

# Effect of Active Radical Production on Plasma Degradation of Phorbol 12-Myristate 13-Acetate in Methanolic and Aqueous Solution

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**ABSTRACT:** In this study, we examine the degradation of phorbol esters with atmospheric pressure plasma and, in particular, the highly tolerant phorbol 12-myristate 13-acetate (PMA) molecule. We determined the degradation mechanism by analyzing the structural information of the by-product obtained using mass spectrometry. The plasma source was also improved to generate more active radicals, thereby enhancing the degradation of PMA.

**KEY WORDS:** Phorbol 12-myristate 12-acetate, atmospheric pressure plasma, degradation, active oxygen radical

## I. INTRODUCTION

Seed oil from the *Jatropha curcas* Linnaeus (*J. curcas*) plant is currently attracting attention for its potential applications in the biodiesel production industry.<sup>1</sup> Mechanical pressing of the *J. curcas* seed yields a rich *J. curcas* oil, which can be used as the raw material for producing biodiesel. However, *J. curcas* seed oil is toxic due to the presence of phorbol esters (PEs).<sup>2</sup> Skin irritation, inflammation, tumor promotion, and other problems arise from these biologically potent PEs,<sup>3</sup> which may also be stable in seed oil at temperatures as high as 200°C.<sup>4</sup> To reduce occupational health risks to workers and make the oil more environmentally friendly, PEs can be extracted from the seed oil by either methanol or ethanol.<sup>5</sup> A PE-enriched extract fraction has been proposed for use as a biopesticide.<sup>6</sup> However, such an application will require long-term evaluation regarding its environmental risks. To address these concerns effectively, a PE inactivation method in a methanolic or aqueous solution must be developed.

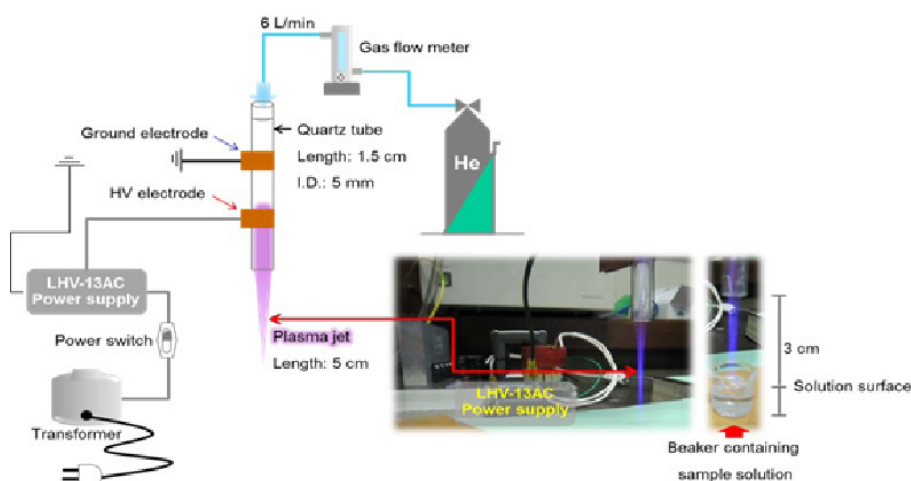
Gamma-ray irradiation from radioisotopes has been regarded as a more promising and efficient method for degrading PEs than biological or chemical methods due to the short treatment time involved and the fact that no additional chemicals are required.<sup>7</sup> Recently, atmospheric pressure helium plasma produced with a dielectric barrier discharge (DBD) was reported to have a similar degradation effect.<sup>8</sup> Both methods produce reactive radicals, such as hydroxy radicals, in the sample solution, which efficiently enhance the PE decomposition process. Degradation efficiency depends not only on the type of solvent used but also on the chemical structure of the PE molecule. The successful deg-

radiation of phorbol 12-myristate 13-acetate (PMA) in aqueous solution is difficult.<sup>8</sup> In our study, we examined in detail the degradation of PMA in methanol by DBD helium plasma and the associated by-products. An argon plasma jet, assisted by corona discharge, was also applied to PMA in water. This new plasma source efficiently produces reactive oxygen radicals and is highly efficient in PMA degradation.

## II. METHODS

The configuration of the DBD plasma system is shown in Fig. 1. Helium served as the working gas and was guided to flow through a quartz tube, which served as a dielectric barrier. The gas flow rate was constantly controlled by a flow meter (Kofloc, Model RK1710). When He gas molecules flow through an electric field area of an electrode supplied with high voltage at low frequency (LF-HV) using a commercial power supply source (Loggy Electronics Ltd, LHV-13AC), a DBD plasma jet is subsequently generated and emitted from the nozzle.

From the results of Kongmany et al.,<sup>8</sup> we expected that a long-term operation would be required to degrade PMA in water using this plasma. As such, this device has some drawbacks with respect to industrial applications. Its running cost is not economical due to the requirement for a huge amount of expensive helium gas for plasma production. Argon gas, which is cheaper than helium, is one possible alternative, but its breakdown voltage is much higher than that of helium. When high voltage is applied, the rapid proliferation of electrons after breakdown would be enhanced, leading to the formation of streamers or a filamentary arc. This result may induce surface damage on the treated samples. By modifying the power electrode to a needle type, Ar plasma can be produced using a lower applied voltage with the help of a corona discharge. Figure 2 shows a pho-



**FIG. 1:** Dielectric barrier discharge plasma system for irradiating the PMA solution

to of the Ar plasma jet produced as a new plasma source for aqueous solution irradiation. The needle of the high-voltage electrode of this plasma source is set at the center of the glass tube. Due to the field concentration effect, discharge between the electrodes starts at a few kilovolts (kV), and the plasma jet is ejected at approximately 5 kV for both the Ar and He working gases.

To monitor the change in the PE concentration and the formation of intermediate products, sample solutions before and after plasma irradiation were analyzed with an ultra-high performance liquid chromatographer equipped with a Shim-pack XR-ODSII (75 mm L  $\times$  0.25 mm I.D, 2.2  $\mu$ m) analytical column, a photodiode array detector, and an electrospray-ionization mass spectrometer. The injection volume was 10  $\mu$ L. A mixture of (A) aqueous ammonium acetate ( $\text{CH}_3\text{COONH}_4$ , 10 mM) and (B) acetonitrile ( $\text{CH}_3\text{CN}$ ) was used as the mobile phase, and injector was operated at a flow rate of 0.2 mL/min in a gradient condition: 20%A/80%B (0–2 min), 10%A/90%B (5 min), 10%A/90%B (8 min), 0%A/100%B (8.01 min), 0%A/100%B (10min), 20%A/80%B (10.10 min), and stop (15 min).

At this point, we assumed that the hydroxyl radical ( $\bullet\text{OH}$ ) would play a major role in decomposing PEs in the sample solution. If an OH radical is produced by plasma irradiation, it oxidizes the organic contaminants in the solution. After treatment, a recombination of  $\bullet\text{OH}$  radicals that do not react with other compounds to form hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) possibly occurs. For this scenario, we employed the KI colorimetric method to

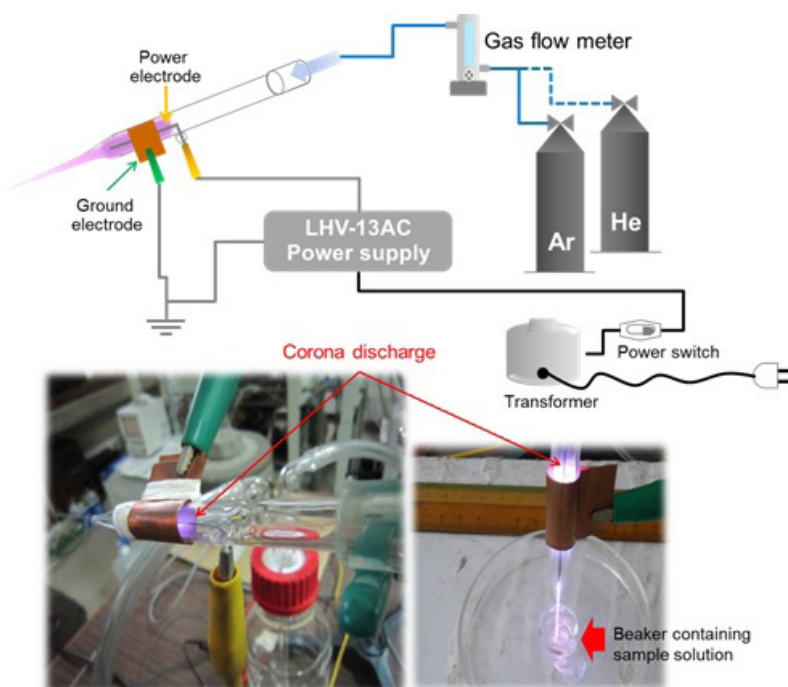


FIG. 2: Corona discharge assists the plasma system to irradiate the PMA solution

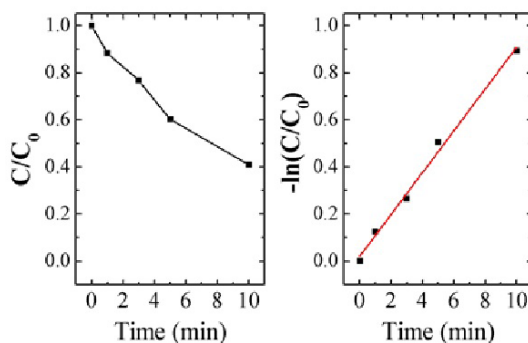
measure the hydrogen peroxide concentration.<sup>9</sup> The principle behind the method is that the  $\text{I}^-$  is oxidized by  $\text{H}_2\text{O}_2$  in a slightly acidic solution with a pH value of approximately 5.85, as shown in the reaction ( $3 \text{I}^- + \text{H}_2\text{O}_2 \rightarrow \text{I}_3^- + 2 \text{OH}^-$ ). The production of  $\text{I}_3^-$  was determined through the 352-nm UV absorption (absorption coefficient:  $2.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) using a UV-Vis spectrometer (Shimadzu UV-3100). The OH radical concentration was tentatively deduced from the measured  $\text{H}_2\text{O}_2$  concentration, which is equal to the  $\text{I}_3^-$  concentration.

### III. RESULTS

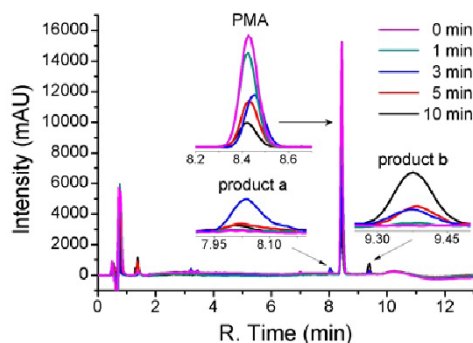
To investigate PMA degradation, 1 mL methanolic PMA solution in a 1.5-mL beaker was irradiated by directly exposing the surface of the solution to the plasma jet. The treatment time was set from 1 to 10 min. The results of the plasma-chemical degradation of PMA (10 mg/L) in methanol are expressed in a  $C/C_0$  ratio (where  $C$  and  $C_0$  are the concentrations of PMA after and before treatment, respectively) versus treatment time (Fig. 3). PMA was exponentially degraded, conforming pseudo-first-order kinetics with a constant of  $1.53 \times 10^{-3} \text{ s}^{-1}$ . The PMA degradation leads to the formation of a new compound, which appears in Fig. 4 as new peaks in the high-performance liquid chromatography (HPLC)/UV chromatogram. The intermediate products exhibit 4–5 nm lower or higher maximum UV absorption bands compared to those of PMA (Fig. 5).

Figure 6 shows that the mass spectra of both PMA and the by-product include a mass difference of  $-288$ ,  $-60$ , and  $-18$  corresponding to the cleavage of myristyl, acetyl, and the hydroxyl groups with protons, respectively. The molecular weight ( $M_w$ ) of the by-product ( $M_w = 648$ ) was higher than that of PMA ( $M_w = 616$ ) by approximately 32 mass units. This may be consistent with the mass of 2 oxygen (O) atoms.

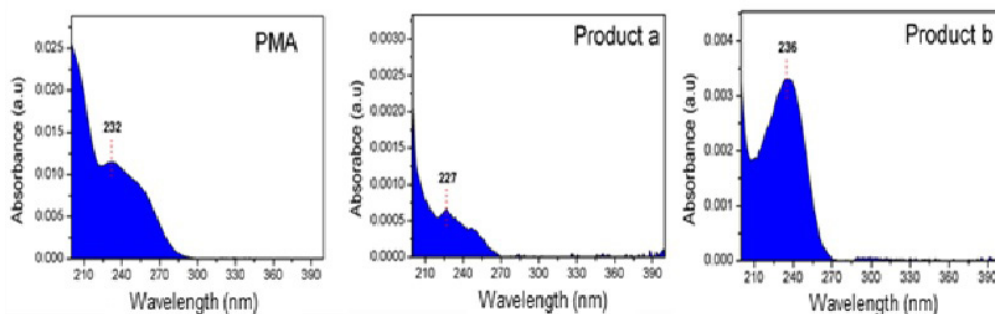
In the new plasma device (Fig. 2), in which a corona discharge around the needle power electrode was employed, the breakdown voltage could be reduced to a level lower than that in the old device (Fig. 1). Furthermore, the discharge current for the same applied voltage is larger. Unfortunately, this large current causes severe heat loading to the ground electrode and the glass tube, making the operation time shorter. We observed that the electrode temperature reached  $>150^\circ\text{C}$ , resulting in a large-scale arc discharge and thermal damage. Figure 7 shows the first results using this new plasma device. The reactive radical concentration in the water was estimated with reference to the hydrogen peroxide concentration measured by the KI colorimetric method, described in the section above. A comparison of the effect of the working gas showed a drastic difference, with a gas flow rate of 1 L/min for the  $\text{N}_2$  gauge and an irradiation time of 5 min. Reactive oxygen radicals were produced much more efficiently using argon plasma rather than helium plasma. PMA degradation efficiency also improved using this new source. Although the plasma treatment time was just one-third of those reported in Kongmany et al.,<sup>8</sup> the degradation ratio doubled. Even though the electrode temperature reached  $>150^\circ\text{C}$ , the temperature of solution was relatively less. Generally, PMA as well as phorbol esters are highly stable at temperatures  $<260^\circ\text{C}$ . Therefore, PMA in solution could not be decomposed by heat.



**FIG. 3:** Degradation of PMA in methanol irradiated with DBD plasma at different treatment times



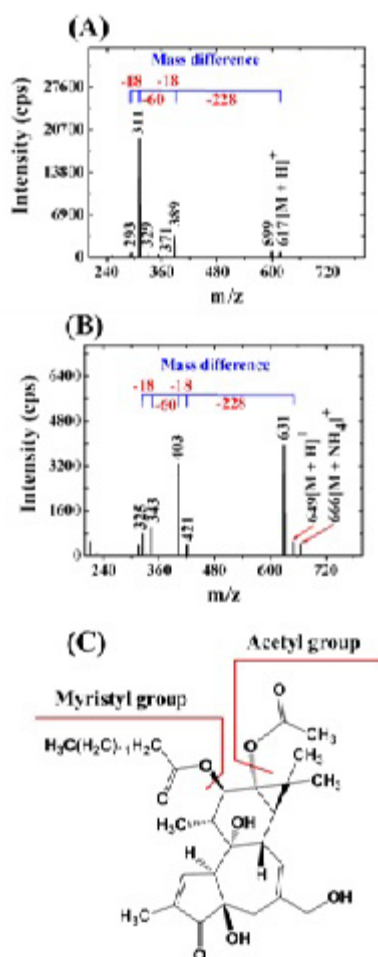
**FIG. 4:** HPLC/UV (250 nm) chromatogram of a methanolic PMA solution treated at times ranging from 0 to 10 min. Peaks a and b are referred to as the peaks of the intermediate products of PMA



**FIG. 5:** UV absorption spectra of PMA and intermediate products

## IV. DISCUSSION

We assume that the 2O atoms added to the PMA molecule may have occurred from a reactive oxygen species, such as a superoxide ion ( $\text{O}_2^-$ ), a hydroxy radical ( $\bullet\text{OH}$ ), or a methoxy radical ( $\text{CH}_3\text{O}\bullet$ ), that was possibly generated during plasma irradiation. Equation (1) shows that this result could be produced by the reaction between a high-energy electron and  $\text{O}_2$  above the solution surface.<sup>10</sup> The interaction of the plasma jet with the methanolic PMA solution could lead to the dissociation of methanol molecules to produce hydroxyl ( $\bullet\text{OH}$ ) and methoxy ( $\text{CH}_3\text{O}\bullet$ ) radicals, according to Equations (2) and (3).<sup>11</sup>



**FIG. 6:** Product-ion-scan mass spectra of (A) PMA and (B) by-product using their ammonium adducts ions,  $[\text{M} + \text{NH}_4]^+$ , as precursor ions: PMA ( $m/z$  634) and the by-product ( $m/z$  666). Collision energy (CE):  $-10$  V. (C) Molecular structure of PMA





posed to other products, as indicated by the small peaks appearing at the retention times of 1.5 and 8 min (Fig. 4).

## V. CONCLUSION

The plasma-chemical degradation of PMA in methanol follows pseudo-first-order kinetics with a constant of  $1.53 \times 10^{-3} \text{ s}^{-1}$  for a conventional DBD helium plasma jet. Epoxide, assumed to be the product initially produced, could be further decomposed. Even though the degradation of PMA in water may be retarded due to the radical scavenging effect of a methanol impurity, an improvement in the plasma source might solve this problem in the near future.

## ACKNOWLEDGMENTS

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