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Evaluation of oxidized buckypaper as material for the solid phase extraction of cobalamins from milk: Its efficacy as individual and support sorbent of a hydrophilic–lipophilic balance copolymer

V. Pérez-Fernández, A. Gentili*, A. Martinelli, F. Caretti, R. Curini

Department of Chemistry, Faculty of Mathematical, Physical and Natural Science, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

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ABSTRACT

This work describes a new analytical method for the determination of four cobalamins (adenosylcobalamin (AdoCbl), methylcobalamin (MeCbl), hydroxocobalamin (OHCbl) and cyanocobalamin (CNCbl)) in cow's milk. The extraction procedure is fast and based on dilution/protein precipitation of a milk sample with 50 mM sodium acetate buffer (pH 4.6), followed by solid phase extraction (SPE) of the filtered supernatant. Relative recoveries higher than 60% have been obtained for all the cobalamins by combining two different types of sorbents in the same SPE cartridge: two disks of buckypaper (BP), a nanoporous felt composed of oxidized multiwalled carbon nanotubes (MWCNTs), separated by a Teflon frit from OASIS HLB (500 mg), a hydrophilic–lipophilic balance copolymer. Before its use as sorbent, BP was characterized in terms of porosity, permeability, surface area, specific adsorption capacity and tested for a potential reuse after adequate chemical regeneration. The analysis of the extracts was performed by liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) on an analytical C_{18} column in less than 10 min. After validation, the method was applied to the determination of the natural content of the four B_{12} homologues in cow's milk samples, providing data lacking in the literature.

1. Introduction

Vitamin B_{12} is a generic term embracing all cobalamins with a potential for antipernicious anaemia activity. Among these, MeCbl and AdoCbl are the forms active as enzyme cofactors in mammals and bacteria. CNCbl can be found in nature, but it is mainly a synthetic form used in pharmaceutical preparations and food supplementation, while OHCbl is the photo-oxidation product of all other forms [1]. Both CNCbl and OHCbl are activated in the human organism in the two coenzymes according to pathways not fully investigated. Fig. 1 shows names, structures and monoisotopic masses of these compounds.

Cobalamins cover a crucial role in nucleoprotein synthesis, haematopoiesis and metabolism of some lipids of myelin and neurotransmitters; therefore, a B_{12} deficiency is closely related to both haematological (pernicious anaemia) and neurological disorders. In fact, in the last years, there is renewed interest in the role of this vitamin in function and disease of the central nervous system at all ages and in its potential use in the prevention of cognitive decline and dementias in elderly people, including Alzheimer's disease [2].

Unlike the other water-soluble vitamins, cobalamins have bacterial origin. One of the main sources is the microbiota in the intestinal lumen of ruminants: after their absorption, cobalamins are stored in animal tissues and then transferred to humans through the food chain. Therefore, all products of animal origin are good sources of vitamin B_{12}, with typical levels ranging from few ng/g (meat, eggs and dairy products) to a hundred of ng/g (liver) [3].

The recommended daily intake (RDA) of vitamin B_{12} is 2.4 μg/day for adults aged over 14 years [4]; specific RDAs and adequate intakes (AI) for other age categories are listed in Table S1. The vitamin B_{12} status may be impaired by the systematic consumption of a vegan diet, by the reduced absorption ability (elderly people and newborns), by the lack of the intrinsic factor (the glycoprotein responsible for the cobalamin transport through the intestine) and by other gastrointestinal malfunctions [5]. Moreover, the bioavailability of this vitamin from foods depends on both form and linkage with proteins: it is averagely estimated to be about 50% for natural cobalamins [5–7] and only 4% for CNCbl [8]. In cow milk, the B_{12} total content (~5–9 μg/L) is not high compared to that of other animal sources or enriched foods

* Corresponding author.
E-mail address: alessandra.gentili@uniroma1.it (A. Gentili)

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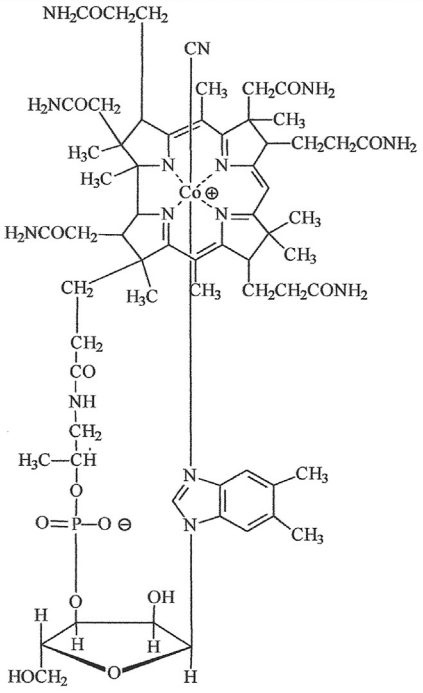
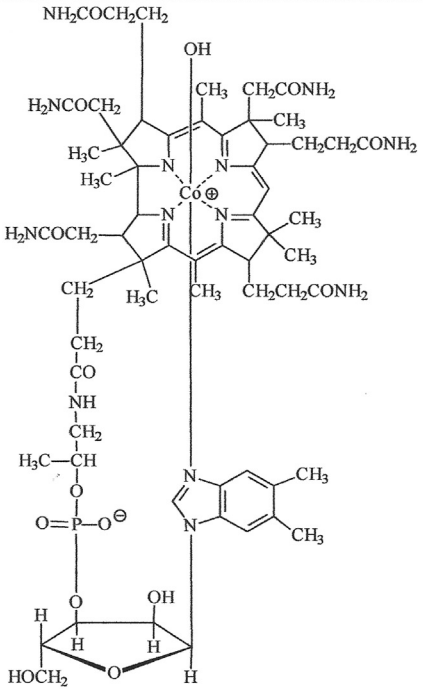
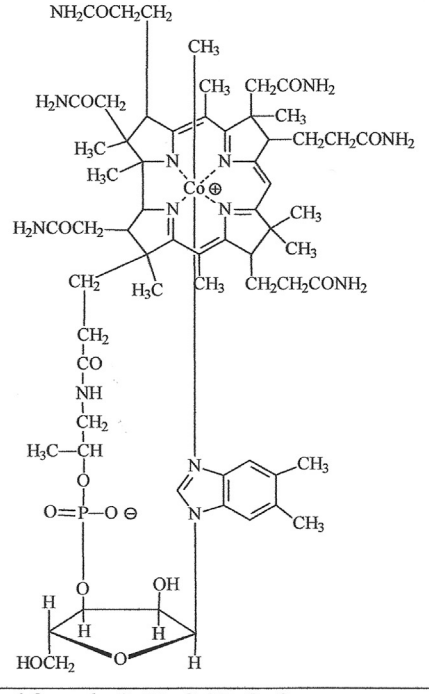
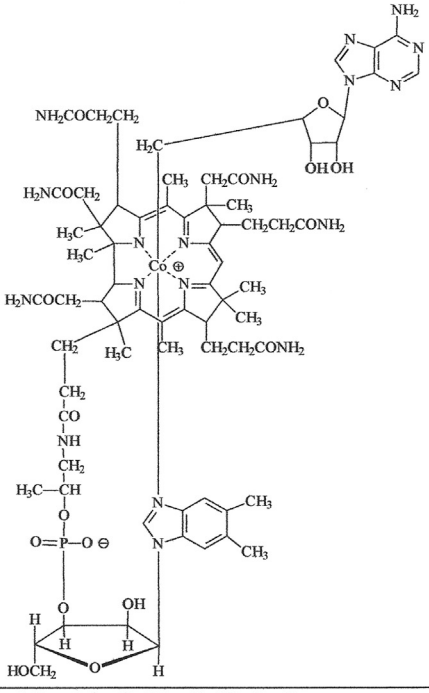
Cyanocobalamin (CNCbl)	Hydroxocobalamin (OHCbl)
	
Empirical formula $C_{63}H_{88}CoN_{14}O_{14}P$	Empirical formula $C_{62}H_{89}CoN_{13}O_{15}P$
Molecular weight 1355.38 u	Molecular weight 1346.37 u
Exact mass 1354.567405 u	Exact mass 1345.567071 u
Methylcobalamin (MeCbl)	**5'-deoxy-adenosylcobalamin (AdoCbl)**
	
Empirical formula $C_{63}H_{91}CoN_{13}O_{14}P$	Empirical formula $C_{72}H_{100}CoN_{18}O_{17}P$
Molecular weight 1344.40 u	Molecular weight 1579.60 u
Exact mass 1343.587806 u	Exact mass 1578.658345 u

Fig. 1. Names, acronyms, structures and masses of the cobalamins selected for this study.

(with about 300 μg/kg of CNCbl), but its bioavailability has been found to be excellent, with absorption percentages up to 88% [6,7]. For these reasons, the elucidation of the natural distribution of the B_{12} homologues in foods, in general, and milk, in particular, is of great interest. Nevertheless, so far, this task has been hampered by the considerable analytical difficulties, mainly related to limited chemical stability and low endogenous concentrations of cobalamins.