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### QUALITATIVE THIN LAYER CHROMATOGRAPHIC IDENTIFICATION AND SEPARATION OF SOME METALPEPTIDOGLYCAN MONOMER COMPLEXES ON CELLULOSE

Lj. Sesartić<sup>a</sup>; O. Hadzija<sup>a</sup>; N. Brajenović<sup>a</sup>

<sup>a</sup> Ruder Boskovic Institute, Zagreb, Croatia

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# QUALITATIVE THIN LAYER CHROMATOGRAPHIC IDENTIFICATION AND SEPARATION OF SOME METAL- PEPTIDOGLYCAN MONOMER COMPLEXES ON CELLULOSE

Lj. Sesartić, O. Hadzija, and N. Brajenović\*

Ruder Boskovic Institute, P. O. Box 180, Bijenicka 54,  
10002 Zagreb, Croatia

## ABSTRACT

In the present work, qualitative thin layer chromatography (TLC) on cellulose of a peptidoglycan monomer (PGM) (full name: GlcNAc-MurNAc-L-Ala-D-isoglutaminnyl-meso-diaminopimelyl-D-Ala-D-Ala),<sup>1,2</sup> some metals and their complexes were tested to find optimal identification and possible separation conditions. An adequate method was developed enabling good color identification and separation of some Me-PGM complexes from their parent metals and PGM by TLC.

## INTRODUCTION

Peptidoglycan monomer (PGM) is a substance of wide biological interest because of its immunostimulating, as well as, anticancerogenic<sup>3,4</sup> properties.

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\*Corresponding author.

PGM can be isolated from the cell walls of different microorganisms; PGM used in this work was isolated from *Brevibacterium divaricatum*.<sup>5</sup> It is known that bivalent metals have a role in the stability, distribution, biotransformation, and elimination of biological active substances.<sup>5</sup> Therefore, the behavior of their complexes is interesting. The coordination of metal with the active hydrophilic groups in PGM may enforce lipophilic parts of the molecule, and thus, enhance their mobility in biological systems.<sup>5</sup> Such complexes were prepared,<sup>5-8</sup> allowing us to develop the method for their identification and separation by TLC.

### EXPERIMENTAL

All chemicals were of analytical grade. The thin layer support was cellulose; commercial plates, 10x10 cm, purchased from "MERCK" (Darmstadt, Germany). Plates were developed in the ascending mode. *n*-Propanol-ammonium hydroxide-distilled water (7:3:1), and *n*-butanol-ethyl acetate-acetic acid-distilled water (1:1:1:1) were applied as mobile phases. Me-PGM complexes and isolated PGM used in this work were provided by Šušković et al.<sup>5</sup> The solvent was allowed to ascend about 8 cm. Detection of Co(II), Ni(II), Zn(II), Cd(II), and Cu(II)-PGM complexes, and the related metals, was performed by dipping the plate in a 0.25% solution of (1-(2-pyridylazo-2-naphthol)) (PAN) in ethanol. For detection of Al(III) and its PGM complex, a saturated ethanolic solution of alizarin was used. Hg(II) and Hg-PGM complex was detected with a 0.05% solution of dithizone in carbon tetrachloride. Detection of PGM was performed by dipping in 0.2% ethanolic ninhydrin solution, and heating at 110°C.

### RESULTS AND DISCUSSION

It was previously concluded, that the synthesized complexes of bivalent metals with PGM are unstable by chromatographing them using silica gel and neutral mobile phase.<sup>5</sup> The purpose of this work was to find the conditions for thin layer chromatographic identification and separation of these compounds. The silica gel support is a very polar sorbent, with strongly acidic functional groups (-OH) that may partially, or completely, influence the decomposition of the complexes. Therefore, we chose cellulose as a slightly polar sorbent. Weakly acid or weakly basic mobile phases were used. These chromatographic systems were chosen to investigate their possible prevention of decomposition of the complexes. The results revealed that no decomposition of the complexes occurred during chromatography. Some good separations were also obtained, as can be seen from Table 1. It can be concluded, that during the chromatographic processes all of the Me-PGM complexes tested were more or less separated from

**Table 1.**  $R_f \times 100$  Values of Metals, Peptidoglycan Monomer, and Their Complexes on Cellulose in Two Solvent Systems

Compounds	$R_f \times 100$	
	Solvent 1	Solvent 2
PGM	44	30
Co(II)	14	23
Co(II)-PGM	24	32
Ni(II)	15	19
Ni(II)-PGM	25	29
Zn(II)	43	25
Zn(II)-PGM	26	34
Hg(II)	0	45
Hg(II)-PGM	30	42
Cd(II)	29	17
Cd(II)-PGM	25	34
Cu(II)	25	32
Cu(II)-PGM	21	41
Al(III)	10	35
Al(III)-PGM	26	27

Solvent Systems:

1. *n*-Propanol-ammonium hydroxide-distilled water (7:3:1), pH=9.5.
2. *n*-Butanol-ethyl acetate-acetic acid-distilled water (1:1:1:1), pH=2.0.

the parent metals and PGM, respectively, and identified by color reactions. Therefore, this method is useful for tracing the complexation processes during synthesis.

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