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The Chitosan as Dietary Fiber: An *in vitro* Comparative Study of Interactions with Drug and Nutritional Substances

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1. Introduction

Obesity is considered a disease that has grown significantly in the last two decades worldwide. The concern with this increase is justified because obesity develops and remains due to different factors, causing several other diseases and sometimes, can lead to death. Several strategies have been searched to control its progress usually through therapies and, in critical cases, surgeries. Despite all efforts, statistical studies show that obesity is still growing consequently generates the necessity of further studies on the subject (Dacome, 2005).

Epidemiological researches have studied the impact of overweight and obesity on the risk of chronic disease, as coronary heart disease, type 2 diabetes mellitus, hypertension, stroke, dyslipidemia, insulin-resistance, glucose intolerance, metabolic syndrome, and cancers of the breast, endometrium, prostate and colon (Aslander-van Vliet et al., 2007). Health consequences and compromised quality of life associated with obesity provide incentives to abate the obesity epidemic. However, despite recognition of these effects, the epidemic of obesity and overweight is not reversed (Johnson et al., 2007).

In general, treatments for obesity are based on regular exercise, nutritional reeducation, pharmacological treatment, behavioral therapy and use of dietary fibers that promote the reduction of fat absorption (Aslander-van Vliet et al., 2007). Much research has been conducted on the dietary supplements that promote the reduction of body weight and fat mass (Saper et al., 2004). These ingredients reportedly act as a fiber to increase satiety and also to decrease the absorption of fat by binding to it (Kumirska et al., 2010).

A natural substance that helps in these anti-obesity treatments that has been highly recommended to control obesity is chitosan (Hennen, 2005). Chemically speaking, chitosan (Figure 1) is a linear polysaccharide of $\beta(1\rightarrow4)$ -linked-2-amino-2-deoxy-D-glucopyranose obtained by deacetylation of chitin, the main component of the exoskeleton of insects and crustaceans (Kumirska et al., 2010). It has many important properties, such as non-toxicity, biocompatibility, biodegradability, antimicrobial activity, chemical reactivity (Cummings et al., 2010), industrial applications (Hennen, 2005), as well as carrier for body fat (Ni Mhurchu et al., 2004; Ni Mhurchu et al., 2005; Jull et al., 2008; Lois & Kumar, 2008), cholesterol and triglyceride (Razdan & Petterson, 1994; Liu et al., 2008; Zhang et al., 2008). Many mechanisms (Tapola et al., 2008; Prajapati, 2009) to explain the carriers and absorptive properties of microenvironment produced by chitosan in solution have been proposed.

However, the use of chitosan is still controversial, and studies in favor and against the use of chitosan have been constantly reported. Many studies have confirmed the hypocholesterolemic activity of chitosan (Sugano et al., 1978; Liao et al., 2007; Yao et al., 2008; Liu et al., 2008; Zhang et al., 2008). The same way, works have reported that the triglyceride and cholesterol absorption have been inhibited and the cholesterol concentration of mice fed with a high fat diet plus chitosan has been decreased (Razdan & Petterson, 1994; Liu et al., 2008; Zhang et al., 2008). Other studies reported that chitosan is efficacious in facilitating the reducing body fat and weight loss in obese individuals (Schiller et al., 2001; Kaats et al., 2006).

On the other hand, studies have shown that oral administration of chitosan has weak action on the reduction of triglyceride and plasma cholesterol in rabbits (Hirano & Akiyama, 1995). Other works have reported that the effect of chitosan on body weight is minimal and unlikely to be of clinical significance (Ni Mhurchu et al., 2004; Ni Mhurchu et al., 2005; Lois & Kumar, 2008, Jull et al., 2008), as well as that the fat trapped was clinically insignificant in studies with overweight adults treated with chitosan capsules before each meal (Pittler et al., 1999; Pittler & Ernst, 2004; Gades & Stern, 2005).

Figure 1. Chemical structure of chitosan.

Is well known that chitosan produces microenvironments with carriers and absorptive properties in acidic aqueous solution. These begin to form above a certain concentration, critical aggregate concentration, CAC (Rodrigues, 2005). The mechanism of solubilization of molecules is well known (Rodrigues, 2005; Rodrigues et al., 2008) however, the process by which chitosan acts as a carrier of fat is not yet fully understood and two mechanisms have been suggested (Prajapati, 2009; Tapola et al., 2008). One of these mechanisms describe the

effect of chitosan fiber network, were chitosan also binds neutral lipids like cholesterol and triglycerides through hydrophobic bonds (Tapola et al., 2008; Prajapati, 2009). In other mechanism, the positive charges (NH_3^+ group generated by stomach acids) on chitosan attract and binds to fatty and bile acids (both negatively charged). This complex is indigestible by the body and excreted in the feces (Tapola et al., 2008; Prajapati, 2009).

Regardless of the solubilization mechanism, nutrients can also be solubilized in chitosan microenvironments, as reported in some studies. Works demonstrated that chitosan causes significant decrease in protein digestibility (Deuchi et al., 1994) and its effect on nutrient digestibility (Ho et al. 2001). Nevertheless, studies on the interaction of chitosan with nutrients are still rare and inconclusive (Gades & Stern, 2005; Hennen, 2005; Kaats et al., 2006; Barbosa et al., 2007; Tapola et al., 2008).

In this context, we present a comparative study of interactions of the chitosan with molecules of two vitamins and one drug. To each molecule, the study was conducted in acid aqueous solution, condition similar to the stomach environment, where occurs formation of chitosan gel responsible for solubilizing molecules.

Drug fluoxetine was chosen for this study. The need for anti-depressive drugs with few side effects, as anticholinergic activity and cardiovascular accidents, boosted the development of new anti-depressant compounds (Böer et al., 2010), as fluoxetine, which inhibits the uptake of serotonin by the neurons in the brain, enhances serotonin neurotransmission and had the longest half-life that other selective serotonin reuptake inhibitors (SSRIs) (Rizo et al., 2011). The precise mechanism of action is not clear but it has less cardiovascular, sedative and anticholinergic effects than the tricyclic antidepressant drugs (Shah et al., 2008). The main indications for the prescription of fluoxetine are for obsessive-compulsive disorder, depression therapy, bulimia nervosa, alimentary disorders and obesity (Suarez et al., 2009). Besides drug, the nutritional reeducation and intake of dietary fibers as chitosan has been recommended in treatments for obesity (Aslander-van Vliet et al., 2007). Based on the possible concurrent use of fluoxetine and chitosan, it is important to evaluate the interactions between both substances.

Vitamins chosen for this study were the B2 and B12. Vitamin B2 or riboflavin is a vitamin B complex that participates in numerous metabolic reactions and physiological functions (United States Pharmacopeia, 2007). Vitamin B12 or cyanocobalamin is an essential component in human diet, plays a key role in cell nucleus, enzymatic processes in the mitochondria, and cytoplasm; it is necessary for the synthesis of red blood cells, for the maintenance of the nervous system, and for the growth and development in children (Wang et al., 2007). Both vitamins are not produced by the body and are consumed only in small quantities (Sommer, 2008); deficiency can cause many diseases (Sun et al., 2007).

The interactions between chitosan-vitamin and chitosan-drug have been verified by monitoring the photophysical properties of these components. For this, fluorescence and UV-Vis absorption measurements were initially evaluated in acid aqueous solution and after in weakly acidic solution of chitosan given information about the interactions between this chemical component in conditions that approaches the stomach chemical environment.

2. Experimental

2.1. Chemicals

Chitosan and fluoxetine hydrochloride were purchased from Aldrich Chemical Co. (St. Louis, MO, USA), and vitamin B2 (riboflavin, 96%) and vitamin B12 (cyanocobalamin,, USP Grade) from Vetec Co. (Duque de Caxias, RJ, Brazil).and Merck Co. (Darmstadt, Hessen, Federal Republic of German), respectively. Other chemicals were ultraviolet/high-performance liquid chromatography grade and used without further purification; ultrapure water was supplied by a Milli-Q system.

2.2. Spectroscopic measurements

Previous studies have shown that the best conditions to solubilize chitosan are: chitosan 1% (w/v) dissolved in aqueous solution of glacial acetic acid 1% (v/v) under stirring (Signini & Campana Filho, 1999; Rodrigues, 2005; Rodrigues et al., 2008), so measurements in presence of chitosan were conducted in these conditions.

Chitosan has no fluorescence emission or absorption in the experimental conditions. The absorption spectra of chemicals (fluoxetine, B2 and B12) were measured using quartz cuvettes with 1 cm of optical pathway. The fluorescence measurements were performed at λ_{excit} = 275 nm and λ_{emis} = 305 nm for vitamin B12 (Li and Chen, 2000); at λ_{excit} = 440 nm and λ_{emis} = 305 nm for vitamin B2 (United States Pharmacopeia, 2007; Association of Official Analytical Chemists, 2005); and at λ_{excit} = 230 nm and λ_{emis} = 290 nm for fluoxetine (United States Pharmacopeia, 2007; Association of Official Analytical Chemists, 2005).

Absorbance measurements were taken at maximum of the absorption spectra and performed at room temperature.

Initially, variations of both fluorescence and absorption spectra of the chemicals (fluoxetine, B12 and B2) were taken as a function of their concentration in acid aqueous solution and after in different concentrations of chitosan in aqueous acid solution, at the same range of chemicals concentration. The variation in the spectra of the chemicals (fluoxetine, vitamins B2 and B12) was also studied by keeping fixed the concentration of vitamin and varying the concentration of chitosan in acid aqueous solution).

3. Results and discussion

Absorption spectra and chemical structures of chemicals are shown in Figure 2. Spectral data in Figure 2.a shows that vitamin B12 absorb significantly within 425–600 nm range, as described in the literature (Zheng & Lu, 1997; British Pharmacopoeia, 1998) and the present work chose the absorption peak at ~550 nm to assess the spectral behavior. Similar graphs have been obtained for vitamin B2 and fluoxetine. Figure 2.b shows that the absorption maximum for the vitamin B2 occurs at ~440 nm, data consistent with the literature ((United States Pharmacopeia, 2007). Figure 2.c. shows the absorption spectrum of fluoxetine

consistent with the literature (Fregonezi-Nery et al., 2008) that exhibits two absorption maxima at 270 and 275 nm. The last one maximum was chosen to monitor the spectral behavior of fluoxetine.

The chitosan-chemicals (fluoxetine, vitamins B2 and B12) interaction have been studied in aqueous acid solution by the monitoring the fluorescence and UV-visible spectra of chemicals, each monitored separately.

In three cases, the increase in concentration of chemical causes an increase in both absorption and fluorescence intensities due to the increase of species that absorb and emit light and this increases is linear profile always indicating that self-aggregation processes are not occurring in this concentration range (data not shown).

Subsequently, chemicals (fluoxetine, vitamins B2 and B12) were studied in the absence and the presence of chitosan, at concentrations 0.050 g.L^{-1} , 0.60 g.L^{-1} and 1.0 g.L^{-1} of polysaccharide, keeping fixed the chemicals concentration ($8.5 \times 10^{-5} \text{ mol.L}^{-1}$). With the increase of chitosan concentration both fluorescence and absorption intensities of chemicals are increased.

Figures 3, 4 and 5 show the behavior of fluorescence intensities to fluoxetine, vitamin B12 and B2, respectively. In all graphics, fluorescence intensities significantly increase when chitosan concentration goes from zero to 1.0 g.L^{-1} . This is a common behavior of fluorescent molecules when they migrate from the solution for environment of different polarity (Kalyanasundaram, 1987) and is due to the influence of microenvironment formed by chitosan on the photophysics of the chemical that is changed due to spatial hindrance that it suffers and due to loss of part of rotational freedom of substituent groups, (Kalyanasundaram & Thomas, 1977; Valeur, 2001). Then, with increase concentration of chitosan, the microenvironment becomes more rigid and the lifetime of the chemicals (fluoxetine, vitamins B2 and B12) in the excited states are living longer (Kalyanasundaram, 1987). However, fluorescence intensities of vitamin B12 and fluoxetine show similar increase rate while vitamin B2 is markedly lower. The increase of fluorescence intensities with the polysaccharide concentration has been observed also to vitamin in pharmaceutical formulations containing dextran (Alda et al., 1996).

Absorption intensities of chemicals (fluoxetine, vitamins B2 and B12) increase with the chitosan concentration similarly to the of fluorescence intensities, Figure 6. The reason for this behavior is the increased stiffness of environment generated by chitosan chains. However, in this case, intensities show the following increasing order: vitamin B2, fluoxetine and vitamin B12.

In chemical structure of all chemicals (fluoxetine, vitamins B2 and B12) there are rings with double bonds and polar groups that can interact strongly with the similar groups of chitosan. There are also OH groups in the molecular structure of chitosan favor hydrogen-bonding type interactions with polar groups of chemicals. These interactions can influence the absorption and the emission processes of radiation of molecules reducing the rotational degrees of freedom of the molecule (Ramamurthy, 1991).

In general way, results demonstrated that three chemicals (fluoxetine, vitamins B2 and B12) are transferred to microenvironment generated by weakly acidic solution of chitosan but in different proportions, due to the structural feature and solubility of each. Table 1 describes the relative increase of the fluorescence and absorption intensities of the chemicals when chitosan concentration ranges from zero to 1.0 g.L⁻¹.

Both vitamins belong to the class of hydro soluble vitamins (Sun et al., 2007), while the fluoxetine drug is slightly soluble in water (Darwish, 2005). The low solubility promotes some molecules of fluoxetine to migrate from aqueous environment to the more rigid environment generated by chitosan (the higher the concentration) causing a proportionately greater increase of absorbance and fluorescence intensities. However, the fluorescence intensities of vitamin B12 also increase in the same proportion and the absorbance intensities in proportion even higher, despite the hydro soluble nature of this vitamin.

Figure 2. Absorption spectra and chemical structures of vitamin B12 (A), vitamin B2 (B) and fluoxetine (C).

These results demonstrate that the transfer process of chemicals from aqueous environment to the chitosan aggregates is influenced by solubility of the molecules in water and/or by the molecular structure. Particularly, the structure molecular of vitamin B12 seems to have an interesting effect in this case. Vitamin B12 belong to the cobalamins, a class of octahedral Co(III) complexes which contain a planar framework called a corrin, with the metal center coordinated in the equatorial position by the four corrin nitrogens. Her energies of excited states are sensitive to the nature of the ligands of center coordinated and are influenced by water content of the surrounding environment (Solheim et al., 2011). These characteristics may be the reason for the more significant increase of the spectral properties of the vitamin B12, with increasing concentration of chitosan (lower water content), compared with the vitamin B2.

In fact, some molecules of fluoxetine or vitamins B2 or B12, are transferred to microenvironment generated by weakly acidic solution of chitosan. Among the three, the vitamin B2 is transferred in a smaller proportion. However, for all of them is expected that its loss in the diet, caused by administration of chitosan, is not so significant.

From our observations, possible risks to the patient should be considered when prolonged treatment with chitosan is prescribed and perhaps extra care should be taken when chitosan and fluoxetine are prescribed together in slimming diets. In the case of vitamins, essential for many physiological functions, there must be some precautions to minimize the impacts generated by this therapeutic, as the replacement of nutrients in the diet of patient.

Figure 3. Fluorescence spectra of fluoxetine in acid aqueous solution of chitosan. Chitosan concentrations: 0.00; 0.050; 0.60 and 1.0 (from the base to the top).

Figure 4. Fluorescence spectra of vitamin B12 in acid aqueous solution of chitosan. Chitosan concentrations: 0.00; 0.050; 0.60 and 1.0 (from the base to the top).

Figure 5. Fluorescence spectra of vitamin B2 in acid aqueous solution of chitosan. Chitosan concentrations: 0.00; 0.050; 0.60 and 1.0 (from the base to the top).

Figure 6. Behavior of absorption intensities of fluoxetine, vitamin B12 and B2.

Chemicals	I	ABS
Vitamin B12	107 %	80 %
Vitamin B2	30 %	14 %
fluoxetine	109 %	40 %

Table 1. Relative increase on the fluorescence (I) and absorption (ABS) intensities of the fluoxetine, vitamins B2 and B12 when chitosan concentration ranges from zero to 1.0 g.L⁻¹.

This paper seeks to warn to possible problems connected with the excessive loss of vitamins and other nutrients by the body during prolonged treatment with chitosan, as well as due the concomitant use of chitosan and fluoxetine.

4. Conclusions

Innumerous studies have described the formation of aggregates in naturals (Kim et al., 2000; Pelletier et al., 2000; Zhbakov et al., 2003), as chitosan ((Rodrigues, 2005; Hennen, 2005; Rodrigues et al., 2008) and synthetic (Kalyanasundaram, 1987; Neumann & Rodrigues, 1994; Neumann et al., 1995; Gomes et al., 2006; Gomes et al., 2007; Sur, 2010) polymers solutions and these molecular structures occur due to intra- and intermolecular interactions.

Chitosan is a polysaccharide precursor of materials suitable to release and/or dissolve drugs into the human body (Hennen, 2005), among other uses. However, the study of spectral properties of chemicals, fluoxetine, vitamins B2 and B12, demonstrated that the microenvironment generated by weakly acidic solution of chitosan also is able to sequester some B2, B12 and fluoxetine molecules, despite the hydro soluble nature of vitamins.

The results described in the current work demonstrated the wide range of possibilities in the studies of interaction between chitosan, used in diets as anti-obesity supplement, and molecules that are present in the human body, as well as with other drugs. Moreover, our work demonstrates the need for more studies on the subject as a means of providing information on the use of chitosan in diets as anti-obesity supplement.

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