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Production of RAFT Imprinted Smart Hydrogel Particles in a Continuous Flow Micro-reactor

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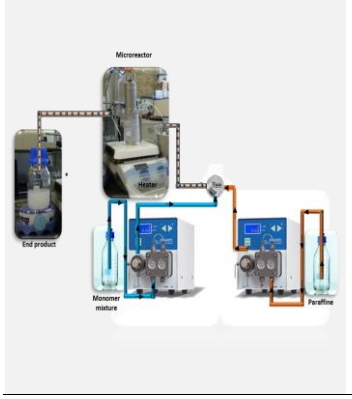

Feasibility of the production of RAFT imprinted smart hydrogel particles in continuous flow micro-reactor is here showed. Microfluidic continuous operation was combined with RAFT polymerization and molecular imprinting tecniques involving selected template molecules. New strategies for the production of advanced materials with tailored properties are thus developed. Particles syntetized in the continuous flow micro-reactor (set-up scheme depicted in the graphical abstract) were purified and characterized using different techniques, namely batch and continuous drugs adsorption and release processes. Particles were packed in small columns allowing the quick testing of these materials using frontal analysis. Therefore, the usefulness of these particles in biotechnology and biomedicine is likely.

Introduction

High surface area/volume enhancing heat transfer, constant product quality at high through-put and small set-up volume are some advantages of continuous flow micro-reactors which have been exploited lately for several purposes, namely process intensification. Moreover, microfluidics devices such as micro-reactors allow the production and manipulation of individual particles and droplets which is an important issue in many technological applications [1]. On the other hand, controlled radical polymerization (e.g. RAFT polymerization [2]) has been considered in several research lines to improve the molecular architectures of vinyl polymers (e.g. aiming at low chain heterogeneity, controlled topologies, etc.). Control of the polymerization mechanisms is also especially important in the formation of gels and hydrogels in order to obtain advanced materials with tailored properties. Imprinted smart hydrogels are a particular class of these polymer networks with important applications in biotechnology and biomedicine [3] due to specific affinity to template molecules (e.g. a drug molecule included in the polymerization process) and the ability to be stimulated by external parameters (e.g. change in pH/temperature).

This work combines these three lines of research: droplet polymerization in microfluidic reactors with controlled radical polymerization (RAFT polymerization is used aiming the improvement of the molecular architecture of networks) and molecular imprinting is here reported. Our final goal is the development of designing tools aiding the synthesis of advanced materials with important applications in many technological/biotechnological fields.

Materials and Methods

Continuous flow micro-reactor was built up using two Knauer HPLC pumps (model Azura P 4. 1S, titanium head) with maximum delivery pressure of 40 MPa and flow rate in the range 0.001 to 10 ml/min. Valco tee devices were used to connect the two lines coming from the pumps with generation of the fed to the micro-reactor. Different T conectors with internal diameters 0.25, 0.5, 0.75, and 1.0 mm were considered. PTFE tubings with different internal diameters (0.2, 0.5, 0.8, 1.0 and 1.5 mm) were used as continuous flow micro-reactors. The maximum lenght of all micro-reactors used is 20 m. The micro-reactor tubing was rolled up on a metallic cylinder and immersed in oil bath with controlled temperature. A container was connected to the end of the reactor in order to collect the carrier fluid (often liquid paraffin) and the aqueous-phase polymer particles. In this container, a polymer precipitating solvent (e.g. methanol or acetone) was also often included and mixing by a magnetic stirrer was also promoted. A scheme of this micro-reactor set-up is provided in the graphical abstract of this paper.

This reserarch is focused on the micro-reactor production of smart hydrogel particles. Within this purpose, water soluble vinyl monomers are considered. Results here presented were obtained with acrylamide (Am), acrylic acid (AA) and

methacrylic acid (MAA) as main monomers and methylene bisacrylamide (MBAm) as crosslinker. V50 (2,2'-azobis(2-methylproprionamidine) dihydrochloride) was selected as water compatible thermal initiator. In this research, RAFT polymerization is combined with operation in micro-reactor aiming the production of networks with improved molecular architecture. Different commercially available RAFT agents are tested, namely 2 - (dodecylthiocarbonothioylthio) - 2 methylpropionic acid (DDMAT), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPA) and cyanomethyl dodecyl trithiocarbonate (CDT). In some polymerization runs, a different solvent was included in the monomer phase (e.g. DMF) in order to deal with the low solubility of RAFT agents in water. In these conditions different thermal initiators were used, namely AIBN (2,2'-azobis(2-methylpropionitrile)).

Different templates molecules were also considered in the screening studies concerning the RAFT imprinting of hydrogels in continuous reactor, namely 5-fluorouracil (5FU), uracil (UR), thymine (THY), caffeine (CAF), isoniazid (INH), ibuprofen (INH), 4-aminopyridine (4AMP), 3-aminopyridine (3AMP), adrenalin (ADR) and norephedrine (NOR). It is important to stress that the polymerization kinetics can be strongly affected (inhibition effects) by the presence of these drugs. This is an important issue in the design of micro-reactor operation (e.g. selection of residence time).

Micro-reactor produced hydrogel particles were isolated and purified through successive cleaning operations. Soxhlet extraction was also used in these purification procedures. Materials were characterized by measuring the swelling ratio of the dried particles. Microscopic images of the particles in the dried/swollen states were also obtained. Performance of the smart hydrogels synthetized was also assessed through batch adsorption studies and frontal analysis. Using this latter technique, was studied the continuous adsorption and release processes involving the produced particles and different drugs. For this purpose, particles were packed in empty columns with bed lengths/internal diameters (mm/mm) of 10/4.6, 33/4.6, 50/4.6 and 33/8. Drugs adsorption/release processes were thus measured using UV continuous monitoring in a GPC pumping system. Complementary information concerning the networks formation processes in batch and continuous micro-reactors were obtained using a SEC system composed of a Viscotek GPCmax VE 2001 integrated solvent and sample delivery module coupled to a tetra detector array (refractive index + light scattering + viscosity + ultraviolet detection).

Results and Discussion

Typical operation conditions used in the studies concerning the production of RAFT imprinted smart hydrogel particles in continuous flow micro-reactor are presented in Table 1. Besides the changes in the initial composition (to assess effects such as imprinting/non-imprinting, FRP/RAFT polymerization), temperature and reactor residence time were also used as screening variables. For each initial polymerization composition, a batch polymerization was performed in the same conditions designed for continuous operation. Batch/continuous products can thus be compared. Measurement of batch gelation time is also a key aspect to assess the feasibility of the continuous polymerization with the selected residence time.
Feasibility of the approach here described is showed in Figures 1 and 2 where the morphology of some particles thus obtained is illustrated (particles in swollen and dried states).

Figure 1. Photography of AA based hydrogel particles produced in micro-reactor. Particles were isolated, dried and poured in aqueous solution (with concomitant sweling).

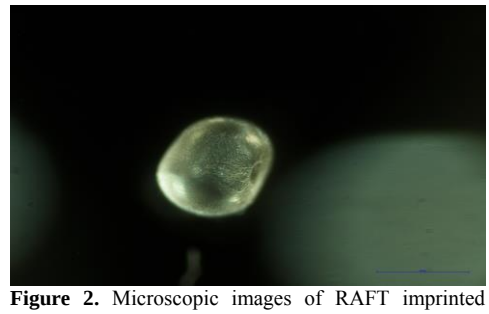

Figure 2. Microscopic images of RAFT imprinted smart hydrogel particles obtained in continuous flow micro-reactor.

Results concerning the testing of the performance of the particles are presented in Figure 3 where adsorption and release of 3AMP in these materials is illustrated.

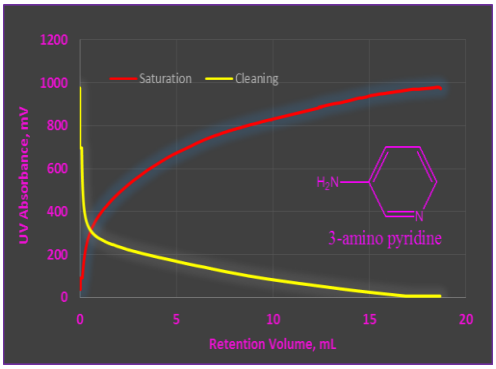

Figure 3. Saturation and release profiles for 3AMP in particles of a RAFT smart hydrogel. Particles are AA based and were synthetized in micro-reactor. Frontal analysis conditions: C_0=0.5 mM, Q=0.5 ml/min in a column containing 11 mg of dried hydrogel.

These data were obtained using frontal analysis in packed columns; they show the huge affinity between AA based RAFT smart hydrogel and the template molecule selected.

Conclusion

Feasibility of the production of RAFT imprinted smart hydrogel particles in a continuous flow micro-reactor has been here demonstrated. The likely usefulness of these particles in biotechnology and biomedicine is also shown through frontal analysis (including sorption and release processes) of selected template molecules in packed columns.

Table 1. Operation conditions used in some runs concerning the production of hydrogel particles in micro-reactor.[a]

M	I	S	Y_M	Y_I	Y_{CL}	$Y_{RAFT/I}$	$Y_{M/RAFT}$	Template	$Y_{T/CL}$	N	T (°C)	τ (min)
AAm	V50	Water	14.5	0.5	-	-	-	-	-	-	50	13
AAm	V50	Water	14.5	0.5	9.5	-	-	-	-	-	50	13
AA	V50	Water	40	0.5	4.0	-	-	-	-	-	50	11
AA	V50	Water	40	0.5	4.0	0.1	2000	-	-	-	50	11
AA	V50	Water	40	1.0	2.0	0.1	1000	-	-	60	50	11
AA	V50	Water	40	1.0	2.0	0.1	1000	3APY	1	-	70	11
AA	V50	Water	40	1.0	2.0	0.1	1000	3APY	1	-	70	50
AA	AIBN	DMF	40	1.0	2.0	0.1	1000	-	-	-	90	30
AA	AIBN	DMF	40	1.0	2.0	0.1	1000	-	-	-	90	20
AA	AIBN	DMF	40	1.0	2.0	0.1	1000	-	-	-	70	12
AA	V50	Water	40	1.0	2.0	1.0	100	-	1	-	60	60
AA	V50	Water	40	1.0	2.0	1.0	100	CAF	1	-	60	60
MAA	V50	Water	40	1.0	2.0	1.0	100	-	1	-	60	60
MAA	V50	Water	40	1.0	2.0	1.0	100	CAF	1	-	60	60

a When applicable, MBAm was used as crosslinker. Y_M (%) represents the mass fraction of monomer in the mixture monomer + solvent. Y_I (%) represents the molar ratio initiator/monomer. Y_{CL} (%) represents the mole fraction of crosslinker in the mixture crosslinker + monomer. $Y_{RAFT/I}$ represents the mole ratio between RAFT agent and initiator. $Y_{M/RAFT}$ represents the mole ratio between monomer and RAFT agent. $Y_{T/CL}$ represents the mole ratio between template molecule and crosslinker. N (%) represents the degree of neutralization of the monomer.

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