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ORIGINAL ARTICLE

Role of humic acid on oral drug delivery of an antiepileptic drug

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Abstract

Context: Humic acid (HA) is omnipresent in natural organic matter that is a macromolecular, negatively charged polyelectrolyte that contains a hydrophobic core. It is also present in a significant amount in Shilajit (used frequently in traditional medicines), which is used in this study as a source of extraction. HA is evaluated for the oral drug delivery of carbamazepine (CBZ). *Objective*: HA is used in this study to increase the dissolution, intestinal permeation, and pharmacodynamic response of CBZ (bio pharmaceutics classification system (BCS) II) by the technique of complexation and other related mechanism reported with humic substances. *Methods*: Different complexation techniques were explored in this study for the entrapment of CBZ, which was authenticated by molecular modeling and conformational analysis. These were further characterized using differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), and X-ray diffraction (XRD). Solubility analysis and dissolution release profile were carried out to access the in vitro parameters. For ex vivo studies, rat gut intestinal permeability was done. And finally pharmacodynamic evaluation (maximal electroshock method) was carried out for optimized complexes. *Results*: Molecular modeling approach and instrumental analysis (DSC, XRD, and FT-IR) confirmed the entrapment of CBZ inside the complexing agent. Increased solubility (~1742%), sustained release (~78%), better permeability (~3.5 times), and enhanced pharmacodynamic responses conferred the best to 1:2 freeze dried (FD) and then 1:2 kneading (KD) complexes compared with pure CBZ. *Conclusion*: Now it could be concluded that HA may be tried as a complexing agent for antiepileptic drug and other classes of low water-soluble drug.

Key words: Carbamazepine, complexation, intestinal permeation, Shilajit, solubility

Introduction

Humic acid (HA) lies in the category of humic substances, with humin and fulvic acid. The source used in this study for humic substances was Shilajit, which is a brown color exudate from steep mountain rocks of the Himalayan and Hindu Kush ranges of the Indian Subcontinent[1,2]. Presence of Shilajit is also reported in Australia, Norway, China, Russia, and so on, and the favorable altitude for its occurrence is 1000–5000 m. It has been used as a rejuvenator and an adaptogen for thousands of years, in one form or the other, as part of the traditional systems of medicine in a number of countries. Humic substances are omnipresent in nature such as soil, river, and sewage water in the form of natural organic matter and are macromolecular, negatively charged polyelectrolytes that contain mainly carboxylic and phenolic functional groups; structural, conformational, and physicochemical differences between them are well reported. Average molecular weight of HA (Figure 1b) is reported to be 6500 Da[3]. It is not soluble in water under acidic conditions (pH < 2) but is freely soluble at higher pH values. Thus, low water-soluble drug molecules can form complexes with it, resulting in increased solubility and bioavailability[4].

Carbamazepine (CBZ) is taken as a model anticonvulsant drug (molecular weight 236) and is widely used in the treatment of simple and complex seizures, trigeminal neuralgia, and bipolar affective disorder[5]. The parameters for its selection as a model candidate were

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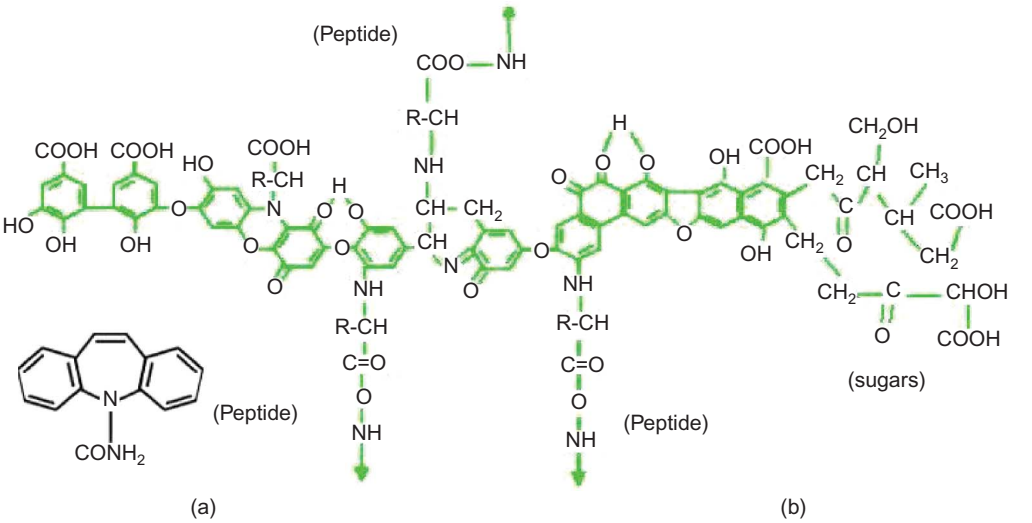

Figure 1. Molecular structure of (a) carbamazepine (molecular weight 236) and (b) humic acid (molecular weight 6500).

its insolubility in water[6] and its dissolution rate-limited absorption[7]. Low aqueous solubility and poor hydrophilicity of the drug contribute to the variability in absorption and hence bioavailability[8,9]. It is generally marketed in a conventional tablet dosage form that yields variable pharmacokinetic profiles. Thus by increasing the dissolution characteristics of the drug we could enhance the rate or extent of its absorption after oral administration. As it is the drug of choice for pediatric seizures[10], a reconstituable suspension of CBZ (Figure 1a) is highly recommended. So, an attempt has been made to complex the CBZ with HA and to increase its different in vitro and ex vivo profiles and to decrease its oral dose with minimizing its side effect.

Materials and methods

Materials

An authentic sample of the rock Shilajit was obtained from Dabur Research Foundation, Ghaziabad, India. CBZ was kindly provided as a gift sample by Novartis Pharmaceuticals Ltd. (Mumbai, Maharashtra, India). All other chemicals and reagents used in the study were AR grade.

Method for obtaining HA from the rock Shilajit

A slightly modified method[11] was used to extract HA. The method consisted of successive extraction of raw Shilajit with hot organic solvents of increasing polarity to remove the bioactive components. The residue (marc) was dissolved in 0.1 N NaOH[12] with intermittent shaking in the presence of nitrogen. The suspension was filtered and the filtrate was acidified to a pH of less than 3 to precipitate out the HA. The filtrate may be further shaken with macroporous ion-exchange resin in order to adsorb the fulvic acid, which was then eluted using 0.1 N aqueous NaOH solutions.

Phase solubility behavior

Phase solubility studies were carried out at room temperature (25°C) in triplicate mode according to the method reported[13]. Excess amount of CBZ was added to distilled water containing various concentrations (0.2–2%, w/v) of HA in a series of stopper conical flasks and shaken for 48 hours on a rotary flask shaker. The suspensions were passed through a membrane filter (0.45 μm) and assayed for CBZ using UV spectroscopy (Shimadzu, UV 1601, Kyoto, Japan) at 285 nm against blanks prepared using the same concentration of HA in distilled water.

Preparation of the inclusion complexes

Complexes of CBZ were prepared with HA extracted from Shilajit by using two different molar ratios 1:1 and 1:2 (drug : HA). The resulting mass was powdered in a glass mortar and pestle and passed through a 100-mesh sieve to obtain a uniformly sized fine powder[1]. Equal amount of the drug was also processed to check the process effect.

Physical mixture

Complexes of CBZ and HA were prepared by grinding the mixture for 60 minutes in a clean, dry glass pestle and mortar and the resulting mass was passed through a 100-mesh sieve to obtain a uniformly sized fine powder[1].

Freeze drying

Weighed amount of CBZ was dissolved in water by using co-solvency (ethanol) and aqueous HA solution was also prepared. Both the solutions were mixed and stirred (200 rpm, 60 minutes) and then sonicated for 1 hour. The solution was frozen for 24 hours in a Lyph-lock apparatus and then freeze dried (FD) (Dry Winner, DW-8-85 Heto Holten, Denmark) for 12 hours. Sucrose solution was (2%, w/v) added as a cryoprotectant. The

resulting mass was then powdered in a glass mortar and pestle and passed through a 100-mesh sieve to obtain a uniform-size fine powder[1].

Solvent evaporation

Calculated amount of the drug was dissolved in water with the help of few drops of ethanol and poured into an aqueous solution of HA. The solution was then sonicated for 1 hour. The solution thus obtained was dried in a rotary evaporator under vacuum (Hs-2001N, Hahn Shin Science Co., Bucheon, Kyungki, South Korea) and passed through a 100-mesh sieve to obtain a uniform-size fine powder[1].

Kneading

Solid complexes of CBZ–HA were prepared in 1:1 and 1:2 molar ratios by following kneading (KD) method[1]. HA was mixed in a glass mortar along with water to obtain a homogeneous paste. Weighed amount of CBZ and HA were triturated for 15 minutes in a clean, dry glass pestle and mortar. During the process, the water content of the paste was empirically adjusted by ethanol and triturated to maintain the consistency of the paste. Trituration was continued until the product started drying on the walls of the mortar. The products were further dried in the hot air oven at 60°C for 30 minutes, powdered, passed through a 100-mesh sieve, and stored in desiccators[4].

Characterization of the solid complexes

Differential scanning calorimetry

For differential scanning calorimetry (DSC) study, samples of the solid complexes, pure drug (CBZ), and HA (10 mg each) were taken in flat-bottomed aluminum pans and heated over a temperature range of 50–400°C at a constant rate of 10°C/min with purging of nitrogen (50 mL/min) using alumina as a reference standard in a differential scanning calorimeter (DSC-7, Perkin Elmer Pyris 6 instrument, USA).

Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) spectra of CBZ, humic, and inclusion complexes were recorded on the Perkin Elmer calorimeter using the potassium bromide (KBr) disc technique. Five mg of previously dried sample was mixed with 100 mg KBr and compressed into a pellet on an IR hydraulic press. Base line was corrected and scanning was done from 4500 to 400 cm^{-1}.

Power X-ray diffraction

XRD of CBZ, humic, and their inclusion complexes were studied using X-ray diffractometer (PW 1830, Phillips, Banglore, Karnataka, India). The samples (1000 mg) on XRD plates were rotated during data collection to reduce orientation effects of particles. XRD patterns of solid complex, pure drug, and HA were recorded between $2\theta = 5°$ and 70° at 35 kV and 30 mA, respectively.

Conformational analysis by computational method

The 3D molecular structures were generated and optimized with Chem 3D-Ultra 8.0 software. All calculations used are for geometric optimization. All the energy minimizations were carried out till the RMS gradient was less than 0.08. Optimized molecular structures and partial atomic charges were used for the molecular modeling of HA and its complex. H-bonding analysis was based on ORTEP III [v1.0.3].

HPLC analysis of CBZ

The concentration of CBZ in in vitro samples was determined using reproduced HPLC method[14] using a Shimadzu LC2010 system consisting of quaternary LC-10A VP pump, SPD-10AVP column oven, variable wavelength programmable UV/VIS detector (285 nm), SCL 10AVP system controller, Rheodyne injector fitted with a 20 µL loop, degasser, and a data processor. Chromatographic separation was achieved using a LiChrospher®100 reversed-phase C-18 column (250 × 4.6 mm) that was packed with 5-µm particles with a mobile phase consisting of water and acetonitrile (60:40 water : acetonitrile). The mobile phase was pumped at a flow rate of 1.0 mL/min at an ambient temperature (25 ± 2°C). The eluent was monitored by ultraviolet absorbance at a wavelength of 285 nm with retention times for CBZ at 4.8 ± 0.35 minutes.

Aqueous solubility determination of solid complexes

Excess amount of complexes was kept in amber-colored bottles containing 10 mL of distilled water and stirred on a thermostated mechanical shaker (Grower enterprises, New Delhi, India) at (25°C) for 5 days. Suspensions were filtered through a 0.22-µm 'Millipore' filter, adequately diluted with distilled water, and analyzed using reported HPLC at $\lambda = 285$ nm[14].

Release of CBZ from complex

Drug release study of active pharmaceutical ingredient (50 mg CBZ solution) and inclusion complexes (equivalent to 50 mg CBZ) was performed using USP II dissolution apparatus (Hanson Research SRS, Chatsworth, CA, USA) in 900 mL of distilled water at 37.5 ± 0.5°C (75 rpm, 3 hours). The study was carried out by transferring the constituted suspension (5 mL) in dialysis bag (Spectra-Por dialysis bag (Sigma Aldrich, St. Louis, MO, USA) with cutoff 12,000–14,000 Da). The concentration of the drug in solution at various time intervals was analyzed using HPLC at 285 nm. All dissolution studies were carried out in triplicate.

In vitro everted intestinal sac permeation study

Rats were anesthetized by ether sprinkled to a piece of cotton wool in a glass container equipped with a lid. After making a midline incision in the abdomen, the small intestine was cut at two positions, at about 18 cm distal to the stomach[15] and at about 30 cm (being the

medial jejunum). This segment was then removed and ligated with silk thread to one end of a glass rod and carefully everted on the rod, rinsed with saline solution, and then cut and secured to the tip of a 1-mL disposable syringe barrel. The gut sac was filled with physiological Krebs–Ringer phosphate buffer (KRPB) solution (pH 7.4) and was then placed inside the bath containing 100 mL of test solution continuously bubbled (95% O_2 and 5% CO_2)[16]. After stabilization, 3 mL (equivalent to about 10 drugs) CBZ (API), 1:2 FD, and 1:2 solvent-evaporated complex solution were added into the sac. The tubes were maintained at 37°C and shaken continuously at 60 rpm with bubbling oxygen supply. Samples of 100 µL were withdrawn at an interval of 0, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours from the dissolution medium and centrifuged at 4000 rpm for 5 minutes. After filtering through Millipore filter (0.45 µm) the samples were analyzed using HPLC[14]. All animal experiments were carried out in accordance with Jamia Hamdard Animal Ethical Committee.

Pharmacodynamic study—anticonvulsant activity

Swiss albino mice with average body weight (20–30 g) of either sex were used for the experiment. The animals were reared in the Central Animal House for 2 weeks in polypropylene cages and fed on standard animal feed and water. The animals were divided into four groups: HA (control), pure CBZ, 1:2 FD complex, and 1:2 KD complex with 6 animals each with an average group weight of 25 g in the maximum electroshock seizure (MES) experiment. Dose of pure CBZ (30 mg/kg body weight of mice) was chosen as per the reports of previous MES study with the same drug[17] and amount of HA–CBZ complexes were taken as per the calculation that contains the said (Table 2) amount of CBZ. The control and different dosages of complexes were given to separate groups of mice 30 minutes before the induction of MES. Then, the stimulus train was applied through an ear-clip electrode (50 mA, 0.2 sec, average voltage 200–250 V) through Electroconvulsiometer (Techno India, Kolkatta, West Bengal, India). The incidence and duration of extensor tonus were noted. The duration of seizures (tonic–clonic convulsions) was recorded[18]. Solutions (qs to 5 mL) of drug and complexes were prepared in glycerin. About 0.2 mL of these solutions were given per mice orally.

Results

Characterization of solid complexes

The phase solubility studies revealed a nonlinear relationship between the aqueous drug solubility with increase in HA concentration (R^2 = 0.9391). The phase solubility diagram showed its characteristics A_L type (Figure 2), according to Higuchi and Connors. Up to a concentration of 1% (w/v) of HA, the relationship was linear but was nonlinear afterward. Thus, the molar

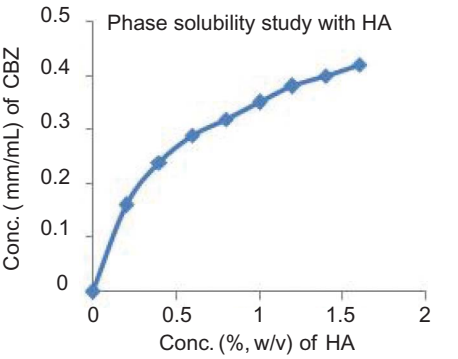

Figure 2. Phase solubility study.

ratios we opted for complexation were 1:1 (steep rising portion) and 1:2 (because it was more inclined toward *x*-axis).

Differential scanning calorimetry

DSC of pure CBZ shows a sharp exothermic peak at 189°C, which is in accordance with the melting point reported in the literature[19]. HA shows blunt endotherm and exotherm in the region of 100–340°C. Complete absence of peaks was observed in nearly all the complexes except 1:1 PM (physical mixture) and 1:2 FD, which is a strong indication of the formation of complex with HA (Figure 3).

Fourier transform infrared spectroscopy

The FT-IR spectrum (Figure 4a) shows characteristic peaks of CBZ at 1752 cm^{-1} (C=O stretching), 3460 cm^{-1}

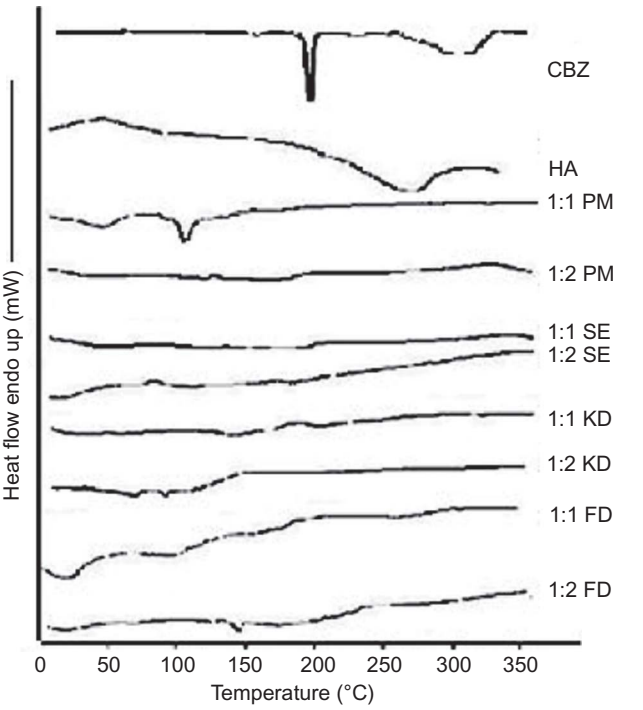

Figure 3. DSC study of CBZ, HA, and different complexes.

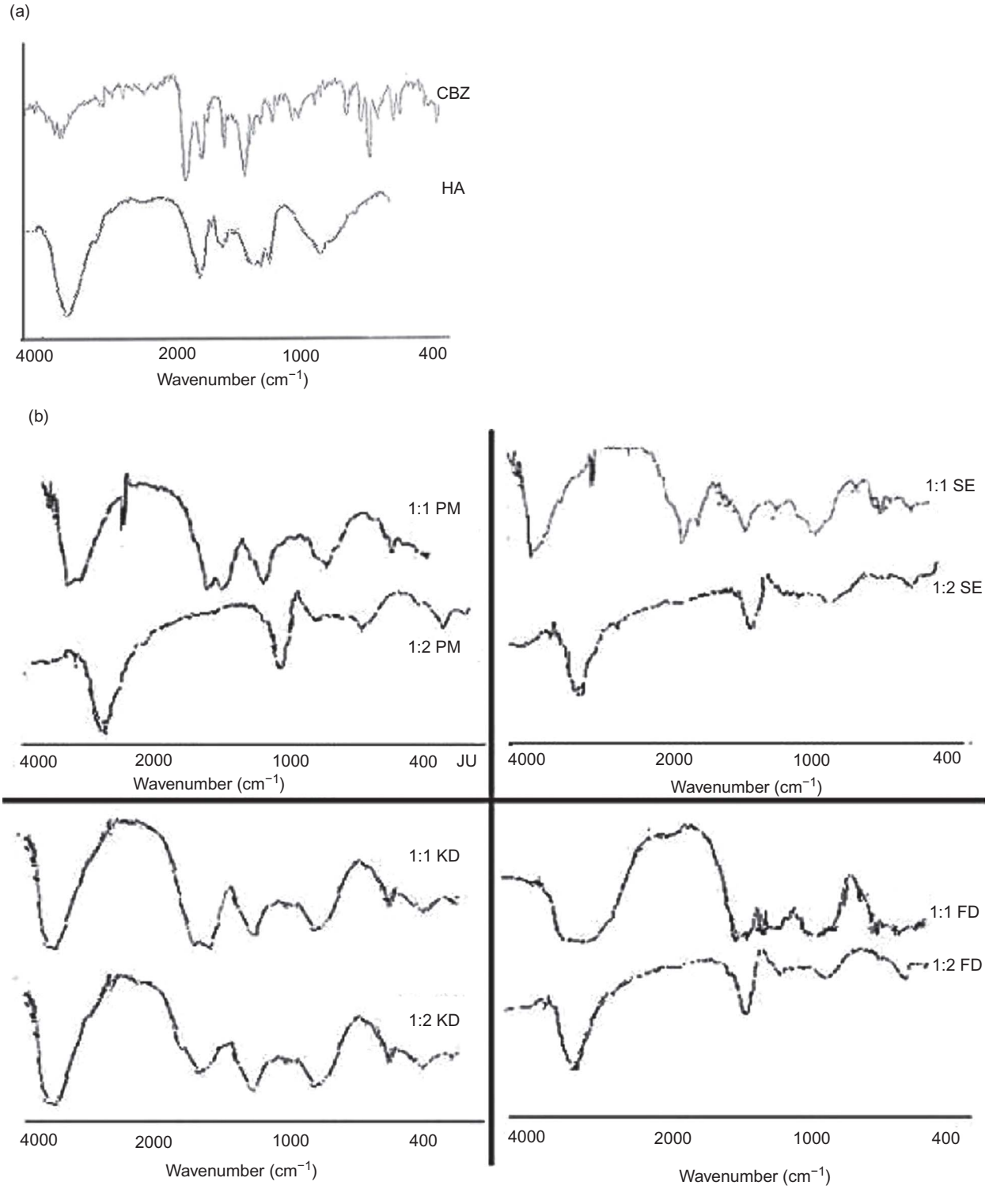

Figure 4. (a) FT-IR of carbamazepine and humic acid. (b) FT-IR study of different complexes.

(N=H vibration), and 1550 cm^{-1} (C=C stretching of phenyl)[19]. FT-IR absorption bands (Figure 4a) of HA extracted from Shilajit were found to be in accordance with those reported in the literature[20]. All the FT-IR spectra of complexes are exhibited in Figure 4a and b. The result obtained further corroborates the inferences of DSC.

X-ray power diffractograms

XRD of CBZ shows various peaks at different angles with most intense peaks at 15.41°(100%) followed by 13.18° (83%), 27.84° (66%), 27.32° (60%) and, 27.66° (56%) (Figure 5), revealing the crystalline nature of CBZ. The XRD patterns of HA show almost amorphous nature.

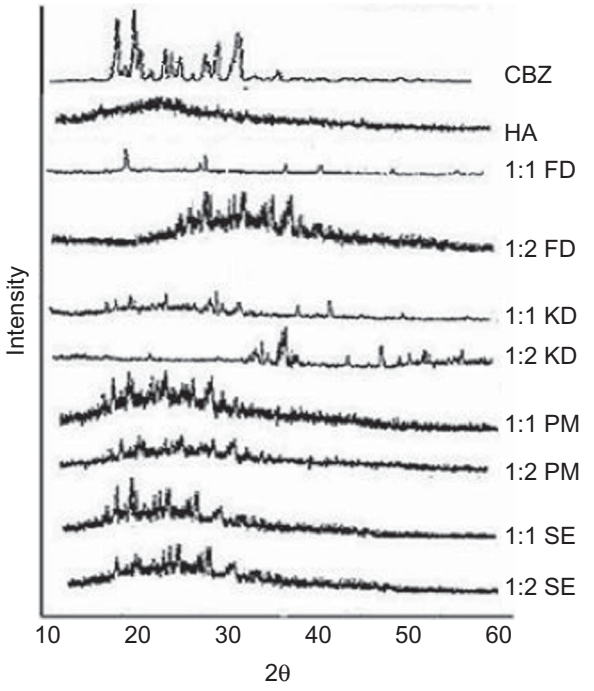

Figure 5. XRD study of carbamazepine, humic acid, and different complexes.

Conformational analysis by computational method

Molecular modeling has shown that complexes of CBZ–HA are stable. It revealed that humic/fulvic acids have ability for inclusion complexation with CBZ. Intermolecular hydrogen bonds observed contribute to the stability of the molecule. In the case of CBZ as shown in Figure 6, amide hydrogen is oriented away from the carbonyl group but is approaching toward one of the aromatic moiety.

Although the exact structure of HA is not yet characterized, a probable structure is modeled in this study. Total potential energy of the HA and the drug molecule are compared. Total potential energy of the HA using Chem 3D-Ultra 8.0 software comes to around −45.896 Kcal/mol (Figure 7) whereas a complex of CBZ and the HA is stabilized at −22.584 (Figure 8), which is more stable as CBZ alone. Energy optimization of CBZ resulted in −6.84 Kcal/mol. In this complex H of amide of CBZ has H-bond with OH of HA.

Aqueous solubility of complexes

CBZ is practically insoluble in water; aqueous saturation solubility of CBZ was found to be 12.65 µg/mL. Complexation of CBZ with HA greatly increased the solubility (Table 1). Maximum percentage increase in solubility was 1742.846 ± 29.73 in FD (1:2) complex whereas the minimum was 504.348 ± 15.38 in 1:1 PM.

Release of CBZ from complex

The release profile of pure CBZ and complexes prepared by different methods are shown in Figure 9. The best release profile was exhibited by 1:2 complexes developed by freeze drying (94.6 ± 0.74% in 1 hour) whereas minimum release was exhibited in case of 1:1 PM complex.

Comparison of anticonvulsant activity

From all the previous mentioned studies it was very much obvious that the complexes developed by KD and freeze-drying methods were showing promising results. So, these were chosen for further pharmacodyanamic study. Study of MES activity showed that because of complexation there was a threefold increase in the potency of CBZ in FD and KD complexes compared with the pure drug (Table 2). The amount of CBZ was chosen as per the recommended dose of the drug[21]. The amount of humic substances present in a dose of suspension was also within the permissible limit, that is, 512 mg/kg body weight[22].

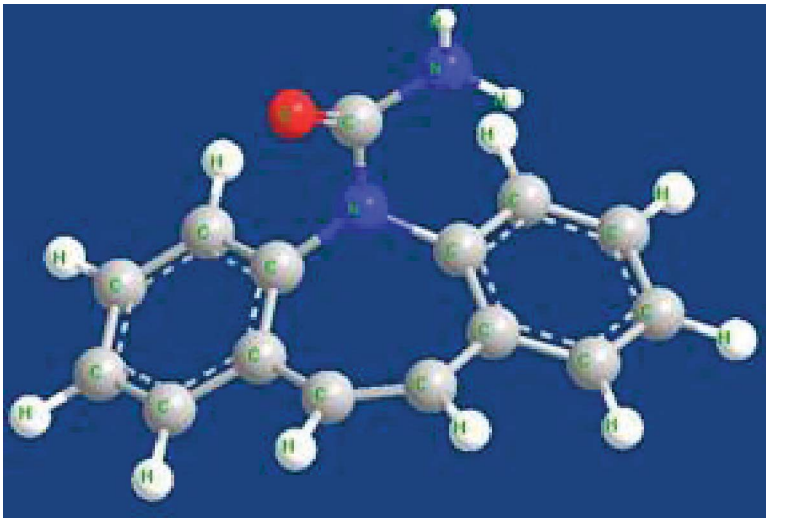

Figure 6. Energy minimized structure of carbamazepine.

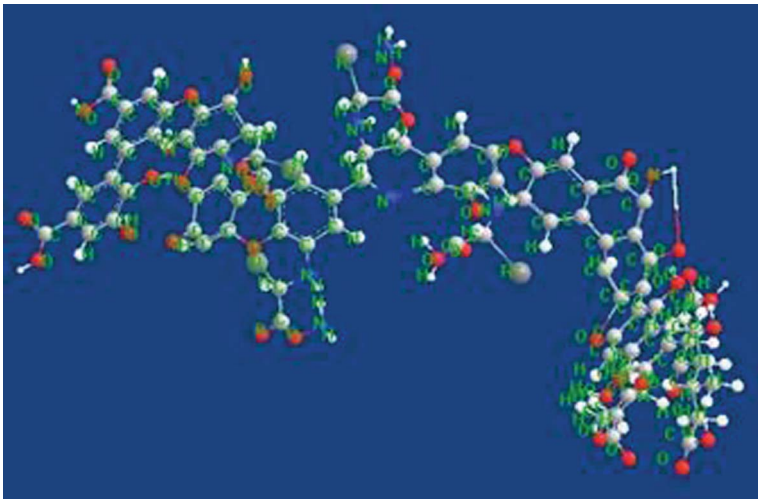

Figure 7. Energy minimized structure of humic acid.

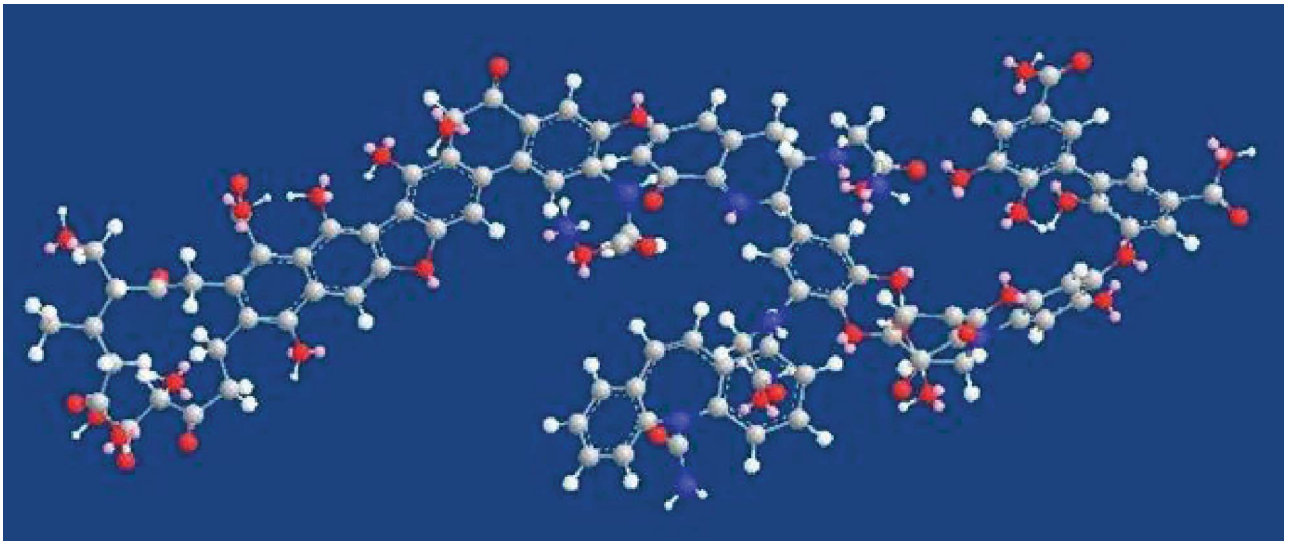

Figure 8. Energy minimized interaction of humic acid and carbamazepine.

Table 1. Solubility of CBZ and CBZ–HA complexes in water at room temperature.

Complexes	Solubility of carbamazepine in water (μg/mL)	Percent increase in solubility of complex (%S_{comp})
1:1 PM	76.45 ± 1.21	504.348 ± 15.38
1:2 PM	84.4 ± 2.17	567.1937 ± 16.19
1:1 KD	123.05 ± 9.63	872.7273 ± 12.59
1:2 KD	135.6 ± 4.19	971.9368 ± 18.93
1:1 SE	141.36 ± 5.21	1017.47 ± 21.13
1:2 SE	157.89 ± 13.13	1148.142 ± 21.19
1:1 FD	176.72 ± 2.02	1296.996 ± 14.2
1:2 FD	233.12 ± 1.35	1742.846 ± 29.73

Note: $\%S_{comp}$ = solubility of CBZ $^{complex} \times 100$/Solubility of CBZ^{API}. PM, physical mixture; KD, kneading; SE, solvent evaporated; FD, freeze-dried.

Permeation study across rat gut sac

The permeability of optimized complexes (1:2 FD and KD complexes) across the gut sac was significantly increased (~2.63 to 3.5 times) compared with CBZ suspension in water in 24 hours (Figure 10). The permeation profile of the complex shows two patterns, that is, in the initial 5 hours there was a sharp increase in permeation but after that a plateau was observed.

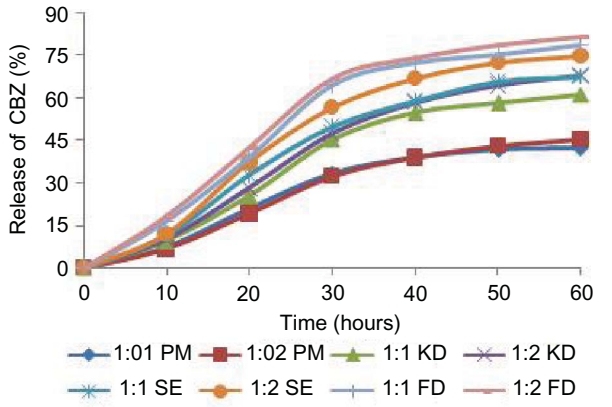

Figure 9. Release profile of carbamazepine and different complexes.

Table 2. Comparative anticonvulsant activity of CBZ and CBZ–HA complexes in swiss albino mice.

Substance	Dose	Percent inhibition
CBZ	30 mg/kg body weight	100
HA	94 mg/kg body weight	0
KD (1:2)	Equivalent to 10 mg/kg body weight of CBZ	75
FD (1:2)	Equivalent to 10 mg/kg body weight of CBZ	75

Note: % inhibition = animals showing positive response × 100/total animals.

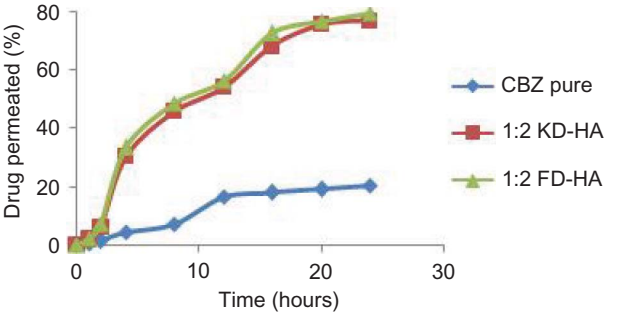

Figure 10. Permeation study of carbamazepine and optimized complexes.

Discussion

Characterization of complexes

Molar ratios opted from the phase solubility study were 1:1 and 1:2. Another finding we could conclude from the data is that at higher concentrations of HA (1.6–2%, w/v), solubility of CBZ exhibits much variable solubility as the deviations are much noticeable. Existence of some other mechanism other than inclusion is also evident from the data obtained. High molecular weight and basic hydrophobicity of humic substances favor formation of 'micelle'-like structures[23,24] with hydrophilic groups on the water side and the hydrophobic nucleus inside the core. This makes it possible to entrap organic moieties inside[25]. There were nearly negligible effects of different complexation process parameters on the pure drug.

Differential scanning calorimetry

Disperse peak of HA may be because of its thermal degradation[26]. Absence of any sharp peak in the DSC thermogram of HA may indicate either the existence of amorphous structure or the presence of impurity. Nearly all complexes were showing the phenomena of complexation. Also with rigorous trituration, interaction with different functional groups to develop complexes has been established[27].

Fourier transform infrared spectroscopy

In FD and KD complex (1:1 and 1:2) there is almost complete absence of peaks in the fingerprint region (Figure 4b). There is interaction of carbonyl peak of CBZ with the carboxylic group of HA, stretching vibration of N=H (3460 cm^{-1}) is interacting with O–H vibration of HA, indicating strong interaction. Even peaks because of C=C are present but are blunt. All these interactions lead to a lesser degree of complex interaction. The spectrum of PM and SE complexes show blunt and diminished peaks in the fingerprint region. Olefinic and carbonyl peaks of the drug are widespread and dispersed indicating weak interaction with similar bands in the complexing agent. In solvent-evaporated complexes, the extent of complexation is lesser than PM and KD. Peaks of the fingerprint regions (1300–400 cm^{-1}) are diminished, indicating interaction between the drug and the complexing agent. Thus, peaks of the FD and KD complexes appear as the best developed complex. A ratio of 1:2 of complexes appears the best.

X-ray power diffractograms

This analysis confirms the inference obtained using DSC and FT-IR analysis and predicts that the HA is amorphous in nature rather having substantial impurity. The complex developed by lyophilization showed nearly complete absence of peak at 15.41°, 13.18°, which were characteristics of CBZ. There are some peaks in the region of 30°–40° but are diminished. These results showed the formation of the complex. KD method-developed complexes showed several characteristic peaks of the drug but in a much reduced form, indicating formation of the complex. Complexes developed by PM and solvent evaporation (SE) show nearly same peaks; characteristic peaks of CBZ are diminished and they appear comparatively less efficient.

Aqueous solubility of complexes

FD (1:2) complex performed the best whereas PM of 1:1 was the poorest among all but was more in solubility than drug alone. All the other complexes were in between these two. The variation in solubility profile may be because of variable entrapment inside the host molecule that could be seen in different characterization spectra.

Results also depict better performance of 1:2 ratios in every method compared with 1:1. The reason behind it indicates the existence of some other mechanism other than complexation, such as micelles formation[28]. Because in a similar study[29], critical micelles concentration (CMC) of humic substances was found to be forming micelles at a concentration of 2 gm/L. This work also reports the amount of drug solubilized by per gram of humic substances, which is in accordance with our findings. Furthermore, humic substances offer both types of interactions, like with metal ion because of presence to various functional groups and inclusion of hydrophobic moieties[30,31].

Release of CBZ from complex

Active pharmaceutical ingredients have an intrinsic dissolution rate that is dependent on its solubility and particle size[32], which was 34% in 60 minutes and attaining plateau then after. A 1:1 PM complex shows the least release but more than the pure drug. In every method opted for complexation, 1:2 ratio exhibited comparatively better release profile than 1:1. The result corroborates the data obtained from solubility analysis and different instrumental analyses (Figures 3–5). We could conclude that better complexing interaction results into more sustained release profile.

Comparison of anticonvulsant activity

Better performance of 1:2 complexes could be correlated with higher proportion of HA. Thus, the existence of some solubilizing mechanism other than inclusion complex cannot be ignored. This may be micelles formation, as humic substances are well known for aggregation properties. HA was also given to mice to check the antiepileptic activity (amount equivalent to HA used in 1:2 ratios of complexes), which showed zero percent inhibition. Our optimized complexes were exhibiting better performance in crossing the blood–brain barrier, which may be attributed to increased solubility and passive diffusion gradient but formation of aggregates in humic material is also well known.

Permeation study across rat gut sac

Two opposing forces (concentration gradient and aggregation of humic material) act against each other. The one that predominates influences the result. Initially, permeation increased steeply because there was an increasing concentration gradient across the sac but after some time (5 hours) it attained plateau. Reason for plateau may be the aggregation of complexed and free HA.

Conclusion

CBZ has a poor bioavailability because of its poor water solubility. Thus, to increase the solubility of the drug, it was complexed with HA in the molar ratio 1:1 and 1:2 by different methods such as PM, KD, SE, and freeze drying. Complexes were characterized using several techniques such as XRD, DSC, and FT-IR. These analyses confirmed the formation of complexes and indicated KD and freeze drying as the optimized methods. Release profile of 1:2 freeze-drying complex was the best. Ex vivo permeation study of KD and freeze drying shows evidence of a good pharmacodyanamic response. Further MES evaluation on mice favors our previous findings. So, complexation of CBZ with HA could be opted as a promising tool for oral drug delivery and needs to be evaluated clinically.

Declaration of interest

The work being presented in this study is original research work done in the mentioned institution. It has not been published anywhere and not been sent simultaneously for publication. There is not any contradiction between authors.

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