ChemPor 2014

U.PORTO

FEUP FACULDADE DE ENGENHARIA UNIVERSIDADE DO PORTO

ORDEM DOS ENGENHEIROS

10 – 12 Sept. 2014

PORTO

PORTUGAL

12th International Chemical and Biological Engineering Conference

BOOK OF EXTENDED ABSTRACTS

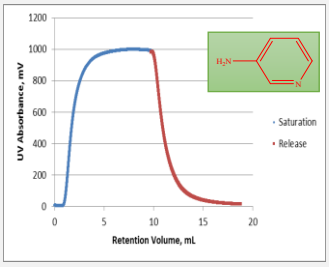
FEUP EDIÇÕES

9 789727 521708

Development of Stimuli-Responsive Smart Hydrogels using Molecular Imprinting and Interpenetrating Polymer Networks

P-CM41

T. Oliveira[1], P. Reitor[1], D. Oliveira[1], P. Kadhirvel[1], R.C.S. Dias[1,], M.R.P.F.N. Costa[2]. [1]LSRE-Instituto Politécnico de Bragança, Quinta de Santa Apolónia, 5300, Bragança, Portuga. [2]LSRE-Faculdade de Engenharia da Universidade do Porto, Rua Roberto Frias s/n, 4200-465, Porto, Portugal. [*]e-mail:* rdias@ipb.pt



Different classes of stimuli-responsive smart hydrogels (SRSH) were synthesized in order to assess the usefulness of molecular imprinting and generation of interpenetrating polymer networks to obtain advanced materials with tailored properties/performance. Reversible Addition-Fragmentation Chain-Transfer (RAFT) polymerisation was exploited as an additional tool to increase the control on the formation process of these materials. Batch adsorption and frontal analysis (*e.g.* for 3-aminopyridine (3AMP) as depicted in the graphical abstract) techniques were used to quantify the affinity of different drugs with the produced SRSH. Stimulated drug release (*e.g.* due to temperature/pH changes) and protein immobilisation/release were also tested. Results obtained show that molecular imprinting and generation of interpenetrating networks are effective routes to obtain tailored materials with a particular affinity to selected template molecules.

Introduction

Stimuli-responsive smart hydrogels (SRSH) find important applications in biotechnology, biomedicine, pharmaceutics or environmental industries. Bio-separations, bio-sensing, tissue engineering and drug or gene delivery are some examples of processes where this class of advanced materials show an increasing importance in the last years [1,2]. Microscopic properties of these polymer networks are sensitive to changes triggered by their surrounding media (*e.g.* aqueous solutions) which can be explored to obtain materials exhibiting specific responses to pH, temperature, ionic strength, presence of biomolecules (*e.g.* glucose), light and electric or magnetic fields.

Nevertheless, the performance of these hydrogels is strongly dependent on their structural topology, which is also affected by synthesis conditions of the networks. Hence, the development of tools linking the production conditions used with the end-use properties of SRSH is an important route to obtain tailored advanced materials. This work reports the development of tools aiding the synthesis of tailored SRSH, namely through the use of molecular imprinting techniques and the production of interpenetrating polymer networks. Moreover, different polymerisation mechanisms are also considered in order to try increasing the control on the formation processes of the polymer networks: SRSH are produced using classical free-radical polymerization (FRP) and also by CRP using Reversible Addition-Fragmentation Chain-Transfer (RAFT) agent. Materials thus obtained are characterized considering batch adsorption studies, frontal analysis in packing columns and also batch drug release processes triggered by external stimuli. Different drug molecules were considered in these studies in order to find particular affinities between the different SRSH produced, template molecules and stimulation.

Materials and Methods

Water-soluble vinyl monomers introducing pH and/or temperature sensibility to the hydrogels were selected in this experimental program. N-isopropylacrylamide (NIPA), N,N-dimethylacrylamide (DMA), 2-(dimethylamino) ethyl methacrylate (DMAEMA), acrylamide (Am), acrylic acid (AA) and methacrylic acid (MAA) are examples of monomers used in this research. Methylene bisacrylamide (MBAm) was considered as crosslinker. Water compatible initiation system was changed when considering different operation conditions, namely the polymerization temperature. The pair ammonium persulfate (APS)/tetramethylethyleneamine (TEMED) was used for redox initiation at low reaction temperature (e.g. 25 °C) whereas 2,2'-azobis(2-methylproprionamidine) dihydrochloride (V50) and 2,2'-azobis[2 - (2 – imidazolin -2-yl) propane] dihydrochloride (VA-044) were selected as thermal initiators (*e.g.* polymerizations at 40 °C

with VA-044 and at 50 °C with V50).

RAFT production of SRSH was also exploited in order to try the improvement of the control on the molecular architectures of the materials. For this purpose, three commercially available RAFT agents are used: 2-(dodecylthiocarbonothioylthio)-2 methylpropionic acid (DDMAT), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPA) and cyanomethyl dodecyl trithiocarbonate (CDT). In order to minimize the issue arising from the low solubility of some RAFT agents in water, binary mixtures (e.g. water/methanol) or organic solvents (e.g. DMF with initiation by AIBN) were also used in the polymerisations [3].

Different drugs were considered in the molecular imprinting synthesis of SRSH and in the loading/release processes of hydrogels: 5-fluorouracil (5FU), uracil (UR), thymine (THY) caffeine (CAF), isoniazid (INH), ibuprofen (INH), 4-aminopyridine (4AMP), 3-aminopyridine (3AMP), adrenalin (ADR) and norephedrine (NOR). These different templates were chosen in order to try to find specific affinities between networks and drugs. Bovine serum albumin (BSA) was used in immobilisation/release studies of proteins with SRSH. Hydrogels resulting from the crosslinking of DNA (salmon testes) with diglycidyl ether monomers were also synthesized in order to compare the performance of pure synthetic networks with those incorporating natural molecules.

Polymer networks thus produced were purified through repeated washing with water. Soxhlet extraction was also used to obtain template-free SRSH products. When needed, hydrogels were dried in vacuum. The swelling ratio of the dried materials was measured in different conditions (*e.g.* aqueous solutions at different pH/temperature values). If needed, dry SRSH were also loaded/reloaded with selected drugs and after submitted to stimulated release testing. Dynamics of drugs and protein release was measured using UV detection.

Batch adsorption studies were also performed considering different combinations between SRSH/templates. Frontal analysis [4] was used to study the continuous adsorption and release processes involving SRSH and different drugs. Materials were packed in small empty columns with bed lengths/internal diameters (mm/mm) of 10/4.6, 33/4.6, 50/4.6 and 33/8. Quick testing involving a small mass of the SRSH was thus possible. A GPC pumping system and UV continuous monitoring were used in these studies.

Molecular architecture of the soluble fraction of SRSH or their linear counterparts was experimentally measured using a SEC apparatus composed of a Viscotek GPCmax VE 2001 integrated solvent and sample delivery module coupled to a tetra detector array including refractive index, light scattering, viscosity and ultraviolet detection. These measurements are especially important to obtain insights on the networks formation dynamics and involved kinetic mechanisms. Mathematical modelling studies of such polymerization processes are thus possible [5].

Results and Discussion

In Tables 1 to 3 are presented some details concerning the synthesis of the different kinds of SRSH materials studied in this research. Note that that the properties/performance of these networks is strongly dependent on the particular synthesis conditions used.

Table 1. Typical conditions used in the synthesis of some imprinted/non-imprinted SRSH.[a]

Monomer	Template	I	RAFT	T
NIPA	5Fu	APS	-	25
NIPA	4AMP	APS	-	40
AA	-	APS	-	20
AA	-	AIBN	DDM	70
AA	3AMP	V50	-	50
DMAEMA /MAA	-	V50	CPA	50

a Temperature (T) in °C. Monomer concentrations and initial ratios Monomer/Initiator/RAFT/Template are important design parameters in this context.

Table 2. Typical conditions used in the immobilisation of BSA in temperature-sensitive SRSH.[a]

NIPA (%)	BSA (%)	APS (%)	NaCl (mol/L)	T
6.4	13	0.2	-	25
6.3	13	0.2	0.69	25
6.4	13	0.2	-	25
6.3	13	0.2	1.01	25
6.4	13	1.1	-	25

a Temperature in °C. NIPA represents the mass fraction of monomer in water/NIPA/crosslinker mixture (crosslinker/NIPA=1.1% mol/mol), BSA the mass fraction of protein in BSA/monomer mixture and APS the molar ratio initiator/NIPA. NaCl was used in order to increase the porosity of the SRSH materials.

Table 3. Typical conditions used in the synthesis of some SRSH interpenetrating polymer networks (IPN).[a]

IPN	M	I	S	T
NIPA/NIPA				
	NIPA	APS	Water	25
	NIPA	APS	Water	25
AA/NIPA				
	AA	V50	Water	50
	NIPA	VA-044	Water	40

a Temperature in °C. For each IPN are described the conditions used in the first and second crosslinking reactions.

In Figures 1 to 4 are illustrated some results concerning the assessment of the performance of the synthetized SRSH, namely frontal analysis for drugs adsorption/release quantification (Figures 1, 2 and also graphical illustration), batch drug release stimulated by the environmental conditions (Figure 3) or release of immobilized BSA (by molecular imprinting) from a temperature-sensitive network.

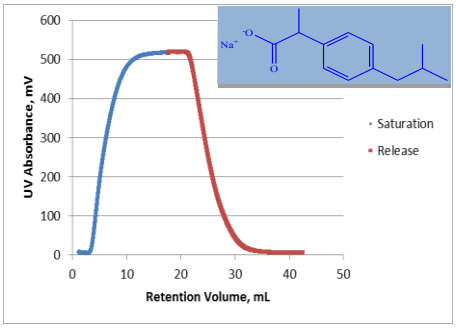


Figure 1. Saturation and release profiles for IBU in an AA based SRSH. Frontal analysis conditions: C_0=0.5 mM, Q=0.33 ml/min in a column containing 15.7 mg of dried hydrogel.

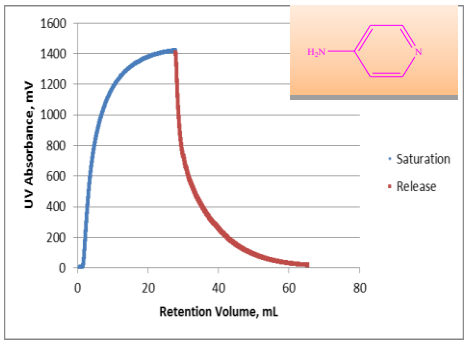


Figure 2. Saturation and release profiles for 4AMP in a NIPA/MAA based SRSH. Frontal analysis conditions: C_0=0.1 mM, Q=0.15 ml/min in a column containing 7.3 mg of dried hydrogel.

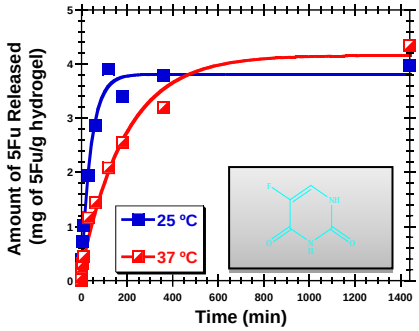


Figure 3. Dynamics of batch release of 5FU from a pre-incubated SRSH (NIPA/5FU MIP) considering temperature stimulation (25 and 37 °C).

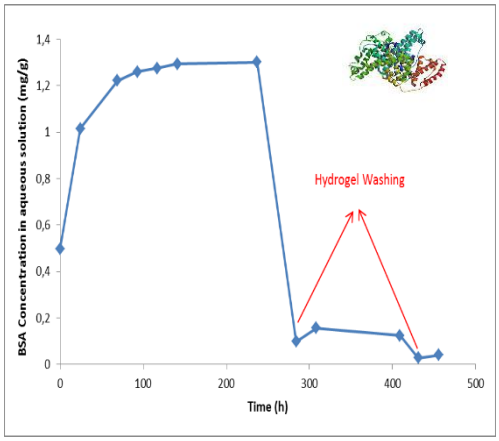


Figure 4. Release of immobilized BSA (by molecular imprinting) from a NIPA hydrogel submitted to successive washing operations with aqueous solutions at 20 °C.

Conclusion

Different classes of SRSH were synthesized and assessed for adsorption/release of different kinds of biomolecules (*ex.* drugs and proteins). Results obtained show that molecular imprinting and generation of interpenetrating networks are effective routes to obtain tailored materials with particular affinity to selected template molecules.

Acknowledgements

The authors thank FCT and FEDER under Programme COMPETE (Project PEst-C/EQB/LA0020/2013), QREN, ON2 and FEDER (Project NORTE-07-0162-FEDER-000050) and QREN, ON2 and FEDER (Project NORTE-07-0124-FEDER- 0000014 - Polymer Reaction Engineering). P.K. thanks NORTE-07-0124-FEDER-0000014 for the Post-Doctoral grant.

References

[1] *Smart Polymers. Applications in Biotechnology and Biomedicine*, 2nd Ed.; I. Galaev, B. Mattiasson, CRC Press, 2007, ISBN 9780849391613.

[2] *Stimuli Responsive Drug Delivery Systems: From Introduction to Application.* A. Bajpai, S. Shukla, R. Saini, A. Tiwari, Smithers Rapra Technology, 2010, ISBN 9781847354167.

[3] M.A.D. Gonçalves, V.D. Pinto, R.A.S. Costa, R.C.S. Dias, J.C. Hernándes-Ortiz, M.R.P.F.N. Costa, *Macromol. Symp.*, 333 (2013) 41.

[4] F. Gritti, W. Piatkowski, G. Guiochon, *J. Chromat. A*, 978 (2002) 8.

[5] M.A.D. Gonçalves, V.D. Pinto, R.C.S. Dias, M.R.P.F.N. Costa, *J. Nanostr. Polym. Nanocomp.*, 9 (2013) 40.