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Multivariate Curve Resolution-Alternating Least Squares for Studying Spectrally Overlapped Valsartan and Amlodipine Release in Drug Delivery

Amin Fozi, Morteza Bahram* and Reza Dadashi

Department of Analytical Chemistry, Faculty of Chemistry, Urmia University, Urmia, Iran

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The combined use of antihypertensive drugs in the treatment of hypertension and prevention of heart attacks has become a very important approach in health care. Valsartan (VAL) and Amlodipine (AML) are two kinds of antihypertensive agents that are used as exforge in the treatment of cardiovascular disease. Clarifying the mechanism and kinetics associated with the release of these drugs is of great importance to establishing an efficient drug delivery system. The aim of this study is to use Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) in the analysis of the *in vitro* kinetic-spectrophotometric data of simultaneous release of AML and VAL from the poly-(acrylic acid-co-2-hydroxyethyl methacrylate) cross-linked by butanediol dimethacrylate P(AA-co-HEMA)-BDMA at pH = 5.5 and 37 °C to obtain the kinetic profiles. Successful loading of drugs on P(AA-co-HEMA)-BDMA was confirmed by investigating the FT-IR spectrums of polymers with and without the loaded drugs. Various mathematical models were exploited to fit the release profile of the drugs. Based on the obtained values for the correlation coefficient (R^2), the release kinetics of both drugs match the Korsmeyer-Peppas model.

Keywords: Simultaneous Drug delivery, Multivariate curve resolution, Valsartan, Amlodipine

INTRODUCTION

Drug delivery systems have been used to treat patients for several years *via* different routes of pharmaceutical agent administrations [1]. In conventional drug delivery systems (CDDSs) which are mostly for oral administration, the release and rapid absorption of the drug is the predominant occurrence. However, CDDSs suffer from short persistence of the drug concentration in blood requiring frequent administration, and the concentration of the drug even can go beyond the therapeutically required range. In order to overcome these issues, controlled drug delivery systems were introduced to maintain the concentration of the medication at the therapeutic level and to release it at a predetermined rate [2]. To this end, liposomes [3], nanoparticles [4], dendrimers [5], polymers [6], and some other drug carriers are often used to release drugs. Polymers are good options among different drug carriers because of the increased drug concentration stability in the blood, decreased immune response, biocompatibility, and other factors [7].

The use of drugs in the combination form that improves therapeutic efficiency has received a lot of attention in recent years [8-11] So far, few studies have been conducted on the simultaneous release of combined drugs in a slow-release form, one of these studies was conducted on the simultaneous release of sumatriptan and naproxen from the polymeric substrate, which has used the chemometrics method to separate the simultaneous release profiles of the drugs from the Kinetic spectrophotometry data [12]. In regard to controlled drug delivery, mathematical models are available to fit the experimental release data and to predict the drug release kinetics. The more accurate the mathematical prediction of the drug release profile, the more efficient development and optimization of new therapeutic products [13,14].

Hypertension is one of the leading causes of heart attack

*Corresponding author. E-mail: m.bahram@urmia.ac.ir

and death worldwide and it is important to keep it under control. The number of people suffering from hypertension rises every day. According to studies, antihypertensive agents used in combination are more effective than drugs used separately in treating hypertension [15,16].

AML (Fig. 1a) is a dihydropyridine calcium antagonist used to treat cardiovascular diseases [17]. Also, VAL (Fig. 1b) is a commonly used nonpeptide receptor that inhibits the formation of angiotensin-II in hypertension treatments [18]. A combination of AML and VAL with complementary mechanisms of action in a single formulation as exforge shows a better effect on reducing blood pressure than each drug alone [16,19]. In previous studies, VAL and AML are loaded onto the polyethylene glycol nanoparticles and mathematical models were used to clarify how the drugs were released *in vitro* [20]. Spectral overlap studies have not been performed on these drugs (AML and VAL) to obtain accurate release profiles of them. For this purpose, the use of powerful chemometrics techniques like MCR-ALS (multivariate curve resolution-alternating least squares) for data analysis can be helpful. MCR-ALS is a variant of multivariate curve resolution (MCR) methods that use a repetitive least squares algorithm to separate the contribution of components in two modes of a multi-component unresolved mixture to real and chemically meaningful bilinear models where each component has a pure contribution along rows and column directions [21-23].

In this work, to load VAL and AML separately, a drug carrier of poly (acrylic acid-co-2-hydroxyethyl methacrylate) cross-linked with Butanediol dimethacrylate P(AA-co-HEMA)-BDMA is used. The FT-IR spectra of intact P(AA-co-HEMA)-BDMA and drugs-loaded ones are examined. UV-Vis spectrophotometry is used to investigate the simultaneous in vitro release of drugs from polymer substrate in 0.01 M phosphate buffer solution (PBS) and plasma at 37 ºC. The Chemometrics method MCR-ALS is applied to the data matrix in order to obtain the drug release profiles. Finally, the mathematical models are used to fit the obtained release profiles to assess the release mechanism.

MATERIALS AND METHODS

Materials

Acrylic acid (>99%), 2-hydroxyethyl methacrylate (>97%), potassium persulphate (>99%), 1,4-butanediol dimethacrylate, hydrochloric acid, phosphoric acid, and ethanol (>99%) purchased from Merck were used in this study. AML and VAL were obtained from Alborzdaroo Company, Tabriz, Iran. Plasma was purchased from Imam Khomeini hospital in Urmia.

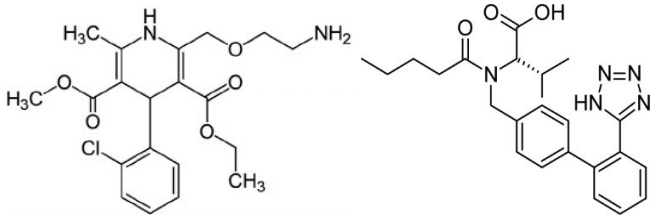

Fig. 1. Chemical structure of AML (a) and VAL(b).

Apparatus and Software

Thermo-Nicolet FT-IR spectroscopy was used to record the spectra of the intact and the drugs-loaded P(AA-co-HEMA)-BDMA polymer. Adjustment of solutions' acidity was performed by Metrohm 750 desktop pH meter. The UV-Vis absorption of samples was obtained by Agilent 8453 spectrophotometer and Chemometrics calculations were carried out in MATLAB (version 8.5).

Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS)

The time-dependent result obtained by the UV-Vis spectrophotometer is second-order data with $m \times n$ dimension. The simultaneous release of drugs in a certain period of time was monitored with a UV-Vis spectrophotometer and resulted in several spectra in the form of a D ($m \times n$) matrix in which m denotes the number of rows in the matrix, or alternatively, the number of recorded spectra, and n denotes the number of columns in the matrix that represents the quantity of wavelengths in each measurement. Decomposition of the matrix D into two matrices of C (with the dimension of ($m \times p$)) and S^T (with the dimension of ($p \times n$) is carried out using MCR-ALS method according to Eq. (1):

$$D = C \times S^T + E \quad (1)$$

where C is the concentration profile and S^T is the pure spectrum of each species and E is the residual matrix that is not explainable by the used model and has the dimension of ($m \times n$) [22,24,25]. The MCR-ALS method in conjunction with mathematical models has been used to resolve kinetic profiles and to predict the release mechanism in melamine-grafted poly-(styrene-alt-maleic anhydride) substrate loaded by Sumatriptan and Naproxen drugs [12].

Mathematical Models

The equation and parameters of a variety of common mathematical models used in the prediction of the drug release kinetics are listed in Table 1. The values of *n* in the Korsmeyer-Peppas model used in the determination of the type of drug release mechanisms are shown in Table 2.

Preparation of P(AA-co-HEMA)-BDMA

The poly (acrylic acid-co-2-hydroxyethyl methacrylate) was synthesized by the method described in the literature [26] and cross-linked with butanediol dimethacrylate. Acrylic acid was mixed with potassium persulphate (initiator) and stirred for 10 min at 300 rpm and 70 °C, and cooled down to room temperature again. Then 2-hydroxyethyl methacrylate and 1,4-butanediol dimethacrylate were added to the solution and stirred for about 2 min at 300 rpm. Finally, the solution was maintained at 80 °C for 3 h and the obtained polymer was cut into discs with 5 mm diameter and washed with distilled water, immersed in a 1:1 mixture of ethanol and water for 24 h, and then dried in the oven at 46 °C.

Loading of Drugs on Polymer

Both drugs are highly soluble in acidic pH of 5.5 so in order to load drugs separately on the polymer, 0.1 M solutions of VAL and AML in PBS (pH = 5.5) were prepared in separate vials. 0.2 g of P(AA-co-HEMA)-BDMA was added to each drug solution and stirred for 24 h at 350 rpm and room temperature. The obtained drug-loaded P(AA-co-HEMA)-BDMA polymers were isolated from the drug solution and dried for 48 h at room temperature.

FT-IR Spectroscopy

The FT-IR spectra of the AML, VAL, P(AA-co-HEMA)-BDMA, VAL- and AML-loaded P(AA-co-HEMA)-BDMA were recorded over the wavelength range of 650-4000 cm^{-1}.

Table 1. Mathematical Models for Estimate Release Kinetics

Mathematical model	Mathematical equation
Zero-order	$Q_t = Q_0 + k_0 t$
First-order	$\ln Q_t = \ln Q_0 - k_1 t$
Higuchi	$Q_t = k_2$
Korsmeyer-Peppas	$\frac{Q_t}{Q_\infty} = k_3 t^n$

Q_0: Initial amount of drug
Q_t: Cumulative amount of drug release at time t
Q_∞: The total amount of drug in its embroidered form
K_0, K_1, K_2, K_3: Constant release rate of the drug
n: Diffusional exponent or release exponent, indicates the echanism of drug release
t: Time

Table 2. Different Values of n Represent the Diffusion Mechanism

The value of n (diffusion exponent or release exponent)	Diffusion mechanism
$n \leq 0.45$	Fickian diffusion
$0.45 \leq n \leq 0.89$	Non-Fickian
$n = 0.89$	Case-II transport
$n > 0.89$	Super case-II transport

Study of *in vitro* Separate Release of Drug

To study the release of each drug from the polymer carrier, 0.01 g of each of the drug-loaded polymers was placed in a dialysis bag immersed in 35 ml of 0.01 M PBS with 3 different pH values of 4.5, 5.5, and 6.8 at 37 °C. At predetermined time intervals, 3 mL of the release medium was withdrawn and simultaneously replaced with PBS to keep the volume of the solution constant, and the absorbance of the sample was recorded with a UV-Vis spectrophotometer at wavelengths ranging from 245 to 450 nm.

Study of *in vitro* Simultaneous Release of Drugs in PBS and Plasma

To study the simultaneous release of the drugs, 0.01 g of

the VAL-loaded polymer was mixed with 0.01 g of the AML-loaded one and placed in a dialysis bag immersed in PBS (pH = 5.5) at 37 °C. At predetermined intervals, 3 ml of the release medium was withdrawn and the absorbance of the sample was recorded with UV-Vis spectrophotometer over the range of 245 to 450 nm, then The withdrawn solution was then returned to the release medium to keep the volume of the solution constant. In order to release the drugs into the plasma, PBS (pH = 5.5) was added to 2.5 ml of plasma to obtain a volume of 35 ml. The simultaneous release of drugs was carried out in a way similar to the method used in their release into PBS. The result of the release process monitoring at 11 specified time intervals in PBS and plasma with UV-Vis spectroscopy leads to a matrix with dimensions of 11 × 156.

Calibration Curve of Valsartan and Amlodipine in PBS

The calibration curves method was used to calculate the concentration of the released drugs in the mediums. Since the release of drugs was carried out in PBS, the calibration curves of the drugs were plotted in PBS. AML shows maximum absorption at 237 nm wavelength, while 365 nm exhibits better linearity in the calibration curve and has less spectral overlap with VAL which appears at 250 nm. So, wavelengths of 365 nm for AML and 250 nm for VAL were chosen to construct their calibration curves. The linearity range of concentration for VAL was seen between 10 and 60 µg ml^{-1} at the wavelength of 250 nm and 10 to 50 µg ml^{-1} at the wavelength of 365 nm for AML.

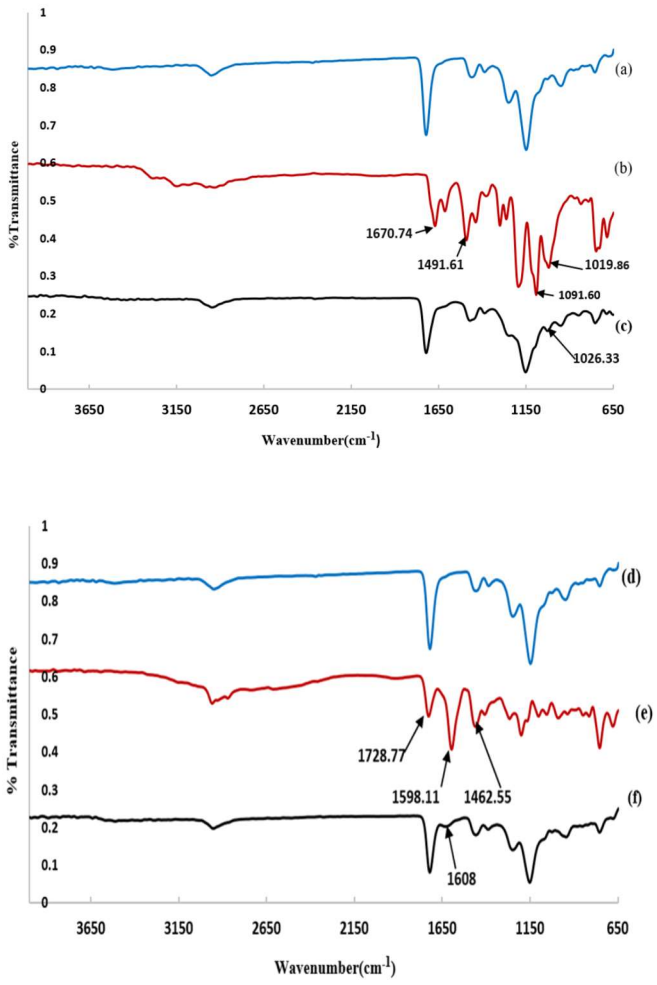

Fig. 2. FTIR spectra of a) P(AA-co-HEMA)-BDMA, b) AML, c) AML-loaded P(AA-co-HEMA)-BDMA, d) P(AA-co-HEMA)-BDMA, e) VAL, f) VAL-loaded P(AA-co-HEMA)-BDMA.

RESULT AND DISCUSSION

FT-IR Spectra Analysis

The loading of each drug on the polymer can be determined by comparing the FT-IR spectra of pure polymer with the spectra of VAL- and AML-loaded one. The absorption peaks of AML (Fig. 2b) appeared at 1670.74, 1491, 1091, and 1019 cm^{-1} are related to C=O stretching, C=C aromatic stretching, C–Cl aromatic stretching, and C–O–C stretching, respectively [27,28]. The absorption peaks of VAL in Fig. 2 appeared in 1728, 1598, and 1462 cm^{-1} can be assigned to the carboxylate stretching, amide carbonyl stretching and stretching of C=C aromatic, respectively [29]. The absorption peak located at 1019 cm^{-1} (assigned to the C–O–C stretching) for AML, shifts to 1026 cm^{-1} and appeasers with less intensity in the spectra of AML-loaded P(AA-co-HEMA)-BDMA (Fig. 2c). The intensive vibration stretching peak of amide carbonyl at 1598 cm^{-1} in VAL also shifts to 1608 cm^{-1} and becomes less intensive in VAL-loaded P(AA-co-HEMA)-BDMA (Fig. 2f). The changes of the spectrum in the drug-loaded polymers with respect to the pure polymer is a good indication that loading on the polymer in both cases is progressed well.

Effect of pH on the Drug Release

In order to reach the efficient absorption of the drug in the gastrointestinal tract, the drug should have good solubility in the desired pH. The observed release, absorption, and efficacy of the drug in the gastrointestinal pH are in the acceptable range [30]. The solubility of VAL is a function of pH where the pH values below 3 are negligible and increase meaningfully in the pH range of 4 to 8 [31]. Regarding this fact, studies were performed at pH values above 3. Contrarily, AML has a good solubility at acidic pH values *i.e.*, 1.5-4.5 [32].

Based on work done by Inglot and co-workers [33], in order to select a proper pH for the solubility of both drugs, the optimal pH of 5.5 is selected based on the evaluation of dissolution at three pH values of 4.5, 5.5, and 6.8 in which the release of drugs was investigated separately at each pH value.

The separate release profiles of the drugs obtained at the different pH values are shown in Fig. 3a for AML and in Fig. 3b for VAL. The best performance is observed at pH 4.5 for AML, however, VAL's release efficiency is poor at this pH and becomes superior at pH = 6.8 while AML release decreases dramatically at this pH. Our observation indicates that pH = 5.5 provides better release efficiency for both drugs compared to 4.5 and 6.8. Therefore, 5.5 was chosen as the optimal pH for the simultaneous release of the drugs.

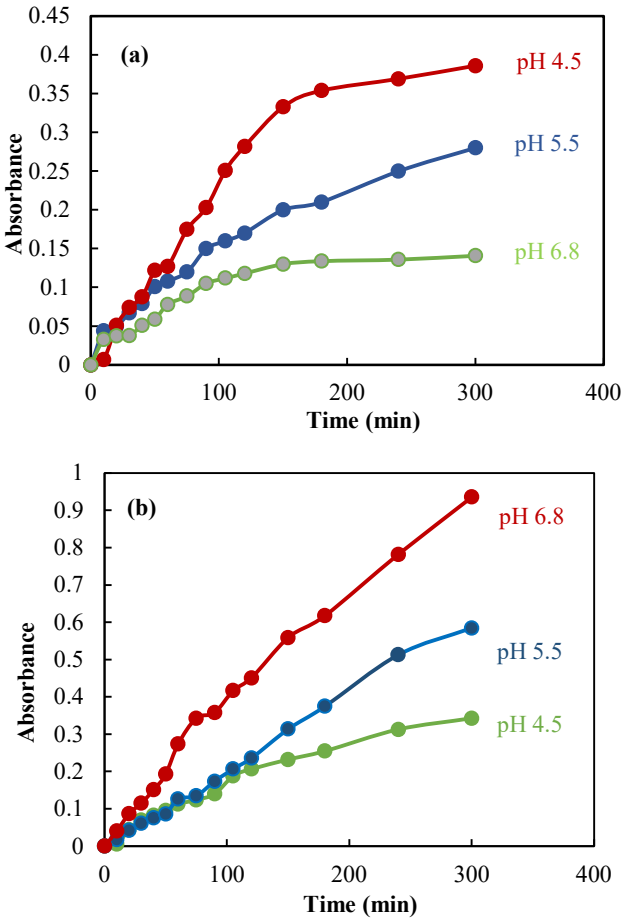

Fig. 3. Separate release profile of AML (Fig. 3a) and Val (Fig. 3b) at pH = 4.5, 5.5, and 6.8.

Applying MCR-ALS to Release Data

Figure 4 shows the broad-range UV spectra collected through the simultaneous release of drugs into the plasma. The MCR-AlS method is applied to the correlated data of the concentration profiles and pure spectra of drugs in order to analyze the release mechanisms. In the performed MCR process, the exact number of species is estimated by the SVD approach [23,34] where the obtained singular values in the PBS and plasma medium are given in Table 3. Based on the analysis, two eigenvalues related to VAL and AML are identified in both release mediums. Non-negativity constraint is applied to the concentration and spectral profiles in each ALS iteration, while unimodality is applied as well to the concentration profile. Kinetic profiles of the drugs were normalized and the pure spectra of drugs were used as initial estimations in the analysis. Figure 5 shows the resolved pure spectra (a) and normalized kinetic profiles of the

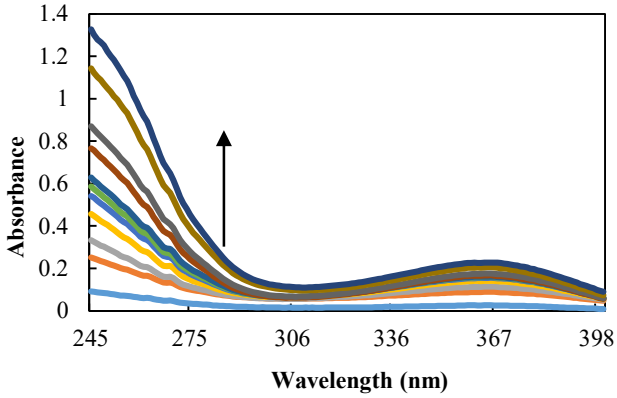

Fig. 4. The total UV spectra recorded during the simultaneous release of drugs in plasma.

Table 3. 10 Singular Values in PBS and Plasma

Singular values	PBS	Plasma
1	8.9	10.5
2	0.50	0.89
3	0.065	0.19
4	0.02	0.02
5	0.01	0.016
6	0.008	0.009
7	0.007	0.006
8	0.005	0.005
9	0.004	0.005
10	0.003	0.004

simultaneous release in PBS (pH = 5.5) (b) and in plasma (c).

Fitting the Release Kinetic Profile to Mathematical Models

The release mechanism can be predicted by fitting the *in vitro* release kinetic profile to mathematical models in which the best fit is taken as the best release model. To this end, the kinetic model with a correlation coefficient closer to unity will be a good representative of the drug release. Korsmeyer-Peppas, zero-order, first-order, and Higuchi models were used in this study to fit the obtained resolved kinetic profiles in PBS and plasma. Figure 6 shows the results of the fitting for the VAL release profile in plasma. The fitting parameters in different kinetic models for the experimental release data are shown in Tables 4 and 5 for VAL and AML, respectively.

Regarding the highest value of the correlation coefficient (R^2) obtained in the Korsmeyer-Peppas model among others, we demonstrate that this model explains best the release mechanism of both drugs in the PBS and plasma. For the release of AML in the two mediums, the values of *n* are obtained at 0.45 and 0.85, therefore, the mechanism of Non-Fickian transport or Anomalous transport can be concluded for the drug that is released by swelling the polymer. The obtained value for *n* in the release of VAL is larger than 0.89, and as a result, its release mechanism is predicted to be Super Case II transport [35].

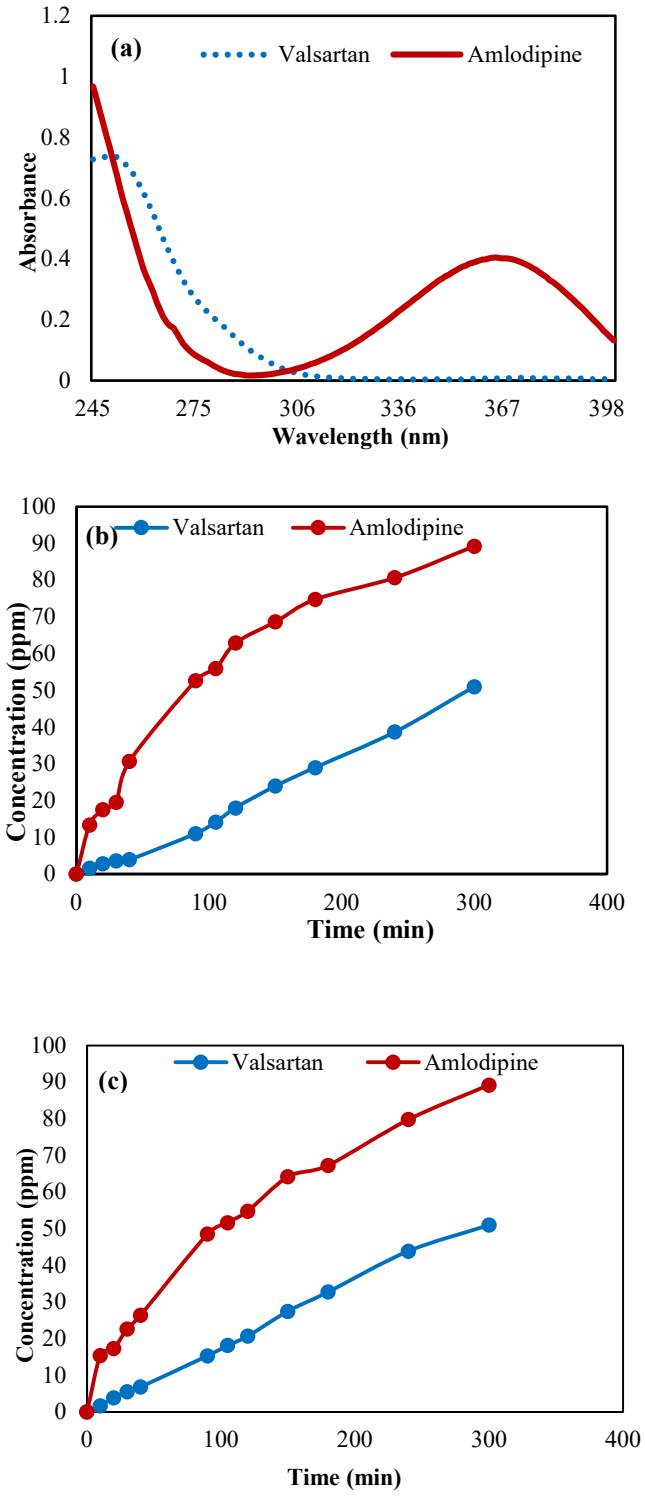

Fig. 5. Resolved pure spectra (a) and release kinetic profile of AM and VAL in PBS (b) and Plasma (c).

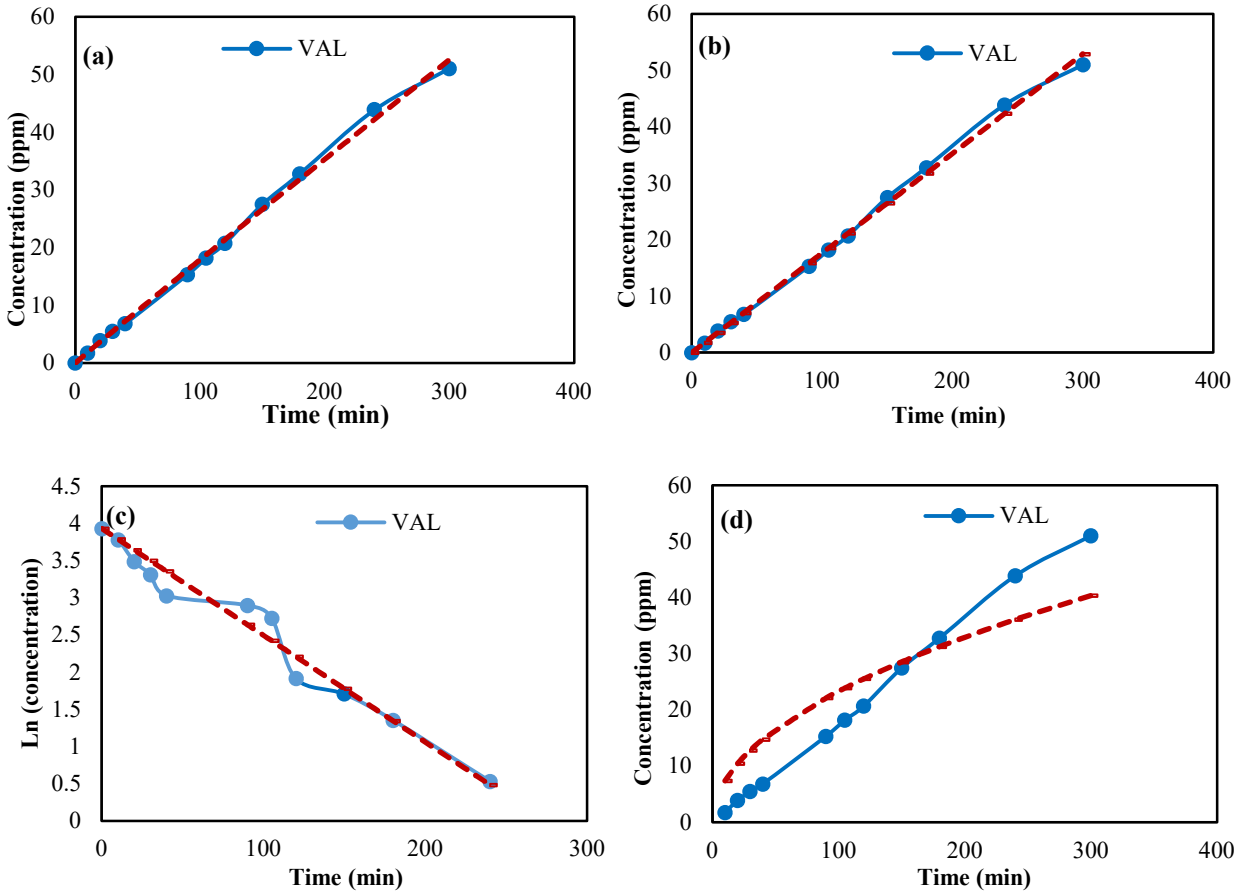

Fig. 6. Fitting of mathematical models with the release profile of VAL in plasma. a) Korsmeyer-Peppas b) Zero-order c) First-order d) Higuchi.

Table 4. Statistical Parameters of VAL Obtained from Fitting

PBS				
Mathematical model	Korsmeyer-Peppas	Zero-order	First-order	Higuchi
k	4.3	0.38	0.01	5.3
n	0.54	-	-	-
R^2	0.98	0.89	0.95	0.97
Plasma				
Mathematical model	Korsmeyer-Peppas	Zero-order	First-order	Higuchi
k	3.6	0.37	0.01	5
n	0.57	-	-	-
R^2	0.99	0.93	0.96	0.96

Table 5. Statistical Parameters of AML Obtained from Fitting

PBS				
Mathematical model	Korsmeyer-Peppas	Zero-order	First-order	Higuchi
k	4.3	0.38	0.008	5.3
n	0.54	-	-	-
R^2	0.98	0.89	0.93	0.97
Plasma				
Mathematical model	Korsmeyer-Peppas	Zero-order	First-order	Higuchi
k	3.6	0.36	0.008	5
n	0.57	-	-	-
R^2	0.99	0.93	0.93	0.96

CONCLUSION

In this study, MCR-AlS as a second-order data analysis method was able to decompose the kinetic-spectrophotometric data matrix, which is related to the in vitro simultaneous release of AML and VAL, into the concentration profiles and pure spectra. The results imply that pH = 5.5 works better for both drugs compared to 4.5 and 6.8 in terms of being released to the target mediums. We concluded that the Korsmeyer-Peppas model predicts best the release of loaded AML and VAL on P(AA-co-HEMA)-BDMA and this polymer shows the amenability of sustained release. The release mechanism of AML and VAL were obtained to be Non-Fickian transport (when $0.43 \leq n \leq 0.89$) and Case II transport (when $n > 0.89$), respectively. Considering the success of the MCR-ALS method coupled with UV-Vis spectroscopy in extracting the concentration profiles of compounds having spectral overlap, it can be demonstrated that this chemometrics technique is well applicable to various combinations of drugs encountering spectral overlap.

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