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Research Article

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Antioxidant Activity Assay *In vitro* of Polysorbate 80 and Dimethyl Sulfoxide (DMSO) through DPPH Method

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ABSTRACT

Excipients are components that help preparing a formulation and are classified according to their purpose. Among the different classes there are the tensoactive ones which are important in various areas of industry for having a capability of generating emulsions. Polysorbate 80 and dimethyl sulfoxide (DMSO) are important tensoactives widely used by industry in different segments to favor the homogenization of immiscible substances. Thus, the aim of this study is to evaluate the antioxidant activity of polysorbate 80 and DMSO through the ability to neutralize free radical DPPH The results were analyzed by logarithmic regression curve to obtain the curve of the function and the curve of the coefficient of determination. Statistical analysis was obtained using the GraphPad Prism® software version 7.0 for Windows®. The different concentrations of polysorbate 80 and DMSO showed significant antioxidant activity in a dose-dependent manner. In this experiment, both substances exhibited antioxidant activity by scavenging free radicals by DPPH, even when used in low concentrations. These results raise concerns in the indiscriminate use of these substances in laboratory research involving antioxidant activity, since the activity can be obtained from use in experiments, DMSO or polysorbate 80. However, it has been emphasized the need for deeper studies that have higher concentrations of the substance, or using different methodologies of antioxidant analysis in vitro and in vivo, in order to establish the performance of these excipients that have a large usability in the pharmaceutical field.

Keywords: Antioxidants activity; Polysorbate 80; Dimethyl sulfoxide (DMSO); 2,2-diphenyl-1-picrylhydrazyl (DPPH)

INTRODUCTION

Excipients are components that help preparing a formulation and are classified according to their purpose. Among the different classes there are the tensoactive ones which are important in various areas of industry for having a capability of generating emulsions. By acting on the reduction of the superficial tension of two imiscible liquids, the tensoactives enable their own dilution [1]. As an example, there is polysorbate 80, and dimethylsulfoxide (DMSO) [2-4].

Polysorbate 80, also known as Tween 80® is used in the food industry, dermocosmetic and medicine as an emulsifier, solubilizer, dispersant, defoamer, wetting agent and surfactant [5]. It has already been reported for introducing toxic characteristics, proved to be a toxicant agent for various vias of administration [6], like in the topical via, causing irritation; and at the oral one diarrhea [7]. *In vivo* tests in mice and rats using the intraperitoneal via, the substance promoted depression in the central nervous system reduced locomotor activity and rectal temperature, ataxia, paralytic activity and potentiation of sleep time induced by phenobarbital. In tests using intravenous via, it was observed an hipotensive efect when administered in dogs, antagonism of the activity of acetylcholine, histamine and barium when administered to rats and myocardial depression when

administered to guinea pigs [8]. Polysorbate 80 also proved toxic to increase the potential for hepatotoxicity observed after amiodarone administration [9]. Another study showed a reduction of the effects of carbamazepine, primidone and after compound administration, as it promotes a reduction in the absorption of drugs. These findings imply that the substance can alter bioavailability by increasing or reducing the toxicity of drugs and experimental materials [10,11]. Dimethylsulfoxide (DMSO) has wide usability in the industrial field, acting as a solvent that allows dissolution of organic molecules such as proteins and steroids [12,13]. The toxicity of DMSO is low when used in topical via. When combined with high toxicity agents, it tends to favor the increase of the toxic effect [12]. *In vivo* studies show that DMSO has clinical activity in animals and humans, having anti-inflammatory and analgesic effects, in addition to their use in muscle damage, reduced intracranial pressure, reduced deposit of amyloid protein in the osteoarticular trauma, burns, edemas and cystitis, both in animals and in humans [14-17]. Many pharmacological and therapeutic properties indicate DMSO as an antioxidant agent including the ability to interact with carbohydrates, lipids, nucleic acids and other drugs without being irreversibly changed in its structures [18]. It has easy distribution in the blood and can be found in all organs of the body about 20 minutes after application [12,19]. The substance has cryoprotectant effect, to be used in the preservation of sperm, embryos, platelets, tumor cells and in cell cultures [20]; radioprotective effect, through the action of removing free radicals [21]; in the healing of ulcers on the skin and reducing inflammation by its ability to inhibit the activity of NLRP3 [22-25], a protein involved in the inflammatory process [21,26-28]. Another feature of DMSO is its action in facilitating permeability of compounds such as antibiotics, antivirals and steroids through the topical way [12,29]. However, despite being an excellent compound for this purpose, it may generate damage when applied to mucosal surfaces [12], as erythema in the stratum corneum and denaturation of proteins, when used at high concentrations. Usually the DMSO is present above 60% as solvent to optimize the efficiency of permeation [19]. Thus, the aim of this study is to evaluate the antioxidant activity of polysorbate 80 and DMSO through the ability to neutralize free radical DPPH in different concentrations, to be widely used in various pharmaceutical preparations.

EXPERIMENTAL SECTION

Sampling

The study has descriptive, quantitative and experimental approach and was conducted at the Laboratory of the *Núcleo de Bioprospecção e Experimentação Molecular Aplicada* (NUBEM). Polysorbate 80, Tween®, and dimethylsulfoxide, imported and distributed by all Chemistry have been used in the study. Both samples were diluted in distilled water at different concentrations. The experiment was carried out in air-conditioned environment and low incidence lighting to reduce change of the compounds used. The free radical DPPH solution was prepared by dissolving in an ethanol-methanol solution (1:1) with concentration of 0.01% DPPH. The DPPH method was created by Blois [30] and adapted by Brand-Williams et al. [31] and is based on the radical capture 2,2-diphenyl-1-picrylhydrazyl (DPPH) by agents with potential antioxidant activity, generating change in the chlorination of the sample detected by a decrease in absorbance at 515-520nm levels [32-34]. The positive control (PC) was analyzed through a solution of vitamin E (α-tocopherol), an antioxidant widely distributed in tissues and in plasma [35,36], at a concentration of 50 mcg / ml. The blank sample was composed of an ethanol-methanol solution (1:1). For the negative control (NC) distilled water was used, the same vehicle used in solubilizing the various concentrations of surfactants.

Calculation of Antioxidant Activity

The antioxidant activity analysis was performed by the spectrophotometer absorbance obtained with 1950 µl DPPH 0.01% added 50 mL of solutions: tocopherol (CP), distilled water (CN), DMSO and polysorbate 80 as demonstrated in Table 1.

Table 1: Concentrations of polysorbate 80 and DMSO solutions before and after mixing with the solution of DPPH free radicals

Polysorbate 80 (P80)		Dimethyl Sulfoxide (DMSO)	
Concentration in 50 µl * (P80 + Water)	**Concentration in 2000 µl ** P80 solution (50 mL) + solution of DPPH (1950 µl)| Concentration in 50mL**	**Concentration in 50 µl (DMSO + Water)**	**Concentration in 2000µl DMSO Solution (50 µl) + DPPH Solution (1950 µl)]**
1.0%	0.025%	10%	0.25%
2.0%	0.05%	20%	0.5%
4.0%	0.1%	40%	1.0%
10%	0.25%	100%	2.5%

The experiment was performed in triplicate to reaffirm the result. The calculation of the percentage of the antioxidant activity (% AA) was performed according to the following formula:

$$AA\% = (A\ control - A\ sample) \times 100 / A\ control$$

Where: A control = absorbance of DPPH solution without the sample; A sample = absorbance of the sample with DPPH.

Statistical Analysis

The results were analyzed by logarithmic regression curve in Excel® 2013 to obtain the curve of the function and the curve of the coefficient of determination. Statistical analysis was obtained using the GraphPad Prism® software version 7.0 for Windows®. The results that follow a parametric distribution were analyzed by Analysis of Variance (ANOVA) followed by Dunnett's test. The values were represented by Mean ± standard error of mean. The significance criterion was $p<0.05$.

RESULTS

Analyses were performed in triplicate and generated an average of the values obtained. From the results a linear regression plot was constructed for the purpose of finding the equation of the line and the coefficient of determination (R^2). The graphs demonstrate that the consumption of DPPH was directly proportional to the concentration of the sample, showing the antioxidant activity in a dose-dependent manner (Figure 1). All samples showed consumption capacity of DPPH absorbance observed at lower values than the negative control solution. Through the different concentrations analyzed it was possible to determine the equation of the logarithmic curve. This allows the calculation of the percentage of antioxidant activity in different concentrations of the analyzed ones. From Figure 2 one can see the confirmation of the test through the Tocopherol 50 mcg/mL solution as positive control (PC). On the negative control (NC) Distilled water was used, confirming no antioxidant activity. The different concentrations of polysorbate 80 and DMSO showed significant antioxidant activity in a dose-dependent manner.

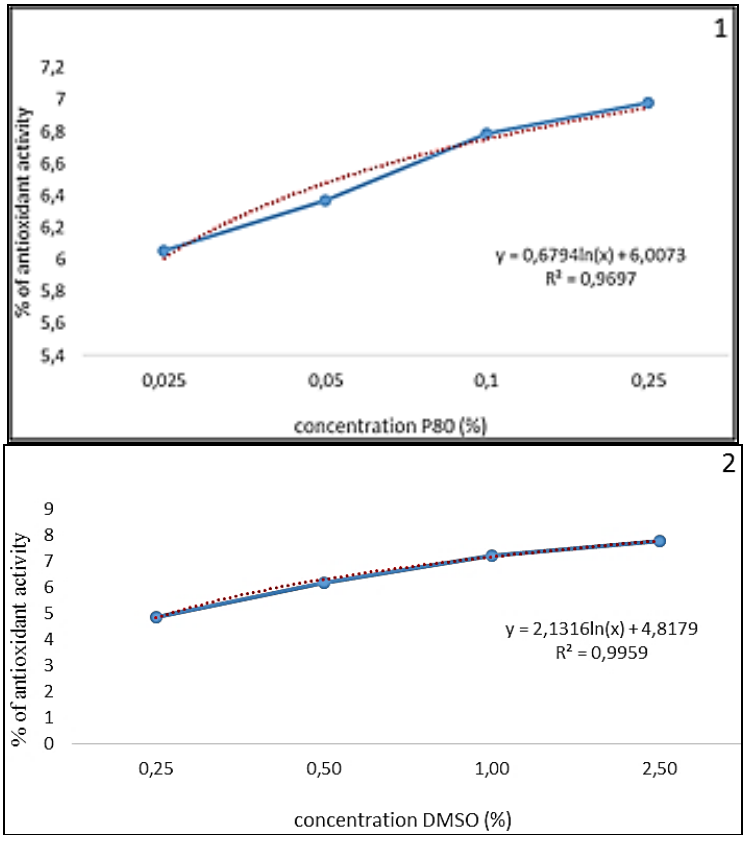
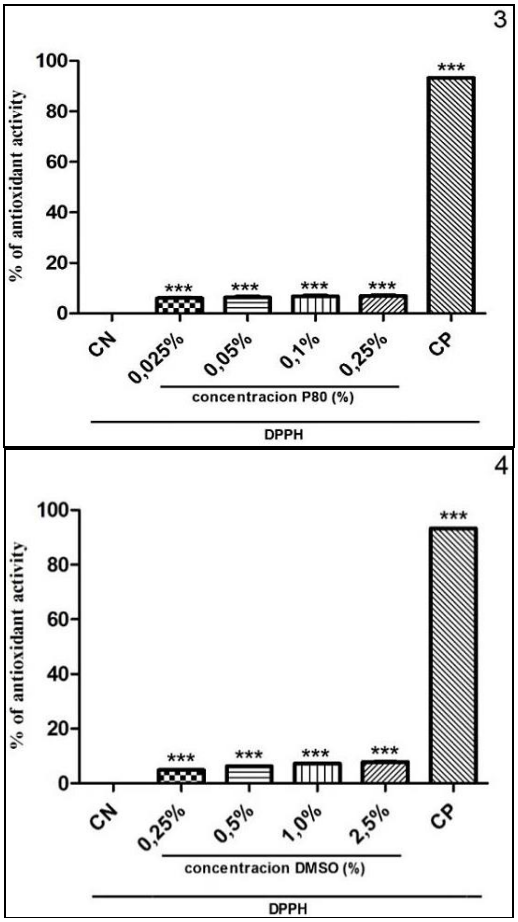


Figure 1: Logarithmic regression analysis with the results of the percentage of antioxidant activity of different concentrations of the surfactant polysorbate 80 (chart 1) and DMSO (c hart 2)



** p <0.001 and *** p <0.0001

Figure 2: Results of the percentage of antioxidant activity in different concentrations of surfactant polysorbate 80 (chart 3) and DMSO (chart 4)

DISCUSSION

Some studies have demonstrated antioxidant activity for DMSO substance as reported by Engelmann [18] and Velasco et al. [17]. These authors reported on the antioxidant capacity related DMSO deriving promoting activity with calcium ions and its ease to interact at the molecular level with various elements such as proteins, lipids, carbohydrates and, consequently, radical stabilizing and reducing the levels of free electrons. For Sturion et al. [12], DMSO acts as a membrane stabilizer to reduce the activity of inflammatory processes by its ability to remove free radicals. There are chances that this event is the result of a possible chelating action of the molecule on the intracellular calcium, since the ion enhances the action of free radicals that end up injuring cell membranes. In the study by Colucci et al. [37], involving pharmacological models of nociception and inflammation, the authors found *in vivo* that DMSO has the potential antinociceptive and anti-inflammatory action. For the experiment, the authors used the 100% DMSO solution and 0.9% NaCl (1:3) in mice by subcutaneous and oral routes. Both Dimethyl sulfoxide concentrations showed these activities. In experiments conducted by Ahn et al. [21] it was observed the DMSO capacity to inhibit inflammasome NLRP3, cytoplasmic multiprotein complex that acts mediating maturation of IL-1 by caspase-1. Through this mechanism, although it is not fully understood, the authors suggest an anti-inflammatory activity of DMSO through *in vitro* and *in vivo* tests. According to Slichter et al. [15] and Sturion et al. [12] DMSO has also been effective in the cryoprecipitation of sperms and cell parts for presenting the ability to generate enzyme inhibition and cryoprotectant effect. The cryoprotection will make the water present within the sample not crystallize completely and enzyme inhibition has characteristics relating to stabilization of reactive hydrogen species present in the enviroment. Thus, there is a removal of free radicals and preservation of cell parts of a possible deterioration. Another study involving DMSO was published by Sanmartín-Suárez et al. [38]. The authors tested the antioxidant effect of the molecule through the lipid peroxidation test and protein oxidation using rat brain homogenate as well as their ability in reducing the production of hydroxyl radicals. In this experiment, the DMSO showed a reduction in lipid peroxidation, protein oxidation and the production of hydroxyl radicals. Few studies report on the antioxidant effect of polysorbate 80. Péres-Rosés et al. [39] tested the antioxidant activity

of the substance using different *in vitro* tests, including free radical stabilization activity DPPH, the production of reactive oxygen species and inhibitory activity of myeloperoxidase. The authors, however, found no antioxidant activity of polysorbate 80 in the DPPH test. Thus, it can be seen that the DMSO can promote antioxidant activity. In this experiment, the substance was able to neutralize to a significant extent, the free radical DPPH, even at low concentrations. The substance has relevant studies with respect to its therapeutic properties, both for humans and for animals, as well as current sources that refer to a substance into focus for experimental work. However, it still needs further clarification about its properties and mechanisms, especially when it relates to its antioxidant capacity which has few explanations in the literature. The amount of experimental studies on antioxidant capacity towards the polysorbate 80 is scarce. In this study, the substance showed potential antioxidant effect by neutralizing DPPH, significantly, agreeing with the experiment of Péres-Rosés et al. [39]. Thus, we emphasize a need to study this great inclusion of substances in pharmaceutical research to generate an updated database and inform about the features and dangers about such compounds.

CONCLUSION

DMSO and polysorbate 80 surfactants are both widely used in pharmacological studies as vehicles for dissolution of chemicals. DMSO has been reported by several authors on their pharmacological activities including its anti-inflammatory, antioxidant, and as a cryoprotectant. On the other hand polysorbate 80 is little reported on its pharmacological activities. In this experiment, both substances exhibited antioxidant activity by scavenging free radicals by DPPH, even when used in low concentrations. These results raise concerns in the indiscriminate use of these substances in laboratory research involving antioxidant activity, since the activity can be obtained from use in experiments, DMSO or polysorbate 80. Although our studies have used low concentrations of surfactants in both tensuatives , the logarithmic regression graph allowed to determine the curve of the function, thus permitting to calculate the percentage of antioxidant activity DMSO and polysorbate 80 at different concentrations of the analyzed ones. However, it has been emphasized the need for deeper studies that have higher concentrations of the substance, or using different methodologies of antioxidant analysis *in vitro* and *in vivo*, in order to establish the performance of these excipients that have a large usability in the pharmaceutical field.

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