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New insights on resveratrol supported by magnesium dihydroxide (Revifast®)

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SHORT COMMUNICATION

Abstract

Based on low solubility in water and high membrane permeability, resveratrol is collocated in the second class of the Biopharmaceutical Classification System, with limited absorption derived from a low dissolution rate. Solid microdispersion of resveratrol supported by magnesium dihydroxide (Resv@MDH, trademark Revifast®) represents a physical mixture of resveratrol (30% w/w) and magnesium dihydroxide (70% w/w) obtained by traditional techniques, such as mixing and micronization under appropriate conditions. Establishing the wide use of Revifast® in food supplements, in the present work we deepen its physicochemical characterization by using diffractometric and infrared analysis. No novel species are found in the Resv@MDH mixture except magnesium dihydroxide and resveratrol extracted from *Polygonum cuspidatum*. The results herein reported strengthened the safety of Revifast® ingredients for resveratrol-based food supplements.

Keywords: dissolution rate; magnesium dihydroxide; microparticles; resveratrol; spectroscopy

Introduction

Resveratrol is a natural compound found in various plants, including grapes, blueberries, and certain types of nuts. Chemically it is a polyphenol (trans-3,5,4'-trihydroxystilbene), initially isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) and later from the roots of *Polygonum cuspidatum*, a plant used in traditional Chinese and Japanese medicines (Baur and Sinclair, 2006). Since 1992, the use of resveratrol as a dietary supplement in the field of nutraceuticals has gained an increasing popularity because of discovery of multiple health benefits associated with the antioxidant properties and in managing cholesterol levels (Su *et al.*, 2022). Nowadays, there is a growing interest to study human health benefits associated with the systematic supplementation of resveratrol in the diet of healthy subjects. Several studies have recently shown that resveratrol could play a significant role in the prevention of diseases, such as cardiovascular disease (CVD), metabolic diseases, cancer-related diseases, and fertility, that are increasingly common in the elderly population (Ragonese *et al.*, 2021; Su *et al.*, 2022; Wu *et al.*, 2022). Again, new evidence showing that resveratrol has a neuroprotective effect opened the way to new therapeutic approaches to treat Alzheimer's disease (Islam *et al.*,

2022). Antiviral activity inhibiting virus entrance in cells and viral replication through different mechanisms was observed as well as an adjuvant in the anti-COVID-19 therapy (Domi *et al.*, 2022). Interestingly, the use of resveratrol on lysosomal storage diseases (LSDs), such as Sanfilippo disease (mucopolysaccharidosis type III [MPS III]), was proposed as a novel therapeutic approach in pathology (Paciotti *et al.*, 2017; Rintz *et al.*, 2023).

From a pharmacokinetic point of view, resveratrol is poorly bioavailable because of reduced absorption, mainly because of its low water solubility and fast metabolism (Baur and Sinclair, 2006). Several strategies have been performed to increase its bioavailability and improve its potential health properties. Among the different strategies, new formulations have been developed that are able to increase its apparent solubility, for example, by using a lipophilic vehicle or through various processes, such as the complexation with cyclodextrins, nanopreparation, or micellar solubilization with biliary acid (Amiot *et al.*, 2013; Amri *et al.*, 2012; Das *et al.*, 2008). *In vitro* studies have demonstrated that increase of apparent solubility of resveratrol allows a partial saturation of the mechanisms that are involved in its metabolism (conjugation) with a subsequent increase of resveratrol's bioavailability (Maier-Salamon *et al.*, 2006). This is in accordance with Biopharmaceutical Classification System (BCS) for molecules class II that increasing resveratrol's apparent solubility produces an improved bioavailability, but in a dedicated study the increased solubility with cyclodextrins doesn't modify its bioavailability (Hurst *et al.*, 2007).

Among the recently developed resveratrol-based ingredients, resveratrol supported by magnesium dihydroxide (Revifast®) has attracted considerable attention in the scientific field because of its results in several clinical trials (Curry *et al.*, 2021). In fact, several scientific publications have shown its potential health benefits and safety in the area of cardiology, metabolism, and fertility. For example, regarding female fertility, as a support in medically assisted procreation cycles and fertility processes, the trial by Gerli *et al.* (2022) demonstrated that a Revifast®-based nutraceutical is able to increase the number of mature follicles. The same supplement was able to increase the number and concentration of spermatozoa in a pilot study comprising 20 idiopathic infertile males (Illiano *et al.*, 2020). Panico *et al.* (2017) and Malvasi *et al.* (2017, 2018) demonstrated the beneficial effects of Revifast® in the related metabolic syndrome, such as cardiovascular risk and polycystic ovary syndrome (PCOS). Specifically, the use of Revifast® if associated with monacolin K or Berberis improved displipidemic profile (Panico *et al.*, 2017) whereas if associated to inositol, it was able to reduce the metabolic deregulation associated with PCOS (Malvasi *et al.*, 2017, 2018). All clinical trials also showed the safety profile of Revifast®. Based on the success of Revifast® in the present work, we investigate physical characterization by using diffractometry and infrared (IR) diffractometric analysis.

Materials and Methods

Materials

Revifast®: Resveratrol 98% (*Polygonum cuspidatum*) extract and magnesium dihydroxide ($Mg(OH)_2$) were purchased from Prolabin & Tefarm srl, Italy. Specifically, the raw materials used in this work were the same as used for the production of Revifast® ingredient.

Sample characterization

X-Ray Powder Diffraction (XRPD)

Revifast® and raw materials: Resveratrol, extracted from *Polygonum cuspidatum*, and magnesium dihydroxide were characterized for structural and phase analyses using XRPD. XRPD patterns were recorded with a Bruker D2 Phaser diffractometer operating at 30 kV and 10 mA, a step size of 0.020 2 degrees/step, and time of 1.00 s per step using copper K-α (Cu K-α) radiation and a multi-strip LYNXEYE SSD160 detector.

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

Infrared spectra were obtained using a Jasco 4600 FTIR spectrophotometer. Each sample was in advance mixed with FTIR-grade potassium bromide (KBr) in 1:100 ratio and pressed into tablets. The spectrum was recorded in a spectral range settled between 4,000 cm^{-1} and 400 cm^{-1} using 100 scans. The spectral resolution was 4 cm^{-1}.

Results

To better describe Revifast®, we applied XRPD and IR spectrometry to analyze Revifast® and the raw materials, magnesium dihydroxide and resveratrol extracted from *Polygonum cuspidatum*, respectively. XRPD pattern of resveratrol 98% (Figure 1, red line) shows several diffraction peaks in the region, 6°–30° 2θ, while magnesium dihydroxide (green line) presents many diffraction peaks in the region, 15°–70° 2θ, according to their crystalline structures. Revifast® XRPD (Figure 1, black line) shows a pattern of diffraction spectra peaks according to overlap of diffraction peaks of crystalline resveratrol and magnesium dihydroxide. No further phases/peaks were associated to new species in this analysis.

Further investigation was performed with FTIR analysis. Initially, we analyzed the signals of raw materials, resveratrol and magnesium dihydroxide, separately. The FTIR

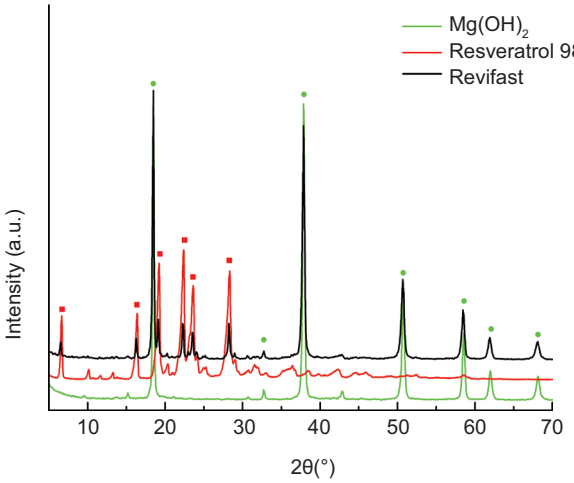

Figure 1. XRPD patterns of magnesium dihydroxide (green line) and resveratrol 98% (red line), compared with Revifast® (black line).

spectrum of resveratrol showed the presence of characteristic bands relative to axial and angular deformations of the chemical bonds representing the functional groups of the molecules studied (Figure 2; Güder *et al.*, 2014). In detail, regarding resveratrol (Figure 2, red line), the broad band at 3192 cm^{-1} refers to O-H stretching of phenolic hydroxyl bonds. The narrow and low-intensity band at 3017 cm^{-1} is related to the =C-H axial stretching of aromatic hydrogen, confirming the presence of unsaturation. The stretching related to C=C bonds of aromatic rings is observed in the bands at 1611, 1583, and 1520 cm^{-1}. Presence of a band at 1155 cm^{-1} confirmed C-O bond stretching for phenolic compounds. Stretching at 1377 cm^{-1} confirmed the O-H phenolic hydroxyl group. The =C-H band at 965 cm^{-1} was characteristic of alkene in the trans-configuration of resveratrol. The stretching at 860–770 cm^{-1} was characteristic of =C-H vibration bands of arene conjugated to the olefinic group (831 cm^{-1}). Deformation bands at 650–500 cm^{-1} corresponded to =C-H of olefinic group (677 cm^{-1}) (Porto Isabel *et al.*, 2018) whereas the FTIR spectrum of magnesium dihydroxide (Figure 2, green line) is characterized by intense absorption at 3695 cm^{-1} related to the -OH stretching and a large band at 470 cm^{-1} ascribed to Mg-O modes. Analysis of the superimposed IR spectra of Revifast® (Figure 2, black line) shows exactly the same absorption peaks of the raw ingredient without the presence of new peaks, indicating that no new chemical bonds were formed during the production process.

Discussion

Revifast® is a physical blend of two food-grade raw materials, one of which is the extract from *Polygonum cuspidatum* titrated at 98% in resveratrol and the other is magnesium dihydroxide. In particular, resveratrol from *Polygonum cuspidatum* is an extract from the plant *Fallopia japonica* and can be used in food supplements, as it has a history of use dating back to prior of 1997 (https://webgate.ec.europa.eu/fip/novel_food_catalogue/). Magnesium dihydroxide is a food additive denoted by E number E528 that has no limit of use. The physical mixture constituting the product Revifast® consists of a main component, magnesium dihydroxide 70% by weight, and a minor component, the extract from *Polygonum cuspidatum* titrated in resveratrol 98%, for about 30% by weight. The product is in fact a solid dispersion of resveratrol by magnesium dihydroxide obtained by traditional techniques, such as mixing and micronization under appropriate conditions.

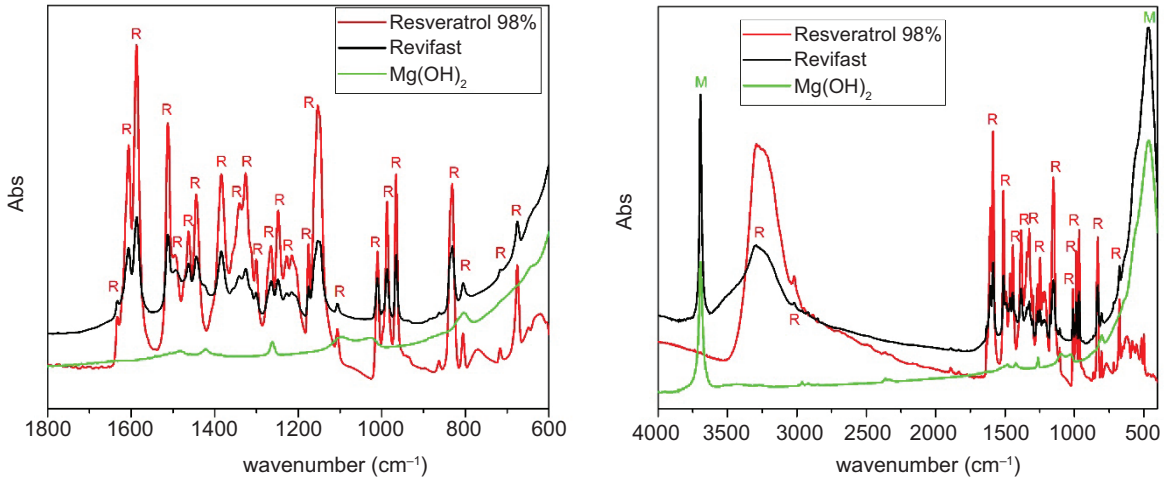

Figure 2. FTIR of resveratrol, magnesium dihydroxide, and Revifast®. Left: FTIR spectra of magnesium dihydroxide (green line) and resveratrol 98% (red line), compared with Revifast (black line). Right: Magnification of the spectral region (1,800–600 cm^{-1}), where all the characteristic absorption bands of resveratrol were present, perfectly overlapping with those of Revifast® spectrum.

The product Revifast® is a registered trademark to distinguish the above-mentioned mixture and has the technical characteristics of improving its properties and use in various pharmaceutical classes used in food supplements, such as tablets, capsules, and granules in sachets in both solid and liquid forms. Revifast® contains no nanomaterial and is not considered a new extract because no solvents are used to produce it.

In this work, we completed the characterization of Revifast® through XRPD and IR analyses, which allowed us to better understand the nature of the ingredients. In particular, XRPD and FTIR spectroscopy provided us respective information regarding crystallographic structure and interactions of the starting materials (resveratrol and magnesium dihydroxide) prior to and after the production process (micronization, mixing, and sieving) to obtain Revifast®. The superimposed XRPD spectra in Figure 1 clearly showed that the Revifast® ingredient had a pattern of diffraction peaks that was exactly overlapping with the diffraction peaks of crystalline resveratrol and magnesium dihydroxide, and there were no further phases attributable to these raw materials. In addition, analysis of the superimposed IR spectra of Revifast® displayed exactly the same absorption peaks of the raw materials without presence of new peaks, thus indicating that no new chemical bonds were formed during the production process. Altogether, the XRPD and IR spectra indicated that the Revifast® ingredient comprised a physical mixture of the two starting raw materials, resveratrol from *Polygonum cuspidatum*/*Fallopio japonica* and magnesium dihydroxide (E528), and the chemical structure of the starting raw materials remained unchanged and no new molecules were formed during the production process.

We hypothesized, on the basis of fluorescence microscopy analysis, the presence of a third microparticle, different from resveratrol and magnesium dihydroxide (Spogli *et al.*, 2018). However, based on the results reported in this work, the presence of this microparticle was rejected. This discrepancy could be considered an artifact, probably due to sample preparation for microscopic analysis, such as the dispersion of Revifast® in glycerol (Spogli *et al.*, 2018).

Furthermore, it was verified through a UV/VIS spectroscopic investigation in a gastric environment that no new chemical species was formed, and no interactions were observed between divalent magnesium and resveratrol, excluding the formation of new complexes (Spogli *et al.*, 2018). Finally, previous granulometric analyses demonstrated the absence of nanomaterials (Spogli *et al.*, 2018). We studied the pharmacokinetics of Revifast® to evaluate the impact of better dissolution rate of resveratrol in gastric fluids (Iannitti *et al.*, 2020). However, no modification was made in bioavailability between Revifast® and resveratrol, but a relative increase of gastric absorption was observed as a consequence of different dissolution kinetic properties (Iannitti *et al.*, 2020; Spogli *et al.*, 2018). These kinetic properties may justify the health benefits observed in clinical trials that successfully used the Revifast®-ingredient (resveratrol-based) food supplements (Curry *et al.*, 2021; also see "Introduction").

Author Contributions

Conceptualization, R.S. and B.F.; validation, A.P. and M.C.; formal analysis, A.P. and F.P.; investigation, R.S., B.F., GB, L.M. and A.P.; resources, R.S., B.F., R.G.I. and G.C.; data curation, A.B., F.P. and M.R.C.; writing—original draft preparation, A.B., P.S., R.S., B.F, and F.P.; writing—review and editing, A.B., P.S., B.F. and R.S.; visualization, F.R., G.B. and L.M.; supervision, B.F. and R.S.; project administration, B.F. and R.S.

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