

고분자의 비파괴 연구를 위한 핵자기 공명 현미영상법

이동훈¹, 고락길, 문찬홍*, 조장희*

배재대학교 물리학과
한국과학기술원 정보 및 통신공학과*

NMR Microimaging for Noninvasive Investigation of Polymers¹

D.H. Lee, R.K. Ko, C.H. Moon*, Z.H. Cho*

Department of Physics, Pai Chai University, Taejon, Korea
Department of Information and Communication Engineering, KAIST, Seoul, Korea*

Abstract

Polymers have been one of the emerging biomedical materials in the area of biomedical research which are applicable to the human body. For human applications, noninvasive characterization of the biomedical polymers has been one of the important topics, and is valuable information. Among others, the swelling rate is one of the important measurements needed for the hydrophilic polymers. NMR imaging has been a suitable method for the noninvasive study of such a material since it is sensitive to many physical and biochemical changes of the specimens. In addition, NMR techniques possess many useful intrinsic properties such as the relaxation and diffusion effects.

The present study has provided a noble and noninvasive method of measuring the process of swelling as well as volumetric changes occurred in polymers and drug delivery processes in a drug delivery system (DDS) together with changes of released drug. This gives information, relating with both water ingress process, volumetric changes of polymer specimens and the visualization of sequential drug delivery process. Also, this study provides more reliable method to ascertain the time dependent swelling process compared to the conventional method. The important aspects is that the proposed method is truly noninvasive and is able to ascertain time dependent processes.

Introduction

NMR has long been utilized for the noninvasive study of condensed materials along with liquids and many kinds of gas since it is sensitive to many physical and biochemical changes of the specimens. As one of the NMR applications, NMR microimaging has become an increasingly important tool in investigating a variety materials such as polymers, petrochemicals, and many biomaterials.[1] This is due to the reason that NMR microimaging has the capability of producing high resolution on the top of the properties that the conventional Magnetic Resonance Imaging(MRI) provides. NMR microimaging makes possible to investigate the dissolving phenomenon of material (drug release) and sequential processes of liquid absorption along with volumetric changes (swelling effect).[2]

In the past decade there have been some experimental works that were aimed at determining the response of the

swelling process to changes in these parameters. However, the swelling effect of polymers is one of the most poorly understood area in polymer science.[3] The conventional method of measuring the degree of swelling of a polymer specimen is to measure the weight change due to the absorbed water in the specimen.[4,5] Unfortunately, this time consuming and intermittent method does not provide the truly noninvasive information necessary for the measurement of the swelling effect in the specimen.

Swelling effect is especially important to understand valuable phenomenon occurring in drug delivery system (DDS). The visualization of drug release and dynamic changes occurring within a DDS could be an important characterization of drug delivery systems, especially when this visualization can be performed noninvasively and sequentially with a certain time interval. This time dependent visualization will provide important information on the mechanism of the drug release.

In the present study we have focused our study on NMR microimaging to characterize the swelling effect of polyvinyl alcohol (PVA) (PVA has been widely used for many biomedical applications such as contact lenses, and artificial vein etc.) and visualization of the drug release as well as volumetric changes of the drug delivery system as a function of time using NMR microimaging.

Materials and Method

Fig.1 displays the experimental setup that comprises a sample vial placed in the solenoidal coil or the saddle coil (respectively for PVA or DDS) located at the center of the 2.0 Tesla superconducting magnet. ¹H NMR microimaging has been performed on a 2.0T whole body MRI scanner as shown in Fig 1. The RF probes were capacitively coupled coils placed in a 6cm diameter gradient coil which is capable of producing the gradient strengths up to 100G/cm. The gradient coil has a conventional goley type. It was encircled by a sample holder, positioned in a glass vial filled with water. 2-dimensional images were acquired on two planes: one plane is perpendicular to the z-axis and is on the xy plane, which is called as the axial plane; the other is on the xz plane, which is specified by the coronal plane for the PVA images while the other plane for the DDS images is on the yz-plane (or the sagittal plane).

The conventional spin echo sequence images (TR/TE=300/30ms and TR/TE=200/30ms, field of view of 20mm and 17mm, and slice thickness of a 2-dimensional image of 1mm; TE -- time to echo: time interval between the

¹ The present study was supported by Korea Science and Engineering Foundation with the 1994 grant as well as the donation of the image analyzer from Bumi Universe Inc.

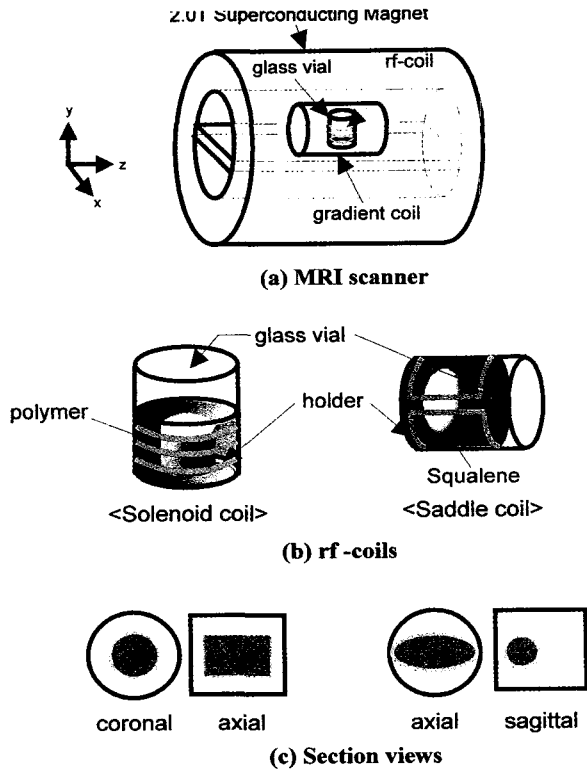


Fig.1 NMR Microimaging setup for the 2.0T superconducting whole body MRI scanner. The direction of the main magnetic field is along the z-axis: a polymer sample is surrounded by an acryl holder positioned in a glass vial filled with water; the glass vial is in the rf coil that is located in the center of the gradient coil.

excitation pulse($\pi/2$ RF *sinc*) and the center of the echo signal; TR -- repetition time: the time interval between one set of the sequence and the next set) were acquired for PVA and squalene. Fig. 2 depicts the timing pulse diagram of the spin echo imaging sequence that was used for the acquisition of 2-dimensional images with an appropriate slice thickness depending on the amplitude of the slice selection gradient (G_z in the case of Fig.2). The basic idea how to obtain spatial information in NMR imaging is relatively simple. The spatial information can be encoded on a NMR signal by using the three magnetic field gradients (G_x , G_y , G_z) whose values vary along the x, y, and z-axes, respectively. The received signal from the selected object can be expressed by the following equation:

$$s_r(t) = \iint m(x,y) \exp(-i\gamma \int_0^t G(\tau) \cdot r d\tau) dx dy \quad [1]$$

where $s_r(t)$ is a received signal, $m(x,y)$ information data for selected object, $G(\tau)$ gradient vector, r location vector and γ gyromagnetic ratio. Then the information can be extracted from the encoded signal by the mathematical tool called Inverse Fourier Transform. Using the imaging sequence shown in Fig. 2, the time dependent 2-dimensional images were acquired along the two different directions and their corresponding image profiles were then extracted. Basic principles behind NMR imaging can be referred to some books and review articles.[6,7]

The experiments are employed for polyvinyl alcohol (PVA) and a DDS. For PVA, three kinds were prepared

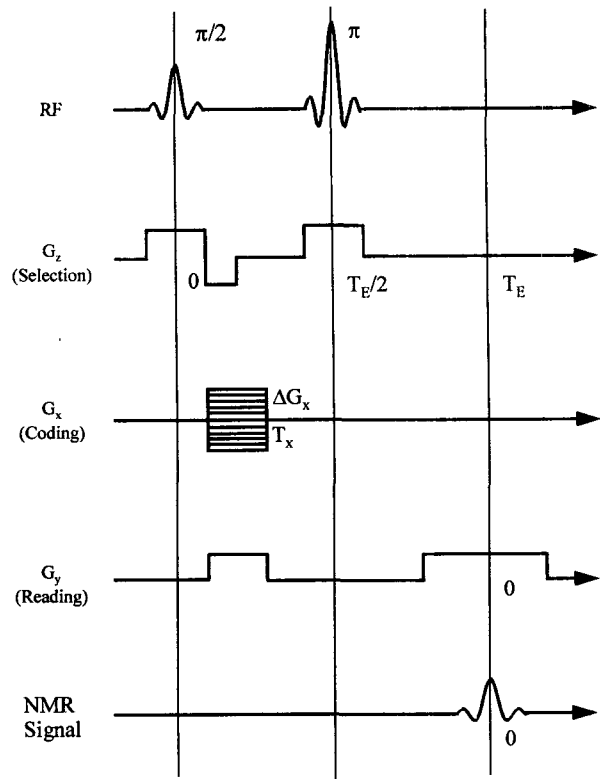


Fig. 2 Timing diagram of spin echo imaging sequence.

with different degrees of cross-link as the following. A 20% of polyvinyl alcohol was dissolved in deionized water, in which H_2SO_4 was added. Different amounts of formaldehyde were deposited in the mixed liquid, depending on the degree of cross-link. Depending on the amount of formaldehyde three specimens were specified as 2g, 3g, and 5g as the number in front of 'g' increases with the amount of formaldehyde to increase the degree of cross-link. The mixture of the liquid was placed in a mold at $60\sim 80^\circ C$ for 5-48 hours until the solidification was completed. The prepared specimens were dried in the mixture of methanol and distilled water. The proportion of water in the mixture was gradually reduced, by increasing methanol concentration, for 6 days until pure methanol remained. The drying process was as the following. Six bottles of water and methanol mixture with different concentration ratios were prepared such that percentage concentrations of water versus methanol were 100:0, 80:20, 60:40, 40:60, 20:80, 0:100, respectively. Each specimen was immersed in the first bottle with 100% of water for 24 hours and was moved to another bottle which had the next concentration ratio and remained for 24 hours. Then the specimen moved again to the bottle with the next concentration ratio. This process was continued until the specimen was reached the last bottle with 100% of methanol. Each dry specimen in the shape of a solid cylinder was positioned in a 13mm sample holder within a glass vial filled with distilled water at $290^\circ K$. The elapsed time was continuously measured since the sample was loaded for the first time in liquid.

Squalene encapsulated by a gelatin capsule was employed as a DDS. These squalene capsules were purchased from Taepyungyang Chemical Inc. Squalene is concentrated oily liquid which is extracted from liners of sharks livering in deeper sea. A capsule of squalene was

attached at the bottom of a 17mm inner diameter glass vial filled with distilled water. The capsule has the shape of a rugby ball. Its long axis was placed in perpendicular to the vial so that both sagittal and axial views can be acquired as shown in Fig.1. Also, pH sensitive drug delivery systems have been imaged with the same setup as that of DDS study and in this case the squalene capsule is placed in distilled water with a pH of 7.

Results

Water ingress behavior is shown in Figure 3a. Furthermore, the expansion process of the coronal plane is displayed in Figure 3b and the expansion rate as a function of time is approximately 0.04mm²/min. Volumetric expansion rate can be anticipated using the plane rates along the two perpendicular directions since the similar geometrical variations may yield the comparable diffusion behavior along the two directions.[8]

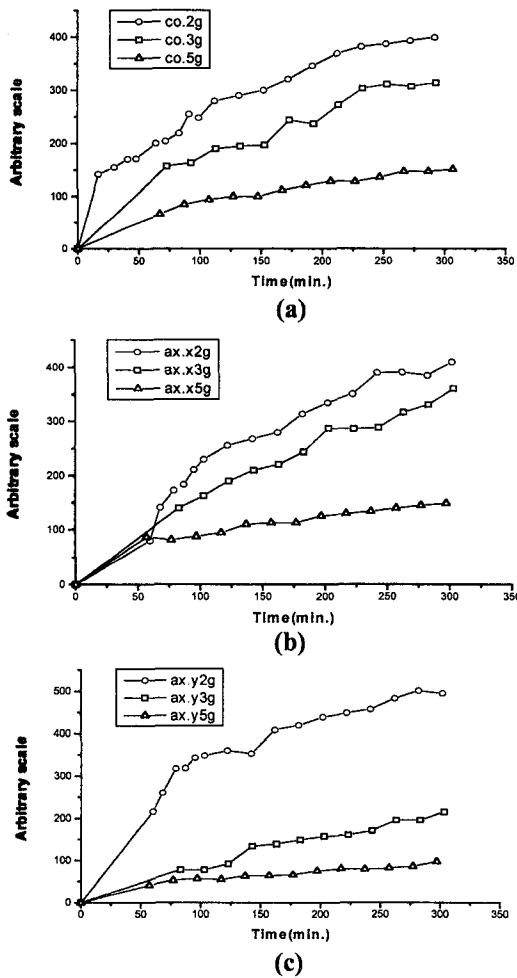


Fig. 3 (a) Water ingress process as a function of time in minutes (vertical axis represents the amount of water diffused through the sample in an arbitrary scale) along the diameter of each coronal image; water ingress process along the x-axis (a) and the y-axis (b) of each axial image.

Fig.4 shows volume expansion process due to the swelling effect of PVA samples immersed in water. The volume expansion is not linearly dependent as a function of time but has an exponential dependence. 2g sample has the lowest degree of cross-link among the three samples and shows most abrupt increase of volume at the initial stage of swelling process. The abruptness on a graph reduces with the increase of degree of cross-link in a sample. Therefore, time constant of each curve may be related with the degree of cross-link.

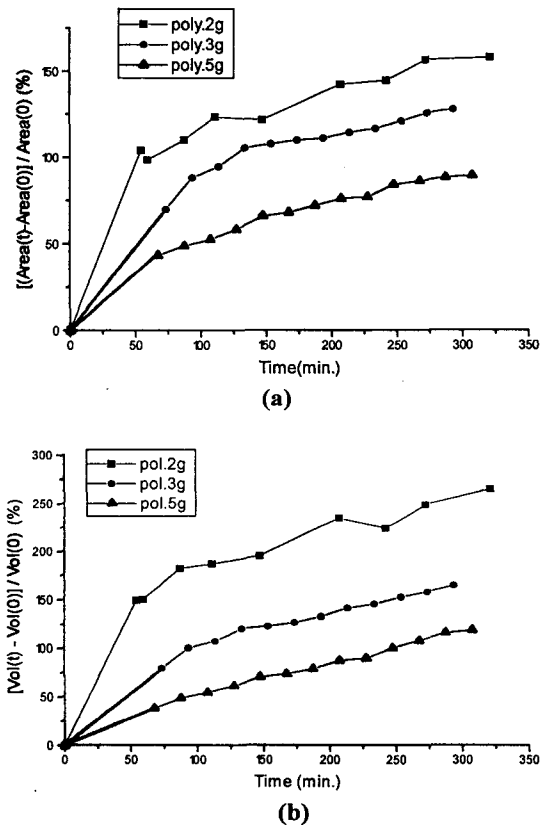


Fig. 4 Time dependent changes of cross sectional area (a) and volume (b) for the three PVA specimens.

Fig.5 displays a series of T1 weighted images of the DDS immersed in distilled water with a pH of 7. Numbers enclosed in parentheses represent hours and minutes elapsed since the DDS was immersed in water. The images shown in Fig.5 clearly delineate the state of drug release as well as the swelling effect of polymer wall (gelatin) which encloses drug. Optical images of this DDS were obtained in Fig.6. For the optical images, a scratch was intentionally made on the surface of a squalene capsule to accelerate the drug release. The number next to each optical image represents the elapsed time in minute since the capsule was immersed in the buffer solution. The liquid drug can not be optically discriminated from the buffer liquid as shown in the optical images of Fig.6. On the other hand, NMR microimaging makes the discrimination possible by adding the relaxation weightings on the images. (in this case all the images were T1 weighted, which means that imaged areas with different spin-lattice relaxation times (T1) are seen with different intensities on the image.) Fig.7 shows the changes of released drug as a

function of time. Net quantity is measured since the drug-release started at the pH of 7. A peak is observed on the graph of Fig.7 approximately 2hours after the immersion and may be attributed to the stress of the vial wall to the swollen DDS.

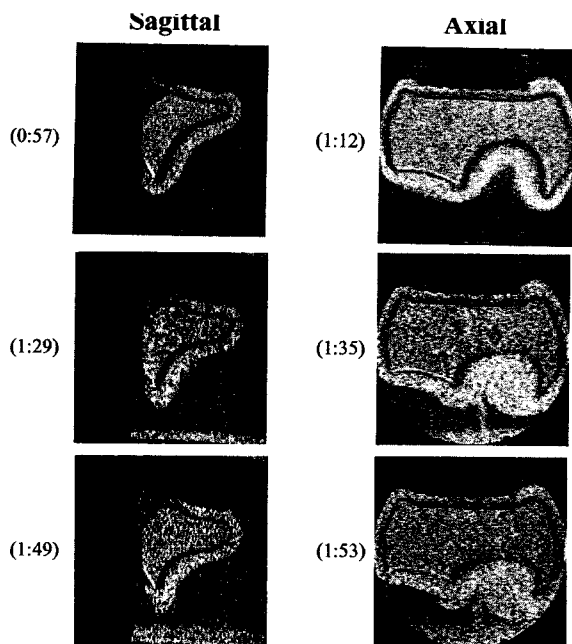


Fig. 5 Time dependent spin echo images of the drug release. Pulse sequence with TR/TE=200/30ms was used with a field of view of 17mm and slice thickness of 1mm. (a) his a series of sagittal views while (b) is a series of axial views, roughly corresponding to the sagittal images shown in (a).

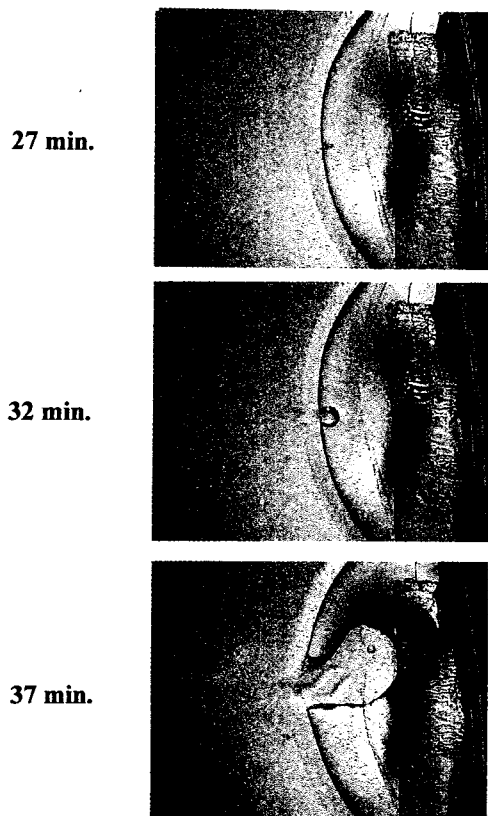


Fig. 6 Optical Images of the drug release.

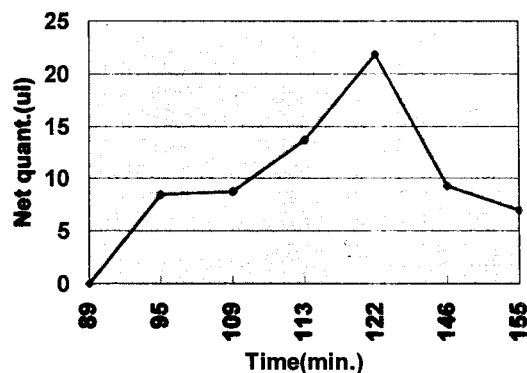


Fig. 7 Changes of released drug as a function of time; net quantity was measured since the drug-release started at the pH of 7.

Discussion and Conclusions

In the present study time dependent NMR microimages were acquired to show noninvasively the ingress of water into PVA specimens and the drug release phenomenon along with their geometrical changes in the DDS. We find that NMR microimaging can be a new and an important analytic tool for the study of polymers noninvasively. In additions, NMR microimaging can evaluate the drug release process for the relaxation weighting, which is impossible for optical microscopy. One problem for PVA specimens is that the specimens investigated in the study are not infinitely long cylinder. Since the diffusion effect relating with the sample geometry varies[9], the limited length of cylindrical sample as in this study may provide different diffusion behavior from that of a long cylindrical sample.

References

1. P.T. Callaghan, "Principles of Nuclear Magnetic Resonance Microscopy", Clarendon Press, Oxford, 1991.
2. D.H. Lee, R.K. Ko, Z.H. Cho and S.S. Kim, "Noninvasive study of the swelling effect for biopolymers using NMR Microimaging", KOSOMBE, 17(1), 223-226(1995).
3. J.U. Sommer, T.A. Vilgis, and G. Heinrich, *J. Chem. Phys.* 100(12), 9181 (1994).
4. A. Hakiki and J. E. Herz, *J. Chem. Phys.*, **101**, 9054, 1994.
5. L. Rogovian, P. Zhou and H.L. Frisch, *J. Polym. Sci. Part A: Polym. Chem.*, **32**, 515 (1993).
6. M.A. Brown and R.C. Semelka, "MRI: basic principles and applications", John Wiley and Sons, 1995.
7. P.T. Callaghan, "Principles of Nuclear Magnetic Resonance Imaging", Clarendon Press, Oxford 1991.
8. Y. Li, and T. Tanaka, *J. Chem. Phys.* **92**, 1365 (1991).
9. Y. Li and T. Tanaka, *J. Chem. Phys.* **92**, 1365- 1990.