Oseltamivir Phosphate–Amberlite™ IRP 64 Ionic Complex for Taste Masking: Preparation and Chemometric Evaluation

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ABSTRACT: The objective of the present work was to evaluate and characterize a pediatric-friendly formulation of a bitter tasting drug, oseltamivir phosphate (drug). Amberlite IRP64 (resin) was used to make ionic complexes for masking its bitterness. Complexes of four drug-to-resin ratios, 1:1, 1:2, 1:4, and 1:6 (w/w), were prepared and characterized. At buccal pH of 6.8, drug–resin complexes of 1:1, 1:2, 1:4, and 1:6 ratios released 42.13%, 23.26%, 4.13%, and 14.94%, respectively, of loaded drug after 20 s. However, at stomach pH of 1.2 (0.1 N HCl), 61.96%, 70.18%, 85.88%, and 91.42% of drug was released from the same complexes in 6 min. Near-infrared (NIR) chemical imaging of the complexes showed homogeneous distribution of drug in the resin. Chemometric partial least squares model using NIR data of the drug showed a high correlation between calibration and predicted data ($R^2 > 0.998$). Overall, these results indicated the complex formation between drug and resin. The pH dependence of drug release from these complexes could minimize drug release in the mouth, whereas immediately releasing it in the stomach. Electronic tongue used to evaluate taste indicated that conductivity taste signals were different from control, suggesting taste masking of the drug. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1800–1812, 2013

Keywords: oseltamivir; ionpair complex; taste masking; Amberlite™; resins; Multivariate Analysis; Principal component Analysis; Complexation; Near Infrared Spectroscopy; FTIR

INTRODUCTION

Oseltamivir phosphate, a prodrug of oseltamivir carboxylate, used in the management of pandemic flu symptoms, acts by inhibiting the viral enzyme neuraminidase and thereby prevents the release as well as the spread of viral progenies from the infected cells.1 Additionally, it has been indicated for the management of pre- or post-viral-exposure-related symptoms in the population ranging from pediatrics of at least 1 year old to adults.2,3 Because a sudden outbreak of viral flu with ever-changing genotype and virulence poses an existential threat to our population, national stockpiling of the finished oseltamivir product is required.2

Despite the importance of this drug for the treatment of the pediatric population in an emergency situation, there is no suitable pediatric dosage form available. Commercially, oseltamivir is available as capsules and powder for oral suspension in doses of 30, 45, 75, and 360 mg oseltamivir base.2,3 However, for pediatric patients below 12 years of age, the dose of the drug is titrated against their body weight, and this requires either metered drug dosing or extemporaneous preparation. In addition to preparatory and administration issues, the oseltamivir drug substance has a bitter taste,2 which poses a palatability challenge, and therefore presents compliance issue for oral administration to children.

Taste is an important factor for drug acceptance and regimen compliance. Therefore, taste modification has been a pragmatic approach to exploit the therapeutic potential of bitter drugs. Various taste-masking formulation strategies that have been employed in the development of palatable dosage forms include microencapsulation; coating granules with polymer; and formation of inclusion complexes, solid dispersions, or drug–resin complexes.4–6 The ultimate objective of these bitterness suppression modalities is to either prevent or minimize drug release to less than 10% during the dwell time of the dosage form
in the oral cavity to avoid/minimize drug–taste bud contact.7–9 Among the various taste-masking strategies, complexation is a simple one because it requires few ingredients, it does not necessarily require organic solvents, the preparation process is simple, and the products can be easy to evaluate.

Previous studies have demonstrated the formation of weak ionic complexes between amine drugs and polycarboxylic acid ion-exchange resins.10,11 In this study, Amberlite™ IRP64, which is a weak acidic cation-exchange resin with an ion-exchange capacity of 10 mEq/g, has been employed to evaluate the feasibility of complexation with oseltamivir phosphate, which is a weak base and is highly soluble in water and methanol (pKₐ = 7.75, logP = 0.36).12 The Amberlite™ resin was selected for its proven taste-masking ability,13–15 as well as its safety for consumption, because it is nonabsorbable.16 It is a water-insoluble polacrilex resin that is a copolymer of methacrylic acid and divinylbenzene. Besides its application as a taste-masking agent, Amberlite™ IRP64 has been used for drug stabilization, as a carrier for cationic drugs, and for preparing controlled-release formulations.17

MATERIALS AND METHODS

Materials

Amberlite™ IRP64 was purchased from Sigma–Aldrich (Saint Louis, Missouri), and oseltamivir phosphate was obtained from Ria International LLC (East Hanover, New Jersey). Hard gelatin capsules of size 000 were purchased from Letco Medical (Decatur, Alabama). Acetonitrile, methanol, and monobasic potassium hydrogen phosphate were purchased from Fisher Scientific Company (Norcross, Georgia). All other chemicals and solvents used were of analytical grade.

Methods

Preparation of Drug–Resin Complexes and Physical Mixtures

Oseltamivir phosphate–Amberlite™ (Sigma–Aldrich) resin complexes were prepared according to the method reported by Tawakkul et al.14 with some modifications. Amberlite™ resin (1, 2, 4, or 6 g; Sigma–Aldrich) and 40 mL of 0.1 N HCl were added to a 125-mL flask and briefly shaken to evenly distribute and wet the resin. Each flask was allowed to stand overnight for the resins to swell and activate. To the swollen and activated resins, specified aliquots of drug in 25 mL of 0.1 N HCl (20 mg/mL) were added to obtain drug–resin ratios of 1:1, 1:2, 1:4, and 1:6. All the flasks were kept at 70°C in a water-bath shaker with a shaking speed of 100 rpm until the drug–resin samples were dry.

The dried samples were then kept in an oven at 50°C for 4–5 h (until constant sample weight) to remove residual solvent. After drying, the samples were passed through a 40 mesh sieve and stored in tightly capped scintillation vials until performing further characterization studies. The drug–resin physical mixtures at ratios of 1:1, 1:2, 1:4, and 1:6 (w/w) were also prepared by geometric dilution, first by mixing with a spatula followed by shaking the mixtures for approximately 100 times in scintillation vials.

Characterization of the Complex

Differential Scanning Calorimetry. Thermal behavior of the samples was collected by differential scanning calorimetry (DSC; Q2000; TA Instruments, New Castle, Delaware) after calibrating it for baseline and temperature with indium. Complexes, physical mixtures, or their pure constituents weighing 2–3 mg were hermetically sealed in an aluminum pan and heated at the rate of 10°C/min from 10°C to 230°C under an inert nitrogen atmosphere at the flow rate of 50 mL/min to prevent oxidative degradation of the samples.

Powder X-Ray Diffraction. Powder X-ray diffraction (PXRD) patterns were collected for the drug, resin, their physical mixtures, and complexes using a Bruker D8 Advance with DaVinci design (Bruker AXS, Madison, Wisconsin) using Cu Kα radiation (λ = 1.5405 Å) at a voltage of 40 kV and current of 40 mA and equipped with the LYNXEYE scintillation detector. Before measurement, the instrument functionality was checked using corundum as an external standard. Each sample was prepared by backloading 500 mg of powder into a stainless steel sample holder, backed by a zero-diffraction single-silicon-crystal plate (MTI Corporation, Richmond, California). Diffraction patterns were collected over the range of 4°–50° 2θ with a step size of 0.01° at 0.5 s per step (4472 total steps). Each sample was subjected to rotation at 15°/min. The PXRD operation, data collection, and data analysis were achieved through Diffract.Suite (V2.2).

Near-Infrared Spectroscopy. Near-infrared (NIR) spectra of the drug complexes, physical mixtures, and the pure components were collected by a NIR spectrophotometer (Model #6500; Foss NIR Systems, Laurel, Maryland). The instrument was fitted with a scanning grating monochromator and a diffuse reflectance apparatus (rapid content analyzer). After the instrument passed the diagnostic tests and reflectance standardization, samples in 25-mL borosilicate glass vials were placed on the sample window and centered with an iris. NIR spectra ranging from 1100 to 2500 nm in 2-nm increments were obtained in sextet from the base of the vial, which was transparent.
to the NIR. The sextet spectra were collected with tapping/shaking of the vial between each iteration to rearrange the powder bed to minimize spectral differences because of any heterogeneity in the powder. Vision software (version 3.2; Foss NIR Systems) was used to collect data, and further qualitative and quantitative analyses of the sample were carried out by Unscrambler X chemometrics software (version 10.1; Camo Process, Oslo, Norway).

**Fourier Transform Infrared Spectroscopy.** Fourier transform infrared (FTIR) spectra of the complexes, physical mixtures, and the pure components were collected using an attenuated total reflectance FTIR spectrometer (ThermoNicolet Nexus 6700 FT-IR; Thermo Scientific, Waltham, Massachusetts). To collect data, a small amount of powder was placed on the diamond disk and pressed with the attached arm to remove air in the powder. The pressed powder was then scanned in the range of 500–4000 cm\(^{-1}\) with a data density of 4 cm\(^{-1}\) using a laser source as incident light on the powder. A total of 50 coadds, which were captured by Omnic data acquisition software (version 6.2, ThermoNicolet; Thermo Scientific) were averaged.

**Scanning Electron Microscopy.** The shape and morphology of the drug–resin complexes at ratios of 1:1, 1:2, 1:4, and 1:6, as well as their corresponding physical mixtures, were visualized using scanning electron microscopy (SEM; JSM-6900 LV; JOEL, Tokyo, Japan) at an applied accelerated voltage and working distance of 5 kV and 12 mm, respectively. Before visualization, samples were gold coated using a sputter coater (Desk V, Denton Vacuum, Moorestown, New Jersey) after applying high vacuum and voltage of 70 mTorr and 30 kV, respectively.

**NIR Chemical Imaging.** Near-infrared chemical images of the complexes, physical mixtures, and the pure components were obtained by a Sapphire imaging system using SapphireGo software (Malvern, Worcestershire, UK). The instrument was equipped with an indium–gallium–arsenide focal-plane array detector, which detects the diffuse reflected light from the sample after being filtered by a liquid crystal tunable filter and produces 320 × 256 pixel images. Before capturing images, the instrument was allowed to condition according to the manufacturer’s specifications. Then, background reference and dark response were collected using Spectralon 99 (Labsphere, North Sutton, New Hampshire) and a clean stainless steel mirror, respectively. The data were obtained in the NIR range of 1400–2450 nm with an increment of 10 nm and eight coadded scans. The data obtained by the instrument were analyzed by ISys chemical imaging software (Malvern). Before analyzing the data, the corrected reflectance data were converted to absorbance by taking the logarithm of the inverse of the reflectance. Data were truncated and normalized by mean centering and scaling to unit variance by spectrum. A library of the two pure components in the complexes was created, and PLS 2 (partial least squares 2) fitting was employed to obtain PLS concentration scores and images.

**Oseltamivir Assay.** Complexes were assayed by dissolving complexes equivalent to 50 mg of drug in 10 mL of either 0.1 N HCl or methanol. The complexes were sonicated for 20, 40, 60, 80, and 120 min to extract the drug. Samples of 0.5 mL were withdrawn, filtered through a 0.45-µm nylon filter, and diluted with the respective media to fit into the HPLC concentration calibration range. The samples were analyzed by the oseltamivir United States Pharmacopeia’s (USP) monograph-validated method using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, California), fitted with a quaternary pump, an autosampler, an ultraviolet detector fixed at 207 nm, and column temperature control (50°C). A reversed-phase Luna C8 (2) 150 × 4.6 mm\(^2\) (5-µm packing, 100 Å) column from Phenomenex (Torrance, California) was used as the HPLC stationary phase along with a C8 guard cartridge. Methanol, acetonitrile, and buffer at pH 6 in the ratio of 245:135:620 were employed as mobile phase, which was isocratically pumped at a flow rate of 1 mL/min, and an injection volume of 15 µL was used.

**Dissolution Studies.** Dissolution studies that simulate conditions of the stomach and the oral cavity were performed in 0.1 N HCl and phosphate buffer at pH 6.8, respectively. For the gastric condition, a USP apparatus II (Vankel VK 7000; Agilent Technologies), connected to autosampler, was used. Hard gelatin capsules of size 000 (Letco Medical) were filled with 500 mg equivalence of the drug in drug–resin complexes or physical mixtures (drug–resin ratios of 1:1, 1:2, 1:4, 1:6). The filled capsules were encased in sinkers to avoid floating and placed into 500 mL of 0.1 N HCl at 37°C with paddle rotation of 50 rpm. Each drug–resin complex or corresponding physical mixtures were studied in triplicates. Samples of 0.5 mL were withdrawn using an autosampler (Agilent Technologies) at time points of 1, 4, 6, 30, and 60 min and filtered through 10-µm filters (QLA, Bridgewater, New Jersey). The samples were analyzed using the HPLC method described in **Taste-Masking Studies.** To simulate conditions of the oral cavity, complexes (1:1, 1:2, 1:4, and 1:6) equivalent to 10 mg of drug were weighed into a conical flask containing 10 mL of phosphate buffer at pH 6.8 and swirled before putting them on a shaker (120/minute) for 5, 10, 15, and 20 s in triplicates. Samples of 0.5 mL were
withdrawn at the end of respective time points, filtered through a 0.45-μm nylon filter, and analyzed by the previously described HPLC method.

**Taste-Masking Studies.** The taste of the ion-complexes was evaluated with an α-Astree liquid and taste analyzer and electronic tongue (E-Tongue) from Alpha MOS (Toulouse, France). Seven sensors represented by ZZ, AB, GA, BB, CA, DA, and JE (Alpha MOS) and an Ag/AgCl reference electrode (Metrohm Ltd., Riverview, Florida) were supplied with instrument for the estimation of the taste in the sample. An autotest of the instrument was performed after starting the instrument and the software control (Alpha Soft, version 12.0) to check software and autosampler configurations. As per the manufacturer’s recommendation, conditioning, calibration, and diagnostic tests were performed. The conditioning step was performed in 0.01 N HCl to hydrate the sensors and check for noise or drift. After the instrument passed the conditioning step, it was calibrated against the manufacturer-specified sets of target values and margins of error in 0.01 N HCl using the troubleshooting analysis method. As suggested by the manufacturer, hydrochloric acid, sodium chloride, and methyl sodium glutamate were employed to evaluate whether the sensors could discriminate between sour, salty, and umami tastes, respectively. The data obtained from the recommended standard solutions can be analyzed either by standard or troubleshooting analysis methods. The troubleshooting analysis method appears to be a liberal method, that is, its passing criterion value for the specified calibration parameters (adjustment criterion, stability criterion, and dispersion criterion conditioning) are wider than the standard analysis method. In this work, the troubleshooting analysis method was selected. Drug or drug–resin complex equivalent to 1 mg/mL was weighed in a conical flask and shaken for 20 s at a speed of 120/min after adding 30 mL of pH 6.8 buffer. Samples of 25 mL were immediately filtered through a 0.45-μm nylon filter into the E-Tongue (Alpha MOS) sample holder. For each sample, signals were collected for 120 s in eight replicates. After signal collection from each sample, the sensors were cleaned for 120 s in water. A library of the acquired data between 95 and 115 s was built. Signals from the sensors ZZ, AB, GA, CA, and DA from the five replicates of the sample were included for conducting principal component analysis (PCA).

**Statistical Analysis**

Statistical analysis of the data including ANOVA and t-test, wherever needed, was performed using JMP software (version 9; SAS Institute Inc., Cary, North Carolina).

**RESULTS AND DISCUSSION**

**Thermal Studies**

Thermal studies of oseltamivir (drug), Amberlite™ IRP64 (resin), their complexes, and their physical mixtures (drug–resin ratios of 1:1, 1:2, 1:4, and 1:6) were performed to potentially evaluate drug–resin interactions (Fig. 1). The DSC thermogram of the drug showed a melting endotherm at 206°C (Fig. 1), whereas the thermogram of the resin demonstrated a baseline transition at approximately 203°C, which likely represents the glass transition temperature of the Amberlite™ resin.

As can be seen from Figure 1, the thermograms of physical mixtures in 1:1, 1:2, 1:4, and 1:6 drug–resin ratios exhibit a different trend than those of the complexes. Endothermic peaks of drug melting are observed in the physical mixtures at ratios of 1:1, 1:2, and 1:4. In the thermogram of the 1:6 physical mixture, this melting peak was not observed, but this is possibly because of the relatively low concentration of drug at this ratio. However, among all thermograms of the complexes, regardless of drug–resin ratio, the melting endotherm of the drug was absent, suggesting that the crystallinity of the drug is reduced during the formulation of the complexes. Of additional note is the presence of broad exothermic transitions within the thermograms of the complexes, but not in those of the physical mixtures. These observed differences in the thermal behavior of complexes versus physical mixtures are consistent with (but do not prove) the formation of complexes via the method described in *Preparation of Drug–Resin Complexes and Physical Mixtures*. To provide further evidence of drug–resin complexation, PXRD and spectroscopic analyses were performed.

**Fourier Transform Infrared**

Infrared (IR) spectroscopy investigates vibrational energy levels of a molecule when irradiated with IR light, which results in characteristic vibrations of various functional groups in the molecule and represents a widely used technique to investigate complexation. The unique vibrations of a molecule form the basis of its identification. For this study, samples were analyzed across the mid-IR region from 700 to 4000 cm⁻¹. As seen from Figure 2, the IR spectrum for oseltamivir demonstrated characteristic peaks at 3349, 3173, 2875, 1720, and 1552 cm⁻¹ representing stretching of –NH₂, the aromatic ring, the aldehyde –C=H, –C=O, and –C=–C functional groups, respectively. In the resin’s spectrum, broad peaks at 2920 and 1705 cm⁻¹ represent carboxylic acid functional groups: O–H stretching and C=O (H-bonded) stretching, respectively. These peaks were present in all the physical mixtures but were absent in all the
complexes. Such absence of peaks in the complexes corroborates the interpretation of the DSC results in which the complexes are different from the physical mixtures of the pure components (Fig. 2). Furthermore, it provides evidence that interaction took place between the drug and the resin.

PXRD Studies

Powder X-ray diffraction was used as an additional approach to investigate the formation of the complex (Fig. 3). The drug exhibited a diffraction pattern with intense peaks located at $5^\circ$, $12.5^\circ$, $13.1^\circ$, $14.4^\circ$, $15.3^\circ$, $16.3^\circ$, $19.4^\circ$, $20.6^\circ$, $24.5^\circ$, and $25.4^\circ$ 2$\theta$, which demonstrated its crystalline character. In the pattern of the resin, no sharp peaks were present; however, the presence of an amorphous halo is consistent with the amorphous nature of this material. X-ray diffraction (XRD) patterns of physical mixtures were practically constituted by the superposition of the spectra of the single components, with the crystalline peaks of the drug emerging on the diffuse background of the resin, indicating no formation of new structure. In contrast, the patterns of formulations prepared by the complexation method did not contain any characteristic drug peaks, indicating the absence of crystalline drug. The results are attributed to drug–resin interactions that prevent crystallization of the drug during the complexation process (Preparation of Drug–Resin Complexes and Physical Mixtures). These interactions resulted in the presence of only amorphous drug in the final product, which consists of a drug–resin complex. Similar complexation between a drug and a resin has been explained based on the XRD studies in the literature.

Scanning Electron Microscopy

Figure 4 contains SEM micrographs for all the collected samples. As can be seen from the micrographs in Figure 4, the resin did not show any crystalline structure, rather a conchoidal surface fracture was present. The drug appeared as very small rod-shaped crystals of less than 10$\mu$m. The drug–resin physical mixtures at ratios of 1:1, 1:2, 1:4, and 1:6 appeared as dense cluster of drug coating the resin surfaces. However, in the images of the complexes, none of the unique structures’ of the pure components could be seen. It could be inferred that the drug was embedded into the resin.

NIR Spectroscopy

Although collection of NIR spectra is rather quick and easy, the data interpretation and estimation of drug content necessitate mathematical treatment and model development because of several reasons, including weak and overlapping NIR peaks, multiplicative scattering because of variation in sample
packing density, baseline shift, and the multivariate nature of the spectra.\textsuperscript{20,21} Therefore, the spectra collected in this study were analyzed with or without mathematical treatment to evaluate the effect of data filtering on the overall improvement in spectral interpretation. The evaluated mathematical treatments were multiple scattering correction, standard normal variate, and first and second derivatives (Savitzky–Golay algorithm). Among these methods, the second-derivative Savitzky–Golay algorithm with 11 points of data smoothing and second polynomial order was found to resolve the overlapping peaks. As seen in Figure 5a, the peaks at wavelengths 1496 and 2036 nm appear to be small bumps in the raw spectra but became distinct after data derivatization. Furthermore, the absence of these peaks at the corresponding wavelengths in complexes supports the idea of the drug–excipient interactions and complexation. After data treatment, the structures of the data were analyzed with data preprocessing using PCA. Preceding model development, PCA was performed as an exploratory step to visualize similarity and differences among spectra and to identify extremes in the data set that might exert more leverage on the overall model. In this approach, a linear combination of the original variables was generated to transform them into latent variables, thereby reducing the dimensionality of the data while explaining the variance in the data set. These latent variables are called principal components (PCs), and usually the first PC describes maximum variability in the data. The successive PC, which is orthogonal to the previous PC, is similarly generated to explain the remaining variance in the data. The number of PCs is chosen based on the eigenvalues (which is a coefficient of eigenvectors' variance–covariance matrices). Each PC is described by its associated score and loading, which provide information about the sample and its associated variables. This information potentially allows the identification of a PC that describes a particular attribute of a sample or its components. As can be seen from the PCA score plot in Figure 5b, there are no extremes in the data. The preprocessed data were found to be well separated into clusters in comparison with raw data. The PC1 and PC2 scores explain 79% and 21% of variance in the data. The trend in the PC score in PC1 strongly suggests that the score value is increasing with increase in drug content in the complex, which has highest drug content in C11 (drug-to-resin ratio, 1:1) to lowest C16 (drug-to-resin ratio, 1:6). Therefore, variation in drug content in the complex could be ascribed to PC1. Similarly, highest drug content showing lowest PC2 score might indicate that PC2 is describing more about the variation in resin. These results were further supported by a loading plot, wherein the influences of variable on

Figure 2. Fourier transform infra-red images of drug, resin, physical mixtures, and complexes at 1:1, 1:2, 1:4, and 1:6 (w/w) ratios. All physical mixtures retained drug peaks at 3350 and 1550 cm\textsuperscript{-1}, whereas complexes lost those peaks. PhyMix, physical mixture; Osel, oseltamivir; Amber, Amberlite.
Figure 3. Powder X-ray diffraction of drug, resin, physical mixtures, and complexes at 1:1, 1:2, 1:4, and 1:6 (w/w) ratios. All physical mixtures retained diffraction peaks of the drug, whereas complexes lost those peaks at all drug to resin ratios. Phy Mixture, physical mixture.

Figure 4. Scanning electron microscopy photomicrograph of drug; resin; drug-to-resin physical mixtures of 1:1, 1:2, 1:4, 1:6; and corresponding complexes at ratios of 1:1, 1:2, 1:4, and 1:6 (w/w).
Figure 5. (a) Original NIR Raw and Savitsky–Golay second-derivative spectra. (b) PC1 and PC2 score plot of second-derivative data of complexes computed by principal component analysis (PCA). (c) PC1 and PC2 loading plot for the second-derivative data of the complexes.

Table 1. PLS Regression Parameters of the Drug–Resin Complexes Prepared at 1:1, 1:2, 1:4, and 1:6 Ratios Using Two PLS Factors and Validated by Cross-Validation Approach

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Calibration</th>
<th>Prediction</th>
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<tr>
<td>Number of samples</td>
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<tr>
<td>Correlation</td>
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<td>0.99</td>
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<td>Offset</td>
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<tr>
<td>Root mean square error</td>
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<tr>
<td>Slope</td>
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<td>1.02</td>
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</table>

98% and 2% variation in complexes data, respectively. As can be seen from the Table 1, the good correlation was shown between the predicted and measured values of the calibration and validation set for the drug as demonstrated by correlation coefficient (>0.99). Moreover, values of root mean square error for calibration and prediction were small (1.65 and 2). The PLS method of modeling divides the data into PLS factors, which correlate data to the physical or chemical properties of interest. Therefore, the PLS loading vectors were compared with individual components (Fig. 6). PLS factor 1 showed negative peaks at 1176, 1350, 1416, 1680, 1910, 2252, and 2296 nm, whereas PLS factor 2 demonstrated negative peaks at 1186, 1382, 1502, 1684, 1882, 2038, 2112, 2262, and 2304 nm, respectively. As the complex contains both of the components, and therefore carries majority of similar peaks in the corresponding pure spectra, the presence of distinctive peaks for the drug at 1496 and 2036 nm could be used as the basis to ascribe PLS factor 2 to drug.

**Chemical Imaging**

Chemical imaging is a powerful, quick, and noninvasive technique, which has found wide application in pharmaceutical industries. It provides spatial distribution of the components of a mixture and has found utility in studying complexes among various other uses. Chemical images are a collection of pixels, which represent three-dimensional stack of data, wherein the data in x,y plane provide information about the location of the pixels and the data in z plane describe about chemically selective spectra. These distinct spectra in each pixel are used to create distribution map of the sample where heterogeneity of the sample can be visually detected. Depending upon the concentration of the drug or resin, each pixel in the image is color coded with variable intensity and represented as color bar. Color-coded bar represents two-color extremes. The extremes representing high-coefficient values indicate higher concentration of the component under consideration, whereas other extremes of low-coefficient values show likely lack of or low concentration of the component under consideration. To obtain PLS image of the complexes, data were preprocessed by normalization to eliminate nonchemical biases from the spectral matrices. These biases could originate from multiple sources that include surface-heterogeneity-associated scattering effect, interferences from external light, and random noises. After the data are preprocessed, the image is classified to identify regions with similar spectral characteristics providing clue in physicochemical properties of the sample, their concentration, and distribution. PLS image of the normalized data of all the samples are shown in Figure 7. As can be seen from the figure, the distribution of drug in the physical mixture is not homogeneous, whereas no separate pocket of pure ingredients or their agglomerates was apparent in the complex images. This fact was further supported by the histogram distribution of drug in PLS images (Table 2), which showed skewness and kurtosis. These statistical parameters represent peakedness (unevenness) in the distribution and were found to be larger in physical mixture than the complexes, which could be taken as heterogeneous distribution of the components around the mean value in the physical mixtures.

**Oseltamivir Assay**

To determine the drug content in the complexes, they were sonicated for 2 h in either 0.1 N HCl or methanol,
**Figure 6.** PLS loading score for PLS factor 1 and PLS factor 2 and loading of pure drug and resin.

**Figure 7.** PLS images showing distribution of drug and resin in the complexes. Homogeneous distribution of drug was seen in the complexes, whereas their distribution is heterogeneous in their corresponding physical mixtures.

**Table 2.** Results of Histogram Distribution of PLS Score Images of Drug–Resin Complexes and Their Corresponding Physical Mixtures

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<th>Complex</th>
<th>Drug–Resin</th>
<th>Number of Pixel</th>
<th>Skewness</th>
<th>Kurtosis</th>
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<tr>
<td>1:2</td>
<td>2484</td>
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<table>
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Table 3. Assay of the Complex

<table>
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<tr>
<th>Drug: Amberlite™ IRP64</th>
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<th>1:4</th>
<th>1:6</th>
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<tr>
<td>Sonication Time (min)</td>
<td>RSD (%)</td>
<td>Mean</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Percent drug (w/w) release in methanol</td>
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<td></td>
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<tr>
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<td>0.09</td>
</tr>
<tr>
<td>120</td>
<td>0.120</td>
<td>69.1</td>
<td>0.231</td>
</tr>
<tr>
<td>Percent drug (w/w) release in 0.1 N HCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.239</td>
<td>54.6</td>
<td>0.230</td>
</tr>
<tr>
<td>40</td>
<td>0.04</td>
<td>57.3</td>
<td>0.054</td>
</tr>
<tr>
<td>60</td>
<td>0.05</td>
<td>61.7</td>
<td>0.054</td>
</tr>
<tr>
<td>80</td>
<td>0.145</td>
<td>59.2</td>
<td>0.194</td>
</tr>
<tr>
<td>120</td>
<td>0.02</td>
<td>63.1</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Figure 8. (a) Dissolution of the complexes at pH 6.8 for 20 s (n = 3). (b) Dissolution complexes and their physical mixtures in 0.1 N HCl (n = 3). t-test performed between the pair of complex and corresponding physical mixtures. p < 0.05 was considered significant. (c) E-Tongue: principal component analysis (PCA) of the drug and complexes at drug-to-resin ratios of 1:1, 1:2, 1:4, 1:6.
and the drug content was analyzed by USP validated method. At 2 h, the drug content on mg/mL basis of the complex sonicated in methanol was found to be significantly ($p < 0.05$, t-test) higher than that in 0.1 N HCl. As can be seen from Table 3, the drug content in the complex equivalent to 1 mg of drug for drug-to-resin ratios of 1:1, 1:2, 1:4, and 1:6 after 2 h were found to be 80.2%, 69.1%, 63.6%, 48.2% in methanol and 67.8%, 63.1%, 59.5%, 42.2% in 0.1 N HCl, respectively. The extracted amount of drug in methanol was therefore considered as assay values. This high extraction of the drug in methanol is in agreement with the product literature report\(^\text{12}\) that indicated high drug solubility in methanol and water.

### Drug Release Studies

The drug release study was performed at pH 6.8 to demonstrate percentage release of the drug from the complex in the oral cavity leading to bitterness. Typically, drug dwell time in the mouth cavity is less than 30 s; therefore, the complex containing drug was agitated at different time point to evaluate the percent drug release from the complex. The drug release at different time points is found to be significantly different for various ratios of the complexes (ANOVA, $p < 0.0001$). As can be seen from Figure 8a, drug release from the drug-to-resin complex ratios 1:1, 1:2, 1:4, and 1:6 after 20 s were 42.13%, 23.26%, 4.13%, and 14.94%, respectively. The gradual slow release of the drug from the complex could be either because of the low exchangeable counterions at pH 6.8 or the drug being embedded into the resin matrix causing the delayed accessibility of the exchanging media as the amount of resin is increasing or because of the differences in diffusion path length.\(^6\)

For dissolution studies, all the complexes and physical mixtures were filled in hard gelatin capsules and dissolution was performed in USP apparatus II, as described in Statistical Analysis. Percent cumulative drug release from the complexes and their corresponding physical mixtures were found to be significantly different ($p < 0.05$, t-test, assuming equal variances; Table 4). As can be seen from Figure 8b, the drug release from the complex was found to be faster than their physical mixtures at drug-to-resin ratios of 1:4 and 1:6, whereas drug release from the complexes at ratios of 1:1 and 1:2 was slower than their physical mixtures in 6 min. At 6 min, dissolution studies demonstrated percent cumulative drug release of 61.96%, 70.18%, 85.88%, and 91.42% at 1:1, 1:2, 1:4, and 1:6 drug to resin complexes and 82.80%, 80.80%, 73.43%, 64.80% at their corresponding physical mixtures, respectively. The dissolution of drug from ion-exchange resin depends upon several factors, which include concentration of ions and their affinity to the ionized polymer matrix and swellability of resin.\(^6\) The higher dissolution of the drug from complexes at 1:4 and 1:6 than their corresponding physical mixtures could be described by a combination of factors which include reduction in crystallinity, development of amorphous structure, surface area, improved wetting, diffusion path length, and concentration of ions in the media.\(^6,14,18,31,32\)

### Taste Masking

Taste-masking efficiency was evaluated by using E-Tongue (Alpha MOS). The data from the sensors were collected and analyzed using chemometric approach. PCA was performed on the selected dataset (section 2.2.k) using two PC (Fig. 8c). This allowed clustering of the data in various groups. PC1 explained about 90% of the variance, whereas PC2 explained about 6% of the variance in the data. A replicate of five samples showed intrasample variance, which is a drawback of the instrument and is reported elsewhere.\(^33,34\) However, the data in Figure 8c indicated that the clusters for all the complexes except 1:1 were far from the drug cluster on PC1 axis, which could be attributed to the different degrees of taste masking as the complexing agent was increased with

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**Table 4.** t-Test Comparison of Dissolution at 6 min Between Drug–Resin Complexes and Their Corresponding Physical Mixtures

<table>
<thead>
<tr>
<th>Formulation Comparison (Complex vs. Corresponding Physical Mixtures)</th>
<th>11</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>p value</td>
<td>0.0009</td>
<td>0.0263</td>
<td>0.0016</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

*p < 0.05 was considered significant differences in dissolution.*

**Table 5.** E-Tongue Distance of the Complex Clusters from the Drug

<table>
<thead>
<tr>
<th>Complexes</th>
<th>References</th>
<th>Distances</th>
<th>p Value</th>
<th>Pattern Discrimination Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>Drug</td>
<td>85</td>
<td>0.07</td>
<td>95.23</td>
</tr>
<tr>
<td>Complex 2</td>
<td>Drug</td>
<td>130</td>
<td>0.05</td>
<td>97.63</td>
</tr>
<tr>
<td>Complex 4</td>
<td>Drug</td>
<td>154</td>
<td>0.02</td>
<td>98.3</td>
</tr>
<tr>
<td>Complex 6</td>
<td>Drug</td>
<td>263</td>
<td>0.01</td>
<td>98.95</td>
</tr>
</tbody>
</table>

*p < 0.5 is considered a significant distance between a complex and the drug.*
respect to the drug amount. It should be noted that the drug amount in all the complexes were kept at the same level; and therefore, the distance between the complex and the drug (Table 5) is not merely because of the concentration differences, but could be attributed to differences in the release rates of the drug from the complexes in 20 s. This is supported by the drug release results (Fig. 8a). Thus, the complex 1:2–1:6, which were far from the drug (Table 5), indicated that the complexation was effective in suppressing the bitter taste of the drug.

CONCLUSIONS

Microscopic, thermal, and spectroscopic studies performed in this study strongly suggest the formation of the drug–resin complexes at all the drug-to-resin ratios (1:1, 1:2, 1:4, and 1:6), whereas their physical mixture at the corresponding ratio did not form complexes. Furthermore, microscopic and NIR chemical imaging provided strong evidence that drug in these complexes is evenly distributed and possibly embedded in the resins structures in contrast to the physical mixture wherein the two components were not evenly mixed. The PLS chemometric model for the drug indicated strong correlation between calibration and validation set, suggesting high predictability of the drug content in unknown sample with minimal error. Lower release of the drug at pH 6.8 in 20 s was indicative of less bitter sensation especially with drug-to-resin ratio of 1:4, which released less than 10% drug. E-Tongue (Alpha MOS) result further corroborates the drug release data at pH 6.8 by demonstrating that taste of the complex is different from the drug. The drug from the complexes, however, would immediately release at stomach pH producing desired therapeutic effects.

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