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Effect of Ultraviolet Light on Absorption Spectra of Carbohydrates

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Kwiecinski *et al.*[1] showed that different carbohydrates after repeated recrystallisations have no specific absorption of ultraviolet light. Marchlewski and Urbánczyk [2] found, however, that hexoses and pentoses treated with alkali got an absorptionmaximum at 270 millimicrons. Starch boiled in alkali gets a band near 280 millimicrons shifting to 255 on acidifying [3].

During investigations of the effect of ultraviolet light upon the biological activity of acid mucopolysaccharides, Balazs and Laurent [4] found that the ultraviolet light absorption spectra of hyaluronic acid and heparin were changed after irradiation and a strong maximum developed at 265 millimicrons. Alkali increased the effect of irradiation.

In the present work we have tried to study the effect of ultraviolet light upon spectra of carbohydrates. That irradiation changes spectra of several other substances is well known. For references see Ellinger [5].

METHODS

For irradiation a mercury lamp with a known emission spectrum was used. Of the energy given off 50 % was localized on the wavelengths under 280 millimicrons. The solutions were irradiated in 0.1 cm thick quartz cuvettes, covered to prevent evaporation. During the irradiation the temperature in the solution rose from 20° C to 52° C in the first 20 minutes; after this time it remained constant. The increase in temperature proved to have a certain influence on the irradiation effect if compared with experiments carried out at constant temperature (20° C). The difference was, however, a relatively small one; therefore the remaining experiments were done without cooling. The adjustment of pH was effected with NaOH and HCl. The solutions were

left to stand some time at 4° C before measuring. All spectra were measured in a Beckman quartz spectrophotometer against a blank of irradiated solvent; we had found, namely, that also NaOH (used for pH-adjustment) showed increased extinction after irradiation, without any absorption maximum, however. The results are expressed as extinction E = -log transmittance.

EXPERIMENTS AND RESULTS

After irradiation of some polysaccharides (dextrin, gum arabic, hyaluronic acid, pectin, chondroitin sulphuric acid) a characteristical change of spectrum was obtained. An absorption maximum appeared at 265 millimicrons, and already a relatively short time of irradiation caused a considerable increase in extinction values. The reasons might be the following:

a. part of the great molecule is broken down and the rise of extinction is due to successively accumulated products, possibly after further action of light.
b. an intramolecular rearrangement, induced by the supplied energy, causes the spectral changes.

That ultraviolet light really causes a depolymerization is certain [4, 6]. After dialysis of an irradiated solution a great deal of the extinction increment disappears, but not all. This suggests a participation of both mentioned points.

We have investigated the spectra of glucose, galactose, mannose, fructose, glucosamine and galacturonic acid. All these substances give after irradiation rise to characteristical absorption with maximum at 265 millimicrons, like the polysaccharides. Here, too, the extinction increases quickly with prolonged irradiation time.

All our preparations had a high degree of purity (commercial pro analysi products, made by different manufacturers and in several different ways) and after computation of the possible extinction values it is obvious that the light absorption cannot be attributed to impurities. Compare, however, Kwiecinski *et al* [1].

The shape and values of the absorption curves received are in the following figures presented as a function of the time of irradiation (Fig. 1), the concentration of the irradiated substance (Fig. 2), and the pH of the solution (Fig. 3).

The influence of acidity can be shortly described in stating that

a. all investigated preparations showed higher light absorption, both irradiated and not irradiated, on rising pH.
b. the irradiation became more effective, as the alkalinity increased.

If a solution first is irradiated in alkaline milieu, and later acidified a shift in the maximum from 265 millimicrons towards 245 occurs and the E maximum

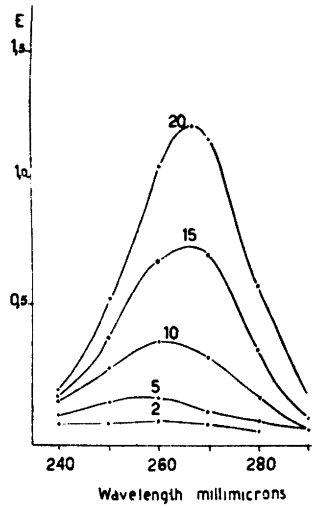

Fig. 1. 50 mg % glucose solution irradiated for 0, 2, 5, 10, 15 and 20 min. pH 9.7.

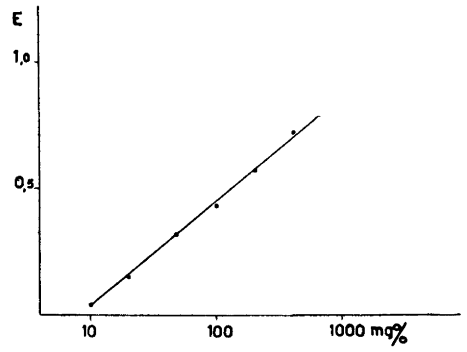

Fig. 2. Glucose in different concentrations irradiated for 10 min. pH 11. Abscissa: glucose concentration (logarithmic scale). Ordinate: Extinction at 265 millimicrons.

sinks considerably. In this connection it is interesting to note that ascorbic acid in weak alkaline solution has a maximum at 265. It shifts on acidifying to 245 [7].

The above is valid for all the monosaccharides tested by us. In addition to that the following holds good for galacturonic acid: after irradiation in

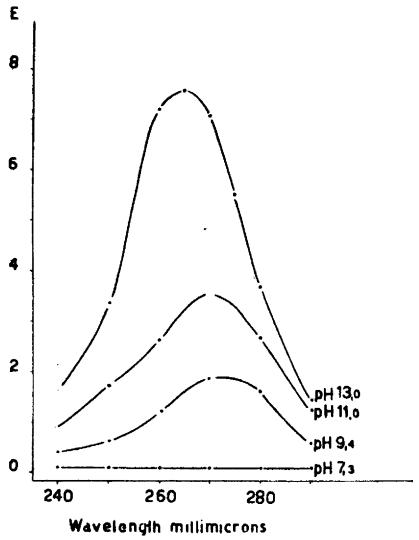

Fig. 3. 50 mg % glucose solution irradiated 30 min. pH 7.3, 9.4, 11.0 and 13.0. Absorption spectra determined without change of pH. E = value read on the photometer × dilution after irradiation.

strongly acid solution (spectrum measured also in acid solution) a maximum near 245 millimicrons. Glucosamine irradiated in acid and measured in alkaline solution gives a maximum near 295 millimicrons.

The postirradiation effect, if any was unsignificant. Experiments carried out in different saline solutions gave somewhat different results, which could be explained, in part, by the absorption of the salts themselves.

No provable CO_2 developed during irradiation.

Changes in absorption spectrum similiar to those just described could also be observed after boiling the sugar solution. Some monosaccharides were treated for 30 minutes in boiling water bath, pH 13. A great rise in extinction and a weak maximum near 265 appeared. This effect was, however, much less than after equal time of irradiation.

The changes in absorption spectra of proteins after ultraviolet irradiation have been observed by several authors. For references see McLaren [8]. Recently an attempt was made to explain those changes in chemical terms as modifications of the aromatic groups [9]. Considering, however, that a great deal of other substances are affected in the same way by ultraviolet irradiation, their interpretation must appear unsatisfactory.

SUMMARY

Ultraviolet irradiation upon alkaline solutions of carbohydrates changes their absorption spectra and forms a maximum at 265 millimicrons. Maximum shifts to 245 millimicrons on acidifying. Spectra for galacturonic acid and glucosamine are changed after irradiation in acid solutions too.

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