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Research article

The use of ^{13}C solid state NMR to elucidate physico–chemical association in Eudragit® RS100 microencapsulated acyl esters of salicylic acid

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Abstract

A series of homogeneous Eudragit® RS100 matrix microspheres containing molecularly dispersed acylated esterified homologues of salicylic acid, (acetylsalicylic acid, valerylsalicylic acid, or caprylsalicylic acid) were prepared in order to investigate the effect of encapsulation on solid-state orientation of the encapsulated molecule. Electrostatic association of the drug with the charged quaternary residues in the polymer may be responsible for the previously observed stability of acetylsalicylic acid (ASA) in aqueous swollen ASA-loaded Eudragit® RS100 microspheres. Evaluation of the ^{13}C nuclear magnetic resonance spectra for evidence of structural association of the incorporated probe molecules indicated that alteration of the microenvironment of the incorporated solutes had occurred. For instance, increasing the aliphatic character of the acyl side chain resulted in an increase in the upfield shift of the acyl bearing aromatic ring carbon, (C2), in the incorporated probe molecule as compared to the unincorporated probe molecule. Similarly, a downfield perturbation in the chemical shift of the free acid bearing aromatic ring carbon, (C1), was also observed. This microenvironment electrostatic shielding in the proximity of the ester carbonyl is attributed to an increase in the association of the probe molecule with the polymer subunits. Thereby, it is postulated that the matrix incorporated probe molecule is essentially shielded from hydrolytic attack until it is liberated into the external aqueous environment. © 1998 Elsevier Science B.V.

Keywords: Eudragit® RS100; Microsphere; Esterified drug; Hydrolysis; ^{13}C solid state nuclear magnetic resonance; Electrostatic association

1. Introduction

Inclusion of esterified molecules susceptible to hydrolysis into a polymeric encapsulating material, such as Eudragit® RS100 (Röhm), may decelerate the reactivity of the encapsulated molecule [1]. The apparent stabilization of the encapsulated molecule was attributed to the nature of the decomposition reaction and the interaction with the encapsulating environment.

Methacrylate copolymers, such as Eudragit® RS100, are widely used in the pharmaceutical industry as solid dosage form adjuvants and coating polymers. The characteristic permeability and solubility of these polymers is dependent on the functional groups present on the copolymer backbone chain, the molecular weight of the polymer and, the nature of the surrounding medium. The RS acrylic resin is a copolymer synthesized from acrylic and methacrylic acid esters. As a result of the incorporation of esterified quaternary ammonium func-

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tional groups, Eudragit® RS possesses a defined swelling capacity and permeability with respect to water which is independent of pH [2].

Therefore the focus of the present work is to examine the extent to which drug structural configuration can be correlated with the potential shielding effect afforded by microencapsulation. This work is facilitated through the use of an analytical technique not frequently reported in the pharmaceutical literature: spin resonance shifts in solid state ^{13}C nuclear magnetic resonance spectroscopy. Molecular conformation and orientation may be assessed by solid state nuclear magnetic resonance (NMR) spectroscopic studies of the individual components and the drug incorporated polymer. Numerous recent advances have enabled high resolution spectra of solids to be made under routine conditions [3]. Solid-state NMR analysis has been demonstrated to be a powerful technique for studying drug-excipient interactions [4], drug inclusion complexes [5] and drug-polymer interactions [6]. The intent of this work is to establish a rationale that adequately describes the observed physico–chemical properties of a Drug-Eudragit® RS100 matrix. Structural analogues of salicylic acid are used to elucidate the relationship between drug compound structure and molecular orientation within a drug-loaded, Eudragit® RS100 polymer microsphere.

2. Background

This study necessitates the preparation of drug-containing microspheres devoid of distinct crystalline regions. The absence of a distinct endotherm for the encapsulated drug in the DSC thermogram suggested the drug was capable of dissolving in the polymer and forming a solid solution [7,8].

The most important reaction contributing to the instability of substituted phenyl esters, as typified by the acyl esters of salicylic acid (SA), in aqueous solution is hydrolysis which yields the parent alcohol moeity, SA, and the corresponding acyl acid. It is postulated that inclusion of a substituted phenyl ester into the polymeric encapsulating material, Eudragit® RS100, may decelerate the reactivity of the encapsulated molecule, with respect to the nature of the decomposition reaction and the encapsulating environment.

Thus in general ester hydrolysis is characteristically pH dependent and can be explained by a hydronium ion-catalyzed reaction in acidic medium and a hydroxide ion-catalyzed reaction in basic solution [9]. The mechanism of ester hydrolysis involves the formation of a rate determining tetrahedral intermediate which includes one molecule of general acid or general base and one molecule of nucleophile (either the solvent or an added nucleophile) in addition to the substrate. Electron withdrawing substituents in either the acyl groups or the alkoxy groups of the ester will facilitate hydrolysis in alkaline solution where the negatively charged tetrahedral intermediate will be stabilized. Consequently, the same type of groups will impede hydrolysis in acidic solution [10]. If a cyclic transition state is necessary for cleavage of the ester group in substituted phenyl esters, then increasing the aliphatic character of the acyl group may result in less conformational freedom in the Eudragit® RS100 encapsulated ester. A number of studies have indicated that where intramolecular catalysis is implicated in ester hydrolysis, structural features that cause the initial conformation to be less like the cyclic transition state will diminish reactivity [11,12]. Increasing the aliphatic character of the acyl portion of substituted phenyl esters may increase the likelihood that polymer matrix incorporated drug molecules associate with the aliphatic portions of the polymer backbone. This preferred association will, in turn, reduce the tendency of the incorporated molecules to assume the necessary spatial conformation to effect hydrolysis. Any factors which affect the proclivity of the ester bond toward hydrolysis or the accessibility of the drug molecule to the hydrolysis medium may affect the drug release behaviour from the encapsulating polymer.

A number of polymer effects are able to modify the hydrolysis of susceptible moieties present on polymer side chains. The local micro-environment imparted by the proximity of the polymer backbone results in 'local medium effects', neighbouring group interactions, and polyelectrolyte effects [13] which are interrelated. Thus it is possible to observe reactions in a polymer which are not greatly affected by a change in bulk solvent properties. This principle may be extended to the reactivity of molecularly incorporated drugs within a polymer environment since hydrolytically susceptible drugs may also be affected by these same 'local medium effects'.

Many hydrolysis reactions of pendant groups in a polymer are subject to large neighbouring group effects such that the kinetics of the process may be described by one of three rate constants, k_0, k_1 or k_2, which characterize the hydrolysis of functional groups with no reacting neighbours, one reacting neighbour or two reacting neighbours, respectively. The theory of the kinetics of a reaction exhibiting this feature has been developed by Keller [14]. A neighbouring group effect may result from direct participation of an adjacent chemical moiety in the reaction or indirectly due to the polarity or hydrophilicity of the neighbouring group. The latter situation is likely to be present when drug compounds are molecularly dispersed in a polymeric matrix. This effect may inhibit or accelerate the hydrolysis reaction taking place within the polymer matrix involving drugs susceptible to such degradation. If the side chains of the polymer backbone contain ionizable

groups, then the pH of the bulk solution will dictate the localized charge distribution around these pendant molecules. In the case of Eudragit® RS100, the quaternary ammonium groups will repel hydronium ions and attract the hydroxide ions. In either case, the unrestricted movement of these potential hydrolysis catalyzing ions will be compromised due to the electrostatic association with the polymer thus inhibiting the acid or base catalysis of ester hydrolysis. These solution effects may be further complicated by polymer/polymer interactions as well.

Several investigations have been published detailing the propensity of pharmaceutically useful polymers to bind drugs [15,16]. Okada et al. [17] studied the adsorption of phenothiazine derivatives on microcrystalline cellulose in buffer solutions. The authors proposed that the electrostatic binding of a cationic drug to the anionic microcrystalline cellulose surface was inhibited by increased ionic strength due to the restriction of the electric double layer around the cationic and/or anionic centre. A certain level of adsorption was maintained even at very high ionic strengths, indicating that non-electrostatic binding due to hydrogen-bonding and van der Waals forces also occurred.

The localization of fixed charges in the Eudragit® RS100 polymer every 40 molecular residues [18] may lead to the binding of counterions at these specific sites. The concept of ion pair formation proposes that for ions of opposite charge the number of ions of charge, z_i, around a reference charge, z_j, passes through a minimum at [19]:

$$r_{\min} = \frac{-e2z_iz_j}{2\epsilon k_B T} \quad (1)$$

where r is the distance between ions, e is the elementary charge, ϵ is the dielectric constant, k_B is Boltzman's constant, T is the absolute temperature of the system. Eq. (1) indicates that all ions which are at a distance smaller than $r_{\min}$ be considered as associated in ion pairs [20]. Fuoss and Chu [21] studied systems of bis-quaternary ammonium salts of the general type $Cl^-[(CH_3)_3N^+-(CH_2)_n-N^+(CH_3)_3]Cl^-$ and found that one of the chloride ions continued to be strongly associated with this di-cation as n was varied from three to five. Thus it is likely that a polyion region will mimic the Eudragit® RS100 polymer chain due to the presence of quaternary ammonium residues such that many ions ($+$ve and $-$ve) will be excluded from this domain owing to the high local concentration of fixed charges. The local electrostatic environment of the polymer may interfere with the degree of conformational freedom available to acyl esterified SA molecules that are closely associated with the polymer. Likewise, the approach of catalytic molecules or ions necessary to achieve hydrolysis of acyl esterified SA may also be retarded. This effect may be likened to the shielding process that occurs in proteins where conformational restriction plays a role in the reactivity of enzymes. For example, in the case of chymotrypsin which catalyzes the hydrolysis of certain amides and esters, the reactivity of a given functional group may be enhanced or reduced by its proximity to another influencing group within the active site of the enzyme. The extent of the enzyme reactivity is a result of the specific defined macromolecular conformation particular to the chymotrypsin polypeptide and is lost when the protein is denatured [22].

3. Materials and methods

Materials were obtained from various commercial suppliers and used as received. Methylene chloride, light paraffin oil (mineral oil), 95% ethanol, acetone, cyclohexane, tetrahydrofuran (THF), and pyridine were certified A.C.S. reagent grade and used as received from Caledon Laboratories, Toronto, Ontario. Glacial acetic acid, sodium acetate, hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium chloride (KCl), and sulfuric acid (H_2SO_4) were of analytical grade and obtained from Fisher, Toronto, Ontario. Deionized water was utilized throughout the experiments.

Salicylic acid (SA), lot number 0995, valeric anhydride, and decanoyl chloride were of high purity grade obtained from Aldrich, Toronto, Ontario. Acetylsalicylic acid USP (ASA), lot 900126, the C2 acyl ester of salicylic acid, was obtained from Ward Robertson, Toronto, Ontario.

Eudragit® RS100, lot number 08-80256, was obtained from Röhm, Darmstadt, Germany. The polymer is a copolymer synthesized from acrylic and methacrylic acid esters with a low content of quaternary ammonium groups present as chloride salts. The molar composition of ethyl acrylate, methyl methacrylate and trimethylammonioethylmethacrylate chloride is 10:20:1 for Eudragit® RS100 with a mean molecular weight of 150 000 [18].

3.1. Structural analogues of salicylic acid

Commercial sources of the C5 and C10 acyl esters of salicylic acid were not readily available. Therefore, the synthesis of these compounds was undertaken in the laboratory. With the exception of acetylsalicylic acid, there is relatively limited information in the chemical literature on the synthesis of higher acyl esters of salicylic acid.

Valerylsalicylic acid (C5 acyl ester) was prepared by acylation of salicylic acid with valeric anyhydride in the presence of a catalytic amount of concentrated sulfuric acid, followed by repeated recrystallization from 35% aqueous ethanol according to a modified procedure from Hofstee [23].

Caprylsalicylic acid (C10 acyl ester) was prepared by acylation of salicylic acid with decanoyl chloride in a mixture of THF and pyridine following a modified procedure from Valenti et al. [24].

Purity of the resulting products was confirmed by thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC), and identity by ultraviolet/visible, infra-red, mass, and nuclear magnetic resonance spectroscopies.

Collectively, with ASA, these compounds form an homologous series of structurally related compounds incorporating acyl ester side chains of varying lengths (C2, C5, C10) at the *ortho*-OH functional group of the root compound, salicylic acid.

3.2. Preparation of microspheres

A solvent partition procedure, described previously [8], was used to prepare Eudragit® RS100 microspheres. An infusion solution (10 ml) of 5:1 polymer to drug ratio in a mixed organic solvent of methylene chloride and acetone (9:1) at a total solids content of 15% w/v was used for this study. Under these conditions, resulting microspheres achieve 10% nominal loading of the active ingredient. Five sets of microspheres were prepared under these conditions; blank microspheres containing no drug, SA loaded microspheres, C2 loaded microspheres, C5 loaded microspheres and C10 loaded microspheres.

3.3. Determination of drug loading

The drug loading, expressed as weight of drug per weight of microspheres, was determined in duplicate for each batch using a UV/Vis spectrophotometer (Perkin-Elmer, Norwalk, CT, model Lambda 2). The samples were analyzed as follows: 50 mg of microcapsules from each batch were dissolved in methylene chloride and the total volume adjusted to 100 ml in a volumetric flask. A standard curve was prepared for the active ingredient and absorbance readings for the samples were recorded at the maxima for this compound. Although the acrylic polymer does not interfere with the absorbance measurements, the absorbance of SA does contribute to that of the parent ester molecule and vice versa. Correction for this interference is achieved through the use of a procedure involving simultaneous equations derived from standard curves [25]. The level of free SA in the microspheres was used as an indicator of ester decomposition during microsphere preparation and was found to be less than 1% of the microsphere in all cases.

3.4. Nuclear magnetic resonance

Approximately 200 mg of the sample was introduced into the probe rotor. Solid state NMR ^{13}C NMR spectra were recorded on a Bruker MSL-100 NMR spectrometer operating at 25.18 MHz with a magnetic field strength of 2.35 T. A ninety degree pulse ^{1}H decoupling field of 15 gauss with a pulse duration of 4 μs and a spinning frequency rate of 4.2 kHz was used to record the cross-polarization magic angle spinning (CP/MAS) spectra. Chemical shifts were referenced to external adamantane with a chemical shift of 38.2 ppm. More than 2400 scans were acquired for each resulting spectrum. All observations were made at temperatures between 20 and 22°C. Nuclear assignments were made according to the typical chemical shifts [26] identified in Fig. 1.

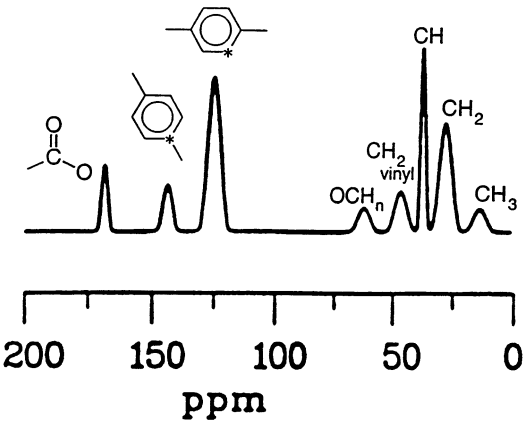


Fig. 1. Schematic overview of ^{13}C isotropic shifts in organic polymers and compounds. Diagrams with the isotropic shift ranges for many moieties may be found in the literature [26].

4. Results and discussion

A previous report [1] has indicated that the observed deceleration in the hydrolysis of ASA located within the Eudragit® RS100 microsphere as compared to the external phase could be ascribed to the shielding of the ester functional group from the attacking nucleophile as the ester molecule electrostatically complexes with ionized moieties in the encapsulating environment. The authors further reported that irreversible binding of ASA within Eudragit® RS100 occurred to the extent of approximately 0.5% w/w of the initial payload, remaining intact and associated with the microsphere phase after prolonged exposure to strongly alkaline aqueous conditions. The same complexing phenomenon may be exerting a modifying influence on the hydrolysis kinetics of other acyl esters of salicylic acid incorporated into Eudragit® RS100 microspheres. The effect of anionic, cationic and nonionic surfactants on the stability of solubilized ASA studied by Nogami et al. [27] indicated that each surfactant suppressed the hydrolysis of unionized ASA, while the hydrolysis of the anionic form of ASA was suppressed only by cationic surfactants.

One of the important factors in the hydrolysis of polymer incorporated esters of SA is the elucidation of the substrate molecule orientation or interaction with the polymer phase. The resultant location of the substrate reaction centre within the polymer matrix would serve to clarify the observed hydrolysis rates. The polymer encapsulation of derivatives of SA containing hydrophobic aliphatic chains was undertaken in an attempt to vary any polymer–drug association that might be occurring within the matrix. Consequently, a physico–chemical change in the system would be reflected in the effective magnetic field environment of the incorporated probe molecules. Some support for this postulation is based on the results obtained by ^{1}H NMR studies conducted by Manohar et al. [28]. These researchers demonstrated that the NMR spectrum of the phenyl ring protons of the salicylic acid anion in the presence of dilute micellar solutions of cetyltrimethylammonium bromide (CTAB) was considerably different from that of unadulterated solutions of sodium salicylate. It was found that the 3-, 4-, and 5-H aromatic resonances shifted to lower δ values in the presence of CTAB while the 6-H resonance was much less shifted. This suggested that the *meta* and *para* protons of sodium salicylate shifted to a more non-polar environment in the presence of CTAB micelles whereas the *ortho* protons essentially had the same polar environment both in the presence and absence of CTAB. Manohar et al. [28] accounted for the observed resonance shifts by proposing a molecular orientation model where the 3, 4 and, 5 carbon atoms of the benzene ring were embedded inside the micelle leaving the rest of the benzene molecule outside of the micelle in the aqueous media. Thus, ^{1}H NMR analysis proved to be a useful tool in ascertaining drug–micelle association in solution.

A fundamental advantage of ^{13}C NMR over ^{1}H NMR is the much broader range of ^{13}C chemical shifts. While most ^{1}H resonances fall within a spectral width of 10 ppm, complete ^{13}C spectra generally occur over a width of 200 ppm. This significant increase in the dispersion shielding range of the carbon atoms is crucial for determining neighbouring group effects in the solid state where spectra lines are broadened as a result of the motionally restricted samples. Neighbouring group effects can include carbon hydridization which may shield or deshield carbons and substituent electronegativities which deshield carbons [29].

A ^{13}C cross-polarization, magic-angle spinning (CP/MAS) NMR spectrum for Eudragit® RS100 in the solid state is shown in Fig. 2. The chemical shift region for the polymer was approximately 10–190 ppm indicative of a polyacrylate polymer [30]. The spectrum consists of two loci; one at 14–60 ppm and the other centred around 176.5 ppm. Although the broad resonance lines can be further resolved by using a single-pulse, magic-angle spinning (SP/MAS) spectrum, Fig. 2 is sufficient for the purposes of identifying the polymer in the probe molecule–polymer matrix samples. Thus, all of the observed resonance signals were assigned as follows; aliphatic methyl group carbons and those corresponding to the quaternary ammonium function resonate between 14 and 20 ppm; aliphatic methylene group carbons appear at 60 ppm; quaternary substituted carbons appear at 44 ppm; α-acyl ester carbons appear at 51 ppm; and carbonyl carbons appear at 176 ppm.

Similarly, ^{13}C CP/MAS spectra for the individual probe molecules (acetyl ester, valeryl ester, capryl ester) were obtained in order to identify the chemical shift regions attributable to these molecules (Figs. 3–5). Chemical shift assignments were made in a manner

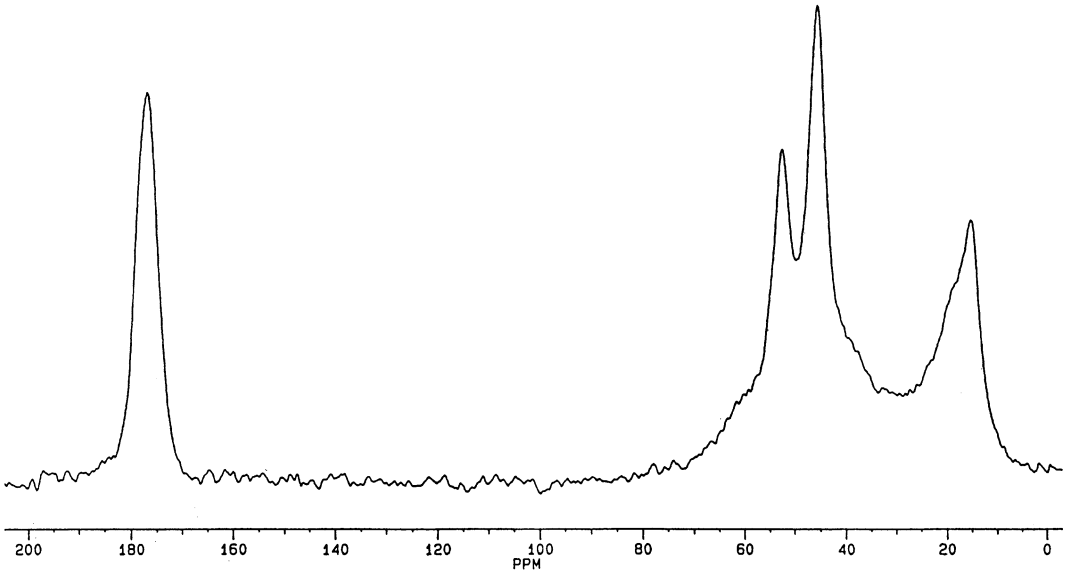


Fig. 2. ^{13}C solid-state NMR of Eudragit® RS100.

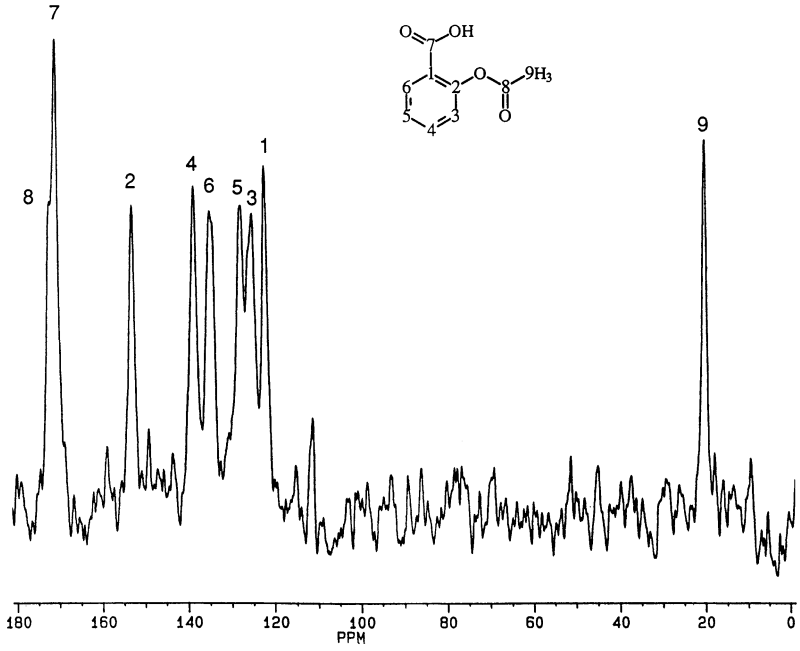


Fig. 3. ^{13}C solid-state NMR of acetylsalicylic acid (C2).

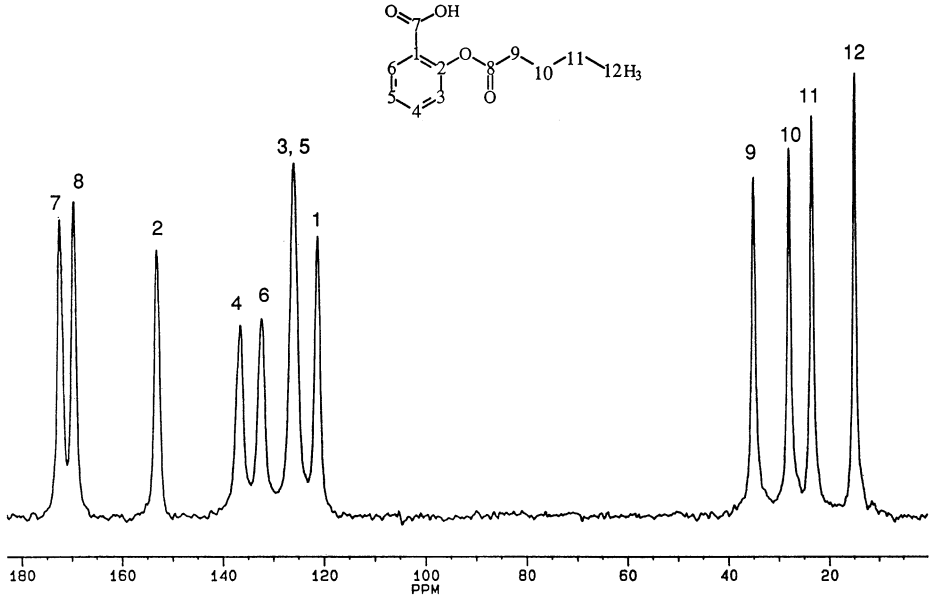


Fig. 4. ^{13}C solid-state NMR of valerylsalicylic acid (C5).

consistent with previously published CP/MAS NMR data for ASA [4]. The aromatic carbons were thought to be of interest since these carbons are particularly sensitive to aromatic ^{13}C shielding originating from local group effects or microenvironment effects. For instance, the carbons of benzene itself resonate at 128.5 ppm and substitution into the ring creates a range of ^{13}C shieldings from 110–170 ppm. This range is particularly amenable to the present drug–polymer association investigation since Eudragit® RS100 displays no resonance activity in this region.

In the absence of molecular interaction, the ^{13}C NMR spectrum for the drug–polymer matrix is expected to be the result of the superposition of the spectra of the two components separately. The spectroscopic behaviour of the Eudragit® RS100 carbons in the complex is virtually identical to that of pure Eudragit® RS100, as can be deduced from a typical spectra of ASA-Eudragit® RS100 in Fig. 6. This indicates that the local structure of the Eudragit® RS100 molecules around the incorporated probe molecules in the solid state matrix have the same orientation as the

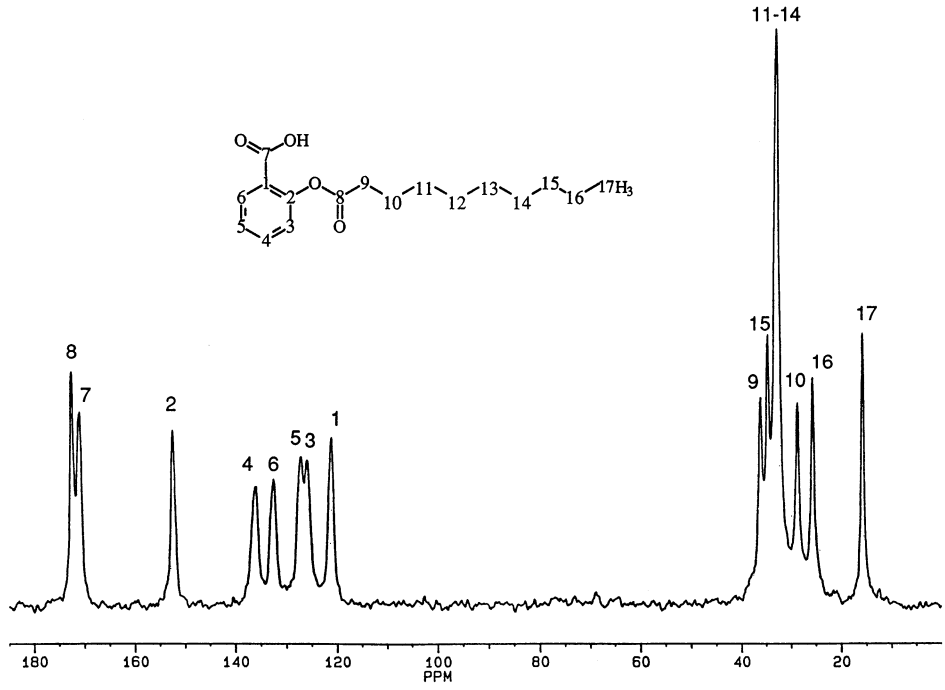


Fig. 5. ^{13}C solid-state NMR of caprylsalicylic acid (C10).

pure bulk polymer. Therefore, incorporating approximately 10% w/w of solute molecules into Eudragit® RS100 polymer does not significantly alter the crystallinity of the polymer. The consequence of this result cannot be understated since the degree of polymer crystallinity on the diffusion rate of polymer incorporated drug molecules has been linked in previous studies [31].

Table 1 lists the ^{13}C chemical shifts experimentally found for ASA when incorporated in Eudragit® RS100 as well as those corresponding to crystalline ASA. As can be inferred from the analysis of the resulting chemical shift changes, the spectroscopic behaviour of ASA carbons in the polymer matrix is similar in relative location to that found in crystalline ASA except for carbons situated in positions 1, 2, 4, 6 and 7. The carbon in position 7 appears electrostatically shielded, showing a substantially lower chemical shift, due to the stabilization of the ASA carboxylic group via intermolecular interaction with the polymer. This closer interaction, in turn, is responsible for the deshielding of the carbon at position 1. The opposite shift is noted for the carbons at positions 4 and 6 primarily as a result of the effects typically experienced by benzene carbons *ortho* and *para* respectively to a substituent. In this case, the electron density field associated with the carbonyl centre at position 7 is delocalized over the adjoining oxygens due to electrostatic bonding with the polymer. Thus the $\pi-\pi$ electron density is shifted from the ring system to this region. The remaining carbons show some degree of up-field or down-field shifts which can be ascribed to changes in the local environment at the various positions in the molecule due to the perturbations noted above. Choi [32] reported comparable chemical field shifts during the study of the molecular recognition of ASA in α-cyclodextrin. He concluded that ASA formed an inclusion complex with the hydrophobic cavity of α-cyclodextrin in the solution state but not in the solid state.

Analysis of the C5 and C10 ester homologues when incorporated into Eudragit® RS100 reveals a similar, but less pronounced, trend as noted for ASA in Eudragit® RS100 (Figs. 7 and 8, respectively and Tables 2 and 3, respectively). Evidently the association of the probe molecule with the polymer favours hydrogen-bonding or van der Waals interactions. This is reflected in a lessening of the electrostatic effects experienced by the carbons at position 1 and 7 as well as a return to normal resonance levels for the remaining carbons of the respective crystalline probe molecule.

It is expected that the increasing hydrophobicity of the homologous series of probe molecules will have a direct influence on the availability of the ester molecule in the aqueous phase within the microsphere. This is of considerable interest since the hydrolysis reaction is necessarily mediated through the solvent phase. Forster et al. [33] were able to show that the aqueous solubilities of a homologous series of alkyl-*p*-hydroxybenzoates, methyl to decyl, followed a linear decrease with increasing alkyl chain length until the chain was over four carbon units long. The effect of adding further

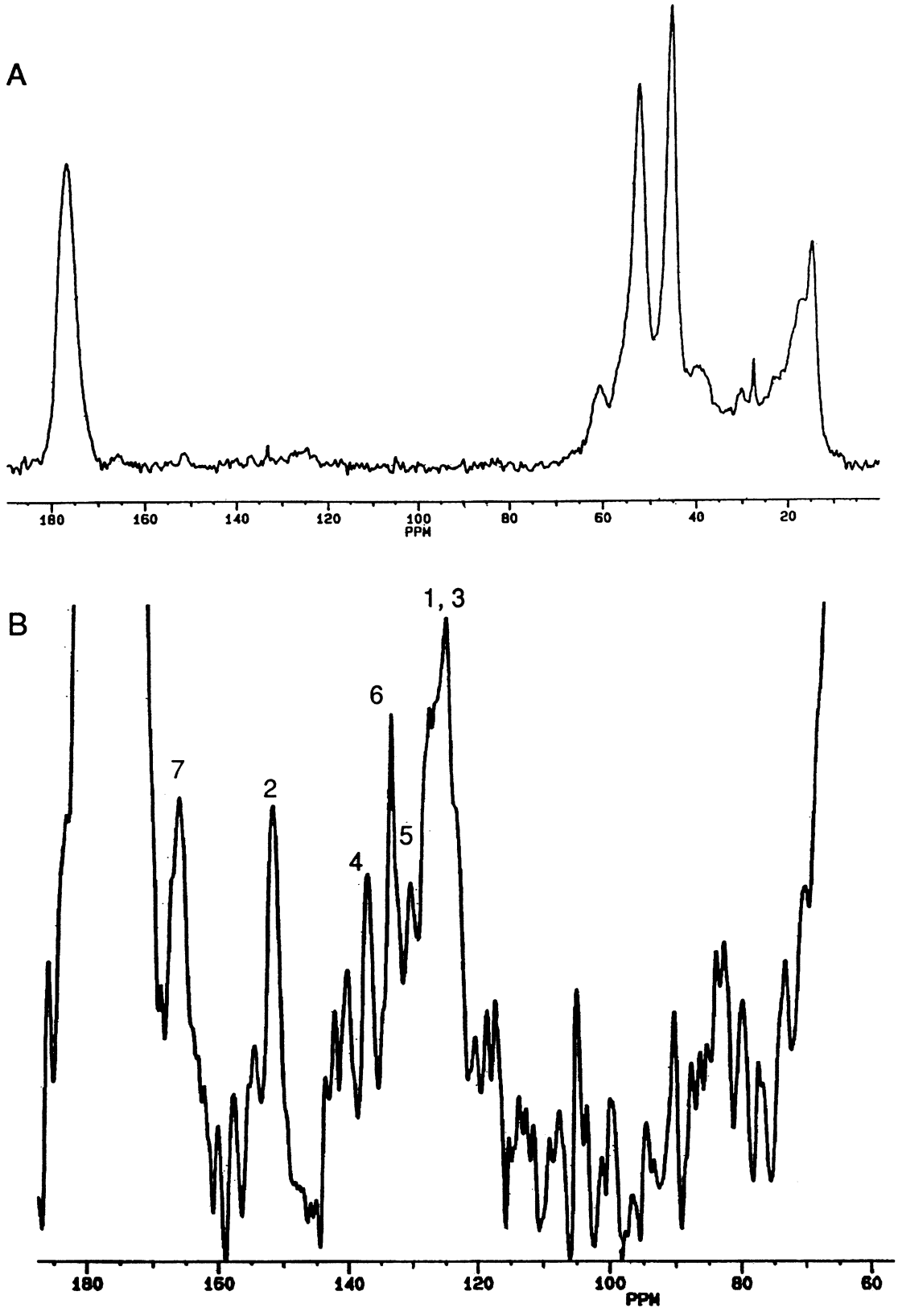


Fig. 6. ^{13}C solid-state NMR of acetylsalicylic acid encapsulated in Eudragit® RS100 microspheres. A, full spectrum; B, close-up of the aromatic carbon region of the spectrum.

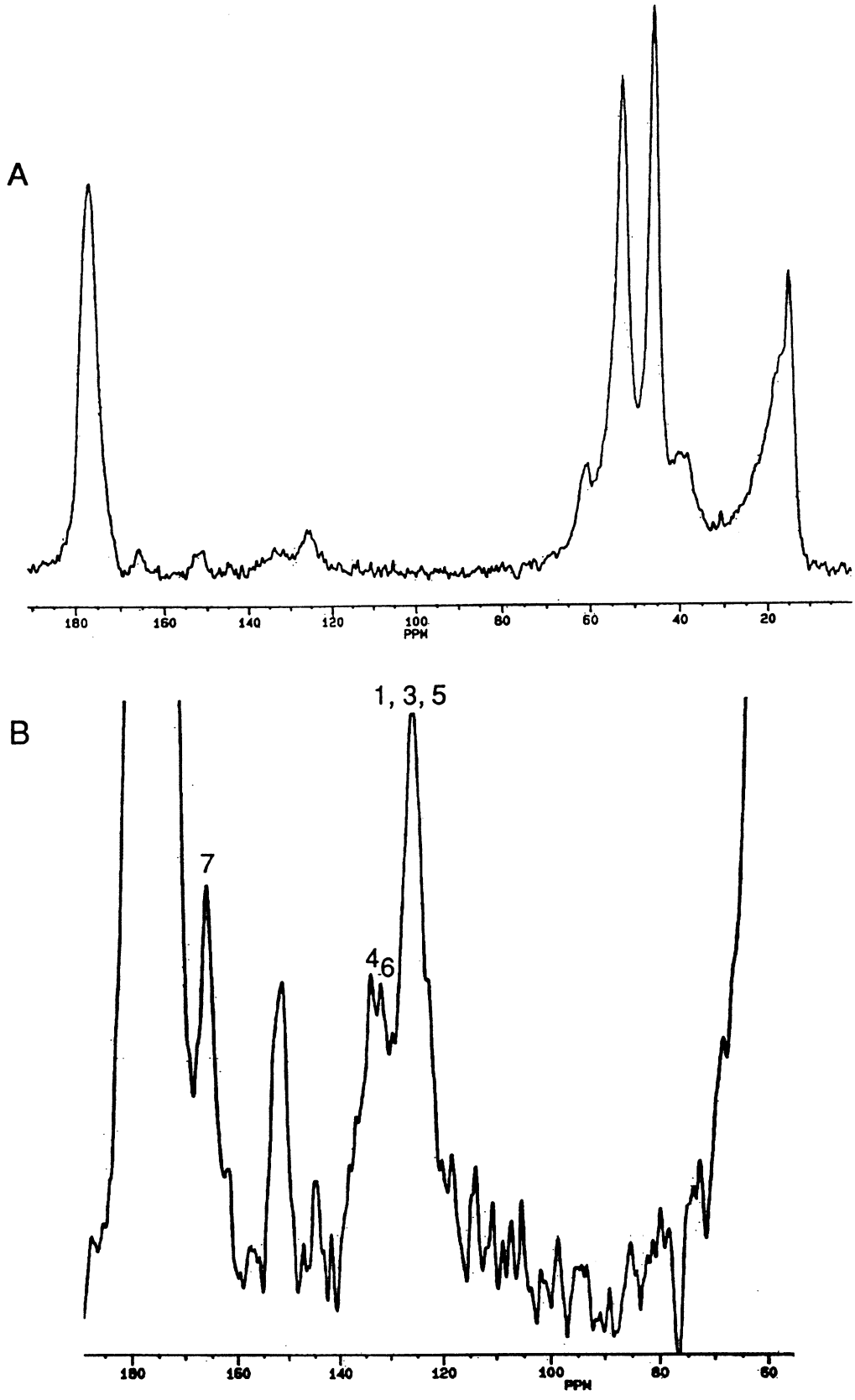


Fig. 7. ^{13}C solid-state NMR of valerylsalicylic acid encapsulated in Eudragit® RS100 microspheres. A, full spectrum; B, close-up of the aromatic carbon region of the spectrum.

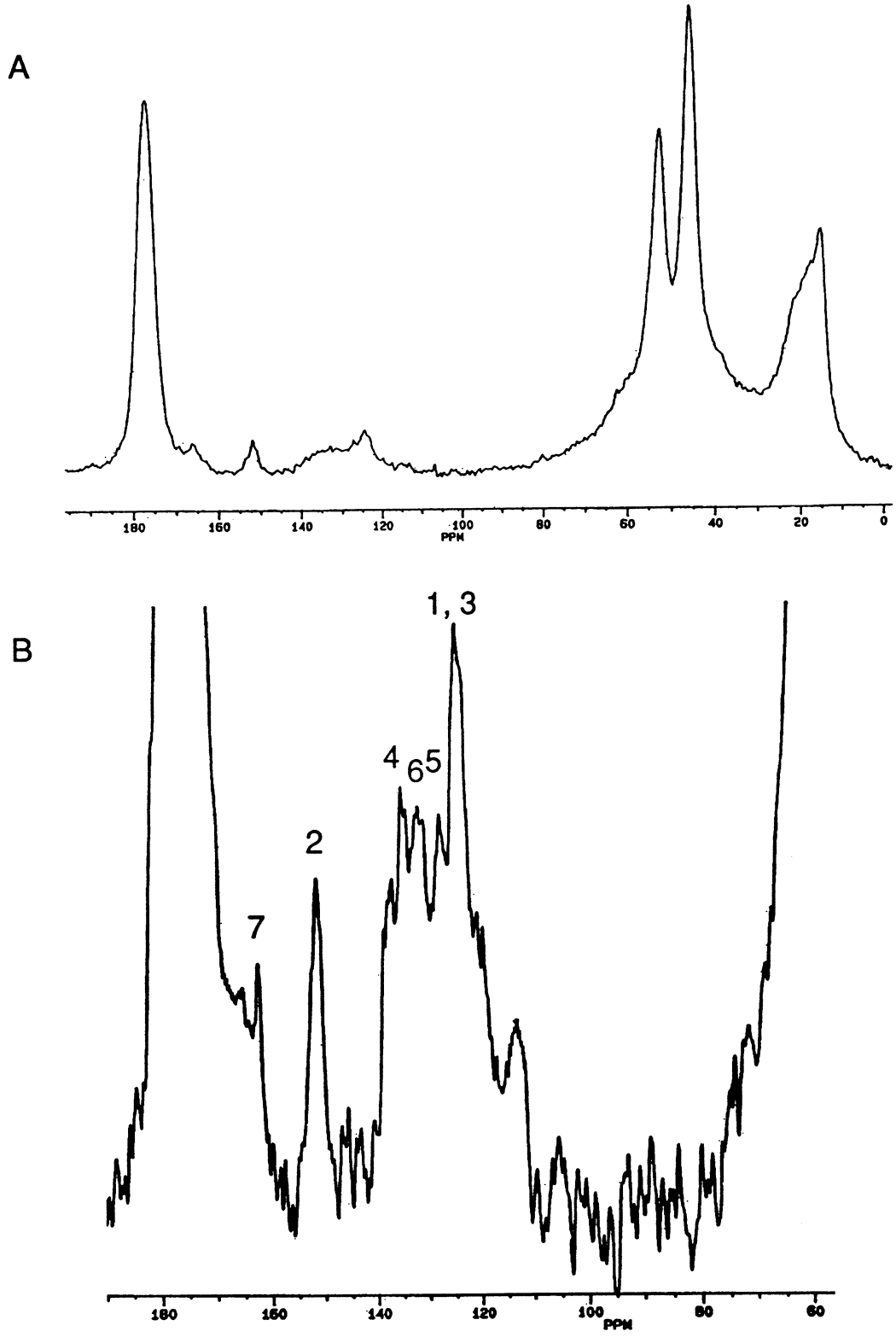


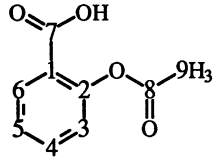
Fig. 8. ^{13}C solid-state NMR of caprylsalicylic acid encapsulated in Eudragit® RS100 microspheres. A, full spectrum; B, close-up of the aromatic carbon region of the spectrum.

methylene groups was significantly lower and resulted in a fluctuation in the solubilities. Since the probe molecules used in the present study are of a similar homologous series (acyl-*o*-hydroxybenzoic acids), it is likely that hydrophobic repulsion as the chain length increases will result in preferential association of the probe molecule with the portion of the Eudragit® RS100 polymer exhibiting alkyl character.

The ionic, hydrogen bonding, and van der Waals association occurring in the drug incorporated Eudragit® RS100 matrix in the solid state dictates the preferred orientation of the solute molecules in this environment. Presumably, the carboxylic group of the probe molecules is so aligned as to be in close proximity with the Eudragit® RS100 pendant molecules. As a result, it is unlikely that the polymer incorporated probe molecule will assume the necessary molecular conformation to effect intramolecular general base catalyzed hydrolysis of the adjacent ester group.

Likewise, this preferred orientation would still occur upon hydration of the polymer and during solute release into the surrounding dissolution medium. In acidic or basic solution, the site of the reaction centre carbonyl located at position 8 is of more direct importance than the alignment of the carboxylic group at position 7. For the hydrolysis reaction to occur, hydroxide ion or hydronium ion must attack the

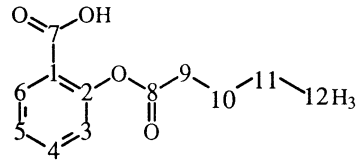
Table 1
Chemical shift comparisons at 25.18 MHz of acetylsalicylic acid and acetylsalicylic acid encapsulated in Eudragit® RS100 microspheres



Carbon	Chemical shift, δ (ppm)		
	ASA	ASA–Eudragit	Difference (Hz)[a]
1	121.97	124.71	−70.0
2	152.64	151.33	+33.0
3	124.88	124.71	+4.3
4	138.24	135.10	+79.1
5	127.47	127.71	−6.0
6	134.61	131.90	+68.2
7	170.52	162.41	+204.2
8	172.01	—[b]	—
9	19.62	—[c]	—

[a] Calulated as: $\delta = \frac{\text{Chemical shift in Hz}}{\text{Radio frequency of the instrument in MHz}}$.
[b] Masked by Eudragit® RS100 carbonyl carbons.
[c] Masked by Eudragit® RS100 alkyl carbons.

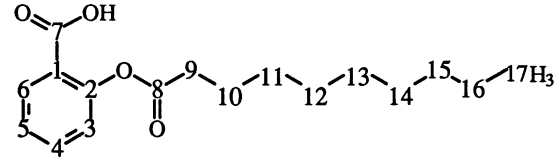
Table 2
Chemical shift comparisons at 25.18 MHz of valerylsalicylic acid and valerylsalicylic acid encapsulated in Eudragit® RS100 microspheres



Carbon	Chemical shift, δ (ppm)		
	ValerylSA	ValerylSA–Eudragit	Difference (Hz)[a]
1	120.91	125.25	−109.3
2	152.90	150.99	+48.1
3	125.60	125.25	+8.8
4	136.29	133.70	+65.2
5	125.60	125.25	+8.8
6	132.04	131.50	+13.6
7	169.40	165.69	+93.4
8	172.23	—[b]	—
9–12	<35.00	—[c]	—

[a] Calulated as: $\delta = \frac{\text{Chemical shift in Hz}}{\text{Radio frequency of the instrument in MHz}}$.
[b] Masked by Eudragit® RS100 carbonyl carbons.
[c] Masked by Eudragit® RS100 alkyl carbons.

Table 3
Chemical shift comparisons at 25.18 MHz of caprylsalicylic acid and caprylsalicylic acid encapsulated in Eudragit® RS100 microspheres



Carbon	Chemical shift, δ (ppm)		
	CaprylSA	CaprylSA–Eudragit	Difference (Hz)[a]
1	120.91	124.51	−90.6
2	152.33	151.29	+26.2
3	125.69	124.51	+29.7
4	135.83	136.60	−19.4
5	126.90	126.90	0.0
6	132.32	132.90	−14.6
7	170.87	165.70	+130.2
8	172.37	—[b]	—
9–17	<36.00	—[c]	—

[a] Calulated as: $\delta = \frac{\text{Chemical shift in Hz}}{\text{Radio frequency of the instrument in MHz}}$.
[b] Masked by Eudragit® RS100 carbonyl carbons.
[c] Masked by Eudragit® RS100 alkyl carbons.

carbonyl group and hence the more sterically hindered this group, the slower the reaction. Patel and Wurster [34] were able to corroborate this effect in a study involving the hydrolysis of an homologous series of naphthyl acyl esters, C2-C6, in CTAB micelles. Increasing the hydrophobic chain length resulted in additional shielding of the carbonyl carbon as it was pulled farther into the micelle. This may explain why the rate of ASA lost from the solvent-swollen microspheres is virtually identical regardless of the pH of the external dissolution medium [1]. In contrast, the loss of ASA alone in these solutions is approximately two orders of magnitude faster in pH 12.1 buffer than in pH 1.2 buffer. Furthermore, the small fraction of polymer incorporated drug that remains permanently bound in the microsphere after long-term dissolution is also a likely consequence of the ionic and hydrogen bonding revealed by ^{13}C NMR. By extension, this interaction mechanism may be responsible for similar Eudragit®-drug adsorption phenomena observed in other studies involving Eudragit® RS/RL [35], Eudragit® L [36] and Eudragit® S [37].

5. Conclusion

Evaluation of the ^{13}C nuclear magnetic resonance spectra of the structural association of incorporated probe molecules, C2, C5 and C10 acyl esters of salicylic acid indicated that alteration of the microenvironment of the incorporated solutes had occurred. The matrix incorporated drug is essentially shielded from hydrolytic attack until it is liberated into the external aqueous environment. Electrostatic association of the drug with the charged quaternary residues in the polymer along with the limiting availability of water within the microsphere may be responsible for the observed stability of phenyl substituted esters in aqueous swollen drug-loaded Eudragit® RS100 microspheres as reported previously [1].

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