Preparation and Characterization of Hydroxypropyl-β-Cyclodextrin Inclusion Complex of Eugenol: Differential Pulse Voltammetry and ^{1}H-NMR

Anuj GARG,[a] Bhavna GUPTA,[b] Rajiv PRAKASH,[b] and Sanjay SINGH*,[a]

[a] *Department of Pharmaceutics, Institute of Technology, Banaras Hindu University; and* [b] *School of Materials Science and Technology, Institute of Technology, Banaras Hindu University; Varanasi–221005, India.*

Received December 31, 2009; accepted July 25, 2010; published online July 30, 2010

The objective of present investigation was to improve the solubility of Eugenol by preparing the inclusion complex of Eugenol with hydroxypropyl-β-cyclodextrin (Hp-β-CD) and characterize the prepared complex by using NMR and differential pulse voltammetry (DPV). Phase solubility curve was plotted using Hp-β-CD in ranging from 0—40 mM of Hp-β-CD and found to be linear. Therefore, inclusion complex was prepared in equimolar ratio of Eugenol and Hp-β-CD by lyophilization method. Fourier transform infrared spectroscopy (FT-IR), ^{1}H-NMR and DPV were performed for Eugenol, Hp-β-CD and prepared inclusion complex of Eugenol. 2D (two dimensional) NMR was also performed for prepared inclusion complex. The proton of phenol moiety of Eugenol experienced a pronounced chemical shift variation in ^{1}H-NMR. The positive sign of the variation for proton in ^{1}H-NMR indicated that the proton was located near to an oxygen atom in the Hp-β-CD cavity and its magnitude showed a strong interaction between –OH proton of Eugenol and Hp-β-CD. 2D NMR confirms the interaction between phenolic group and hydrogen atoms of Hp-β-CD. A well defined anodic peak current corresponding to oxidation of Eugenol in non-encapsulated and Hp-β-CD-Eugenol inclusion complex in phosphate buffer (pH 6.8) was obtained at about 0.35 V and 0.40 V, respectively. The positive shift in oxidation potential indicated the formation of complex *via* hydrophobic interactions. The oxidant power of Eugenol was retained in complex form as indicated by DPV results. Thus, its oxidation dependent pharmacological property such as antimicrobial activity is not affected after complexation with Hp-β-CD. Thus, ^{1}H-NMR, 2D-NMR and DPV techniques can be used as valuable tools to determine the mechanism of complexation and state of electrochemical active drug in inclusion complex.

Key words Eugenol; hydroxypropyl-β-cyclodextrin; inclusion complex; NMR; differential pulse voltammetry

The mounting cost of developing new chemical entities (NCEs) is clogging the system of drug development by shrinking the pipeline of new drugs. Consequently, research on plant extract or fraction are increased and also showed the efficacy almost comparable to that of synthetic counterpart. Therefore, for the diseases where current drugs are either little efficacious or are ineffective, plant extract may be alternative to NCEs. The rapid propagation of antimicrobial drug resistance has propelled the investigators to use plant as a reliable source for discovery of active antimicrobial agents. Essential oils and their components exhibit myriad medicinal properties including antimicrobial (antibacterial and antifungal), analgesic and antioxidant activity.[1—3] Antimicrobial properties of the essential oils have been recognized and used since ancient times for food preservation as well as for medicinal purpose because of their antimicrobial and antifungal effects. The antifungal effect of essential oils (EO) has been noted in several studies.[4,5] Specific anticandidal activity of some essential oil is also well established.[6—8] The antifungal activity of EO, have been due to its phenolic components.[9]

Eugenol a phenolic major component of clove oil is interesting molecule due to its wide spectrum of activities such as analgesic, anti-inflammatory, antibacterial, antifungal, antiviral and antihypertensive. Surprisingly, eugenol apart from this peripheral action also acts at central level and can be useful in stress, anxiety and depressive disorders, as well as it has antipyretic activity.[10—12] Antifungal activity of Eugenol (a phenolic constituent of clove oil) was investigated by using an experimental model of oral and vaginal candidiasis in immunosuppressed rats.[13] Eugenol is also widely used as analgesic in dentistry. Analgesic activity of Eugenol can be attributed to its ion channel blocking properties. Furthermore, Eugenol has been reported to inhibit nitric oxide (NO) production in lipopolysaccharide (LPS) stimulated RAW 164.7 cell lines and to block the release of interleukin 1-β, tumor necrosis factor α and prostaglandin E_2 from LPS stimulated macrophages showing its ability to act as an anti-inflammatory agent.[14,15] Eugenol in dose of 5 μg/kg has been shown to reduce tongue edema in mice induced by *Dieffenbachia picta*, a wild African plant.[16]

However, some limitations are associated with Eugenol such as low solubility, degradation, irritation property towards the mucosa and pungent taste which make unsuitable to use as such. It is well established that cyclodextrin inclusion complexes can eliminate the unwanted effects such as irritating effect (or toxicity) to the cornea, mucosa or skin. Hydroxypropyl-β-cyclodextrin (Hp-β-CD) is the highly hydrophilic cyclodextrin and reported to reduce the skin toxicity. The natural cyclodextrin, β-CD, is restricted in its pharmaceutical applications due to its limited aqueous solubility (1.85 g/100 ml). Therefore, chemically modified β-CDs are used to overcome this problem. Among β-CD derivatives, only Hp-β-CD has pharmacopoeial monographs in current European Pharmacopeia and British Pharmacopeia.[17] Therefore, HP-β-CD is selected to prepare the inclusion complex of eugenol. Moreover, improved solubility due to highly hydrophilic cyclodextrin *i.e.* Hp-β-CD is associated with enhanced bioavailability for the topical formulations.[18,19] The increase in bioavailability is mainly due to higher concentration at the site of administration, caused rather by

* To whom correspondence should be addressed. e-mail: drsanjaysingh@rediffmail.com

© 2010 Pharmaceutical Society of Japan

higher aqueous solubility and thus improved availability onto the tissue surface.[20,21)]

Therefore, the objective of present investigation is to improve the solubility of Eugenol by preparing the inclusion complex of Eugenol with Hp-β-CD and characterize the prepared complex by using NMR and differential pulse voltammetry (DPV). Phase solubility study was performed to determine the moles of Hp-β-CD required for a mole of Eugenol. In the study, inclusion complex was prepared by lyophilization method and characterized by using Fourier transform-infra red (FT-IR), ^{1}H-NMR and differential pulse voltammetry (DPV) technique. FT-IR study indicates the formation of complex but ^{1}H-NMR study relates to the functional group involved in the complexation. The chemical shift values in ^{1}H-NMR also indicate about the mechanism of complexation. Study of electrochemical behavior of Eugenol after formation of inclusion complex is very important as oxidant effect of Eugenol is responsible for most of the medicinal properties of Eugenol such as anti-microbial activity.[2)] Cyclodextrin complexation could result in loss of pharmacological activity of compound.[22)] Therefore, in the present work electrochemical behaviour of Eugenol was determined by using DPV. Differential pulse voltammetry is an excellent electroanalytical technique to evaluate the antioxidant power of drug molecules or natural compounds due to their quickness, simplicity and low cost.[23)] Thus, this technique can provide more insight into the availability of drug molecules for the redox reactions (oxidation-reduction) after preparing of inclusion complex which in turns also provide valuable information regarding its pharmacological activity.[24,25)] *In-vitro* release studies of the inclusion complexes were also carried out.

Experimental

Materials Eugenol and Hp-β-CD is kindly donated by Prime Dental Pvt. Ltd., Mumbai and ISP (Hong-Kong Ltd.), Hyderabad, respectively. A dialysis bag with a 12000 molecular weight cutoff was purchased from Sigma-Aldrich Chemicals Private Ltd. (Bangalore). All other chemicals are of analytical grade.

Phase Solubility Study Phase solubility study was carried out according to Higuchi and Connors.[26)] An excess amount of Eugenol was added to 5 ml of water containing different concentration of Hp-β-CD (0—40 mM). The suspensions were shaken in 10 ml screw capped vials under magnetic stirring for 48 h at 25 °C. The content of each vial was centrifuged (C-24, Remi Instruments) at 5000 rpm to separate insoluble oil and filtered through a syringe filter (pore size 0.45 μm). The filtered solution was analyzed by UV spectrophotometer (Hitachi UV-Spectrophotometer). All data were the average of at least three determinations.

The apparent stability constant was determined from the slope of the linear portion of the phase solubility diagram according to Eq. 1[26)]:

$$K = \text{slope}/S_0(1-\text{slope}) \quad (1)$$

where S_0 is the aqueous solubility of Eugenol or intercept of plot.

Preparation of Inclusion Complexes The inclusion complexes were prepared at 1 : 1 molar ratio of Eugenol to Hp-β-CD. Typically, 1.38 g (1 mM) of Hp-β-CD was dissolved in 50 ml water to which 150 mg of Eugenol (1 mM) was added and stirred for 24 h. Any undissolved amount of drug was separated by filtration after that the solution was lyophilized (Decibel Instruments) at −40 °C to obtain lyophilized complex.

Fourier Transform-Infra Red (FT-IR) Spectroscopy FT-IR spectra were obtained on a FT-IR spectrometer (Series 8000, Shimadzu, Japan) by the conventional KBr pellet method. The samples were ground gently with anhydrous KBr and compressed to form pellet. The scanning range was 400—4000 cm^{-1} and the resolution was 4 cm^{-1}.

Nuclear Magnetic Resonances ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on JEOL AL300 FT-NMR spectrometer at 300 MHz at 293 K, using dimethyl sulfoxide (DMSO) as solvent and tetra methylsilane (TMS) a internal reference. 2D-NMR rotating frame Overhauser enhancement spectroscopy (ROESY) experiments were carried out in D_2O.

Differential Pulse Voltammetry Differential pulse voltammetry (DPV) experiments were carried out using a potentiostat/galvanostat model CHI 7041C (CH Instruments Inc., U.S.A.) attached to a computer with proper software for total control of the experiments, data acquisition and treatment. An electrochemical cell with platinum disc as working electrode, a platinum gauge as counter electrode, and an Ag/AgCl as reference electrode was used for the measurements.

***In-Vitro* Release Study** *In-vitro* release studies were performed by using Franz diffusion cell. Inclusion complex of eugenol with Hp-β-CD was incorporated in 1% w/v carbopol gel. Gel (1 g) equivalent to 2 mg of eugenol was placed on dialysis membrane which acts as donor chamber. Phosphate buffer (pH 6.8) containing 40% v/v PG was taken as dissolution media in receiver chamber. Then dissolution studies were carried out for 12 h at 37 °C under magnetic stirring. Samples were withdrawal at predetermined time interval and equal amount of fresh dissolution media was placed in receiver chamber. Samples were diluted and analyzed by using spectrophotometer at 281 nm. *In vitro* release studies were performed in triplicate. Time to release 40% of eugenol from gel ($T_{40\%}$) was calculated by using graphical method to compare the results.

Results and Discussion

Phase Solubility Study Phase solubility graph was plotted between the concentration of Hp-β-CD and soluble amount of eugenol at that concentration of Hp-β-CD. The increase in eugenol solubility occurred as a linear function of Hp-β-CD concentration in range of 0—40 mM (Fig. 1), corresponding to the A_L-type profile defined by Higuchi and Connors.[26)] This relationship suggests a first order kinetics on the complex formation between Eugenol and Hp-β-CD. Therefore, Eugenol and Hp-β-CD were taken in equimolar amount during preparation of complex. The value of apparent stability constant (K) was found to be 1206.4 M^{-1}. The value of apparent stability constant (K) within the range of 200—5000 M^{-1} is considered adequate to improve the bioavailability of poorly soluble drugs.[27)]

Fourier Transform-Infra Red (FT-IR) Spectroscopy FT-IR spectroscopy allowed the analysis of changes in the spectral features of the guest molecule with respect to those of the host oligosaccharide (Hp-β-CD). FT-IR spectra of eugenol, Hp-β-CD, and prepared inclusion complexes of eugenol with Hp-β-CD are shown in Fig. 2. The Eugenol spectrum exhibited the peak in the 3300—3600 cm^{-1} wave number regions. It indicates the presence of –OH stretching (Fig. 2a). Absorption peak in the region of 1600—1680 cm^{-1}

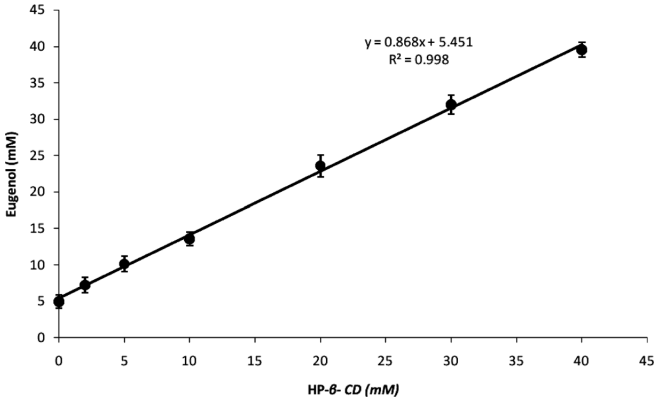

Fig. 1. Phase Solubility Diagram of Eugenol in Presence of Hydroxypropyl-β-Cyclodextrin in Phosphate Buffer (pH 6.8) at 25 °C

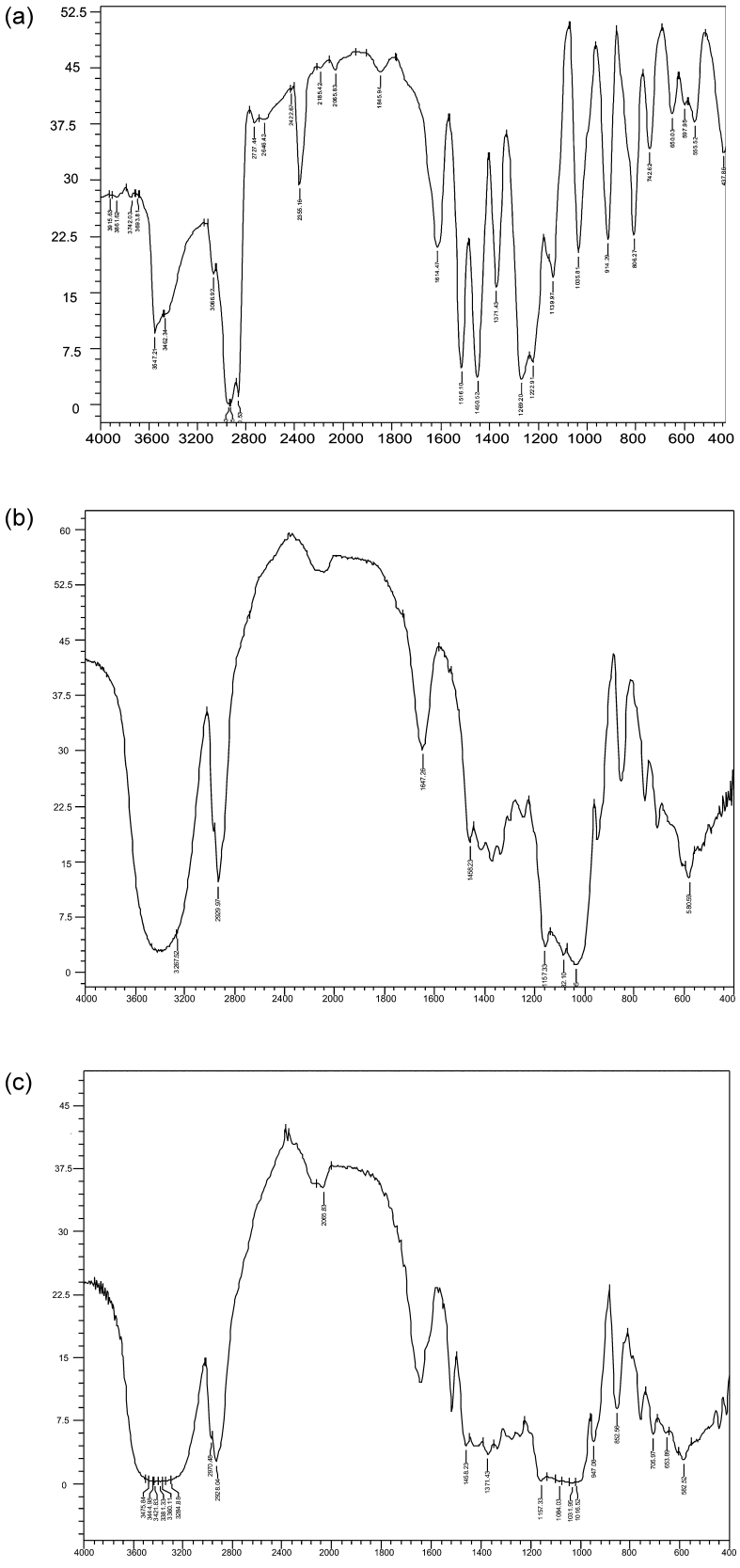

Fig. 2. FT-IR Spectra of (a) Eugenol, (b) Hydroxypropyl-β-Cyclodextrin, (c) Inclusion Complex of Eugenol and Hydroxypropyl-β-Cyclodextrin

can be assigned to alkene C=C and aromatic ring present in eugenol.[28] The spectrum within the range 1200—1020 cm^{-1} can be attributed to the presence of the C–O stretching band present in hydroxypropyl-β-cyclodextrin. The hydroxypropyl-β-cyclodextrin spectrum indicated –OH group of the Hp-β-CD in the range of 3200—3600 cm^{-1} wave number regions (Fig. 3b). FT-IR spectra of prepared inclusion complex showed the broad peak in 3200—3600 cm^{-1} and 1200—

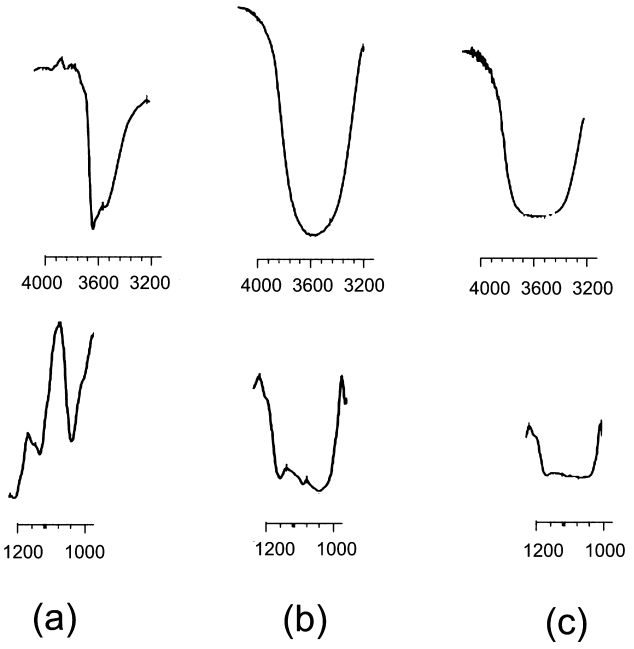

Fig. 3. FT-IR Spectra of (a) Eugenol, (b) Hp-β-CD, (c) Complex of Eugenol and Hp-β-CD in Range of 3600—3200 and 1200—1000 cm^{-1}

1020 cm^{-1} wave number region indicating the possibility of hydrogen bonding of phenolic –OH group of Eugenol with C–O bond of hydroxypropyl-β-cyclodextrin (Fig. 3c). The broadening and reduction of peak intensity confirms the formation of inclusion complexation by lyophilization method.[29] Further, ^{1}H-NMR technique could be helpful to elucidate the mechanism of complexation.

Nuclear Magnetic Resonances (NMR) Spectroscopy ^{1}H-NMR technique has been used as important tool for investigating the group involved in interaction and mechanism of complexation. Eugenol, Hp-β-CD and inclusion complex of eugenol and Hp-β-CD in 1 : 1 molar ratio were analyzed for ^{1}H-NMR (Figs. 4a—c). The absence of new peak in the spectra of inclusion complex suggested that the inclusion complexation of eugenol with Hp-β-CD is a fast exchange process. The spectra revealed that under the present condition only changes in chemical shift occurred (shown by arrow in Fig. 4c). Only ^{1}H-chemical shift changes of eugenol protons resonance were analyzed in the ^{1}H-NMR studies of the interaction between eugenol and Hp-β-CD. As the individual Hp-β-CD protons, especially H-3 and H-5 could not be assigned to resonance signals.[30,31] The molecular structure of eugenol with labeling of protons (denoted as a—g) is shown in Fig. 4d and corresponding chemical shift values of each proton are enlisted in Table 1.

The effect of Hp-β-CD on the chemical shift of Eugenol protons is also presented in Table 1. The effect of Hp-β-CD on the ^{1}H-NMR chemical shifts of eugenol are split into two groups, one shifted upfield and the other downfield. A downfield displacement of the drug protons indicate that they are close to an electronegative atom, oxygen.[32] An upfield shift displacement is probably due to a variation in local polarity when the protons are inside the cavity of Hp-β-CD and indicates weaker interaction with hydrogen atoms (shielding effect due to van der Waals forces between the drug and carbohydrate chains.[33—35] The proton of phenol moiety (H_d) experienced a pronounced chemical shift variation. The positive sign of the variation for proton 'd' indicates that it is located near to an oxygen atom in the Hp-β-CD cavity and its magnitude showed a strong interaction between d (H_d) proton and Hp-β-CD (Table 1). Thus, ^{1}H-NMR results indicated that phenolic ring of Eugenol goes inside the Hp-β-CD cavity. Results of FT-IR can be well correlated with the results of ^{1}H-NMR.

Table 1. ^{1}H-Chemical Shift Corresponding to Eugenol in the Presence and Absence of Hp-β-CD

Eugenol proton	$\delta_{(free)}$	$\delta_{(complex)}$	$\Delta\delta$
H_a	3.305	3.404	0.099
H_b	3.871	3.725	−0.146
H_c	5.034	5.01	−0.024
H_d	5.470	5.712	0.242
H_e	5.902	5.87	−0.032
H_f	6.683	6.688	0.005
H_g	6.824	6.708	−0.116

2D-NMR ROESY (two dimensional) spectroscopy was performed for Eugenol-Hp-β-CD prepared complex to evaluate and confirm the mode of complexation and spatial arrangement between host and guest atoms obtained from ^{1}H-NMR studies. Cross peaks in 2D-NMR ROESY spectrum (Fig. 5) of Eugenol-Hp-β-CD inclusion complex showed the intermolecular nuclear Overhauser effect (NOE) between phenolic proton 'd' and H atoms of Hp-β-CD.[36] Thus, 2D-NMR confirmed the results obtained with ^{1}H-NMR which also suggested the interaction of phenolic group of Eugenol with hydrogen atom of Hp-β-CD.

Differential Pulse Voltammetry (DPV) The electrochemical behavior of non-encapsulated and encapsulated eugenol in hydroxypropyl-β-cyclodextrin (pH 6.8) on gold electrode in phosphate buffer pH 6.8 solution was determined. The electrochemical behavior of phosphate buffer pH 6.8 alone and polymeric (cyclodextrin) solution was studied which indicates absence of anodic peak (Fig. 6a). A well defined anodic peak current corresponding to oxidation of eugenol in non-encapsulated and Hp-β-CD-Eugenol inclusion complex in phosphate buffer (pH −6.8) was obtained at about 0.35 V and 0.40 V, respectively (Figs. 6b—c). The anodic oxidation peak was shifted to the slightly positive side and peak current was decreased in presence of Hp-β-CD. Bard *et al.* reported that positive peak potential shift of complex indicated the binding *via* hydrophobic interactions.[37] The decrease of current intensity also indicated the smaller diffusion coefficient of the bulk cyclodextrin complex as compared to that of free guest. Thus, DPV study showed that complexation of eugenol with Hp-β-CD occurs *via* hydrophobic interactions and also demonstrated that Eugenol molecules were electrochemically active after formation of inclusion complex with Hp-β-CD.

***In-Vitro* Release Study** *In-vitro* release profile of carbopol gel (1%) containing inclusion complex of Eugenol with Hp-β-CD and free Eugenol (not complexed) was plotted (Fig. 7). As Eugenol is poorly soluble in water, it is difficult to disperse in the gel. Inclusion complex of Eugenol with Hp-β-CD improves the solubility in water and retard the dif-

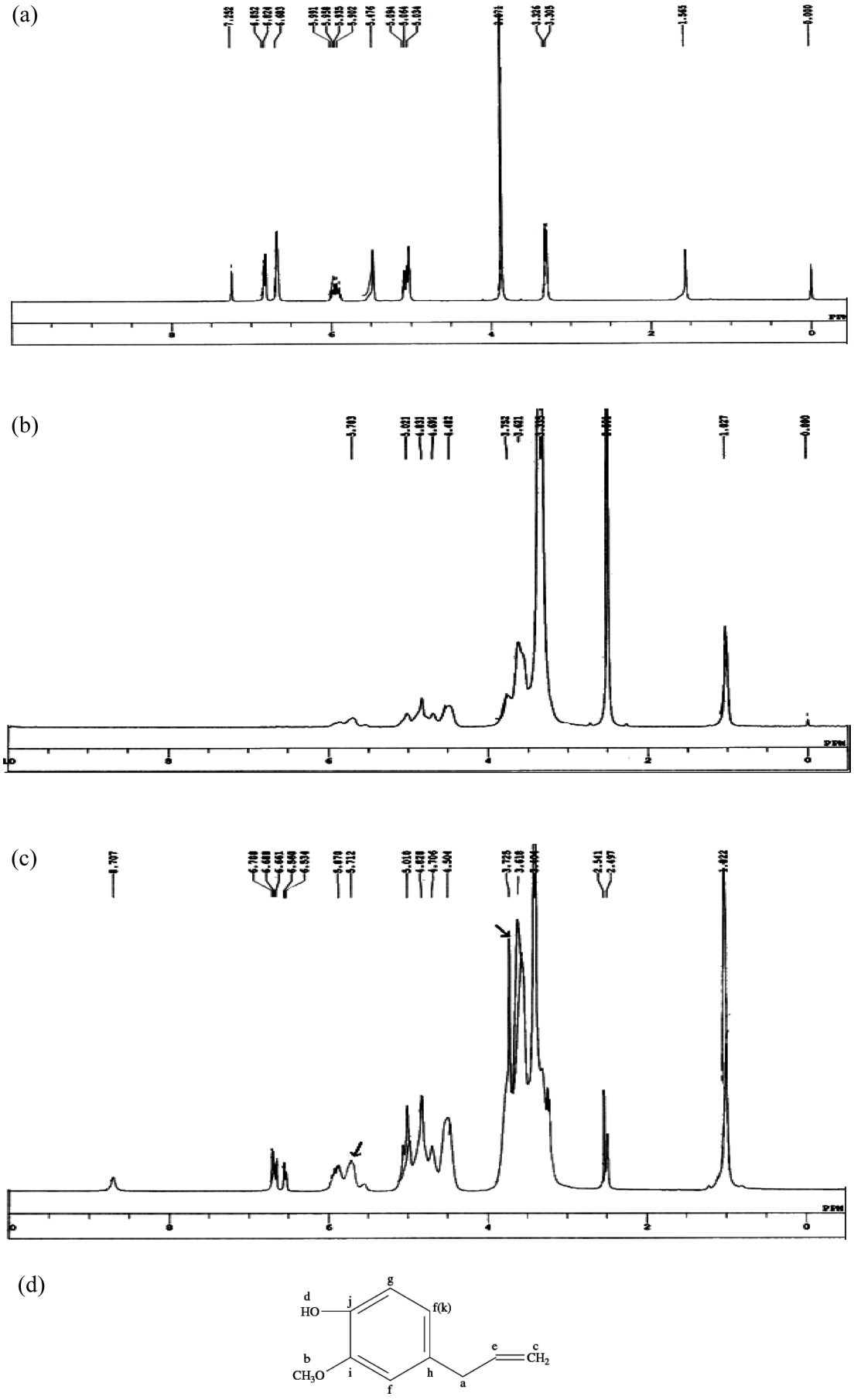

Fig. 4. ^{1}H-NMR Spectra of (a) Eugenol, (b) Hydroxypropyl-β-Cyclodextrin, (c) Inclusion Complex of Eugenol and Hydroxypropyl-β-Cyclodextrin, (d) Molecular Structure of the Eugenol with Labeling of Protons

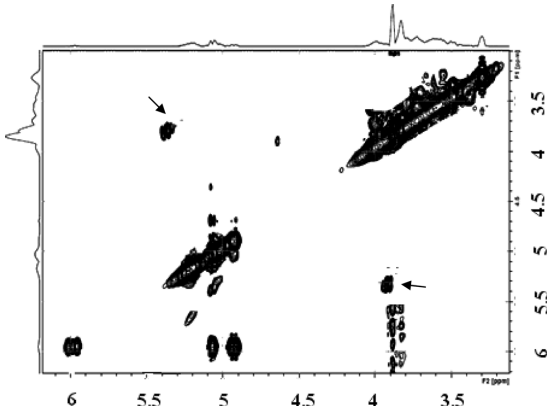

Fig. 5. 2D-NMR (ROESY) Spectrum of Eugenol-Hp-β-CD Inclusion Complex in D_2O

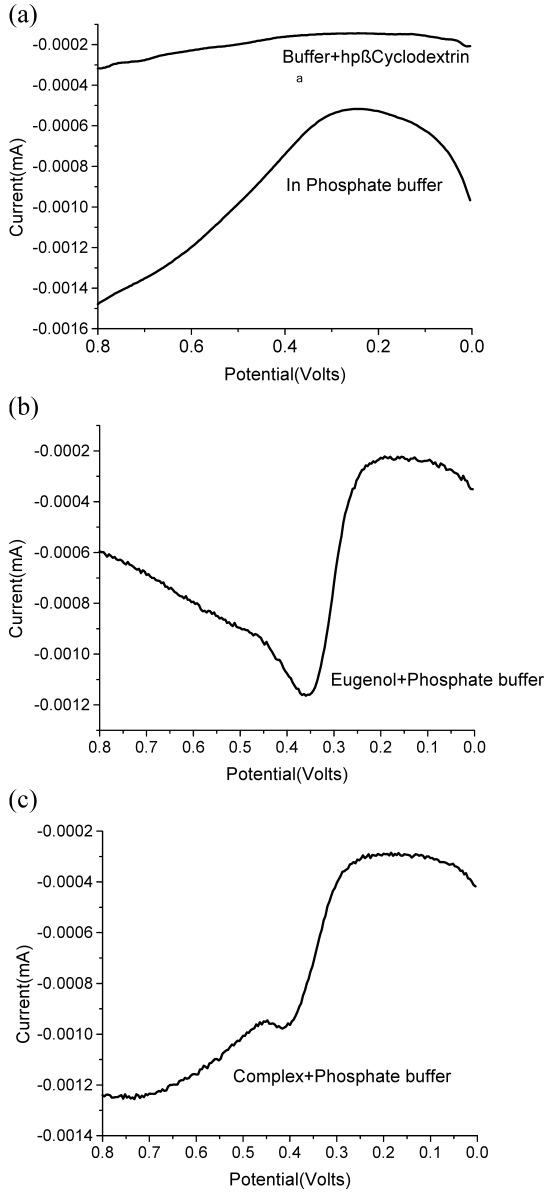

Fig. 6. Differential Pulse Voltammetry Curves of (a) Phosphate Buffer pH 6.8 and Hydroxypropyl-β-Cyclodextrin in Phosphate Buffer (pH 6.8), (b) Eugenol, (c) Inclusion Complex of Eugenol and Hydroxypropyl-β-Cyclodextrin

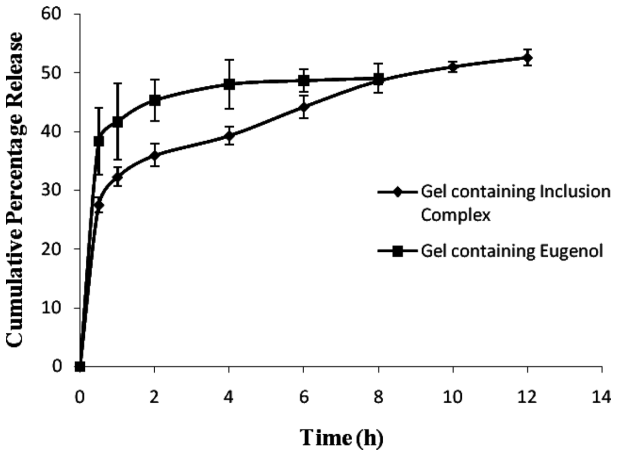

Fig. 7. *In-Vitro* Release Profile of 1% w/v Carbopol Gel Containing Free Eugenol and Inclusion Complex of Eugenol with Hydroxypropyl-β-Cyclodextrin

fusion of Eugenol over the hydrated swollen polymer of gel.[38] Only free drug can permeate through membrane and thus, inclusion complex present in gel acts as reservoir of free drug. Thus, the difference in the dissolution profile is due to the inclusion complex formation. $T_{40\%}$ of gel containing inclusion complex of Eugenol with Hp-β-CD and free Eugenol was found to be 4.4 h and 0.6 h, respectively. $T_{40\%}$ clearly indicated that Gel containing inclusion complex showed slower and sustained release of Eugenol through dialysis membrane as compared to the gel containing free Eugenol.

Conclusion

The results of study suggested that inclusion complex of Eugenol with Hp-β-CD can be successfully prepared by lyophilization method. The present study also indicated that ^{1}H-NMR, 2D-NMR and DPV techniques can be used as valuable tools to determine the mechanism of complexation and state of electrochemical behavior of drug in inclusion complex, respectively. The oxidant power of Eugenol was retained in complex form as indicated by DPV results. Thus, its oxidation dependent pharmacological property such as antimicrobial activity is not affected after complexation with Hp-β-CD. Thus, DPV can be employed to determine the oxidant potential of other antioxidants as well and obviate the need to reassess their pharmacological activity after formation of complexes. DPV is a quick, simple and inexpensive method as compared to other chemical and biochemical methods to determine the oxidant potential.

Acknowledgements The authors are grateful to University Grants Commission, New Delhi, India for the financial assistance for the present work.

References

1) Bakkali F., Averbeck S., Averbeck D., Idaomar M., *Food Chem. Toxicol.*, **46**, 446—475 (2008).
2) Shokeen P., Bala M., Singh M., Tandon V., *Int. J. Antimicro. Agents*, **32**, 174—179 (2008).
3) Chami N., Bennis S., Chami F., Aboussekhra A., Remmal A., *Oral Microbiol. Immunol.*, **20**, 106—111 (2005).
4) Knobloch K., Pauli P., Iberl B., Weigand H., Weiss N., *J. Essential Oil Res.*, **1**, 119—128 (1989).
5) Salgueiro L. R., Cavaleiro C., Pinto E., Pina-Vaz C., Rodrigues A. G., Palmeira A., Tavares C., Costa-de-Oliveira S., Gonçalves M. J., Martinez-de-Oliveira J., *Planta Med.*, **69**, 871—874 (2003).

6) Nenoff P., Haustein U. F., Brandt W., *Skin Pharmacol.*, **9**, 388 (1996).
7) Hammer K. A., Carson C. F., Riley T. V., *J. Antimicrob. Chemother.*, **42**, 591—595 (1998).
8) Suresh B., Sriram S., Dhanaraj S. A., *J. Ethnopharmacol.*, **55**, 151—159 (1997).
9) Viollon C., Chaumont J. P., *Mycopathologia*, **128**, 151—153 (1994).
10) Sen P., Maiti P. C., Puri S., Ray A., Audulov N. A., Valdman A. V., *Ind. J. Exp. Biol.*, **30**, 592—596 (1992).
11) Irie Y., Itokazu N., Anjiki N., Ishige A., Watanabe K., Keung W. M., *Brain Res.*, **1011**, 243—246 (2004).
12) Norte M. C. B., Cosentino R. M., Lazarini C. A., *Phytomedicine*, **12**, 294—298 (2005).
13) Chami F., Chami N., Bennis S., Trouillas J., Remmal A., *J. Antimicrob. Chemother.*, **54**, 909—914 (2004).
14) Tung Y., Chua M., Wang S., Chang S., *Bioresource Technol.*, **99**, 3908—3913 (2008).
15) Lee Y., Hung S., Pai S., Lee Y., Yang S., *J. Endod.*, **33**, 698—702 (2007).
16) Dip E. C., Pereira N. A., Fernandes P. D., *Toxicon*, **43**, 729—735 (2004).
17) Challa R., Ahuja A., Ali J., Khar R. K., *AAPS Pharm. Sci. Tech.*, **6**, E327—E329 (2005).
18) Jug M., Becirevic-Lacan M., Kwokal A., Cetina-Cizmek B., *Acta Pharm.*, **55**, 223—236 (2005).
19) Babu R. J., Pandit J. K., *Int. J. Pharm.*, **271**, 155—165 (2004).
20) Arima H., Miyaji T., Irie T., Hirayama F., Uekama K., *Chem. Pharm. Bull.*, **44**, 582—586 (1996).
21) Jutunen J., Jarvinem T., Niemi R., *J. Pharm. Pharmacol.*, **57**, 1153—1157 (2005).
22) Durán N., Justo G. Z., Melo P. S., De Azevedo M. B. M., Souza Brito A. R. M., Almeida A. B. A., Haun M., *Can. J. Physiol. Pharmacol.*, **81**, 387—396 (2003).
23) Cosio M. S., Buratti S., Mannino S., Benedetti S., *Food Chem.*, **97**, 725—731 (2006).
24) Barros L., Falcão S., Baptista P., Freire C., Vilas-Boas M., Ferreira C. F. R. I., *Food Chem.*, **111**, 61—66 (2008).
25) Radi A., El-Shahawi M. S., Elmogy T., *J. Pharm. Biomed. Anal.*, **37**, 195—198 (2005).
26) Higuchi T., Connors A. K., *Adv. Anal. Chem. Instrum.*, **4**, 117—212 (1965).
27) Vandelli M. A., Ruozi B., Forni F., Mucci A., Salvioli G., Galli E., *J. Inclusion Phenomena Macrocyclic Chem.*, **37**, 237—251 (2000).
28) Haiyee Z. A., Saim N., Said M., Illias R. M., Mustapha W. A. W., Hassan O., *Food Chem.*, **114**, 459—465 (2009).
29) Yuwono M., Siswandono, Hafid A. F., Poemomo A. T., Agil M., Indrayanto G., Siegfried E., *Analytical Profiles of Drug Substances and Excipients*, **29**, 149 (2002).
30) Müller B. W., Albers E., *Int. J. Pharm.*, **79**, 273—288 (1992).
31) Veiga F. J. B., Fernandes C. M., Carvalho R. A., Campos-Geraldes C. F. G., *Chem. Pharm. Bull.*, **49**, 1251—1275 (2001).
32) Ganza-Gonzalez A., Vila-Jato J. L., Anguiano-Igea S., Otero-Espinar F. J., Blanco-Méndez J., *Int. J. Pharm.*, **106**, 179—185 (1994).
33) Djedaïne F., Lin S., Perly B., Wouessidjewe D., *J. Pharm. Sci.*, **79**, 643—646 (1990).
34) Zornoza A., Martín C., Sánchez M., Vélaz I., Piquer A., *Int. J. Pharm.*, **169**, 239—244 (1998).
35) Zhang D. D., Zhao P. Y., Huang N. J., Wu Y. L., Zhai Y. M., "Minutes of the 5th Int. Symp. on Cyclodextrins," ed. by Duchêne D., Editions de Santé, Paris, 1990, pp. 146—149.
36) Beni S., Szakács Z., Csernák O., Barcza L., Noszál B., *Eur. J. Pharm. Sci.*, **30**, 167—174 (2007).
37) Bard A. J., Cater M. T., Rodoriguez M., *J. Am. Chem. Soc.*, **111**, 8901—8911 (1989).
38) Jug M., Becirevic-Lacan M., *Eur. J. Pharm. Sci.*, **21**, 251—260 (2004).