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Molecularly imprinted acrylic-based microspheres for colonic delivery of 5-aminosalicylic acid

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Feasibility of molecularly imprinted polymeric microspheres (MIPs) has been investigated for colonic delivery of 5-aminosalicylic acid (5-ASA). 5-ASA imprinted microspheres were prepared by a single step precipitation polymerization of 2-(diethylamino) ethyl methacrylate (DEAEMA; functional monomer) and trimethylolpropane trimethacrylate (TRIM; crosslinker). The release profiles of 5-ASA imprinted and non-imprinted microspheres were evaluated. We present a precipitation polymerization method for preparing uniform molecularly imprinted microspheres in micron range, quickly and cleanly. Monodisperse polymer particles with good spherical shapes and smooth surfaces were obtained. Furthermore, the imprinted microspheres have a slower 5-ASA release in the initial stages than the non-imprinted microspheres, because of the interaction of the drug molecules with the recognition sites in the imprinted microspheres. This result showed that molecular imprinting may have a potential for controlled delivery of drugs.

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1. Introductıon

One of the recent advances in the field of drug delivery is achieved by introducing molecular recognition sites into the polymers. The product is called as "recognitive drug delivery system". The polymers that carry these recognition sites are known as molecularly imprinted polymers (MIPs) (1, 2). Molecularly imprinted polymers are created in the presence of a template molecule that forms recognition sites providing shape and functional group complementary to this molecule. In general, molecularly imprinted polymers are prepared by the copolymerization of an appropriate functional monomer with a crosslinker in the presence of a template molecule. The functional monomer and the template molecule interact and form a complex during the crosslinking reaction. Then the removal of the template leaves the recognition sites (binding sites) within the polymer as shown in Fig 1.

Molecular imprinting can provide the polymers used in controlled delivery of drugs; enhanced reloading, sustained release, enantioselective release and responsive release (2). The applicability of MIPs in controlled drug delivery systems was presented by many researchers. Allender et al. (3) prepared MIPs for propranolol using methacrylic acid as a functional monomer for a transdermal controlled release device. They reported that the permeation of propranolol was slower from the imprinted polymers than from non-imprinted ones because of their affinity to the MIPs.

Again, the imprinted polymer microspheres exhibited prolonged sulfasalazine release relative to the non-imprinted system, as demonstrated by Puoci et al. (4). In the study of Hiratani et al. (5) timolol loading capacity of the polymer for ophthalmic delivery was improved by the molecular imprinting method. Therefore, recognitive drug delivery system seems to be a potential for modifying drug loading and release behavior.

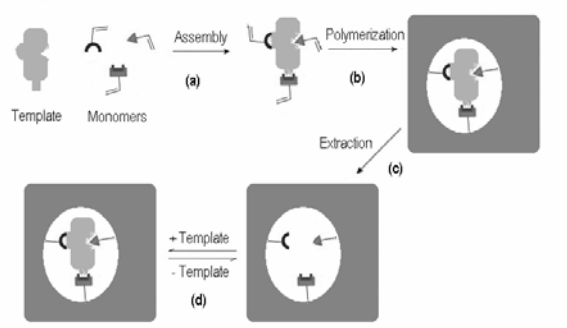

Fig. 1. Schematic representation of molecular imprinting process: a) orientation of three different functional groups of monomers to the template, b) formation of template-monomer complex, c) formation of rigid polymer matrix, d) removal of template leaving a functionalized cavity in polymer.

On the other hand, small size and large specific surface area with narrow distribution of uniformity of the microspheres make them an important class of polymeric materials to allow controlled delivery of drugs (6). They can be prepared by different heterogeneous polymerization methods, for example emulsion, suspension, dispersion polymerization etc.

Emulsion polymerization method produces polymer particles of 0.01-1 μm in diameter. A stabilizer, an ionic or non-ionic surfactant or protective colloid is required to

prevent coagulation of the particles. Suspension polymerization can be utilized to prepare larger polymer beads in a wide range of 5-1000 μm diameter. Again surfactants are used to prevent coagulation as in the emulsion polymerization. On the other hand dispersion polymerization yields polymer microspheres in the range of 1-15 μm which is characterized by its initially homogeneous polymerization medium in which the insoluble polymer is stabilized by the surfactants.

However, it is diffucult to get monodisperse highly crosslinked microspheres in small sizes by the above discussed conventional methods. Precipitation polymerization is a simple and attractive method to prepare highly crosslinked, monodisperse polymer microspheres (7, 8). Also, it is a suitable method to fabricate molecularly imprinted polymers since neither surfactant nor stabilizers are required in this technique. The precipitation polymerization method, which involves polymerization of monomers in a dilute solution (total monomer concentration of about 2-5 % v/v relative to solvent), produces monodispersed spherical particles from one nanometer to a few micrometer in diameter.

In the present study, recognitive drug delivery systems were prepared and examined as potential carriers for 5-aminosalicylic acid (5-ASA), an active agent used in the treatment of ulcerative colitis and Crohn's disease (9). Molecularly imprinted poly(diethylamino ethyl methacrylate-co-trimethylolpropane trimethacrylate), poly(DEAEMA-co-TRIM) microspheres were prepared using 5-ASA as template by precipitation polymerization method. The release characteristics of 5-ASA imprinted and non-imprinted microspheres were determined and molecular imprinting effect on release behaviors was evaluated.

2. Experimental

Materials

2-(Diethylamino) ethyl methacrylate (DEAEMA) was purchased from Aldrich (USA) and purified by vacuum distillation prior to use in order to remove inhibitors. Trimethylolpropane trimethacrylate (TRIM) was supplied by Aldrich (Germany) and purified by using inhibitor remover replacement packing for HQ and MEHQ obtained from Aldrich (USA). Azobisisobutyronitrile (AIBN) and trisodium phosphate (Na_3PO_4) were obtained from Merck (Germany). Acetonitrile (HPLC grade), hydrochloric acid (37%) and toluene (analytical grade) were supplied by Riedel (Germany). 5-Aminosalicylic acid (5-ASA) (99%) was purchased from Sigma (Germany). Diaysis Tubing Cellulose Membrane (size: 25 mm x 16 mm, Mw:12,400) was from Sigma-Aldrich (Germany).

Preparation of 5-ASA imprinted and non-imprinted microspheres

Generally, precipitation polymerization was done with 2-5 vol % monomer concentration relative to solvent (10). In our previous studies, copolymerization of DEAEMA and TRIM were well studied by precipitation polymerization under various parameters. It was found that the total monomer concentration relative to the volume of solvent should be 1 vol % in order to avoid aggregation (11). Using these information, 5-ASA imprinted microspheres have been prepared as follows: 2 mmol DEAEMA, 0.2 mmol 5-ASA and 2 mmol TRIM were solvated in acetonitrile, and then 20.9 mg initiator; AIBN (2 % wt of total monomer), was added. After 15 min purging with N_2, the solution was sealed in an ampoule under vacuum and then heated to 60^0C for 24 hours. After the polymerization was complete, 5-ASA was removed by solvent extraction with methanol containing 10 % (v/v) acetic acid. The microspheres were washed several times with this solution and then dried under vacuum. Non-imprinted microspheres were prepared under the same conditions without addition of 5-ASA as control.

In molecular imprinting technique, an important part of the process is the stabilization of the monomer-template complex. This was achieved by high crosslinking so that 50 % of the total monomer was TRIM. In addition, the molar ratio of template and functional monomer is another critical factor to obtain high number and the quality of the recognition sites (12, 13). Low monomer-template ratios afford less than optimal complexation of insufficient functional monomer and too high monomer-template ratio yields non-selective binding polymer matrix.

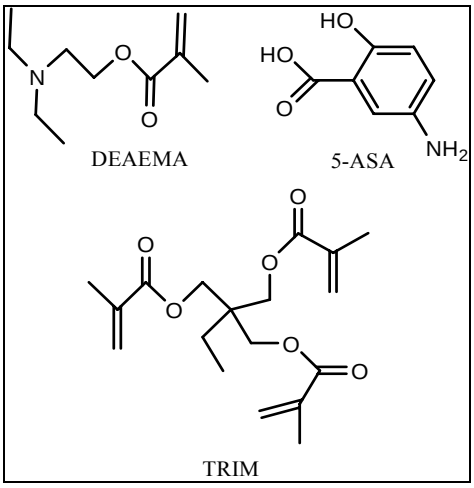

Fig. 2. The structures of compounds used in this study. Possible interactions of carboxylic acid, amine and hydroxyl groups of 5-ASA with the functional groups of DEAEMA was expected to be by hydrogen bonding.

It is noteworthy that the molar ratio of 5-ASA relative to the functional monomer; DEAEMA was kept as 1/10 to obtain stable microspheres. Since, higher template-monomer ratios were prepared; these ratios induced aggregation of the microspheres. Therefore, we strictly used 1/10 ratio in our studies.

Characterization of 5-ASA imprinted and non-imprinted microspheres

The surface area, pore volume and average pore diameter of the polymer particles in the dry state were measured by surface area and pore size analyzer (Quantachrome, Nova 2200e). The average size and size distribution curves of microspheres were obtained in acetonitrile by using a particle size analyzer (Nano-S, Laser Particle Size Analyzer). The morphologies of the samples were examined by scanning electron microscope (SEM, Jeol 6400), after coating the samples with gold.

5-ASA loading

5-ASA was reloaded to imprinted and non imprinted polymeric microspheres using the soaking method. In brief, 40 mg of polymeric microspheres were immersed in 10 ml of a mixture of acetonitrile and HCl solution at pH 1.2 (19/1, v/v) with 5-ASA (1 mg/mL) and soaked for 6 hours at room temperature. The mixture was continuously stirred and at the end of the time the solvent was removed. Then the microspheres were dried under vacuum overnight at 40°C. For % loading calculation, 1 mL of samples were withdrawn from removed solvent and analyzed for 5-ASA with UV-visible spectrophotometer (Agilent 8453) at 300 nm.

5-ASA Release Studies

To the determine drug release, 20 mg of microspheres (5-ASA reloaded) were placed in dialysis tubing and suspended 2.5 mL of 0.1 N HCl and then placed in 200 mL of 0.1 N HCl solutions (pH 1.2, simulated gastric fluid) for 2 hours. At the end of 2 hours, by adding Na_3PO_4, pH of the solution was adjusted to 6.8 (simulated intestinal fluid). Microspheres suspensions were continuously shaken at 200 rpm on horizontal water bath shaker at 37°C. At certain time intervals, 1 mL of samples were withdrawn (replaced with fresh medium) and analyzed for 5-ASA by UV-visible spectrophotometer (Agilent 8453) at λ= 300 nm for pH 1.2 and λ=330 nm for pH 6.8. Each experiment was conducted in triplicate.

3. Results and discussion

Highly crosslinked, monodisperse polymeric microspheres have unique applications because of their superior strength, thermal and solvent resistances. In this study, acrylic-based molecularly imprinted microspheres were prepared by precipitation polymerization of DEAEMA and TRIM in the presence of 5-ASA. Non-imprinted microspheres were also prepared by the same method for control purposes. Precipitation polymerization yields monodisperse, spherical polymer particles in the micron range, quickly and cleanly as shown in Fig. 3.

Average diameters of 5-ASA imprinted and non-imprinted polymers were found as 4.5 μm and 2.5 μm, respectively. Size distribution of 5-ASA imprinted (PDI = 0.259) and non-imprinted microspheres (PDI = 0.416) shows the narrow range enough to consider these microspheres as monodisperse in size, because the PDI is lower than 1.05 (14) (Fig. 4).

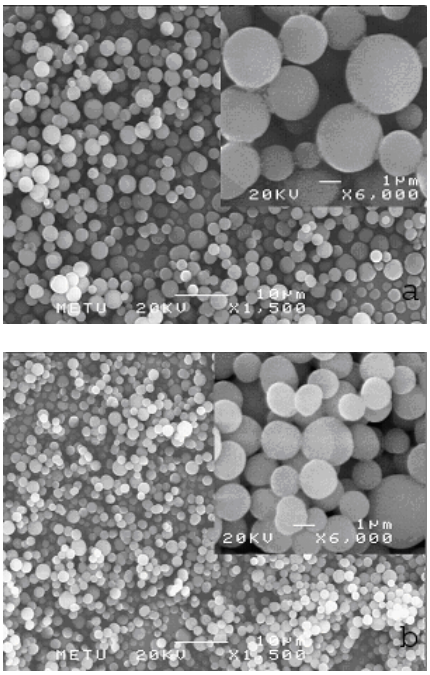

Fig. 3. SEM of Poly(DEAEMA-co-TRIM) microspheres obtained by the precipitation polymerization of 1% (v/v) total monomer concentration relative the volume of acetonitrile with 2 % (w/w) AIBN as initiator (relative to total monomer) in acetonitrile (a) 5-ASA imprinted microspheres (average diameter of 4.5 μm), *Molar ratio of 5-ASA:DEAEMA is 1/10. (b) non-imprinted microspheres at the same composition with 5-ASA imprinted in the absence of 5-ASA (2.5* μm).

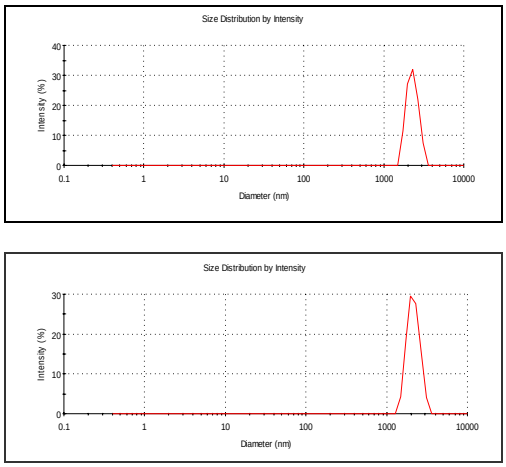

Fig .4. Particle size distribution of 5-ASA imprinted (upper figure) and non-imprinted microspheres (lower figure) in acetonitrile were obtained by using the particle size analyzer (Nano-S, Laser Particle Size Analyzer). Both of microspheres are monodisperse (PDI < 1.05).

The porosities of the 5-ASA imprinted and non-imprinted microspheres were characterized by their specific surface area, pore volume, and average pore diameter as given in data of Table 1.

Table 1. Porosity analysis results of non-imprinted and imprinted poly(DEAEMA-co-TRIM) microspheres.

Microsphere	Non-Imprinted	5-ASA Imprinted
Specific Surface Area S (cm^2/g)	4.98×10^4	3.39×10^4
Pore Volume V (cm^3/g)	1.00×10^{-2}	6.11×10^{-3}
Average Pore Diameter DP (nm)	42.80	43.58

The average pore diameter of 42.80 nm and 43.58 nm, the specific surface areas of 4.98×10^4 and 3.39×10^4 cm^2/g were found for non-imprinted and 5-ASA imprinted microsphes, respectively. These data indicate that the presence of 5-ASA in the precipitation polymerization slightly influences the polymer morphology. The difference in porosity may be due to imprinting effect.

Furthermore, both types of microspheres are mesoporous. In terms of site accessibility, large surface area and accessible meso and macro-pores, which provide rapid mass transfer, are preferred in MIPs.

Importantly, the 5-ASA loading was almost identical in non-imprinted (86 %) and 5-SA imprinted microspheres (84 %) irrespective of the imprinting effect and particle size. This may be explained as follows: 5-ASA is hydrophilic in nature, (5-ASA solubility in water is 0.1 g/100 mL at 21^0C), and therefore, HCl solution was added to acetonitrile (19/1, v/v) in order to increase 5-ASA solubility.

Thus, the interactions between a template molecule and functional monomer were hampered during loading process. On the other hand the reloading of 5-ASA in polymer matrix may lead to uniform distribution because of possible interaction of 5-ASA and functional monomers.

The release profiles of 5-ASA by the imprinted and the non-imprinted microspheres are shown in Fig. 5. The resultant release profiles illustrated that 5-ASA released faster from the non-imprinted microspheres than imprinted microspheres at the first 5 hours. Therefore it is possible to note that imprinted microspheres showed a better controlled release of 5-ASA than non-imprinted microspheres. The extended release profile may not be obtained with 5-ASA imprinted microspheres; however it is remarkable that the initial release was lowered with 5-ASA imprinted microspheres. Firstly, this observation can be explained by the molecular imprinting effect as follows: 5-ASA released slowly from the imprinted microspheres because of their functional group interaction with the recognition sites in the imprinted microspheres. Carboxylic acid, amine and hydroxyl groups of 5-ASA interact with the functional groups of DEAEMA through hydrogen bonding as expected.

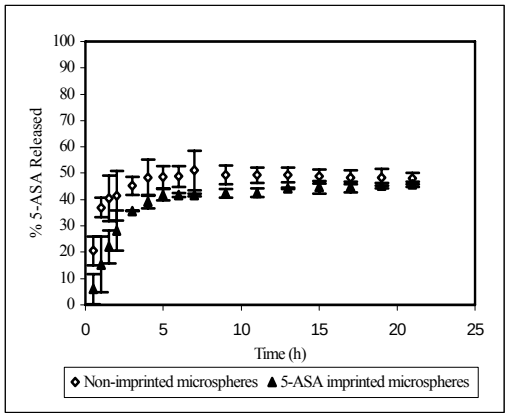

Fig. 5. Release profiles of 5-ASA from imprinted and non-imprinted microspherse. 5-ASA release was observed first for 2 hours at pH 1.2 and then at pH 6.8.

Secondly, microsphere size is a critical factor determining the drug release rates. In many cases, smaller microspheres release faster due to increased surface area/volume ratio. In other words, diffusion plays a major role in the control of drug release from higly crosslinked and non-degradable systems. An increase in microsphere size is important and it is expected to results in reduced relative rates due to increased length of the diffusion pathways and thus, decreased drug concentration gradients.

4. Conclusions

Monodisperse, highly crosslinked 5-ASA imprinted and non-imprinted poly(DEAEMA-co-TRIM) microspheres were successfully prepared by precipitation polymerization for the first time and evaluated as recognitive drug carrying systems. We have demonstrated that molecular imprinting can be applied to this system and it was observed that the 5-ASA imprinted microspheres released the drugs slower than the non-imprinted ones in the initial stages. However, molecular imprinting did not show substantial effect on the release of 5-ASA. That is imprinting neither extended the release of 5-ASA nor increased the percent drug loading. From our point of view, the preparation of MIPs is simple, however the rational design of MIPs is very complicated because of number of experimental variables, for example functional monomer(s), nature of template, ratio of functional monomer(s) to template, crosslinker(s), ratio of functional monomer(s) to crosslinker(s), solvent(s), initiator, methodology polymerization parameters and solubility of

drug in polymerization mixture etc. Therefore detailed studies on drug delivery systems are needed to investigate the effects of various parameters and to make comparison among them by using several drugs for further progress.

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