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ZSM-5
A promising Drug Delivery Platform for Podophyllotoxin

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Abstract

In pursuit of development of a drug carrying platform for delivery of Podophillotoxin, an anti-cancer drug, a porous metal–organic frameworks (MOFs), ZSM-5, was synthesized and used. The unique structure of ZSM-5which are built of inorganic nodes and organic ligands lead to successful encapsulation of different ions and molecules. Following our recentstudy, ZSM-5was prepared and characterizedusing variety of analytical methods containing FTIR, FESEM, and EDS.The loading and releasing profile of Podophyllotoxin in the synthesized platform ZSM-5were evaluated. The in vitro cytotoxicity results revealed ZSM-5- Podophyllotoxin was able to increase cytotoxicity compared to that of Podophyllotoxin on HT-29 cancerous cells indicating the remarkable role of this drug delivery system.

Keywords: ZSM-5, MOF, drug delivery, Podophyllotoxin, Cytotoxicity

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Fig1. Placement of drug in the structure of MOF.

1. Introduction

Cancer as the most prevalent diseases worldwide is one of the main public health concerns. In spite of intensive efforts for treatment of cancer, the necessity of developing effective agents isn't ignorable [1]. Designing an ideal drug delivery system for targeting cancer cell is considered as a hot topic in life science research. MOFs with crucial features including high drug loading capacity, high surface area, as well as tunable pore size is used for drug delivery intensively[2]. MOFs plays an important role as an carriers in drug delivery because they are non-toxic as well as the uptake of drugs and getting across the cell membrane has been facilitated via controlling the size of MOFs[3].

Podophyllotoxinis anticancer drugs which is able to induces cytotoxic and increase DNA damage[4]. Although, 5 FU frequently applied, developed drug resistance and severe side effects affected its clinical application [5]. Encapsulate of Podophyllotoxinusing various DDS could be an effective idea[6]. In present work, the drug loading capacity of i for 5-FU as an anticancer drug was evaluated. Upon exposure by Podophyllotoxinthe in vitro cytotoxicity against cancer cells were assessed.
Finally but contrary to the original goal of this project, which was to use a muff, because of the simpler and faster synthesis, we carried out this project with a MOF.

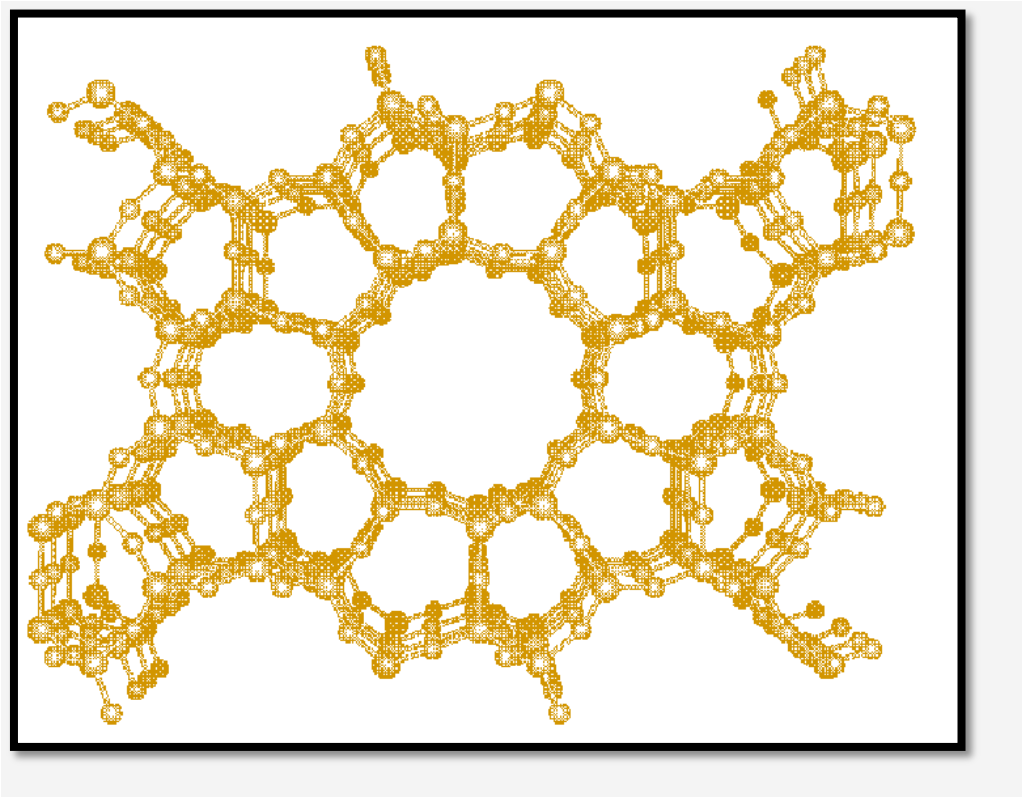

Fig2. The framework structure of ZSM-5.

NanoComposite

Nanocomposite is a multiphase solid material where one of the phases has one, two or three dimensions of less than 100 nanometers (nm) or structures having nano-scale repeat distances between the different phases that make up the material. [7]
The idea behind Nanocomposite is to use building blocks with dimensions in nanometre range to design and create new materials with unprecedented flexibility and improvement in their physical properties. In the broadest sense this definition can include porous media, colloids, gels and copolymers, but is more usually taken to mean the solid combination of a bulk matrix and nano-dimensional phase(s) differing in properties due to dissimilarities in structure and chemistry. [8]
The mechanical, electrical, thermal, optical, electrochemical, catalytic properties of the nanocomposite will differ markedly from that of the component materials. Size limits for these effects have been proposed.

1. <5 nm for catalytic activity
2. <20 nm for making a hard magnetic material soft
3. <50 nm for refractive index changes
4. <100 nm for achieving superparamagnetism, mechanical strengthening or restricting matrix dislocation movement [9].

Nanocomposites are found in nature, for example in the structure of the abalone shell and bone. [10] The use of nanoparticle-rich materials long predates the understanding of the physical and chemical nature of these materials. Some researchersinvestigated the origin of the depth of color and the resistance to acids and bio-corrosion of Maya blue paint, attributing it to a nanoparticle mechanism. From the mid-1950s nanoscale organo-clays have been used to control flow of polymer solutions (e.g. as paint viscosifiers) or the constitution of gels (e.g. as a thickening substance in cosmetics, keeping the preparations in homogeneous form). By the 1970s

polymer/clay composites were the topic of textbooks, although the term "nanocomposites" was not in common use. [11]

In mechanical terms, nanocomposites differ from conventional composite materials due to the exceptionally high surface to volume ratio of the reinforcing phase and/or its exceptionally high aspect ratio. The reinforcing material can be made up of particles (e.g. minerals), sheets (e.g. exfoliated clay stacks) or fibers (e.g. carbon nanotubes or electrospun fibers). [12] The area of the interface between the matrix and reinforcement phase(s) is typically an order of magnitude greater than for conventional composite materials. [13] The matrix material properties are significantly affected in the vicinity of the reinforcement. Some scientists be aware that with polymer nanocomposites, properties related to local chemistry, degree of thermoset cure, polymer chain mobility, polymer chain conformation, degree of polymer chain ordering or crystallinity can all vary significantly and continuously from the interface with the reinforcement into the bulk of the matrix. This massive quantity of reinforcement surface area means that a relatively small amount of nanoscale reinforcement can have an observable effect on the macroscale properties of the composite. [14]

Zeolite

Zeolites are a group of crystalline materials made up of evenly sized pores and tunnel systems. When purifying VOCs and hydrocarbons, we use a synthetic hydrophobic zeolite. When the contaminated air passes through the material, the hydrocarbons are adsorbed. The material can adsorb a certain amount of hydrocarbons before needing to be regenerated. [16], [17]

A smaller flow of hot air is then directed through the material so that the hydrocarbons release from the zeolite in a higher concentration. This enables more cost-effective incineration. One of its strengths is that it is non-combustible–meaning it can withstand very high temperatures. [18] This means that we are also able to purify volatile hydrocarbons such as fumes emitted from vulcanization, plastic smoke and styrene, all of which require very high temperatures during regeneration. The resistance to high temperatures and the structure of the material also allows the zeolite to be completely regenerated – meaning that the VOCs completely release from the zeolite when heated. This means that the system maintains its high purification rate year after year and that the material does not have to be replaced, which gives it a long lifespan and a minimal need for maintenance. [19] Our systems have an availability of over 99% and a lifespan exceeding 25 years. Combining the benefits of zeolite with our 30 years of experience in working with air purification gives our customers a supremely sustainable and customized system with low operating costs and high availability.

ZSM-5 is composed of several pentasil units linked together by oxygen bridges to form pentasil chains. A pentasil unit consists of eight five-membered rings. In these rings, the vertices are Al or Si and an O is assumed to be bonded between the vertices. The pentasil chains are interconnected by oxygen bridges to form corrugated sheets with 10-ring holes. Like the pentasil units, each 10-ring hole has Al or Si as vertices with an O assumed to be bonded between each vertex. Each corrugated sheet is connected by oxygen bridges to form a structure with "straight 10-ring channels running parallel to the corrugations and sinusoidal 10-ring channels perpendicular to the sheets." Adjacent layers of the sheets are related by an inversion point. The estimated pore size of the channel running parallel with the corrugations is 5.4–5.6 Å. The crystallographic unit cell of ZSM-5 has 96 T sites (Si or Al), 192 O sites, and a number of compensating cations depending on the Si/Al ratio, which ranges from 12 to infinity. The structure is orthorhombic (space group Pnma) at high temperatures, but a phase transition to the monoclinic space group $P2_1/n.1.13$ occurs on cooling below a transition temperature, located between 300 and 350 K.

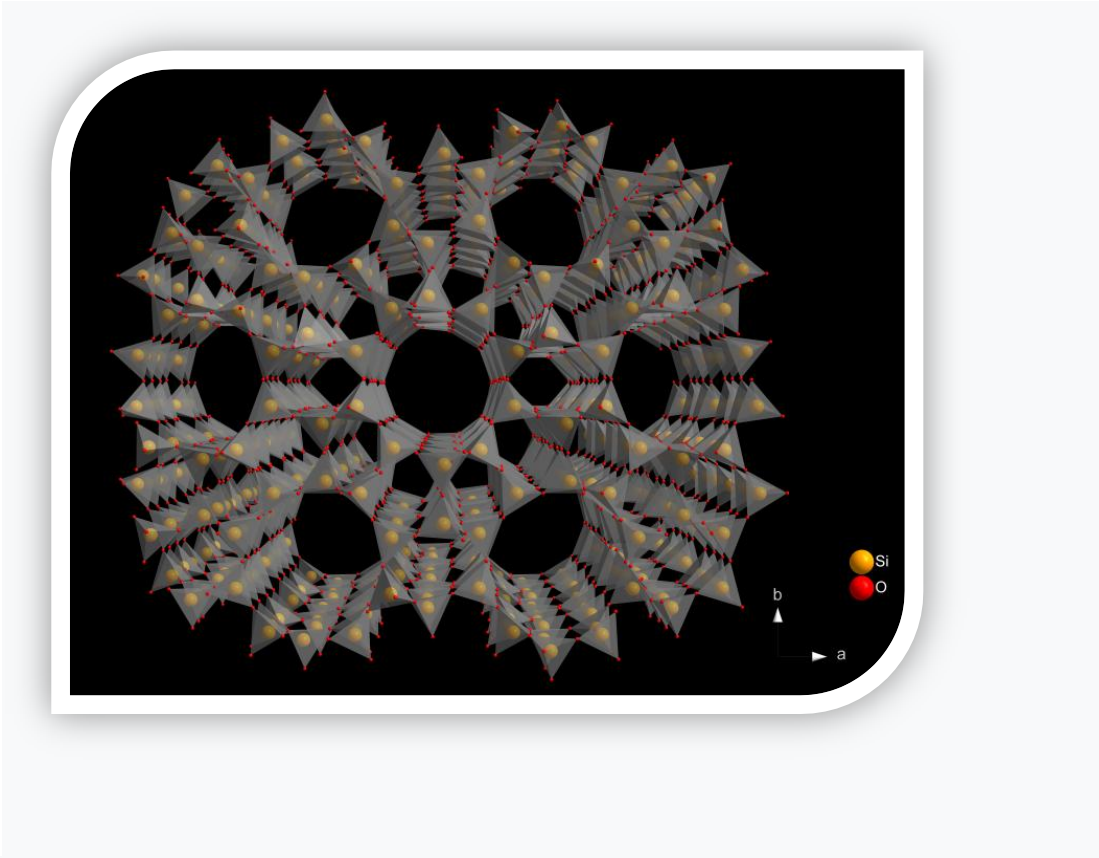

Fig3. The molecular structure of ZSM-5 zeolite, showing well defined pores and channels in the zeolite. Yellow balls represent Si and red balls represent O.

ZSM-5 catalyst was first synthesized by Argauer and Landolt in 1969. It is a medium pore zeolite with channels defined by ten-membered rings. The synthesis involves three different solutions. The first solution is the source of alumina, sodium ions, and hydroxide ions; in the presence of excess base the alumina will form soluble $Al(OH)_4^-$ ions. The second solution has the tetrapropylammonium cation that acts as a templating agent. The third solution is the source of silica, one of the basic building blocks for the framework structure of a zeolite. Mixing the three solutions produces supersaturated tetrapropylammonium ZSM-5, which can be heated to recrystallize and produce a solid.

Background of the invention

Pentasil-zeolites are defined by their structure type, and more specifically by their X-ray diffraction patterns. ZSM-5 is the trade name of a pentasil-zeolite.

As early as 1967, Argauer and Landolt worked out parameters for the synthesis of pentasilzeolites, particularly those relating to the following molar ratios: OH^-/SiO_2 = 0.07–10, SiO_2/Al_2O_3 = 5–100, H_2O/SiO_2 = 1–240. However, the Argauer and Landolt procedure succeeded in synthesizing a reasonably pure phase ZSM-5 zeolite only if organic amines with a structure-giving function (i.e. template function), such as tetrapropyleneammonium compounds were used. Subsequent publications have disclosed methods of conducting the synthesis of pentasil-zeolites without requiring the very expensive, toxic and easily inflammable organic amine templates. Still other subsequent publications have disclosed substitutes for these amines. In addition to their expense, toxicity and flammability, such amines are disfavored because they are subject to thermal decomposition which can destroy the zeolite structure. Further publications have disclosed modifications of the Argauer and Landolt process directed towards improving the reactivity of the SiO_2 and Al_2O_3 starting materials.

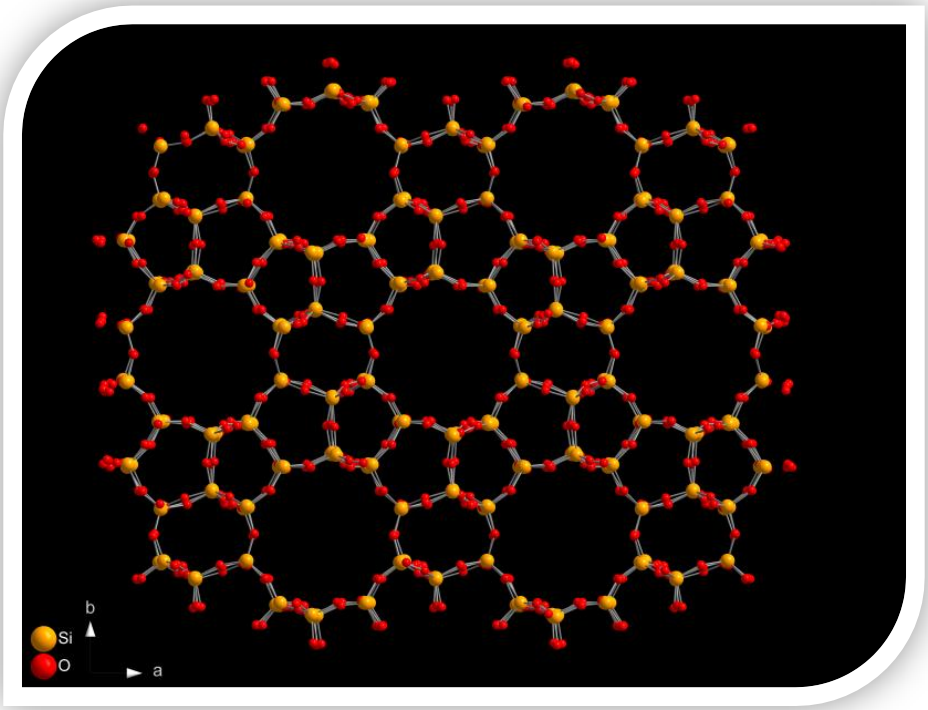

Fig4. The molecular structure of ZSM-5 zeolite, showing well defined pores and channels in the zeolite. Yellow balls represent Si and red balls represent O.

Synthesis

ZSM-5 is a synthetic zeolite, closely related to ZSM-11. There are many ways to synthesize ZSM-5; a common method is as follows:
An aqueous solution of silica, sodium aluminate, sodium hydroxide, and tetrapropylammonium bromide are combined in appropriate ratios

$$SiO_2 + NaAlO_2 + NaOH + N(CH_2CH_2CH_3)_4Br + H_2O \rightarrow \text{ZSM-5} + \text{analcime} + \text{alpha-quartz}$$

ZSM-5 is typically prepared at high temperature and high pressure in a Teflon-coated autoclave and can be prepared using varying ratios of SiO_2 and Al containing compounds.

Uses

ZSM-5 has a high silicon to aluminum ratio. Whenever an Al^{3+} cation replaces a Si^{4+} cation, an additional positive charge is required to keep the material charge-neutral. With proton (H^+) as the cation, the material becomes very acidic. Thus the acidity is proportional to the Al content. The very regular 3-D structure and the acidity of ZSM-5 can be utilized for acid-catalyzed reactions such as hydrocarbon isomerization and the alkylation of hydrocarbons. One such reaction is the isomerization of meta-xylene to para-xylene. Within the pores of the ZSM-5 zeolite, para-xylene has a much higher diffusion coefficient than meta-xylene. When the isomerization reaction is allowed to occur within the pores of ZSM-5, para-xylene is able to traverse along the pores of the zeolite, diffusing out of the catalyst very quickly. This size-selectivity allows the isomerization reaction to occur quickly in high yield.[9]

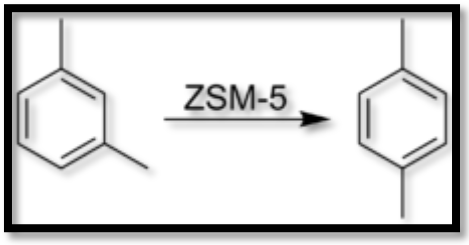

Fig5. Isomerisation of meta-xylene to para-xylene on passing through a ZSM-5 catalyst.

ZSM-5 has been used as a support material for catalysis. In one such example, copper is deposited on the zeolite and a stream of ethanol is passed through at temperatures of 240 to 320 °C as a vapor stream, which causes the ethanol to oxidize to acetaldehyde; two hydrogens are lost by the ethanol as hydrogen gas. It appears that the specific pore size of ZSM-5 is of benefit to this process, which also functions for other alcohols and oxidations. The copper is occasionally combined with other metals, such as chromium, to fine tune the diversity and specificity of the products, as there is likely to be more than one. Acetic acid is an example of one possible byproduct from hot copper oxidation.
ZSM-5 is also used to convert alcohols directly into gasoline. One such process is known as the Methanol to Gasoline (MTG) process, patented by Mobil.

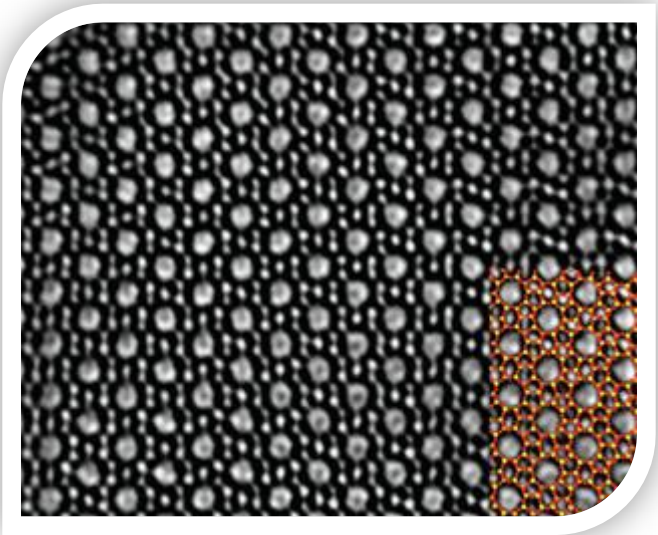

Fig6. Bragg-filtered HR-TEM image of MFI nanosheet with the overlaid crystal structure along [010] direction.

Podophyllotoxin

Podophyllotoxin (PPT) is the active ingredient in Podofilox, which is a medical cream that is used to treat genital warts and molluscum contagiosum. It is not recommended in HPV infections without external warts. It can be applied either by a healthcare provider or the person themselves. It is a non-alkaloid toxin lignin extracted from the roots and rhizomes of Podophyllum species. A less refined form known as podophyllum resin is also available, but has greater side effects.

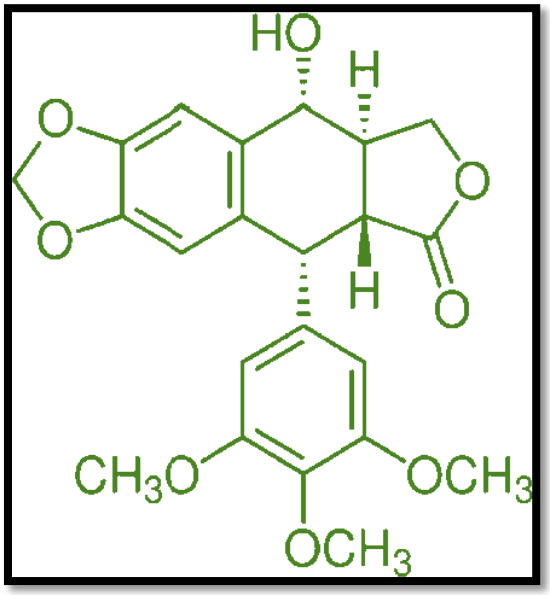

Fig5. Podophyllotoxin structure

Medical uses

Podophyllotoxin possesses a large number of medical applications, as it is able to stop replication of both cellular and viral DNA by binding necessary enzymes. It can additionally destabilize microtubules and prevent cell division. Because of these interactions it is considered an antimitotic drug. Podophyllotoxin and its derivatives are used as cathartic, purgative, antiviral agent, vesicant, antihelminthic, and antitumor agents. Podophyllotoxin derived antitumor agents include etoposide and teniposide. These drugs have been successfully used in therapy against numerous cancers including testicular, breast, pancreatic, lung, stomach, and ovarian cancers.

Podophyllotoxin cream is commonly prescribed as a potent topical antiviral. It is used for the treatment of HPV infections with external warts as well as molluscum contagisum infections. 0.5% PPT cream is prescribed for twice daily applications for 3 days followed by 4 days with no application, this weekly cycle is repeated for 4 weeks. It can also be prescribed as a gel, as opposed to cream. PPT is also sold under the names condyline and warticon.

Adverse effects

The most common side effects of podophyllotoxin cream are typically limited to irritation of tissue surrounding the application site, including burning, redness, pain, itching, and swelling. Application can be immediately followed by burning or itching. Small sores, itching and peeling skin can also follow, for these reasons it is recommended that application be done in a way that limits contact with surrounding, uninfected tissue

Neither podophyllin resin nor podophyllotoxin lotions nor gels are used during pregnancy because these medications have been shown to be embroytoxic in both mice and rats. Additionally, antimitotic agents are not typically recommended during pregnancy. Additionally, it has not been determined if podophyllotoxin can pass into breast milk from topical applications and therefore it is not recommended for breastfeeding women.

Podophyllotoxin cream is safe for topical use; however, it can cause CNS depression as well as enteritis if ingested. The podophyllum resin from which podophyllotoxin is derived has the same effect.

Mechanism of action

Podophyllotoxin destabilizes microtubules by binding tubulin and thus preventing cell division. In contrast, some of its derivatives display binding activity to the enzyme topoisomerase II (Topo II) during the late S and early G2 stage. For instance, etoposide binds and stabilizes the temporary DNA break caused by the enzyme, disrupts the reparation of the break through which the double-stranded DNA passes, and consequently stops DNA unwinding and replication. Mutants resistant to either podophyllotoxin, or to its topoisomerase II inhibitory derivatives such as etoposide (VP-16), have been described in Chinese hamster cells. The mutually exclusive cross-resistance patterns of these mutants provide a highly specific means to distinguish the two kinds

of podophyllotoxin derivatives. Mutant Chinese hamster cells resistant to podophyllotoxin are affected in a protein P1 that was later identified as the mammalian HSP60 or chaperonin protein. Furthermore, podophyllotoxin is classified as an arytetralin lignan for its ability to bind and deactivate DNA. It and its derivates bind Topo II and prevent its ability to catalyze rejoining of DNA that has been broken for replication. Lastly, experimental evidence has shown that these arytetralin lignans can interact with cellular factors to create chemical DNA adducts, thus further deactivating DNA.

Chemistry

Structural characteristic

The structure of podophyllotoxin was first elucidated in the 1930s. Podophyllotoxin bears four consecutive chiral centers, labelled C-1 through C-4 in the following image. The molecule also contains four almost planar fused rings. The podophyllotoxin molecule includes a number of oxygen containing functional groups: an alcohol, a lactone, three methoxy groups, and an acetal.

Biosynthesis

The biosynthetic route of podophyllotoxin was not completely eludicidated for many years; however, in September 2015, the identity of the six missing enzymes in podophyllotoxin biosynthesis were reported for the first time. Several prior studies have suggested a common pathway starting from coniferyl alcohol being converted to (+)-pinoresinol in the presence of a one-electron oxidant through dimerization of stereospecific radical intermediate. Pinoresinol is subsequently reduced in the presence of co-factor NADPH to first lariciresinol, and ultimately secoisolariciresinol. Lactonization on secoisolariciresinol gives rise to matairesinol.
A sequence of enzymes involved has been reported to be dirigent protein (DIR), to convert coniferyl alcohol to (+)-pinocresol, which is converted by pinocresol-lariciresinol reductase (PLR) to (-)-secoisolariciresinol, which is converted by sericoisolariciresinol dehydrogenase (SDH) to (-)-matairesinol, which is converted by CYP719A23 to (-)-pluviatolide, which is likely converted by Phex13114 (OMT1) to (-)-yatein, which is converted by Phex30848 (2-ODD) to (-)-deoxypodophyllotoxin. Though not proceeding through the last step of producing podophyllotoxin itself, a combination of six genes from the mayapple enabled production of the etoposide aglycone in tobacco plants.

Chemical synthesis

Podophyllotoxin has been successfully synthesized in a laboratory; however, synthesis mechanisms require many steps, resulting resulted in low overall yield. It therefore remains more efficient to obtain podophyllotoxin from natural sources.
Four routes have been used to synthesize podophyllotoxin with varying success: an oxo ester route, lactonization of a dihydroxy acid,cyclization of a conjugate addition product, and a Diels-Alder reaction.

Natural abundance
Podophyllotoxin is present at concentrations of 0.3% to 1.0% by mass in the rhizome of the American mayapple (Podophyllum peltatum). Another common source is the rhizome of Sinopodophyllum hexandrum Royle (Berberidaceae).
It is biosynthesized from two molecules of coniferyl alcohol by phenolic oxidative coupling and a series of oxidations, reductions and methylations.

2. Results And Discussion
Characterization
The chemical structure of the zsm-5-Podophyllotoxinwas characterized with different analytical methods such as XRD, SEM & TEM.

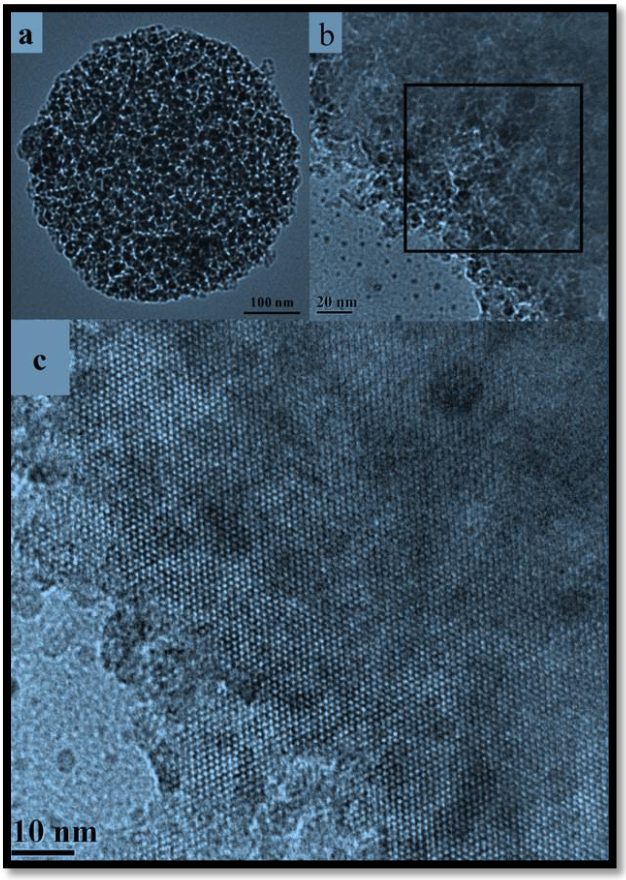

Fig7. TEM images (a, b) of mesoporous ZSM-5 microsphere of different magnifications and the HR-TEM image (c) from the area marked by a black square in (b).

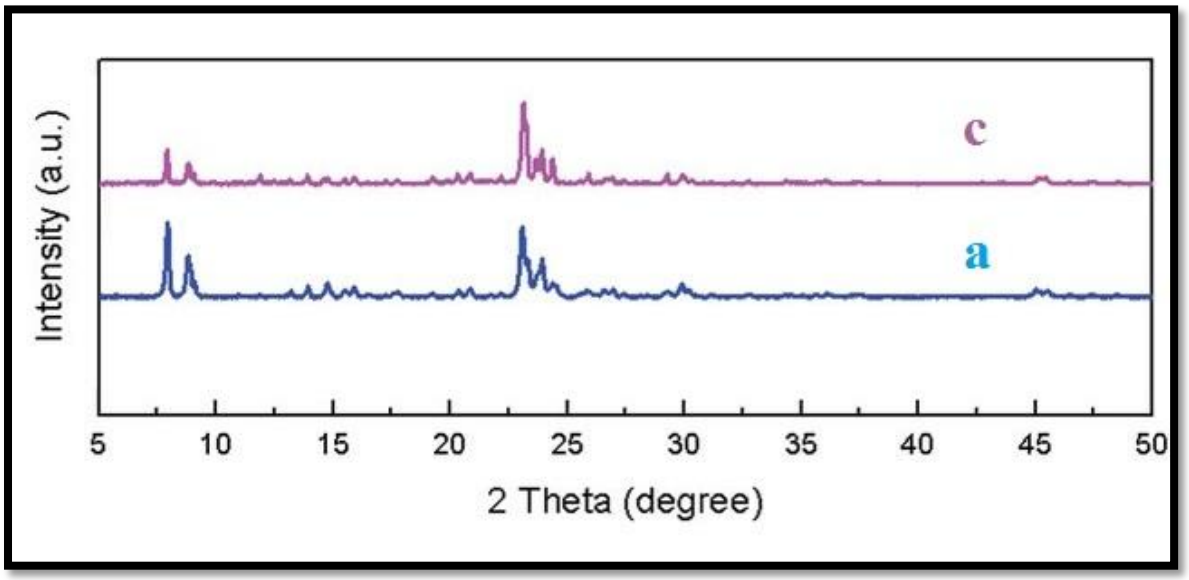

Fig8. XRD patterns of ZSM-5 samples obtained Podophyllotoxin:
(a) ZSM-5 (c) ZSM-5-Podophyllotoxin.

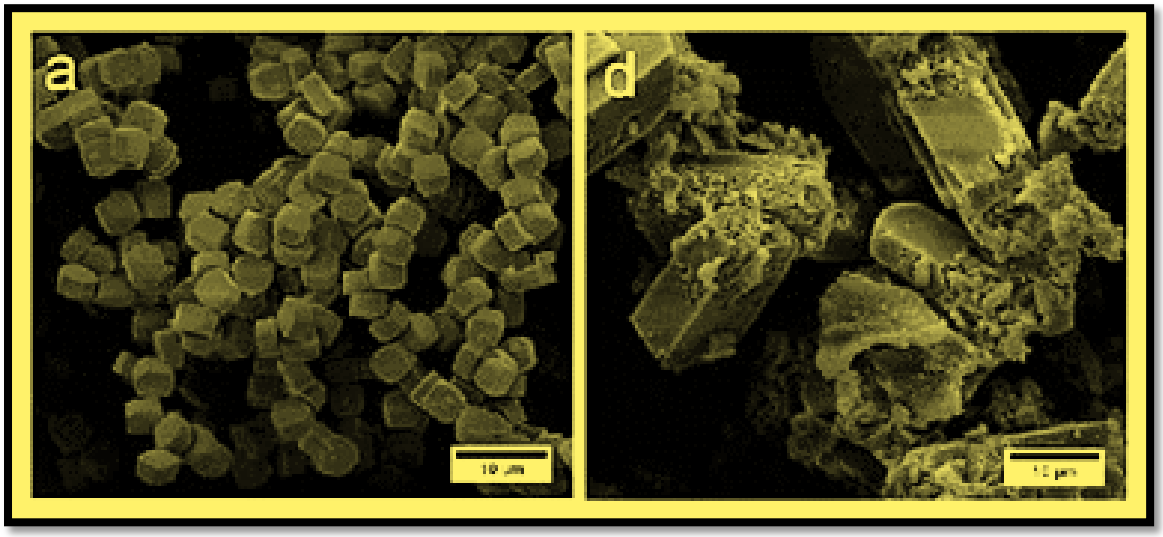

Fig9. SEM images of ZSM-5 samples obtained Podophyllotoxin:
(a)ZSM-5, (d) ZSM-5- Podophyllotoxin.

3. Conclusion

In this study, ZSM-5 as a drug carrier was applied for delivery ofPodophyllotoxin. The obtained nanostructure poses spherical morphology with an average diameter of 39-52 nm. The results showed the high loading capacity (73%) and sustained drug release behavior for Podophyllotoxin after 48h. In addition, upon exposure by ZSM-5-Podophyllotoxin, the growth inhibition was increased compared to those for ZSM-5 &Podophyllotoxin drug against HT-29 cells. Collectively, ZSM-5 may could beused asa promising drug deliverysystem for Podophyllotoxin.

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