Functional Cellulose Beads: Preparation, Characterization, and Applications

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Functional Cellulose Beads: Preparation, Characterization, and Applications

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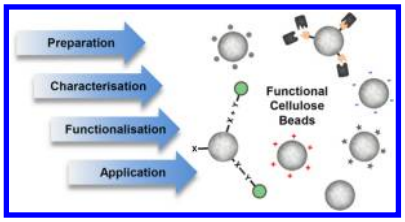

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1. INTRODUCTION

The preparation of cellulose-based materials is an area of increasing interest for scientists working in chemistry, chemical engineering, biochemistry, and many other related areas involving the chemical design of biobased materials.[1−4] Polysaccharides provide excellent mechanical and chemical properties combined with the features of a bioresource, for example, abundance, biocompatibility, and sustainability.[5−8] Furthermore, the ease of chemical modification enables tuning of cellulose's properties from hydrophilic to hydrophobic and from noncharged to anionic or cationic.[9−11]

Cellulose beads are spherical particles with diameters in the micro- to millimeter scale (Figure 1 a), which are used in many advanced applications ranging from chromatography over solid-supported synthesis and protein immobilization to retarded drug release. Over the last decades, various procedures for the preparation of cellulose beads have been reported, including the use of different solvents, shaping techniques, and technical devices for large batch production. Functional materials for specific applications have been prepared by introducing numerous chemical functionalities or blending cellulose with organic and inorganic compounds. In addition, commercial cellulose bead products with defined properties are available (Figure 1b and c).[12−14]

The aim of the present Review is to provide a systematic overview of the preparation of cellulose beads, characterization of their physical properties by different analytical techniques, the preparation of functional cellulose beads by chemical modification, and potential fields of application for these versatile materials. Special emphasis was placed on current and future developments brought to the field with the recent discovery of novel cellulose solvents such as ionic liquids and aqueous systems. These solvents show promising features with respect to the environmental, economical, and safety concerns of currently applied dissolution processes, which has resulted in a tremendous increase in research regarding the preparation of advanced cellulose materials and has fuelled interest in cellulose beads.[15]

Cellulose beads have frequently been named: microspheres, pellets, cellulose gels, pearl cellulose, or beaded cellulose. To avoid confusion caused by different terminologies, cellulose beads are henceforward defined as (i) spherical particles with diameters ≥10 μm (ii) that are prepared via the dissolution, shaping, and regeneration of cellulose (or a derivative that is subsequently converted to cellulose) and (iii) that are exclusively composed of cellulose and fixed in their spherical

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Figure 1. Cellulose beads of different sizes (a). Commercially available cellulose beads (b and c).[12,13]

shape by re-establishment of the hydrogen bonding network and typical cellulose−cellulose interactions. Thus, cellulose granules, prepared by extrusion−spheronization and subsequent exposure to mechanical abrasion to obtain a spherical shape, are explicitly excluded.[16] These materials may possess the same macroscopic appearance as beads, but they are processed from cellulose suspensions not solutions, which implies a different type of microscopic morphology, especially regarding the pore structure.

The term "functional cellulose beads" refers to materials prepared by heterogeneous chemical modification of cellulose beads or by the inclusion of inorganic and organic materials into them. The latter may include other polysaccharides and polysaccharide derivatives, but the matrix (i.e., the major component that is responsible for mechanical stability) remains cellulose. Thus, beads exclusively composed of a cellulose derivative as well as polysaccharide blend beads that are stabilized predominantly by ionic interactions, for example, calcium alginate gels, are not covered in this article.

2. PREPARATION OF CELLULOSE BEADS

The preparation of spherical cellulose beads was described for the first time in 1951.[17] The materials, then named cellulose pellets, were simply prepared by hand-dropping a viscose solution into an aqueous coagulation bath. Since that report, various procedures for obtaining cellulose beads with diameters ranging from about 10 μm to 1−3 mm have been developed using different solvents and techniques to obtain spherical particles (Table 1). In principle, bead production can be simplified into three steps: (i) dissolution of cellulose (or a cellulose derivative), (ii) shaping of the polysaccharide solution into spherical particles, and (iii) sol−gel transition and solidification of the solution particles to beads. In addition, several post- and pretreatments can be applied to fine-tune certain properties. To obtain a systematic overview, these points were discussed separately in the following chapters.

2.1. Shaping into Spherical Particles

Despite the fact that various starting polysaccharides, solvents, and regeneration methods can be applied for the preparation of cellulose beads, all procedures have in common the fact that the shaping of the beads from a polysaccharide solution is either achieved by dropping (Figure 2a−d) or dispersion techniques (Figure 2e). This separation is reasonable since it is not only based on technological aspects but likewise a rough macroscopic separation into cellulose beads with a size above (dropping techniques) or below (dispersion) 250−500 μm.

2.1.1. Dropping Procedures. Beads can be obtained by the formation of spherical droplets of a polysaccharide solution and solidification of these droplets in a coagulation bath of a nonsolvent (Figure 2a). Upon pressing the solutions through a thin opening, like a syringe nozzle, a droplet is formed when the combined forces of gravity and pressure used for ejection exceed a certain value that is determined by the surface tension of the solution and capillary forces at the outlet.[18] The formation of smaller droplets can be forced by using vibrating nozzles or air jets, aimed at the tip of the capillary from which the solution protrudes.[19−21] In general, the diameter of cellulose beads obtained by dropping techniques is limited to a range of approximately 0.5−3 mm, since it is restricted to the size of droplets that can be prepared. The form of the beads, that is, the length to width factor, is determined by the mechanical strain that the droplets encounter when hitting the surface of the coagulation bath. When the stability of the droplets is low compared to the applied force, flattening of the beads may occur resulting in a disk-like shape. Moreover, the droplets require a certain time to transform from a "tear-like" shape, directly after ejection from the solution, to a spherical one. Optimization of ejection speed, falling height, and solution viscosity are consequently important for the preparation of cellulose beads using a dropping technique.[22,23]

Different technical devices can be applied to obtain droplets of a defined size and shape. Especially for large batch productions, the utilization of automated systems that work at higher operation speeds is favorable. A high number of droplets can be prepared within a short time by ejecting cellulose solutions through a rotating cylindrical vessel with small outlets (spinning drop atomization, Figure 2c).[24−26] Adjusting the rotational speed, geometrical parameters of the cup, and size of its outlets allows particle sizes to be controlled efficiently.[27,28] As a result of the high forces applied to the cellulose solution, it is also possible to obtain droplets of comparably small diameters of 500 μm or less. Another potential technique for droplet formation is "spinning disc atomization" (Figure 2d). Here, a thin film of the polymer solution is constantly spread onto a rotating disc and droplets are ejected from the edge as a result of centrifugal forces.[29,30]

When cellulose solutions are ejected through a thin opening at a high velocity, a constant stream is obtained instead of droplets. By passing this stream through a rotating knife apparatus, it can be cut into spherical particles, which subsequently fall into a coagulation bath (jet cutting).[31,32] Thereby, ejection and cutting of the stream may also be performed directly into the coagulation medium (underwater pelletizing).[33] Using these techniques, the size and shape of the beads can be controlled by the ejection speed, diameter of the jet nozzle, and geometry and rotation speed of the knife.[34] In addition to the above-described procedures, spraying techniques have also been applied for obtaining fine spherical

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Table 1. Procedures for the Preparation of Cellulose Beads Using Different Shaping Techniques, Starting Materials, and Solvents

technique	starting material[a]	solvent[b]	additives	dispersion medium	solidification	size[c]	references
dropping	cellulose	DMA/LiCl			coagulation in water/alcohol	~0.5 mm	21, 70, 71
		ionic liquids	chitosan		coagulation in water	n.a.	103
		ionic liquids	lipase + polysaccharides		coagulation in water	~2 mm	171
		$NaOH_{aq}$			coagulation in water	~1 mm	23
		$NaOH/urea_{aq}$			coagulation in $HNO_{3\ aq}$	~2 mm	22
		$NaOH/urea_{aq}$	Fe_2O_3-nanoparticles, active carbon		coagulation in water	~2 mm	88
		N-ethylpyridinium chloride/dipolar aprotic cosolvent			coagulation in water	~1 mm	243
		NMMO			solidification at 10 °C, washing with water	~2 mm	75
		NMMO	chitosan		coagulation in water	n.a.	76
	CA	acetone/DMSO			coagulation in water, saponification with $NaOH_{aq}$	~1 mm	35, 36
		DMSO			coagulation in HCl_{aq}, saponification with $NaOH_{aq}$	n.a.	55
	CXA	$NaOH_{aq}$			coagulation in water, solidification at 90 °C	~2 mm	17
		$NaOH_{aq}$	$CaCO_3$		coagulation in HCl_{aq},	~4 mm	125
(jet cutting)	cellulose	ionic liquids			coagulation in water	n.a.	33
		NMMO			solidification at 10 °C, washing with water/DMSO	50−1000 μm	31
	CC	$NaOH_{aq}$			coagulation in $H_2SO_{4\ aq}$	~0.5 mm	32
(spinning drop atomization)	cellulose	$NaOH_{aq}$			coagulation in $H_2SO_{4\ aq}$	~0.5 mm	28
	cellulose	$NaOH/urea/sulfourea_{aq}$	azodicarbonamide		coagulation in HCl_{aq}	~300 μm	26
	CXA	$NaOH_{aq}$			coagulation in HCl_{aq}	0.5−1 mm	24, 25
		$NaOH_{aq}$			coagulation in $H_2SO_{4\ aq}$	~0.5 mm	27
(spraying)	CA	acetone/organic solvent mixtures			coagulation in water, saponification	~0.5 mm	35, 36
dispersion	cellulose	caden		benzene	coagulation with acetic $acid_{aq}$	~100 μm	44, 45
		$Ca(SCN)_{2aq}$		dichlorobenzene	coagulation with methanol	n.a.	244
		cuoxam		benzene	coagulation with benzoic $acid_{aq}$	~100 μm	44, 45
		cuoxam		toluene/oil	coagulation with benzoic $acid_{aq}$	~50 μm	63
		Fe-(II)-tartrate		benzene	coagulation with acetic $acid_{aq}$	~100 μm	44, 45
		ionic liquids		cyclohexane/oil	coagulation with water	~10 μm	98
		ionic liquids	tungsten carbide	oil	coagulation with ethanol	100−300 μm	78
		ionic liquids	bovine serum albumin	poly(propylene glycol)	solidification at 40 °C, washing with alcohol/water	250−1000 μm	100
		ionic liquids	chitosan, Fe_3O_4-particles	oil	solidification at 40 °C and by coagulation with ethanol	~200 μm	101, 102
		$NaOH/urea_{aq}$		oil	coagulation with HCl_{aq}	10−1000 μm	90
		$NaOH/urea_{aq}$	Fe_3O_4-nanoparticles	oil	coagulation with HCl_{aq}	~10 μm	120
		$NaOH/urea_{aq}$	Fe_2O_3-nanoparticles	oil	coagulation with HCl_{aq}	~200 μm	89
		$NaOH/thiourea_{aq}$	konjac glucomannan		coagulation with $CaCl_{2\ aq}$, and HCl_{aq}	~100 μm	147
		NMMO		oil	solidification at 35 °C, washing with water/alcohol	10−1000 μm	74
		NMMO		oil	solidification at low temperature	~100 μm	77
		NMMO	tungsten carbide	oil	solidification at 10 °C, washing with water/alcohol	75−300 μm	99
	CA	CH_2Cl_2		water	solvent evaporation, saponification with $NaOH_{aq}$	~100 μm	52, 54
		ethylacetate/methanol mixtures		water	solvent evaporation, saponification with $NaOH_{aq}$	1−10 μm	53, 245, 246
	CAB	CH_2Cl_2	hydroxyalkyl cellulose	water	coagulation with acetic acid	~100 μm	247, 248

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Table 1. continued

technique	starting material[a]	solvent[b]	additives	dispersion medium	solidification	size[c]	references
	CC	$NaOH_{aq}$		chlorobenzene	solidification at 90 °C or coagulation with ethanol/acid mixture	n.a.	50
	CXA	$NaOH_{aq}$		benzene	coagulation with acetic acid	~100 μm	44, 45
		$NaOH_{aq}$		ethylene dichloride	cross-linking and subsequent coagulation with acetic acid	~50 μm	46
		$NaOH_{aq}$		chlorobenzene	solidification at 90 °C	10–300 μm	47, 48
		$NaOH_{aq}$		chlorobenzene/CCl_4	solidification at 90 °C	~100 μm	139
		$NaOH_{aq}$	ferrite powder	oil	solidification at 90 °C	20–2000 μm	119
		$NaOH_{aq}$	$CaCO_3$	chlorobenzene	solidification at 90 °C	n.a.	123
		$NaOH_{aq}$	$CaCO_3$	oil	solidification at 90 °C	~100 μm	124
		$NaOH_{aq}$	FeOOH	chlorobenzene/oil	solidification at 90 °C	~100 μm	122
		$NaOH_{aq}$	Ni powder	chlorobenzene/oil	solidification at 95 °C	~100 μm	115
		$NaOH_{aq}$	starch, tungsten carbid	chlorobenzene/oil	solidification at 95 °C	50–250 μm	116
		$NaOH_{aq}$	steel powder	oil	solidification at 90 °C	60–180 μm	114
		$NaOH_{aq}$	TiO_2	chlorobenzene/oil	solidification at 95 °C	n.a.	113
	TMSC	hexane or CH_2Cl_2		aqueous gelatin solutions	solvent evaporation, desilylation with HCl_{aq}	100–300 μm	56

[a]CA, cellulose acetate; CAB, cellulose acetate butyrate; CC, cellulose carbamate; CXA, cellulose xanthate; TMSC, trimethylsilyl cellulose. [b]Caden, cadmium tris(ethylenediamine) hydroxide; cuoxam, cuprammonium hydroxide; DMSO, dimethylsulfoxide; NMMO, *N*-methylmorpholine *N*-oxide monohydrate. [c]Approximate diameter/diameter range. n.a.: No information available.

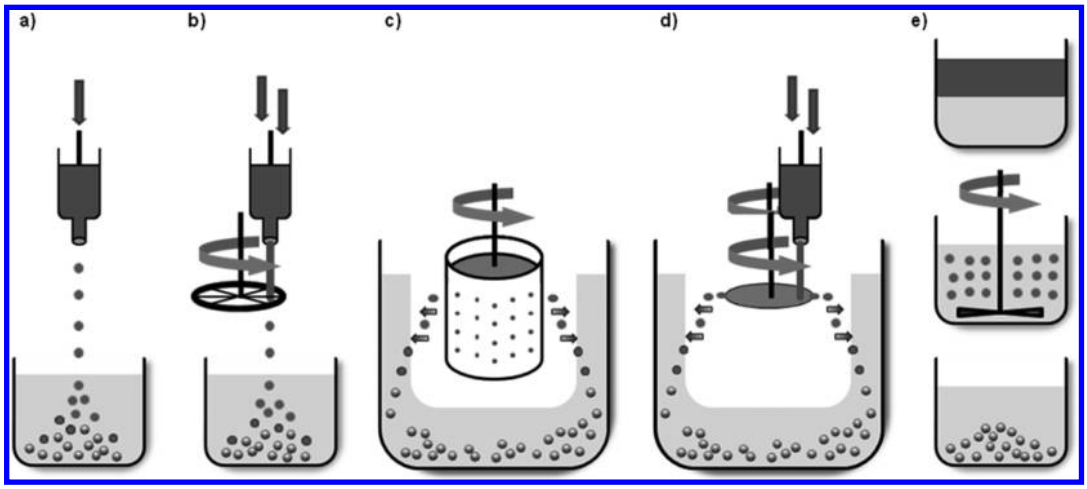

Figure 2. Schematic drawings of different procedures for the preparation of cellulose beads by different techniques: dropping (a), jet cutting (b), spinning drop atomization (c), spinning disc atomization (d), and dispersion (e).

particles.[35,36] However, strong deformation of the droplets can be expected in this case.

2.1.2. Dispersion Procedures. Dispersion of a solution of cellulose or a cellulose derivative in an immiscible solvent of opposite polarity under high rotational speed results in the formation of emulsions that can be stabilized with the aid of surfactants.[37] These emulsions contain droplet particles of the dissolved polysaccharides that can be solidified to beads of the same size, as described below. The diameter of the droplets within the dispersion ranges from about 10 to several 100 μm and is determined by the mixing speed, type and amount of surfactant, ratio of hydrophobic to hydrophilic solvent, and viscosity of the dispersion medium and cellulose solution.[38,39] Consequently, cellulose beads prepared by dispersion are roughly 10-times smaller compared to those prepared by dropping techniques (Table 1). In contrast to procedures using dropping techniques, no special equipment is required to obtain products with reproducible properties. Thus, bead preparation via dispersion has already been commercialized and products with different sizes, from about 30–250 μm, are available on the market (Figure 1a and b).[12,13]

2.2. Dissolution and Regeneration of Cellulose

It is a well-known fact that cellulose is insoluble in water and common organic solvents, presumably because of the formation of an exceptionally strong inter- and intramolecular hydrogen bonding network.[5,40] Different solvent systems that can dissolve cellulose have been intensively studied in the past decades with aim to shape the polysaccharide.[15] In general, three different types can be distinguished (Figure 3): Non-derivatizing solvents dissolve cellulose by physical interactions without the chemical conversion of its hydroxyl groups. Regeneration of the polysaccharide is achieved by disturbing these interactions, for example, by coagulation in an excess of protic nonsolvent. In contrast to that, derivatizing solvents temporarily convert cellulose into derivatives that are, in most cases, only metastable

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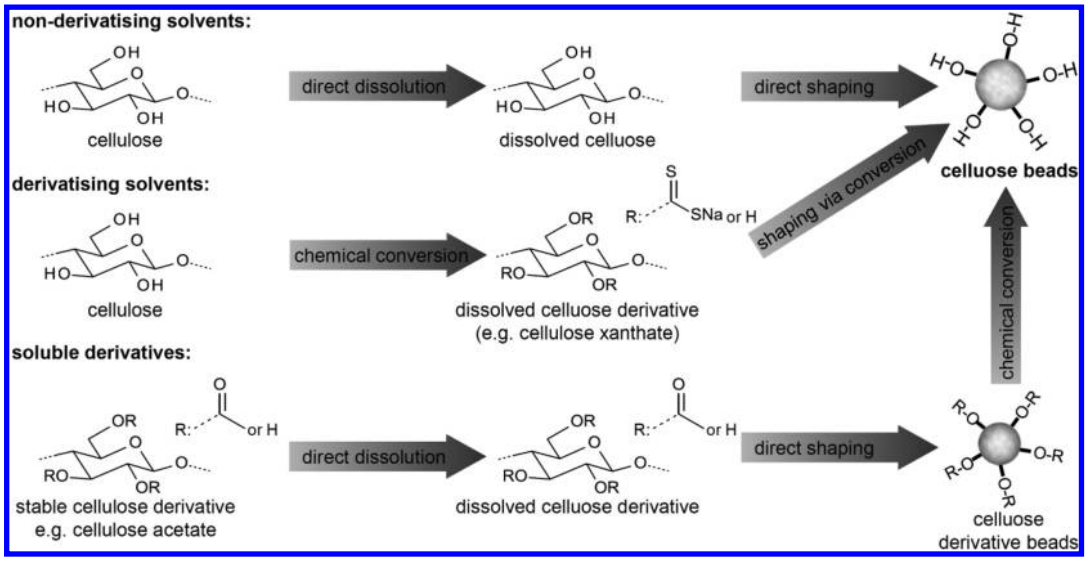

Figure 3. General routes for dissolution of cellulose and shaping into beads.

under the dissolution conditions. Thus, cellulose can be regenerated and shaped into objects, such as fibers or beads by cleavage of the intermediate derivatives, which can be induced by adding water or a change in pH or temperature.

Another option for preparing shaped cellulosic objects is the utilization of stable cellulose derivatives that are soluble in common organic solvents and often commercially available. After shaping of the solution into particles, the derivative is regenerated by coagulation or evaporation of the solvent in the presence of a nonsolvent. In contrast to regeneration of cellulose from derivatizing solvents, the "auxiliary" substituent is not hydrolyzed during this process. Thus, an additional process step is required in order to convert the shaped derivatives into the corresponding cellulose beads.

2.2.1. Derivatising Cellulose Solvents. The most prominent example for the utilization of a derivatizing cellulose solvent is the viscose process.[41−43] By a sequence of alkalization, aging, and CS_2 treatment, cellulose is converted into cellulose xanthate (CXA), which is soluble in aqueous NaOH. In contrast to the derivatives described in section 2.2.2, CXA is not isolated from the reaction mixture but directly processed. After the solution was brought into the desired shape, for example, fibers or beads, the polysaccharide is regenerated by cleavage of the xanthate substituent. This can be achieved by acid treatment or by increasing the temperature (90 °C for several minutes). Despite environmental concerns, the viscose process is still an important routine for the commercial production of cellulosic fibers. In addition, it has been the most intensively studied dissolution process used for the preparation of cellulose beads; employed for the first time in 1951 when dropping CXA solution into an aqueous coagulation bath.[17] In the following decades, the preparation has been modified first by using dispersion techniques and later by exploiting temperature-induced cleavage of the xanthate group instead of acid hydrolysis.[44−47] Centrifugation (spinning drop atomization) has also been applied to prepare cellulose beads from viscose solutions.[24,25,27] Nowadays, cellulose beads prepared from viscose by dispersion techniques are commercially available.[12,48] However, the viscose process has several drawbacks due to the use of highly toxic CS_2. Thus, environmentally friendly alternatives such as aqueous NaOH/urea and ionic liquids (IL) have attracted significant interest in recent years (see section 2.2.3).

In the presence of excess molten urea (>130 °C), cellulose that has been activated with caustic soda forms cellulose carbamate (CC), which is soluble in aqueous NaOH.[49] Comparable to the viscose process, the derivative is hydrolyzed in the presences of acids and thus CC solutions can be used to shape cellulose into fibers. Also, the preparation of cellulose beads from CC via dropping and dispersion techniques has been reported.[32,50]

2.2.2. Stable Cellulose Derivatives. Cellulose acetate (CA) is another frequently applied derivative for cellulose bead preparation. However, the procedures differ from those using derivatizing solvents in two important ways. CA is a rather stable cellulose derivative that can be isolated and stored after preparation without the need of an immediate processing. In contrast to the xanthate and carbamate groups, the acetyl moiety is not cleaved during the regeneration process. CA is precipitated by displacing the organic solvent with a nonsolvent, such as water, which implies that an additional processing step is required to convert cellulose derivative beads into cellulose beads.

CA can be produced with different degrees of substitution (DS) by esterification of cellulose with acetic acid anhydride or chloride in different homogeneous or heterogeneous systems.[9,51] Commercially available products, which have been used so far for bead preparation, usually possessed a DS > 2.0 and are consequently insoluble in water but readily dissolve in nonpolar solvents, such as chlorinated hydrocarbons or ethyl acetate. After the formation of emulsions of CA solutions in water, the polysaccharide is regenerated by evaporation of the volatile solvents and coagulation in the remaining aqueous nonsolvent.[52,53] Despite the requirement for an "auxiliary substituent", this procedure has been brought to commercial application.[13,54] In addition, beads have been prepared by dropping a CA solution into water.[35,55] Analogous to CA, trimethylsilyl cellulose (TMSC) has been utilized for the preparation of cellulose beads by the dispersion technique.[56] In both cases, beads of a cellulose derivative are obtained that need to be converted into cellulose by saponification with aqueous NaOH (CA) or by cleaving the acid labile silyl ether group by HCl treatment (TMSC). The additional processing

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steps required for introducing and finally removing acetyl or silyl moieties are the main drawback of procedures employing cellulose derivatives as a starting material.

2.2.3. Non-derivatizing Cellulose Solvents. Procedures that involve temporary conversion into soluble derivatives (see sections 2.2.1 and 2.2.2) are commonly used for shaping of cellulose because they yield good product properties. However, to dissolve cellulose, an "auxiliary substituent" is first introduced and finally removed again from the end product. Derivatization and conversion back into cellulose require additional efforts and imply the formation of high amounts of chemical waste. Thus, the direct dissolution of cellulose, without chemical modification of the polysaccharide, and subsequent coagulation in a nonsolvent is the most convenient method for producing cellulose beads.

Different "classical" non-derivatizing solvents for cellulose have been well-known for decades.[57,58] Cuprammonium hydroxide (cuoxam) and certain other aqueous metal salt solutions of copper, cadmium, nickel, iron, and cobalt that contain coligands, such as ammonia, ethylene diamine, or tartaric acid, can dissolve cellulose via complexation of its hydroxyl groups.[59,60] These solvents have been among the first reported for the dissolution of cellulose.[61,62] Although they have been studied with regard to the formation of beads, their application nowadays is strongly restricted because of the involvement of heavy metals.[44,63]

Cellulose dissolves in *N*,*N*-dimethylacetamide (DMA) containing LiCl, which has been intensively exploited for the chemical derivatization of the polysaccharide under homogeneous conditions.[64,65] This solvent could also be exploited for the shaping of cellulose and cellulose/polymer blends into films, fibers, and highly porous materials.[66−69] The preparation of beads by dropping cellulose/DMA/LiCl solutions into protic coagulation media has also been reported.[21,70,71] Despite its usefulness regarding dissolution power and the possibility to prepare blend materials, DMA/LiCl has found little use for processing of cellulose beyond academic research, which is mainly due to the high costs of the solvent and the limited recyclability.

In the field of cellulose fiber production, *N*-methylmorpholine *N*-oxide monohydrate (NMMO) is the most frequently applied non-derivatizing cellulose solvent.[72] It is intensively studied as an alternative to the viscose process, which needs to be replaced because of safety and environmental concerns related to the use of CS_2. Upon cooling, cellulose/NMMO solutions crystallize at temperatures around 20−40 °C.[73] Thus, cellulose beads could be prepared by the formation of spherical cellulose/NMMO droplets, either by dropping or dispersion techniques, solidification via temperature decrease, and removal of NMMO residues from the preshaped particles by washing with water.[31,74−78] Although commonly used for the fabrication of cellulosic fibers in large scales, NMMO has the disadvantage of being thermally unstable, especially upon the addition additives such as activate carbon, magnetic particles, or other polysaccharide derivatives.[79,80] Consequently, there is still a growing demand for safe and environmentally friendly cellulose solvents that can be used for shaping of the polysaccharide in general and for the preparation of beads in particular.

In recent years, novel cellulose solvents have drawn significant attention in the field of polysaccharide research and have fuelled research on the preparation of cellulose beads. Aqueous NaOH solutions, in combination with different additives that prevent gelation, for example, urea, thiourea, or ZnO, gained much interest in this context.[81−84] They bear huge potential because the ingredients are cheap, environmentally friendly, and nontoxic. Upon cooling to temperatures around −10 °C, the aqueous solvents rapidly dissolve cellulose and yield clear solutions from which cellulose can be regenerated by coagulation in diluted acids. From a thermodynamic point of view, aqueous NaOH solutions are rather poor cellulose solvents. The dissolution process is somewhat limited in terms of maximum cellulose concentration and degree of polymerization (DP). Nevertheless, aqueous cellulose solutions are well suited for bead preparation because the shaping step itself, in particular via dropping techniques, already requires rather low solution viscosities, that is, low polymer concentration and DP. Moreover, efficient pretreatment routines have been developed to improve the solubility of cellulose.[85−87] Cellulose beads have been prepared successfully from aqueous NaOH solutions, with and without additives, for example, urea and thiourea, by dropping techniques, including spinning drop atomization.[23,28,88] In addition, dispersion techniques have been applied.[89,90]

ILs, which are defined as molten organic salts with melting points below 100 °C, are another promising type of novel cellulose solvents.[91−93] They have been intensively studied in recent years toward spinning of fibers, biorefinery and conversion of lignocellulosic biomass, and chemical modification of polysaccharides.[94−97] In particular, imidazolium-based ILs have also been employed for the preparation of cellulose beads via dispersion and dropping techniques.[33,98,99] Also, other polysaccharides, such as chitosan and agarose, as well as various artificial polymers, easily dissolve in ILs, which could be exploited for obtaining cellulose composite beads (see section 4.2).[100−103] One of the main drawbacks, however, is the relatively high viscosity of cellulose/IL solutions.[104,105] Sophisticated technical devices, such as an "underwater pelletizer", might be required for their processing.[33] Efficient recycling strategies have to be implemented in the bead preparation process to make it profitable and sustainable.[97,106] Moreover, complete removal of IL residues from regenerated cellulose needs to be ensured.[107]

Interestingly, the first descriptions of the dissolution of cellulose in molten organic salts date back to the early 1930s.[108,109] The *N*-alkyl-pyridinium chlorides applied are, strictly speaking, not ILs because their melting points lie about 20 K above the limit of 100 °C and pyridine has been added to enable dissolution at ambient temperature. Mixtures of *N*-ethylpyridinium chloride and dipolar aprotic cosolvents have even been exploited as solvents for the preparation of cellulose beads.[110] Although these early findings did not raise significant attention during their time, they deserve mention for the sake of completeness and because cosolvents are nowadays frequently applied to reduce IL viscosity.[111,112]

2.3. Property Tuning

The size of cellulose beads is mainly determined by the technique used for forming spherical solution particles. Their physical bead properties, for example, density, specific area, and pore size structure, are dictated by the coagulation process. By altering the process parameters, transition from the dissolved polysaccharide to solid particles and consequently the bead morphology can be controlled.[22] For example; beads prepared from higher cellulose concentrations will provide less porous beads, whereas the temperature and composition of the coagulation medium influence morphology, internal surface

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area, and pore size distribution. If the dependence of bead properties on individual process parameters is well understood, tailoring of beads for specific applications is possible.

By the chemical modification of cellulose beads, it is possible to tune chemical and adsorption properties in particular (see section 4). A possibility for modifying physical properties is to include inorganic substances in cellulose beads. The compounds are directly added to the cellulose (or cellulose derivative) solution prior to the shaping process. Upon regeneration of the polysaccharide, the particles are trapped within the beads due to their large size, relative to the mean pore diameter.

The density of cellulose beads, which is usually about 1 g/cm^3 because of the high porosity of the material, has been increased up to 2.4 g/cm^3 by including dense particles of TiO_2, tungsten carbide, nickel, or stainless steel powder.[78,99,113−116] These high values are required for expanded bed applications to guarantee the formation of a fluidized bed (see section 5.1).[117,118] Magnetic cellulose beads have been prepared by adding iron, iron oxide powders, or nanoparticles to the cellulose solution prior to bead formation by dropping or dispersion techniques (Figure 4).[88,89,119,120] Those beads, which can be further functionalized by chemical modification, are useful, for instance for the selective removal of compounds, since they can easily be separated using a magnet.[121] It is also possible to entrap inorganic materials subsequent to the bead formation. ß-FeOOH-particles have been incorporated by slowly increasing the pH-value of a mixture of cellulose beads and an aqueous $FeCl_3$ solution.[122] These loaded beads could be used for the removal of arsenic from water.

If entrapped macroscopic particles are removed from the cellulose beads, void cavities are generated that result in highly porous materials. Cellulose beads with macroscopic pores have been prepared by the inclusion of inorganic salts with defined particle sizes, for example, Na_2SO_4 or $CaCO_3$, and subsequent removal by washing with aqueous solutions.[55,123−125] In a comparable approach, starch has been incorporated into cellulose beads and was finally removed again by amylase treatment generating a porous material.[116] In addition, enzymatic hydrolysis of pure cellulose beads with cellulase has been carried out to increase the pore diameter.[126] However, it is reasonable to assume that enzymatic degradation of cellulose starts at the edge of the beads and only slowly progresses into the interior. An uneven pore size distribution along the lateral cross section should be the result. Another possibility for producing highly porous cellulose beads is to add "blowing agents", such as $NaHCO_3$ or azodicarbonamide to the cellulose solutions.[26] During the coagulation process, these compounds decompose under the liberation of inert gases, which results in the formation of macroscopic pores.

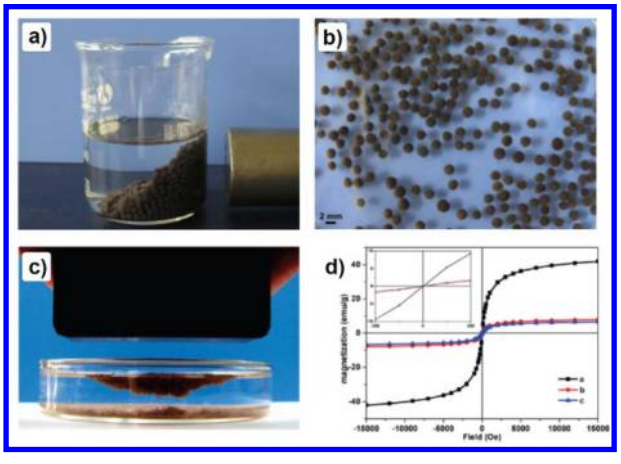


Figure 4. Magnetic cellulose beads, prepared by dropping (a, b) or dispersion technique (c). Magnetic hysteresis loops (d): γ-Fe_2O_3 nanoparticles (black curve), magnetic cellulose composite beads (red curve), and magnetic cellulose composite beads "activated" with epichlorohydrin (blue curve). Panels a and b are adapted with permission from ref 88. Copyright 2009 Elsevier, Inc. Panels c and d are adapted with permission form ref 89. Copyright 2010 American Chemical Society.

3. CHARACTERIZATION OF CELLULOSE BEADS

The performance of cellulose beads in specific applications is determined by the attached chemical functionalities (see chapter 4), and various physical properties, such as size, shape, and morphology. The following chapter is devoted to techniques that are useful for the comprehensive characterization of cellulose beads. Knowledge on the physical bead properties and how these properties can be tuned, for example, by altering process parameters during the preparation process, is crucial for developing tailor-made materials for specific applications.

3.1. Size and Shape

The size of cellulose beads is predetermined mainly by the preparation technique (section 2.1) and can be controlled within certain ranges to obtain particle diameters that may cover a wide range from about 10 μm up to 2−3 mm. Thus, different analytical tools are required to assess the dimensions of beads prepared by dropping or dispersion techniques.[127] It has to be noted that cellulose beads within one batch are not monodisperse in size but possess a certain size distribution. Size fractions can be separated by sieving cellulose beads through different meshes and weighting the fraction proportions.[27] Depending on the number of meshes used, sieving can provide a rough estimation of the size distribution, but it lacks detailed information on the physical dimensions. Nevertheless, the technique is particularly useful for large scale productions. Particle size analyzers, which exploit the principle of laser light diffraction, provide rapid information on the size distribution.[53] On the basis of the technical specifications of the particular devices, the upper limit that can be determined is in the order of several millimeters, meaning that they are of limited use for the characterization of cellulose beads that were prepared by dropping techniques. On the contrary, laser light diffraction is well suited for evaluation of the size distribution of beads prepared by dispersion techniques.

In the methods described above, beads are treated as perfect spheres. In practice, cellulose beads are more likely to have an elliptical shape, which results from mechanical deformation of the droplets hitting the surface of a coagulation bath (dropping technique) or by applying high centrifugal forces (dispersion technique) for instance. Direct information on particle size distribution and shape can be gained by analysis of images from optical or scanning electron microscopy with the aid of an imaging software (Figure 5).[22,128] The method is relatively laborious in the beginning because it might require adjustments of the contrast and brightness to obtain well-separated beads and remove undesired objects, for example, agglomerates, shades, or light reflections. However, if established, software aided image analysis is a very descriptive technique that directly

dx.doi.org/10.1021/cr300242j | Chem. Rev. 2013, 113, 4812−4836

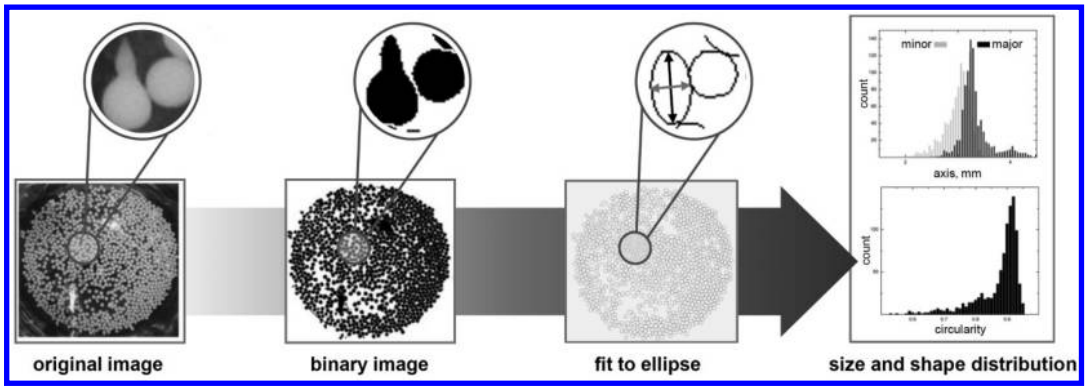


Figure 5. Determination of size distribution (gray, minor axis; black, major axis) and shape of cellulose beads via image analysis.

correlates size and shape of cellulose beads with an image of the particles.

3.2. Morphology

Scanning electron microscopy (SEM) is the most commonly used technique to characterize the morphology of cellulose beads (Figure 6). It enables the qualitative evaluation of the pore structure of the bead surface.[98] Information on the lateral morphology can be obtained from SEM images of cellulose bead cross sections. As an example; it could be demonstrated for beads prepared from aqueous NaOH/urea solutions by dropping technique that low acid concentrations in the coagulation bath yield a uniform morphology, whereas cellulose beads with a dense skin and more porous core are obtained at high concentrations.[22] SEM has been used, as well for the characterization of cellulose blend beads, to evaluate the incorporation of inorganic material within the particles.[116] In addition, analysis of SEM pictures with the aid of imaging software can yield information on bead size and shape as well.

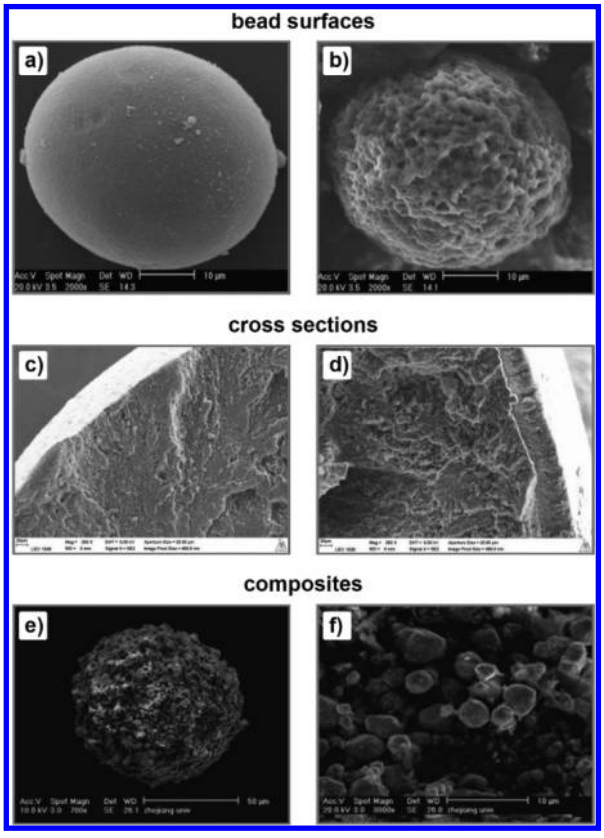


Figure 6. Scanning electron microscope images of the surfaces of cellulose beads with different porosity (a, b), the cross sections of cellulose beads with different skin-core morphology (c, d), and surface and inner morphology of cellulose/tungsten carbide blend beads (e, f). Panels a and b are adapted with permission from ref 98. Copyright 2010 Elsevier, Inc. Panels c and d are adapted with permission from ref 22. Copyright 2011 Springer Science+Business Media B.V. Panels e and f were adapted with permission from ref 116.Copyright 2007 Elsevier, Inc.

To prepare cellulose bead samples for SEM, as well as for the determination of porosity and inner surface area, an appropriate drying technique is required. Evaporation of water, either under vacuum, by heating, or simply by storing the beads in an open vessel, is not recommended since it results in "hornification" of the bead surface and loss of their huge porosity and surface area. Lyophilization cannot be applied as well because the volume expansion of water upon freezing and the growth of ice-crystals will result in a collapse of the micro- and mesopore in cellulose beads.[32] Critical point (CP) drying has been found to be a gentle technique that preserves the bead morphology and enables advanced characterization.[22,32] Thereby, water is stepwise exchanged by ethanol, acetone, and finally liquid CO_2, which is removed under supercritical conditions.

One of the trademarks of cellulose beads and the reason for their usefulness in plenty of applications is their high porosity. In general, pores are categorized as micro- (<2 nm), meso- (2–50 nm), or macropores (>50 nm).[129] Different pore types can be distinguished that will differently affect the pore size distribution gained by individual analytic tools (Figure 7). Thus, it is important to realize the limitations of these techniques and, if need be, utilize additional techniques to gain complementary information. Cylindrical, closed-ended pores are the ideal case when determining the size distributions and pore volumes. Conical pores may give distorted information, since they might contribute to smaller or bigger pore diameters. Depending on the particular method, ink-bottled and closed pores (pockets) may not be measured at all. In addition, ink-bottled pores may feign a too high fraction of pores with a small diameter. Open-ended pores form pore networks, which can also effect the distribution measurements by changing the desorption isotherm.[130]

Total porosity, that is, the total amount of liquid in a particle compared to the dry mass can be determined by measuring the difference between the wet mass of never dried cellulose beads and the dry mass.[22,116] The "apparently" opposite way, that is, immersing already dried beads in aqueous solution and

dx.doi.org/10.1021/cr300242j | Chem. Rev. 2013, 113, 4812–4836

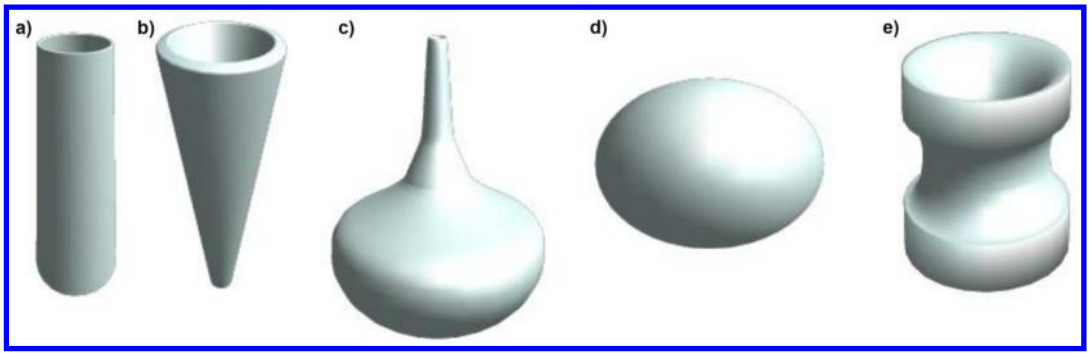


Figure 7. Schematic representation of different pore types: cylindrical (a), conical (b), inkbottle (c), closed pore (e), and open-ended pore (d).

monitoring the weight gained, has been applied as well.[55] However, the adverse effect of drying (in particular by the evaporation of water) on porosity has to be considered in that case. Either way, total porosity is only an indication of the "empty space" in beads, but it does not necessarily correspond to the amount of accessible pores or their size distribution.

Pore size distribution can be measured by mercury intrusion or nitrogen sorption.[32,126] Both techniques can also be used to determine the total inner surface area, which plays a key role, for instance, for the chemical functionalization of beads and their application as supports in solid-phase synthesis. They work as a complementary tools; mercury intrusion can measure pores from 2 nm up to several hundreds of μm and nitrogen sorption from 0.3 to 300 nm. For both techniques, CP drying of the cellulose beads is required. Pores are filled with mercury or liquid nitrogen, and pressure is applied to fill pores against the surface tension of the filling substance. Especially in the case of mercury intrusion, high pressures are applied, which can either destroy some of the pore structures or compress the small or closed pores in a sample, resulting in false values, for example, a too large fraction of small pores.[131]

Although CP drying is a rather gentle technique for preparing samples for porosity measurements, it might nonetheless slightly affect the morphology of cellulose beads. Thus, alternative methods, where pores sizes are measured directly in the wet state, can provide additional complementary information. Small angle X-ray scattering (SAXS) has been used to determine pore sizes and surface areas of never dried cellulose beads.[32,132] The technique is based on the measurement of different electron densities between the pore wall and the water phase, and it likewise assesses closed pores and pore sizes from 2 to 200 nm, meaning that comparison with the above-mentioned techniques is difficult. However, dry-state measurements are possible as well, which enables evaluation of the effect of different drying procedures on pores of different sizes.[132]

Another method that can be applied for measuring pore size distribution of cellulose beads in the wet state is the spin echo NMR technique.[133] This exploits the delayed diffusion of water molecules near the pore walls compared to the molecules further away from the wall.[134] Interpretation of the results can be rather laborious and literature references are infrequent. In comparison to previous techniques, spin echo NMR measurements do not provide information about the surface area. In return, NMR experiments do not require specific sample preparations or affect the sample itself, as is the case for SAXS measurements.

Solute exclusion using dextran or polyethyleneglycol (PEG) macromolecules of defined molecular weight has been used for direct quantification of the accessibility of the pores of different sizes within cellulose beads.[22,135] A disadvantage of solute exclusion is the fact that it is limited to the available sizes of macromolecules, for example, 1−56 nm for dextran and 0.7−5.7 nm for PEGs, and thus provides a discontinuous distribution. However, direct information on size exclusion properties are gained that are valuable, for example, for applications in chromatography.

3.3. Mechanical Stability

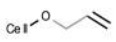
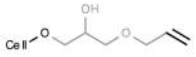
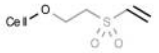
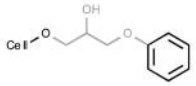
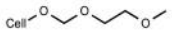
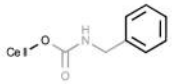
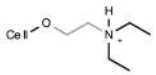
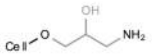
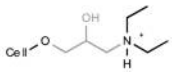
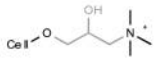
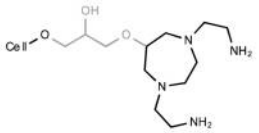
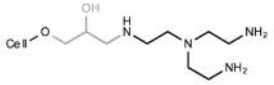
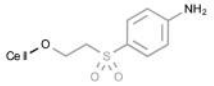
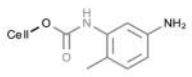
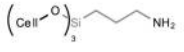
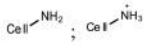
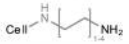
Mechanical stability is an important factor for practical handling. Moreover, it determines the maximum flow rate that can be applied in chromatographic column systems. With increasing flow rates, the pressure drop along the column first increases linearly but then rises exponentially when cellulose beads start to be crushed; this has been exploited to quantify the mechanical stability of cellulose beads.[70,136] In particular for beads with diameters in the millimeter-scale, mechanical stability can be assessed by placing them between parallel plates and measuring the normal force upon compression.[137]

Cellulose beads are not rigid but are elastic and compressible to a certain extent. Their shape might be deformed permanently even before breaking. This aspect has not been studied yet, although it might influence the performance in certain applications, in particular when beads are reused several times. Combining a method for applying defined forces to the beads, for example, centrifugation with microscopic image analysis, might provide useful information on the mechanical stability and "softness" of cellulose beads.

3.4. Biocompatibility

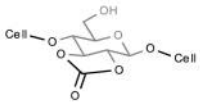
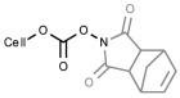
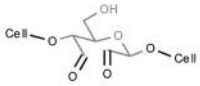
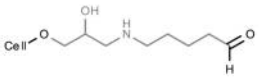
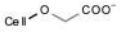
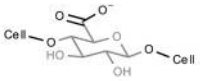
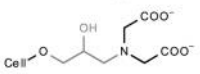
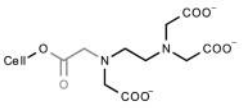
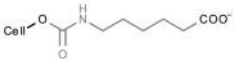
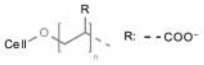
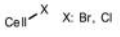
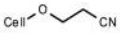
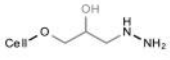
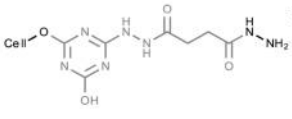
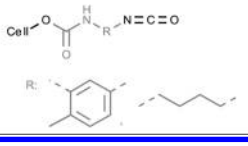
It is well-known that pure cellulose does not cause any diseases or symptoms in digestive systems after ingestion.[138] However, for the use of cellulose beads in specific biomedical applications, evaluation of their biocompatibility, cytotoxicity, and other related properties might be required, for example, when beads are exposed to living tissues or blood components. For this purpose, advanced testing routines are required that are specific to the particular application and should be performed according to standardized and approved norm procedures. As an example; the use of modified cellulose beads as adsorbents in hemodialysis requires evaluation of the blood compatibility, for example, by means of a hemolysis test, which measure the release of hemoglobin from ruptured blood cells, and platelet adhesion tests, in which the platelet concentration in blood samples is measured before and after passing through a packed column.[139]

Table 2. Overview of Cellulose Beads, Chemically Modified with Different Classes of Functional Groups for Specific Applications

entry	functional group	classification	preparation notes	application	references
	alkenes:				
1		reactive group	etherification of beads with allyl bromide	scavenging of Br_2, *in situ* conversion to **37** by bromine water and subsequent reaction with R-NH_2 or R-SH	114, 142, 174, 176
				coupling with R-SH groups	149, 225
2		reactive group	etherification of beads with allyl glycidyl ether	n.a.	142
3		reactive group	etherification of beads with divinyl sulfone	coupling with R-SH groups	149, 226
	alkyl/aryl/silyl ethers:				
4		hydrophobic group	etherification of beads with phenyl glycidyl ether	protein adsorption/purification	221-223
5		hydrophobic group	etherification of beads with methoxyethoxymethyl triethylammonium chloride	purification of blood cells	249
6		hydrophobic group	conversion of **21** with benzylamine	protein adsorption/purification	205
7		hydrophobic group	silylation of beads	size exclusion chromatography	147
	amines/hydrazines/ammonium compounds:				
8		reactive group	reduction of **33**	coupling with R-COOH (peptide synthesis)	236-237
9		ionic group	etherification of beads with diethylaminoethyl chloride	weak anion exchanger (protein adsorption/ purification)	24, 35, 52, 98, 124, 126, 140-141, 219
10		reactive group	conversion of **37** with $NH_{3\ aq}$	coupling/scavenging of R-CHO	177
			n.a.	coupling of R-CHO (e.g., protein immobilisation via dialdehydes)	185-188
11		ionic group	conversion of **37** with diethylamine	protein adsorption/purification	113
12		ionic group	n.a.	strong anion exchanger	152
13		affinity group	conversion of **37** with diazacycloheptan derivative	chelate ligand for metal ions	123
14		reactive group	*in situ* activation of **1** to **37** and conversion with amines	scavenger for R-NCO	174-175
15		reactive group	n.a.	diazotation and coupling with aromatic compounds (e.g., protein immobilization via tyrosine)	190-192
				solid support synthesis	235, 250, 190-191
16		reactive group	hydrolysis of **36**	diazotation coupling with aromatic compounds (e.g., tyrosine in proteins), coupling with O-methylisourea	182, 251
17		reactive group	silanisation of beads	coupling of R-CHO (e.g., protein immobilisation via dialdehydes)	182
18		ionic group	conversion of **47** with NH_3	weak anion exchanger	140
19		reactive group	conversion of **31** with diamines	coupling of R-CHO (e.g., protein immobilisation via dialdehydes)	196

dx.doi.org/10.1021/cr300242j | *Chem. Rev.* 2013, 113, 4812−4836

Table 2. continued

entry	functional group	classification	preparation notes	application	references
	carbonates:				
20		reactive group	conversion of beads with ethyl chloroformate	protein immobilisation	202
21		reactive group	conversion of beads with carbonochloridate derivative	coupling with R-NH_2 (e.g., diamines, proteins)	203-205
	carbonyls:				
22		reactive group	oxidation of beads with $NaIO_4$	coupling with R-NH_2 (e.g., diamines, hydrazines, proteins)	141, 143, 158-161, 180-181, 192
				sulfonation with $NaHSO_3$	189
23		reactive group	n.a.	coupling with R-NH_2 (e.g., protein immobilization)	183-184
24		reactive group	conversion of beads with glutardialdehyde	coupling with R-NH_2 (e.g., protein immobilization)	182
	carboxylates:				
25		ionic group	carboxymethylation of beads	weak cation exchanger	24, 52, 126, 140-143
		reactive group		coupling with R-NH_2 via carbodiimide activation	178
26		ionic group	TEMPO mediated oxidation of beads	weak cation exchanger (e.g., adsorption of metal ions and cationic polymers)	165
27		affinity group	conversion of **37** with iminodiacetic acid	chelate ligand for metal ions	141, 160, 228
28		affinity group	esterification of beads with EDTA-bis anhydride	chelating sorbent for metal ions	156
29		reactive group	activation of beads with carbonyldiimidazole and conversion with aminocarboxylic acids	coupling with R-NH_2 via carbodiimide activation	178, 206
30		ionic group	grafting of acrylic acid onto beads	protein adsorption	166
	halogens/pseudo halogens:				
31		reactive group	halogenation of beads with *N*-halosuccinimides	nucleophilic displacement of halide groups (e.g., with amines)	196
32		reactive group	conversion of beads with cyanogens bromide	coupling with R-NH_2 (e.g., protein immobilisation)	70, 182
33		reactive group	cyanoethylation of beads	reduction to **8**	236-237
				n.a.	24
	hydrazines:				
34		reactive group	conversion of **37** with hydrazine	coupling/scavenging of R-CHO	177
35		reactive group	conversion of **49** with succinic acid dihydrazide	coupling with R-CHO (e.g., protein immobilisation)	199
	isocyanates:				
36		reactive group	conversion of beads with diisocyanates	coupling with R-NH_2 (e.g., protein immobilisation)	35, 182

dx.doi.org/10.1021/cr300242j | *Chem. Rev.* 2013, 113, 4812−4836

Table 2. continued

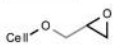
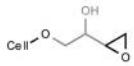
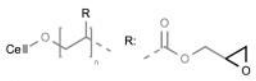
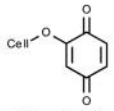
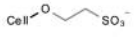
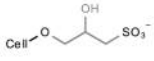
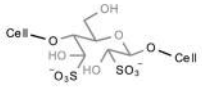
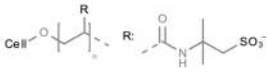
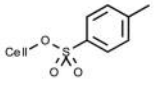
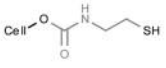
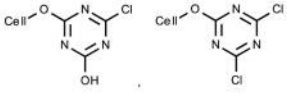
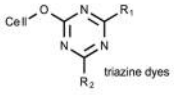
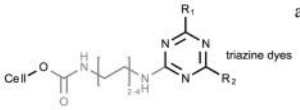
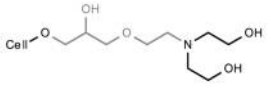
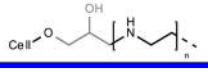
entry	functional group	classification	preparation notes	application	references
	oxiranes:				
37		reactive group	conversion of beads with epichlorohydrin	coupling with R-NH_2, R-SH, R-OH	89, 139, 141, 160, 177-179
				coupling with Cell-OH (cross-linking)	24, 52
			formed *in situ* when reacting **1** with bromine or *N*-bromosuccinimides in water	coupling with R-NH_2	174-176
38		reactive group	conversion of beads with bisepoxide	coupling with R-NH_2, R-SH	142
39		reactive group	grafting of acrylic acid derivative onto beads	n.a.	167
	phosphates:				
40		ionic group	esterification of beads with phosphorus oxychloride	medium cation exchanger	140, 143
	quinones:				
41		reactive group	conversion of beads with benzoquinone	coupling of R-NH_2 (e.g., protein immobilisation)	200-201
	sulfates/sulfonates/sulfonic acid esters:				
42		ionic group	sulfation of beads	strong cation exchanger, adsorption of viruses	153-154
43		ionic group	etherification of beads with chloroethane sulfonate	strong cation exchanger	140
44		ionic group	etherification of beads with hydroxychloropropane sulfonate	strong cation exchanger	121, 144
45		ionic group	sulfonation of **22** with $NaHSO_3$	n.a.	189
46		ionic group	grafting of acrylamide derivative onto beads	cation exchanger (e.g., amino acid adsorption/ purification)	77
47		reactive group	conversion of beads with tosylchloride	nucleophilic displacement of tosyl groups (e.g., with amines, proteins)	140, 155
	thiols:				
48		reactive group	activation of beads with carbonyldiimidazole and conversion with mercaptoethanolamine	coupling with R-Cl (e.g., scavenger for electrophiles)	208
	triazines:				
49		reactive group	conversion of beads with cyanuric chloride	coupling with R-NH_2 (e.g., proteins)	135, 192, 197-199
50		affinity group	conversion of beads with reactive triazine dyes	adsorption/purification of proteins (dye-ligand chromatography)	151, 214-215
51		affinity group	conversion of **21** with NH_2-functionalized triazine dye	adsorption/purification of proteins (dye-ligand chromatography)	203
	others:				
52		n.a.	conversion of **37** with triethanol amine	n.a.	140
53		affinity group	conversion of **37** with poly(ethyleneimine)	adsorption of DNA	216

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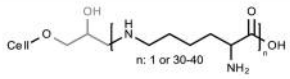
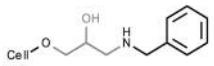
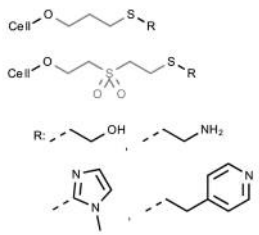
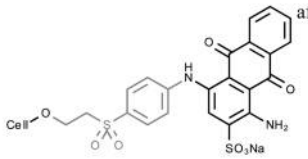
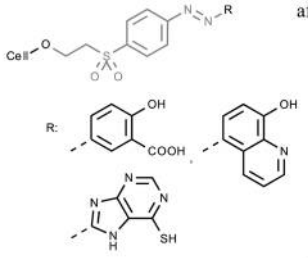
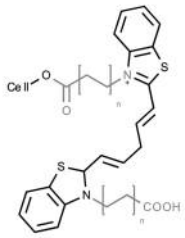
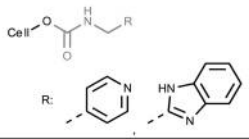
Table 2. continued

entry	functional group	classification	preparation notes	application	references
54		affinity group	conversion of **37** with lysine/poly(lysine)	adsorption of endotoxins, DNA	139, 216
55		hydrophobic group	*in situ* activation of **1** to **37** and conversion with benzyl amine	protein adsorption/purification (hydrophobic charge induction chromatography)	114
56		hydrophobic group	conversion of **1** or **3** with R-SH	protein adsorption/purification (hydrophobic charge induction chromatography)	149, 225-226
57		affinity group	etherification of beads with reactive dye	adsorption/purification of proteins (dye-ligand chromatography)	150-151
58		affinity group	azo coupling of **15** with aromatic compounds	selective adsorption of metal ions (e.g., Al^{3+}, Hg^{2+})	190-191
59		affinity group	esterificaction of beads with reactive dye	adsorption/purification of proteins (dye-ligand chromatography)	157
60		hydrophobic group	activation of beads with carbonyldiimidazole and conversion with amine	protein adsorption/purification (hydrophobic charge induction chromatography)	207

4. FUNCTIONALIZATION OF CELLULOSE BEADS

4.1. Chemical Modifications of Beads

By introducing different chemical groups, functional cellulose beads with tailored properties can be prepared for various types of applications. Heterogeneous modification of cellulose subsequent to the shaping is by far the most frequently applied approach for the preparation of such functional cellulose beads, because of its simplicity. Cellulose possesses three hydroxyl groups per repeating unit that are accessible for chemical modification via the reactions described below. Table 2 provides information on the various chemical groups that could be attached to cellulose beads. Different classes of functionalities were distinguished according to their role in potential applications (see section 4.3). In most cases, the substituents were attached in a two-step procedure or contained a linker between the cellulose backbone and the essential structural feature. For ease of recognition, only the latter were highlighted in the chemical structures.

4.1.1. Etherification. Cellulose beads can be etherificated by conversion with halogen or vinyl compounds (alkylation) as well as oxiranes (hydroxyalkylation) under alkaline conditions. Especially compared to ester bonds, the advantage of ether linkages is their stability in aqueous systems even at low/high pH-values. One of the most frequently applied methods for the chemical modification of cellulose beads is carboxymethylation with chloroacetic acid, which yields anionic materials for ion exchange applications (Table 2; **25**).[24,126,140−143] Functionalization with sulfoalkyl moieties has been performed in a comparable way and for the same purpose (**43** and **44**).[140,144] On the other hand, weak cationic ion exchange resins (**9**) have been prepared by the etherification of cellulose beads with 2-chloroethyl diethylamine.[24,98,124,126,140,141] Cellulose reacts with trimethylsilyl chloride or hexamethyldisilazane to yield the trimethylsilyl ethers.[145,146] This reaction has been exploited in

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order to prepare hydrophobic cellulose beads for size exclusion chromatography (SEC).[147]

Etherification of cellulose beads with reagents carrying a second reactive group is commonly used to "activate" the beads for further functionalization with compounds that do not react directly with the polysaccharide (see section 4.3.1). The most prominent example for this approach is the conversion of cellulose beads with epichlorohydrin under alkaline conditions, which proceeds in two steps.[148] First, the epoxide reacts under ring-opening with hydroxyl groups of the polysaccharide to form a halohydrin substituent that subsequently eliminates HCl to yield a novel oxirane moiety. Derivatization of cellulose beads with allyl bromide and divinyl sulfone has been performed to introduce reactive alkene groups.[142,149] The reaction of cellulose beads with reactive remazole dyes also proceeds via a vinyl sulfonyl intermediate.[150,151]

4.1.2. Esterification. Esterification of cellulose beads with inorganic acid derivatives yields charged materials, for example, with sulfate (**42**) or phosphate groups (**40**), which could be used for ion exchange or affinity adsorption chromatography.[152−154] Cellulose beads have been converted with tosylchloride to make them more accessible for nucleophilic displacement reactions.[140,155] The tosyl moiety is an excellent leaving group and can be substituted, for instance by amines and ammonia. Reports on the preparation of functional cellulose beads by esterification with carboxylic acid derivatives are scarcely found in the literature, which might be due to the limited stability of the ester linkages under aqueous alkaline conditions.[156,157]

4.1.3. Oxidation. Oxidation with $NaIO_4$ has been applied frequently to activate cellulose beads, in particular for the coupling of enzymes.[141,158−161] The reaction leads to the selective cleavage of the C2−C3-linkage of the anhydroglucose ring in the cellulose repeating unit, yielding two reactive carbonyl moieties (**22**).[162,163] The primary hydroxyl group of cellulose can be selectively converted to yield 6-deoxy-6-carboxy-cellulose via oxidation mediated by piperidine oxoammonium salts (TEMPO).[164] The resulting beads are anionic and could be used for the adsorption of metals or polycations.[165]

4.1.4. Polymer Grafting. Grafting of poly(acrylic acid) derivatives onto cellulose has been applied to produce functionalized bead materials.[77,166,167] Chain growth is usually induced by Ce^{4+} ions, which are assumed to form a chelate complex with cellulose that finally decomposes to generate free oxygen radicals that initiate the polymerization of acrylic acid monomers.[168,169] The mechanism includes the oxidation of one of the chelating OH-groups in C-2 and C-3 position, which results in cleavage of the anhydroglucose ring, comparable to oxidation with $NaIO_4$.

4.2. Blending with Other Polysaccharides

The subsequent modification of beads is rather easy to perform and generally applicable. However, it has to be ensured that the particle properties, in particular their pore structure, as well as chemical- and mechanical stability, are not changed to their detriment under the partly harsh reaction conditions, which may include high temperatures and the use of organic solvents. In addition, it has to be considered that the heterogeneous reaction course influences the distribution of functional groups along the lateral cross section. While the outmost hydroxyl groups at the edge of the beads are readily accessible, derivatization of those located within the internal pores is limited by diffusion of the reagent into the bead interior. Thus, the density of functional groups should decrease when approaching the interior of the beads, especially when highly reactive reagents are employed. In contrast, uniform distribution of functionalities can be expected for beads, prepared from blends of cellulose, as the structure forming matrix, and a second polysaccharide derivative, which carries the desired functional group. The different distribution patterns should have a significant influence on the bead properties but up to now, the localization of substituents within lateral cross sections has not been studied.

Functional cellulose blend beads can be easily prepared according to the same general procedure described above, with the exception that a mixed solution of cellulose and a polysaccharide derivative with specific functionalities in an appropriate solvent is used. However, despite the ease of this approach, only few examples could be found in the scientific literature. Mixed beads of cellulose and different cellulose esters have been prepared by dissolving both polysaccharides in DMA/LiCl and dropping the solution into a nonsolvent.[170] In case of cellulose laurate, the beads showed only a small decrease in mechanical stability, even when 60% of the cellulose matrix was replaced by the ester. Amino group containing composite beads of cellulose and chitosan, a polysaccharide consisting mainly of β-(1−4)-linked D-glucosamine, have been prepared from NMMO solutions via the dropping technique in order to obtain functional beads.[76]

Novel, efficient cellulose solvents are required not only for the large scale preparation of cellulose beads but also for obtaining functional materials via blending. ILs appear to be favorable solvents for that purpose regarding the fact that cellulose, as well as a variety of different polymers, including other polysaccharides, can easily be dissolved. Composite cellulose/chitosan beads have been successfully prepared from mixed IL solutions via dropping and dispersion techniques.[101−103] The materials could be used as biosorbents for heavy metal ions as well as for the immobilization of enzymes. Beads prepared from mixed solutions of cellulose and chitosan or agarose have been utilized for physical enzyme entrapment and showed higher loadings compared to beads solely composed of cellulose.[171] Aqueous NaOH/urea is suited for preparation of composite beads as well. Cellulose/alginate beads could be obtained via dispersion technique from aqueous solutions of the mixed polysaccharides.[172] However, these carboxylate group-containing materials were additionally stabilized by formation of an insoluble calcium alginate gel and are consequently close to the restrictions of the above assigned definition of cellulose beads.

4.3. Classification of Functional Groups

4.3.1. Reactive Groups. Direct binding of a specific functional group to cellulose is not always possible when the desired molecule does not sufficiently react with hydroxyl groups. Thus, different reactive groups that show higher reactivity or selectivity toward the target molecule have been attached to cellulose beads. In most cases, the "activated" intermediates are not isolated but are directly converted into the desired functional cellulose beads.

4.3.1.1. Alkenes. After activation with a peroxide initiator, alkene moieties (Table 2, **1**−**3**) readily react with thiols via a free radical addition mechanism (thiol-ene reaction).[173] Thus, cellulose beads have been converted with allyl bromide or divinyl sulfone, to subsequently attach various mercapto

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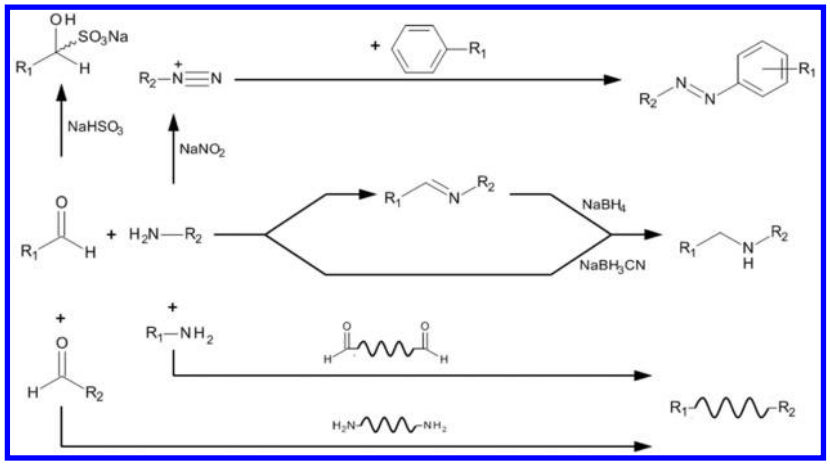

Figure 8. Schematic representation of possible reactions of "reactive" amine- and carbonyl groups. R_1 and R_2 represent either cellulose beads or target molecules.

compounds. In addition, alkene groups rapidly react with bromine or *N*-bromosuccinimide. In the presence of water, the halogenation yields bromohydrin, which eliminates HBr to give an oxirane.[174,175] This activation method has been applied, for instance to couple beads with amino group-containing substituents (see section 4.3.1.3).[114,176]

4.3.1.2. Oxiranes. Attaching oxirane moieties (**37**−**39**) to the cellulose backbone is one of the most commonly applied procedures for activating beads for further functionalization. It can be performed starting from allyl cellulose beads, as described above, or in a more direct approach by heterogeneous etherification with epichlorohydrin.[139,160] Also, conversion of beads with bisepoxides as well as by grafting of glycidyl methacrylate has been reported.[142,167] Functionalization of beads with oxiranes has been performed mainly in order to subsequently convert them with amines, imines, or hydrazines.[139,141,160,177,178] The reaction has been exploited also for the immobilization of proteins by coupling via their amino moieties.[89,179] Oxirane moieties bound to the polysaccharide backbone may also react with hydroxyl groups of the same or neighboring cellulose chains, which can lead to cross-linking (section 4.3.2). To prevent this loss of reactive groups, activation of cellulose beads with epichlorohydrin is usually carried out at lower temperatures (≤40 °C) and/or shorter reaction times.

4.3.1.3. Amines and Carbonyls. Amines (**8**, **10**, **17**, and **19**) and carbonyl moieties (**22**−**24**) are very versatile reactive groups (Figure 8). They can react with each other to form imines, which are usually directly reduced to amines in order to increase chemical stability of the linkage (reductive amination).[158] Hydrazine derivatives and hydroxylamines may react in a comparable way to give the corresponding hydrazones or oximes. These reactions have been exploited in many cellulose bead applications. By attaching one of these functionalities to the cellulose backbone, the functionalized beads can readily react with target molecules carrying the corresponding counterpart.

Carbonyl functionalized beads have been applied for coupling with amines, diamines, amino acids, and hydrazine derivatives.[143,180,181] Moreover, these coupling reactions have been exploited for direct immobilization of enzymes via their amino groups.[141,159−161,182−184] The same target molecules can also be immobilized onto amino group-containing beads with help of bifunctional aldehyde linkers.[185−188] It can be expected that a considerable amount of functional groups is consumed in this case due to cross-linking reactions. In addition to the formation of imines, cellulose beads functionalized with carbonyl groups have been reacted with sodium sulfite to introduce anionic sulfonic acid moieties (**45**).[189] Aromatic amines can be converted into the corresponding diazonium salts by oxidation with $NaNO_2$ under acidic conditions. The resulting reactive species have been used to couple aromatic substituents (**58**) to cellulose beads.[190,191] Proteins have been immobilized by this path via their aromatic amino acid residues.[182,192]

4.3.1.4. Leaving Groups. The hydroxyl groups of cellulose itself are not readily substituted in nucleophilic displacement reactions. The reactivity can be increased by conversion into tosyl or halogen moieties, which are better leaving groups.[193,194] Reactive deoxy-halo cellulose beads (**31**) have been prepared by heterogeneous reaction with *N*-halosuccinimides.[195] The introduced halogen groups could be substituted by diamines to yield functional beads for enzyme immobilization (**19**).[196] Thereby, significant conversion was only observed for substitution of bromide functionalized beads in DMSO. In a similar manner, tosylated cellulose beads (**47**) have been converted with liquid NH_3 to yield amino-deoxy beads (**18**).[140]

4.3.1.5. Other Reactive Groups. Reactive beads that can immobilize proteins and other amino group containing target molecules have been prepared by activation with cyanuric chloride (**49**).[135,197−199] Cellulose beads have also been converted with benzoquinones for the same purpose (**41**).[200,201] By reaction of beads with diisocyantes, isocyanate groups have been introduced (**36**).[35,182] These highly reactive moieties can either be hydrolyzed to amines or used to immobilize proteins via their amino groups. Amines could be coupled with cellulose beads containing carboxylic acid groups.[178] However, amide formation is not quantitative and requires expensive coupling reagents such as carbodiimides. The conversion of cellulose into highly reactive carbonate or carbamate intermediates (**20** and **21**) has been performed in order to activate beads for subsequent modification.[141,202−207] Also in these cases, the applied activation reagents are expensive and highly reactive, which implies that undesired side reactions may occur. Finally, thiol substituents have been attached to cellulose beads (**48**) to obtain materials that are reactive toward chlorinated compounds.[208]

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4.3.2. Cross-Linkable Groups

To prevent excessive swelling or dissolution of the partly modified beads, which may especially occur in aqueous systems, cellulose can be cross-linked subsequent to regeneration.[24,52] The most commonly applied reagent for this purpose is epichlorohydrin. As described above, it first reacts with hydroxyl groups and introduces oxirane moieties that can further react with hydroxyl groups of the same or another cellulose chain in close vicinity (Figure 9).[148] Compared to the activation of cellulose beads with epichlorohydrin, cross-linking is usually performed at higher reaction temperatures (≥50 °C) and longer reaction times. Also, other compounds bearing two reactive groups such as epoxy-, carbonyl-, or carboxyl moieties have been applied for cross-linking, for example, glycol diglycidyl ether, glutardialdehyde, citric acid, and maleic acid derivatives.[120,209,210]

It should be noted that cross-linking is not always desired. In case the introduced reactive groups may react with hydroxyl groups as well as the desired target molecule, a certain degree of cross-linking is inevitable. Especially when bi- or multifunctional reagents of high reactivity are employed (e.g., **3**, **14**, **19**, **28**, **35**, **36**, **49**, and **52**), it can be expected that a certain amount of potential binding sites is consumed by undesired cellulose−cellulose or target−target coupling. These side reactions have to be taken into account, especially when high loading capacity and efficiency is desired.

4.3.3. Ionic Groups. Cellulose beads have been modified with different anionic and cationic groups, mostly in order to obtain ion exchangers of different strength. Beads carrying carboxylate groups are comparably weak acidic ion exchangers due to the high pK_A of the corresponding carboxylic acids. They have been prepared in most cases by carboxymethylation (**25**) but also by oxidation (**26**) and grafting reactions (**30**).[140,165,166] Sulfate (**42**) and sulfonate moieties (**43** and **44**), which have been attached to beads by esterification or sulfoalkylation, are comparably strong acidic groups.[140,144] Cellulose phosphate beads (**50**) can be settled in between.[140,143] Cationic cellulose beads have been prepared by attaching tertiary amines to the polysaccharide backbone (**9** and **11**).[98,113,140] At common pH-values (4−7), these moieties are only partly protonated, meaning that they are only partly charged. Strong cationic ion exchangers have been prepared from permanently charged quaternary ammonium groups (**12**).[152]

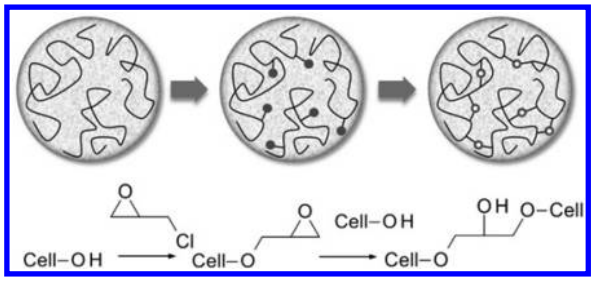

Figure 9. Schematic representation of the cross-linking of cellulose beads with epichlorohydrin.

4.3.4. Affinity- and Hydrophobic Groups. The term "affinity groups" describes moieties that have been attached to cellulose beads in order to improve their affinity toward adsorption of specific target molecules. As an example; multidentate chelate ligands (**13**, **27**, **28**, and **58**) enable the strong adsorption of metal ions, whereas several dye molecules (**50**, **51**, **57**, and **59**) are known to bind proteins in a very selective manner. In addition, cellulose beads have been functionalized with hydrophobic groups (**4**-**7**, **55**, and **56**), either in order to improve hydrophobic−hydrophobic interactions or to suppress hydrophilic−hydrophilic ones.

5. APPLICATIONS OF FUNCTIONAL CELLULOSE BEADS

5.1. Chromatography

Because of their spherical shape, cellulose beads are excellent filling materials for chromatographic applications. They are easy to handle and to pack into columns, provide less flow resistance than powders, and can support even faster flow of the eluent than cross-linked dextran particles without deformation or breaking.[21,70,211] Thus, cellulose beads have been employed as the stationary phase in many chromatographic systems for the adsorption and purification of various substances, exploiting different types of interactions between the matrix and the molecules to be separated.

Porous cellulose beads have been applied for size exclusion chromatography (SEC) and gel filtration (desalting).[21,212] In SEC, specific interactions between the stationary phase and the macromolecules are undesired. Molecules are exclusively separated according to their difference in hydrodynamic radii, which determine the time required to flow through the total porous volume of the cellulose beads. It has been proposed that performance in polymer fractionation can be improved by hydrophobization of the cellulose bead surfaces via trimethylsilylation.[147]

In contrast to SEC, the basic separation mechanism of affinity chromatography is a difference in the adsorption of the molecules that needs to be separated from one another onto the stationary phase (cellulose beads). In general, undesired impurities are eluted first, while the desired substances are either delayed or completely adsorbed and subsequently removed from the column by washing with an eluent of a different composition, for example, in terms of pH or salt concentration. Unmodified cellulose beads only enable the nonspecific adsorption of molecules by interaction with hydroxyl groups. However, by introducing different types of functionalities, very specific sorbents, in particular for the purification of biomolecules, have been prepared (Figure 10).

In "dye-ligand chromatography", the specific interaction of surface bound dye molecules with nucleotide-dependent enzymes is exploited (Figure 10 a). The affinity ligands interact with certain protein binding sites that are usually occupied by coenzymes, e.g., NAD(P)H and flavins, because the dyes mimic the structure of these naturally occurring heterocyclic compounds.[213] To obtain affinity materials for protein adsorption and purification, cellulose beads have been modified with different dyes, such as reactive cibacron, procion, and remazol dyes that are commonly applied for staining of cellulosic textiles (**50**, **51**, **57**, and **59**).[151,157,214,215] In particular, dehydrogenases, that is, enzymes that depend on $NAD(P)^+$ coenzymes for H^+/e^- transfer, could be removed selectively from animal tissue extracts by adsorption onto functional cellulose beads.[150,203] Desorption of proteins from the solid support can be achieved by adjusting pH, ionic strength, or temperature of the eluent, or by using competitive free ligands, for example, buffer solutions containing excess NAD^+ or water-soluble dextran-dye ligands.[214]

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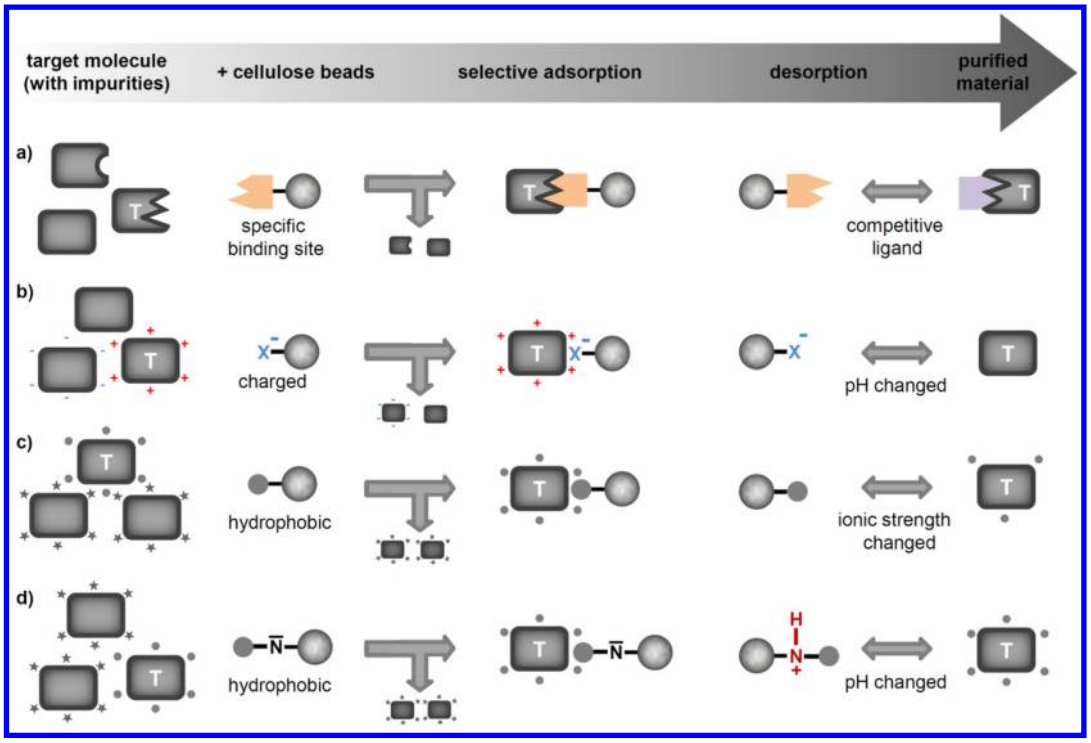

Figure 10. Scheme for chromatographic separation of target molecules from impurities via different techniques using functional cellulose beads: dye-ligand/specific affinity chromatography (a), ion exchange chromatography (b), hydrophobic interaction chromatography (c), and hydrophobic charge induction chromatography (d).

In addition to dye molecules, other functionalities with high affinity toward certain biomolecules have been attached to cellulose beads. Beads modified with lysine, poly(lysine) (**54**), or poly(ethyleneimine) (**53**) have been applied for the chromatographic removal of endotoxin and DNA from biological samples, including blood.[139,216] Prepacked columns of cellulose beads with immobilized dextran sulfate are commercially supplied for blood purification.[217] Beads of cellulose sulfate (**42**) have been found to be highly effective in binding various types of viruses and virus-like particles.[153,154] Both, sulfated dextran and cellulose mimic the interaction of viruses with naturally occurring polysaccharide sulfates, such as heparin and heparan sulfate, which are known to possess antiviral activities.

Instead of exploiting the specific interaction of biomolecules with the stationary phase, ion exchange chromatography separates molecules according to their charge (Figure 10 b).[218] Proteins contain acidic, as well as basic residues; the ratio of both determines their isoelectric point. Thus, the net charge and consequently the retention time of proteins depend on the pH of the eluent. Cationic cellulose beads have been prepared by functionalization with tertiary amines (**9** and **11**). These weak ion exchangers have been applied for chromatographic protein purification, for example, bovine serum albumin and glucose isomerase, and proved to be more efficient then commonly applied columns packed with cationic cellulose in powder form because of the better flow properties.[98,124,219] Also, anionic cellulose beads have been utilized as weak (carboxylate based, **25**) or strong cation exchangers (sulfonate based, **44**), for instance for the purification of γ-globulin and lysozyme.[52,121]

Proteins usually possess hydrophobic regions where non-polar amino acids are concentrated on the surface, which enables their separation by hydrophobic interaction chromatography (HIC, Figure 10c).[220] Cellulose beads, modified with hydrophobic alkyl or aryl moieties (**4**–**6**), have been successfully applied for HIC.[205,221−223] The advantage of this method over dye-ligand chromatography is the ease of protein removal from the column. The adhesion strongly depends on the salt concentration. Adsorption requires a rather high salt content whereas desorption of the purified sample is achieved by decreasing the concentration. No competitive ligands are required. Hydrophobic charge induction chromatography (HCIC) follows a comparable concept.[224] In addition to the hydrophobic moieties that are responsible for protein adsorption, the hydrophobic moieties contain nitrogen atoms (**55** and **60**).[114,207] These are deprotonated or protonated according to the pH value of the eluent, which enables switching of the stationary phase from neutral (adsorption) to charged (desorption). This mechanism can be exploited to remove the proteins from the functional cellulose beads after separation from impurities (Figure 10d). HCIC is not dependent on the salt concentration and can be applied for protein adsorption from low, as well as high, ionic strength feedstock. Several HCIC groups that have been attached to cellulose beads additionally contained sulfur atoms (**56**), which improved protein adsorption.[149,225,226]

Common chromatography systems utilize packed columns with a fixed bed of bead materials that offers only a small void volume. To prevent plugging, large particles have to be removed prior to the separation process. In expanded bed chromatography (EBC), the bed material is allowed to rise in an upward directed eluent stream (Figure 11).[117] As a result, the columns volume increases, which creates void space and allows large particles to pass the bed without plugging it. This enables the direct purification of proteins from crude feedstocks that usually contain suspended materials, such as cell fragments.[118] Time consuming clarification techniques such as

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centrifugation or filtration can be omitted. To operate EBC systems with considerable flow rates, the bed materials are required to have an appropriate particle density, size, and size distribution. Too large/heavy particles require high flow velocities and too small/light ones would result in over-expansion of the bed. Moreover, for a stable expanded bed without back mixing within the column, the size ratio between the biggest and smallest particles should be at least 2.2.[117] Cellulose beads guarantee high adsorption capacity, because of the high porosity, and can meet the requirements in terms of size and size distribution, but their density is relatively low. Thus, blend beads of cellulose with dense inorganic materials, such as TiO_2, tungsten carbide, nickel, or stainless steel powders have been prepared and applied for EBC.[78,99,113−116] In principle, these high density blend beads can be functionalized in the same way as common cellulose beads, in order to improve protein adsorption.[113,114]

5.2. Metal Ion Exchange and Water Treatment

Pollution of water resources with harmful metals, such as lead, mercury, cadmium, copper, iron, and chromium, is a serious threat for human beings, as well as flora and fauna.[227] Cellulose beads modified with anionic sulfate (**42**), sulfonate (**43**, **44**), phosphate- (**40**), or carboxylate groups (**25**, **26**) are strong, medium, or weak cation exchangers.[140,144,152] Depending on the amount of functional groups, these materials can consequently adsorb high amounts of metals from aqueous solutions. Thus, functional cellulose beads have been studied as biocompatible sorbents for water treatment. Cellulose beads carrying carboxylate groups have been reported to possess high adsorption capacity for calcium, copper, silver, and lead.[165] Depending on the metal and the procedure used to introduce the anionic group (carboxymethylation vs TEMPO mediated oxidation), the adsorption ratios of metal per carboxylic group were found to be 70−100%. Beads have also been prepared by blending cellulose with alginate or chitosan for the removal of cadmium and copper.[76,172] Thereby, cellulose represented the matrix whereas the additional polysaccharide provided the functionalities for metal adsorption via ionic (carboxylate in alginate) or metal−ligand interaction (amino in chitosan).

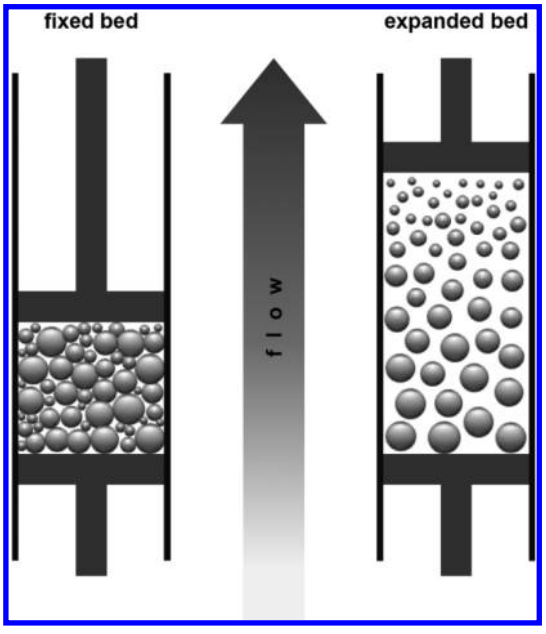

Figure 11. Schematic comparison of fixed bed and expanded bed chromatographic columns.

Strong fixation of metal ions, especially multivalent ones, to cellulose beads has been achieved by coupling later with specific ligands that possess multiple functionalities for complexation of the metals (**58**).[190,191] The obtained materials have been utilized for the fractionation of aluminum from water samples as well as the quantitative determination of mercury in a river. Also, chelating ligands such as diamines (**13**), iminodiacetic acid (**27**), or ethylenediaminetetraacetic acid (**28**) have been coupled to cellulose beads.[123,141,156,228] As a result of the high stability of the metal-chelate complexes, the latter ones could be used to immobilize proteins in an oriented manner (see section 5.3).[160]

5.3. Protein Immobilization

Immobilization of enzymes on a solid support is often a prerequirement for their utilization in commercial scale processes because it simplifies purification procedures and enables reuse of the rather expensive biocatalyst.[229,230] Cellulose beads are promising materials for immobilizing enzymes since they are biocompatible, sustainable, and can be chemically modified. The spherical shape guarantees excellent flow properties in column systems, and because of the high porosity of the matrix, a large surface area is available for immobilization. Moreover, the enzymes are protected from contact with external surfaces, for example, gas bubbles, mechanical stirrers, and walls of the reaction vessel, which increases their operational and storage stability.

Functional cellulose beads with different reactive groups have been studied intensively for the immobilization of various enzymes (Table 3). Some of them are of great industrial interest, such as amylase (glucose production from starch), galactosidase (production of lactose free dairy products), and invertase (saccharose hydrolysis).[179,183,198] In most cases, proteins have been immobilized by reaction of their amino groups, which are present in the residues of many amino acids, with reactive groups on functional cellulose beads. The general basics of the corresponding coupling reactions were described in section 4.3.1. Functional cellulose beads of high reactivity, for example, oxiranes and isocyanates, may additionally react with hydroxyl or thiol moieties within the protein. A very selective immobilization technique is the frequently applied coupling of cellulose bound diazonium salts with the aromatic residue of tyrosine.[182,192,198]

By incorporation of cellulose-enzyme beads into analytical devices, sensors for the determination of ethanol, glucose, glutamate, lactate, and glycerol in food products have been prepared.[185−187] The basic principle of these systems is the enzymatic conversion of the analytes that yields either hydrogen peroxide or NADH as byproducts, which can be quantified amperometrically. Enzymes that were stabilized by immobilization to cellulose beads have been proposed as therapeutics, for example, for cancer treatment.[184] Also, other types of proteins have been immobilized on cellulose beads, such as antibodies and protein inhibitors, which possess well-defined binding sides for very specific proteins. Bioaffinity matrices for purification of either the antibody or its target have been prepared by coupling the corresponding counterpart to cellulose beads.[141,155,160,197,199,201]

In general, immobilization of proteins proceeds by more or less random coupling of their functional amino acid residues with reactive groups on the cellulose beads. However, proteins

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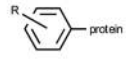
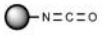
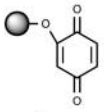
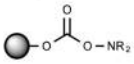
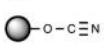
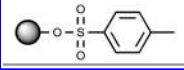
Table 3. Overview of Cellulose Beads with Different Functionalities Employed for Protein Immobilization

cellulose bead		immobilized protein			references
functionality	coupling method	functionality	example	application	
	imine coupling (reductive amination)	H_2N–protein	IgG-antibodies	affinity chromatography	252
			albumins	general immobilisation study	159
			amylases	glucose production from starch	183
			anti-tumour necrosis factor-α antibodies	affinity chromatography	160
			cytosine deaminase	stabilized enzymes for therapeutic use	184
			galactosidase	general immobilisation study	192
			human globulins	affinity chromatography	141, 155
			lysozyme	general immobilisation study	159
			methaemoglobin	affinity chromatography	141
			trypsin, chymotrypsin	specific fragmentation of proteins	161, 180
	coupling with diamines/ dihydrazides		glycosylated human serum albumin ($NaIO_4$ oxidized)	general immobilisation study	181
	imine coupling (reductive amination), hydrazone coupling		anti-chymotrypsin antibody ($NaIO_4$ oxidized)	oriented immobilisation of chymotrypsin	197
			invertase ($NaIO_4$ oxidized)	hydrolysis of saccharose	198
			ovalbumin ($NaIO_4$ oxidized)	affinity chromatography	199
	glutardialdehyde coupling	H_2N–protein	alcohol oxidase	sensor for ethanol monitoring	185
			alkaline phosphatase	general immobilisation study	196
			inulinase	fructose production from inulin	188
			glucose oxidase	sensor for glucose monitoring	185
			glutamate oxidase	sensor for glutamate monitoring	186
			glycerol dehydrogenase	sensor for glycerol monitoring	187
			lactate oxidase	sensor for lactate monitoring	185
	azo coupling		invertase	hydrolysis of saccharose	198
			galactosidase	general immobilisation study	192
			glucoamylase, glucose isomerase, glucose oxidase	general immobilisation study	182
	activation with oxirane	HY–protein Y: O, S, NH	galactosidase	hydrolysis of lactose in dairy products	179
			human γ-globulin	affinity chromatography	141
			penicillin acylase	production of aminopenicillanic acid	89, 141
	activation with cyanuric chloride	HY–protein Y: O, S, NH	anti-lysin antibody	immobilisation of chymotrypsin	197
			galactosidase	general immobilisation study	192
			human immunoglobulin	affinity chromatography	155
			invertase	hydrolysis of saccharose	198
	isocyanate coupling	HY–protein Y: O, S, NH	glucoamylase, glucose isomerase, glucose oxidase	general immobilisation study	182
	activation with benzoquinone	H_2N–protein	galactosidase	general immobilisation study	200
			trypsin inhibitor	affinity chromatography	201
	activation with ethyl chloroformate	HY–protein Y: O, S, NH	chymotrypsin A	general immobilisation study	202
	activation with carbonochloridate derivative	HY–protein Y: O, S, NH	albumins, anti- human immunoglobulin G antibody, human globulins, lectins, protein A, trypsin	affinity chromatography, general immobilisation study	141, 204
	activation with cyanogens bromide	H_2N–protein	anti-human protein C monoclonal antibody	general immobilisation study	70
			glucoamylase, glucose isomerase, glucose oxidase	general immobilisation study	182
			human immunoglobulin	affinity chromatography	155
	coupling by nucleophilic displacement	H_2N–protein	human immunoglobulin	affinity chromatography	155

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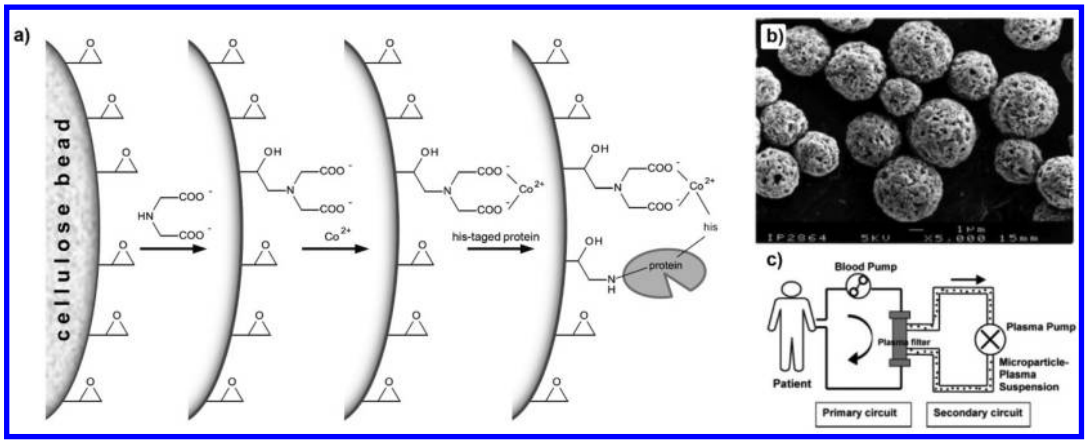

Figure 12. Scheme for the oriented fixation of histidine (his)-tagged antibodies on cellulose beads (a). Scanning electron microscopy image of cellulose beads used for protein immobilization (b). Scheme of a detoxification system utilizing cellulose beads with immobilized antibodies for blood plasma purification (c). Adapted with permission from ref 160. Copyright 2005 American Chemical Society.

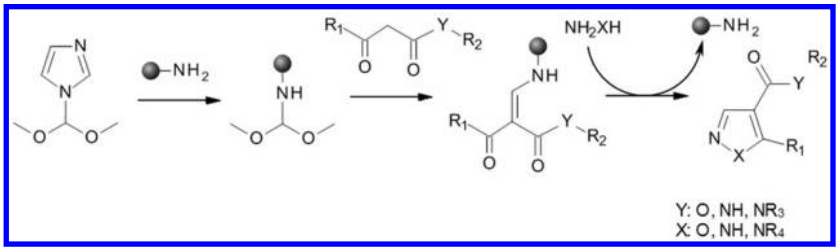

Figure 13. Reaction scheme for the solid-supported synthesis of pyrazoles and isoxazoles using functionalized cellulose beads as a catch and release anchor. Adapted with permission from ref 235. Copyright 2003 American Chemical Society.

are three-dimensional molecules with a very specific tertiary, even quaternary structure. In many cases, enzymatic activity and substrate specificity is attributed to a certain domain within the enzyme. Consequently, random protein immobilization might result in blocking of these active sites, which reduces catalytic activity. Also, the flexibility of enzymes, which is determined by the number of linkages between the cellulose surface and an individual enzyme, as well as the length of the spacer group, is an important parameter.[230] This influences the orientation of the catalytic domain relative to the substrate molecule. Oriented immobilization is achieved, when only a specific part of a protein is coupled to the cellulosic surface. Glycoproteins possess sugar moieties that are not essential for the activity itself and can thus be oxidized with $NaIO_4$ to generate carbonyl groups. Subsequently, the activated biomolecules can be coupled selectively with cellulose beads carrying amino groups.[197−199] In a comparable approach, proteins have been glycosylated with $NaIO_4$ oxidized mono- and disaccharides and have subsequently been immobilized on cellulose beads carrying carbonyl groups via dihydrazide linkers.[181] A unique way to immobilize enzymes in a specific manner is to genetically engineer proteins carrying cellulose-binding domains.[231,232] Moreover, the strong binding of biotin by the protein avidin has been exploited for selective enzyme immobilization on cellulose beads.[233]

It is possible to influence the protein alignment relative to the cellulose surface by introducing secondary interactions. For this purpose, Co^{2+} has been immobilized on beads via a chelating ligand in the first step followed by a histidine-tagged antibody afterward (Figure 12).[160] The additional interaction of the amino acid residue with the metal ion resulted in fixation of the antibodies in a specific orientation, which improved the affinity toward its counterpart, tumor necrosis factor-α.

5.4. Solid-Phase Synthesis Supports

Organic synthesis on solid-phase matrices is commonly applied in combinatorial chemistry because of the ease of product purification.[234] Resins based on polystyrene, partly cross-linked with divinylbenzene, are widely used for reagent coupling, but their application in polar solvents is limited due to insufficient swelling.[175] Functional cellulose beads may overcome these difficulties. They could be used for solid-supported synthesis of libraries of pyrazoles and isoxazoles in high yields.[235] Thereby, amino groups (**15**), attached to the beads, acted as a "catch and release anchor" for the reaction intermediates (Figure 13). In addition, amino group functionalized cellulose beads (**8**), prepared by cyanoethylation (**33**) and subsequent reduction, have been employed for the multistep synthesis of peptides according to the procedure developed by Merrifield.[236,237]

Functional cellulose beads may act as solid-support scavenger for electrophiles. Beads functionalized with amino groups (**14**) have been applied for product purification in the preparation of amides or urea derivatives from amines.[174] Subsequent to the initial reaction, they were added to bind the excess electrophilic reagents. Beads carrying allyl groups (**1**) could be used for the removal of bromine from reaction mixtures.[175] In addition to their use in organic synthesis, scavenger beads have been proposed for water treatment because they can chemically bind and remove hazardous compounds. Thiol-functionalized beads (**48**) have been tested for the removal of metolachlor, a representative for a number of herbicides with electrophilic moieties, from polluted water.[208]

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5.5. Drug Loading and Release

Cellulose, encoded in Europe as E460, is a nontoxic compound approved for use in food and pharmaceutical applications. For oral delivery systems, the drugs are usually mixed with cellulose powder and several other fillers and granules, also referred to as pellets, are prepared by extrusion and spheronisation.[16] Uniform distribution of the drugs is crucial in this step in order to prevent the release of high local dosages.

Cellulose beads provide several advantages over conventional granulate materials. Their internal surface area has been found to be at least one order of magnitudes higher, which indicates that beads can be loaded uniformly with high amounts of drugs.[22,238] Gradual release of the drugs has been reported for these materials.[239,240] Porosity of the matrix is an important factor for the release profile.[241] Thus, it is possible to meet the requirements of different pharmaceutical applications by controlling bead properties via variations of the process parameters. Depending on the type of drug (hydrophobic or hydrophilic, charged or noncharged), additional fillers, film formers, or solubilizers can be added during the cellulose bead preparation process.[240,242] These compounds can influence the release by facilitating the uniform distribution of a drug within the matrix, enhancing its adsorption, or preventing its crystallization. Moreover, functional cellulose beads may provide numerous functionalities for specific drug loading, for example, by ionic or hydrophobic interactions. Anionic cellulose beads with carboxymethyl- or phosphate groups have been used as tablet matrix for the gradual release of prazosin hydrochloride, which improved the overall release of the poorly water-soluble drug (Figure 14).[143] In addition, drug

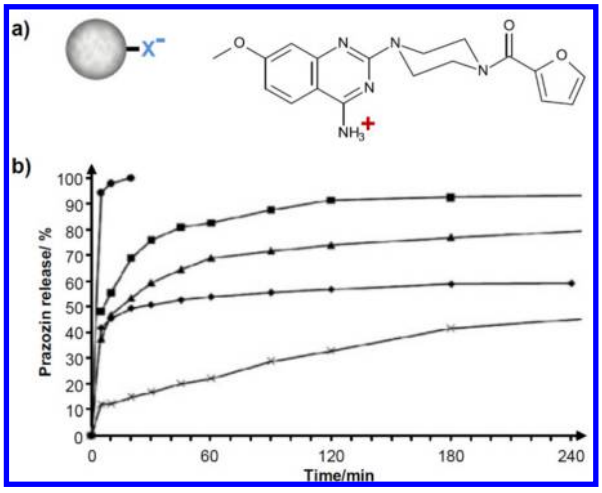

Figure 14. Schematic representation of prazosin attached to anionic cellulose beads (a). Prazosin release into buffer solution (b): prazosin loaded cellulose phosphate beads (-●-), prazosin powder tablet (-♦-), prazosin loaded cellulose carboxymethyl beads/ethanol dried (-■-), prazosin loaded cellulose carboxymethyl beads/water dried (-▲-), prazosin hydrochloride (-x-). Adapted with permission from ref 143. Copyright 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

immobilization by covalent binding is feasible. The linkage can be cleaved under defined conditions, for example, a certain pH, or by the action of a particular enzyme, which enables target-specific drug delivery. Despite the potential that cellulose beads bear for pharmaceutical applications, little research has been performed in that area so far. This may change with the recent discovery of novel cellulose solvents for bead preparation. In particular, aqueous solvents, such as NaOH/urea/water, which are cheap and nontoxic, show significant advantages over the currently employed viscose process.

6. CONCLUSION AND OUTLOOK

Functional cellulose beads are versatile materials for many applications. Since their first description in the literature, the techniques for their preparation, characterization, and especially chemical modification have been improved continuously over the past decades. However, compared to other advanced cellulosic materials, such as fibers, films, and membranes, cellulose beads have long been considered to be niche products mainly because of the limitations of conventional processes for the dissolution and shaping of cellulose. This assessment has changed in recent years with the increasing interest in biobased materials and the rapid advances in the field of polysaccharide research. New solvents and advanced procedures for the efficient dissolution of cellulose have emerged and were exploited for shaping the polysaccharide into cellulosic objects with defined properties. Ionic liquids, and especially aqueous solvents, bear great potential for the preparation of functional cellulose beads. Consequently, further developments in the field of functional cellulose beads can be expected regarding the enormous interest that novel polysaccharide solvents currently receive in scientific, applied, and business oriented research. A deeper understanding of the mechanism involved in the dissolution and coagulation of cellulose will not only make the bead preparation more efficient but also enables tailoring of their properties by adjusting the process parameters. Research in that field is closely related to the development or improvement of technical devices for the low-cost production of high batches with constant product quality. Moreover, functional hybrid materials prepared by blending cellulose with highly engineered polysaccharide derivatives bear great potential but are almost completely unexplored.

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Notes

The authors declare no competing financial interest.

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Martin Gericke, born in 1980, joined Åbo Akademi University, Laboratory of Fibre and Cellulose technology in June 2011 as postdoctoral researcher. Before that, from November 2010, he worked at the Thuringian Institute for Textile and Plastics Research

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Pedro Fardim, born in 1966, obtained his Ph.D. in physical chemistry in 1999 at Campinas State University (UNICAMP) in Brazil. In 2000, he became associated with the Department of Chemical Engineering at Åbo Akademi University (ÅA) as postdoctoral researcher and was appointed Adjunct Professor in 2004. In August 2005, he was appointed full Professor in the topic of Biomass Chemical Engineering at ÅA. From 1992 to 1999, he worked as a supervisor in the R&D centre of a top-level biomaterials-based company in Brazil. His company-driven project activities covered the entire field from plant genetics to biobased polymers and materials. Pedro Fardim's main research interests lie within the area of chemistry and topochemistry of biomass-based materials including pretreatment, fractionation, and functionalization of biomolecules and biostructures and shaping into advanced materials. Topochemical interactions in solid−liquid interfaces and their role in biomass processing are also part of his research activities. He is the elected vice-president of the European Polysaccharide Network of Excellence (EPNOE), a nonprofit organisation dedicated to promoting the investigation and innovative utilisation of polysaccharides and is a Fellow of the International Academy of Wood Science. He is also member-at-large of the Cellulose and Renewable Materials Division of the American Chemical Society.

Jani Trygg, born in 1981, joined Professor Fardim's group at Åbo Akademi, Laboratory of Fibre and Cellulose technology in 2009 as a doctoral student and researcher. Before that he studied chemistry at the University of Turku, Laboratory of Materials Chemistry and Chemical Analysis. He graduated in 2008, writing his thesis about spectroscopic properties of lanthanide oxisulfides and thermokinetic reactions. Currently, under the Future Biorefinery (FuBio) program, he is doing his research on functionalisation of cellulose beads, including the pretreatment and dissolution of cellulose. Additionally, his personal interests also lie in educational systems and the field of pedagogy.

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REFERENCES

(1) Johansson, C.; Bras, J.; Mondragon, I.; Nechita, P.; Plackett, D.; Šimon, P.; Svetec, D. G.; Virtanen, S.; Baschetti, M. G.; Breen, C.; Clegg, F.; Aucejo, S. *Bioresource* **2012**, *7*, 2506.
(2) Habibi, Y.; Lucia, L. A.; Rojas, O. J. *Chem. Rev.* **2010**, *110*, 3479.
(3) Eichhorn, S.; Dufresne, A.; Aranguren, M.; Marcovich, N.; Capadona, J.; Rowan, S.; Weder, C.; Thielemans, W.; Roman, M.; Renneckar, S.; Gindl, W.; Veigel, S.; Keckes, J.; Yano, H.; Abe, K.; Nogi, M.; Nakagaito, A.; Mangalam, A.; Simonsen, J.; Benight, A.; Bismarck, A.; Berglund, L.; Peijs, T. *J. Mater. Sci.* **2010**, *45*, 1.
(4) Jawaid, M.; Abdul Khalil, H. P. S. *Carbohydr. Polym.* **2011**, *86*, 1.
(5) Klemm, D.; Heublein, B.; Fink, H.-P.; Bohn, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 3358.
(6) Shen, L.; Patel, M. *J. Polym. Environ.* **2008**, *16*, 154.
(7) Stevens, E. S. *Green Plastics: An Introduction to the New Science of Biodegradable Plastics*; Princeton University Press: Princeton, NJ, U.S.A., 2002.
(8) John, M. J.; Thomas, S. *Carbohydr. Polym.* **2008**, *71*, 343.
(9) Heinze, T.; Liebert, T.; Koschella, A. *Esterification of Polysaccharides*; Springer Verlag: Berlin-Heidelberg, Germany, 2006.
(10) Heinze, T.; Liebert, T. *Prog. Polym. Sci.* **2001**, *26*, 1689.
(11) Fox, S. C.; Li, B.; Xu, D.; Edgar, K. J. *Biomacromolecules* **2011**, *12*, 1956.
(12) Iontosorb Home Page. http://www.iontosorb.cz (accessed November, 2011).
(13) JNC Corporation Home Page. http://www.jnc-corp.co.jp/fine/en/cellufine/index.html (accessed June, 2012).
(14) Rengo Co., Ltd. Home Page. http://www.rengo.co.jp/english/products/functional/biscp.html (accessed October, 2011).
(15) *Cellulose Solvents: For Analysis, Shaping and Chemical Modification*; Liebert, T., Heinze, T., Edgar, K. J., Eds.; American Chemical Society: Washington, DC, U.S.A., 2010.
(16) Dukić-Ott, A.; Thommes, M.; Remon, J. P.; Kleinebudde, P.; Vervaet, C. *Eur. J. Pharm. Biopharm.* **2009**, *71*, 38.
(17) O'Neill, J. J., Jr.; Reichardt, E. P. U.S. Patent 2,543,928, 1951.
(18) Wilkes, E. D.; Phillips, S. D.; Basaran, O. A. *Phys. Fluids* **1999**, *11*, 3577.
(19) Su, H.; Bajpai, R.; Preckshot, G. *Appl. Biochem. Biotechnol.* **1989**, *20−21*, 561.
(20) Mazzitelli, S.; Tosi, A.; Balestra, C.; Nastruzzi, C.; Luca, G.; Mancuso, F.; Calafiore, R.; Calvitti, M. *J. Biomater. Appl.* **2008**, *23*, 123.
(21) De Oliveira, W.; Glasser, W. G. *J. Appl. Polym. Sci.* **1996**, *60*, 63.
(22) Trygg, J.; Fardim, P.; Gericke, M.; Mäkilä, E.; Salonen, J. *Carbohydr. Polym.* **2011**, DOI: 10.1016/j.carbpol.2012.03.085.
(23) Sescousse, R.; Gavillon, R.; Budtova, T. *J. Mater. Sci.* **2011**, *46*, 759.
(24) Ishimura, D.; Morimoto, Y.; Saito, H. *Cellulose* **1998**, *5*, 135.

(25) Ishimura, D.; Morimoto, Y.; Saito, H. *Cellulose* **1998**, *5*, 135. (b) Morimoto, Y.; Tsukida, N.; Saga, H.; Saito, H. EP Patent 850,979 A2.
(26) Wang, H.; Li, B.; Shi, B. *BioResources* **2008**, *3*, 3.
(27) Rosenberg, P.; Suominen, I.; Rom, M.; Janicki, J.; Fardim, P. *Cellul. Chem. Technol.* **2007**, *41*, 243.
(28) Rosenberg, P.; Rom, M.; Janicki, J.; Fardim, P. *Cellul. Chem. Technol.* **2008**, *42*, 293.
(29) Senuma, Y.; Hilborn, J. G. *Polym. Eng. Sci.* **2002**, *42*, 969.
(30) Teunou, E.; Poncelet, D. *J. Food Eng.* **2005**, *71*, 345.
(31) Beyer, C.; Meister, F.; Michels, C.; Riedel, B.; Taeger, E. DE Patent 19,755,353 C1, 1999.
(32) Pinnow, M.; Fink, H.-P.; Fanter, C.; Kunze, J. *Macromol. Symp.* **2008**, *262*, 129.
(33) Braun, M.; Guentherberg, N.; Lutz, M.; Magin, A.; Siemer, M.; Swaminathan, V. N.; Linner, B.; Ruslim, F.; Fernandez, R. G. A. U.S. Patent 20,100,331,222 A1, 2010.
(34) Prüße, U.; Bruske, F.; Breford, J.; Vorlop, K.-D. *Chem. Eng. Technol.* **1998**, *21*, 153.
(35) Chen, L. F.; Tsao, G. T. *Biotechnol. Bioeng.* **1976**, *18*, 1507.
(36) Tsao, G. T.; Chen, L. F. DE Patent 2,717,965 A1, 1977.
(37) Karbstein, H.; Schubert, H. *Chem. Eng. Process.* **1995**, *34*, 205.
(38) Maggioris, D.; Goulas, A.; Alexopoulos, A. H.; Chatzi, E. G.; Kiparissides, C. *Chem. Eng. Sci.* **2000**, *55*, 4611.
(39) Kotoulas, C.; Kiparissides, C. *Chem. Eng. Sci.* **2006**, *61*, 332.
(40) Jarvis, M. *Nature* **2003**, *426*, 611.
(41) Fink, H. P.; Ebeling, H.; Vorwerg, W. *Chem. Ing. Tech.* **2009**, *81*, 1757.
(42) Perepelkin, K. *Fibre Chem.* **2008**, *40*, 10.
(43) Weber, H. *Lenzinger Ber.* **2005**, *84*, 8.
(44) Determann, V. H.; Rehner, H.; Mitarbeit, T. W.; Meyer, v. N.; Wente, F. *Makromol. Chem.* **1968**, *114*, 263.
(45) Determann, H.; Wieland, T. U.S. Patent 3,597,350, A, 1971.
(46) Chitumbo, K.; Brown, W. *J. Polym. Sci., Part C: Polym. Symp.* **1971**, *36*, 279.
(47) Peska, J.; Stamberg, J.; Hradil, J.; Ilavský, M. *J. Chromatogr., A* **1976**, *125*, 455.
(48) Peska, J.; Stamberg, J.; Blace, Z. Patent CS 172,640 B1, 1977.
(49) Loth, F.; Schaaf, E.; Weigel, P.; Fink, H.-P.; Gensrich, H. J. WO Patent 2003,099,871 A1, 2003.
(50) Lang, H.; Loth, F.; Bertram, D. DD Patent 259,533 A3, 1988.
(51) Klemm, D.; Philipp, B.; Heinze, T.; Heinze, U.; Wagenknecht, W. *Comprehensive Cellulose Chemistry 2. Functionalization of Cellulose*; Wiley-VCH: Weinheim, Germany, 1998.
(52) Motozato, Y.; Hirayama, C. *J. Chromatogr., A* **1984**, *298*, 499.
(53) Thümmler, K.; Fischer, S.; Feldner, A.; Weber, V.; Ettenauer, M.; Loth, F.; Falkenhagen, D. *Cellulose* **2011**, *18*, 135.
(54) Motozato, Y.; Ishibashi, H. EP Patent 25,639 A1, 1981.
(55) Bai, Y.-X.; Li, Y.-F. *Carbohydr. Polym.* **2006**, *64*, 402.
(56) Loth, F.; Stein, A.; Heinze, T.; Klemm, D. DD Patent 295,861 A5, 1991.
(57) Liebert, T. In *Cellulose Solvents: For Analysis, Shaping and Chemical Modification*; Liebert, T., Heinze, T., Edgar, K. J., Eds.; American Chemical Society: Washington, DC, U.S.A., 2010; pp 3−54.
(58) Klemm, D.; Philipp, B.; Heinze, T.; Heinze, U.; Wagenknecht, W. *Comprehensive Cellulose Chemistry 1. Fundamentals and Analytical Methods*; Wiley−VCH: Weinheim, Germany, 1998.
(59) Saalwächter, K.; Burchard, W.; Klüfers, P.; Kettenbach, G.; Mayer, P.; Klemm, D.; Dugarmaa, S. *Macromolecules* **2000**, *33*, 4094.
(60) Burchard, W.; Habermann, N.; Klüfers, P.; Seger, B.; Wilhelm, U. *Angew. Chem., Int. Ed.* **1994**, *33*, 884.
(61) Schweizer, E. *J. Prakt. Chem.* **1857**, *72*, 109.
(62) Jayme, V. G.; Neuschäffer, K. *Makromol. Chem.* **1957**, *23*, 71.
(63) Adikane, H. V.; Nene, S. N. IN Patent 2000DE00,214 A, 2008.
(64) McCormick, C. L.; Callais, P. A. *Polymer* **1987**, *28*, 2317.
(65) Heinze, T.; Liebert, T. F.; Pfeiffer, K. S.; Hussain, M. A. *Cellulose* **2003**, *10*, 283.
(66) Nishio, Y.; Manley, R. S. J. *Macromolecules* **1988**, *21*, 1270.
(67) Davé, V.; Glasser, W. G. *J. Appl. Polym. Sci.* **1993**, *48*, 683.
(68) Marsano, E.; Conio, G.; Martino, R.; Turturro, A.; Bianchi, E. *J. Appl. Polym. Sci.* **2002**, *83*, 1825.
(69) Duchemin, B. J. C.; Staiger, M. P.; Tucker, N.; Newman, R. H. *J. Appl. Polym. Sci.* **2010**, *115*, 216.
(70) Kaster, J. A.; de Oliveira, W.; Glasser, W. G.; Velander, W. H. *J. Chromatogr., A* **1993**, *648*, 79.
(71) Velander, W. H.; Kaster, J. A.; Glasser, W. G. U.S. Patent 5,328,603 A, 1994.
(72) Fink, H. P.; Weigel, P.; Purz, H. J.; Ganster, J. *Prog. Polym. Sci.* **2001**, *26*, 1473.
(73) Biganska, O.; Navard, P.; Bédué, O. *Polymer* **2002**, *43*, 6139.
(74) Beyer, C.; Meister, F.; Michels, C.; Riedel, B.; Taeger, E. WO Patent 9,931,141 A2, 1999.
(75) Niemz, F.-G.; Vorbach, D. WO Patent 2,002,057,319 A2, 2002.
(76) Twu, Y.-K.; Huang, H.-I.; Chang, S.-Y.; Wang, S.-L. *Carbohydr. Polym.* **2003**, *54*, 425.
(77) Liu, M.; Huang, J.; Deng, Y. *Bioresour. Technol.* **2007**, *98*, 1144.
(78) Shi, F.; Lin, D.-Q.; Phottraithip, W.; Yao, S.-J. *J. Appl. Polym. Sci.* **2011**, *119*, 3453.
(79) Rosenau, T.; Potthast, A.; Sixta, H.; Kosma, P. *Prog. Polym. Sci.* **2001**, *26*, 1763.
(80) Konkin, A.; Wendler, E.; Meister, F.; Roth, H. K.; Aganov, A.; Ambacher, O. *Spectrochim. Acta, Part A* **2008**, *69*, 1053.
(81) Zhang, L.; Ruan, D.; Gao, S. *J. Polym. Sci., Part B Polym. Phys.* **2002**, *40*, 1521.
(82) Jin, H.; Zha, C.; Gu, L. *Carbohydr. Res.* **2007**, *342*, 851.
(83) Egal, M.; Budtova, T.; Navard, P. *Cellulose* **2008**, *15*, 361.
(84) Liu, W.; Budtova, T.; Navard, P. *Cellulose* **2011**, *18*, 911.
(85) Trygg, J.; Fardim, P. *Cellulose* **2011**, *18*, 987.
(86) Rosenberg, P.; Budtova, T.; Rom, M.; Fardim, P. In *Cellulose Solvents: For Analysis, Shaping and Chemical Modification*; Liebert, T., Heinze, T., Edgar, K. J., Eds.; American Chemical Society: Washington, DC, U.S.A., 2010; pp 213−226.
(87) Qi, H.; Yang, Q.; Zhang, L.; Liebert, T.; Heinze, T. *Cellulose* **2011**, *18*, 237.
(88) Luo, X.; Zhang, L. *J. Hazard. Mater.* **2009**, *171*, 340.
(89) Luo, X.; Zhang, L. *Biomacromolecules* **2010**, *11*, 2896.
(90) Luo, X.; Zhang, L. *J. Chromatogr., A* **2010**, *1217*, 5922.
(91) El Seoud, O. A.; Koschella, A.; Fidale, L. C.; Dorn, S.; Heinze, T. *Biomacromolecules* **2007**, *8*, 2629.
(92) Pinkert, A.; Marsh, K. N.; Pang, S. S.; Staiger, M. P. *Chem. Rev.* **2009**, *109*, 6712.
(93) Wang, H.; Gurau, G.; Rogers, R. D. *Chem. Soc. Rev.* **2012**, *41*, 1519.
(94) Kosan, B.; Michels, C.; Meister, F. *Cellulose* **2008**, *15*, 59.
(95) Stark, A. *Energy Environ. Sci.* **2011**, *4*, 19.
(96) Sun, N.; Rodriguez, H.; Rahman, M.; Rogers, R. D. *Chem. Commun.* **2011**, *47*, 1405.
(97) Gericke, M.; Fardim, P.; Heinze, T. *Molecules* **2012**, *17*, 7458.
(98) Du, K.-F.; Yan, M.; Wang, Q.-Y.; Song, H. *J. Chromatogr., A* **2010**, *1217*, 1298.
(99) Phottraithip, W.; Lin, D.-Q.; Shi, F.; Yao, S.-J. *J. Appl. Polym. Sci.* **2011**, *121*, 2985.
(100) Turner, M. B.; Spear, S. K.; Holbrey, J. D.; Daly, D. T.; Rogers, R. D. *Biomacromolecules* **2005**, *6*, 2497.
(101) Liu, Z.; Wang, H.; Liu, C.; Jiang, Y.; Yu, G.; Mu, X.; Wang, X. *Chem. Commun.* **2012**, *48*, 7350.
(102) Liu, Z.; Wang, H.; Li, B.; Liu, C.; Jiang, Y.; Yu, G.; Mu, X. *J. Mater. Chem.* **2012**, *22*, 15085.
(103) Sun, X.; Peng, B.; Ji, Y.; Chen, J.; Li, D. *AIChE J.* **2009**, *55*, 2062.
(104) Gericke, M.; Schlufter, K.; Liebert, T.; Heinze, T.; Budtova, T. *Biomacromolecules* **2009**, *10*, 1188.
(105) Sescousse, R.; Le, K. A.; Ries, M. E.; Budtova, T. *J. Phys. Chem. B* **2010**, *114*, 7222.
(106) *Ionic Liquids in Synthesis*, 2nd ed.; Wasserscheid, P., Welton, T., Eds.; Wiley-VCH: Weinheim, Germany, 2008.
(107) Ha, S. H.; Mai, N. L.; An, G.; Koo, Y.-M. *Bioresour. Technol.* **2011**, *102*, 1214.

(108) Gesellschaft für Chemische Industrie in Basel CH 153,446, 1932.
(109) Graenacher, C. U.S. Patent 1,943,176, 1934.
(110) Linko, Y.-Y.; Viskari, R.; Pohjola, L.; Linko, P. *Appl. Biochem. Biotechnol.* **1977**, *2*, 203.
(111) Gericke, M.; Liebert, T.; Seoud, O. A. E.; Heinze, T. *Macromol. Mater. Eng.* **2011**, *296*, 483.
(112) Rinaldi, R. *Chem. Commun.* **2011**, *47*, 511.
(113) Lei, Y.-L.; Lin, D.-Q.; Yao, S.-J.; Zhu, Z.-Q. *J. Appl. Polym. Sci.* **2003**, *90*, 2848.
(114) Gao, D.; Yao, S.-J.; Lin, D.-Q. *J. Appl. Polym. Sci.* **2008**, *107*, 674.
(115) Xia, H.-F.; Lin, D.-Q.; Yao, S.-J. *J. Appl. Polym. Sci.* **2007**, *104*, 740.
(116) Xia, H.-F.; Lin, D.-Q.; Yao, S.-J. *J. Chromatogr., A* **2007**, *1175*, 55.
(117) Anspach, F. B.; Curbelo, D.; Hartmann, R.; Garke, G.; Deckwer, W.-D. *J. Chromatogr., A* **1999**, *865*, 129.
(118) Chase, H. A. *Trends Biotechnol.* **1994**, *12*, 296.
(119) Lenfeld, J. *Angew. Makromol. Chem.* **1993**, *212*, 147.
(120) Luo, X.; Liu, S.; Zhou, J.; Zhang, L. *J. Mater. Chem.* **2009**, *19*, 3538.
(121) Safarik, I.; Sabatkova, Z.; Tokar, O.; Safarikova, M. *Food Technol. Biotechnol.* **2007**, *45*, 355.
(122) Guo, X.; Chen, F. *Environ. Sci. Technol.* **2005**, *39*, 6808.
(123) He, Y.-B.; Meng, L.-Z.; Wu, C.-T.; Qian, T. *J. Appl. Polym. Sci.* **1999**, *74*, 1278.
(124) Wang, D.-M.; Hao, G.; Shi, Q.-H.; Sun, Y. *J. Chromatogr., A* **2007**, *1146*, 32.
(125) Sakurai, A.; Itoh, M.; Sakakibara, M.; Saito, H.; Fujita, M. *J. Chem. Technol. Biotechnol.* **1997**, *70*, 157.
(126) Buschle-Diller, G.; Fanter, C.; Loth, F. *Cellulose* **1995**, *2*, 179.
(127) Bowen, P. *J. Dispersion Sci. Technol* **2002**, *23*, 631.
(128) Hentschel, M. L.; Page, N. W. *Part. Part. Syst. Char* **2003**, *20*, 25.
(129) McNaught, A. D.; Wilkinson, A. *IUPAC. Compendium of Chemical Terminology*, 2nd ed.; Blackwell Scientific Publications: Oxford, U.K., 1997.
(130) Conner, W. C.; Cevallos-Candau, J. F.; Weist, E. L.; Pajares, J.; Mendioroz, S.; Cortes, A. *Langmuir* **1986**, *2*, 151.
(131) Johnston, G. P.; Smith, D. M.; Melendez, I.; Hurd, A. J. *Powder Technol.* **1990**, *61*, 289.
(132) Thünemann, A.; Klobes, P.; Wieland, C.; Bruzzano, S. *Anal. Bioanal. Chem.* **2011**, *401*, 1101.
(133) Ek, R.; Henriksson, U.; Nyström, C.; Ödberg, L. *Powder Technol.* **1994**, *81*, 279.
(134) Webber, B. *Physics* **2012**, 5.
(135) Grznárová, G.; Yu, S.; Stefuca, V.; Polakovic, M. *J. Chromatogr., A* **2005**, *1092*, 107.
(136) Kavoosi, M.; Lam, D.; Bryan, J.; Kilburn, D. G.; Haynes, C. A. *J. Chromatogr., A* **2007**, *1175*, 187.
(137) Degen, P.; Leick, S.; Rehage, H. *Z. Phys. Chem.* **2009**, *223*, 1079.
(138) Anderson, R. L.; Owens, J. W.; Timms, C. W. *Cancer Lett.* **1992**, *63*, 83.
(139) Fang, H.; Wei, J.; Yu, Y. *Biomaterials* **2004**, *25*, 5433.
(140) Peška, J.; Štamberg, J.; Hradil, J. *Angew. Makromol. Chem.* **1976**, *53*, 73.
(141) Boeden, H. F.; Pommerening, K.; Becker, M.; Rupprich, C.; Holtzhauer, M.; Loth, F.; Müller, R.; Bertram, D. *J. Chromatogr., A* **1991**, *552*, 389.
(142) Burton, S. C.; Harding, D. R. K. *J. Chromatogr., A* **1997**, *775*, 29.
(143) Volkert, B.; Wolf, B.; Fischer, S.; Li, N.; Lou, C. *Macromol. Symp.* **2009**, *280*, 130.
(144) Matějka, Z.; Štamberg, J.; Beneš, M. J. *React. Polym.* **1984**, *3*, 33.
(145) Köhler, S.; Liebert, T.; Heinze, T. *J. Polym. Sci., Part A Polym. Chem.* **2008**, *46*, 4070.
(146) Mormann, W.; Demeter, J. *Macromolecules* **1999**, *32*, 1706.
(147) Xiong, X.; Zhang, L.; Wang, Y. *J. Chromatogr., A* **2005**, *1063*, 71.
(148) Gough, B. M. *Starch-Stärke* **1967**, *19*, 240.
(149) Xia, H.-F.; Lin, D.-Q.; Wang, L.-P.; Chen, Z.-J.; Yao, S.-J. *Ind. Eng. Chem. Res.* **2008**, *47*, 9566.
(150) Mislovičová, D.; Gemeiner, P.; Kuniak, L.; Zemek, J. *J. Chromatogr., A* **1980**, *194*, 95.
(151) Mislovicová, D.; Gemeiner, P.; Breier, A. *Enzyme Microb. Technol.* **1988**, *10*, 568.
(152) Štamberg, J. *Sep. Purif. Method.* **1988**, *17*, 155.
(153) Yamamoto, S.; Miyagawa, E. *J. Chromatogr., A* **1999**, *852*, 25.
(154) Ohtaki, N.; Takahashi, H.; Kaneko, K.; Gomi, Y.; Ishikawa, T.; Higashi, Y.; Todokoro, M.; Kurata, T.; Sata, T.; Kojima, A. *J. Virol. Method.* **2011**, *174*, 131.
(155) Peng, L.; Calton, G. J.; Burnett, J. W. *J. Biotechnol.* **1987**, *5*, 255.
(156) Kahovec, J.; Matějka, Z.; Štamberg, J. *Polym. Bull.* **1980**, *3*, 13.
(157) Boto, R. E. F.; Anyanwu, U.; Sousa, F.; Almeida, P.; Queiroz, J. A. *Biomed. Chromatogr.* **2009**, *23*, 987.
(158) Turková, J.; Vajcner, J.; Vancurova, D.; Stamberg, J. *Collect. Czech. Chem. Commun.* **1979**, *44*, 3411.
(159) Gemeiner, P.; Pašteka, M. *Appl. Biochem. Biotechnol.* **1983**, *8*, 381.
(160) Weber, V.; Linsberger, I.; Ettenauer, M.; Loth, F.; Höyhtyä, M.; Falkenhagen, D. *Biomacromolecules* **2005**, *6*, 1864.
(161) Korecká, L.; Bílková, Z.; Holèapek, M.; Královský, J.; Benes, M.; Lenfeld, J.; Minc, N.; Cecal, R.; Viovy, J.-L.; Przybylski, M. *J. Chromatogr., B* **2004**, *808*, 15.
(162) Rahn, K.; Heinze, T. *Cellul. Chem. Technol.* **1998**, *32*, 173.
(163) Sirvio, J.; Hyvakko, U.; Liimatainen, H.; Niinimaki, J.; Hormi, O. *Carbohydr. Polym.* **2011**, *83*, 1293.
(164) Tahiri, C.; Vignon, M. R. *Cellulose* **2000**, *7*, 177.
(165) Hirota, M.; Tamura, N.; Saito, T.; Isogai, A. *Cellulose* **2009**, *16*, 841.
(166) Yu, H.; Fu, G.; He, B. *Cellulose* **2007**, *14*, 99.
(167) Wang, D.-M.; Sun, Y. *Biochem. Eng. J.* **2007**, *37*, 332.
(168) McDowall, D. J.; Gupta, B. S.; Stannett, V. T. *Prog. Polym. Sci.* **1984**, *10*, 1.
(169) Littunen, K.; Hippi, U.; Johansson, L.-S.; Österberg, M.; Tammelin, T.; Laine, J.; Seppälä, J. *Carbohydr. Polym.* **2011**, *84*, 1039.
(170) De Oliveira, W.; Glasser, W. G. *J. Appl. Polym. Sci.* **1996**, *61*, 81.
(171) Kim, M. H.; An, S.; Won, K.; Kim, H. J.; Lee, S. H. *J. Mol. Catal. B-Enzym.* **2012**, *75*, 68.
(172) Zhang, L.; Cai, J.; Zhou, J.; Tang, Y. *Sep. Sci. Technol.* **2004**, *39*, 1203.
(173) Hoyle, C. E.; Bowman, C. N. *Angew. Chem., Int. Ed.* **2010**, *49*, 1540.
(174) Chesney, A.; Steel, P. G.; Stonehouse, D. F. *J. Comb. Chem.* **2000**, *2*, 434.
(175) Chesney, A.; Barnwell, P.; Stonehouse, D. F.; Steel, P. G. *Green Chem.* **2000**, *2*, 57.
(176) Burton, S. C.; Harding, D. R. K. *J. Chromatogr., A* **1997**, *775*, 39.
(177) Yamamoto, M.; Izuhara, Y.; Kakuta, T.; Takizawa, S.; Fujita, A.; Higaki, T.; van Ypersele de Strihou, C.; Miyata, T. *Peritoneal Dial. Int.* **2007**, *27*, 300.
(178) Burton, S. C.; Haggarty, N. W.; Harding, D. R. K. *Biotechnol. Bioeng.* **1997**, *56*, 45.
(179) Roy, I.; Gupta, M. N. *Process Biochem.* **2003**, *39*, 325.
(180) Gemeiner, P.; Breier, A. *Biotechnol. Bioeng.* **1982**, *24*, 2573.
(181) Gemeiner, P.; Viskupič, E. *J. Biochem. Biophys. Methods* **1981**, *4*, 309.
(182) Chen, L. F.; Tsao, G. T. *Biotechnol. Bioeng.* **1977**, *19*, 1463.
(183) Ohnishi, M.; Iwata, K.; Tomita, T.; Nishikawa, U.; Hiromi, K. *Starch-Stärke* **1990**, *42*, 486.
(184) Katsuragi, T.; Shibata, M.; Sakai, T.; Tonomura, K. *Agric. Biol. Chem.* **1989**, *53*, 1515.

(185) Matsumoto, K.; Matsubara, H.; Hamada, M.; Ukeda, H.; Osajima, Y. *J. Biotechnol.* **1990**, *14*, 115.
(186) Matsumoto, K.; Sakoda, K.; Osajima, Y. *Anal. Chim. Acta* **1992**, *261*, 155.
(187) Matsumoto, K.; Matsubara, H.; Hamada, M.; Doi, T.; Osajima, Y. *Agric. Biol. Chem.* **1991**, *55*, 1055.
(188) Nakamura, T.; Ogata, Y.; Shitara, A.; Nakamura, A.; Ohta, K. *J. Ferment. Bioeng.* **1995**, *80*, 164.
(189) Dash, R.; Ragauskas, A. J. *J. Biobased Mater. Bioenergy* **2010**, *4*, 440.
(190) Matús, P.; Kubová, J. *J. Inorg. Biochem.* **2005**, *99*, 1769.
(191) Diviš, P.; Szkandera, R.; Brulík, L.; Dočekalová, H.; Matúš, P.; Bujdoš, M. *Anal. Sci.* **2009**, *25*, 575.
(192) Kéry, V.; Kucár, S.; Matulová, M.; Haplová, J. *Carbohydr. Res.* **1991**, *209*, 83.
(193) Rahn, K.; Diamantoglou, M.; Klemm, D.; Berghmans, H.; Heinze, T. *Angew. Makromol. Chem.* **1996**, *238*, 143.
(194) Nakamura, S.; Amano, M.; Saegusa, Y.; Sato, T. *J. Appl. Polym. Sci.* **1992**, *45*, 265.
(195) Furuhata, K.; Aoki, N.; Abe, H.; Taniguchi, T.; Sakamoto, M. *Sen'i Gakkaishi* **1993**, *49*, 563.
(196) Aoki, N.; Taniguchi, T.; Arai, N.; Furuhata, K.-i.; Sakamoto, M. *Sen'i Gakkaishi* **1994**, *50*, 520.
(197) Bílková, Z.; Churáček, J.; Kučerová, Z.; Turková, J. *J. Chromatogr., B* **1997**, *689*, 273.
(198) Štefuca, V.; Gemeiner, P.; Báleš, V. *Enzyme Microb. Technol.* **1988**, *10*, 306.
(199) Turková, J.; Petkov, L.; Sajdok, J.; Káš, J.; Beneš, M. J. *J. Chromatogr., A* **1990**, *500*, 585.
(200) Chun, M.; Dickopp, G.; Sernetz, M. *Appl. Biochem. Biotechnol.* **1980**, *5*, 211.
(201) Kucera, J. *J. Chromatogr.* **1981**, *213*, 352.
(202) Kennedy, J. F.; Barker, S. A.; Rosevear, A. *J. Chem. Soc., Perk. Trans. 1* **1973**, 2293.
(203) Naumann, M.; Reuter, R.; Metz, P.; Kopperschläger, G. *J. Chromatogr., A* **1989**, *466*, 319.
(204) Marx, A.; Giersch, T. *Anal. Lett.* **1995**, *28*, 267.
(205) Schwidop, W.-D.; Klossek, P.; Müller, R.; Claus, R. *J. Chromatogr., A* **1990**, *520*, 325.
(206) Burton, S. C.; Haggarty, N. W.; Harding, D. R. K. *J. Chromatogr., A* **1991**, *587*, 271.
(207) Burton, S. C.; Harding, D. R. K. *J. Chromatogr., A* **1998**, *814*, 71.
(208) Willems, H. P. L.; Berry, D. F.; Samaranayake, G.; Glasser, W. G. *Environ. Sci. Technol.* **1996**, *30*, 2148.
(209) Braun, M.; Dyllick-Brenzinger, R.; Maase, M.; Stein, F.; Turner, M.; Uerdingen, E. Int. Patent WO 2009,037,146 A1, 2009.
(210) Rojas, J.; Azevedo, E. *Int. J. Pharm. Sci. Rev. Res.* **2011**, *8*, 28.
(211) Gemeiner, P.; Polakovic, M.; Mislovicová, D.; Stefuca, V. *J. Chromatogr., B* **1998**, *715*, 245.
(212) Vincent, P.; Compoint, J.-P.; Fitton, V.; Santarelli, X. *J. Biochem. Biophys. Methods* **2003**, *56*, 69.
(213) Denizli, A.; Pişkin, E. *J. Biochem. Biophys. Methods* **2001**, *49*, 391.
(214) Mislovičová, D.; Gemeiner, P.; Stratilová, E.; Horváthová, M. *J. Chromatogr., A* **1990**, *510*, 197.
(215) Pai, A.; Gondkar, S.; Lali, A. *J. Chromatogr., A* **2000**, *867*, 113.
(216) Sakata, M.; Nakayama, M.; Yanagi, K.; Sasaki, M.; Kunitake, M.; Hirayama, C. *J. Liq. Chromatogr. Relat. Technol.* **2006**, *29*, 2499.
(217) Kobayashi, A.; Nakatani, M.; Furuyoshi, S.; Tani, N. *Ther. Apher.* **2002**, *6*, 365.
(218) Kopaciewicz, W.; Rounds, M. A.; Fausnaugh, J.; Regnier, F. E. *J. Chromatogr., A* **1983**, *266*, 3.
(219) Gong, C.-S.; Chen, L. F.; Tsao, G. T. *Biotechnol. Bioeng.* **1980**, *22*, 833.
(220) Queiroz, J. A.; Tomaz, C. T.; Cabral, J. M. S. *J. Biotechnol.* **2001**, *87*, 143.
(221) Gemeiner, P.; Hrabarova, E.; Zacharova, M.; Breier, A.; Beneš, M. J. *Collect. Czech. Chem. Commun.* **1989**, *54*, 2375.
(222) Gemeiner, P.; Špánik, V.; Šnajdrová, A.; Stratilová, E.; Horváthová, M.; Hagarová, D.; Markovič, O. *Folia Microbiol.* **1991**, *36*, 283.
(223) Murao, S.; Oyama, H.; Nomura, Y.; Tono, T.; Shin, T. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 177.
(224) Ghose, S.; Hubbard, B.; Cramer, S. M. *Biotechnol. Prog.* **2005**, *21*, 498.
(225) Burton, S. C.; Harding, D. R. K. *J. Chromatogr., A* **1998**, *796*, 273.
(226) Subramanian, A.; Martinez, B. C. *Int. J. Bio-Chromatogr.* **2000**, *5*, 31.
(227) Kumar Sharma, R.; Agrawal, M.; Marshall, F. *Ecotox. Environ. Safe* **2007**, *66*, 258.
(228) Yeomans-Reina, H.; Ruiz-Manriquez, A.; Wong, B. R.; Mansir, A. T. *Biotechnol. Prog.* **2001**, *17*, 729.
(229) Sheldon, R. A. *Adv. Synth. Catal.* **2007**, *349*, 1289.
(230) Mateo, C.; Palomo, J. M.; Fernandez-Lorente, G.; Guisan, J. M.; Fernandez-Lafuente, R. *Enzym. Microb. Technol.* **2007**, *40*, 1451.
(231) Fishman, A.; Levy, I.; Cogan, U.; Shoseyov, O. *J. Mol. Catal. B: Enzym.* **2002**, *18*, 121.
(232) Karpol, A.; Kantorovich, L.; Demishtein, A.; Barak, Y.; Morag, E.; Lamed, R.; Bayer, E. A. *J. Mol. Recognit.* **2009**, *22*, 91.
(233) Janolino, V. G.; Swaisgood, H. E. *J. Food Biochem.* **2002**, *26*, 119.
(234) Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, *100*, 2091.
(235) De Luca, L.; Giacomelli, G.; Porcheddu, A.; Salaris, M.; Taddei, M. *J. Comb. Chem.* **2003**, *5*, 465.
(236) Englebretsen, D. R.; Harding, D. R. K. *Int. J. Pept. Prot. Res.* **1992**, *40*, 487.
(237) Englebretsen, D. R.; Harding, D. R. K. *Int. J. Pept. Prot. Res.* **1994**, *43*, 546.
(238) Balaxi, M.; Nikolakakis, I.; Malamataris, S. *J. Pharm. Sci.* **2010**, *99*, 2104.
(239) Wolf, B.; Finke, I. *Pharmazie* **1992**, *47*, 35.
(240) Wolf, B. *Int. J. Pharm.* **1997**, *156*, 97.
(241) Gómez-Carracedo, A.; Souto, C.; Martínez-Pacheco, R.; Concheiro, A.; Gómez-Amoza, J. L. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 675.
(242) Wolf, B. *Drug Dev. Ind. Pharm.* **1998**, *24*, 1007.
(243) Linko, Y.-Y.; Viskari, R.; Pohjola, L.; Linko, P. *J. Solid Phase Biochem.* **1977**, *2*, 203.
(244) Kuga, S. *J. Chromatogr., A* **1980**, *195*, 221.
(245) Wagenknecht, W.; Fanter, C.; Loth, F. EP Patent,750,007 A1, 1996.
(246) Ettenauer, M.; Loth, F.; Thümmler, K.; Fischer, S.; Weber, V.; Falkenhagen, D. *Cellulose* **2011**, *18*, 1257.
(247) Edlund, O. H.; Andreassen, B. A. Patent DE 2,138,905 A1, 1972.
(248) Edlund, O. H.; Andreassen, B. A. U.S. Patent 3,731,816, 1973.
(249) Shibusawa, Y.; Suzuki, K.; Kinoshita, H.; Matsumoto, U. *J. Chromatogr., B* **1995**, *666*, 233.
(250) De Luca, L.; Giacomelli, G.; Porcheddu, A.; Salaris, M.; Taddei, M. *C. R. Chim.* **2003**, *6*, 607.
(251) Dickensheets, P. A.; Chen, L. F.; Tsao, G. T. *Biotechnol. Bioeng.* **1977**, *19*, 365.
(252) Sato, H.; Kidaka, T.; Hori, M. *Appl. Biochem. Biotechnol.* **1987**, *15*, 145.