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# Addendum

# Recent advances in plasma techniques for biomedical and drug engineering*

Masayuki Kuzuya‡, Shin-ichi Kondo, and Yasushi Sasai

*Laboratory of Pharmaceutical Physical Chemistry, Gifu Pharmaceutical University, 5-6-1, Mitahora-Higashi, Gifu 502-8585, Japan*

*Abstract*: Plasma-induced surface radicals formed on a variety of organic polymers have been studied by electron spin resonance (ESR), making it possible to provide a sound basis for future experimental design of polymer surface processing (i.e., plasma treatment). On the basis of the findings from such studies on the nature of radical formation and radical reactivity, several novel bioapplications in the field of biomedical and drug engineering have been developed. Applications derived from the nature of plasma-induced surface radical formation include the preparation of a reservoir-type drug delivery system (DDS) of sustained and delayed release, and a floating drug delivery system (FDDS) possessing gastric retention capabilities, the combined findings leading to preparation of a novel "patient-tailored DDS" administered under consideration of the fact that the environment (pH and transit time, etc.) in the gastrointestinal (GI) tract varies with each patient. Applications derived from the reactivity of plasma-induced surface radicals include the preparation of composite powders applicable to a matrix-type DDS by making a mechanical application to the surface radical-containing polymer powders with drug powders, plasma-assisted immobilization of *oligo*-nucleotides (DNA) onto polymer surfaces applicable to constructing a DNA diagnosis system, and basic study of plasma-assisted preparation of a novel functionalized chemo-embolic agent of non-crosslinked hydrogel, vinyl alcohol-sodium acrylate copolymer (PVA-PAANa).

*Keywords*: drug delivery systems; plasma-induced surface radicals; biomedical engineering; drug engineering.

## INTRODUCTION

Cold plasmas are being used for an ever-increasing number of applications. This is related to the many advantages associated with the use of plasmas for modification of materials. These techniques contain environmental benefits owing to dry chemistry, thus, they do not produce contaminated aqueous waste water. One of the characteristics of plasma treatment is that it is surface-limited (ca. 500–1000 Å) so that only the surface properties can be changed without affecting the bulk properties.

Cold plasma of inert gas emits intense UV and/or VUV rays to cause an effective energy transfer to solid surface and gives rise to a large amount of stable free radicals on the polymer surface. In view of the fact that surface reactions of plasma treatment are initiated by plasma-induced radicals, study of the resulting radicals is of utmost importance for understanding the nature of plasma treatment.

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‡Corresponding author

However, the detailed studies of such plasma-induced surface radicals have not been worked out. Thus, we have undertaken plasma irradiation of a wide variety of polymers, synthetic and natural, and the surface radicals formed were studied in detail by electron spin resonance (ESR) coupled with the aid of systematic computer simulations. On the basis of the findings from a series of such studies, we were able to open up novel plasma-assisted bioapplication works.

In the present article, the state-of-the-art of our novel bioapplication works especially in the field of pharmaceutical engineering by plasma techniques are described, which include: (1) For the drug engineering field, preparations of multilayered tablets applicable to a reservoir-type drug delivery system (DDS) of sustained and delayed release, the development of an intragastric floating drug delivery system (FDDS) for oral controlled-release dosage forms possessing gastric retention capabilities, all these devices leading to a novel "patient-tailored DDS", and functionalized composite powders applicable to a matrix-type DDS by mechanical applications of plasma-irradiated polymer powder. Figure 1 shows a conceptual illustration for the preparation of these devices. (2) For the biomedical engineering field, plasma-assisted immobilization of biomolecules onto polymer surfaces and initial study on preparation of functionalized PVA-PAANa hydrogel for chemo-embolic agent, as well as brief overviews of ESR studies on plasma-induced surface radicals of several organic polymers relevant to the present study.

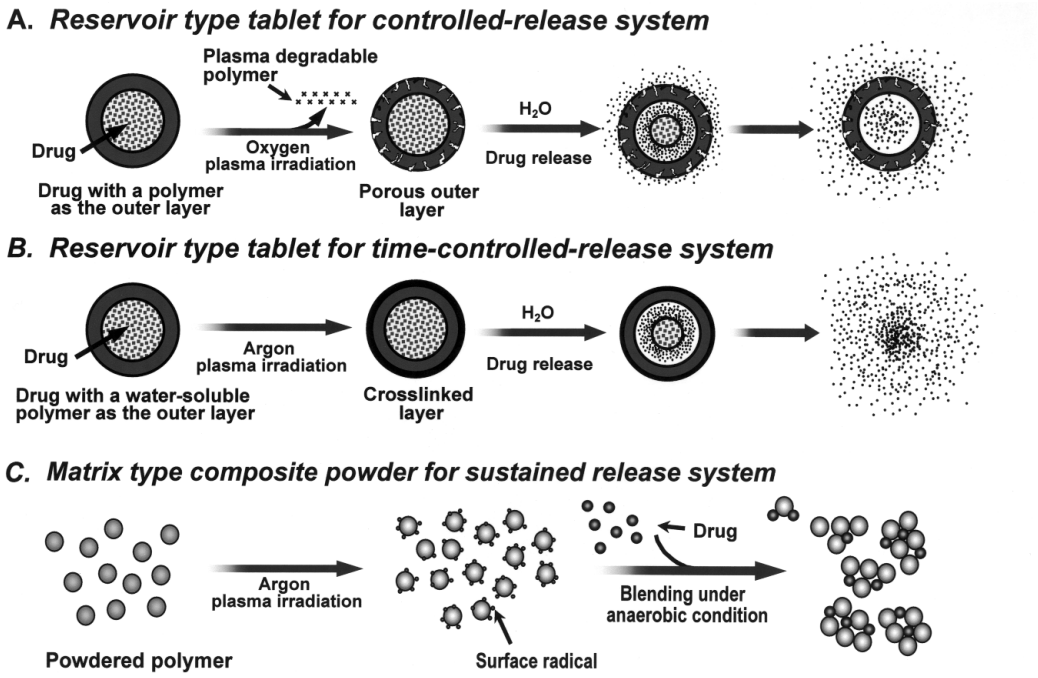


**Fig. 1** Conceptual illustration for preparation of DDS by plasma techniques.

## NATURE OF PLASMA-INDUCED POLYMER RADICALS [1–14]

Over the years, we have been working on the structural identifications of plasma-induced surface radicals of various kinds of organic polymers as studied by ESR spectra coupled with the systematic computer simulations. One of the advantages of plasma irradiation over other types of radiations for the study of the polymer radicals is that the radical formation can be achieved with a brief plasma duration by a simple experimental apparatus, so that polymer radicals can be studied without a significant change of polymer morphology. The experimental set-up for the plasma irradiation and ESR spectral measurement is schematically shown in Fig. 2.

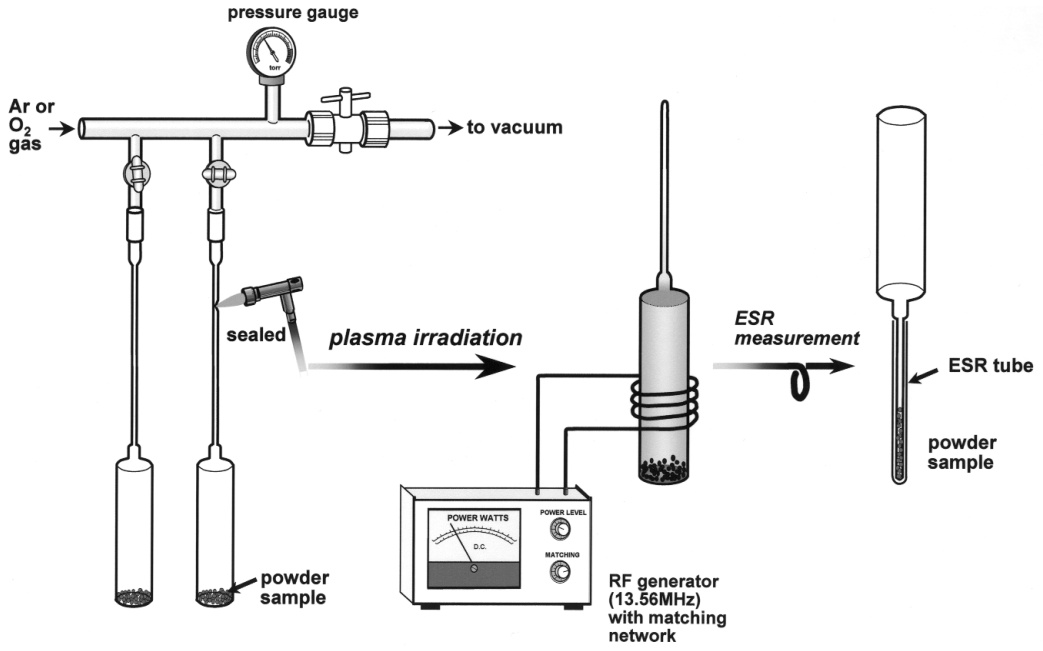


**Fig. 2** Schematic representation for plasma irradiation and ESR spectral measurement.

Figure 3 shows the observed ESR spectra of plasma-induced surface radicals formed on several selected polymers relevant to the present study, together with the corresponding simulated spectra shown as dotted lines. Based on the systematic computer simulations, all the observed spectra in addition to those shown here were deconvoluted, and the component radical structures have been identified.

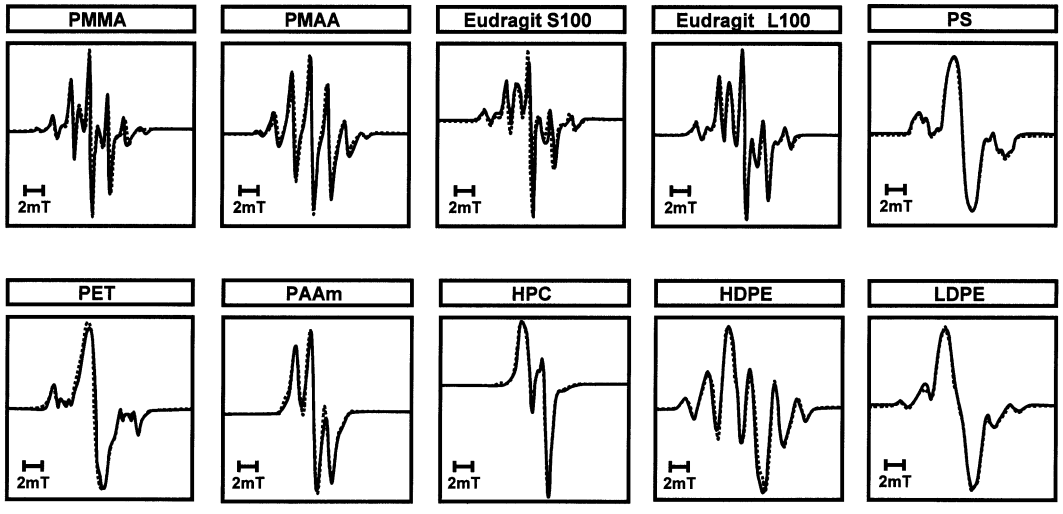


**Fig. 3** Room-temperature ESR spectra of plasma-induced radicals in organic polymers relevant to present work. Plasma operational conditions: power: 40 W; Ar pressure: 0.5 Torr; duration: 1–3 min; PMMA: poly(methyl methacrylate); PMAA: poly(methacrylic acid); Eudragit S100: copolymer of methacrylic acid and methylmethacrylate (4:6); Eudragit L100: copolymer of methacrylic acid and methylmethacrylate (3:7); PST: polystyrene; PET: poly(ethylene terephathalate); PAAm: polyacrylamide; HPC: hydroxylpropyl-cellulose; HDPE: high-density polyethylene; LDPE: low-density polyethylene.

Based on a series of this work, we were able to establish the general relationship between the structure of radicals formed and the polymer structural features. Crosslinkable polymers give the mid-chain alkyl radical as a major component radical, while degradable polymers give the end-chain alkyl radical as a major component radical, and if polymers are of branched structure or contain the aromatic ring, the crosslink reactions occur preferentially on these moieties. And, one of the common features is that dangling bond sites (DBSs) are more or less formed in all plasma-irradiated polymers resulting from the occurrence of CASING (crosslinking by activated inert gas).

## DRUG ENGINEERING FOR DDS PREPARATION BY PLASMA TECHNIQUES

For the most suitable therapy, development of sustained- and controlled-release systems for drug delivery is one of the most active areas today in the entire field of drug research. A wide variety of approaches to controlled-release DDSs have been thus far investigated for oral application. Oral drug delivery is the most desirable and preferred method of administrating therapeutic agents for their systematic effects such as patient acceptance, convenience in administration, and cost-effective manufacturing. We have developed plasma-assisted preparation of multilayered tablets applicable to an oral DDS. Figure 4 illustrates the schematic representation for preparation of double-compressed tablets and a drug dissolution test including the experimental set-up for plasma irradiation on the tablets.

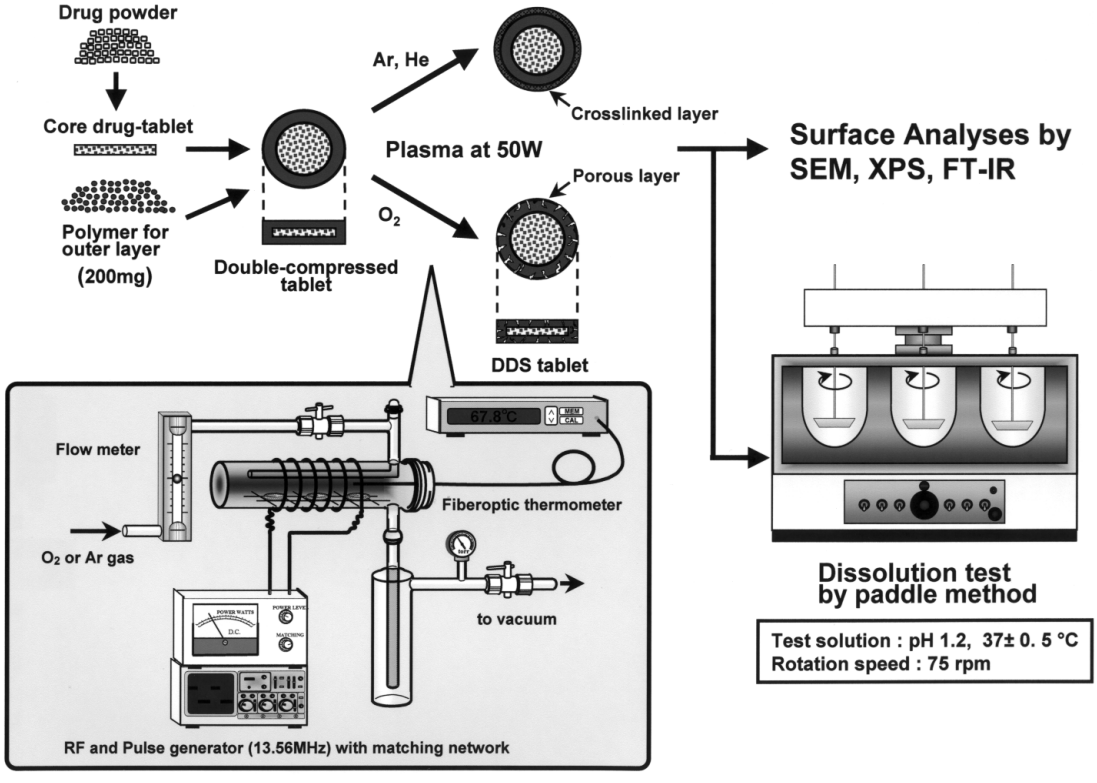


**Fig. 4** Schematics for preparation of double-compressed tablets including the experimental set-up for plasma irradiation.

### Preparation of a sustained-release DDS from plasma-irradiated double-compressed tablets

When oxygen plasma was irradiated to the outermost layer of the double-compressed tablet, which consists of a drug as a core material and a mixture of plasma-crosslinkable and plasma-degradable polymer powders as a wall material, plasma-degradable polymers could be selectively eliminated and simultaneously the crosslinkable polymer undergoes the rapid crosslink reaction to result in the formation of the porous outer layer of the tablet. As a result, the drugs could be released from the tablet through the resulting micropore.

Figure 5 shows the effect of oxygen plasma duration on theophylline release from the double-compressed tablet using a mixed powder of polystyrene (PS) and polyoxymethylene (POM) for the outer layer as the representative example of the release test. The release rate of theophylline increases as plasma duration increases, while the blank tablet did not exhibit any appreciable release of theophylline even with longer dissolution time [15,19]. Thus, the release profile of theophylline from a double-compressed tablet can readily be controlled by the selection of plasma operational tunings. Based on the fact that the value of weight loss shown in parentheses increases as the plasma duration increases, it is apparent that plasma-degradable POM could be selectively eliminated by oxygen plasma irradiation, while plasma-crosslinkable PS undergoes the crosslink reaction, to result in the formation of the porous outer layer of the tablet. Then, theophylline could be released from the tablet through the resulting micropore evidenced by the scanning electron micrograph (SEM) pictures.

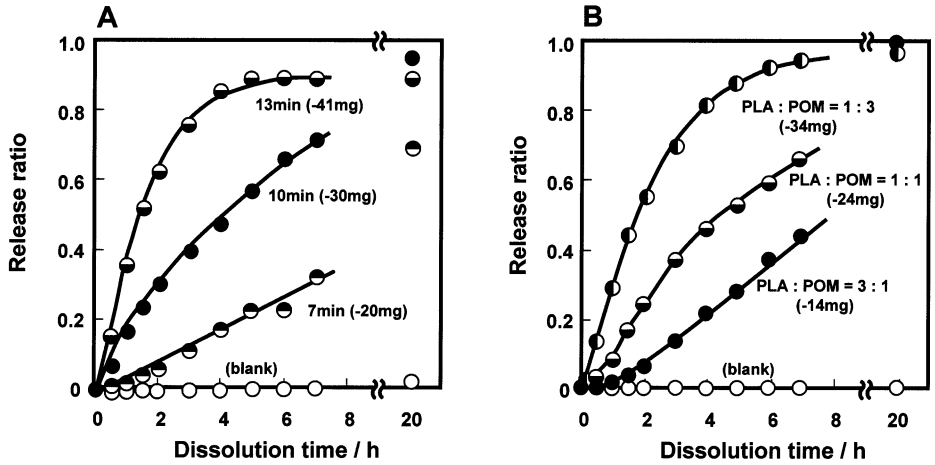


**Fig. 5** Effect of oxygen plasma irradiation on theophylline release from double-compressed tablet (200 kg/cm$^2$ for 30 s). The values shown in parentheses denote the weight loss of the tablets after plasma irradiation. Core tablet: 100 mg (theophylline). For (A) outer layer: 80 mg (PS:POM = 1:1); plasma operational conditions: power: 50 W, pressure: 0.5 Torr; $O_2$ 50 ml/min. For (B) outer layer: 80 mg (PLA:POM = 1:3, 1:1, 3:1); plasma operational conditions: duration: 2 h; power: 6 W; pressure: 0.5 Torr; $O_2$ 50 ml/min.

The work similar to the above has further been extended to preparation of the controlled release tablet by oxygen plasma irradiation on the outer layer of double-compressed tablets with a variety of polymers as a single wall material [16–22]. When a water-soluble polymer, poly(methacrylic acid) (PMAA) or polyacrylamide (PAAm), is used for a wall material of the double-compressed tablet, the rapid drug release rate from the tablet was suppressed by argon plasma-induced crosslink reactions and changed into the slow release with a sigmoid release pattern due to a decrease in the solubility of water-soluble polymers [21].

### Preparation of a time-controlled drug release system by plasma techniques [23–25]

Today, the therapy based on the factor of biorhythmic time is becoming more and more important in the progress toward an aging society in many countries, in addition to customary controlled-release systems. A time-controlled release system has a function of timer, so that the main technical point for the development of this system is how to control lag time and drug release after lag time.

It is well known that methacrylic-acrylic acid copolymers, including their derivatives with various combinations and composition ratios of the monomers, have been used as pharmaceutical aids for enteric coating agents commercially known as a series of Eudragits. These Eudragit polymers turn out to be water-soluble in a certain specific pH solution, and they show a different dissolution rate. The structures and the dissoluble pH values of several Eudragit polymers are shown in Fig. 6.

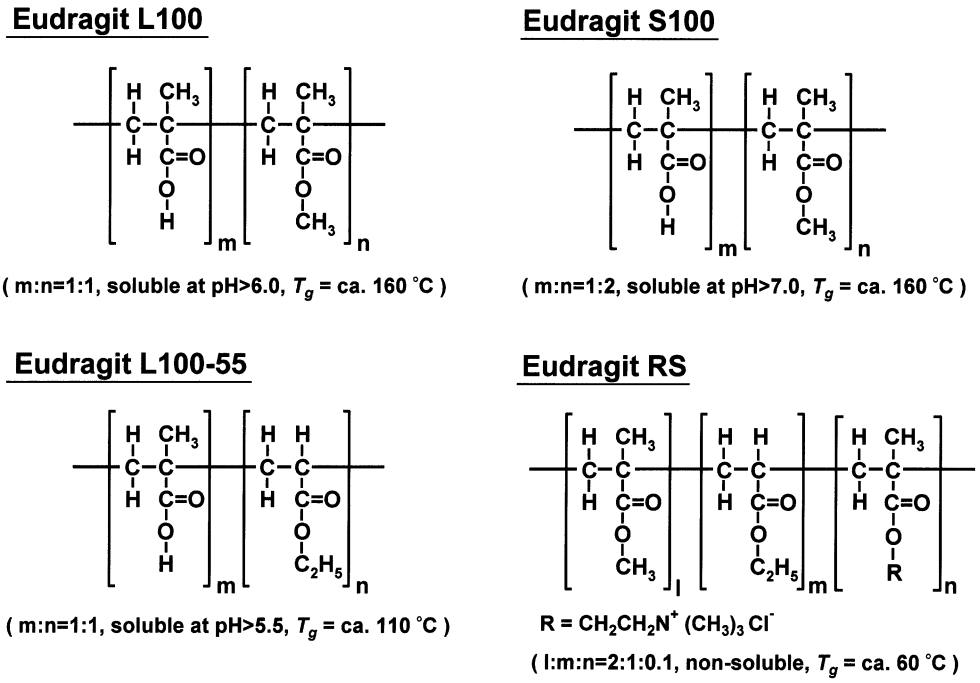


**Fig. 6** Commercial enteric coating agents licensed for human use.

Since plasma-crosslinkable acrylic monomers are one of the component polymers in Eudragit L100-55, argon plasma irradiation would lead to the suppression of Eudragit L100-55 solubility even in a dissoluble pH-value solution (pH > 5.5), owing to the occurrence of the surface crosslink reactions. Thus, when Eudragit L100-55 is used as a wall material of the double-compressed tablet, the initial drug release could be completely sustained for a certain period of time.

With this expectation in mind, we have undertaken argon plasma irradiation to examine the possibility of a rapid-release, double-compressed tablet of Eudragit L100-55, being converted into a delayed-release tablet, i.e., the time-controlled DDS.

Figure 7 shows the effect of argon plasma irradiation on theophylline release profiles in pH 6.5 buffer solution and the SEM pictures of the surface of the Eudragit L100-55 tablet before and after argon plasma irradiation. Figure 7 shows that the Eudragit L100-55 tablets plasma-irradiated for 3 and 5 min produce prolongation of lag time for theophylline release.

The SEM pictures demonstrate that the tablet surface with 5 min irradiation has converted into the rather smooth surface with clogging of the crack presenting at particle–particle interfaces by softening of Eudragit L100-55, and into the porous outer layer with 10 min irradiation. It is considered that

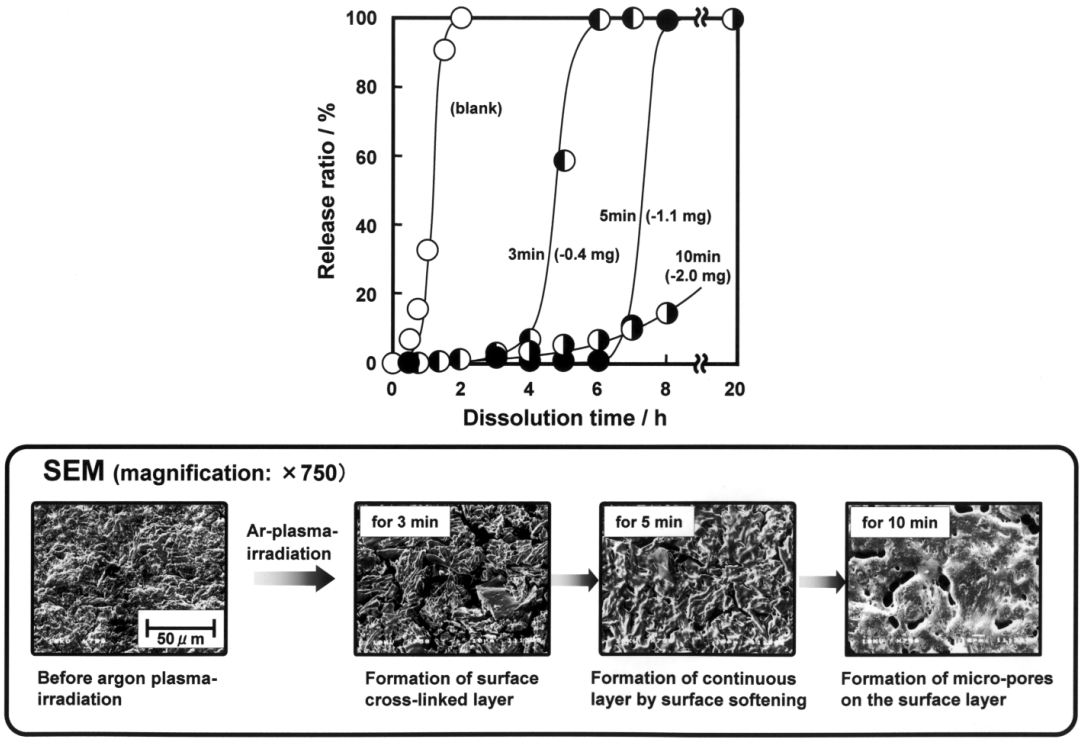


**Fig. 7** Effect of plasma duration on release properties of theophylline from plasma-irradiated, double-compressed tablets of Eudragit L100-55 (100 mg) in pH 6.5 buffer solution and the SEM pictures before (A) and after plasma irradiation for 5 min (B) and 10 min (C). Plasma operational conditions: power: 30 W; Ar pressure: 0.5 Torr; flow rate: 50 ml/min.

the porous layer was formed not only by the effect of plasma irradiation, but also by physical actions such as evolved gas scattering, accompanied by softening of the Eudragit L100-55 owing to the plasma heat fusion.

## Preparation of intragastric FDDS by plasma techniques

Intragastric FDDS has been noted as an orally applicable system for the prolongation of the gastric emptying time [27]. Hence, this system is useful for obtaining a sufficient bioavailability and an effective plasma level, especially for having a limited absorption site in the GI tract.

In the course of our study on plasma-assisted DDS preparation, we found that carbon dioxide was trapped in the tablet when argon plasma was irradiated onto the surface of double-compressed tablet composed of plasma-crosslinkable polymers possessing carboxyl group as an outer layer. It was considered that such tablets could be applicable to FDDS.

In fact, we have obtained the intragastric FDDS by plasma irradiation when the double-compressed tablet was prepared using the outer layer so as to trap evolved carbon dioxide. Figure 8 shows the floating property on the simulated gastric fluid and the release property of 5-fluorouracil (5-FU) from argon plasma-irradiated double-compressed tablet using methyl vinyl ether-maleic acid copolymer (VEMAC), and a mixture of VEMAC and hydroxypropyl methylcellulose phthalate (HPMCP) as an outer layer.

It is seen that the plasma-irradiated tablet remains buoyant in the simulated gastric fluids for a prolonged period of time, and the 5-FU release is considerably suppressed, giving an indication that the

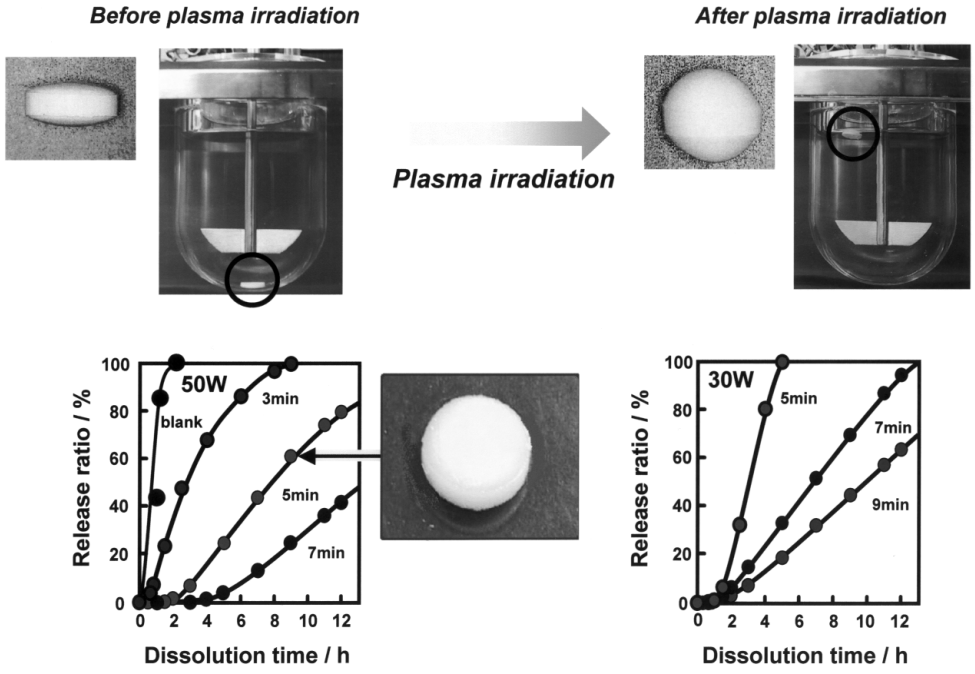


**Fig. 8** Photos of double-compressed tablet prepared for FDDS before and after plasma irradiation, and the effect of plasma duration on 5-FU release properties. Plasma conditions: 0.5 Torr, Ar 50 ml/min. Outer layer: VEMAC/HPMCP (1:1).

drug release can be controlled at a desired rate from the tablets by selecting the plasma operational tunings [28].

## PATIENT-TAILORED DDS FOR LARGE INTESTINE TARGETED-RELEASE PREPARATIONS

With most of today's oral DDS devices, it is difficult for all patients to obtain the expected therapeutic effects of drugs administered, owing to the individual difference in the environment, such as pH value and the transit time in the GI tract, which causes the slippage of time-related and positional timing of drug release. Thus, from the viewpoint of the real optimization of drug therapy, the "patient-tailored DDS" (tailor-made DDS) should be developed and administered based on the diagnosis of each patient's GI environment, which can be obtained by direct monitoring using a diagnostic system of the pH-sensitive radio telemetry capsule, the so-called "pH-chip".

We have fabricated an experimental set-up for the simulated GI tract for large intestine targeting, the dissolution test solution being changed in pH value corresponding to stomach (pH 1.2), small intestine (pH 7.4), and large intestine (pH 6.8), and examined the drug release test of plasma-irradiated, double-compressed tablet in the simulated GI tract.

Figure 9 shows the preliminary result of the theophylline dissolution test in pH 6.8 test solution on the double-compressed tablets using a mixture of Eudragit L100-55/RSPO (7:3) as outer layer. It is seen that the lag time has increased with the extension of plasma irradiation time. The lag time has not been largely affected by treatment in pH 1.2 and pH 7.4 test solution, which indicated the possibility for the development of the "patient-tailored DDS" targeting the large intestine. We are now elaborating these initial studies aimed at more rapid drug release right after the drug preparations reaches the prescribed pH value of the large intestine, owing to contents of semi-solid nature in the large intestine.

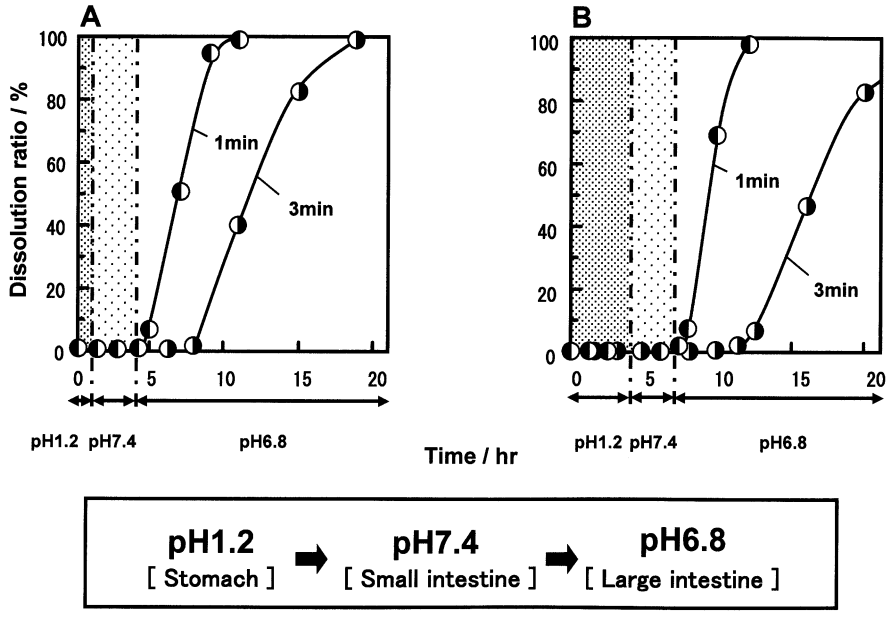


**Fig. 9** Release property of theophylline from plasma-irradiated double-compressed tablets using Eudragit L100-55/RS (7:3) as outer layer in the simulated GI tract. (A) for 1 h in pH 1.2; (B) for 4 h in pH 1.2.

### Preparation of functionalized composite powders applicable to matrix-type DDS

The recombination of solid-state radicals is significantly suppressed owing to the restriction of their mobilities, unlike radicals in the liquid or gas phase. Interactions between radicals at solid–solid interfaces do not occur under a normal condition [8].

We present the occurrence of mechanically induced surface radical recombination of plasma-irradiated polymers. As shown in Fig. 10, plasma-irradiated polyethylene (PE) powder, low-density polyethylene (LDPE), and high-density polyethylene (HDPE), were applied to mechanical vibration in a Teflon twin-shell blender for the prescribed period of time at room temperature under strictly anaerobic conditions, and submitted to ESR measurement.

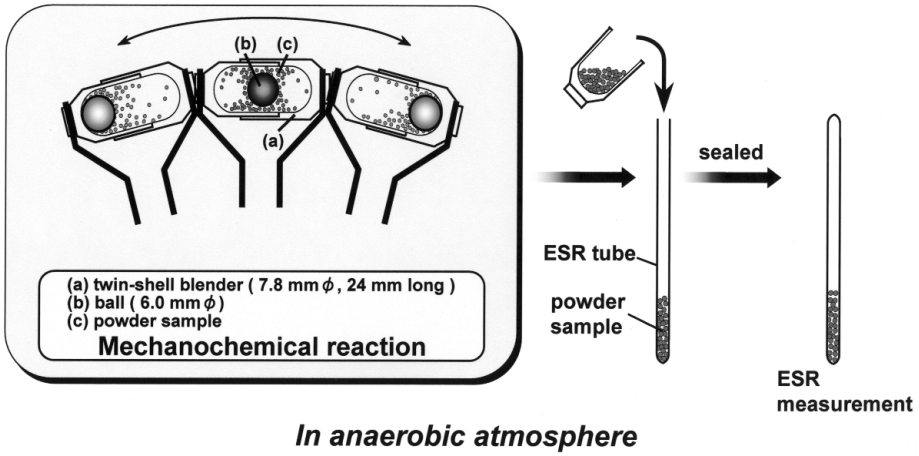


**Fig. 10** Schematic representation for mechanical vibration and ESR spectral measurement.

As shown in Fig. 11, the spectral intensity gradually decreased, with a change of the spectral pattern for the case of LDPE, as the duration of mechanical vibration increased. This clearly indicated that plasma-induced surface radicals of PE underwent effectively the solid-state radical recombination in intra- and inter-particle fashion on its mechanical vibration, since the spectral intensity did not appreciably decrease on standing at room temperature, so long as it is kept under anaerobic conditions.

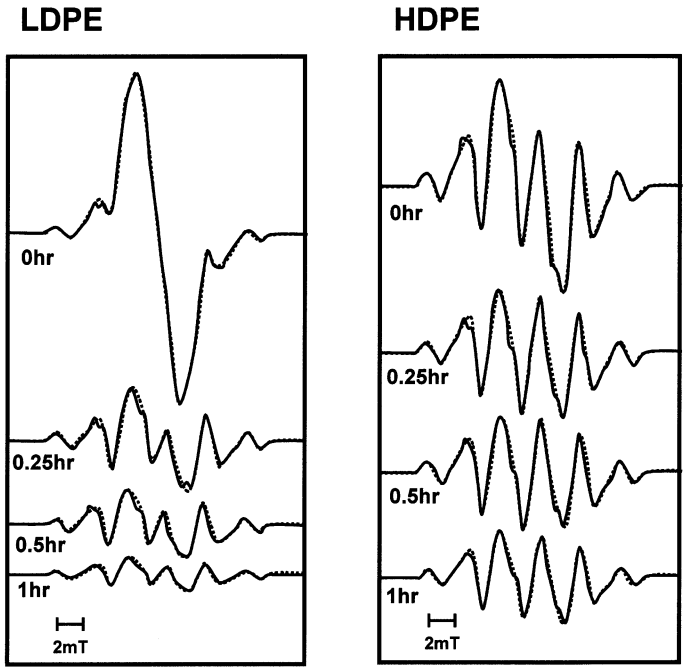


**Fig. 11** Progressive changes of observed ESR spectra of 10 min plasma-irradiated LDPE and HDPE powders with various duration of mechanical vibration (60 Hz) in Teflon twin-shell blender, together with the simulated spectra shown as dotted lines.

For the matrix-type DDS preparation, the mechanical vibration of plasma-irradiated PE powder was carried out in the presence of theophylline powder so as to immobilize the theophylline powder into PE matrix formed by interparticle linkage of PE powder. Examples of the theophylline release from the resulting composite powders of LDPE and HDPE are shown in Fig. 12. It is seen that the theophylline release is apparently suppressed from each of the plasma-irradiated PE powders, being proportional to the spin number of the surface radicals, owing to trapping theophylline powder into the PE matrix [29]. It should be noted here that the theophylline release is further retarded from the tablet prepared by compressing the above composite PE powders.

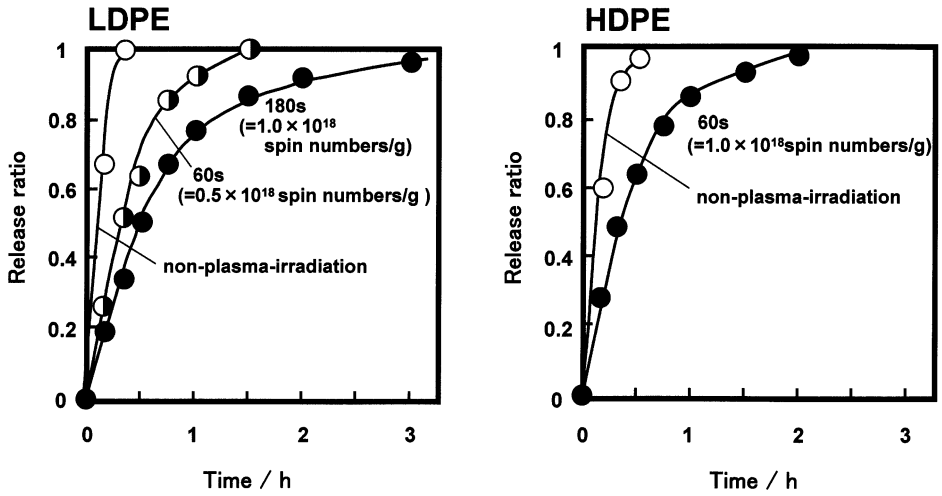


**Fig. 12** Theophylline release profiles from the composite powder composed of theophylline and Ar plasma-irradiated polyethylenes, LDPE and HDPE. LDPE plasma-irradiated for 60 s: $0.5 \times 10^{18}$ spin/g, for 180 s: $1.0 \times 10^{18}$ spin/g. HDPE plasma-irradiated for 60 s: $1.0 \times 10^{18}$ spin/g. Plasma operational conditions: power: 40 W; Ar pressure: 0.5 Torr; duration: 1 min.

## BIOMEDICAL ENGINEERING BY PLASMA TECHNIQUES

The wettability of polymer surface is an important characteristic relating to biocompatibility for biomaterials. It is known, however, that the wettability introduced by plasma treatment decays with time after treatment. The mechanism has been ascribed to several reasons such as the overturn of hydrophilic groups into the bulk phase for crosslinkable polymers, and detachment of the hydrophilic lower-molecular-weight species from the surface for degradable polymers.

### Plasma-assisted immobilization of *oligo*-nucleotides (DNA) onto polymer surfaces

Considerable interest has been focused on the immobilization of several important classes of biomolecules such as DNA, enzyme, and protein, onto the water-insoluble supports. The development of DNA chips, on which many kinds of *oligo*-DNA are immobilized, has revolutionized the fields of genomics and bioinformatics [30,31]. However, all the current biochips are disposable and lack reusability, in part because the devices are not physically robust [32].

We have recently reported a novel method to introduce a durable surface wettability and minimize its decay with time on several hydrophobic polymers—poly(ethylene naphthalate) (PEN), LDPE, and Nylon-12 [33,34]. The method involves a sorption of VEMA into the surface layer and its immobilization by plasma-induced crosslink reaction, followed by hydrolysis of maleic anhydride linkage in VEMA to generate durable hydrophilic carboxyl groups (VEMACs) on the surface (Fig. 13).

The method has further been extended to application to the plasma-assisted immobilization of single-stranded *oligo*-DNA onto the LDPE-VEMAC sheet by the reaction of 5'-aminolinker *oligo*-DNA with a condensation reagent. The 5'-aminolinker *oligo*-DNA, which possesses an aminohexyl group as a 5'-terminal group of DNA, is considered to be able to react with the carboxyl group on the surface of the LDPE-VEMAC sheet. It was found from the measurement of fluorescence with a confocal laser microscope that the resulting LDPE-VEMAC-DNA sheet was able to detect several complementary *oligo*-DNAs by effective hybridization.

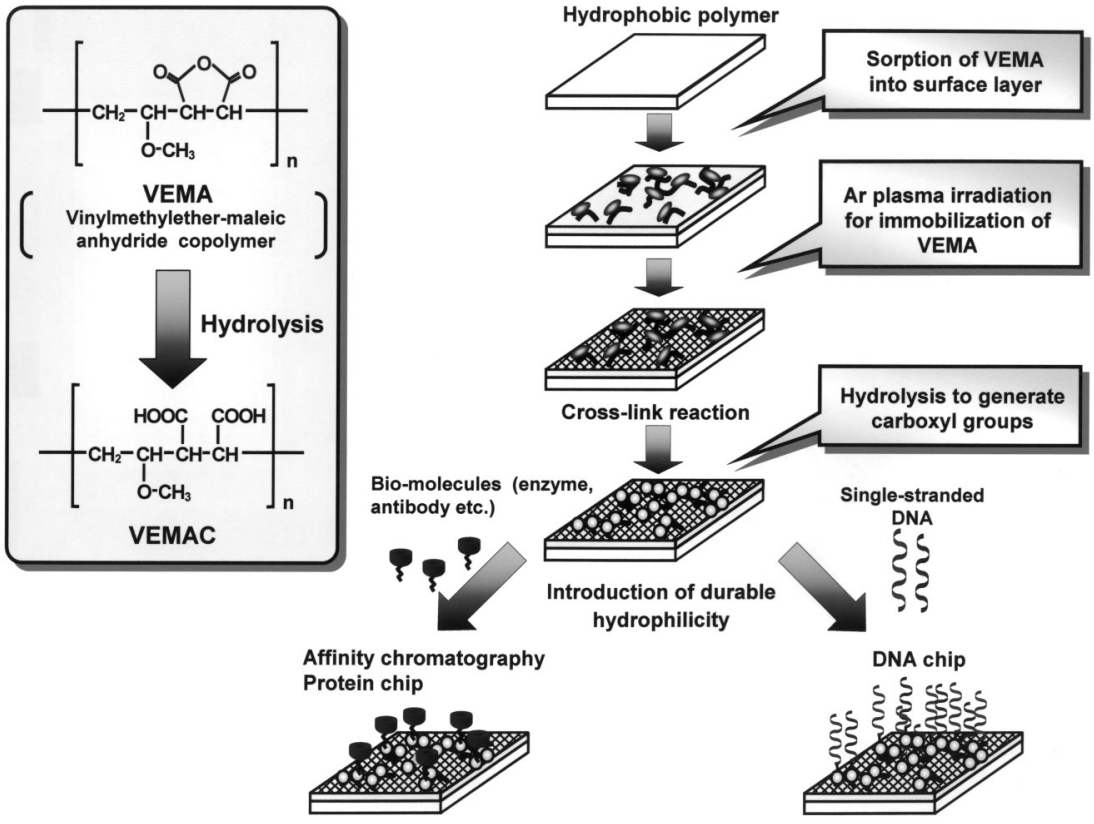


**Fig. 13** Conceptual illustration for immobilization of biomolecules onto hydrophobic polymer surface.

To examine the reusability of the LDPE-VEMAC-DNA sheet, we have repeatedly conducted the hybridization and dehybridization of perfect matching (PM) on the same LDPE-VEMAC-DNA sheet, according to the general procedure to remove bounded target DNA from the chip (washing with hot water, 90 °C, for 5 min). Figure 14 shows the result of reusability test based on the confocal laser microscope images of the LDPE-VEMAC-DNA sheet. The fluorescence is observed nearly at the same level of intensity even after the repetition several times of the hybridization and dehybridization of PM. The result indicates that the LDPE-VEMAC-DNA sheet obtained by the present method would be reusable [35].

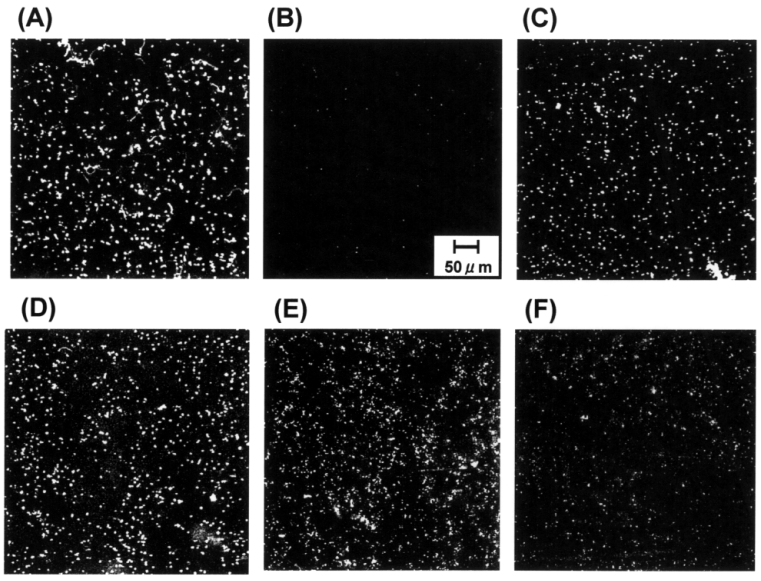


**Fig. 14** Scan image of the fluorescence intensity of LDPE-VEMAC-DNA sheet for reusability test. (A) Hybridization of *oligo*-DNA-PM; (B) After hot water rinse of sheet (A) for 5 min. Hybridization of *oligo*-DNA-PM on the same sheet (C) 2 times; (D) 5 times; (E) 7 times; (F) 8 times.

## Basic study on preparation of functionalized PVA-PAANa hydrogel for chemo-embolic agent

Vinyl alcohol-sodium acrylate copolymer (PVA-PAANa) is well known as a noncrosslinked hydrogel (water-absorbent polymer) owing to the intense hydrogen-bonding network among the hydroxyl groups of PVA moiety. The PVA-PAANa microsphere (ca. 100 μm) swells ca. 3.5 times in diameter larger than its original size in human serum within a few minutes and can pass through a microcatheter. Recently, its microsphere has been applied to the chemo-embolic agent used for transcatheter arterial embolization (TAE) in clinical trials on patients [36–38]. The PVA-PAANa microsphere is shape-adjustable in nature according to the surrounding blood pressure because of the noncrosslinked structure, so that it has been shown to occlude the blood vessel much more effectively than any other conventional embolic agents such as gelatin sponge and lipiol.

In order to seek the possibility of further functionalization of PVA-PAANa, such as a capability of controlling the ratio and rate of swelling by plasma processing, we carried out argon plasma irradiation onto the PVA-PAANa microsphere and the surface radicals formed were studied by ESR on its comparison with those of vinyl alcohol-acrylic acid copolymer (PVA-PAA) as well as its respective component homopolymer, PVA, PAA, and its sodium salt (PAANa). In fact, it was found that the ESR spectra have shown the vast difference in pattern between PVA-PAANa and PVA-PAA, demonstrating the strong sodium salt effect on the nature of plasma-induced surface radical formation (Fig. 15). The systematic computer simulations of the ESR spectra revealed that the major spectral component was the radicals derived from PVA site for PVA-PAANa and the ones from PAA site for PVA-PAA. The SEM pictures indicated that the observed site-selectivity for the surface radical formation has been derived from the difference in the surface morphology between PVA-PAANa and PVA-PAA (Fig. 16). PVA-PAANa forms the microphase separation structure with the condensed domain of PAANa site so as to reduce the effective surface area for the surface radical formation. The present result provides a basis for the future experimental design for giving an additional performance to PVA-PAANa, including the sustained drug-release function at the occluding site.

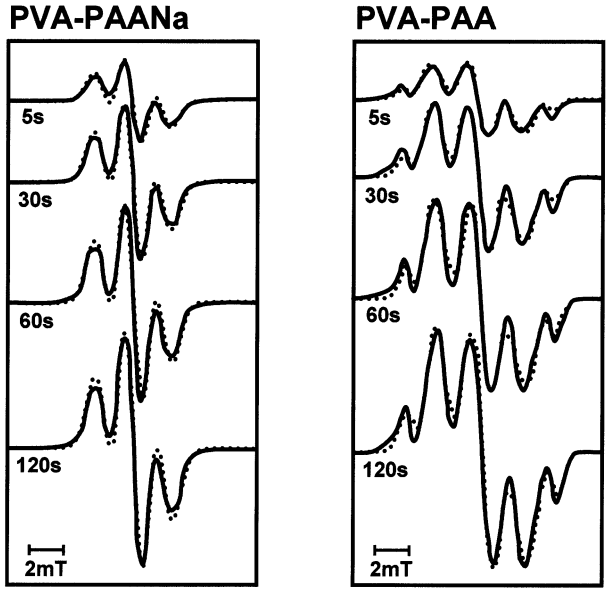


**Fig. 15** Progressive changes in observed ESR spectra of plasma-irradiated PVA-PAANa and PVA-PAA together with the simulated spectra shown as dotted lines.

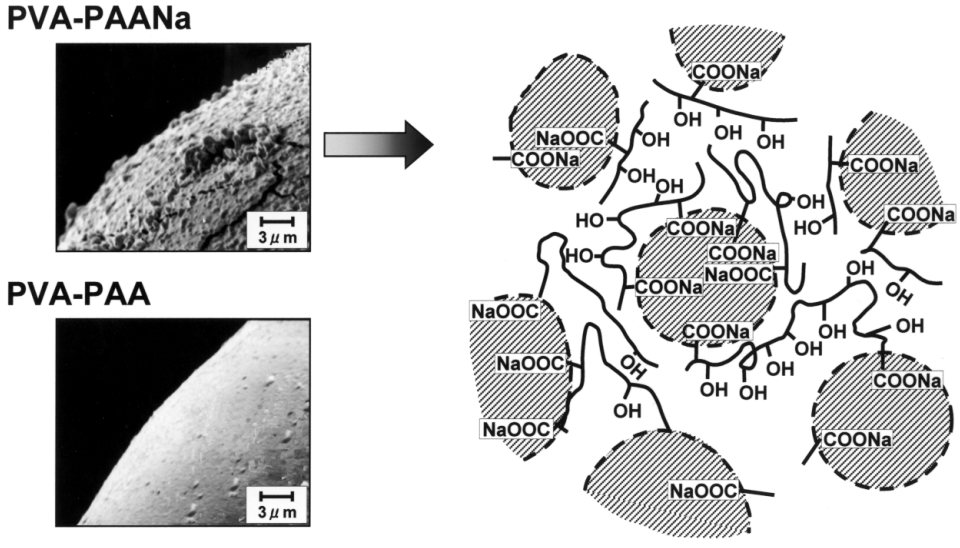


**Fig. 16** SEM pictures of PVA-PAANa and PVA-PAA, and the conceptual illustration of microphase separation structure of PVA-PAANa.

## CONCLUSION

The present results have clearly shown that one can prepare a variety of desired DDS devices if one selects the tailored polymers for wall materials of double-compressed tablets as well as plasma operational conditions. And the method of plasma-assisted DDS preparation contains several advantages: (1) it is a totally dry process, (2) the polymer surface is modified without affecting the bulk properties, (3) direct plasma exposure to drugs is avoided, and (4) there is versatile control of drug release rates. Thus, it is hoped that more practical applications will be developed in the course of attempts now in progress.

It should be noted, however, that we have restricted use of the organic polymers so as to manipulate the existing pharmaceutical aids licensed for practically patient use, since the pharmaceutical aids containing lower than 0.1 % level impurity can be used without the structural identification of the impurities, in accordance with the harmonized tripartite guideline among the European Union, Japan, and the United States. Otherwise it is very cost- and time-consuming to obtain the approval and license for manufacturing new drug and quasi-drug substances for human use, unlike the approval of industrial substances.

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