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Research Paper

Ionization States in the Microenvironment of Solid Dosage Forms: Effect of Formulation Variables and Processing

Ramprakash Govindarajan,[1,5] Andrey Zinchuk,[2] Bruno Hancock,[3] Evgenyi Shalaev,[4] and Raj Suryanarayanan[1]

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Purpose. Evaluation of the effect of formulation composition and processing variables on the microenvironment in solid dosage forms, based on ionization of indicator probes.
Materials and Methods. Sulfonephthalein indicators were intimately mixed with individual excipients, binary excipient mixtures or multi-component blends by the solvent deposition method. Diffuse reflectance visible spectroscopy of these solids provided a measure of indicator ionization extent. Indicator solution studies yielded equations relating solution pH to the ratio of the absorbance signals of the ionized to that of the unionized form, for each indicator. These equations and the spectral data of the indicator-treated solids were used to calculate an acidity function, 'pH_{eq}' for the solids. The ionization of incorporated probes was also monitored during various stages of simulated pharmaceutical processing *viz*. wet and dry mixing.
Results. The pH_{eq} provided a measure of the physicochemical environment experienced by the probe in the solid. The surface nature of formulation components and their surface area available for interaction influenced the overall properties of the final blend. The extent of probe ionization varied at different stages of a simulated wet mixing–drying process. The pH of the excipient suspension was not a good predictor of the probe ionization in the final dried solid. Indicator ionization is expected to be influenced by the microenvironmental acidity, polarity and ionic strength. Individual excipient properties contributed to the overall microenvironment in powder mixtures even when dry mixed at low water contents.
Conclusions. The environment experienced by a drug in the final solid dosage form will be influenced by the nature of the excipients, the extent of their surfaces available for interaction, surface modification during processing and the amount and nature of solvent used.

KEY WORDS: diffuse reflectance spectroscopy; excipients; microenvironmental acidity; processing; solid dosage forms; sulfonephthalein indicators; surface acidity.

INTRODUCTION

Control of particulate microenvironmental acidity in solid dosage forms, attained by use of pH modifiers has enabled enhancement of solid-state stability of various active pharmaceutical ingredients (API) (1–4). It is suggested that the mechanism of action of these pH modifiers in the "near dry" solid state is based on their ability to influence the pH of the sorbed water on the solid surface (5). This provides a microenvironment conducive to drug stability. Therefore, the effectiveness of the pH modifier is facilitated by the use of water during processing and residual water in the final dosage form. Similar approaches have been employed to manipulate drug release from dosage forms (6,7). Dissolution of the pH modifier in the invading fluids provides the desired acidity in the microenvironment around the drug and in the diffusion channels. This allows control over the release of drugs which exhibit pH dependent solubility.

The surface properties of excipients are expected to influence the microenvironment in the final solid formulation and have been reported to exert both stabilizing and destabilizing effects, depending on the nature of the excipient surface and the stability characteristics of the API. Takahashi and Yamamoto, in a series of papers, evaluated the influence of surface acidity of solid excipients on the isomerization of vitamin D_2 (8,9). The acidic nature of the excipient surface catalyzed the degradation reaction. Interestingly, although exposure to water is normally expected to be detrimental to stability, exposure of the vitamin D_2-excipient mixtures to high relative humidity enhanced the stability of the active. It was suggested that the surface acidity decreased due to sorption of water and this stabilized the vitamin. When *n*-butylamine was chemisorbed on the surface of the solids, the reaction rates decreased with an increase in the amine content on the surface. This suggests that the chemical nature of the surfaces play an important role in the determination of the surface acidity (8). The presence of Brönsted or Lewis

[1] Department of Pharmaceutics, University of Minnesota, 308 Harvard St. SE, Minneapolis, MN 55455, USA.
[2] University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06030, USA.
[3] Pfizer Global R&D, Groton, CT 06340, USA.
[4] Pfizer Global R&D, Groton, CT 06340, USA.
[5] To whom correspondence should be addressed. (e-mail: govin002@umn.edu)

acid/base sites on the excipient surface can therefore significantly influence the microenvironmental acidity.

In addition to vitamin D_2, the effect of excipient surface acidity on the stability of several other drugs has been investigated (10–13). The microenvironmental acidity in solid dosage forms can affect the rates of acid/base catalyzed degradation reactions. For example, the stability of DMP-754, an acetate salt of an ester prodrug, was dependent on the 'microenvironmental pH' in solid dosage forms. The use of a mesylate salt (DMP-755) increased the microenvironment 'acidity' in the dosage form, thereby increasing the stability of the API in the formulation (2).

The physicochemical properties of the microenvironment will also affect drug ionization. The immediate environment of the drug in a formulation can influence salt to free acid/base conversions thereby causing instability or affecting product performance (14,15). Often, the free acid/base forms of drugs are inherently unstable and hence salt forms have to be used in formulations (1,16). Minimizing exposure to water during processing and the use of an alkalinizer minimized the conversion of a stable salt to an unstable free acid, thereby improving stability during the process and storage (1). Rohrs *et al.* have reported the conversion of delaviridine mesylate to the free base. This dissociation, a consequence of an acid-base interaction with croscarmellose sodium in the formulation, was found to adversely affect *in vitro* drug release (15).

The effective microenvironment might be affected by the formulation composition as well as the processing variables. For example, the stability of the API was different in formulations of identical composition but made by different procedures *viz*. dry and wet granulation (2–4,17). The API, a benzenesulphonate salt of a weakly basic triazine derivative, caused a highly acidic microenvironment in solid formulations; resulting in hydrolytic drug degradation (3). While wet granulation *per se* was predictably detrimental to drug stability, optimal stabilization by a pH modifier (sodium carbonate), required a wet granulation process. Sodium carbonate by dissolving in the granulating fluid may be more uniformly distributed on particle surfaces, than when it is dry mixed. This process therefore increases the effectiveness of the pH modifier in altering the microenvironment (3).

Similarly, excipients which can change the 'microenvironmental pH' in a direction detrimental to API stability would be more effective if a wet process is used. When the API constitutes a very low weight fraction of the dosage unit, wet granulation process facilitates content uniformity. Dissolution in the granulating fluid, followed by recrystallization during drying, could result in fine particles of API, uniformly distributed in the excipient matrix. Thus a high surface area of the API will be exposed to the microenvironment. If the microenvironmental conditions favor drug decomposition, the wet granulation process could therefore lead to a decrease in shelf-life.

The extent of ionization of probe molecules incorporated into formulations may be used as a measure of the microenvironmental properties. The use of sulfonephthalein indicators, as probes, to evaluate the surface acidity of excipients has been reported (18–20). The ionization of the probe intimately mixed with individual excipients or formulation blends, would provide a measure of the physicochemical environment which a drug molecule would experience in the formulation. In this study, the ionization state of an indicator, solvent-deposited on the solid samples, was studied using reflectance spectroscopy. The effect of process variables could also be evaluated by monitoring ionization of the incorporated probe during selected processing stages.

The overall goal of this project was to investigate the influence of excipient properties as well as process variables on the microenvironment of the final formulation. The specific objectives were as follows. (i) In multi-component formulations, determine the extent of influence of each formulation component on the effective microenvironment. (ii) Evaluate the influence of a lubricant, magnesium stearate, at a low concentration of 1% w/w, on probe ionization. (iii) Determine the effects of 'dry' and 'wet' mixing of excipients on the microenvironment of the final dried blend. (iv) Using the extent of ionization of the probe as a measure, determine if the suspension pH reflected the ionization in the final dried formulation.

MATERIALS AND METHODS

Materials

All grades of Microcrystalline Cellulose NF (MCC; Avicel® PH 101, 102 and 105, FMC corporation), Lactose Monohydrate NF (Lactose Fast Flo® 316, Foremost Farms USA), Dibasic Calcium Phosphate, anhydrous USP (ADCP, obtained from both Sigma Chemical Company and A-TAB® granules from Rhodia Pharma Solutions, NJ), Magnesium stearate NF (Vegetable source, Mallinckrodt Laboratory Chemicals), sodium starch glycolate NF (Explotab®, Edward Mendell Co, Inc, Penwest, CT), and the Calcium Carbonate USP grades Vicron® 75-17-FG and Vicality® Medium PCC (both from Specialty Minerals Inc., Bethlehem, PA) Calcipure® GCC 300 (Particle Dynamics Inc., MO) and Precarb® 150 (Mutchler Inc., NJ) were used as received.

The solvents used were methanol (HPLC grade, Fisher Scientific) and deionized water, which was freshly boiled and cooled. The sulfonephthalein indicators, bromocresol green (*BG*), bromophenol blue (*BB*), bromocresol purple (*BP*), thymol blue (*TB*) and phenol red (*PR*) were obtained as monosodium salts from Sigma Chemical Company, St. Louis, MO. Chlorophenol red (*CR*) sodium salt was obtained from Lancaster Synthesis Inc, Pelham, NH. Figure 1 contains the structures of the sulfonephthalein indicators. The ionized and unionized forms of these indicators exhibit distinct absorption bands in the visible spectra. Visible absorption spectroscopy was therefore used to study the ionization of these indicators in solution and when they were solvent-deposited onto excipients and formulation blends.

Indicator Solutions

Indicator solutions were prepared over a series of pH values on either side of their pK_a, using hydrochloric acid buffer (21), 20 mM citrate buffer or 50 mM phosphate buffer (21) depending on the desired solution pH. The solution pH values were measured using a pH meter (Oakton pH500 Series), which was calibrated using standard buffer solutions (pH 4.01, 7.00 and 10.01, Oakton Instruments, Vernon Hills, IL). The concentration of each indicator (listed in Table I) was so selected that the absorbance of the ionized as well as

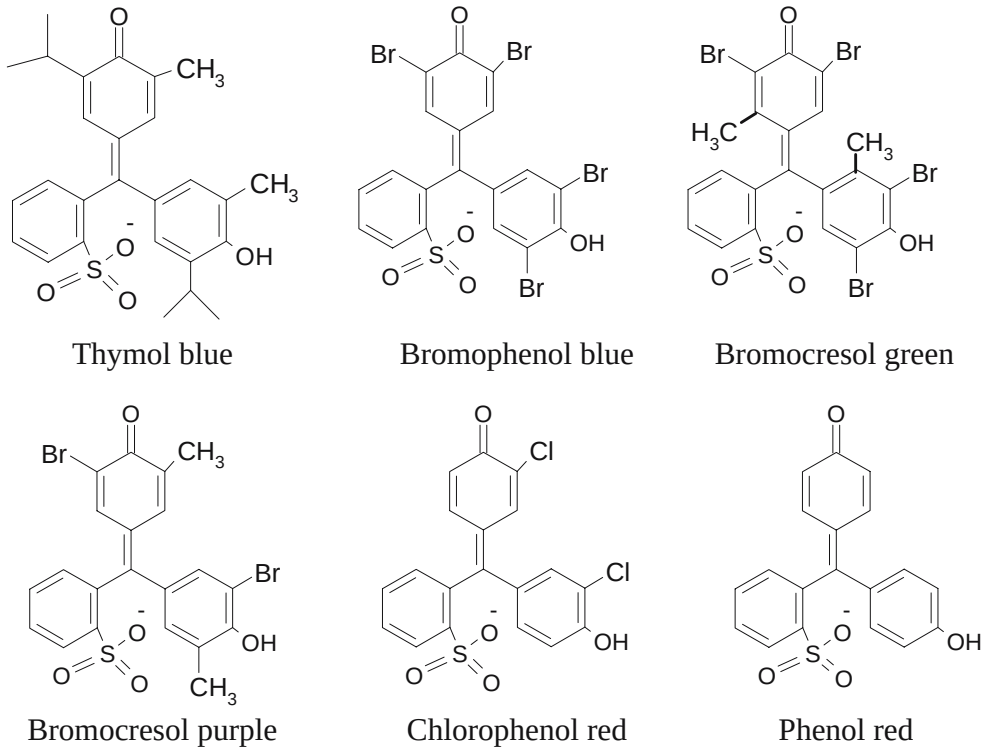


Fig. 1. Chemical structures of sulfonephthalein indicators represented as the anions of the monosodium salts. In case of thymol blue, both the ionization steps were relevant in the acidity range studied. In all other cases, the second ionization—that of the phenolic group—was of interest.

the unionized forms were within the linear range of the Beer–Lambert Law. The visible spectra of these solutions were recorded using a UV–Visible spectrophotometer (Model 100 Bio, Cary). Table I lists the experimental details.

Solid Samples

Sample Preparation

Single excipient. Each excipient was weighed and mixed with a methanolic solution of a suitable indicator, to yield the desired final indicator concentration (Table II). The choice of solvent was based on the solubility of the probe used and the "insolubility" of the excipient. For the purpose of this study methanol was used as a common solvent for all the solid samples as it satisfied the solvent property requirements for all the excipients. The indicator concentrations were based on earlier reports (18,19) and theoretical indicator coverage calculations reported in an earlier publication (20). Sufficient methanol was added to permit effective mixing of the indicator with the excipients. The total methanol quantity varied between 3 and 6 ml per 10 g of the solid, depending on the excipient. While avoiding grinding, the wet mass was gently mixed in a mortar to obtain a homogeneous distribution of the indicator and dried under reduced pressure at 50°C for

Table I. Indicator Solution Studies: Experimental Details and Results

Indicator and Concentration	pK_a	Buffer	pH Range	Equation[a]	R^2
Thymol blue (20 μg/ml) [b]	1.6 [c]	HCl [d]	1.64 to 2.73	$y = 0.91x - 1.82$	0.997
Bromophenol blue (15 μg/ml) [e]	4.0 [f]	Citrate	2.57 to 4.26	$y = 1.02x - 3.64$	0.994
Bromocresol green (20 μg/ml) [e]	4.7 [f]	Citrate	3.38 to 4.84	$y = 0.92x - 4.05$	0.999
Chlorophenol red (10 μg/ml) [e]	6.0 [g]	Citrate	4.42 to 6.08	$y = 1.00x - 5.53$	0.990
Bromocresol purple (10 μg/ml) [e]	6.3 [f]	Citrate	4.66 to 6.28	$y = 1.02x - 5.87$	0.999
Phenol red (8 μg/ml) [e]	7.9 [f]	Phosphate [d]	6.04 to 7.92	$y = 0.94x - 6.97$	0.996
Thymol blue (20 μg/ml) [e]	9.0 [h]	Phosphate [d]	7.76 to 8.90	$y = 0.92x - 7.89$	0.995

[a] $y = mx - n$; x = solution pH, $y = \log_{10}$ (peak ratio)
[b] First ionization.
[c] T.M. Ramirez, P. Balderax-Hernandez, A. Rojas-Hernandez, A. Gutierrez, *Talanta* 46 (1998) 1439–1452.
[d] Since the objective was only to derive a relation between the pH of solution and the ionization of the indicators, the pH range of phosphate and hydrochloric acid buffers were extended beyond that in the USP.
[e] Second ionization.
[f] S. Budavari (Ed) 1996. The Merck Index. 12th ed.: Merck Research Labs, Merck & Co.
[g] B. Cohen, *Pub Health Repts* 41 (1926) 3051–3074.
[h] H. Yamazaki, R.P. Sperline, H. Freiser, *Anal. Chem*. 64 (1992) 2720–2725.

Table II. Excipient pH_{eq} Values Based on Indicator Probe Ionization[a]. Aqueous Suspension pH Values are also Provided

Excipient	Probe	Probe Concn. (mg/g)	pH_{eq}	5% w/v Suspension pH	10% w/v Suspension pH
Dibasic calcium phosphate anhydrous USP (A-TAB® granules)	TB	0.1	2.21 ± 0.01	5.48	5.24
Dibasic calcium phosphate anhydrous USP (Sigma Chemicals)	BB	0.2	3.59 ± 0.00	7.01	6.92
Microcrystalline cellulose NF (Avicel® PH102)	BG	0.2	4.03 ± 0.03	5.99	ND
Microcrystalline cellulose JP (Avicel® PH101)	BG	0.2	4.07 ± 0.01	5.70	ND
Microcrystalline cellulose NF (Avicel® PH105)	BG	0.2	4.14 ± 0.02	6.10	ND
Lactose monohydrate NF (Fast Flo® 316, spray-dried)	BG	0.2	4.24 ± 0.00	ND	ND
Sodium starch glycolate NF (Explotab®)	BG	0.2	4.77 ± 0.00	ND	ND
Calcium carbonate USP (Vicron® 75-17-FG)	PR	0.2	6.58 ± 0.05	9.52	ND
Calcium carbonate USP (Calcipure® GCC 300)	PR	0.2	7.20 ± 0.02	9.62	9.72
Magnesium stearate NF	PR	0.2	7.45 ± 0.00	9.43	9.57
Calcium carbonate USP (Precarb® 150)	PR	0.2	7.69 ± 0.03	9.59	ND
Calcium carbonate USP (Vicality® Medium PCC)	TB	0.4	8.07 ± 0.08	ND	ND

[a] Methanolic solution of indicator was used.
ND Not determined.

at least 12 h. The dried sample was gently mixed to break down aggregates, and if necessary, dried further under reduced pressure and stored over anhydrous calcium sulfate (RH ~ 0%) until analyzed. Each excipient, treated similarly but without the indicator, served as a 'blank' in the spectral measurements.

Depending on the spectrum obtained, an indicator with a higher or lower pK_a was chosen, until an appropriate indicator was identified, which was partially ionized when deposited on the solid sample. The color produced in the mixture also provided a visual indication of indicator suitability.

Excipient mixtures. In case of excipient blends, the initial indicator choice was based on the mixture composition. As a first step in evaluating complex pharmaceutical compositions, binary mixtures of model excipients were prepared. These powder blends were then treated with indicator as described earlier. The influence of mixture composition on the probe ionization was evaluated. Three calcium carbonate grades (Calcipure® GCC 300, Vicron® 75-17-FG and Vicality® Medium PCC) were evaluated in binary mixtures with microcrystalline cellulose (Avicel® PH101). Multi-component powder blends were prepared by dry blending in a mixer (T-urbula®) for 15 min. Four different formulation compositions were evaluated (Table III). Each composition was studied without a lubricant, and also after blending for 3 min with magnesium stearate (1% w/w). Methanol was used as solvent to incorporate the indicator in all the powder mixtures.

Instrumentation

Diffuse reflectance visible spectroscopy was used to record the spectra of the indicators in the solid samples. A UV visible spectrophotometer (Cary 100 Bio), equipped with a diffuse reflectance accessory (Labsphere, model DRA-CA-30I) having an integrating sphere and an inbuilt photo-multiplier tube, was used. The inner surface of the sphere is coated with poly-(tetrafluoroethylene). A "zero-degree" wedge in the sample port ensured that the powder sample surface was always perpendicular to the incident light. As a result, reflection back through the sample beam entrance port eliminated the specular component of the reflected light. The integrating sphere collects the light diffusely reflected by the sample and presents an integrated signal to the detector.

Theory

The spectra of the indicators deposited on the solids were recorded as Kubelka Munk function, $F(R)$ *vs.* wavelength plots. $F(R)$ is a function of the fraction of light diffusely reflected by the powder bed, R. It is directly proportional to the analyte concentration as defined in Eq. 1 (22).

$$F(R) = \frac{\varepsilon' c}{s} \tag{1}$$

where ε' and c are, respectively, the extinction coefficient and the concentration of the analyte, and s is the scattering coefficient of the powder bed. When both the unionized and ionized species are present, the ratio of the Kubelka–Munk functions at the corresponding absorption peaks can be represented as follows.

$$\frac{F(R)_i}{F(R)_u} = \frac{\left(\varepsilon_i' c_i s_u\right)}{\left(\varepsilon_u' c_u s_i\right)} \tag{2}$$

Table III. Composition of Multi-Component Excipient Blends After Lubrication with Magnesium Stearate (in % w/w)

Ingredients	Formulation I	Formulation II	Formulation III	Formulation IV
Microcrystalline cellulose NF (Avicel® PH102)	63.8	48.0	63.8	48.0
Lactose monohydrate NF (Lactose Fast Flo®)	32.2	48.0	–	–
Calcium phosphate, dibasic anhydrous USP (A-TAB®)	–	–	32.2	48.0
Sodium starch glycolate NF (Explotab®)	3.0	3.0	3.0	3.0
Magnesium stearate NF (vegetable grade)	1.0	1.0	1.0	1.0

where the subscripts 'u' and 'i' refer to the unionized and ionized species, respectively, ε_i' and ε_u' are the extinction coefficients of the two species at the respective peaks and s_i and s_u are the scattering coefficients at the respective peak positions.

When the wavelength of the radiation<<powder particle size (as is the case for the samples investigated) the scattering coefficient is independent of the wavelength (22), and Eq. 3 simplifies to:

$$\frac{F(R)_i}{F(R)_u} = \frac{(\varepsilon_i' c_i)}{(\varepsilon_u' c_u)} \quad (3)$$

The ratio of the signals at the peaks (peak ratio, Eq. 3) is therefore a measure of the concentration ratio of the two forms and hence of probe ionization extent.

Surface Area Determination

The specific surface area of each excipient was determined -'as is', and after subjecting them to typical sample preparation procedures ('processed samples'), by the BET method, using nitrogen as the adsorbate (Tristar 3000 Gas Adsorption Analyzer, Micromeritics, Norcross, GA). The samples were initially degassed at 40°C under a nitrogen purge for 16 h.

Suspension pH

The pH of aqueous excipient suspensions (5 and 10% w/v) were measured using a calibrated pH meter (Oakton PH500 series) at ambient temperature (25 ± 2°C).

Processing Variables: Effect on Probe Ionization

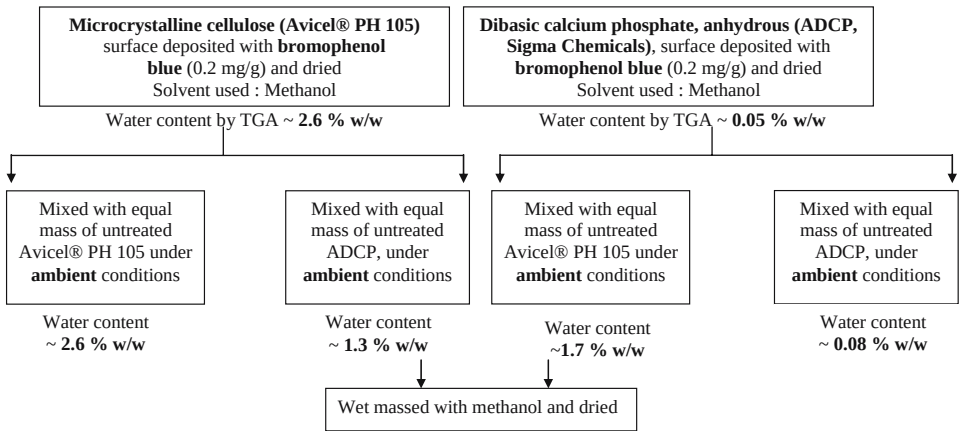
In the previous experiments, the probe was solvent deposited onto premixed powder blends. The resulting extent of ionization would therefore be a measure of the overall microenvironmental properties of the final blend. In an attempt to evaluate the influence of processing and to monitor changes in microenvironmental properties during processing steps, probe ionization was measured during simulated mixing procedures as detailed below.

Dry Mixing

The change in the ionization of a probe, which was deposited on an excipient, was investigated, after dry mixing with another untreated excipient. Two model systems were investigated: (a) Microcrystalline cellulose (MCC, Avicel® PH105)–anhydrous dibasic calcium phosphate (ADCP, Sigma) and (b) Calcium carbonate (Precarb® 150)–ADCP (Sigma).

In the first set of experiments (a), Avicel® PH105, which had relatively high water content (~2.7 w/w), was used and the mixing operations were carried out under ambient laboratory conditions. This was done to simulate the conditions during a typical pharmaceutical mixing operation involving an excipient with high water content. In the second set of experiments (b), excipients with very low water content (< 0.1% w/w) were selected. In order to minimize sorption of atmospheric water, mixing was conducted under dry nitrogen atmosphere in a glove box.

The experimental protocol for the dry mixing studies is shown in Scheme 1. The dried indicator-treated excipient (for example, MCC in system (a)) was mixed gently with an equal weight of the same untreated excipient (MCC). The indicator-treated excipient (MCC) was also mixed with an equal weight of the other untreated excipient (ADCP). Similarly, the indicator was deposited on ADCP and dry mixed with either untreated ADCP or MCC. The amount of solvent present in the powder blend could influence the changes in the ionization of the probe. Therefore, the total amount of solvent (methanol + water) was determined by thermogravimetric analysis (TGA), in each component before the dry mixing, and in the powder blend immediately after mixing. It was expected that the residual methanol concentration would be minimal and the TGA weight loss would be a measure of the water content of the powder. All the mixtures were stored overnight at 0% RH, prior to spectral measurements.



Scheme 1. Schematic representation of the dry mixing experiment for the Avicel® PH 105–ADCP (Sigma) system. The dry mixing was carried out under ambient laboratory conditions. The results are shown in Fig. 6. The experimental design for the calcium carbonate (Precarb® 150)–ADCP (Sigma) system (Fig. 7) was similar, except that the mixing in this case was carried out in a glove box under a dry nitrogen purge (Relative humidity< 5%) and the water contents of all the individual components as well as the powder blends was always< 0.1% w/w.

The binary systems obtained by dry-mixing different excipients were mixed with sufficient methanol, dried and re-evaluated by diffuse reflectance spectroscopy. A similar protocol was used for the calcium carbonate (Precarb® 150)–ADCP (Sigma) system.

Wet Mixing

Three model excipient–indicator systems *viz*. (i) calcium carbonate (Calcipure® GCC 300)–phenol red (ii) MCC (Avicel® PH101)–bromocresol green and (iii) calcium phosphate (ADCP, Sigma)–thymol blue, were evaluated. The ionization of each indicator was studied during three simulated stages of a wet mixing–drying procedure of the corresponding excipient, using water as solvent. (a) The ionization of the indicator was evaluated in aqueous solution and (b) in a 5% w/v excipient suspension. (c) The indicator was also deposited on the excipient from an aqueous solution, and the dried solid was evaluated by diffuse reflectance visible spectroscopy.

Very often, non-aqueous solvents are used during pharmaceutical wet processing operations. The solvent nature will affect the ionization of drugs during processing and may also influence the ionization in the final dried solid. In order to evaluate the effect of solvent nature, methanol, a model solvent of lower polarity, was used in place of water, in a similar experiment on the Calcipure® GCC 300–phenol red system.

RESULTS AND DISCUSSION

Indicator–Spectral Measurements

The structures of the sulfonephthalein indicators used are given in Fig. 1. All the indicators are diprotic acids and were used as monosodium salts. They undergo ionization in two stages, represented as:

$$InH_2 \underset{pK_{a1}}{\overset{-H^+}{\rightleftharpoons}} InH^- \underset{pK_{a2}}{\overset{-H^+}{\rightleftharpoons}} In^{2-} \qquad (4)$$

In case of thymol blue, the first ionization resulted in an ionized species (InH^-), which exhibited an absorption peak at a lower wavelength than InH_2. For all the other indicators listed in Table I, the second ionization (pK_{a2}) was of interest and the lower wavelength peak corresponded to the monovalent anion (InH^-). For all these indicators, in the pH range around pK_{a2}, the first ionization was essentially complete. The second ionization was usually of interest. In these cases, for the sake of convenience, the monovalent anion (InH^-) has been referred to as the unionized species and the divalent anion (In^{2-}) as the ionized species.

Indicator Solutions

The ratio of the absorbance value at the peak of the ionized form to that of the unionized form (peak ratio) was measured at each solution pH. An increase in the solution pH resulted in an increase in the peak ratio due to an increase in the extent of ionization as seen in Fig. 2 for bromocresol purple. For each indicator, a linear relationship was obtained between the peak ratio (log scale) and the solution pH, over a pH range close to the pK_a (Table I).

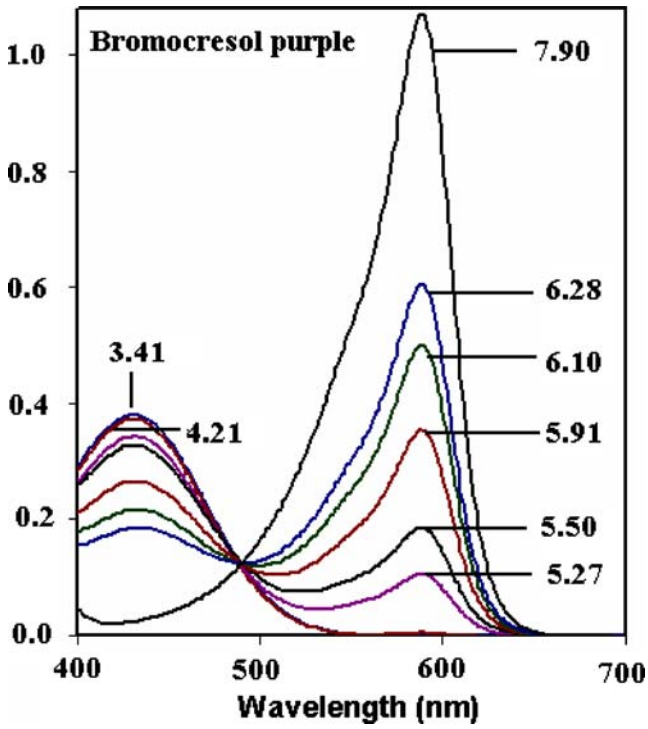


Fig. 2. Spectra of bromocresol purple solutions buffered with citrate buffer to different pH values. The lower wavelength peak corresponds to the unionized form (InH^-) and the higher peak to the ionized form (the divalent anion, In^{2-}).

pH_{eq} Calculation

The peak ratios in the solid samples are a measure of the extent of the indicator ionization and hence a measure of the physicochemical environment in the sample. These peak ratios were substituted in the calibration equations obtained for the corresponding indicator, in solution (Table I). Thus, the pH value obtained from the equation is a measure of the solid sample properties and it was referred to as 'pH_{eq}', as reported earlier (18,19). The pH_{eq} of a solid sample, for the purpose of this study, can be defined as the solution pH at which a given indicator has the same peak ratio of ionized to unionized, as in the diffuse reflectance spectrum of the solid, treated with the same indicator.

It was expected that water content in the sample would significantly influence the microenvironmental properties and hence the ionization of the indicator. Preliminary experiments were carried out using microcrystalline cellulose (Avicel® PH101), which is expected to sorb significant amounts of water. Water contents, ranging from 0.9 to 4.8% w/w (measured by Karl Fischer titrimetry) were obtained by storing the indicator-treated samples in chambers maintained at RH values ranging from 0 to 33%, respectively. These samples were quickly filled into the sample holder and their spectra were immediately recorded. The pH_{eq} values ranged from 4.2 (0.9% w/w water) to 4.3 (4.8% w/w water). At higher water contents, obtained by storage at RH > 33%, the samples exhibited a decrease in peak ratio and a consequent decrease in pH_{eq}. This was in agreement with earlier reports for Avicel® PH101 (19). During our experiments, however, the samples were always stored in a desiccator over anhydrous calcium sulfate (0% RH) prior to measurement.

Samples were filled into the sample holder by backfilling and the sample chamber was sealed with a spring-loaded metal disc. In case of hygroscopic samples like MCC, the filling was done in a glove box under dry nitrogen purge. As a result, the sample water content was expected to be low and in a narrow range. Small variations in water content during sample preparation and handling were not expected to cause big changes in the value of the pH_{eq}. *It is however recognized that the water content is a major determinant of the probe ionization and hence will influence the microenvironmental properties*. For example, the ionization of BB dispersed in an amorphous trehalose–citrate buffer matrix increased from 24 to 31% with an increase in the matrix water content from 1.4 to 9.8% w/w (23).

Moreover, the location of the water in the solid sample (surface adsorbed or absorbed into the disordered regions) and its location with respect to the indicator would also influence the measured probe ionization. These factors have been discussed later.

Limitations of pH_{eq}

In all the indicators, the species exhibiting a peak at the higher wavelength had a weak but measurable absorbance at the lower wavelength peak position. As a result, the lower wavelength peak has a small contribution from absorption due to the other species. This contribution varied as a function of solution pH (extent of ionization) and in some cases also resulted in a shift in the lower wavelength peak position. This contribution could be accounted for in solution and amorphous freeze-dried sugar matrices (23), and concentration-proportional signals could be obtained. However, the same could not be accurately done when the indicators were solvent deposited on the powder samples. Therefore, this small contribution was ignored and the signals were measured at the points of maximum absorbance, irrespective of peak position, in solutions as well as solids. Besides, the ratios of the extinction coefficients in solution may be different from that on solid surfaces. Therefore, the pH_{eq} will not be an exact measure of the indicator ionization extent.

The proton activity in the microenvironment will be influenced by the acidic/basic nature of the solid surfaces as well as the high concentration of formulation components in the surface water. The proton activity would have a major influence on the ionization of the indicator. It is also recognized that the local *ionic strength* and the *polarity* would affect the ionization by influencing the apparent pK_a of the indicator. Therefore, although the pH_{eq} provides a measure of the environment within the solid sample, which is influenced by several factors, it is *not an absolute measure of the acidity of the microenvironment.*

The pH_{eq} is however expected to provide a relative measure of the nature of different excipient surfaces. It would also provide valuable insight into the effect of various pharmaceutical processing steps and formulation factors on the effective microenvironment in the final dosage form. If the extinction coefficient ratio ($\varepsilon'_u/\varepsilon'_i$, in Eq. 3) of an indicator deposited on a solid is the same as that in solution, and the measured peak ratio is proportional to the ratio of the concentrations of the ionized to the unionized species, then the calculated pH_{eq} will be equal to the *Hammett acidity function* of the solid surface (24). Application of the Hammett function and its limitations in defining the environment in amorphous formulations has been described in an earlier publication (23).

Solid Samples

Single Excipient

The pH_{eq} provided a convenient measure of the excipient properties, based on which they could be rank-ordered (Table II). For example, the results show that there might be a considerable difference in the surface properties of different grades of calcium carbonate.

As mentioned earlier, the location of water in the solid sample is of critical importance. Since we have evaluated crystalline excipients as well as materials with substantial disorder, we recognize that the location of water as well as that of the indicator would vary from system to system. In crystalline excipients (assuming negligible levels of lattice disorder), the water is expected to be predominantly adsorbed on the crystal surface. Depending on the specific surface area of the excipients and the water content in these samples, several layers of adsorbed water might be present. Even when the level of lattice disorder is low, the water is expected to preferentially distribute into the disordered regions. In excipients that are predominantly amorphous, most of the water would be sorbed into the bulk. The indicator may also be distributed between the surface water and the bulk amorphous regions. As a result, the measured ionization of the probe is an averaged effect of the overall environment experienced by the probe.

Binary Excipient Mixtures

The ionization states of deposited probes, as a function of binary mixture composition, were evaluated using MCC (Avicel® PH101)–calcium carbonate mixtures. Three calcium carbonate grades, differing in their pH_{eq} values and specific surface area, were used (Tables II and IV). The components of the binary mixture were chosen in such a way that they differed significantly in their pH_{eq}. This was done in order to evaluate the ability of the technique to yield a measure of the microenvironmental properties over a wider range of pH_{eq} values.

The indicator selection, as mentioned earlier, was based on trial and error. The mixtures were first made and a given indicator was mixed with the powder blend as a methanolic solution. Most blends were therefore evaluated using multiple probes (one at a time) until a suitable probe was identified which was partially ionized in the final dried solid. The results of the experiments with different indicators were always in qualitative agreement. For example, bromocresol green (pK_a = 4.7) was partially ionized when mixed with Avicel® PH101, and phenol red (pK_a = 7.9) was partially ionized when mixed with Calcipure® GCC-300. In case of a binary mixture containing 25% w/w Avicel® PH101 and 75% Calcipure® GCC-300 (pH_{eq} 6.65 using PR), bromocresol green was found to be completely ionized. One could assume that a substantial fraction of the indicator would be interacting with the "surface" or the bulk disordered phase of Avicel®, where it would be expected to be partially ionized. This gives rise to the possibility that the indicator ionization in the solid sample is not simply a function of the fraction of the indicator molecules interacting with a given surface. Similarly, phenol

Table IV. Specific Surface Area (SSA) of Excipients Determined by the BET Method. Column B Contains the SSA of Samples Subjected to Processing (Sample Preparation Procedure). The Values in the Last Column (B–A) Reflect the Effect of Sample Processing on the SSA

Excipient	SSA (m^2/g)		
	As-is (A)	Processed (B)	(B–A)
Microcrystalline cellulose JP (Avicel® PH101)	1.2	2.9	1.7
Microcrystalline cellulose NF (Avicel® PH102)	1.2	3.4	2.2
Microcrystalline cellulose NF (Avicel® PH105)	2.3	3.3	1.0
Calcium carbonate USP (Calcipure® GCC 300)	1.2	1.2	0.0
Calcium carbonate USP (Vicron® 75-17-FG)	0.7	0.6	−0.1
Calcium carbonate USP (Vicality® Medium PCC)	6.0	5.9	−0.1
Calcium carbonate USP (Precarb® 150)	4.2	4.1	−0.1
Dibasic calcium phosphate anhydrous USP (Sigma)	1.1	1.2	0.1
Dibasic calcium phosphate anhydrous USP (A-TAB®)	14.3	14.3	0.0
Lactose monohydrate, spray dried NF (Fast Flo® 316)	0.2	0.5	0.3

red was found to be completely unionized when mixed with a blend containing 25% w/w Calcipure® GCC-300 and 75% w/w Avicel® PH101 (pH_{eq} 4.68 using BG), although it would be expected that there would be some ionization when it interacts with calcium carbonate. It seems possible that each component of the blend affects the ionization by influencing the microenvironment around the indicator, with water playing a facilitating role. The probe ionization extent can therefore be a measure of the interparticulate microenvironment. It will be affected by the proton activity, polarity and ionic strength experienced by the probe and may therefore provide a measure of the chemical reactivity in the formulation.

The pH_{eq} values of all the binary mixtures were measured and are reported in Fig. 3. The pH_{eq} of the binary mixtures were also calculated based on the mixture composition and the pH_{eq} of the components. The calculated value was obtained as a weighted average using Eq. 5 and the averaging was done based on both weight fraction and the surface area of each component.

$$pH_{eq(calc)} = \sum pH_{eq(i)} X_i \quad (5)$$

$pH_{eq(i)}$ is the measured pH_{eq} of component i and X_i is its weight fraction or fractional surface area in the mixture. This exercise was carried out in order to determine if the contribution of a component to the overall microenvironment in the blend was influenced by its weight fraction or by its surface area. It was expected that the surface area would be a more critical factor and hence averaging based on 'processed surface area' would provide the value closest to the experimental value. The calculated and experimentally determined values were compared (Fig. 3). For all the binary mixtures, the rank ordering of the surface acidity, based on calculated and measured values were identical. Overall, with an increase in the calcium carbonate content, there was an increase in the pH_{eq} (calculated as well as measured). This reflected a decrease in the 'acidity' of the environment within the mixture. In formulations, an increase in the proportion of an excipient having low surface acidity (for example, calcium carbonate), would decrease the microenvironmental 'acidity' with a possible increase in the extent of ionization of an acidic drug. This could also lead to stabilization of drugs which are susceptible to acid-catalyzed degradation. The calcium carbonate grade with the highest specific surface area (Vicality® Medium PCC, Table IV) was found to exert the maximum influence on the microenvironment in the mixture (Fig. 3).

As mentioned earlier, the surface area of *individual* excipients were measured, before and after processing, by the BET method using nitrogen as adsorbate. This formed the basis for the calculations (Eq. 5). The surface area of some of the excipients, *viz*. Avicel® grades and Lactose Fast Flo® 316, were found to increase significantly upon processing (Table IV). Therefore, the processed surface area values were used to calculate the relative contributions of each excipient to the microenvironmental properties of the mixture. This treatment was based on the assumption that the BET surface areas of the processed excipients would provide a measure of (i) their relative surface areas in the mixture and (ii) the extent of their influence on the environment within the powder blend.

Interestingly, the calculations based on weight fractions (dark bars in Fig. 3), overestimated the contribution of Vicron® 75-17-FG (lower specific surface area than Avicel® PH101, Table IV) to the overall properties of the mixture. The contribution of Vicality® Medium PCC (much higher surface area than Avicel® PH101) was underestimated. This indicates that as the surface area of a component increases, its influence on the overall microenvironment of the formulation is higher, as might be expected. Therefore, a microenvironmental alkalinizer, such as calcium carbonate, might be most effective in a solid formulation when used in a form with the highest surface area.

Multi-Component Excipient Blends

Four model multi-component blends were prepared, using typical tablet excipients (Table III). Each model blend was evaluated before and after lubrication with magnesium stearate. In order for magnesium stearate to serve as an effective lubricant, a high specific surface area is desired (25). As per the manufacturer's specifications (Mallinckrodt Inc.), 90% of the particles should be < 30 μm and 50% should be < 10 μm in size. The surface area ranges between 6 and 12 m^2/g. In powder blends, magnesium stearate is localized on the particle surfaces where it exerts its lubricating action. The large surface area of the lubricant, its low surface acidity

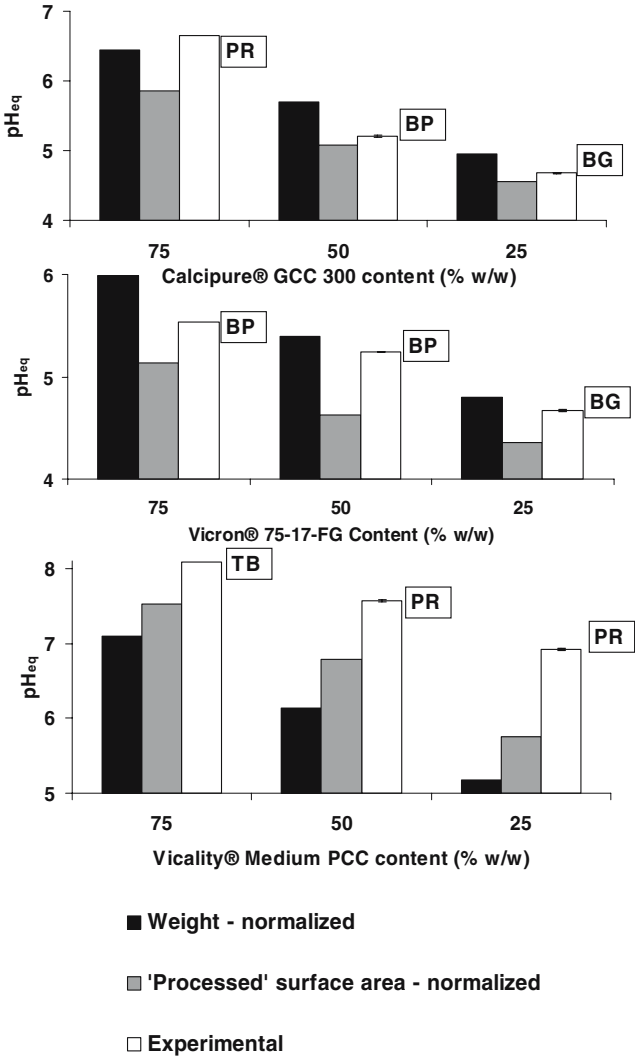


Fig. 3. Comparison of experimentally determined pH_{eq} of Avicel® PH101–calcium carbonate mixtures with those calculated by different averaging techniques. The calcium carbonate grades used were Calcipure® GCC 300, Vicron® 75-17-FG and Vicality® Medium PCC. For each component, its weight fraction or the fractional 'processed' surface area were used for calculation of the pH_{eq}. The indicator used in each case has been reported in the figure. *TB* Thymol blue, *PR* phenol red, *BP* bromocresol purple, *BG* bromocresol green.

(Table II), and its presence on particle surfaces in lubricated blends can have a major influence on the microenvironmental properties of the formulation. This was investigated by evaluating the pH_{eq} of the mixtures before and after lubrication with magnesium stearate.

ADCP (A-TAB® granules) has a low pH_{eq} of 2.21. Its presence therefore resulted in a much lower pH_{eq} value for formulations III and IV. This might reflect a higher microenvironmental 'acidity' in these formulations than in formulations I and II which contained lactose (pH_{eq} = 4.24) instead of ADCP.

The indicator used for each unlubricated blend exhibited a pronounced increase in ionization when deposited on the surface of the corresponding lubricated blend, reflecting a change in the environment. This made the probe unsuitable for evaluation of the lubricated blends and an indicator with a higher pK_a had to be used. There was a consequent increase in the pH_{eq} value following lubrication (Fig. 4). Magnesium stearate is a salt of fatty acids (weakly acidic, pK_a ~5) and a strong base, magnesium hydroxide (pK_a = 11.4). Its basic nature is expected to decrease the overall 'acidity' of the blend. Magnesium stearate is known to form a film on the surface of the particles and thereby exerts its lubricating action (26). The surface coverage therefore significantly influences the particle surface microenvironment where the indicator is present (Fig. 4). Thus, by influencing the surface properties of the particles; magnesium stearate significantly lowers the overall microenvironmental 'acidity' in the blend. It was reported earlier that magnesium stearate-lubrication of Avicel® PH101, previously treated with bromocresol green, caused a gradual increase in the ionization of the indicator, during the process (20).

Thus it is important to consider the effect of interaction between the solids and surface modification during processing on the effective environment in the solid. To support this point, let us consider a few examples from literature, related to the influence of certain excipients, at low concentrations, on API stability in solid dosage forms. Small concentrations of ionic pH modifiers deposited on the surface of excipients have enabled control of the microenvironmental properties in the formulation. Use of small quantities of disodium citrate (~2.5% w/w) in a tablet formulation enhanced drug stability. During a wet granulation process, the disodium citrate, which was previously reported to have a pH_{eq} of 3.76 based on the ionization of bromophenol blue (18), is expected to dissolve and deposit homogeneously on the surface of the excipients thereby providing the desired microenvironment within the tablet (4). Sodium carbonate exhibited a high pH_{eq} of 8.58 based on the ionization of thymol blue (18). Use of a low concentration of sodium carbonate (5% w/w) and a wet granulation technique provided a microenvironment of lower acidity in an API-Avicel® PH102-magnesium stearate formu-

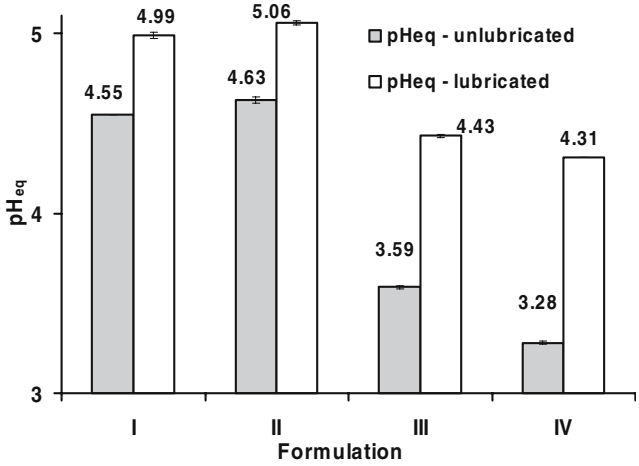


Fig. 4. The measured pH_{eq} values of model pharmaceutical compositions. The values for unlubricated blends and blends lubricated with 1% w/w magnesium stearate are compared. While bromophenol blue was suitable for determining the pH_{eq} of unlubricated formulations **III** and **IV**, it was unsuitable for the lubricated blends, which were evaluated using bromocresol green. Chlorophenol red was used to evaluate the lubricated formulations **I** and **II**, whereas bromocresol green was used for the corresponding unlubricated samples.

lation thereby stabilizing the drug against acid catalyzed degradation (3).

Anhydrous calcium phosphate granules have been reported to exhibit 'acidic' surface properties based on probe ionization (10,20) as well as chemical reactivity of aspirin and bisoprolol fumarate (10,13). It was also demonstrated that the surface properties of calcium phosphate can be significantly altered and manipulated by treating it with buffer solutions followed by filtration of the slurry and drying. These modifications are reflected not only in the ionization of surface-deposited probes but also in the rates of hydrolysis of surface-deposited aspirin (10). It was also hypothesized that magnesium stearate, present in dry granulated formulations of DMP-754 and lactose, may coat the surface of lactose particles, thereby stabilizing the drug against lactose-catalyzed amidine hydrolysis (4).

The averaging technique (Eq. 5) in the earlier section was used to investigate the influence of surface area of excipients on the microenvironment in mixtures. Such a prediction of microenvironmental properties of multi-component systems using the surface areas and pH_{eq} values of the individual components (Eq. 5) would assume that the relative surface areas are retained in the final samples after processing and that the surfaces are unmodified, *i.e.* the components are non-interacting. This clearly would not be the case in the examples discussed above where the process results in deposition or coating of one or more excipient on the surfaces of the other components. Hence simple averaging techniques would not be suitable to predict the microenvironmental properties in these formulations.

Processing Variables: Effect on Probe Ionization

Wet Mixing

The ionization of an indicator during different stages of a wet mixing–drying process was evaluated with several model excipients. This experiment was expected to provide an insight into excipient effects, during various processing stages, on the ionization of the probe. Figure 5 contains the spectra of the indicator, (i) in aqueous solution, (ii) in supernatant of the excipient suspension, and (iii) surface-deposited on the excipient from aqueous solution.

A comparison of the spectra of aqueous indicator solution with that of the supernatant of an excipient suspension, containing the indicator, reveals the effect of the excipients on probe ionization (Fig. 5, a *vs.* b, d *vs.* e). The chemical nature of the excipient and its aqueous solubility will influence pH of the medium. The measured pH value of the aqueous suspension showed qualitative agreement with the extent of indicator ionization. For example, the measured pH of calcium carbonate (Calcipure® GCC-300) suspension was 9.45. While phenol red (pK_a = 7.9) was completely unionized in aqueous solution (pH 4.1, Fig. 5a), it was almost completely ionized in the suspension (Fig. 5b). In the suspensions, the indicator was predominantly in the aqueous phase of the suspension and the settled solid did not exhibit any color indicating little or no indicator uptake by the solid.

Finally, the probe ionization in the suspension was compared with that in the dried solid (Fig. 5b *vs.* c for

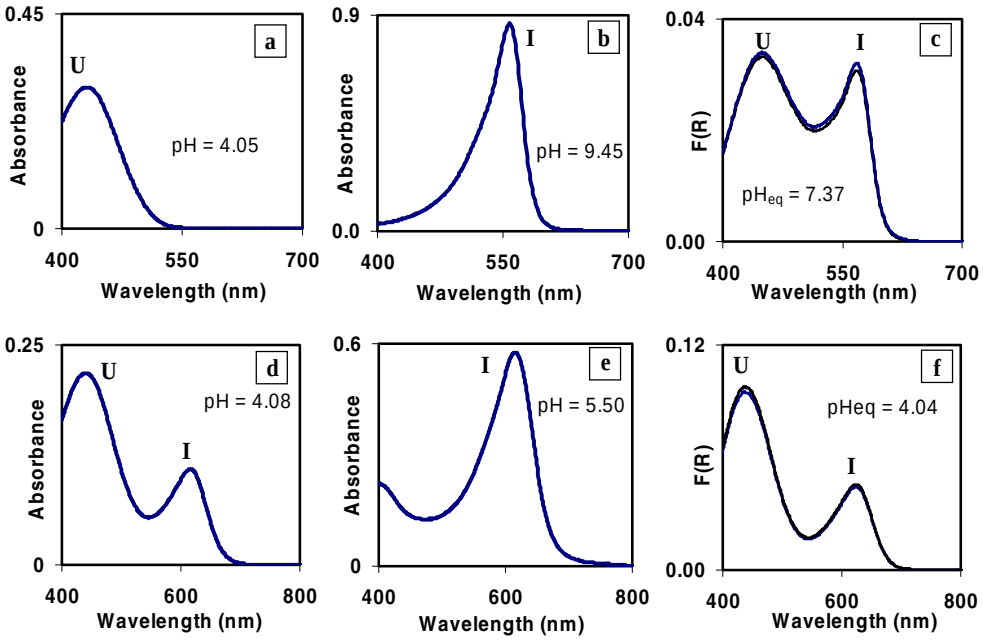


Fig. 5. *Top panel* (a, b, c): Ionization of PR at different stages of sample preparation (processing) with calcium carbonate (Calcipure® GCC-300). *Bottom Panel* (d, e, f): Ionization of BG at different simulated stages of processing with Avicel® PH101. The solvent used was water. a Aqueous solution of PR (5 μg/ml). b Supernatant of a 5% w/v Calcipure® GCC-300 suspension containing 5 μg/ml of PR. c PR solvent-deposited on the surface of Calcipure® GCC-300 at a concentration of 0.1 mg/g. d Aqueous solution of BG (10 μg/ml). e Supernatant of a 5% w/v Avicel® PH101 suspension containing 10 μg/ml of BG. f BG surface-deposited on Avicel® PH101 at a concentration of 0.2 mg/g. The letter U represents the peak of the unionized form (InH^-) and the letter I the peak of the ionized form (In^{2-}).

Calcipure® GCC-300 and 5e *vs.* f for Avicel® PH101). With both PR and BG, the ionization extent decreased when the indicator was deposited on the excipient by removal of water. Similar effects were observed for the thymol blue (TB)–ADCP (A-TAB® granules) system (data not shown). TB was completely ionized (first ionization) in aqueous solution as well as in an aqueous suspension of the excipient, since the pH values in both cases were much higher than the pK_a (pK_{a1} = 1.6). When it was deposited on the surface of A-TAB®, the diffuse reflectance spectrum indicated the presence of unionized species, H_2In.

Suspension pH vs. pH_{eq}. As we compare the probe ionization in the suspension with that in the dried solid, it is instructive to recognize that the ionization will be affected not only by the proton activity but also the polarity and the ionic strength in the immediate environment of the probe. The concentration of the residual sorbed water in the dried solid will also affect these microenvironmental properties. Impurities in the excipients can get highly concentrated in the small amount of water present in the microenvironment. Thus, they can influence probe ionization in the 'near dry' state even when they are present at very low concentrations in the excipient. While their influence on the suspension acidity may be negligible, they can exert a pronounced effect on the microenvironment in the dried state. In light of these factors the suspension pH may not be a good indicator of drug ionization in the final solid dosage form.

The pH of a suspension or a saturated solution of an excipient has been widely used to predict or explain the effect of the excipient on the microenvironment in a solid dosage form (1–4,13,27). We compared the ability of the two approaches (pH *vs.* pH_{eq}) to describe the nature of the microenvironment in the final solid. In this context, it was demonstrated that the measured pH of calcium phosphate (A-TAB® granules) decreases from a value of 6.3 for a 1% w/w suspension to 5.3 for a 40% w/w suspension (13). The value extrapolated from this trend, to a solid containing 1% water, was found to be ~5, which would, in a way, provide a measure of the microenvironmental properties near the solid surface (13). Although, from our data (Table I), no conclusive inferences could be drawn and extrapolations to the microenvironment containing very low amounts of water were not made, it might indicate that the 'pH' of the highly concentrated residual water in a solid dosage form may not be reflected by a suspension or slurry pH.

In Table II, the suspension (5% w/v and 10% w/v) pH values have been compared with the pH_{eq} values for the corresponding solids. There was a broad agreement between the pH_{eq} and suspension pH values, for example, both scales revealed that the calcium carbonate samples and magnesium stearate were the most basic excipients. However, a rank ordering of the excipients, based on these two approaches, did not show good agreement. As discussed earlier, the physicochemical environment on the 'near-dry' solid surface is expected to be different from that in the suspension. For example, while two grades of calcium carbonate, Vicron® 75-17-FG and Precarb® 150, have very similar suspension pH values (9.52 and 9.59, respectively), the respective pH_{eq} values of 6.58 and 7.69 were markedly different.

The suspension pH values of the Avicel® PH101–Calcipure® GCC-300 mixtures were compared with the measured pH_{eq} values of the solids (Fig. 6). Presence of calcium carbonate in the mixture increased the suspension pH. For example, replacement of 25% of the Avicel® PH 101 with calcium carbonate causes a large increase in suspension pH from 5.7 to 9.05. However, the influence of calcium carbonate content on the pH_{eq} value of the powder blends was much less pronounced (Fig. 6). Although a low weight fraction of calcium carbonate in the powder blend significantly influenced the suspension pH, its influence on the microenvironment within the dry solid, measured in terms of probe ionization was more gradual (Figs. 3 and 6). Similarly, the effects of surface modification (*e.g.* magnesium stearate lubrication, Fig. 4) on the final formulation properties, reflected by the pH_{eq} measurements, may also not be predicted by the pH of formulation slurries.

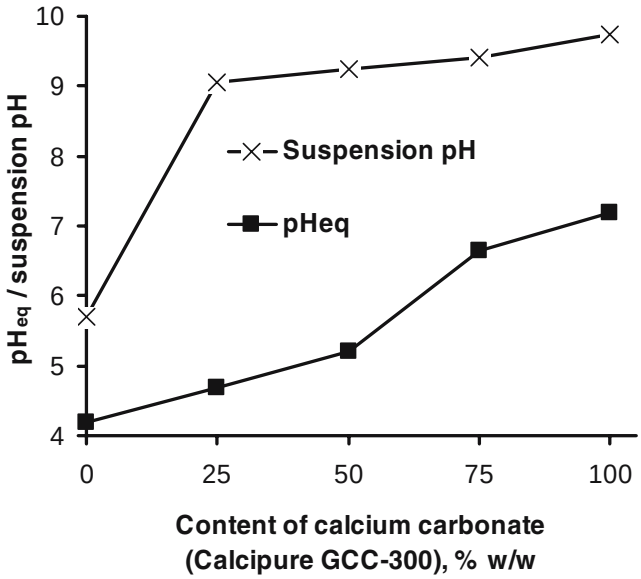


Fig. 6. Comparison of the pH values of 5% w/v aqueous suspensions, with the pH_{eq} measured for Avicel® PH101 (microcrystalline cellulose)–Calcipure® GCC-300 (calcium carbonate) binary mixtures, as a function of mixture composition. The pH_{eq} values have been taken from the data in Fig. 3.

Effect of solvent. In order to evaluate the effect of solvent nature on the ionization of the probe, methanol was used as solvent in place of water to study the Calcipure® GCC-300-phenol red system (Fig. 7). The ionization state of PR in methanolic and aqueous solutions was qualitatively similar, with the indicator predominantly unionized in both the media. However, when methanol was used as the solvent to evaluate the GCC-300 suspension, significant amount of the incorporated indicator was found to be associated with the solid phase. The methanolic dispersion medium of the suspension and the final supernatant was yellow in color, indicating that the indicator was unionized in the supernatant, as was confirmed by the spectrum of the supernatant (Fig. 7b). This was contrary to what was seen in the aqueous process. The solid phase on the other hand was distinctly pink in color, indicating the presence of ionized PR. The solid was separated by decantation and centrifugation and was dried in a vacuum oven at 40°C. The diffuse reflectance spectrum of the residue shown in Fig. 7c, reveals significant amount of ionization of the indicator on the surface (pH_{eq} = 7.48). When deposited on the surface of the same excipient from a methanolic solution, the

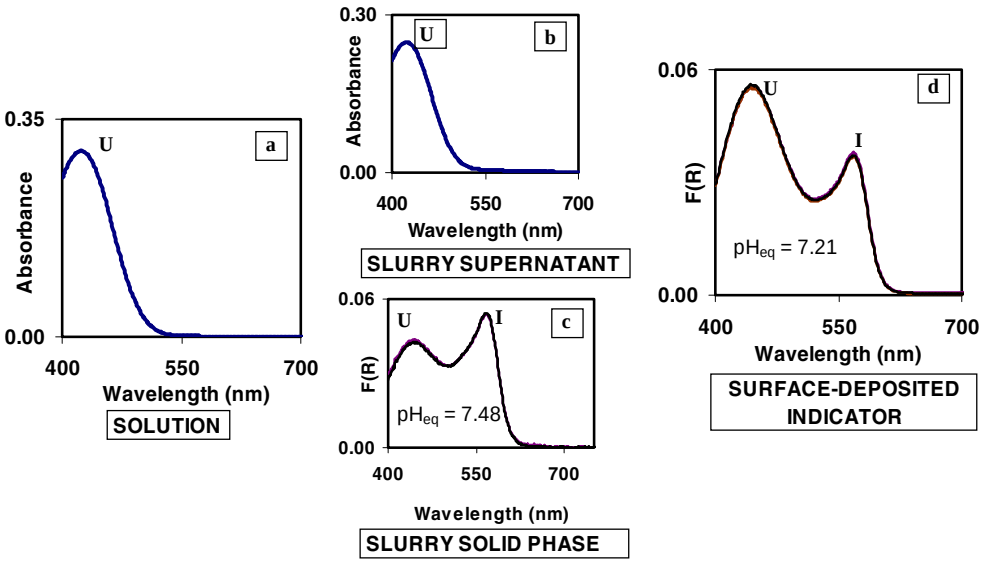


Fig. 7. Ionization of phenol red indicator during various stages of processing with calcium carbonate (Calcipure® GCC-300), using methanol as solvent. a Methanolic solution of PR (5 μg/ml). b Supernatant of 5% w/v Calcipure® GCC-300 slurry in methanol containing 5 μg/ml of PR. c The *solid phase* of the above slurry separated by decantation and dried. d PR deposited on the surface of Calcipure® GCC-300 at a load of 0.1 mg/g of the excipient, using methanol as solvent. The letter U represents the peak of the unionized form and the letter I the peak of the ionized form.

indicator was found to be partially ionized and the calculated pH_{eq} value was 7.21 ± 0.01, which was similar to the value reported in Table II.

These experiments and the data from the characterization of the individual excipients allowed a comparison of the pH_{eq} values determined using different solvents. The pH_{eq} values obtained using water and methanol as solvents for Avicel® PH101 (4.07 and 4.04, respectively) and calcium carbonate, Calcipure®GCC-300 (7.37 and 7.20, respectively), were reasonably similar. This is in good agreement with earlier reports of similar pH_{eq} values obtained for Avicel® PH101 using aqueous or methanolic solution of bromophenol blue (18). The small differences could arise possibly due to differences in the residual solvent, different effects of the two solvents on the particle surfaces and differences in distribution of the indicator. It can be argued that a part of these differences can be attributed to the retention of a "memory" of the ionization of the probe from the wet mixing stage. Removal of this "memory", if any, is expected to be facilitated in the presence of residual water. A more detailed study using solvents with different polarities and salt and free acid forms of the probe is warranted.

In this context, it must be mentioned that dissolution of excipient during sample preparation is an inherent problem in the solvent deposition method. However, this method was chosen to bring about an intimate mixture of the probe with the solid sample and thereby mimic certain pharmaceutical operations. It is also recognized that sample surface modification due to dissolution can influence the microenvironmental properties. However, the final ionization behavior of the probe would reflect the microenvironment near the surface of the excipient which has been processed with a similar solvent.

Dry Mixing

Water (solvent) used during pharmaceutical processing can act as a medium for transfer of protons and facilitate acid-base reactions. Drug stability may therefore be enhanced, by avoiding water during pharmaceutical processing. However, the presence of water in formulations cannot be completely avoided. There might therefore be enough water to facilitate proton transfer, thereby influencing drug stability. As described in the experimental section, two different model systems were studied to investigate the effects of "dry" mixing of excipients on the ionization states of an incorporated probe.

In the first model system, Avicel® PH105 was selected as an excipient which possessed relatively higher water content. All the water present in Avicel® PH105 would not be on the particle surface, since water is expected to be absorbed into the amorphous regions. However, upon dry mixing with calcium phosphate, a possible redistribution of the water in the binary mixture might make water available to facilitate the effects of both excipients on probe ionization.

Dry mixing of Avicel® PH105 pretreated with BB, with equal weight of untreated Avicel® PH105 resulted in no appreciable change in the ionization state of the probe. The signals obtained in the diffuse reflectance spectra were however approximately halved due to probe dilution (Fig. 8, a→c). Similarly, dilution of indicator-treated ADCP with equal weight of the same untreated excipient resulted in no significant change in the ionization of the probe (Fig. 8, b→f). However, mixing of untreated ADCP with indicator-deposited Avicel® PH105 resulted in a decrease in probe ionization, seen as a small relative increase in the peak signal of the unionized form (Fig. 8a→d). On the other hand, dry mixing

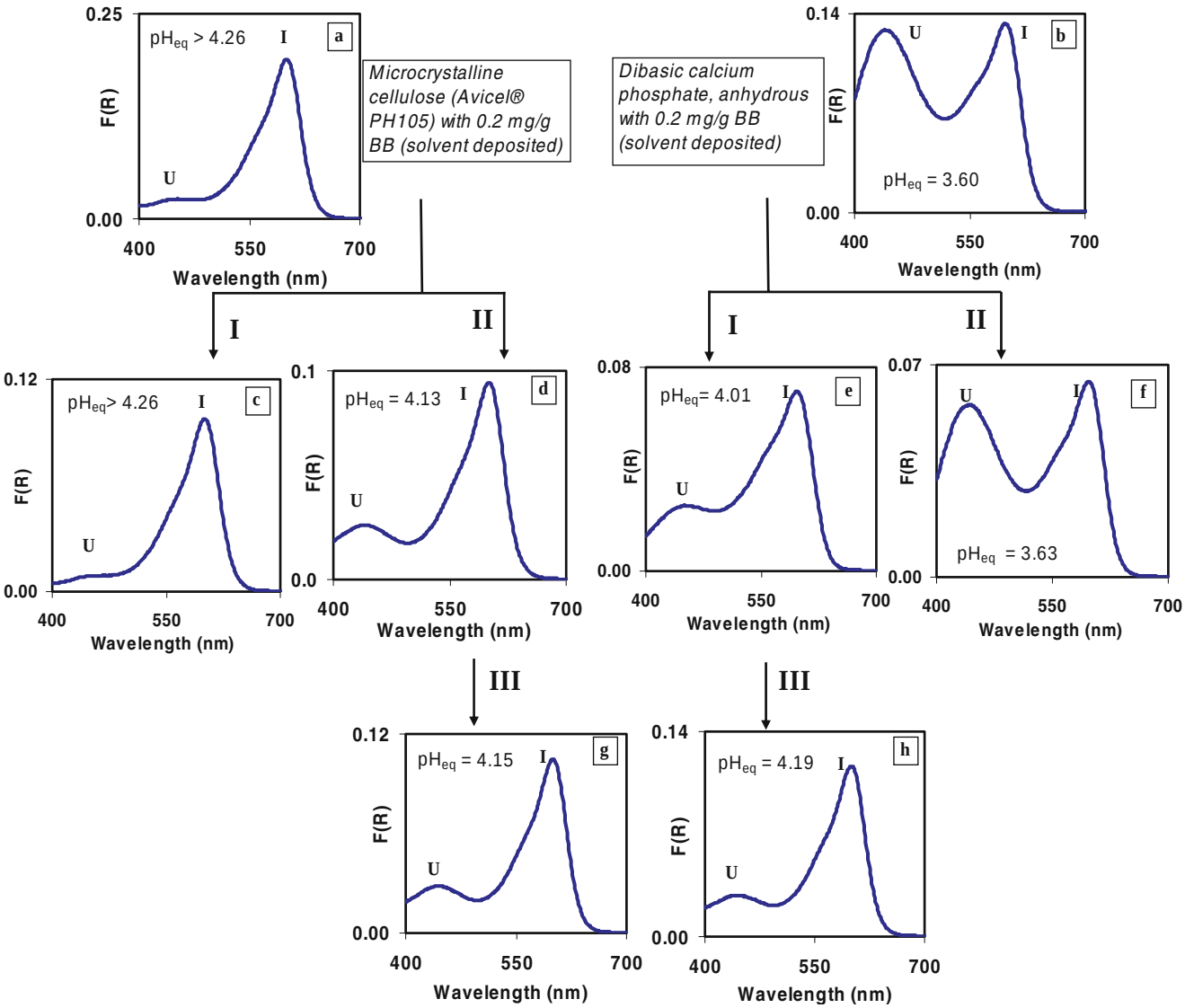


Fig. 8. Ionization of BB during different stages of dry mixing of microcrystalline cellulose (Avicel® PH105) and ADCP (Sigma). The compositions at stages a and b are given in figure. Process I refers to dry mixing with equal mass of untreated Avicel® PH105, process II to dry mixing with equal mass of untreated anhydrous ADCP and process III to wet massing with methanol followed by drying. The letter U represents the peak of the unionized form and the letter I the peak of the ionized form.

of untreated Avicel® PH 105 with indicator treated ADCP resulted in an increase in probe ionization (Fig. 8b→e).

The relatively high water content of MCC (Avicel® PH105; water content of ~2.7% w/w) resulted in MCC–$CaHPO_4$ mixtures with 1.3 to 1.7% w/w water (determined by TGA). Under these conditions, after dry mixing with the second excipient, there was a marked change in the ionization state of the probe (Fig. 8, a→d and b→e). Thus, the microenvironmental acidity in a formulation is significantly influenced even by dry mixing of excipients and this is expected to be facilitated by the presence of residual water.

In the second system, calcium carbonate–ADCP mixtures, the water content of the individual components as well as the final blends was consistently< 0.1% w/w. In this system as well, the addition of one excipient influenced the ionization of the probe deposited on the other (as described below). When phenol red was deposited on ADCP (Sigma), it was completely unionized (Fig. 9b). Dry mixing with calcium carbonate revealed the influence of the surface properties of the latter, seen as the appearance of ionized phenol red (In^{2-}) peak (Fig. 9, b→e). Interestingly, when PR was deposited on calcium carbonate (Precarb® 150; Fig. 9a) and dry mixed with untreated ADCP, there was no perceptible change in probe ionization (Fig. 9 a→d). From these two observations, it seems that calcium carbonate surface properties influence probe ionization to a much greater extent than that of ADCP (Sigma). Dry mixing of an indicator-treated excipient with the same untreated excipient (Fig. 9, a→c; b→f), as seen in the earlier case (Fig. 8), only resulted in indicator dilution and no significant change in the ionization.

In the calcium carbonate–ADCP blends, the binary mixtures d and e (Fig. 9) were of identical composition. However, in one case, (Fig. 9d) the indicator was first deposited on calcium carbonate while in the other case (Fig. 9e), it was deposited on ADCP. The probe ionization in

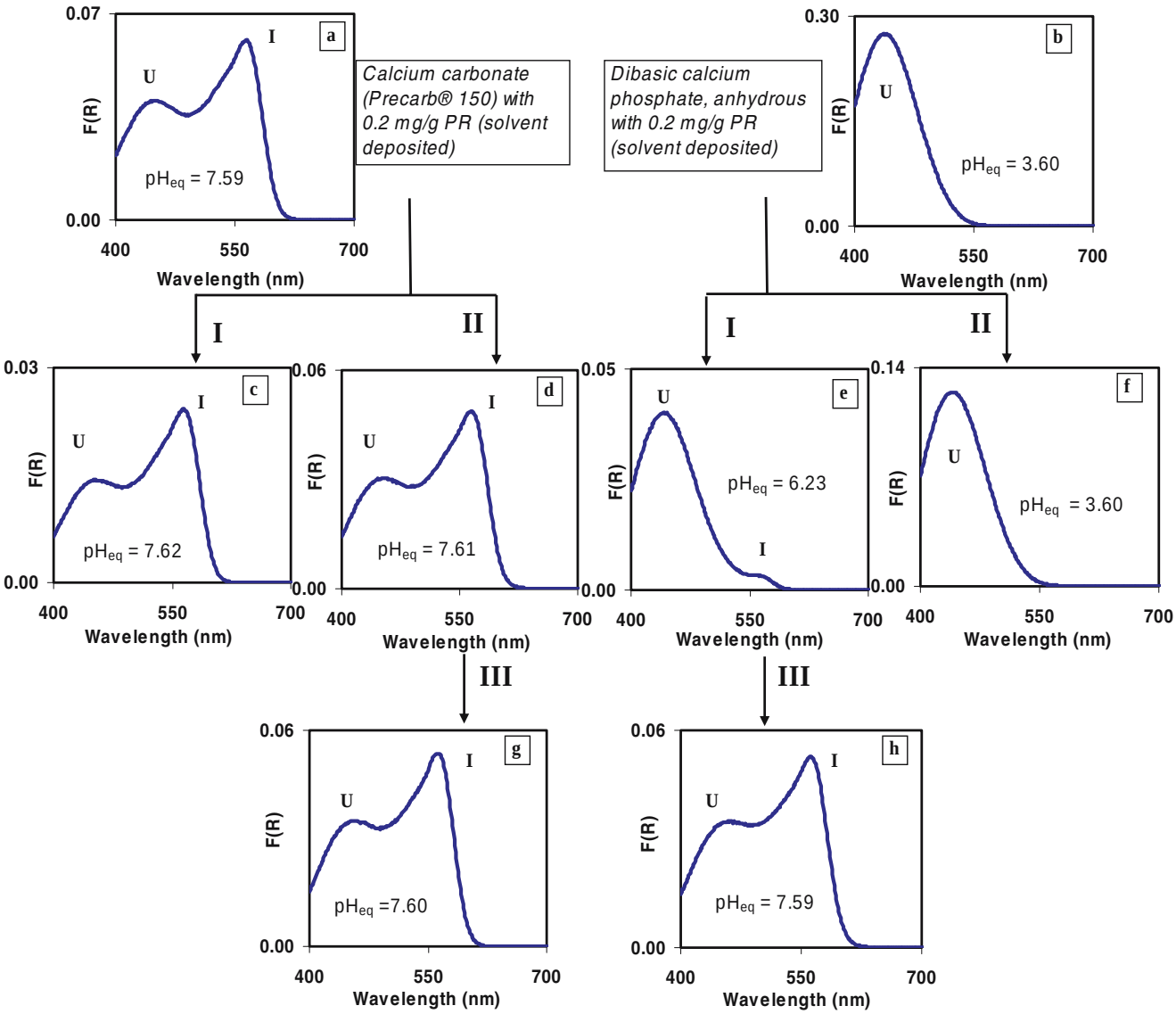


Fig. 9. Ionization of PR during different stages of dry mixing of calcium carbonate (Precarb® 150) and ADCP (Sigma chemicals). The compositions at stages a and b are given in figure. Process I refers to dry mixing with equal mass of untreated calcium carbonate, process II to dry mixing with equal mass of untreated ADCP and process III to wet massing with methanol followed by drying. The letter U represents the peak of the unionized form and the letter I the peak of the ionized form. The pH_{eq} values reported in panels 9b and 9f were determined using bromophenol blue as the indicator.

the blends was markedly different (compare Fig. 9d & e and the pH_{eq} values). Similar differences were observed in the MCC–$CaHPO_4$ systems (Fig. 8d and e). In mixtures of similar composition, over the experimental time-scales, the ionization of the probe therefore depends on the sample preparation process. Greater interaction of the probe with one of the excipients in the mixture results in higher influence of the surface of that excipient on the environment experienced by the probe. In order to investigate if presence of solvent influenced/altered the probe ionization in these systems, the binary blends (Figs. 8d, e, 9d and e) were wet-massed with methanol (enough methanol to permit uniform mixing), dried and their spectra were obtained (Figs. 8 and 9, d→g and e→h). The observed differences in probe ionization (Figs. 8 and 9; d *vs.* e) were minimized after methanol treatment. The resulting spectra were very similar (Figs. 8 and 9; g and h). The influence of the surface properties of each excipient, on the ionization of the incorporated probe, is facilitated by methanol. Methanol causes redistribution of the probe molecule over the excipient surfaces and also acts as a medium for proton transfer reactions, which would influence the ionization of the probes.

In the Precarb® 150-ADCP system, both the binary mixtures exhibited significant ionization of PR after methanol treatment (Fig. 9g and h; pH_{eq} = 7.60 and 7.59). The spectra were very similar to that when PR was deposited on Precarb® 150 (Fig. 9a, pH_{eq} = 7.59). Therefore, these results also suggest a greater influence of Precarb® 150 on the microenvironmental properties. This can possibly be explained by the much higher surface area of Precarb® 150, (4.2 m^2/g) compared to ADCP (1.1 m^2/g, Table IV).

Low dose drugs are often solvent deposited on excipients and are further processed with other formulation components so as to achieve uniform distribution. This approach also

enables stabilization of labile drugs. For example, acid-labile drug calcium pantothenate is deposited on the surface of basic excipients such as magnesium oxide (5). However even dry processing with other excipients could significantly alter the microenvironment and drug ionization. Our dry mixing experiments with indicator probes mimic this low dose drug-loading process and provide an insight into the changing environment experienced by the drug during processing.

CONCLUSIONS

Diffuse reflectance visible spectroscopy enabled the study of the ionization of sulfonephthalein indicators, intimately mixed with solid excipients or powder blends. Based on these measurements, excipients and formulations could be characterized and rank-ordered based on an acidity function 'pH_{eq}'. The properties of all the components of a formulation influenced the microenvironment in the final blend. The contribution of each component to the overall properties of the final solid seemed to be influenced by its surface area available for interaction.

The effective environment experienced by the API in the final solid can also be influenced by the process employed. Presence of solvent during processing enhanced the effectiveness of microenvironmental pH-modifiers. The pH of the excipient suspension, which is often used to characterize the effect of a formulation component (2,13), may not always provide a good measure of the microenvironment in the final solid formulation. Solid surface properties of excipients can influence the physicochemical properties of the formulation microenvironment even when processed at water contents encountered during typical "dry" pharmaceutical processes. The probe ionization provided an insight into the changing environment experienced by the drug during processing. The effective microenvironment in a solid dosage form, and hence API stability, will therefore be influenced by the formulation composition, the particulate properties of the components in the final product, as well as the processing conditions employed.

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