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Electronic Supplementary Information

Development of Functionalized Hollow Microporous Organic

Capsules Encapsulating Morphine – In-Vitro and In-Vivo Study

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Experimental Details Preparation of silica nanoparticles

The silica nanoparticles were synthesized in ethanol according to the Stöber method. Ethanol (500 ml) and TEOS (40 ml) were mixed by vigorous mechanical agitation in a 1000 ml four-neck round bottom flask and followed by adding $NH_3 \cdot H_2O$ (40 ml). After 24h stirring, mixture of MPS and ethanol (4ml: 26 ml) was dropped into the dispersion of SiO₂ spheres in ethanol. The reaction was further agitated for 24h and SiO₂-MPS were obtained by three cycles of centrifugation and redispersion with ethanol, dried in vacuum oven at 60°C for 24h.

Synthesis of PMAA microspheres

The monodispersed PMAA microspheres has been synthesized using the distillation-precipitation polymerization¹ in acetonitrile with AIBN as an initiator. In a typical recipe, MAA (2.0ml) is added to 80ml of acetonitrile in a two neck round bottom flask fitted with a distillation condenser and a receiving flask. AIBN (0.04g, 2% of MAA) was used to initiate the polymerization. The reaction mixture was heated from ambient temperature to the boiling state over a period of 20 min. Afterwards the solvent began to be distilled from the reaction system. The milky white dispersion was appeared after half volume of acetonitrile was distilled from the reaction system. The resultant white product (PMAA microspheres) was centrifuged (10,000 rpm for 20 min) and washed repeatedly by sonicating in acetonitrile.

Morphine.HCl loading and release

The loading of the drug was carried out by the immersion of HMOCs in aqueous solution of Morphine.HCl with a certain concentration. A typical procedure of loading Morphine.HCl in HMOCs was as follows: 100 mg of HMOCs was suspended in 10 ml of 500 mg/ml Morphine.HCl(aq) under stirring for 96 h. The drug-loaded sample was separated from the solution by vacuum filtration, and washed with copious amount of water to get rid of unbound drug molecules. The drug loaded HMOCs were dried at 60°C in a vacuum oven. Filtrate was used to

determine the drug-loading amount by UV-Vis spectrophotometer and encapsulation efficiency was calculated by following equation²:

 $Encapsulation \ efficiency(\%) \\ = \frac{[Morphine]Total - [Morphine]supernatant}{[Morphine]Total} \times 100$

Similarly, the drug loading capacity was determined by applying the following equation³:

 $Drug \ Loading \ (\%) = \frac{[Morphine]Total - [Morphine]supernatant}{Weight \ of \ HMOCs} \times 100$

To determine the release kinetics of HMOCs, the drug-loaded samples (100 mg) was transferred to semipermeable bag, the release rate was obtained by soaking the drug-loaded sample in 50 ml of simulated body fluid (PBS, pH = 7.4, buffer solution, 37°C). Thus, at predetermined time intervals, 3ml samples of buffer solution was withdrawn and replenished immediately. The amount of Morphine.HCl was analyzed by UV-Vis spectrophotometer at 285 nm and release kinetics was evaluated by using the equation² as under:

$$Cumulative \ release(\%) = \frac{Total \ Morphine \ Released}{Initial \ amount \ of \ Morphine \ in \ HMOCs} \times 100$$

The drug loading and release kinetics for pure PMAA microspheres had been carried out following the above mentioned methodology. It is obvious from the fig. S6, that the drug encapsulation efficiency (42%) of PMAA is much lower than HMOCs. It might be attributed to the capsular and microorous architecture of HMOCs. Similarly the release behavior of morphine.HCl has shown a burst release initially and almost 57% of drug has been liberated over a period of 5h while no obvious released had been observed over a prolonged time span. As PMAA has a considerable higher colloidal stability in water due to the presence of -COOH functionality. The maximum liberation at initial duration has been attributed to the physical adsorption of drug molecules on the PMAA microspheres. Due to the hydrophilicity of Morphine as well as PMAA, the higher content of the drug had been released in a small duration at pH 7.4. It is well

noticed that PMAA polymeric chains are more prone to ionization at higher pH value that may enhances the liberation of encapsulated molecule.

Zeta potential measurements

The zeta potential of HMOCs was measured in a capillary cell (Malvern). Water dispersion of the samples was injected slowly into the cell avoiding air bubbles. When the cell was inserted into the Zeta sizer, electrophoresis was carried out by the voltage supplied by the electrode positioned on either side of the cell holder. The instrument automatically calculated the Zeta potentials and determined the electrophoretic mobility using the Henry equation.

Zeta potential measurement is a robust and rapid technique to assess the surface – COOH density. Thus average potential obtained reflects the overall surface charged groups on the surface of HMOCs. The results of the measurements are incorporated into the Table S1. For the purpose of comparison, zeta potential measurement has been done for PS-DVB microspheres, expressing no charged densities on their surface displayed a value closed to zero.

Thermogravimetric analysis

TGA of HMOCs had been carried out to determine the total percentage of –COOH functionality. The powdered sample was employed to carry out the TG curves in a temperature range of 20-800°C under inert environment (nitrogen atmosphere) at a heating rate of 20°C/ min (Fig.S5). Comparative study has been carried out for the modified and unmodified HMOCs. It is evident from the TG curves that both samples have shown thermal stability up to 350-380°C and mass loss of 100 percentages. Moreover the determination of total COOH content by TG measurement is not obvious. It might be attributed to the copolymerization of MAA on the PS microspheres, which may inhibit the mass loss at initial stages and enhances the stability of resulting microspheres.



Figure S1. Generalized trend of neutralization of carboxylated HMOCs in a conductometric titration, utilizing H_2SO_4 (0.4N) and NaOH (0.005N) at room temperature



Figure S2. General colloidal dispersion of HMOCs-15%.



Figure S3. Drug encapsulation efficiency of HMOCs demonstrated by UV-Vis spectrophotometer.



Figure S4. Cumulative release (%) of morphine.HCl from HMOCs-1 HMOCs-2.5, HMOCs-5, HMOCs-10.



Figure S5. Thermogravimetric curves of HMOCs-MAA and HMOCs in a temperature range of 20-800°C under Nitrogen.



Figure S6. Cumulative release (%) of morphine.HCl from PMAA microspheres.

| Samples | Zeta Potential |
|------------------|----------------|
| | mV(±3mV) |
| Unmodified HMOCs | -4.17 |
| Modified HMOCS | |
| 0.5% | -31.3 |
| 1.0% | -32.2 |
| 2.5% | -31.9 |
| 5.0% | -30.6 |
| 10.0% | -32.5 |
| 15.0% | -31.4 |

Table S1: Summarized zeta potential measurement

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