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Spirin A S. Spektrofotometrichesloe opredelenie summarnogo kolichestva nukleinovykh kislot (Spectrophotometric determination of total nucleic acids). *Biokhimiya (USSR)* **23**:656-62, 1958.
[A.N. Bakh Institute of Biochemistry, Academy of Sciences of the USSR, Moscow, USSR]

The paper describes a simple universal method of determining nucleic acids in biological materials based on the measurement of the difference in optical densities of the hot $HClO_4$ extract at two wavelengths, 270 mμ and 290 mμ. [The *SCI*® indicates that this paper has been cited in over 760 publications.]

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In 1955-1957 I was a postgraduate student in A.N. Belozersky's laboratory working on the content and base composition of nucleic acids of various bacteria.[1] In the course of the work, a quick and precise determination of the total nucleic acid content in biological materials was required, and, naturally, I turned my attention primarily to spectral methods[2] applied to hot $HClO_4$ extracts of the cells.[2-4] I noticed that though there were big differences in the absorbance spectra of different nucleotides, their molar extinction coefficients in an acidic medium differ relatively little at 270 mμ. So I began to employ, and also urged my colleagues working with me to do the same, the standard coefficient of molar extinction $\varepsilon_{270} = 10{,}000$ in hot $HClO_4$ extracts of nucleic acids regardless of the type (DNA or RNA) and base composition of the nucleic acids under investigation.

Subsequently, a modification had to be made to reduce the effect of extracted ultraviolet-absorbing materials of a nonnucleic acid nature; instead of a direct measurement of optical density at 270 mμ, I suggested using the difference of optical densities at two close wavelengths, 270 mμ and 290 mμ (the difference in optical densities at 270 mμ and 290 mμ divided by 0.19 gives the number of nucleic acid phosphorus micrograms per milliliter). The method proved to be very convenient and yielded precise results at testing on a large number of objects of animal and plant origin. (However, when applied to bacteria, the method in this differential variant gave some under- or overestimation in the cases of extreme GC or AT types of their DNA, respectively.)

Under strong pressure from my colleagues, I sent a two-page typewritten description of the method to *Biokhimiya* (USSR). The manuscript was rejected as unsubstantiated. This was one of the first lessons for me as a beginning scientist. Then I supplied the manuscript with formulae, equations, spectra, and so on, and this scientifically equipped paper was accepted.

The method proved to be so simple, precise, and widely applicable that it is used up to the present and is frequently cited even though it is not well known to the international scientific community, having been published only in Russian. Among Soviet biochemists, it is the most popular method for determining nucleic acids.

1. **Belozersky A N & Spirin A S.** A correlation between the compositions of deoxyribonucleic and ribonucleic acids. *Nature* **182**:111-12, 1958. (Cited 65 times.)
2. **Ogur M & Rosen G.** The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. *Arch. Biochem.* **25**:262-76, 1950. (Cited 1,095 times since 1955.)
3. **Schneider W C, Hageboom G H & Ross H E.** Intracellular distribution of enzymes. VII. The distribution of nucleic acids and adenosinetriphosphatase in normal mouse liver and mouse hepatoma. *J. Nat. Cancer Inst.* **10**:977-82, 1950. (Cited 120 times since 1955.)
4. **Ogur M, Minkler S, Lindegren G & Lindegren C C.** The nucleic acids in polyploid series of *Saccharomyces*. *Arch. Biochem. Biophys.* **40**:175-84, 1952. (Cited 140 times since 1955.)

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