# Enhanced Drug Uptake and Retention by Surface Phosporylated Polyvinyl Alcohol

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**ABSTRACT:** Polyvinyl alcohol (PVA) is one of the widely used synthetic polymers for a variety of medical applications. Surface modification of polymers is often used to improve the surface-mediated interactions with the bioenvironment. When phosphate groups are introduced onto the surface of PVA, the modification enhances the hydrophilicity of the polymer. A severalfold enhancement in the extent of uptake of antibacterial drugs such as ampicillin occurs as a result of the phosphorylation. It is also found that drug retention is prolonged significantly by the modification process. Interestingly, the modification did not alter bulk features like the crystallinity of the PVA. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 651–656, 2004

**Key words:** PVA; phosporylation; ampicillin

## INTRODUCTION

Hydrogels are prominent materials with extensive applications in contemporary health care management. This class of polymers in different forms is involved in fabrication related to ophthalmic products, wound dressings, sustained drug release formulations, etc.[1–3] Polyvinyl alcohol (PVA) is a prominent member of the hydrogel family. PVA has been used in many studies related to drug delivery systems, scaffolds for cells, and wound care formulations.[4–7] The commercial availability, the relatively better biocompatibility, and the feasibility of crosslinking the polymer using a wide range of difunctional entities are the major factors for the wide popularity of this polymer.

Infection is a major concern of devices based on synthetic materials.[8,9] The ability of microorganisms to adhere to the surface of the material is the primary factor in the initiation of device-related infection.[10] Unfortunately, the device-related infection is often resistant to treatment due to formation of a biofilm, which reduces the effectiveness of antibacterial drugs.[11,12] Apparently, treatment of biofilm-associated infections pose a significant challenge to clinicians. The simple and straightforward approach to control material-related infections is coating the surface of the device such as the catheter or wound dressing with antibacterial agent-like antibiotics. This approach, in fact, has widely been used in many studies.[13–15] The serious limitation of this process is the rapid loss of the drug, curtailing the long-term effectiveness.[16] The material modifications to enhance the retention of the drug often affect the material adversely. Simple modifications that do not affect the inherent features of the materials capable of enhancing the drug lodability and control the release profile appear to be advantageous. Such a possibility is demonstrated in this article using PVA. Phosphate groups can easily be incorporated into PVA without affecting its salient features. The enhanced uptake of a representative drug, ampicillin, as a result of this modification as well as the improved release profile is discussed in this article. To this investigator's knowledge such a study has not been reported.

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## EXPERIMENTAL

### Materials

Hot-water-soluble PVA with an average molecular weight of 70,000 was obtained from Sigma Chemicals Co. (St. Louis, MO). All other chemicals were from E. Merck (Mumbai, India).

### Preparation of the polymer film

About 2 g PVA was placed in about 30 mL distilled deionized water and heated to 70°C under stirring. The solution obtained was poured into a petri dish and the water was allowed to evaporate at room temperature (30°C). The film obtained was subsequently dried in a vacuum oven at 50°C. Strips of dimensions

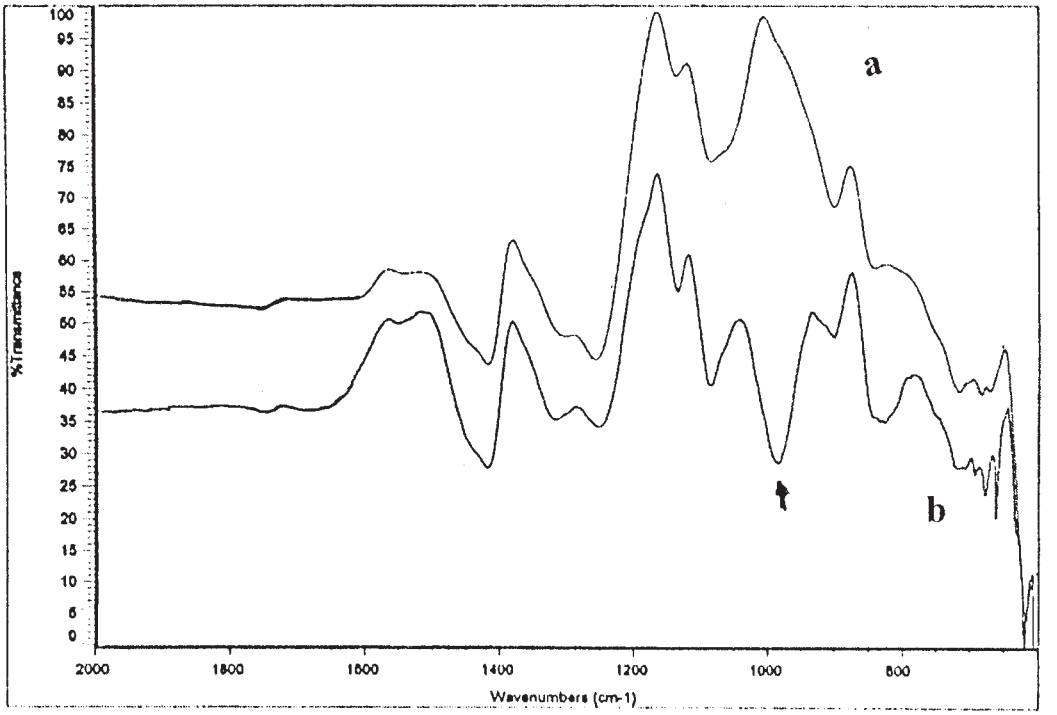



**Figure 1** (a) FT-ATR-IR spectrum of PVA; (b) FT-ATR-IR spectrum of phosphorylated PVA (M-PVA).

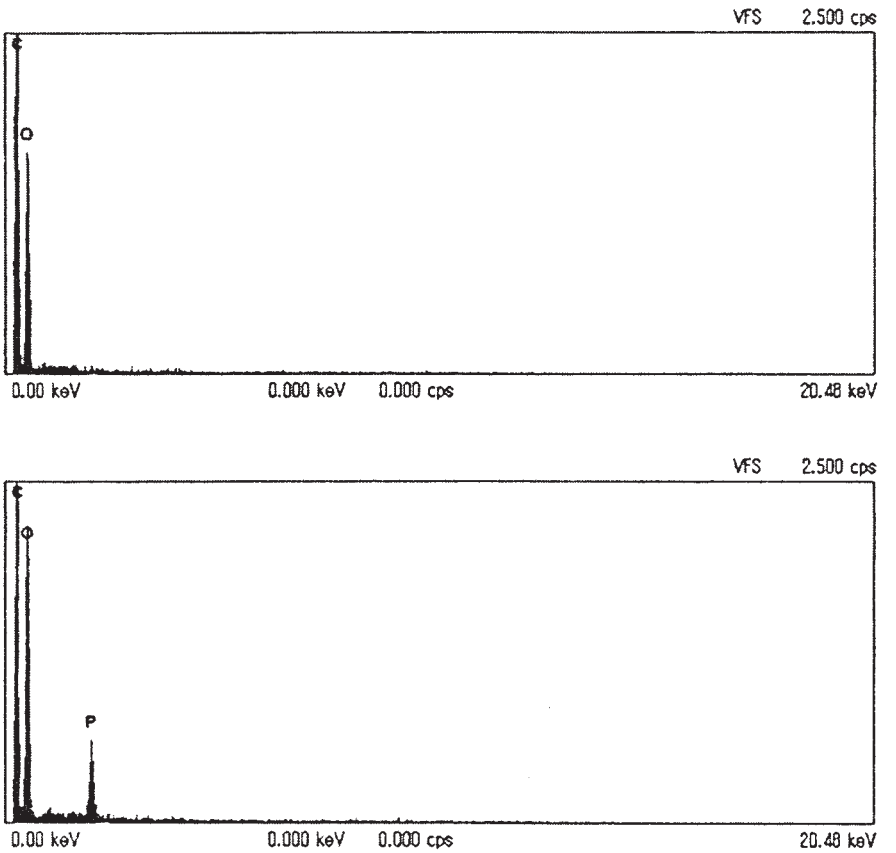



**Figure 2** (a) Energy dispersive X-ray analysis spectrum of PVA; (b) energy dispersive X-ray analysis spectrum of M-PVA.

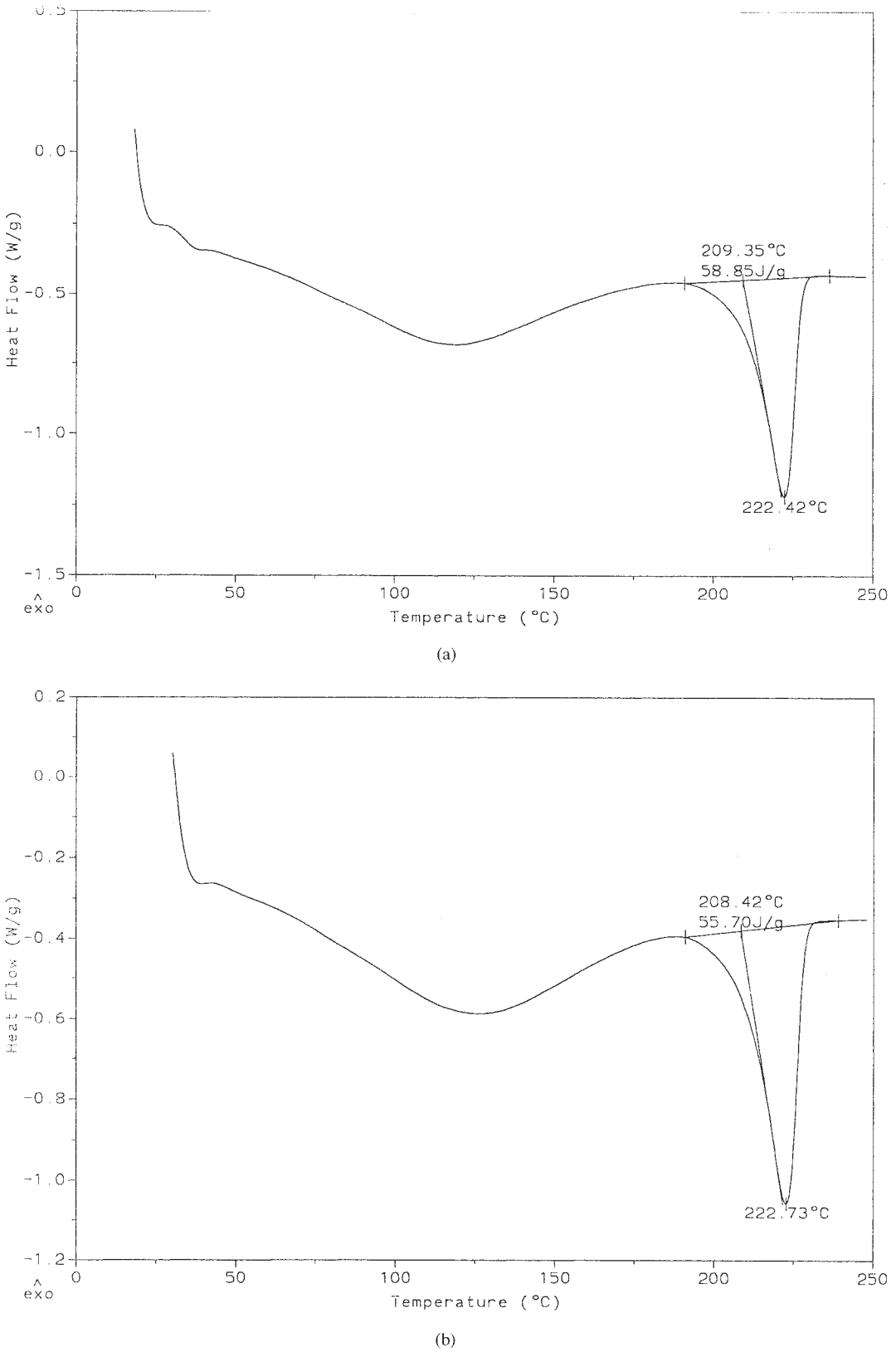



**Figure 3** (a) Differential scanning calorimetric scan of PVA; (b) differential scanning calorimetric scan of M-PVA.

$4 \times 1$ cm$^2$ with a thickness of 0.2 mm were subjected to phosporylation as reported elsewhere.[17] The strips were immersed in the solution for 24 h at room temperature (30°C). The strips were then washed extensively in distilled water and then dried in an air oven at 50°C.

**TABLE I**
**Water and Drug Uptake by the Polymers**

| Material | Equilibrium absorption of water (%) | Equilibrium uptake of the drug($\mu g/cm^2$) |
|---|---|---|
| PVA | 220 ± 5 | 40 ± 2 |
| M-PVA | 310 ± 4 | 382 ± 4 |

### Drug loading

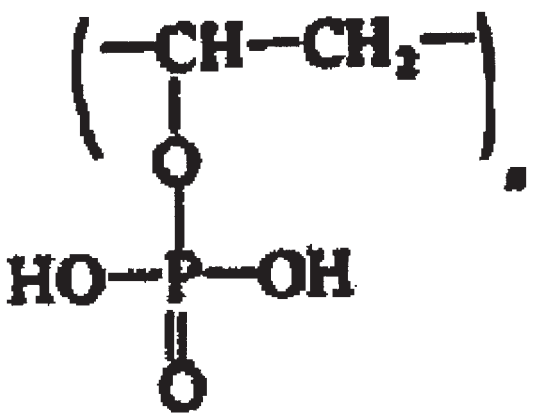
The phosporylated and control strips were immersed in 10 mL solution of ampicillin (0.2 mg/mL). The pH of the solution was around 7. The OD of the solution at 254 nm before placing the strips was measured. After every hour, the OD was measured until it was constant, indicating the equilibrium absorption of the drug by the polymer. The quantity of drug absorbed by the polymer was estimated from a calibration plot constructed between the OD and concentrations of standard drug solutions. In a similar fashion, the extent of uptake of the drug by unmodified polymer was also estimated.

### Swelling of the polymer

A known weight of dried polymer strip was placed in distilled deionized water (pH ~ 7) and the weight of the film was measured at regular time intervals. The equilibrium swelling ($S$) was estimated from $S = (W - W_0/W_0)\ 100$, where $W$ is the equilibrium weight, and $W_0$ is the initial weight.

### Drug release profile

The dry drug loaded polymer strip was placed in 10 mL distilled deionized water. At regular time intervals water was completely drawn out and OD was measured. A total of 10 mL fresh water was again added. A plot was constructed between the amount of drug released and time in hours. All experiments were performed in triplicate and points in the plot are the average of the three readings.



**Scheme 1**

### Cytotoxicity evaluation

L929 cells were cultured in Dulbecco's minimum essential medium (Hi Media) supplemented with 10% calf serum in standard tissue culture flasks (Nune) in a humidified atmosphere containing 5% carbon dioxide. To prepare the test well, cells were detached from the culture flasks using a trypsin/EDTA solution (0.25%) and resuspended as a single suspension in culture medium at a concentration of $2 \times 10^5$ cells/mL. Cells were seeded into the wells of a 24-well plate at a density of $\sim 1 \times 10^5$ cells/well. Cells were allowed to grow to a monolayer. Once the cells were grown as a monolayer, the materials were kept in contact with the cells.

After 24 h of contact with the materials, cells were examined using a Leitz phase contrast inverted microscope. Morphology of the cells was assessed in comparison with negative and positive control materials. Materials were considered nontoxic when the cells showed the same morphology as cells in contact with negative control.

### Instrumental

Infra red spectra were obtained using a Nicolet, Inc. (Madison, WI) model Impact 410 FT-IR spectrophotometer with a horizontal ATR accessory. The resolution was 2 $cm^{-1}$ and the total number of scans was 50. Energy dispersive X-ray analysis was performed on EDS Model 6051SP (Oxford Instruments, UK) attached to a Hitachi model S-2400 scanning electron microscope.

### Differential scanning calorimetry

The DSC analysis was performed on TA (DE) Model 2920 DSC cell. The samples were hermetically sealed in aluminum pans and scanned from 25 to 250°C under a dynamic atmosphere of nitrogen at a heating rate of 10°C/min.

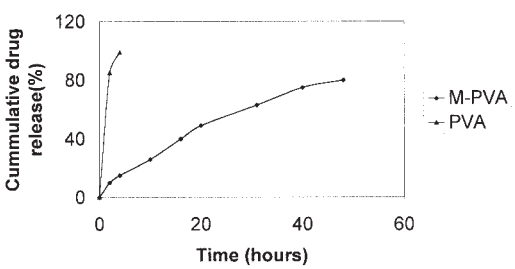
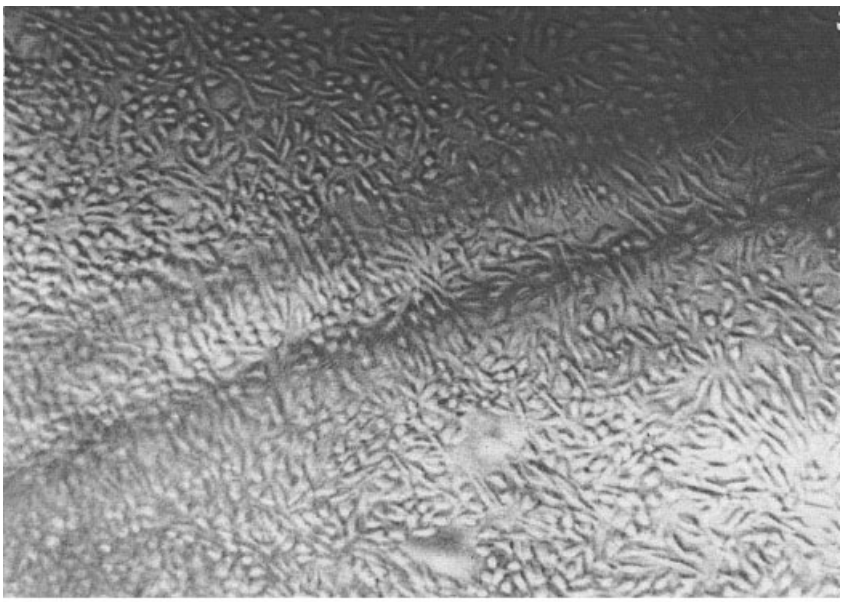
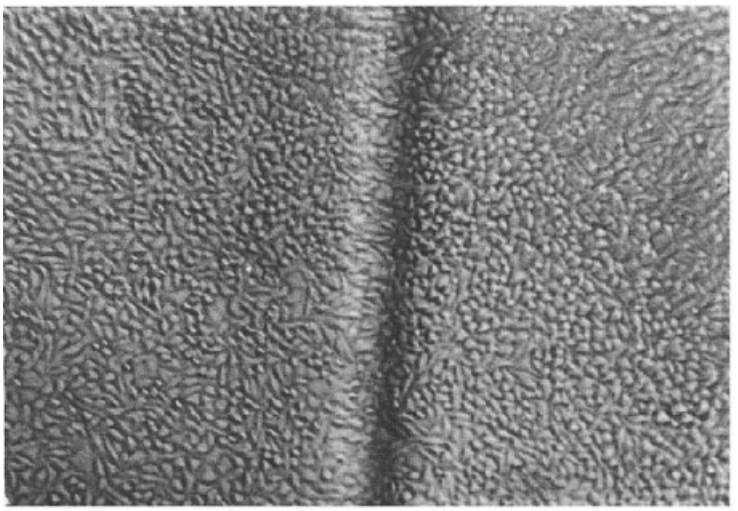



**Figure 4** Release pattern of the drug from M-PVA and PVA.



(a)



(b)

**Figure 5** (a) L929 cells in contact with M-PVA; (b) control cells.

## RESULTS AND DISCUSSION

Figure 1a and b shows the FT-ATR-IR spectra of PVA and phosporylated PVA (M-PVA), respectively. A strong peak, characteristic of P–O–C stretching, can be seen around 990 $cm^{-1}$, indicating the presence of phosphate groups. EDX scans (Fig. 2 b) show the presence of P at 2.01 eV in addition to C and O, while it is absent in PVA (Fig. 2a).

Figure 3a and b depicts the DSC scans of PVA and M-PVA. A broad endotherm centered on 100°C is assigned to the evaporation of traces of water. The endotherm centered around 223°C is associated with the melting of the crystalline phase.[18] The same feature is exhibited by M-PVA, indicating that the morphology is almost unaffected by the modification. This is expected, in the sense that the phosphrylation is largely confined to the surface of the film.

Table I summarizes the equilibrium water and the drug (ampicillin) uptake by the materials. It is interesting to see that M-PVA absorbs more water than PVA, indicating that modification enhances the hydrophilic nature of PVA. The structure of PVA after the phosphorylation is shown in Scheme 1.[19]

The presence of additional –OH groups due to the presence of phosphates groups presumably could engage in hydrogen-bonding interactions with water molecules, resulting in enhanced absorption. The equilibrium absorption of the drug by PVA is 40 $\mu g/$

$cm^2$ while that of M-PVA is 382 $\mu g/cm^2$. Phosphorylation results in a nearly 10-fold increase in the drug uptake. The presence of phosphate groups seems to be responsible for the increased absorption of the drug.

Figure 4 depicts the release profile of the drug from the polymers. It is interesting to see that the drug is desorbed rapidly from the unmodified polymer while it is delayed considerably from the M-PVA, again reflecting the enhanced interaction between the drug and the phosphate groups.

The minimum inhibitory concentration (MIC) of drugs such as ampicillin for pathogens (e.g., *Staphyloccocus aureus*) is 0.05 $\mu g/mL$. However, for certain pathogens like *Pseudomonas aeruginosa*, the MIC is around 200 $\mu g/mL$.[20] In such cases only modified PVA can deliver the required quantity of drugs. The extent of uptake of the drug by M-PVA is 382 $\mu g/cm^2$, indicating that the material is capable of controlling the bacterial growth to a significant time period and it is a suitable material for a variety of application such as wound dressings and hydrophilic coating.

The mandatory requirement of any material intended for medical applications is nontoxicity. The optical microphotograph shown in Figure 5a is that of L929 cells in contact with the material. The cell morphology exactly matches that of the control cell (Fig. 5b), indicating that M-PVA is noncytotoxic.

The prevention and control of biomaterial-related infection has been the subject of many investigations. The majority of these studies used methods of incorporating antimicrobial agents into the medical device itself. A widely followed safe approach is to coat the surface of the device with an appropriate material. It is highly desirable that the coated material should possess good biocompatibility in addition to being capable of releasing the antimicrobial agent in a sustained way. The method reported here seems to have these features, considering the fact that PVA is widely known for its tissue-loving properties.[21]

## CONCLUSION

The surface phosphorylation of PVA enhances the hydrophilicity while retaining the bulk morphological features such as crystallinity. This simple modification, apart from increasing the drug uptake, prolonged the drug retention, reflecting the suitability of the material for antibacterial and related applications.

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