IN VITRO AND *IN VIVO* REMOVAL OF ORAL ANTIDIABETIC AGENTS (METFORMIN) USING ACTIVATED CARBONS

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Introduction

Diabetes is the most worldwide common chronic disease, according the International Diabetes Federation [1], more than 32 million citizens living in the European Union have diabetes, representing nearly 10% of the population, to which we should add equal number of people suffering from impaired glucose tolerance. Diabetes prevalence is growing at alarming rate worldwide, being of particular relevance the type 2 diabetes. Nowadays 285 million people worldwide live with diabetes and it is expected that this numbers will increase by 20% until 2030 due to obesity and the ageing of the population [1].

This growth leads to an increasing consumption of drugs such as oral antidiabetics. Metformin is one of the active principles most commonly used for this purpose being among the pharmaceuticals with the highest production numbers worldwide to treat type 2 diabetes because is cheap, has high level of tolerance and when used in the prescribed dosage is very secure with minimal side effects. However, in case of overdose of metformin upon a ingestion of more than 10 times the prescribed dosage, accidentally or on propose, lactic acidosis and low blood pressure can occur. Overdoses with metformin are relatively uncommon, but may have serious consequences, if medical attention is not given on time, it may lead to coma and ultimately death Because of its spread use another problem must be taken into consideration, which needs to be addressed, the occurrence of metformin residues in sewage and surface waters due to improper discharge of the non-used tablets to regular garbage [2]. This situation is becoming a serious problem of environmental pollution and public health.

This paper reports the use of activated carbon produced from biomass for the removal of metformin in 2 different settings. On one hand, from aqueous solutions and, in another hand, from simulated biological fluids (gastric and intestinal) conjugated with *in vivo* testing.

Experimental

The activated carbon (AC) samples were produced by carbon dioxide activation at 700ºC from vine shoots (V739), and 800ºC from coffee endocarp (Cf840), esparto grass (E853) and Eucalyptus pulp (P827). The last 2 digits stand for the burn-off level, in wt%. The experimental details can be seen elsewhere [3]. Batch adsorption experiments were carried out in a series of Erlenmeyer flasks inserted in a shaking thermostat at 25ºC for 120 minutes using 15mL of aqueous solutions of metformin, with concentration 1.6 to 550mg/L, and 0.05g of AC. Another set of experiments were done at 37ºC using simulated body fluids, pepsin omitted, namely intestinal fluid (SIF, pH=7.5) and gastric fluid (SGF, pH=1.2). The metformin quantification was done by UV-Vis spectrophotometry at 233nm.

Samples P827 and E853 were selected to perform the *in vivo* experiments. This work was done in 16 Wistar rats weighing 198 to 368 g, which were distributed in three groups. To Group 1 the metformin was administered at a dose 600 mg/kg that corresponds to approximately 60% LD50. To Group 2 the same dose of metformin and activated carbon were administered, and finally to the third group only activated carbon was given to the animals, in the same dosage as Group 2. After oral administration the animal behaviour was observed. We have analyzed the serum and urine collected from the rats in order to study a number of biochemical markers, such as amylase, lipase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine. The glycaemia of the animals were analysed at pre-determined time intervals.

Results and Discussion

The principal properties of the ACs can be seen in table 1. The materials are all basic with point of zero charge (pzc) close to 10 but with different porous structure. Although, the apparent BET surface area range from 598 to 1122m^2g^{-1} and the pore volume (V_s) from 0.31 to 0.46cm^3g^{-1} all ACs are essentially microporous, as indicated by the N_2 adsorption isotherms (not shown here), which are all type I according the IUPAC classification.

Table 1. Textural and chemical characterization of ACs

Sample	BET S_{BET} /m^2g^{-1}	Alfa s V_s /cm^3g^{-1}	Alfa s S_{ext} /m^2g^{-1}	DR V_0 /cm^3g^{-1}	pzc
E853	1122	0.46	84	0.47	9.52
P827	649	0.29	25	0.26	10.7
Cf840	598	0.32	11	0.32	10.2
V739	708	0.31	49	0.30	9.96

The *in vitro* metformin maximum adsorption capacity is shown in table 2. First of all for the adsorption from aqueous solutions, which evaluates the ability of the tested ACs to be used in water treatments, we can observe that the highest adsorption capacity was observed for samples P827 and E853, which reach 30 and 33mg/g, respectively. It seems that the adsorption, in this case, is determined by the porous development of the samples.

Regarding the adsorption from simulated body fluids, it is visible that the metformin adsorption from SGF (pH=1.2) presents a totally different behaviour with adsorption capacity

much higher than the other experimental conditions tested. The maximum capacity to remove metformin was achieved for samples V739, P827 and E853 with approximately 130mg/g. This high capacity to capture metformin at gastric conditions is crucial to prevent the absorption of the drug, which occurs mainly at the intestinal tract. The experiments done using SIF (pH=7.3) show similar results to those performed with aqueous solutions (pH=7.5) probably due to the similarity of the pH.

It is also interesting to observe that at low pH all ACs show almost the same adsorption capacity, regardless the pore volume or apparent BET area, thus it seems that for SGF conditions the driving force for adsorption is the chemical interactions between the surface of the ACs and the totally ionised metformin molecule.

Table 2. Maximum adsorption capacity (mg/g) for the *in vitro* study

Sample	Aqueous Sol.	Simulated body fluids	
		SIF	SGF
E853	32.8	41.2	132.6
P827	29.5	23.4	130.2
Cf840	23.0	11.8	126.7
V739	15.3	15.8	130.9

The *in vivo* studies have shown that ACs did not introduce any behavioural change on the animal of the group 3. This indicates a high biocompatibility of the ACs samples tested without any visible toxic effects.

The animals of group 1 had suffered inflammation and renal dysfunction, as indicated by clearance of the creatinine, urea and the presence of leucocytes in the urine. On the opposite, the animals of group 2 did not shown symptoms of renal malfunction, which indicates that the activated carbons prevent this to occur by the adsorption of the metformin. The glycaemia values of the animals in groups 1, 2 and 3 show similar trend after the administration of a glucose bolus at time zero, as can be seen in figure 1.

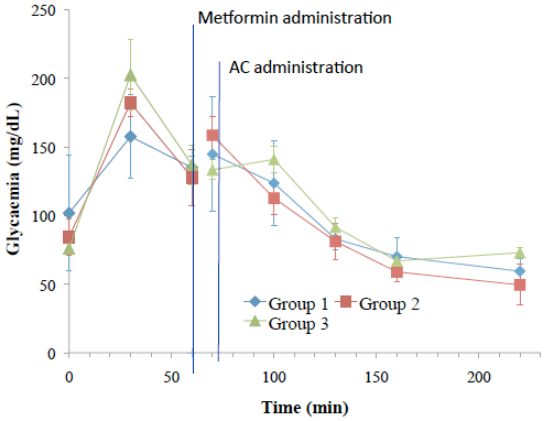

Fig. 1 Glycaemia analyses

Other biochemical markers such as amylase and lipase, ALT, AST showed values for animals in groups 1, 2 and 3 indicating the non-existence of serious injuries at pancreas, hepato-biliary disorders, acute myocardial infarction, respectively.

Conclusions

1) The ACs tested can be efficiently used to remove metformin from water streams, in particular samples E823 and P827.

2) The in vitro adsorption on simulated body fluids has shown that adsorption is dependent on the pH and constitution of the solution.

3) The maximum adsorption capacity is achieved at gastric conditions, which is fundamental to prevent the drug absorption at intestinal tract.

4) The in vivo studies supported the non-toxic properties of ACs and the its capability to prevent the toxic impact of a metformin overdose.

5) It is viable to use the ACs samples for the treatment of drug poisoning due to its high biocompatibility and adsorption capacity.

6) If a person was to ingest 10 pills of 850mg of metformin and this was to mix with 1L of stomach fluid, a drug concentration of 8.5g/L would result, which is in the range of an expected overdose. Taking into account the maximum adsorption capacity of sample E853 we can estimate a dose of 140g of activated carbon slurry to treat the person.

7) Sample E823 showed the best performance in all settings studied.

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