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Application of Riboflavin Photochemical Properties in Hydrogel Synthesis

Gabriela Ionita and Iulia Matei

Abstract

Riboflavin, known as vitamin B2, belongs to the class of water-soluble vitamins with redox, fluorescence, and photosensitizing properties. Riboflavin contains a fragment of 7,8-dimethyl-10-(1'-D-ribityl) isoalloxazine with a system of conjugated double bonds that is responsible for its photochemical properties. In the presence of light and oxygen, riboflavin generates reactive oxygen species that can be further involved in the oxidation of biological molecules such as amino acids, proteins, nucleotides, and lipids. The chapter focuses on the photochemical application of riboflavin in (1) cross-linking of structural proteins such as collagen and (2) synthesis of hydrogels. The involvement of riboflavin in such processes has already found application in medicine, especially in the treatment of ophthalmic diseases and in tissue engineering.

Keywords: riboflavin, hydrogel, cross-linking, extracellular matrix, polysaccharides

1. Introduction

Riboflavin (**Figure 1**), known as vitamin B2, has been intensively investigated since it was discovered and separated, initially from cow milk by Blyth and, almost 50 years later, from brewers' yeast. Riboflavin belongs to the flavin class and its structure (7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4-dione) has been proven by chemical synthesis by Kuhn and Karrer. It consists of a ribityl side chain attached to an isoalloxazine ring with a system of conjugated double bonds that is responsible for the photochemical properties of riboflavin [1–4].

As a vitamin, riboflavin fulfills a number of essential biological functions, being involved in redox and photoreactions with nucleic acids, in the destruction of tumor cells and inactivation of viruses or bacteria [5, 6]. Riboflavin is a structural component of two flavoproteins with coenzyme role, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (**Figure 1**). These take part in various and complex enzymatic processes such as the synthesis, conversion, and recycling of vitamins B1, B6, and folate and the synthesis of heme proteins, in fatty acid metabolism and in the regulation of thyroid hormones [7–9]. Riboflavin is thus involved in the citric acid and methionine cycles. Moreover, riboflavin and its metabolites have antioxidant properties and participate in the glutathione redox cycle [7, 10].

Figure 1.

Structures of riboflavin and its derivatives flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Obviously, the involvement of riboflavin in biochemical processes is due to the flavin core. In aqueous solution at pH 7, the UV-Vis absorption spectrum of riboflavin is characterized by the presence of four bands with maxima at 223, 267, 373, and 444 nm [11, 12]. All bands have high molar extinction coefficients ($> 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) that are indicative of π - π^* electronic transitions. The band positions and molar extinction coefficients are sensitive to the particularities of the environment sensed by the flavin chromophore. In water at pH 7, riboflavin emits yellow green fluorescence at 520 nm [13]. The fluorescence quantum yield and lifetime of the first excited singlet state S_1 are 0.26 [14] and 5 ns [15, 16], respectively. These values are influenced by the polarity and protic/aprotic character of the solvent, as well as by the pH value. The decrease in solvent polarity determines a hypsochromic shift of the emission band and an increase in the fluorescence quantum yield [17, 18], and this has been exploited to study different chemical systems formed with cyclodextrins and serum albumins [19–21]. Although neutral riboflavin presents strong fluorescence, the protonated species forming at pH < 4 are nonfluorescent, while the anionic species at pH > 9.7 are weakly fluorescent [22]. Flavoenzymes containing FMN as prosthetic group have similar emission intensity with riboflavin, while those containing FAD show a drastic drop in emission caused by the presence of adenosine [23].

Riboflavin undergoes various reactions under thermal or chemical treatment and under exposure to UV light [11]. Under irradiation, riboflavin can undergo photolysis to various degradation products and can produce reactive oxygen species that may further alter the structure and biological activity of proteins, lipids, and, to a lesser extent, carbohydrates [24]. Significant for this review are the processes that can be controlled by the reactive radical species formed in a system in the presence of riboflavin and UV light.

Photophysical and photochemical studies involving riboflavin are important for understanding the biological processes taking place in the presence of light and mediated by flavin derivatives and their further applications. In the presence of light, riboflavin is excited to a singlet state of higher energy, followed by nonradiative intersystem crossing to an excited triplet state. From this state, riboflavin can be involved in a photosensitized oxidation process following one of two mechanisms schematically presented in **Figure 2**. In type I mechanism, riboflavin transfers the energy to a substrate and generates riboflavin free radical species that further

Figure 2.
 Scheme of the photosensitized oxidation mechanisms mediated by riboflavin.

interact with molecular oxygen in the ground state to yield oxidation products. This mechanism occurs preferentially at low oxygen concentration. In type II mechanism, riboflavin transfers the energy to molecular oxygen in the ground state to generate the more reactive singlet molecular oxygen. The latter, in reaction with a substrate, leads to the final oxidation products [25].

The property of riboflavin to be involved in biological redox processes is currently exploited for the photodegradation of water pollutants including pesticides, herbicides, phenol, toluene, and toluene derivatives [26, 27]. Another research field that is expanding in what concerns the application of the photochemical properties of riboflavin regards the cross-linking process of the components of the extracellular matrix (ECM). Photoinduced collagen cross-linking is nowadays a method applied for the treatment of various diseases that involve components of the ECM, such as varicose veins [28], degraded dentine [29–31], and a series of ophthalmic diseases (keratoconus, pellucid marginal corneal degeneration, post-LASIK ectasia, infectious keratitis, bullous keratopathy) [32–35].

2. Collagen cross-linking in the presence of riboflavin and UVA light

The photochemical properties of riboflavin in the presence of UVA light are nowadays applied in the treatment of some ocular diseases, including keratoconus, in order to induce cross-linking of collagen that improves the biomechanical stability of corneal stroma and increases the resistance to enzymatic digestion [32–34]. The mechanism of corneal collagen cross-linking involves excitation of riboflavin to the triplet state, followed by generation of reactive oxygen species— $\text{HO}^\bullet$, $\text{O}_2^{\bullet -}$, and $\text{O}_2^{\bullet -}$ in the case of type I mechanism—or production of singlet oxygen ($^1\text{O}_2$) that can further generate peroxide and hydroxyl radicals, in the case of type II

mechanism [12, 36, 37]. The schematic representation of this process is shown in **Figure 3**. In fact, both mechanisms depicted in **Figure 2** lead to the formation of reactive oxygen species. Formation of such reactive species favors reactions with carbonyl groups from the protein chain, which further increase the protein cross-linking. The production of reactive radical species can be evidenced by electron paramagnetic resonance (EPR) spectroscopy, in spin trapping experiments.

There are some studies reported in literature regarding the radical species formed in systems that contain collagen in the presence of riboflavin and UV light. In water solution of riboflavin, an environment exposed to oxygen, the formation of hydroxyl radicals was evidenced under the influence of UV radiation. This radical was also present in a solution of collagen and riboflavin [38]. Later, another EPR study was reported, aiming to monitor the formation of radical species generated in the presence of riboflavin in solutions of H_2O_2 and catalase or bovine serum albumin [39]. It was found that hydroxyl radicals are formed both in the presence of catalase and BSA, as the DMPO-OH adducts gave the characteristic signal with four lines in the intensity ratio of 1:2:2:1. The stable radical species such as TEMPO (2,2,6,6-tetramethylpiperidine 1-oxyls) derivatives can report on the changes in the tear protein compositions during the treatment of dry eye syndrome that is often associated with keratoconus disease [40]. Obviously, during the UVA-riboflavin collagen cross-linking treatment, the tears collected from patients contain reactive radical species. In a preliminary EPR study aiming to evidence radical species formed in solutions containing proteins that are present in a significant amount in tears, the spin trapping experiments evidenced the presence of the DMPO-OH adduct (the lines are marked with red dots in **Figure 4**) but also a carbon-centered radical. This second component of the spectrum (the corresponding lines are marked with blue dots in **Figure 4**) demonstrates that some other radicals can form during riboflavin/UVA treatment.

The EPR studies clearly demonstrate a radical mechanism of the processes occurring in the ECM protein components in the presence of riboflavin, mainly referring to collagen cross-linking. Changes in the collagen structure of human sclera tissues induced by riboflavin-UVA irradiation were evidenced by Raman spectroscopy and atomic force microscopy (AFM) [41]. Raman spectroscopy revealed changes in the position of some bands associated with specific vibration modes: (CCO), (CCC) stretch, and (S-S) stretch. Another feature observed in the Raman spectra of human sclera tissue after riboflavin/UVA irradiation treatment was the increase in the intensity and sharpness of the bands. After the sclera collagen cross-linking occurred, the AFM images revealed arrangements of interlocked

Figure 3.
Schematic representation of the collagen cross-linking process in the presence of riboflavin and UVA light.

Figure 4.
 DMPO adducts of $\text{HO}^\bullet$ (red) and carbon-centered (blue) radicals formed in collagen solution exposed to UVA light in the presence of riboflavin.

collagen fibrils. Both Raman spectra and AFM images proved the formation of interfibrillar cross-links in sclera tissues.

The ECM is responsible for the enhancement of cell proliferation, differentiation, and cell-to-cell interaction. Therefore, the information obtained on the structure and properties of collagen in natural tissues can be used as reference for building new networks that can mimic the ECM and can find application in tissue engineering, with implantology as target. Many studies revealed that the self-assembled collagen artificial networks are mechanically weaker compared to natural collagen in vivo [42]. However, some studies reported that riboflavin cross-linking improves the physicochemical properties of collagen scaffolds [43]. The collagen matrix built in vivo under the action of riboflavin and UV light has properties similar to those of natural collagen and is able to retain water for a longer time.

The collagen fibril formation is favored by the presence of glycosaminoglycans such as chondroitin sulfate and hyaluronic acid, both of them being parents of the natural ECM with a common function to control tissue hydration and to act as lubricants. Therefore, it is not surprising that these polysaccharide polymers are used to generate various hydrogels that can be used in medicinal applications.

3. Synthesis of hydrogels with medicinal applications mediated by riboflavin

Presently, the photochemistry of riboflavin can be exploited to control the physicochemical properties of new gel materials generated by the assembly of polymers that are similar to those found in the natural ECM. These novel materials are dedicated to medicinal applications such as tissue engineering involving space-filling agents, as vehicles for bioactive molecule delivery, for cell encapsulation, and wound healing.

Hydrogels can be classified according to the processes involved in the generation of their solid network. Supramolecular hydrogels result either through non-covalent assembly of most often small molecular weight gelators, while polymeric hydrogels are obtained through covalent polymer cross-linking. Supramolecular gels are more dynamic and sensitive to physical factors that generate their network, while

polymeric structures have a more robust frame. The biomedical applications of hydrogels depend on their biocompatibility and physicochemical properties. For instance, it is expected for supramolecular hydrogels to find applications in drug delivery, while polymeric hydrogels can be used in tissue engineering. Synthetic polymers like poly(ethylene oxide) (PEO), poly(vinyl alcohol) (PVA), or poly(acrylic acid) (PAA) can be used in this specific biochemical application, although the naturally derived polymers present in the ECM—hyaluronic acid, chondroitin sulfate, collagen, and gelatin, are more suitable because the resulting materials show a better mimicking of the natural tissues [44].

The number of reports on the supramolecular gels resulted by assembly of low-molecular gelators involving riboflavin is relatively small. Formation of this type of gels often involves the supramolecular assembly of two gelators [45]. Saha et al. [46, 47] reported data on hydrogels that have riboflavin as gelator. They reported in a communication the assembly of riboflavin with melanin into a gel network [46], and the mechanistic aspects of this supramolecular process have been discussed later in a full paper [47]. Both molecules can be involved in multiple hydrogen bonds and π - π interactions of their aromatic moieties, which are in fact the pillars of the gel network formation. The ribityl chain of riboflavin ensures the water solubility of the riboflavin-melanin complex due to the hydroxyl groups present in the structure [46]. Polar solvents like water and hydrogen bond formation decrease the photophysical parameters of riboflavin [17, 18, 48]. Surprisingly, Saha et al. found that in the temperature interval corresponding to the gel phase of riboflavin/melanin, the photoluminescence spectra show increased intensity [46]. Electron microscopy techniques revealed that the gel fibrils of these gel systems have a helical pattern, which sustains the initial hypothesis of π - π stacking interactions. These results were corroborated with FT-IR, circular dichroism, and photoluminescence data [47]. Other hydrogels built on riboflavin and aromatic structures with functional groups that can be involved in hydrogen bonding, such as uric acid [49], gallic acid [50], quinazolinone derivatives [51], salicylic acid, dihydroxy benzoic acid, and acetoguanamine [52], have been reported. These studies regarding different pairs of gelators revealed that the gel properties depend on the complementarity between gelators.

The studies on polymeric hydrogels that contain riboflavin or are generated making use of the photochemical properties of riboflavin are more numerous. The ability of riboflavin to generate peroxide radicals in the presence of UV light and oxygen represents a nontoxic way to initiate acrylamide- or methacrylate-based hydrogel networks [53, 54]. Batchelor et al. [55] have reported the formation of a hydrogel through the reaction of multi-arm polyethylene glycol, functionalized with norbornene moieties, with dithiothreitol (DTT) as cross-linker, in the presence of riboflavin excited by blue light. In this way, they have generated the thiyl radical that reacts rapidly with the double bond from norbornene to generate a carbon-centered radical. Following the propagation scheme, this carbon-centered radical reacts with another thiol group. The gelation process is dependent on the initiator concentration and light intensity [55].

The spectral properties of riboflavin depend on the pH, and this feature has been used by Patra et al. to monitor the release of two drugs, ciprofloxacin and metronidazole, from a biocompatible hydrogel obtained by cross-linking poly(methacrylic acid) with glycogen that was further labeled with riboflavin. The resulted hydrogel was characterized by physicochemical methods that indicated a reversible swelling behavior and pH-dependent drug release properties [56].

Wank et al. [57] investigated the recognition interactions in an interpenetrating polymer network based on poly(acrylic acid) and poly(vinyl alcohol). This hydrogel has been imprinted with 1-(4-methoxyphenyl)-5-methyl-1,2,3-triazol-4-carboxylic

acid (MMTCA), and its adsorption properties have been determined. The ability of this gel network to load MMTCA has been compared with those to uptake of aspirin and riboflavin. Competitive loading from mixed solutions has shown that MMTCA is selectively uptake relative to aspirin and riboflavin. The results have been explained by analyzing different structural factors including the ability of hydroxyl and carboxyl groups to form hydrogen bonds with the gel network, as well as stereo-shape aspects of the uploaded compounds.

Glycol chitosan functionalized with methacrylate groups can generate a hydrogel network in the presence of riboflavin under exposure to visible blue light [58, 59]. The gelation can occur in situ; therefore, this system can be considered for applications in tissue engineering. The chitosan hydrogel can incorporate chondroitin and type II collagen—two components of the cartilaginous ECM that can themselves be used as scaffolds for tissue engineering. In this way, the instability and rapid enzymatic degradation of these ECM components are reduced. This chitosan-based hydrogel supports the growth of chondrocytes [58], and the incorporation of collagen and chondroitin sulfate increases chondrogenesis and favors cellular condensation [59]. The chitosan hydrogel described is stable if incubated at body temperature for 42 days in the absence of lysozyme but is degraded in the presence of lysozyme at concentrations that are found in human cartilage (up to 3 mg/g) [59]. Chondroitin and collagen II are retained into the hydrogel during the incubation time, although initially a small quantity has been released. All these results recommend this chitosan derivative hydrogel as a biomaterial that can find biomedical applications in cartilage regeneration.

Functionalization of natural polysaccharides with high occurrence in different tissues can generate biomaterials that have a stronger adhesion to body tissues. In this sense, Donnelly et al. [60] have reported the photo-hydrogelation of tyramine-derivatized hyaluronate by UV exposure in the presence of various concentrations of riboflavin. The network of hydrogel is the result of cross-linking processes following the reactions depicted in **Figure 5**. Functionalization of hyaluronate with aromatic residues of tyramine allows a hydrophobic interaction with the tyrosine residues of collagen from ECM. Although the material is not suitable for cell encapsulation, it can be used for various other applications like filling small fissure

Figure 5.
 Schematic representation of photo-cross-linking of TA-polymer in the presence of riboflavin.

defects, ensuring an interface between osteochondral grafts and host tissues in order to prevent edema, or acting as glue between the graft and the tissue.

The chitosan/riboflavin pair can be used in filling demineralized dentin substrates, due to the ability of riboflavin to generate, under UV light, radicals that initiate the cross-linking of collagen fibrils from dentin and chitosan. Fawzy et al. [61] have studied in detail the changes in dentin morphology by treating demineralized dentin with chitosan solutions in various concentrations, in the presence of riboflavin, over a period of 6 months. Their results showed improved mechanical properties of dentin and an enhanced stability of the collagen network.

Hyaluronic acid, as biocompatible polymer, is often used in drug formulations. Derivatives of hyaluronic acid such as methacrylate hyaluronic acid and thiolated hyaluronic acid are also biocompatible. In the presence of riboflavin and blue light (458 nm), slow gelation occurs [62], a process that has a different kinetic compared to the system described in Ref. 45. The induced gelation in a solution of these two hyaluronic derivatives occurs in 15 min at body temperature, and this delay in reaching the gel state can be exploited for in situ applications in ophthalmology and stomatology. The systems were characterized by rheological methods and FT-IR, and the ability to encapsulate and release bovine serum albumin and their cyto-compatibility were tested. Interestingly, in the absence of thiolated hyaluronic acid, the gelation process does not occur, while in the absence of riboflavin and light, the transition from sol to gel occurs even slower (in 24 h), and the resulting gel is weaker (the gel moduli have lower values). The gelation process is the result of two reactions: the oxidation of thiol groups leading to the formation of disulfide bonds (evidenced by IR spectroscopy) and the addition of the thiol group to the double bond of the methacrylate group.

A similar gel was obtained by reaction of methacrylated dextran with L-arginine in the presence of riboflavin, L-arginine initiating the photo-cross-linking [63]. The authors found that the riboflavin concentration influences the rate of gelation, an optimum value being in the range 0.2–1.0 wt%. At low riboflavin concentration, the resulting materials are brittle, while at higher concentration, they are compliant. The pH also affects the gelation process, a neutral pH being more favorable. The presence of arginine was essential as cross-linker, since gelation did not occur in its absence. The concentration of arginine was found to influence the properties of the gel. Thus, the weight ratio of arginine/methacrylate precursor in the range 0.8–1.2 led to gels with desirable properties in terms of shape stability, stickiness, and compliance. Microscopic images (SEM) indicated the honeycomb structure of the hydrogel in the swollen state.

4. Conclusions

In conclusion, in this review, we presented the general pathways for the generation of reactive species in the presence of riboflavin and UV-Vis light. The use of riboflavin as photoinitiator for polymerization processes presents the advantage of it being nontoxic and biocompatible. The research papers mentioned in this review also highlight the potential medicinal applications of gels formed in the presence of riboflavin.

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Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Northrop-Clewes CA, Thurnham DI. The discovery and characterization of riboflavin. *Annals of Nutrition and Metabolism*. 2012;**61**:224-230. DOI: 10.1159/000343111
- [2] Bacher A, Eberhardt S, Eisenreich W, Fischer M, Herz S, Illarionov B, et al. Biosynthesis of riboflavin. *Vitamins and Hormones*. 2001;**61**:1-49. DOI: 10.1016/S0083-6729(01)61001-X
- [3] Revuelta JL, Ledesma-Amaro R, Lozano-Martinez P, Díaz-Fernández D, Buey RM, Jiménez A. Bioproduction of riboflavin: A bright yellow history. *Journal of Industrial Microbiology and Biotechnology*. 2017;**44**(4-5):659-665. DOI: 10.1007/s10295-016-1842-7
- [4] Schwechheimer SK, Park EY, Revuelta JL, Becker J, Wittmann C. Biotechnology of riboflavin. *Applied Microbiology and Biotechnology*. 2016;**100**(5):2107-2119. DOI: 10.1007/s00253-015-7256-z
- [5] Reddy HL, Dayan AD, Cavagnaro J, Gad S, Li J, Goodrich RP. Toxicity testing of a novel riboflavin-based technology for pathogen reduction and white blood cell inactivation. *Transfusion Medicine Reviews*. 2008;**22**:133-153. DOI: 10.1016/j.tmr.2007.12.003
- [6] Insińska-Rak M, Sikorski M. Riboflavin interactions with oxygen—A survey from the photochemical perspective. *Chemistry—A European Journal*. 2014;**20**:15280-15291. DOI: 10.1002/chem.201403895
- [7] Kennedy DO. B Vitamins and the brain: Mechanisms, dose and efficacy—A review. *Nutrients*. 2016;**8**:68-96. DOI: 10.3390/nu8020068
- [8] Edwards AM. General properties of flavins. In: Silva E, Edwards AM, editors. *Flavins Photochemistry and Photobiology*. Cambridge: The Royal Society of Chemistry; 2006. pp. 1-11. DOI: 10.1039/9781847555397-00001
- [9] Massey V. Chemical and biological versatility of riboflavin. *Biochemical Society Transactions*. 2000;**28**:283-296. DOI: 10.1042/bst0280283
- [10] Ashoori M, Saedisomeolia A. Riboflavin (vitamin B2) and oxidative stress: A review. *The British Journal of Nutrition*. 2014;**111**:1985-1991. DOI: 10.1017/S0007114514000178
- [11] Sheraz MA, Hafeez Kazi S, Ahmed S, Anwar Z, Ahmad I. Photo, thermal and chemical degradation of riboflavin. *Beilstein Journal of Organic Chemistry*. 2014;**10**:1999-2012. DOI: 10.3762/bjoc.10.208
- [12] Beztsinna N, Sole M, Taib N, Bestel I. Bioengineered riboflavin in nanotechnology. *Biomaterials*. 2016;**80**:121-133. DOI: 10.1016/j.biomaterials.2015.11.050
- [13] Weber G. Fluorescence of riboflavin and flavin adenine dinucleotide. *The Biochemical Journal*. 1950;**47**:114-121. DOI: 10.1042/bj0470114
- [14] Koziol J. Studies on flavins in organic solvents-I. Spectral characteristics of riboflavin, riboflavin tetrabutylate and lumichrome. *Photochemistry and Photobiology*. 1966;**5**:41-54. DOI: 10.1111/j.1751-1097.1966.tb05759.x
- [15] Sikorska E, Khmelinskii IV, Komasa A, Koput J, Ferreira LFV, Herance JR, et al. Spectroscopy and photophysics of flavin related compounds: Riboflavin and iso-(6,7)-riboflavin. *Chemical Physics*. 2005;**314**:239-247. DOI: 10.1016/j.chemphys.2005.03.005

- [16] Wahl P, Auchet JC, Visser AJWG, Müller F. Time resolved fluorescence of flavin adenine dinucleotide. *FEBS Letters*. 1974;**44**:67-70. DOI: 10.1016/0014-5793(74)80307-8
- [17] Grodowski MS, Veyret B, Weiss K. Photochemistry of flavins-II. Photophysical properties of alloxazines and isoalloxazines. *Photochemistry and Photobiology*. 1977;**26**:341-352. DOI: 10.1111/j.1751-1097.1977.tb07495.x
- [18] Naman SA, Tegnér L. Decay kinetics of the triplet excited state of lumiflavin. *Photochemistry and Photobiology*. 1986;**43**:331-333. DOI: 10.1111/j.1751-1097.1986.tb05612.x
- [19] Morrison PW, Connon CJ, Khutoryanskiy VV. Cyclodextrin-mediated enhancement of riboflavin solubility and corneal permeability. *Molecular Pharmaceutics*. 2013;**10**:756-762. DOI: 10.1021/mp3005963
- [20] Neacsu MV, Ionita G, Topala C, Oprea E, Tecuceanu V, Matei I. Poly(ethylene glycol)/ β -cyclodextrin covalent gel networks: Host matrices for studying radical processes in plant extract-riboflavin systems following UV irradiation. *Chemical Papers*. 2017;**71**:607-616. DOI: 10.1007/s11696-016-0047-x
- [21] Voicescu M, Angelescu DG, Ionescu S, Teodorescu VS. Spectroscopic analysis of the riboflavin - Serum albumins interaction on silver nanoparticles. *Journal of Nanoparticle Research*. 2013;**15**:1555. DOI: 10.1007/s11051-013-1555-z
- [22] Drossler P, Holzer W, Penzkofer A, Hegemann P. pH dependence of the absorption and emission behaviour of riboflavin in aqueous solution. *Chemical Physics*. 2002;**282**:429-439. DOI: 10.1016/S0301-0104(02)00731-0
- [23] Heelis PF. The photophysical and photochemical properties of flavins (isoalloxazines). *Chemical Society Reviews*. 1982;**11**:15-39. DOI: 10.1039/CS9821100015
- [24] Ahmad I, Vaid FHM. Photochemistry of flavins in aqueous and organic solvents. In: Silva E, Edwards AM, editors. *Flavins Photochemistry and Photobiology*. Cambridge: The Royal Society of Chemistry; 2016. Chapter 2. DOI: 10.1039/9781847555397-00013
- [25] Choe E, Huang R, Min DB. Chemical reactions and stability of riboflavin in foods. *Journal of Food Science*. 2005;**70**:R28-R36. DOI: 10.1111/j.1365-2621.2005.tb09055.x
- [26] Cui H, Hwang HM, Zeng K, Glover H, Yu HT, Liu YM. Riboflavin-photosensitized degradation of atrazine in a freshwater environment. *Chemosphere*. 2002;**47**:991-999. DOI: 10.1016/S0045-6535(02)00006-1
- [27] Haggi E, Bertolotti S, Miskoski S, Amat-Guerri F, Garcia N. Environmental photodegradation of pyrimidine fungicides. Kinetics of the visible-light-promoted interactions between riboflavin and 2-amino-4-hydroxy-6-methylpyrimidine. *Canadian Journal of Chemistry*. 2002;**80**:62-67. DOI: 10.1139/v01-192
- [28] Frullini A, Manetti L, Di Cicco E, Fortuna D. Photoinduced collagen cross-linking: A new approach to venous insufficiency. *Dermatologic Surgery*. 2011;**37**:1113-1118. DOI: 10.1111/j.1524-4725.2011.02047.x
- [29] Cai J, Palamara JEA, Burrow MF. Effects of collagen crosslinkers on dentine: A literature review. *Calcified Tissue International*. 2018;**102**:265-279. DOI: 10.1007/s00223-017-0343-7
- [30] Fawzy AS, Nitisusanta LI, Iqbal K, Daood U, Neo J. Riboflavin as a dentin

crosslinking agent: Ultraviolet A versus blue light. *Dental Materials*. 2012;**28**:1284-1291. DOI: 10.1016/j.dental.2012.09.009

[31] Liu XQ, Zhou JF, Chen L, Yang Y, Tan JG. UVA-activated riboflavin improves the strength of human dentin. *Journal of Oral Science*. 2015;**57**:229-234. DOI: 10.2334/josnusd.57.229

[32] Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. *Experimental Eye Research*. 1998;**66**:97-103. DOI: 10.1006/exer.1997.0410

[33] Wollensak G, Iomdina E, Dittert DD, Salamatina O, Stoltenburg G. Cross-linking of scleral collagen in the rabbit using riboflavin and UVA. *Acta Ophthalmologica Scandinavica*. 2005;**83**:477-482. DOI: 10.1111/j.1600-0420.2005.00447.x

[34] Spoerl E, Wollensak G, Seiler T. Increased resistance of crosslinked cornea against enzymatic digestion. *Current Eye Research*. 2004;**29**:35-40. DOI: 10.1080/02713680490513182

[35] Hsu KM, Sugar J. Keratoconus and other corneal diseases: Pharmacologic cross-linking and future therapy. *Pharmacologic Therapy of Ocular Disease*. 2017;**242**:137-161. DOI: 10.1007/164_2016_23

[36] Silva E, Jopia M, Edwards AM, Lemp E, Lissi E. Protective effect of Boldo and tea infusions on the visible light-mediated pro-oxidant effects of vitamin B2, riboflavin. *Photochemistry and Photobiology*. 2002;**75**:585-590. DOI: 10.1562/0031-8655(2002)075<0585:PEOBAT>2.0.CO;2

[37] Besaratinia A, Kim SI, Bates SE, Pfeifer GP. Riboflavin activated by ultraviolet A1 irradiation induces oxidative DNA damage-mediated

mutations inhibited by vitamin C. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**:5953-5958. DOI: 10.1073/pnas.0610534104

[38] Constantin MM, Corbu C, Ionita G. EPR study on the role of riboflavin used in photo-oxidative collagen cross-linking. *Revista de Chimie*. 2010;**61**:495-497

[39] Sel S, Nass N, Pötzsch S, Trau S, Simm A, Kalinski T, et al. UVA irradiation of riboflavin generates oxygen-dependent hydroxyl radicals. *Redox Report*. 2014;**19**:72-79. DOI: 10.1179/1351000213Y.0000000076

[40] Constantin MM, Corbu CG, Tanase C, Codrici E, Mihai S, Popescu ID, et al. Spin probe method of electron paramagnetic resonance spectroscopy – a qualitative test for measuring the evolution of dry eye syndrome under treatment. *Analytical Methods*. 2019;**11**:965-972. DOI: 10.1039/C8AY02783H

[41] Jung GB, Lee HJ, Kim JH, Lim JI, Choi S, Jin KH, et al. Effect of cross-linking with riboflavin and ultraviolet A on the chemical bonds and ultrastructure of human sclera. *Journal of Biomedical Optics*. 2011;**16**:125004. DOI: 10.1117/1.3662458

[42] Rich H, Odlyha M, Cheema U, Mudera V, Bozec L. Effects of photochemical riboflavin-mediated crosslinks on the physical properties of collagen constructs and fibrils. *Journal of Materials Science. Materials in Medicine*. 2014;**25**:11-21. DOI: 10.1007/s10856-013-5038-7

[43] Chan BP, So KF. Photochemical crosslinking improves the physicochemical properties of collagen scaffolds. *Journal of Biomedical Materials Research. Part A*. 2005;**75**:689-701. DOI: 10.1002/jbm.a.30469

- [44] Drury JL, Mooney DJ. Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials*. 2003;**24**:4337-4351. DOI: 10.1016/S0142-9612(03)00340-5
- [45] Hirst AR, Smith DK. Two-component gel-phase materials—highly tunable self-assembling systems. *Chemistry--A European Journal*. 2005;**11**:5496-5508. DOI: 10.1002/chem.200500241
- [46] Manna S, Saha A, Nandi AK. A two component thermoreversible hydrogel of riboflavin and melamine: Enhancement of photoluminescence in the gel form. *Chemical Communications*. 2006;**41**:4285-4287. DOI: 10.1039/b608234c
- [47] Saha A, Manna S, Nandi AK. A mechanistic approach on the self-organization of the two-component thermoreversible hydrogel of riboflavin and melamine. *Langmuir*. 2007;**23**:13126-13135. DOI: 10.1021/la7026219
- [48] Penzer GR, Radda GK. The chemistry and biological function of isoalloxazines (flavines). *Quarterly Reviews, Chemical Society*. 1967;**21**:43-65. DOI: 10.1039/QR9672100043
- [49] Anderson KM, Day GM, Paterson MJ, Byrne P, Clarke N, Steed JW. Structure calculation of an elastic hydrogel from sonication of rigid small molecule components. *Angewandte Chemie, International Edition*. 2008;**47**:1058-1062. DOI: 10.1002/anie.200703785
- [50] Saha A, Roy B, Garai A, Nandi AK. Two-component thermoreversible hydrogels of melamine and gallic acid. *Langmuir*. 2009;**25**:8457-8461. DOI: 10.1021/la900156w
- [51] Roy B, Saha A, Esterrani A, Nandi AK. Time sensitive, temperature and pH responsive photoluminescence behaviour of a melamine containing bicomponent hydrogel. *Soft Matter*. 2010;**6**:3337-3345. DOI: 10.1039/C0SM00036A
- [52] Saha A, Roy B, Esterrani A, Nandi AK. Effect of complementary small molecules on the properties of bicomponent hydrogel of riboflavin. *Organic & Biomolecular Chemistry*. 2011;**9**:770-776. DOI: 10.1039/C0OB00670J
- [53] Oster G, Yang NL. Photopolymerization of vinyl monomers. *Chemical Reviews*. 1968;**68**:125-151. DOI: 10.1021/cr60252a001
- [54] Encinas MV, Rufs AM, Bertolotti S, Previtali CM. Free radical polymerization photoinitiated by riboflavin/amines. Effect of the amine structure. *Macromolecules*. 2001;**34**:2845-2847. DOI: 10.1021/ma001649r
- [55] Batchelor RR, Kwandou G, Spicer PT, Stenzel MH. (–)-Riboflavin (vitamin B2) and flavin mononucleotide as visible light photoinitiators in the thiol–ene polymerisation of PEG-based hydrogels. *Polymer Chemistry*. 2017;**8**:980-984. DOI: 10.1039/c6py02034h
- [56] Patra P, Seesala VS, Soni SR, Roy RK, Dhara S, Ghosh A, et al. Biopolymeric pH-responsive fluorescent gel for in-vitro and in-vivo colon specific delivery of metronidazole and ciprofloxacin. *European Polymer Journal*. 2019;**114**:255-264. DOI: 10.1016/j.eurpolymj.2019.02.037
- [57] Wang B, Liu MZ, Liang R, Ding SL, Chen ZB, Chen SL, et al. MMTCA recognition by molecular imprinting in interpenetrating polymer network hydrogels based on poly(acrylic acid) and poly(vinyl alcohol). *Macromolecular Bioscience*. 2008;**8**:417-425. DOI: 10.1002/mabi.200700176

[58] Hu J, Hou Y, Park H, Choi B, Hou S, Chung A, et al. Visible light crosslinkable chitosan hydrogels for tissue engineering. *Acta Biomaterialia*. 2012;**8**:1730-1738. DOI: 10.1016/j.actbio.2012.01.029

[59] Choi B, Kim S, Lin B, Wu BM, Lee M. Cartilaginous extracellular matrix-modified chitosan hydrogels for cartilage tissue engineering. *ACS Applied Materials & Interfaces*. 2014;**6**:20110-20121. DOI: 10.1021/am505723k

[60] Donnelly PE, Chen T, Finch A, Brial C, Maher SA, Torzilli PA. Photocrosslinked tyramine-substituted hyaluronate hydrogels with tunable mechanical properties improve immediate tissue-hydrogel interfacial strength in articular cartilage. *Journal of Biomaterials Science. Polymer Edition*. 2017;**28**:582-600. DOI: 10.1080/09205063.2017.1289035

[61] Fawzy AS, Nitisusanta LI, Iqbal K, Daood U, Beng LT, Neo J. Chitosan/riboflavin-modified demineralized dentin as a potential substrate for bonding. *Journal of the Mechanical Behavior of Biomedical Materials*. 2013;**17**:278-289. DOI: 10.1016/j.jmbbm.2012.09.008

[62] Lee HJ, Fernandes-Cunha GM, Myung D. In situ-forming hyaluronic acid hydrogel through visible light-induced thiolene reaction. *Reactive and Functional Polymers*. 2018;**131**:29-35. DOI: 10.1016/j.reactfunctpolym.2018.06.010

[63] Kim SH, Chu CC. Fabrication of a biodegradable polysaccharide hydrogel with riboflavin, vitamin B2, as a photo-initiator and L-arginine as coinitiator upon UV irradiation. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2009;**91B**:390-400. DOI: 10.1002/jbm.b.31414