A Spectroscopic Investigation of Riboflavin in the Dextran and Dextrin Solvents

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ABSTRACT

Corneal collagen cross-linking (CXL) treatment has been proven to strengthen the weak corneal structure in keratoconus. This method works by increasing collagen cross-linking, which are the natural "anchors" within the cornea by means of a highly localized photo-polymerization using UVA light and a photosensitizer riboflavin (vitamin B_2)-dextran solution drops which is routinely used in the keratoconus treatment [1-3]. In this study, the usability of riboflavin – dextrin solution to achieve the CXL of collagen fibrils of the cornea has been investigated instead of riboflavin – dextran solution which is routinely used in the keratoconus treatment.

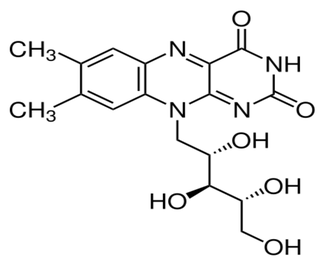
In this work, we prepared dextran- riboflavin and dextrin- riboflavin solvents in saline solutions with various concentrations of dextran and dextrin from 0.01 to 1% weight, respectively. The absorption, excitation and emission spectrum of the various concentrations of dextran and dextrin - riboflavin solutions were performed by the UVA and steady state fluorescence techniques, respectively.

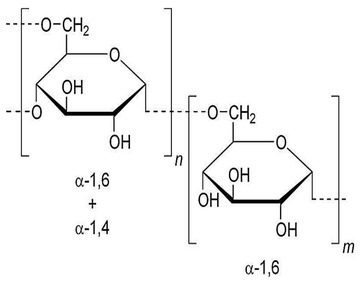
We investigated that dextrin- riboflavin solution which is more economic one can be used as an alternative to routinely used dextran- riboflavin solution in the keratoconus treatment. And also, these solutions can be developed for biological tissues of the body such as collagen fibrils which are generated from fibroblasts around tooth bud and the collagen fibrils in tendon, bone and muscle.

OBJECTIVES & METHODS

Both dextran and dextrin are polysacharite and also have apprioprite properties to exist crosslinking between collagen fibres in cornea. In routinely used keratonus treatment, UVA light is used to situmulate the crosslinking reaction to provide binding the collagen fibres in cornea [1,4]. Absorbtion, excitation and emission properties of the solutions is critical for the eye treatment due to the UVA light has detrimental effects to the eye. That's why, we investigate the spectroscopic properties of both solutions.

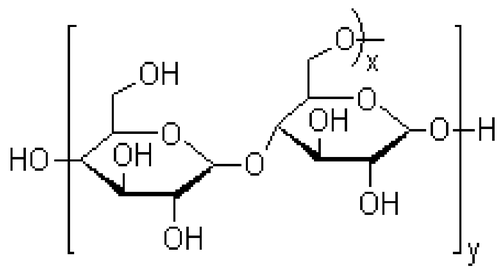
Riboflavin [2,4] ($C_{17}H_{20}N_4O_6$) is the coenzyme of flavoenzymes which play an important role as cofactors in enzymes and as chromophores in blue-light sensitive biological photoreceptors.





Dextran ($H(C_6H_{10}O_5)_xOH$) is a bacterial polysaccharide composed of (1→6) linked α-D-glucopyranosyl residues, and it has three hydroxyl groups per anhydroglucose unit. Upon light exposure, a higher degree of substitution results in higher crosslinking density which in turn decreases the degree of swelling and the water content.

Dextrins ($(C_6H_{10}O_5)_n$) are mixtures of polymers of D-glucose units linked by α-(1,4) or α-(1,6) glycosidic bonds and partially or fully water-soluble, yielding optically active solutions of low viscosity



Dextran – riboflavin solutions has prepared including 20% dextran and different concentrations of riboflavin. Dextran is solved in salin solution which includes 0,9% distiled water. Riboflavin is solved by distiled water. Dextrin – riboflavin solutions is prepared as the same way, only difference is that using dextrin instead of dextran.

Dextran – Riboflavin Solution			Dextrin – Riboflavin Solution		
		H_20			H_20
Riboflavin	0,0025 g	10 ml	**Riboflavin**	0,002 g	10 ml
NaCl	0,36 g	40 ml	**NaCl**	0,36 g	40 ml
Dextran	10 g		**Dextrin**	10 g	
Total		50 ml	**Total**		50 ml

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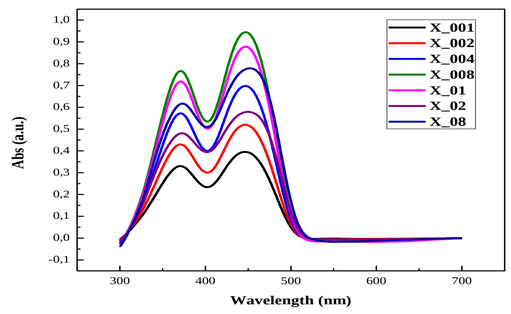
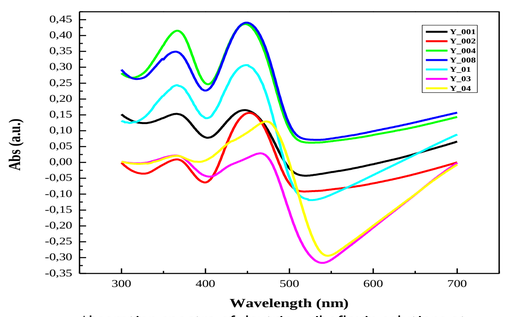
RESULTS

ABSORPTION SPECTRA

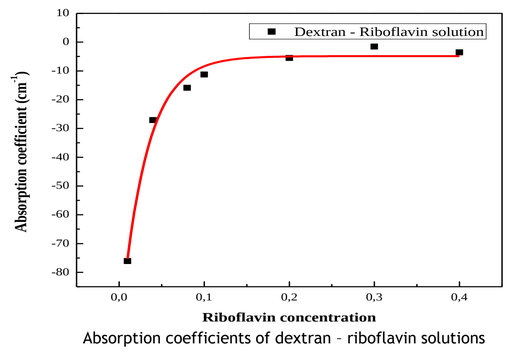
Absorption spectrums of both dextran – riboflavin solutions and dextrin – riboflavin solutions are measured using UV-Absorbance spectraphotometer. Dextran – riboflavin solutions' absorption is in the 371 nm and 450 nm. Similarly, dextrin – riboflavin solutions absorb the light at 365 nm and 450 nm; however, in higher concentrations of the solutions the peaks of the spectra are shifted to higher wavelengths.



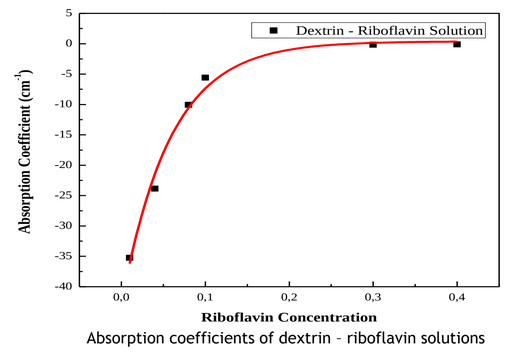
Absorption specra of dextran – riboflavin solution at different concentrations.



Absorption spectra of dextrin – riboflavin solutions at different concentrations.



Absorption coefficients of dextran – riboflavin solutions

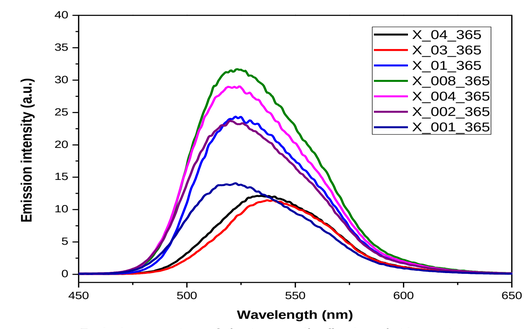


Absorption coefficients of dextrin – riboflavin solutions

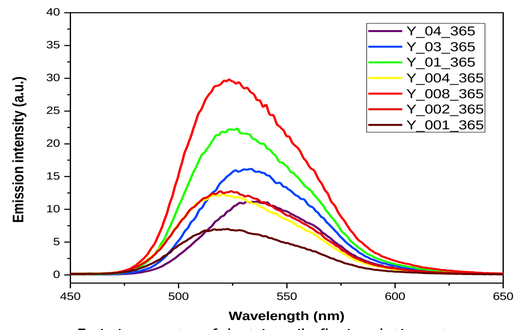
As seen in the graphes , absorption coefficients of the solutions do not change in higher concentrations. This situation is consequent for not only dextran – riboflavin solution but also dextrin – riboflavin solutions. The increment in absorption coefficient become steady after a critical concentration which is 0,1% for both dextran – riboflavin solution and dextrin – riboflavin solution.

FLUORESCENCE SPECTRA

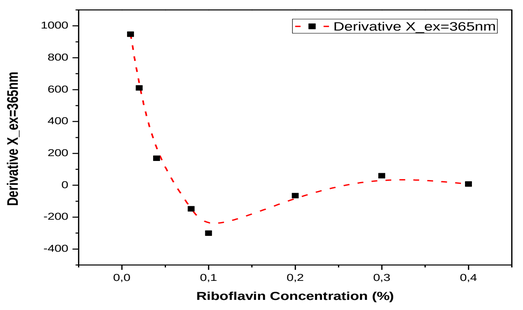
Emission spectra of the solutions are measured using fluorescence spectrophotometer. The solutions at different concentrations are excited with the light at 365 nm. Both dextran – riboflavin solutions and dextrin – riboflavin solutions have emission at 520 nm. The emission peaks shifted to the higher wavelength at higher concentrations for both solutions.



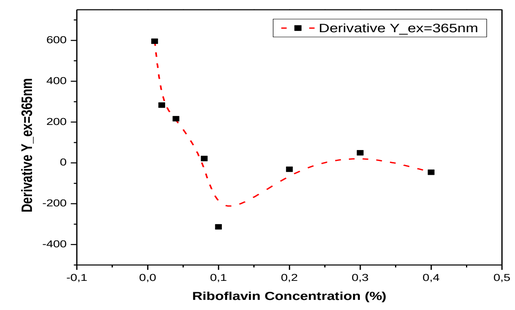
Emission spectra of dextran – riboflavin solutions at different concentrations.



Emission spectra of dextrin – riboflavin solutions at different concentrations.



Differentiation of emission pick of dextran – riboflavion solution.



Differentiation of emission pick of dextrin – riboflavion solution.

First derivative of the emission peak at each concentration give the increasingly changeable point which is the critical concentration in our case [5].

CONCLUSION

Riboflavin – Dextran solution is already used routinely in keratoconus disease treatment. In this treatment, dextran is used source to crosslinking of collagen fibrilles in cornea. Furthermore, it provides the viscosity of the solution. Dextrin seems to have the similar properties to exist crosslinking, therefore, we investigate their spectroscopic properties whether dextrin – riboflavin solution is appropriate for the crosslinking treatment of keratoconus disease or not.

In this study, we show that dextrin – riboflavin solution has similar spectroscopic properties with dextran – riboflavin solutions. Dextran – riboflavin solution has absorption at 371 nm and 450 nm and emission at 520 nm. Whereas, dextrin – riboflavin solutions has absoprtion at 365 nm and 450 nm and emission at 520 nm. Furthermore, we show that critical concentrations are both solutions are at 0,1% which is proved by both absorption measurements and fluorescence measurements.