Characterization technique	Measured parameters	Gradient type	Merits/Limitations
XPS	elemental distribution of the hydrogel surface - element ratios/mole fractions of monomer - cross-linking density qualitative + quantitative technique	Chemical + physical	− surface characterization − preliminary pretreatment of the samples (applies only for the lyophilized samples)
FLUO	characterization of inner environment or structural changes using fluorescent probes qualitative + quantitative technique	Chemical	+ applies in "wet" state + possible tracking of gradient formation in real time −sensitive to pH, ionic strength and temperature changes + applies in "wet" state − preliminary pretreatment of the sample (labelling by hydrophilic fluorescent probe)
RAMAN	chemical structure and identity, phase and polymorphism in high-water content media - cross-linking density qualitative + quantitative technique	Chemical + physical	+ insensitive to aqueous absorption bands + doesn't need sample preparation − large fluorescence background (can suppress the smaller Raman signals)
CLSM	three-dimensional imaging of samples (by selectively accessing the fluorescence signals from different planes) -distribution of functional groups labelled by fluorescent dye - cross-linking density	Chemical + physical	− preliminary pretreatment of the sample (labelling by fluorescent probe) − limited depth of field + applies in "wet" state
FTIR	presence of the specific chemical/functional groups or inorganic compounds	Chemical + physical	− sensitive to aqueous absorption bands − preliminary pretreatment of the samples

	(alterations in characteristic patterns of absorption bands indicate change in the material composition) qualitative technique		(applies for the lyophilized samples or samples dissolved in organic solvent)

Characterization technique	**Measured parameters**	**Gradient type**	**Merits/Limitations**
SEM	imaging of hydrogel surface and internal structure - pore sizes, shapes and distribution - pore interconnectivity -cross-linking density - wall thickness - fiber orientation qualitative technique	Physical	+ high resolution (in contrast to − preliminary pretreatment of the samples (applies only for the lyophilized samples) − structural artifacts (freeze-drying process could cause changes in morphology)
POM	composition and three-dimensional structure - direction of polymer alignment/orientation of rigid molecules based on optical anisotropy of the material - absorption color and optical path boundaries between layers qualitative technique	Physical	− anisotropic hydrogels
micro-CT	3D imaging of internal structure of hydrogels - porosity - cross-linking density - wall thickness	Physical	+ 3D volume reconstruction − demand labeling or contrasting the hydrogel chemically − limited resolution − preliminary pretreatment of the samples

	- fiber orientation qualitative technique		(applies for the lyophilized samples
AFM	imaging of hydrogel topography - roughness - pore sizes, shapes and distribution - wall thickness qualitative + quantitative technique	Physical	− surface characterization + applies in “wet” state