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Activated Carbons: In Vitro Affinity for Aflatoxin B_1 and Relation of Adsorption Ability to Physicochemical Parameters

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ABSTRACT

Affinity in vitro tests were conducted of the efficacy of 17 activated carbons (ACs) in binding aflatoxin B_1 from solution. Relationships between adsorption ability and physicochemical parameters of the ACs (surface area, iodine number, methylene blue index, and surface acidity) were tested. Using 5 ml of a 4 µg/ml aqueous solution of aflatoxin B_1 and 2 mg of an AC, adsorption abilities ranged from 44.47% to 99.82%. Four ACs showed very high adsorption abilities, binding more than 99% of the available aflatoxin B_1. In comparative testing five ACs showed a greater ability to bind aflatoxin B_1 than hydrated sodium calcium aluminosilicate (HSCAS). Three ACs also showed high adsorption abilities (ca. 99%) at increasing aflatoxin B_1 concentrations (50 and 250 µg/ml) whereas HSCAS adsorption ability greatly declined. With the exception of three ACs, aflatoxin B_1 adsorption was significantly correlated with all the physicochemical parameters, confirming a close relationship between molecule trapping and the surface physicochemical adsorption process. The methylene blue index was more reliable than iodine number and surface area in predicting AC adsorptive ability. The results suggested that ACs with a high methylene blue index and low surface acidity have a very high in vitro affinity for aflatoxin B_1; however, their efficacy in protecting against aflatoxicosis should be verified further by in vivo tests.

Key words: Activated carbons, aflatoxin B_1, binding, detoxification

Detoxification of aflatoxin-contaminated foods and feeds is a current problem, as aflatoxins are toxic, carcinogenic, mutagenic and teratogenic in animals including humans *(7)*.

According to Piva et al. *(20)*, although the principal chemical detoxification methods are very effective, they do not seem able to fulfill all the requirements, especially those concerning the safety of reaction products and the safeguarding of the nutritional characteristics of the treated foods and feeds.

The addition of sorbents in contaminated feeds to reduce aflatoxin absorption in the intestine is one of the most important approaches aimed at reducing the risk of aflatoxicosis or in limiting decreases in animal performance and toxic metabolite carryover in milk, meat, and eggs.

Some in vitro tests *(17)* showed that hydrated sodium calcium aluminosilicates (HSCASs) are capable of binding aflatoxin B_1 (AFB_1) in aqueous solution. Definitive information on the mechanism cannot be furnished yet, even if surface physicochemical adsorption phenomena should occur. The AFB_1 molecule could easily remain imprisoned within the typical complex stratified-reticular structure of the HSCAS. Phillips et al. *(18)* suggested that the molecular mechanism of AFB_1 binding may involve the chelation of metal ions in HSCASs with the β-dicarbonyl moiety in AFB_1. Evidence of the validity of this binding action arises from numerous in vivo studies *(20)*. Other sorbents such as zeolites, bentonites, and modified phylloaluminosilicates were investigated in detoxification tests. In vivo tests on weaning piglets *(24)*, dairy cows *(19)*, and broilers of domestic fowl *(23)* showed contrasting data on the efficacy of zeolites, whereas both in vitro *(21, 32)* and in vivo *(28, 29)* studies found bentonites to be efficacious sequestering agents.

Activated carbons (ACs) are another important group of sorbents. They are a family of carbonaceous substances manufactured by activation processes aimed at developing a highly porous structure *(1)*. Because of their remarkable adsorptive properties they are customarily used in decolorization processes, wastewater treatment, vapor-phase treatment of toxic air emissions, and heterogeneous catalysis.

Dalvi and Ademoyero *(5)* and Dalvi and McGowan *(6)* reported a low ability of an activated charcoal to reduce AFB_1 toxic effects in chickens. Kubena et al. *(12)* similarly showed no efficacy for protection and also an increased toxic effect. Studies on mink *(3)*, duckling *(22)*, and chickens *(11, 27)* reported variable efficacy of activated charcoals in prevention of aflatoxicosis.

Nevertheless, because of the wide typology of carbonaceous substances and activation processes numerous ACs with quite different properties are available. Unfortunately,

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there is no single universal test of the adsorptive properties of ACs; therefore they must be selected under application conditions. However, to well characterize ACs several physicochemical parameters can be considered *(2, 30, 31)*. It is known that the adsorption properties are roughly correlated with the total surface area (SA) measured by adsorption of a very small molecule (nitrogen) by the method of Brunauer et al. *(4)*. However, the pore-size distribution of AC is an important property and general information about the accessibility of the internal carbon surface can be obtained from such a distribution. As diffusion effects inside the pores can slow down the adsorption process *(25)*, the effective pore-size distribution of AC can influence adsorption as a function of the molecular size of the adsorbate. Information about mesopores and macropores can be obtained by mercury porosimetry, which allows determination of the size distribution of pores of inside diameter (i.d.) greater than 75 Å (7.5 nm). Iodine number (IN) is a relative indicator of AC microporosity and it is often used as an approximation of SA because of the simple equipment required. However, it must be realized that any relationship between SA and IN can not be generalized. The methylene blue index (MBI) is a test which covers predominantly the medium-size-pore (mesopores) range and is an important indicator in practice of AC ability to adsorb organic molecules of medium-large size from a solution *(16)*. In this study the MBI was used because of the similarity of the molecular sizes of methylene blue and AFB_1. Recently also the importance of surface properties of ACs, such as the presence of acidic or basic functional groups, has received growing attention in attempting to improve AC performance. However, different source materials, preparation methods, and chemical treatments can greatly modify the surface characteristics of ACs *(9, 15)*.

The objective of the present investigation was to evaluate the AFB_1 affinity in vitro of ACs and compare them with that of an HSCAS. Moreover, the wide variability of physicochemical properties of ACs suggested we investigate the relationships between the ability to bind AFB_1 and some specific physicochemical parameters (PCPs) of ACs.

MATERIALS AND METHODS

Sorbents

The following sorbents were tested: 13 experimental activated carbons (EACs) from exhausted olive residues (AF), peach stones (PEP2), and almond shells (MAP2) obtained with laboratory equipment of the Chemistry Department of the University of Catania, by several experimental activation processes appropriately selected to obtain the desired PCP; four commercial AC (CAC) produced in industrial processing equipment; an HSCAS demonstrated to have an high affinity in vitro for AFB_1 *(17)* and to reduce aflatoxin toxicity *(8, 12–14, 17)*. All the sorbents were finely pulverized.

Activated carbon: physicochemical parameters and measurement equipment

The PCP of the ACs are shown in Table 1. SA (m^2/g) was determined by a Sorptomatic 1800 (Carlo Erba, Milan, Italy) using N_2 adsorption at 77° K and the two-parameter equation of Brunauer et al. *(4)*. Mercury porosimetry was performed up to a pressure of 2,000 kg/cm^2 using a Carlo Erba Porosimeter (model 2000) equipped with a macropore unit. Determination of IN (mg of I_2 adsorbed by 1 g of AC) was carried out according the ASTM method D4607-86 *(1)*. MBI (mg of methylene blue adsorbed by 1 g of AC) was measured by adsorption tests carried out at 298°K in aqueous solution followed by UV-VIS analysis at 620 nm (Hewlett Packard 8452A, Hewlett Packard Co., Palo Alto, CA, USA) *(16)*.

TABLE 1. *Physicochemical parameters (PCP) of experimental (EAC) and commercial (CAC) activated carbons*

Activated carbon[a]	Surface area (m^2/g)	Iodine number (mg/g)	Methylene blue index (mg/g)
EAC			
AF13	561.1	728.0	34.0
AF24	291.0	437.0	16.2
AF29	433.7	566.0	14.0
AF32	787.2	931.0	109.6
AF33	260.3	237.0	1.41
AF34	309.0	292.0	3.8
AF35	309.9	162.0	4.8
AF37	403.0	450.0	NA[b]
AF45	493.1	563.0	NA
AF47	482.0	575.0	NA
AF48	865.0	966.0	239.4
PEP2	943.0	811.0	206.0
MAP2	1254.0	1082.0	256.0
CAC			
CAC1	1122.0	1100.0	200.0
CAC2	1116.0	1250.0	200.0
CAC3	905.0	1350.0	260.0
CAC4	1059.0	1100.0	283.0

[a] Sources of ACs: AF, olive residues; PEP, peach stones; MAP, almond shells.
[b] NA, not assayed.

AC surface acidity was measured in a conventional apparatus by the temperature-programmed desorption technique (TPD) of adsorbed NH_3 *(26)*. Samples (ca. 0.7 g) were flushed with He at 600°C, then exposed to a mixture of 1.5 vol % of NH_3 in He at room temperature. The physisorbed NH_3 was removed in flowing He and then TPD experiments were run from room temperature to 600°C with a heating rate of 5°C/min using He as carrier gas (30 cm^3/min). NH_3 desorption was followed using a gas chromatograph equipped with a thermal conductivity detector.

Samples of each sorbent were individually weighed into glass tubes (three replicates per sample) and amounts of AFB_1 (Sigma Chemical Co., St. Louis, MO, USA, purity > 99%) in aqueous solution were added. After a reaction time of 1 h at 25°C, with mixing at 15-min intervals, all the tubes were centrifuged for 10 min at 1,500 rpm and then 100 µl of supernatant were collected for dehydration of the liquid phase by absolute ethanol and derivatization by trifluoroacetic acid. Finally, AFB_1 (as hydroxy AFB_2) determination was carried out by high-performance liquid chromatography (HPLC).

Four adsorption tests were carried out, varying the amounts of AFB_1 and AC. In test 1, 1.0 ml of a solution containing 10 µg of AFB_1 was added to 100 mg of each sorbent. In test 2, 5 ml of a solution containing 4 µg of AFB_1 was added to 2 mg of each sorbent. In this test, relationships between AFB_1 adsorption and the PCPs of the ACs were investigated. To differentiate AC adsorption ability and determine the saturation limit, in tests 3 and 4, respectively, 5 ml of two solutions containing 10 and 50 µg of AFB_1

were added to 2 mg of each of the ACs capable of binding close to 100% of AFB_1 in test 2, and to 2 mg of HSCAS for comparison.

HPLC apparatus and procedure

The HPLC instrument used was a Perkin-Elmer Series 3B (Perkin-Elmer Corp., Norwalk, CT, USA) equipped with ISS 100 sampling system and LCI 100 computing integrator; detector: Perkin-Elmer LS 4 fluorescence spectrophotometer at excitation and emission wavelengths of 365 nm (slit 15 nm) and 440 nm (slit 20 nm), respectively; stationary phase: LiChrosorb RP-18 (250 by 4 mm (i.d.) column) 7 µm (Merck, Darmstadt, Germany); mobil phase: acetonitrile (A)—water (B). The program was 20A plus 80B to 50A plus 50B, 0 to 12 min; 100A plus 0B, 13 to 14 min; 20A plus 80B, 15 to 21 min; with a flow rate of 1.5 ml/min. Retention time for AFB_1 was 8.4 to 8.5 min.

Statistics

AFB_1 adsorption data are presented as means ± SEM of three replicates per sample and $P < 0.05$ values were considered to be significant (Duncan multiple range test). Relationships between AFB_1 adsorption and PCPs were tested by the best fit to linear and nonlinear function models (Fig. P®, Biosoft®, Ferguson, MO, USA).

RESULTS

In test 1, all ACs showed very high abilities to bind AFB_1 (ca. 100%) and no difference between their adsorption abilities was found (data not shown). In test 2, CAC1, CAC2, AF48, and AF32 showed very high adsorption abilities (Figure 1), binding more than 99% of the available AFB_1. CAC4 showed a remarkable adsorption ability too, adsorbing 95.52% of AFB_1. All of these ACs showed a significantly higher abilities to bind AFB_1 than HSCAS (89.34%).

In tests 3 and 4, the ACs also showed overall high adsorption abilities as AFB_1 increased (Table 2). CAC1, CAC2 and AF48 adsorbed more than 99% of the available

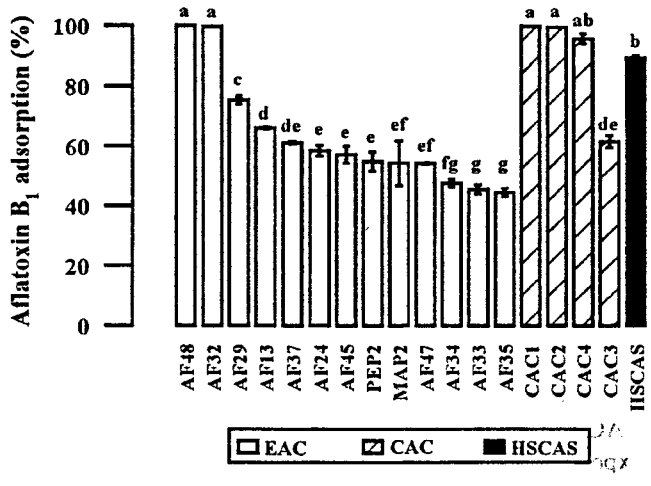

FIGURE 1. *Aflatoxin B_1 adsorption from aqueous solution by experimental activated carbons (EAC), commercial activated carbons (CAC), and hydrated sodium-calcium aluminosilicate (HSCAS). Each bar represents the mean,* n = *3; error bars denote standard error of the mean; different letters on tops of bars mean significantly different,* $P < 0.05$.

TABLE 2. *Adsorption ability of activated carbons and HSCAS at increasing AFB_1 concentrations*

	Adsorption of available AFB_1, (%)[a]		
	AFB_1 (µg/ml)[b]		
Sorbents[c]	4	10	50
CAC1	99.80 ± 0.00A	98.72 ± 0.70A	98.72 ± 0.32A
CAC2	99.57 ± 0.06A	98.69 ± 0.79A	98.10 ± 0.38A
AF32	99.65 ± 0.03A	99.28 ± 0.58A	89.79 ± 0.73B
AF48	99.97 ± 0.01A	99.63 ± 0.06A	99.44 ± 0.10A
HSCAS	89.34 ± 0.91B	77.10 ± 1.77B	62.97 ± 1.50C

[a] Values indicate group mean ± SEM; $n = 3$. Different letters in columns represent significant differences ($P < 0.05$).

[b] Five ml of solution added to 2 mg of sorbent.

[c] CAC, commercial AC; AF, olive residue AC; HSCAC, hydrated sodium-calcium aluminosilicate.

AFB_1, from 10-µg/ml (50 µg of AFB_1) and 50-µg/ml (250 µg of AFB_1) solutions. AF32 adsorption ability slightly decreased from 99.28% (10-µg/ml solution) to 89.79% (50-µg/ml solution). The adsorption ability of HSCAS declined greatly as AFB_1 increased (77.10% and 62.97% from 10-µg/ml and 50-µg/ml solutions, respectively) and was significantly lower than those of the ACs.

AFB_1 adsorption was correlated with SA and IN respectively according to the linear equations: AFB_1 adsorption = 0.07 SA + 32.28 ($r^2 = 0.84$) (Figure 2); AFB_1 adsorption = 0.07 IN + 31.03 ($r^2 = 0.90$) (Figure 3).

The best-fitting equation model of regression for the MBI of AFB_1 adsorption was monoexponential (1st-order)

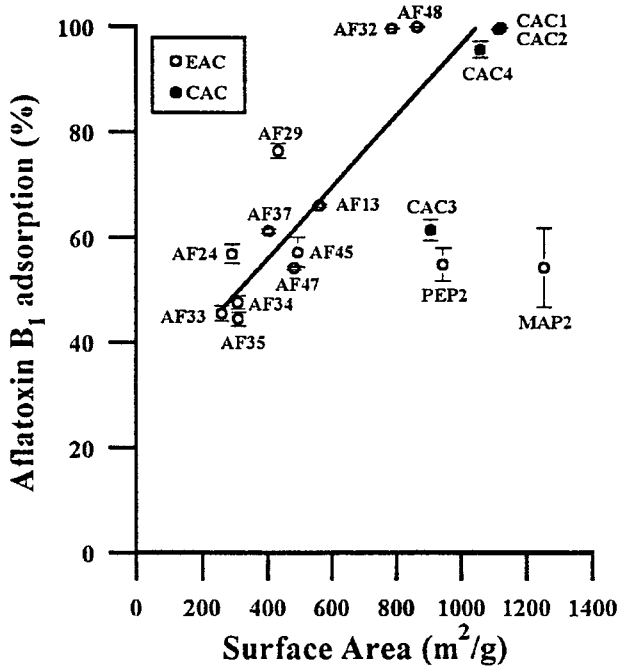

FIGURE 2. *Relationship between surface area of experimental (EAC) and commercial (CAC) activated carbons and adsorption ability for aflatoxin B_1. Each point represents the mean,* n = *3; error bars denote standard error of the mean. CAC3, PEP2, and MAP2 were not considered as data for the regression.*

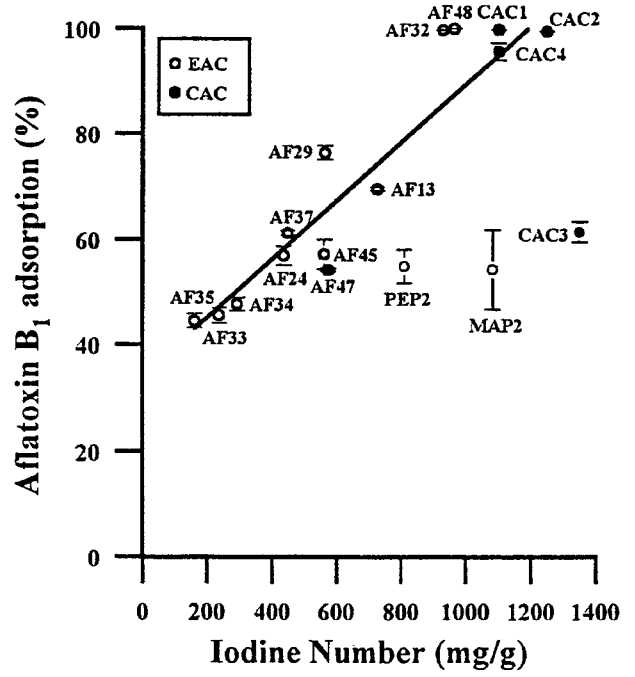

FIGURE 3. *Relationship between iodine number of experimental (EAC) and commercial (CAC) activated carbons and adsorption ability for aflatoxin B_1. Each point represents the mean,* n = *3; error bars denote standard error of the mean. CAC3, PEP2, and MAP2 were not considered as data for the regression.*

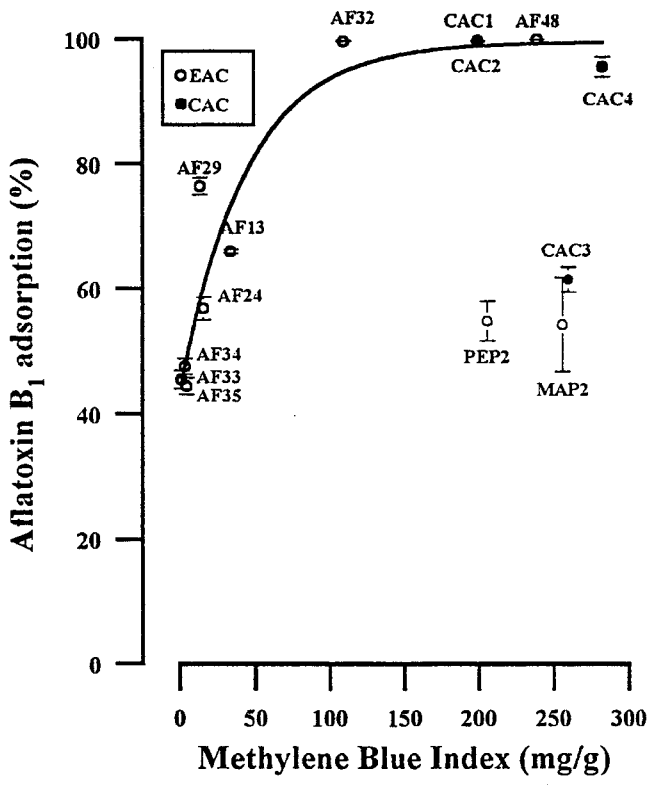

FIGURE 4. *Relationship between methylene blue index of experimental (EAC) and commercial (CAC) activated carbons and adsorption ability for aflatoxin B_1. Each point represents the mean,* n = *3; error bars denote standard error of the mean. CAC3, PEP2, and MAP2 were not considered as data point of the regression.*

decay with residual (Figure 4) ($Y = a^{-kX} + R$, where the variables were $a = -56.4$; $k = -0.02$; $R = 99.57$). CAC3, MAP2, and PEP2 data were not considered in the regressions.

DISCUSSION

All data showed a very high AC affinity in vitro for AFB_1. Phillips et al. *(17)* demonstrated the in vitro ability of various argiles, zeolites, silicates, and HSCAS to sorb AFB_1 using 0.1 μg of AFB_1/mg of sorbent and reporting adsorption ability ranging from 1.2% to 98.1%. In test 1, this latter AFB_1-to-sorbent ratio was adopted but no significant differences in all AC adsorption levels of AFB_1 (close to 100%) were observed. This fact suggested selection in test 2 of a 50-fold smaller amount of each sorbent (10 μg of AFB_1/mg of sorbent). Under these conditions sorbent ability ranged from 44.47% to 99.97%. Particularly CAC1, CAC2, CAC4, AF32, and AF48 showed a significantly greater adsorption abilities for AFB_1 than HSCAS. In tests 3 and 4, the ACs showed high adsorption ability at increasing AFB_1 as well. Probably the saturation conditions were not reached for CAC1, CAC2 and AF48. The saturation limit should be over 125 μg of AFB_1/mg of sorbent whereas the HSCAS saturation limit was ca.79 μg of AFB_1/mg of sorbent.

Regressions showed that AFB_1 adsorption was significantly correlated with all the PCPs, confirming the close relationship existing between molecule trapping and the physicochemical surface adsorption process.

With increasing SA, IN, and MBI a corresponding positive trend in AFB_1 adsorption was observed. Therefore an overall highly porous structure must be necessary to allow AC surface-active sites to bind the AFB_1 molecule. As SA, IN, and MBI are reciprocally correlated it is not easy to distinguish their roles in the adsorption process. Nevertheless, although the linear equations between AFB_1 adsorption and both SA and IN were highly significant, the MBI was more reliable than SA and IN in predicting adsorption ability for AFB_1. The exponential equation model tending to reach an asymptotic plateau (over 100 to 150 MBI values) (Figure 4) seems more appropriate to describe the trend of the adsorption than the linear model. Since the MBI is strictly correlated with mesoporosity, mesopores (i.d. from 20 to 500 Å [2.0 to 50.0 nm]) must be directly involved in AFB_1 adsorption and could increase adsorption ability because of a better fit to the AFB_1 molecule, whose width ranges from 10.83 to 12.78 Å (1.083 to 1.278 m) *(18)*. On the contrary, micropores (i.d. < 20 Å [2.0 nm]) could hinder the diffusion of the AFB_1 molecules inside the AC *(25)* and decrease the adsorption ability.

This trend should be confirmed by the behavior of CAC3 (Figures 2 to 4), whose performance was far from that expected by the fitted equations. Further investigations showed that CAC3 had a higher microporosity than the other ACs as confirmed by higher IN compared to its other PCPs and by mercury porosimetry, which revealed a very low amount of mesopores and macropores (spectra not shown). Moreover, temperature-programmed desorption of NH_3 (Figure 5) revealed that the surface acidity of CAC3 was higher

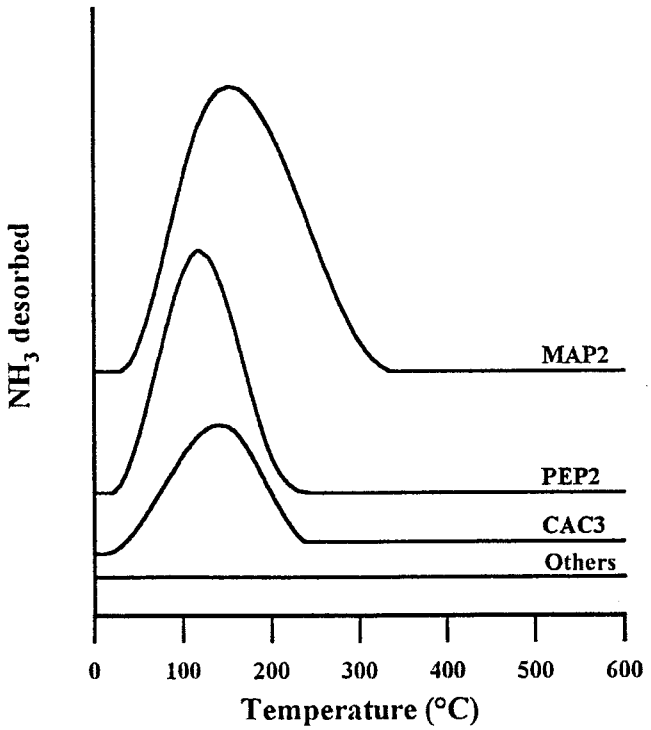

FIGURE 5. *Temperature-programmed desorption curves of ammonia adsorbed by activated carbons MAP2, PEP2, CAC3, and others.*

than those of the other ACs (no detectable ammonia was adsorbed on these latter samples).

These results indicated that both high surface acidity and microporosity could account for the different behavior of CAC3. To discriminate between these two parameters, two EACs (PEP2 and MAP2) were prepared using an experimental technique (impregnation of peach stones or almond shells with H_3PO_4, followed by activation) which leads to ACs with high surface acidity *(15)*. TPD curves (Figure 5) showed that the amount of NH_3 desorbed by PEP2 and MAP2 was much higher than for the other ACs. The presence of acidic groups on the surface of these carbons was further confirmed by Fourier-transform infrared spectroscopy, which demonstrated the presence of bands attributed to carboxylic and phosphate groups.

AFB_1 adsorption tests clearly showed that CAC3, PEP2, and MAP2 exhibited poor adsorption abilities for AFB_1, despite their highly porous structure. For this reason they were excluded from curve-fitting procedures. It also has to be noted that mercury porosimetry carried out on PEP2 and MAP2 revealed on these samples a large amount of mesopores and macropores (spectra not shown). Therefore it should be concluded that surface acidity strongly hinders adsorption ability towards AFB_1, independently from pore-size distribution, probably because of the acidity of the AFB_1 molecule. Moreover a steric hindrance effect of surface functional groups which block pore mouths has been reported in the literature *(10)*; however the chemical nature of the AC surface seems to be a fundamental physicochemical parameter in determining the ability of ACs to bind AFB_1. At the present time, a study on the possibility of improving adsorptionperformance using ACs with basic surface groups *(10)* is in progress in our laboratory.

In summary, our results demonstrate that ACs with high MBI and low surface acidity have greater affinity for AFB_1 in vitro than HSCAS and are potential protective agents against aflatoxicosis. Further in vivo investigations on ACs should establish the concentration in feed that can be efficacious and verify undesired preferential adsorption of useful organic molecules (e.g., vitamins or minerals). No toxic effect should occur, as ACs are currently used in water treatment and allowed for use in pharmacological preparations.

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