., Wu C. and Chang J. Dual drug release from electrospun poly (lactic-co-glycolic acid)/mesoporous silica nanoparticles composite mats with distinct release profiles. Acta Biomater., 2012, 8, 1901-1907.
9. Goenka S., Sant V. and Sant S. Graphene-based nanomaterials for drug delivery and tissue engineering. J. Control. Release, 2014, 173, 75-88.
10. Cirillo G., Hampel S., Spizzirri U. G., Parisi O. I., Picci N. and Iemma F. Carbon nanotubes hybrid hydrogels in drug delivery: a perspective review", Biomed Res Int. 2014.
11. Low S. P., Williams K. A., Canham L. T. and Voelcker N. H. Evaluation of mammalian cell adhesion on surface-modified porous silicon. Biomaterials, 2006, 27, 4538–4546.

12. Zhang H., Liu D., Shahbazi M. A., Mäkilä E., Herranz-Blanco B., Salonen J., Hirvonen J. and Santos H. A. Fabrication of a Multifunctional Nanoin-micro–Drug Delivery Platform by Microfluidic Templated Encapsulation of Porous Silicon in Polymer Matrix, Adv. Mater. 2014, 26, 4497–4503.
13. Wang C. F., Mäkilä E. M., Kaasalainen M. H., Liu D., Sarparanta M. P., Airaksinen A. J., Salonen J., Hirvonen J. T. and Santos H. A. Copperfree azide–alkyne cycloaddition of targeting peptides to porous silicon nanoparticles for intracellular drug uptake, Biomaterials, 2014, 35, 1257-1266.
14. Santos H. A. and Hirvonen J. Nanostructured porous silicon materials: potential candidates for improving drug delivery, Nanomedicine, 2012, 7, 1281–4.
15. Paulis L, Mandal S., Kreutz M. and Figdor C. Dendritic cell-based nanovaccines for cancer immunotherapy, Curr. Opin. Immunol. 2013, 25, 389-395.
16. Chang-Fang W., Mäkilä E. M., Bonduelle C., Rytkönen J., Raula J., Almeida S., Närvänen A., Salonen J., Lecommandoux S., Hirvonen J. T. and Santos H. A. Functionalization of Alkyne-Terminated Thermally Hydrocarbonized Porous Silicon Nanoparticles with Targeting Peptides, ACS Appl. Mater. Interfaces, 2015, 7, 2006−2015.
17. Salonen J., Laitinen L., Kaukonen A., Tuura J., Björkqvist M., Heikkilä T., Vähä-Heikkilä K., Hirvonen J. and Lehto V. P. Mesoporous silicon microparticles for oral drug delivery: Loading and release of five model drugs', Journal of Controlled Release, 2005, 108, 362–374.
18. Maniya N. H., Patel S. R. and Murthy Z. V. P. Development and in vitro evaluation of acyclovir delivery system using nanostructured porous silicon carriers. Chemical engineering research and design, 2015, 104, 551–557.
19. Hamidi M., Azadi A. and Rafiei P. Hydrogel nanoparticles in drug delivery Adv. Drug Deliv. Rev., 2008, 60, 1638–1649.
20. Wang X., Chi N. and Tang X. Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. European Journal of Pharmaceutics and Biopharmaceutics. 2008, 70, 735–740.

21. Andersen T., Bleher S., Flaten G. E., Tho I., Mattsson S. and Škalko-Basnet N. Chitosan in Mucoadhesive Drug Delivery: Focus on Local Vaginal Therapy. Mar Drugs. 2015, 13, 222–236.
22. Wang X., Zheng C., Wu Z., Teng C., Zhang X., Wang Z. and Li C. Chitosan-NAC Nanoparticles as a Vehicle for Nasal Absorption Enhancement of Insulin. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2009.
23. Tamilselvan N. and Raghavan C. V. Formulation and characterization of anti-alzheimer's drug loaded chitosan nanoparticles and its In vitro biological evaluation. Journal of Young Pharmacists. 2015,7.
24. Dasha M., Chiellini F., Ottenbrite R. M. and Chiellini E. Chitosan—A versatile semi-synthetic polymer in biomedical applications. Progress in Polymer Science. 2011, 36, 981-1014.
25. Md S., Khan R. A., Mustafa G., Chuttani K., Baboota S., Sahni J. K. and Ali J. Bromocriptine loaded chitosan nanoparticles intended for direct nose to brain delivery: Pharmacodynamic, Pharmacokinetic and Scintigraphy study in mice model. European Journal of Pharmaceutical Sciences, 2013, 48, 393–405.
26. Vazzana M., Andreani T., Fangueiro J., Faggio C., Silva C., Santini A., Garcia M. L. Silva A. M. and Souto E. B. Tramadol hydrochloride: Pharmacokinetics, pharmacodynamics, adverse side effects, co-administration of drugs and new drug delivery systems. Biomedicine & Pharmacotherapy, 2015, 70, 234-238.
27. McCarberg B. Tramadol extended-release in the management of chronic pain, Ther. Clin. Risk Manage. 2007, 3, 401.
28. Brvar N., Mateovic-Rojnik T. and Grabnar I. Population pharmacokinetic modelling of tramadol using inverse Gaussian function for the assessment of drug absorption from prolonged and immediate release formulations. International Journal of Pharmaceutics. 2014, 473, 170–178.

2. JUSTIFICATION

Based on the advantageous properties of biodegradability, biocompatibility and mucoadhesiveness of the designed composites (UnPSip/CH and TOPSip-OH/A/G/CH), the option of developing new hybrid materials as transport and delivery vehicles for therapeutic agents. In this way, by modifying the synthesis parameters of the composites, the physicochemical and morphological properties can be modulated that allow control of the release, improve the bioavailability and efficacy of the therapeutic agents.

3. HYPOTHESIS

Functional PSip/chitosan composites designed with careful control of their particle size and pore size, surface chemistry, shape, and porosity, impregnated with tramadol will be able to act as sustained release systems. The biodegradability, biocompatibility and mucoadhesive properties of the composites will allow a safe and sustained release of tramadol and will ensure greater permanence and permeability of tramadol at the target site. In addition, using the PSip will increase the adsorption of tramadol and will have a double control of release, from inside the PSip and through the chitosan chain.

4. GENERAL OBJECTIVE

Design, synthesize and characterize last generation functional composites based on nanostructured PSip/chitosan and evaluate their potential application as transport and release vehicles for tramadol.

5. SPECIFIC OBJECTIVES

- Determine the synthesis protocol to obtain PSip and determine the effect of the synthesis parameters on morphology, porosity, particle size and pore size.

- Synthesize and determine the methodology for obtaining and functionalizing the PSip, with the different reagents (thermal oxidation, APTES-GTA, UA), and optimizing the factors that intervene and directly modify the size (pore and particle), thickness and porosity of the PSip.
- Determine the morphological and physicochemical properties of PSip, using characterization techniques such as HRSEM, HRTEM, FTIR, etc.
- Determine the protocol to perform the loading of tramadol in PSip, evaluate the loading percentage, and determine the in vitro release kinetics by simulating the local environment of the gastrointestinal system.
- To evaluate the TOPSip-OH / TR nanostructured composites in carrageenan-induced hyperalgesia and edema models.
- Optimize the APTES functionalization of PSip parameters.
- Incorporate the chitosan to the PSip by chemical binding using APTES-GTA and UA as bridges and establish the synthesis methodology.
- Determine the morphological and physicochemical properties of the UnPSip/CH and TOPSip-OH/A/G/CH composites, using characterization techniques such as HRSEM, HRTEM, FTIR, etc.
- Evaluate the mucoadhesive properties of the UnPSip/CH and TOPSip-OH/A/G/CH composites by mucin adsorption.
- Evaluate the percentage of tramadol loading in the composites and monitor the release kinetics in vitro simulating the gastrointestinal system.
- Obtain the in vitro release kinetics modeling of PSip and composites.

CHAPTER 1

POROUS SILICON MICROPARTICLES AS NANOVEHICLES FOR THE SUSTAINED RELEASE OF TRAMADOL: KINETIC, PHYSICOCHEMICAL AND BIOLOGICAL EVALUATION

Cándida A. Cisneros-Covarrubias[a,b], César F. A. Gómez-Durán[a,b], Patricia Aguirre-Bañuelos[c], Rosa Alejandra Hernández-Esquivel[c], Gabriela Palestino[a,b*]

[a]Laboratorio de Biopolímeros y Nanoestructuras, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, C.P. 78210 San Luis Potosí, México

[b]Sección de Biotecnología, Centro de Investigación en Ciencias de la Salud y Biomedicina, Universidad Autónoma de San Luis Potosí, C.P. 78210 San Luis Potosí, México

[c]Laboratorio de Farmacología, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, C.P. 78210 San Luis Potosí, México

CHAPTER 2

OPTIMIZED MICROWAVE-ASSISTED FUNCTIONALIZATION AND QUANTIFICATION OF SUPERFICIAL AMINO GROUPS ON POROUS SILICON NANOSTRUCTURED MICROPARTICLES

Cándida Anahy Cisneros-Covarrubias,[a] Gabriela Palestino,[ab] César F. A. Gómez-Durán,[a] Sergio Rosales-Mendoza[b] and María de Lourdes Betancourt-Mendiola[*b]

[a]Laboratorio de Biopolímeros y Nanoestructuras, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, C.P. 78210 San Luis Potosí, México.

[b]Sección de Biotecnología, Centro de Investigación en Ciencias de la Salud y Biomedicina, Universidad Autónoma de San Luis Potosí, C.P. 78210 San Luis Potosí, México.

Published in: Analytical Methods

Year: 2021

DOI: 10.1039/d0ay02083d.

CHAPTER 3

Design of functional PSi/CH composites: synthesis, characterization, and evaluation in therapeutics

GENERAL CONCLUSIONS

In recent years, the use of composite materials applied to the controlled release of therapeutic agents grew exponentially due to the advantageous properties they present, in addition, composite materials can be designed by modifying the synthesis parameters with the main objective of obtaining the optimal characteristics that improve the dosage of therapeutic agents. In this research work, two different types of biodegradable and biocompatible drug delivery systems were proposed based on the combination of porous silicon microparticles and chitosan for the transport and sustained release of TR. The synthesis and study of these tramadol release systems was carried out in three stages.

As a first stage, the development of bare thermally oxidized porous silicon microparticles (TOPSip) with different particle sizes, pore sizes and porosity were proposed. A highly efficient top-down approach was used to synthesize the TOPSip and it was shown that the synthesis parameters played an essential role in the materials design. The modified synthesis parameters were current density, concentration of HF relative to ethanol, ultrasonication time, material architecture, and number of perforations. Through the characterization techniques of HRSEM and FT-IR, the successful synthesis of TOPSip was demonstrated. Average size, thickness, and pore dimensions were calculated, measuring at least 150 PSi microparticles randomly selected in the HRSEM images. Modifying these parameters showed that TOPSip with large particle sizes and small pore sizes were produced by using high concentration of HF relative to ethanol (3:1) and low ultrasonication times (15 min) of monolayers. In contrast, TOPSip with smaller particle size and larger pore size were obtained using a low concentration of HF relative to ethanol (3:7) and high ultrasonication time (60 min) of multilayers of 40 defects. With respect to porosity, porosity was determined from gravimetric measurements. High porosities (86 – 89 %) were obtained using low concentrations of HF relative to ethanol. FT-IR, ζ-potential and UV-Vis spectroscopy demonstrated that TR molecules were successfully adsorbed on the TOPSip porous structure by intermolecular forces (ion-ion electrostatic forces and hydrogen bonding)

created between the TR and the TOPSip inorganic surface. Percentages of TR loading capacity of 10 – 31 % were obtained, which was mainly mediated by pore dimension. TOPSip with bigger pore size showed a higher TR loading capacity (31 %), instead, the lowest drug loading capacity (10 %) was found governed by particles with smaller pore size. The in vitro TR cumulative release profiles study was performed at 37 °C in two simulated fluids: gastric and intestinal, simulating the TR process desorption in the human organism. This study showed that TR cumulative release was mediated by the particles pore size, solvent diffusion, and pH. TOPSip with smallest pore size and largest particle dimensions achieved a sustained TR release over 24 h in both simulated fluids with a non-significant burst effect compared to the rest of the nanostructured TOPSip. Due to the high affinity between TR (positive charge) and TOPSip inorganic surface (negative charge) promoted in simulated intestinal fluid, TR sustained release was obtained. On the other hand, in simulated gastric fluid an initial burst release from TOPSip was obtained, which can be explained in terms of microparticles low stability and low binding affinity between TOPSip inorganic surface (positive charge) and TR (positive charge) in strong acid media. The analysis of TR release kinetics for a time of 24 h showed that TR transport is mainly controlled by drug concentration gradient (Fickian diffusion). Finally, the in vivo evaluation using TOPSip microcarriers was performed for the first time. Models of carrageenan-induced inflammation and withdrawal threshold by mechanical stimulation in rats were used. These studies showed that the TOPSip did not cause any adverse effects in rats and evidence of better and sustainable anti-nociceptive and anti-inflammatory effects were obtained when using TOPSip-OH/TR composites compared with TR alone. In addition, it is essential to highlight that the treatment with TOPSip-OH/TR composite at the lowest TR dosage increased the anti-inflammatory and antinociceptive effect compared to TR alone at its highest dose.

However, despite the results of in vivo studies showing that TOPSip microcarriers with reasonable particle size and pore size enhanced the pharmacokinetic effect of TR, in vitro release studies showed an initial burst effect. Based on this, it was proposed to chemically binding CH to the PSip inorganic surface,

to obtain more controlled drug release profiles. The chemical binding of CH to the PSip was carried out using UA and APTES-GTA as bridges. The functionalization protocol of native PSi layers with UA has already been established in our research group, however, the APTES functionalization of PSip has not been fully established. It was observed in the literature that there are many functionalization conditions of PSip with APTES, therefore, as a second part of the work, the APTES functionalization of PSip under different reaction conditions using the traditional and MW-assisted silanization methods was studied. In this study, the modified variables were reaction time (5 - 30 min), reaction temperature (80 - 120 °C), TOPSip chemical surface (silanol and siloxane groups), APTES solution concentration (2 and 5 %) and silanization method (traditional and MW-assisted). Our studies showed that the MW-assisted method had a significant reduction in reaction time (26 min) when compared to the traditional method (24 h), moreover APTES surface coverage increased by 39 % using MW irradiation. Surface modification was evidenced by FT-IR, STEM, and elements mapping; as well as ζ-potential measurements. It was found that silanol groups (Si-OH) in TOPSip-OH increased 3-fold the amino surface coverage when using a 5 % APTES solution. Compared to using TOPSip with siloxane groups (SiO^-) and the same APTES solution. In addition, it was found that using a 5 % APTES solution and TOPSip-OH increased the amino surface coverage compared to a 2 % APTES solution on the same PSip. The optimization and modeling of APTES functionalization on TOPSip-OH with 5% APTES solution and MW-assisted method using a composite central design was proposed. APTES surface functionalization was found to strongly depend on the reaction time, reaction temperature and percentage of APTES. The optimal functionalization conditions were obtained at middle-temperature values (95 ºC), using 5 % APTES solution in dry toluene for 26 min. Finally, the ninhydrin method was adapted to quantify amino functional groups in PSip. The concentration of amino functional groups quantified by the ninhydrin method was confirmed by TGA. Comparing both results, it was concluded that ninhydrin method is an excellent alternative for amino groups quantification. Another important advantage of the ninhydrin method is that this method involves direct reaction with amino groups;

therefore, quantification is primarily directed at accessible amino groups. Which is relevant since the quantified amino groups are used in subsequent functionalization reactions.

Finally, as the third stage of the research work, the synthesis of biodegradable and biocompatible composites based on chitosan and PSip was carried out: UnPSip/CH and TOPSip-OH/A/G/CH. The native PSi layers were functionalized with UA (UnPSip), functionalization conditions established in the literature and implemented in our research group were used, and a concentration of carboxylic groups of 1.6 mEq/g of UnPSip was obtained. On the other hand, APTES functionalization of TOPSip-OH (TOPSip-OH/A) was carried out using the optimal functionalization conditions obtained in the second stage. Subsequently, the functionalization of the amino groups with GTA (TOPSip-OH/A/G) was carried out, obtaining a reaction's efficiency of 81%. CH was chemically linked to the aldehyde groups of the TOPSip-OH/A/G and to the carboxylic acid groups of the UnPSip. FTIR spectroscopy and ξ-potential demonstrated the chemical modifications carried out at each step of the synthesis of composites. TR loading capacity was lower in composites compared to bare TOPSip. This is the result of the prolonged exposure time (24 h) of the UnPSip/TR and TOPSip-OH/A/G/TR with the medium used in the chemical binding of CH (buffer pH 5). TR is highly soluble in aqueous media, therefore, while CH was chemically bound to UnPSip/TR and TOPSip-OH/A/G/TR, TR diffused from the particles into the medium, causing large amount of TR will be removed in the supernatant. TR release of UnPSip/TR/CH and TOPSip-OH/A/G/TR/CH composites were mediated by stiffness to the polymeric chain and intermolecular forces (electrostatic and/or van der Waals) created between composites surface and TR. Through this strategy, a sustained release of TR was achieved along 30 h. TR release was also mediated by pH. UnPSip/TR/CH and TOPSip-OH/A/G/TR/CH composites showed a high burst effect in SGF due to electrostatic repulsion forces between composites and TR. On the other hand, in SIF, a sustained release of TR was obtained due to the high affinity between TR (positive charge) and UnPSip/CH and TOPSip-OH/A/G/CH composites (negative charge) promoted in this physiological condition. The analysis of TR release kinetics for a time of 30 h showed

that TR transport is mainly controlled by drug concentration gradient (Fickian diffusion). To determine the absorption in the intestine and effectiveness of TR, the mucoadhesive properties were studied by the adsorption of mucin. Mucoadhesive properties were higher at pH 4.6 (duodenum pH) compared with SGF and SIF. These results suggest that mainly the composites will remain for an extended period in duodenum, where the absorption of TR begins. Finally, UnPSip/CH is a promising TR delivery system for oral therapy, increasing TR residence in intestine and maintaining a sustained release.

APPENDIX A

Published articles, Congresses and Awards certificates

Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2021, 13, 516

Optimized microwave-assisted functionalization and quantification of superficial amino groups on porous silicon nanostructured microparticles

Cándida Anahy Cisneros-Covarrubias,[a] Gabriela Palestino,[ab] César F. A. Gómez-Durán,[a] Sergio Rosales-Mendoza[b] and María de Lourdes Betancourt-Mendiola[*b]

Received 10th November 2020
Accepted 23rd December 2020

DOI: 10.1039/d0ay02083d

rsc.li/methods

This work presents an optimized microwave (MW)-assisted method for the chemical functionalization of porous silicon particles (PSip). 3-(Aminopropyl)triethoxysilane (APTES) was grafted on previously stabilized PSip. The functionalization efficiency was studied and optimized in terms of reaction time (Rt) and reaction temperature (RT) using a central composite design (CCD). The effect of MW irradiation on the surface coverage was found to strongly depend on the PSip surface chemistry, Rt, RT, and percentage of APTES. Quantification of grafted amino groups was performed by the ninhydrin method (NHIM); confirming the results by thermogravimetric analysis (TGA). Reacting with 5% APTES solution at 95 °C for 26 min was the best functionalization conditions. The efficiency of PSip–APTES prepared under the optimized conditions was compared to those functionalized by the traditional method; MW irradiation increases by 39% the number of functional groups grafted onto the PSip surfaces with the additional benefit of having a drastic reduction in Rt.