Scientific paper

Feasibility of Clinoptilolite Application as a Microporous Carrier for pH-Controlled Oral Delivery of Aspirin

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Received: 03-12-2013

Abstract

Clinoptilolite is a natural zeolite which due to high surface area/volume ratio has found many applications in industries and medicine. Aspirin is a non-steroidal anti-inflammatory drug which is currently used as an anticoagulant, antinociceptive, antipyretic, and anti-inflammatory drug. It is an acidic drug which induces gastric irritation due to inhibition of cyclooxygenase I located in gastric mucosa. In the present work, adsorption and desorption of aspirin on Iranian clinoptilolite micronized particles were studied in acidic and relatively alkaline pHs. Effect of particle size of clinoptilolite was also investigated on adsorption and desorption of aspirin. Specific surfaces, particle sizes, and zeta potentials of clinoptilolite particles were also determined. Scanning electron micrograph was used to study the morphology and crystallinity of clinoptilolite particles.

The results showed that adsorption and desorption of aspirin on clinoptilolite are particle size- and pH-dependent. The present work proposes clinoptilolite as an inexpensive, efficient, and non-toxic natural available microporous material for aspirin oral delivery.

Keywords: Clinoptilolite, aspirin, adsorption, desorption, oral drug delivery.

1. Introduction

Zeolites are micoporous hydrated aluminosilicate materials which are available as naturally occurring materials or as synthetically manufactured substances. They are used in industries and medicine for their high surface area/volume ratio. Clinoptilolite as a natural zeolite due to its high availability, low cost, and high surface area/volume ratio has found many applications in agriculture,[1,2] animal husbandry,[3] industries,[2,4] and medicine.[5–10] It has been found to be an efficient adsorbent of cations,[4,11] and a nontoxic carrier for drugs.[5]

Aspirin as a non-steroidal anti inflammatory drug is currently used as an anticoagulant, antinociceptive, antipyretic, and anti-inflammatory drug.[12] It is an acidic drug with a pKa of 3.5 at 25 °C.[13] According to Henderson-Hasselbalch equation, at gastric pH (about 2.0), about 97% of aspirin, exists as unionized, protonated, and lipophile form which can irritate gastric mucosa by inhibition of cycooxygenase I located in gastric mucosa while at intestine pH (about 6.5), about 99.9% of it, exists as ionized and hydrophile drug.[14,15]

The present work investigated the adsorption and desorption of aspirin on clinoptilolite surface with 3 different particle sizes at acidic (2.0 and 6.5) pHs. The main objective of this work is to study the feasibility of clinoptilolite application as a carrier for pH-dependent aspirin oral delivery.

2. Experimental

Pure aspirin (Sigma), potassium dihydrogen phosphate (Merck), di-potassium hydrogen phosphate (Merck), ortho-phosphoric acid 85% (Merck), miconized (particle size ≤ 37 μm) Iranian clinoptilolite (Anzymite®) powder (Afrand Tooska, Tehran, I.R. Iran), stainless steel mesh sieves (1400, 800, and 400 mesh equivalent to 8.7, 16, and 37 μm opening diameters respectively) (Anping County Resen Screen Co., Hengshui, China), and magnet stirrer (Alpha, Iran) were used in the present study.

Firstly, clinoptilolites with 3 different particle sizes were prepared by suspending micronized clinoptilolite powder (particle size ≤ 37 μm) in distilled water (DW) followed by passing the suspension from a sequence of stainless steel sieves (1400, 800, and 400 mesh equivalent to 8.7, 16, and 37 μm openings diameters respectively). The passed fraction of powder from 1400 mesh sieve (sieved fraction 1), passed fraction of powder from 800 mesh sieve which retained by 1400 mesh sieve (sieved fraction 2), passed fraction of powder from 400 mesh sieve which retained by 800 mesh sieve (sieved fraction 3) were used in the present study. Before use, the sieved fractions were washed 3 times with DW, centrifuges at 5000 rpm, and dried in an oven at 250 °C for 24 hrs.[16]

Calibration curves of aspirin at pHs 2.0 and 6.5 were separately constructed by depicting absorption at 228 nm versus different concentrations of aspirin (2, 4, 8, 16, and 20 μg/ml).

For study of adsorption of aspirin by clinoptilolite sieved fractions, following steps were performed on each of above fractions:

1.5 mg pure aspirin was dissolved in 50 ml of 0.1 M phosphate buffer (pH = 2.0) (30 mg/L aspirin final concentration) followed by addition of 1 gm of clinoptilolite powder under continuous stirring at room temperature. After 5, 15, 30, 60, 90, and 120 min time intervals, 1 ml of suspension was sampled and centrifuged at 5000 rpm for 5 min followed by measurement of UV absorption of supernatant at 228 nm. Amount of adsorbed aspirin on clinoptilolite particles was determined by interpolation of UV absorption of supernatant at 228 nm over aspirin tabulated concentrations on aspirin calibration curve followed by multiplication by respective volume of clinoptilolite suspension and subtraction from original aspirin amount (1.5 mg). Adsorption of aspirin by clinoptilolite different particle sizes at pH = 6.5 was also studied according to abovementioned method except that 0.1 M phosphate buffer (pH = 6.5) was used.

For study of desoprtion of aspirin from aspirin-loaded clinoptilolite particles, following steps were performed on each of clinoptilolite fractions:

Aspirin-loaded clinoptilolite was prepared as follows: 3 mg of purified aspirin was dissolved in 3 ml of DW and 1 gm clinoptilolite powder was added to it and left alone at room temperature for 1 hr. The suspension was then carefully divided to two equal volumes, centrifuged at 5000 rpm for 5 min, supernatants were removed and adsorbed amount of aspirin on clinoptilolite particles was determined by interpolation of UV absorption of each supernatant at 228 nm over aspirin tabulated concentrations on aspirin calibration curve followed by multiplication by respective volume of supernatant and subtraction from original aspirin amount (1.5 mg for each suspension). Due to high concentration of aspirin in supernatant which caused spectrophotometer absorption over-range at 228 nm, dilution of supernatant was done by DW. Hence, the dilution factor was used in final calculation of adsorbed amount of aspirin to clinoptilolite. One precipitate was used for study of aspirin desorption at pH=2.0 while another one used for study of aspirin desorption at pH = 6.5. For study of desoprtion of aspirin from aspirin-loaded clinoptilolite particles, the following steps were performed on precipitates: one precipitate was dissolved in 50 ml of 0.1 M phosphate buffer pH = 2.0 while another one dissolved in 50 ml of 0.1 M phosphate buffer pH = 6.5 under continuous stirring at room temperature followed by 1 ml sampling from suspensions after 5, 15, 30, 60, 90, and 120 min time intervals. The samples were then centrifuged at 5000 rpm for 5 min followed by measurement of UV absorption of supernatants at 228 nm. Amount of desorbed aspirin from aspirin-loaded clinoptilolite particles was determined by interpolation of UV absorption of supernatant at 228 nm over aspirin tabulated concentrations on aspirin respective calibration curve followed by multiplication by respective volume of clinoptilolite suspension.

Particle size and zeta potential of clinoptilolite sieved fractions in DW were determined by a particle size analyzer (Mastersizer Hydro 2000S, Malvern Instruments Ltd., UK) and a Zetasizer (Nano ZS, Malvern Instruments Ltd., UK) respectively. Clinoptilolite sieved fraction 1 was photographed by a field-emission scanning electron microscope (FE-SEM) (Hitachi, Model S-4160, Japan) to study the morphology and crystallinity of clinoptilolite particles. Specific surfaces of clinoptilolite sieved fractions were also determined by Nano SORD (Toseye Hesgarsazan Asia Co., Iran).

All adsorption and desorption experiments were repeated 5 times.

Difference between adsorption and desorption of aspirin on 3 different clinoptilolite particle sizes at pHs 2.0 and 6.5 and different time intervals were statistically analyzed by one-way analysis of variance (ANOVA) followed by Scheffe Post-Hoc on SPSS statistical package. *P*-values less than 0.05 were statistically considered significant. Graphs were depicted by SigmaPlot 12.0.

3. Results and Discussion

Figures 1 and 2 show UV spectra of 20 μg/ml solutions of aspirin in 0.1 M phosphate buffer at pHs 2.0 and 6.5 respectively.

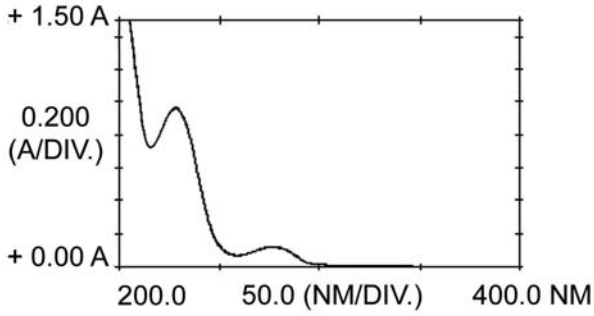

Figure 1. UV spectrum of 20 μg/ml aspirin in 0.1 M phosphate buffer pH = 2.0.

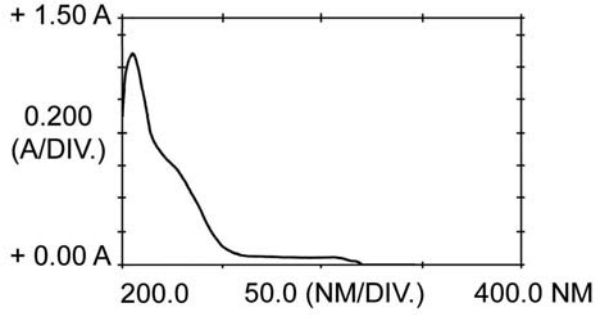

Figure 2. UV spectrum of 20 μg/ml aspirin in 0.1 M phosphate buffer pH = 6.5.

Calibration curves of aspirin in 0.1 M phosphate buffer for pHs 2.0 and 6.5 are shown in figures 3 and 4 respectively.

Each standard curve showed good linearity over the range of aspirin concentrations examined. The specific surfaces (BET) of sieved fractions 1, 2, and 3 were 39.33, 36.99, and 33.79 m^2/g respectively. Zeta potentials of sieved fractions 1, 2, and 3 were –32.7, –29.0, and –27.9 mV respectively. FE-SEM photomicrograph of sieved fraction 1 is shown in figure 5. Sieved clinoptilolite shows some degrees of crystallinity. In fact, it shows a semi-crystalline morphology.

Particle sizes of sieved fractions 1, 2, and 3 were 1.5–10 μm, 5–16 μm and 11–40 μm respectively (figure 6).

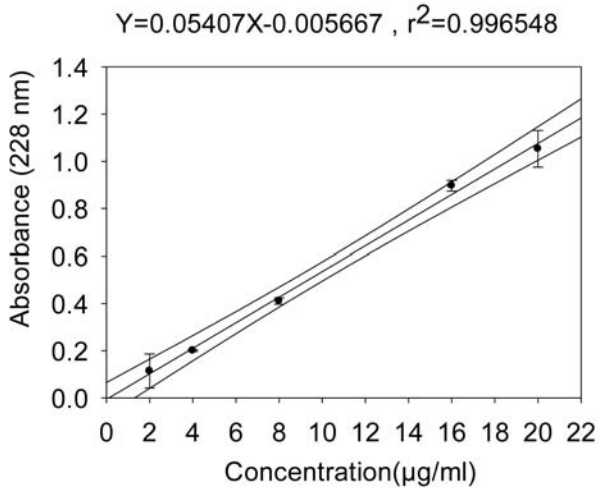

Figure 3. Aspirin calibration curve in 0.1 M phosphate buffer pH = 2.0. The results represent the mean ± SD (n = 5).

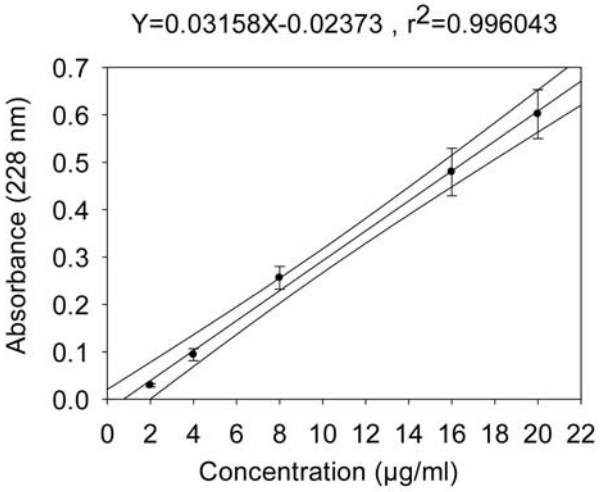

Figure 4. Aspirin calibration curve in 0.1 M phosphate buffer pH = 6.5. The results represent the mean ± SD (n = 5).

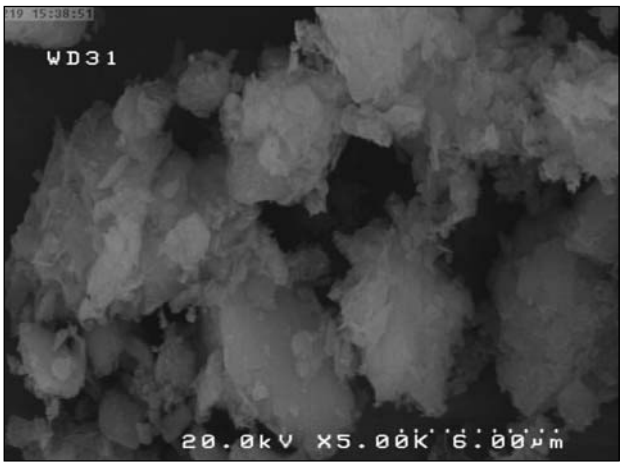

Figure 5. FE-SEM photomicrograph of smallest particle size sieved fraction (≤ 37 μm).

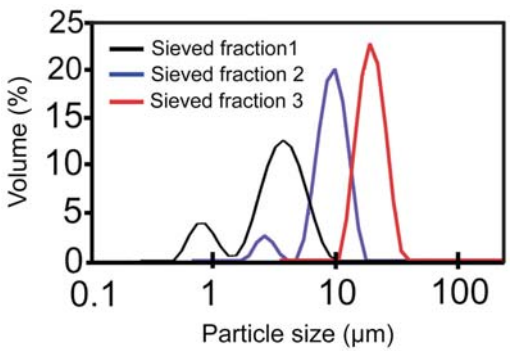

Figure 6. Particle size distributions of sieved fractions.

Adsorption of aspirin by different sizes of clinoptilolite particles at pHs 2.0, and 6.5 are shown in figures 7 and 8 respectively.

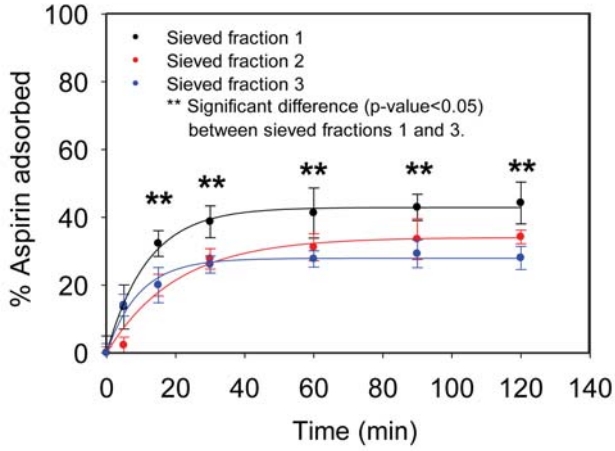

Figure 7. Adsorption of aspirin by different sizes of clinoptilolite particles at pH 2.0.

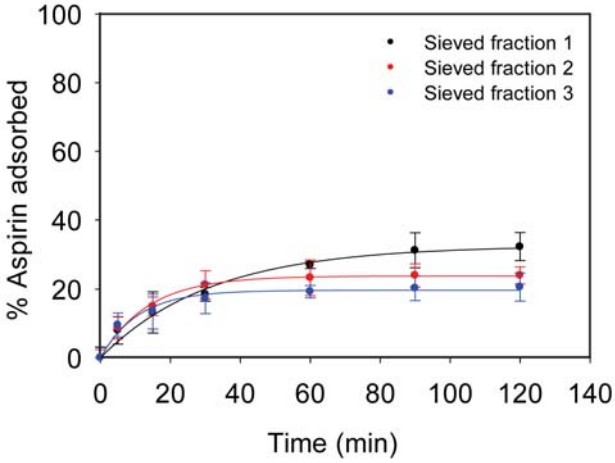

Figure 8. Adsorption of aspirin by different sizes of clinoptilolite particles at pH 6.5.

Sieved fraction 1 significantly (P<0.05) adsorbed more aspirin than sieved fraction 3 at pH = 2.0 at 15, 30, 60, 90, and 120 minutes. There were no significant differences between sieved fractions 1 and 2, and 2 and 3 in aspirin adsorption at pH = 2.0 at 5, 15, 30, 60, 90, and 120 minutes time intervals. There were no significant differences between 3 sieved fractions in aspirin adsorption at pH=6.5 at 5, 15, 30, 60, 90, and 120 minutes time intervals. Desorption of aspirin from different sizes of clinoptilolite particles at pHs 2.0, and 6.5 are shown in figures 9 and 10 respectively.

There were significant differences (P<0.05) between sieved fractions 1 and 2, 1 and 3, and 2 and 3 in aspirin desorption at pH = 2.0 at 15, 30, 60, 90, and 120 minutes time intervals. Sieved fraction 1 significantly (P<0.05) desorbed more aspirin than sieved fraction 3 at pH = 6.5 at 5, 15, 30, 60, 90, and 120 minutes. There were no significant differences between sieved fractions 1 and 2. Sieved fraction 2 significantly (P<0.05) desorbed more aspirin than sieved fraction 3 at pH = 6.5 at 5, 15, and 30 minutes.

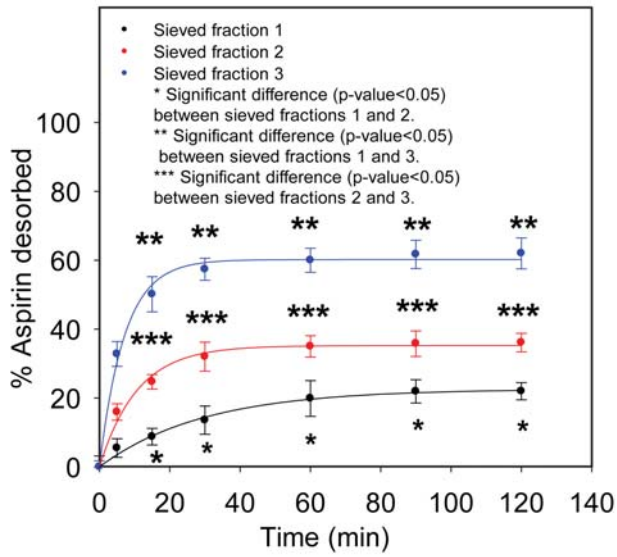

Figure 9. Desorption of aspirin from different sizes of clinoptilolite particles at pH 2.0.

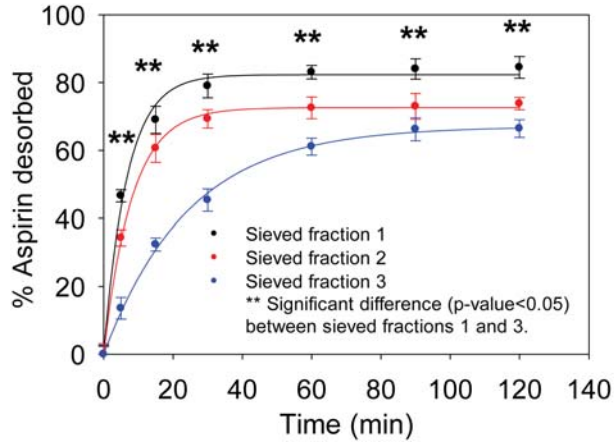

Figure 10. Desorption of aspirin from different sizes of clinoptilolite particles at pH 6.5.

Clinoptilolite has been shown to be a potent adsorbent of organic compounds.[17] It is a moderate adsorbent of heavy metals due to its cation exchange property.[4,11,18] Moreover, it is used as a molecular sieve.[19,20] It is a highly porous material with high surface area/ volume ratio which make it a potent adsorbent.

Higher specific surface of sieved fraction 1 (39.33 m^2/g) than those of sieved fractions 2 (36.99 m^2/g) and 3 (33.79 m^2/g) indicates higher adsorptive property of the

latter than the formers. This well explains why sieved fraction 1 adsorbs more aspirin than sieved fractions 2 and 3.

Previously, theoretical feasibility of aspirin physical adsorption by natural clinoptilolite was explained.[21] This study theoretically showed that aspirin interacts with the clinoptilolite windows through three principal groups of aromatic, ester, and carboxyl.[21] It also showed that the interaction between aspirin and clinoptilolite is only of physical nature.[21]

Physical adsorption of several drugs such as metronidazole and sulfamethoxazole by natural clinoptilolite has been investigated and fully described.[5] It has been shown that clinoptilolite efficiently adsorbs metronidazole. Moreover, theoretical feasibility of metronidazole physical adsorption by natural clinoptilolite was explained.[22] Adsorption and desorption of ketoprofen on synthetic Zeolites A and X were also shown to be pH-dependent so that they adsorb more drug at acidic pH while they desorb more drug at alkaline pH.[23]

The present study indicated that increase in pH of the aspirin solution, decreases the amount of drug adsorbed by clinoptilolite. This fact is related to the aspirin equilibrium as a function of the solution pH. At pHs below the aspirin's pKa value (3.5), the molecule is protonated. Therefore, the interaction between the aspirin molecule and the oxygen atoms of the zeolite framework will be favored at pH smaller than the pKa value, when the molecule is positively charged. The protonated drug could also be exchanged with cations present in the pore mouths.[5]

It should be noted that the overall results of the present study are in agreement with those reported regarding aspirin-clinoptilolite interaction studies through quantum mechanical calculations.[21]

The present results show that clinoptilolite sieved fraction 1 (with the smallest particle size) adsorbs more aspirin than those of sieved fractions 2 and 3 at pH = 2.0. This may be firstly due to higher surface area/volume ratio and hence higher surface energy of the latter than those of the formers and secondly due to more unionized form of aspirin at this pH which clinoptilolite can adsorbs. The results also showed that at pH = 6.5 there are no significant differences between sieved fractions in aspirin adsorption over 120 minutes time period which may be due to formation of more unadsorbable ionized form of aspirin.

Desorption of aspirin at pH = 2.0 was lesser by sieved fraction 1 than those of sieved fractions 2 and 3. This may be due to more potent bond between clinoptilolite adsorbent active sites and aspirin unionized form which may be due to higher surface energy of fraction 1 than those of fractions 2 and 3 resulting from particle size reduction. Desorption of aspirin at pH = 6.5 was greater by sieved fraction 1 than those of sieved fractions 2 and 3 which may be due to easier release of aspirin ionized form. Release of ionized aspirin from inner parts of larger sizes clinoptilolite (fractions 2 and 3) is more difficult which may be due to trapping of aspirin in inner parts of clinoptilolite particles.

Natural clinoptilolite has been shown to have an anti-acid property.[24] Thus, it is predicted that aspirin-loaded clinoptilolite has a reduced gastric irritating effect than that of aspirin alone. The results of present work propose that the best dosage form for oral delivery of aspirin-loaded clinoptilolite is capsule. This dosage form is predicted to allow slow dispersion of aspirin-loaded clinoptilolite particles through gastrointestinal tract so that aspirin is released in a pH-dependent manner.

4. Conclusion

The present work investigated interaction between natural clinoptilolite and aspirin at pH_s 2.0 and 6.5 which mimic pHs of stomach and small intestine respectively. The results showed that adsorption and desorption of aspirin on clinoptilolite are particle size-and pH-dependent. It proposes clinoptilolite as a non-toxic, highly available, and low cost microporous material carrier for oral delivery of aspirin. Particularly, we found that less than 20% of the drug was released from sieved fraction 1 at the gastric pH, a result that clearly indicates the effectiveness of this system in reducing the adverse effects commonly accompanying oral administrations of aspirin. The results of the present work recommend sieved fraction 1 (1.5–10 μm particle size) for oral delivery of aspirin. They also showed maximum adsorption and desorption of aspirin by this fraction at pH_s 2.0 and 6.5 respectively which mimic pHs of stomach and small intestine respectively in human.

5. Acknowledgment

The authors gratefully thank Afrand Tooska Co. for supplying micronized clinoptilolite powder (Anzymite®) used in the present work. This research has been supported by Tehran University of Medical Sciences & health Services grant (No. 12327, April 19, 2011).

6. References

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Povzetek

Klinoptilolit je naravni zeolit, ki se zaradi dobrega razmerja med površino in volumnom veliko uporablja v industriji in tudi medicini. Aspirin je nesteroidno zdravilo, ki se uporablja tako kot antikoagulant kot tudi kot protibolečinsko, protivročinsko in protivnetno zdravilo.
Zaradi inhibicije ciklooksigenaze v želodčni sluznici lahko draži želodec. V tem delu smo proučevali adsorpcijo in desorpcijo aspirina na klinoptilolitu (iz Irana) z različnimi velikosti delcev v kislem in alkalnem mediju. Izkazalo se je, da bi bil klinoptilolit kot poceni, učinkovit in nestrupen mikroporozni material primeren tudi kot dostavni sistem za aspirin.