# Serbian Biochemical Society



## Proceedings

**Editor:** Ivan Spasojević
**Technical support:** Jovana Trbojević-Ivić, Milena Dimitrijević, Tijana Ćulafić
**Cover design:** Zoran Beloševac
**Publisher:** Faculty of Chemistry, Serbian Biochemical Society
**Printed by:** Colorgrafx, Belgrade
**No of printed copies:** 130

# Serbian Biochemical Society
# Twelfth Conference

International scientific meeting

September 21-23, 2023, Belgrade, Serbia

"***Biochemistry in Biotechnology"***

# Simple two-step semi-preparative isolation and purification of transferrin from human serum

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Human transferrin (Tf) is a bilobal 76 kDa iron-binding glycoprotein present in human serum. Each lobe has the ability to bind one ferric ion ($Fe^{3+}$) and a single synergistic bicarbonate anion. The main role of Tf is to transport $Fe^{3+}$ ions through the circulation to cells, via interaction with transferrin receptor (TFR) on the cell surface. Previously described methods for Tf isolation and purification are either very time-consuming or provide Tf of lower final purity. Here we describe a fast and simple FPLC method for the isolation and purification of Tf from human serum. Serum samples were prepared by precipitation, while protein purification was performed on FPLC system, using an anion-exchange column. Several different buffers at the same pH were tested. Tf purified by this method was analyzed by Western blot, followed by immunodetection, as well as with silver staining after SDS PAGE. Its functionality was tested with respect to iron-binding capacity (ferozzine method) and its ability to interact with TFR by immunofluorescent staining. The conformation of purified Tf was analyzed by recording intrinsic fluorescent emmision spectra originating from Trp residues. The method itself is highly reproducible (intra- and interday), easy to perform (only two steps) and fast (under an hour), yielding 98% to 99% pure Tf with all buffers. Purified Tf was shown to have retained its iron-binding capacity, as well as the ability to interact with TFR. Purified Tf also retained its native three-dimensional structure. Described method for the isolation and purification of Tf is fast, simple and highly reproducible, yielding a functional Tf of high purity in its native state while offering the flexibility of using different buffer systems. All of these features make this protocol a method of choice for the isolation and purification of Tf on a semi-preparative scale.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2022-14/200019).

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

577.1(048)

**SERBIAN Biochemical Society. International scientific meeting (12 ; 2023 ; Beograd)**
"Biochemistry in Biotechnology" : [proceedings] / Serbian Biochemical Society, Twelfth Conference, International scientific meeting, September 21-23, 2023, Belgrade, Serbia ; [editor Ivan Spasojević]. - Belgrade : Faculty of Chemistry : Serbian Biochemical Society, 2023 (Belgrade : Colorgrafx). - 156 str. ; 23 cm

Tiraž 130. - Bibliografija uz većinu apstrakata.

ISBN 978-86-7220-140-6 (FOC)

a) Биохемија -- Апстракти

COBISS.SR-ID 124201993

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