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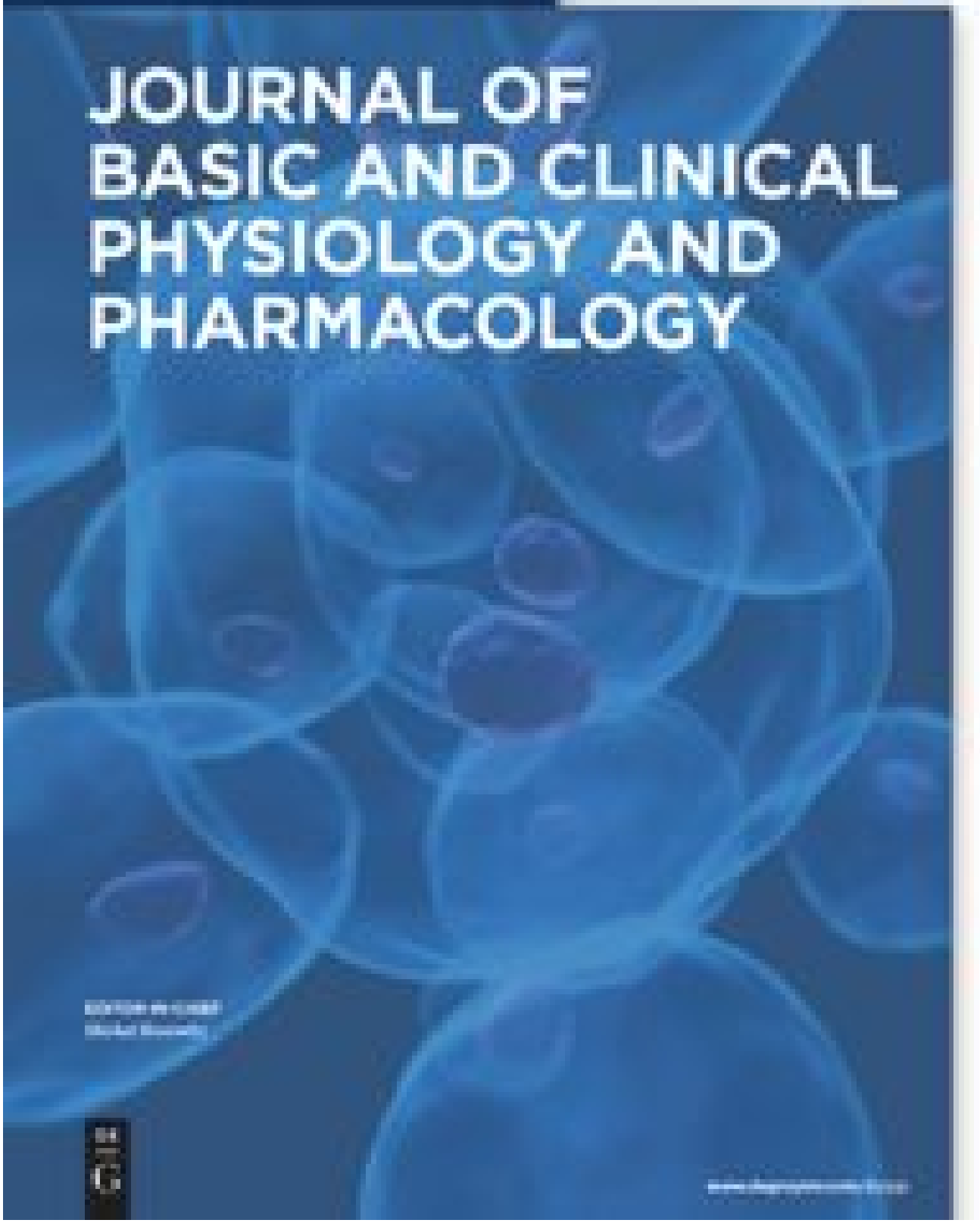

JOURNAL OF BASIC AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

Published since December 1, 1986

Journal of Basic and Clinical Physiology and Pharmacology

ISSN: 2191-0286

Editor-in-chief: Ugo Oliviero

Managing Editor: Alberto Marra

Editorial

Editorial Office:
E-mail: jbcpp.editorial@degruyter.com

(Deutsch)

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Online ISSN: 2191-0286
Type: Journal
Language: English
Publisher: De Gruyter
First published: December 1, 1986
Publication Frequency: 6 Issues per Year
Audience: researchers and health professionals in the field of clinical physiology and pharmacology

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Published by De Gruyter

Volume 32 Issue 4 - INTERNATIONAL CONFERENCE OF PHARMACY AND HEALTH SCIENCES: The 3rd JOINT CONFERENCE UNAIR - USM; Guest Editors: Suciati & Andang Miatmoko

July 2021

Issue of Journal of Basic and Clinical Physiology and Pharmacology

CONTENTS JOURNAL OVERVIEW

Original Articles

Requires Authentication June 25, 2021

Green tea and its active compound epigallocathechin-3-gallate (EGCG) inhibit neuronal apoptosis in a middle cerebral artery occlusion (MCAO) model

Abdulloh Machin, Imam Susilo, Djoko A. Purwanto

Page range: 319-325

More Cite this

Requires Authentication June 25, 2021

The effects of quercetin on nicotine-induced reward effects in mice

Mahardian Rahmadi, Dian Suasana, Silvy Restuning Lailis, Dinda Monika Nusantara Ratri, Chrismawan Ardianto

Page range: 327-333

More Cite this

Requires Authentication June 25, 2021

Resveratrol ameliorates physical and psychological stress-induced depressive-like behavior

Chrismawan Ardianto, Aniek Setiya Budiatin, I Nengah Budi Sumartha, Nurrahmi Nurrahmi, Mahardian Rahmadi, Junaidi Khotib

Page range: 335-340

More Cite this

Requires Authentication June 25, 2021

Translation and cross-cultural adaption of an instrument measuring patient's well-being under treatment for schizophrenia

Julaeha Julaeha, Umi Athiyah, Margarita Maria Maramis, Agus Sugianto, Andi Hermansyah

Page range: 341-347

More Cite this

Requires Authentication June 25, 2021

Quercetin promotes behavioral recovery and biomolecular changes of melanocortin-4 receptor in mice with ischemic stroke

Tuhfatul Ulya, Chrismawan Ardianto, Putri Anggreini, Aniek Setiya Budiatin, Dwi Setyawan, Junaidi Khotib

Page range: 349-355

More Cite this

Requires Authentication June 25, 2021

Knowledge and attitudes of healthcare professionals on prescribing errors

Desak Ketut Ernawati, Ida Ayu Alit Widhiartini, Endang Budiarti

Page range: 357-362

More Cite this

Requires Authentication June 25, 2021

Inhibition of Ras and STAT3 activity of 4-(*tert*-butyl)-*N*-carbamoylbenzamide as antiproliferative agent in HER2-expressing breast cancer cells

Aguslina Kirtishanti, Siswandono Siswodihardjo, I Ketut Sudiana, Desak G. A. Suprabawati, Aristika Dinaryanti

Page range: 363-371

More Cite this

Requires Authentication June 25, 2021

Predicting the molecular mechanism of glucosamine in accelerating bone defect repair by stimulating osteogenic proteins

Maria Apriliani Gani, Ahmad Dzulfikri Nurhan, Aniek Setiya Budiatin, Siswandono Siswodihardjo, Junaidi Khotib

Page range: 373-377

More Cite this

Requires Authentication June 25, 2021

Larvicidal toxicity and parasporal inclusion of native *Bacillus thuringiensis* BK5.2 against *Aedes aegypti*

Salamun, Fatimah, Ahmad Fauzi, Seling N. Praduwana, Ni'matuzahroh

Page range: 379-384

More Cite this

Requires Authentication June 25, 2021

Synthesis, ADMET predictions, molecular docking studies, and *in-vitro* anticancer activity of some benzoxazines against A549 human lung cancer cells

Melanny Ika Sulistyowaty, Retno Widyowati, Galih Satrio Putra, Tutuk Budiati, Katsuyoshi Matsunami

Page range: 385-392

More Cite this

Requires Authentication June 25, 2021

Thymoquinone and its derivatives against breast cancer with HER2 positive: *in silico* studies of ADMET, docking and QSPR

Adinda Adelia Wulandari, Achmad Aziz Choiri, Fitria, Tri Widiandani

Page range: 393-401

More Cite this

Requires Authentication June 25, 2021

Assessment of patient understanding of their conventional cardiac medicines and herbal prepared/derived products: preliminary survey and interviews with selected community-dwelling elderly patients in the Philippines

Jay P. Jazul, Trisha Michaela G. Arciga, Mary Angelie C. Ante, Danavin Gwyneth B. Berlin, Loise Francoise L. Ravana, Samantha A. Reyes, Jashanjit Singh

Page range: 403-413

More Cite this

Requires Authentication June 25, 2021

The development and validation of the health belief model questionnaire for measuring factors affecting adherence in the elderly with hypertension

Rodhiyatul Fithri, Umi Athiyah, Elida Zairina

Page range: 415-419

More Cite this

Requires Authentication June 25, 2021

Analysis of the side effect of QTc interval prolongation in the bedaquiline regimen in drug resistant tuberculosis patients

Denny Ardhianto, Suharjono, Soedarsono, Umi Fatmawati

Page range: 421-427

More Cite this

Requires Authentication June 25, 2021

Shallot skin profilling, computational evaluation of physicochemical properties, ADMET, and molecular docking of its components against P2Y12 receptor

Juni *Ekowati, Kholidah Febriani, Itsna N. A. Yaqin, Adinda A. Wulandari, Indra H. Mulya, Kholis A. Nofianti, Achmad Syahrani*

Page range: 429-437

More Cite this

Requires Authentication June 25, 2021

Analysis of HMGB-1 level before and after providing atorvastatin standard therapy in coronary artery disease patients with type-2 diabetes mellitus compared to without type-2 diabetes mellitus

Widya Handayani, Suharjono, Mohammad Yogiarto

Page range: 439-446

More Cite this

Requires Authentication June 25, 2021

Analysis of matrix metalloproteinase-9 levels among acute heart failure patients with ACE inhibitor therapy (Dr. Soetomo Regional General Hospital, Surabaya)

Ira Purbosari, Bambang Zubakti Zulkarnain, Muh Aminuddin, Umi Fatmawati

Page range: 447-451

More Cite this

Requires Authentication June 25, 2021

The correlation between self-related adherence, asthma-related quality of life and control of asthma in adult patients

Elida Zairina, Gesnita Nugraheni, Gusti Noorrizka Veronika Achmad, Arie Sulistyarini, Yunita Nita, Arief Bakhtiar, Muhammad Amin

Page range: 453-458

More Cite this

Requires Authentication June 25, 2021

Providing counseling through home pharmacy care (HPC) for hemodialysis patients with hypertension in lowering blood pressure

Rahmiyati Daud, Bambang Subakti Zulkarnain, Ivan Virnanda Amu

Page range: 459-465

More Cite this

Requires Authentication June 25, 2021

Community knowledge and attitude in recognizing asthma symptoms and using medication for asthma attacks: a cross-sectional study

Arina Dery Puspitasari, Bindaria Mutmaina Prabawati, Alfian Nur Rosyid

Page range: 467-472

More Cite this

Requires Authentication June 25, 2021

A study of anticoagulant therapy in patients with coronary artery disease

Arina D. Puspitasari, Daniel Dwi Christiananta Salean, Didik Hasmono, Rudy Hartono, Meity Ardiana

Page range: 473-478

More Cite this

Requires Authentication June 25, 2021

The association of FKBP5 polymorphism with asthma susceptibility in asthmatic patients

Sura F. Alsaffar, Haider A. Rasheed, Jabbar H. Yenzeel, Haider F. Ghazi

Page range: 479-484

More Cite this

Requires Authentication June 25, 2021

Gastroprotective effect of fluvoxamine and ondansetron on stress-induced gastric ulcers in mice

Mahardian Rahmadi, Nily Su'aida, Pratiwi Yustisari, Wahyu Agung Dewaandika, Elma Oktavia Hanaratri, Mareta Rindang Andarsari, Sumarno, Toetik Aryani

Page range: 485-490

More Cite this

Requires Authentication June 25, 2021

Osteoblast iron genes: real time PCR and microarray hybridization approach under hyperoxia

Prihartini Widiyanti, Hartmut Kuehn, Soetjipto Soetjipto

Page range: 491-496

More Cite this

Requires Authentication June 25, 2021

Attenuation of hyperplasia in lung parenchymal and colonic epithelial cells in DMBA-induced cancer by administering *Andrographis paniculata* Nees extract using animal model

Aniek Setiya Budiatin, Ilham Bagus Sagitaras, Ika Putri Nurhayati, Nismatun Khairah, Khoirotin Nisak, Imam Susilo, Junaidi Khotib

Page range: 497-504

More Cite this

Requires Authentication June 25, 2021

N-nitrosodiethylamine induces inflammation of liver in mice

Devy Maulidya Cahyani, Andang Miatmoko, Berlian Sarasitha Hariawan, Kusuma Eko Purwantari, Retno Sari

Page range: 505-510

More Cite this

Requires Authentication June 25, 2021

AST/ALT levels, MDA, and liver histopathology of *Echinometra mathaei* ethanol extract on paracetamol-induced hepatotoxicity in rats

Angelica Kresnamurti, Dita Nurlita Rakhma, Amitasari Damayanti, Septiyan Dwi Santoso, Enggar Restryarto, Wifqi Hadinata, Iwan Sahrial Hamid

Page range: 511-516

More Cite this

Requires Authentication June 25, 2021

Development, characterization, molecular docking, and *in vivo* skin penetration of coenzyme Q10 nanostructured lipid carriers using tristearin and stearyl alcohol for dermal delivery

Ni Luh Dewi Aryani, Siswandono Siswodihardjo, Widji Soeratri, Nadia Fitria Indah Sari

Page range: 517-525

More Cite this

Requires Authentication June 25, 2021

The effect of *Camellia sinensis* (green tea) with its active compound EGCG on neuronal cell necroptosis in *Rattus norvegicus* middle cerebral artery occlusion (MCAO) model

Abdulloh Machin, Ramidha Syaharani, Imam Susilo, Muhammad Hamdan, Dyah Fauziah, Djoko Agus Purwanto

Page range: 527-531

More Cite this

Requires Authentication June 25, 2021

Hepatoprotective effect of ethanolic extract of sugarcane (*Saccharum officinarum* Linn.) leaves

Ika P. Dewi, Rifdah B. Kwintana, Jihan U. Ulinnuha, Fadhillah Rachman, Fransiska M. Christianty, Diana Holidah

Page range: 533-540

More Cite this

Requires Authentication June 25, 2021

Correlation between the exposure time to mobile devices and the prevalence of evaporative dry eyes as one of the symptoms of computer vision syndrome among Senior High School students in East Java, Indonesia

Rozalina Loebis, Bambang Subakti Zulkarnain, Nadhifa Zahra

Page range: 541-545

More Cite this

Requires Authentication June 25, 2021

The effect of various high-fat diet on liver histology in the development of NAFLD models in mice

Mahardian Rahmadi, Ahmad Dzulfikri Nurhan, Eka Dewi Pratiwi, Devita Ardina Prameswari, Sisca Melani Panggono, Khoirotin Nisak, Junaidi Khotib

Page range: 547-553

More Cite this

Requires Authentication June 25, 2021

Fabrication and characterization of bovine hydroxyapatite-gelatin-alendronate scaffold cross-linked by glutaraldehyde for bone regeneration

Samirah, Aniek Setiya Budiatin, Ferdiansyah Mahyudin, Junaidi Khotib

Page range: 555-560

More Cite this

Requires Authentication June 25, 2021

Health related quality of life among postmenopausal woman with hormone responsive HER2- breast cancer in Indonesia

Ria Etikasari, Tri Murti Andayani, Dwi Endarti, Kartika Widayati Taroeno-Hariadi

Page range: 561-565

More Cite this

Requires Authentication June 25, 2021

Gender differences in the blood glucose type 2 diabetes patients with combination rapid and long acting insulin therapy

Dinda M. N. Ratri, Arina D. Puspitasari, Cahyo W. Nugroho, Budi Suprapti, Suharjono, Christoper P. Alderman

Page range: 567-570

More Cite this

Requires Authentication June 25, 2021

Correlation of dietary iron intake and serum iron with thyroid stimulating hormone (TSH) and free thyroxine (FT4) levels in adult hyperthyroid patients

Utami Harjantini, Yulia Lanti Retno Dewi, Diffah Hanim, Ida Nurwati

Page range: 571-576

More Cite this

Requires Authentication June 25, 2021

The effect of pillbox use and education by pharmacist toward medication adherence in diabetes mellitus patients in a Primary Health Care Center in Mataram

Mahacita Andanalusia, Yunita Nita, Umi Athiyah

Page range: 577-582

More Cite this

Requires Authentication June 25, 2021

Variation concentration effect of propyleneglycol, glycerin, and polyethyleneglycol 400 to physical properties and dissolution rate of loratadine liquisolid tablet

Mikhania Christiningtyas Eryani, Esti Hendradi, Siswandono

Page range: 583-587

More Cite this

Requires Authentication June 25, 2021

Role of *Centella asiatica* and ceramide in skin barrier improvement: a double blind clinical trial of Indonesian batik workers

Sylvia Anggraeni, Menul Ayu Umborowati, Damayanti Damayanti, Anang Endaryanto, Cita Rosita Sigit Prakoeswa

Page range: 589-593

More Cite this

Requires Authentication June 25, 2021

Secondary metabolite and antipyretic effects of Maja (*Crescentia cujete* L.) in fever-induced mice

Teodhora, Munawarohthus Sholikha, Asniatul Ania, Ika Maruya Kusuma

Page range: 595-601

More Cite this

Requires Authentication June 25, 2021

Hydration effect on kidney function and serum electrolyte in children with tumor lysis syndrome (TLS) and risk of TLS

Yulistiani, Claudia Tiffany, I. Dewa Gede Ugrasena, Mariyatul Qibtiyah

Page range: 603-609

More Cite this

Requires Authentication June 25, 2021

Drug utilization study and cost analysis of adult β-thalassemia major patient therapy at Dr. Soetomo General Hospital Surabaya

Hasna Qatrunnada, Suharjono, Siprianus Ugroseno Yudho Bintoro, Siti Wahyuni

Page range: 611-616

More Cite this

Requires Authentication June 25, 2021

The role of hyperbaric oxygen to platelet aggregation in noninsulin-dependent diabetes mellitus (NIDDM)

Prihartini Widiyanti, Purnomo Suryohudoyo

Page range: 617-621

More Cite this

Requires Authentication June 25, 2021

Cocrystal formation of loratadine-succinic acid and its improved solubility

Dwi Setyawan, Firdaus Rendra Adyaksa, Hanny Lystia Sari, Diajeng Putri Paramita, Retno Sari

Page range: 623-630

More Cite this

Requires Authentication June 25, 2021

The role of chondroitin sulfate to bone healing indicators and compressive strength

Herry Wibowo, Prihartini Widiyanti, Syaifullah Asmiragani

Page range: 631-635

More Cite this

Requires Authentication June 25, 2021

The effects of quercetin on the expression of SREBP-1c mRNA in high-fat diet-induced NAFLD in mice

Jamal Nasser Saleh Al-maamari, Mahardian Rahmadi, Sisca Melani Panggono, Devita Ardina Prameswari, Eka Dewi Pratiwi, Chrismawan Ardianto, Santhra Segaran Balan, Budi Suprapti

Page range: 637-644

More Cite this

Requires Authentication June 25, 2021

Analysis of stress ulcer prophylaxis drug regimentation in surgical patients

Dhani Wijaya, Suharjono, Fendy Matulatan, Elfri Padolo

Page range: 645-649

More Cite this

Requires Authentication June 25, 2021

The stability and irritability study of the chitosan–*Aloe vera* spray gel as wound healing

Dini Retnowati, Retno Sari, Esti Hendradi, Septiani Septiani

Page range: 651-656

More Cite this

Requires Authentication June 25, 2021

Effectiveness of citicoline in pediatric patients with refractive amblyopia in Surabaya, East Java, Indonesia

Rozalina Loebis, Bambang Subakti Zulkarnain, Fitri Amalia Siswanto

Page range: 657-661

More Cite this

Requires Authentication June 25, 2021

The thermodynamic study of *p*-methoxycinnamic acid inclusion complex formation, using β-cyclodextrin and hydroxypropyl-β-cyclodextrin

Dewi Isadiartuti, Noorma Rosita, Juni Ekowati, Achmad Syahrani, Toetik Ariyani, M. Ainur Rifqi

Page range: 663-667

More Cite this

Requires Authentication June 25, 2021

The effect of chitosan type and drug-chitosan ratio on physical characteristics and release profile of ketoprofen microparticles prepared by spray drying

Muhammad A. S. Rijal, Hanah Masitah, Fanny Purvitasari, Retno Sari

Page range: 669-673

More Cite this

Requires Authentication June 25, 2021

The maximum dose and duration in the therapy single use methotrexate to achieve remission by rheumatoid arthritis patients through disease activity score 28 (DAS28)

Anisyah Achmad, Tika Yasmin Rahmayanti, Bagus Putu Putra Suryana

Page range: 675-680

More Cite this

Accessible June 25, 2021

Knowledge, attitudes, and practices (KAP) towards COVID-19 among university students in Pakistan: a cross-sectional study

Shah Faisal, Junaidi Khotib, Elida Zairina

Page range: 681-686

More Cite this Download PDF

Requires Authentication June 25, 2021

The impact of glutaraldehyde on the characteristics of bovine hydroxyapatite-gelatin based bone scaffold as gentamicin delivery system

Aniek Setiya Budiatin, Maria Apriliani Gani, Chrismawan Ardianto, Samirah, Sahrati Yudiaprijah Daeng Pattah, Fitroh Mubarokah, Junaidi Khotib

Page range: 687-691

More Cite this

Requires Authentication June 25, 2021

Analysis of the use of antibiotics profile and factors of surgical site infections study on digestive and oncology surgeries

Lisa Narulita, Suharjono, Kuntaman, Mohammad Akram

Page range: 693-700

More Cite this

Requires Authentication June 25, 2021

Second internal transcribed spacer (ITS-2) as genetic marker for molecular characterization of *Sarcoptes scabiei* in rabbits from several areas of East Java, Indonesia

Nunuk Dyah Retno Lastuti, Nur Rusdiana, Poedji Hastutiek

Page range: 701-705

More Cite this

Requires Authentication June 25, 2021

Design of gossypetin derivatives based on naturally occurring flavonoid in *Hibiscus sabdariffa* and the molecular docking as antibacterial agents

Nuzul W. Diyah, Isnaeni, Shabrina W. Hidayati, Bambang T. Purwanto, Siswandono

Page range: 707-714

More Cite this

Requires Authentication June 25, 2021

Discovery of new targeting agents against GAPDH receptor for antituberculosis drug delivery

Muhammad Amirul Asyraf Noh, Siti Sarah Fazalul Rahiman, Habibah A Wahab, Amirah Mohd Gazzali

Page range: 715-722

More Cite this

Requires Authentication June 25, 2021

The effect of red passion fruit (*Passiflora edulis* Sims.) fermentation time on its activity against Extended Strain Methicillin-Resistant (ESBL) *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Iif Hanifa Nurrosyidah, Ni Made Mertaniasih, Isnaeni

Page range: 723-727

More Cite this

Requires Authentication June 25, 2021

Antibiotic use on acute respiratory tract infection nonpneumonia and nonspecific diarrhea in Primary Health Care Centre in Banjarbaru City, South Kalimantan, Indonesia

Rizky Liestya Wardani, Suharjono, Kuntaman, Agus Widjaja

Page range: 729-735

More Cite this

Requires Authentication June 25, 2021

Screening of anti-HIV activities in ethanol extract and fractions from *Ficus fistulosa* leaves

Siti Qamariyah Khairunisa, Dwi Wahyu Indriati, Lidya Tumewu, Aty Widyawaruyanti, Nasronudin Nasronudin

Page range: 737-742

More Cite this

Requires Authentication June 25, 2021

The characteristics of lactic acid bacteria isolated from fermented food as potential probiotics

Victoria Yulita Fitriani, Budi Suprapti, Muhammad Amin

Page range: 743-749

More Cite this

Requires Authentication June 25, 2021

Profile of gyrA gene mutation in clinical isolate of levofloxacin resistant *Escherichia coli*

Alifia Risma Fahmi, Suharjono, Kuntaman

Page range: 751-754

More Cite this

Requires Authentication June 25, 2021

Antimicrobial activity of *Centella asiatica* and *Gigantochloa apus*

Siti Mudaliana

Page range: 755-759

More Cite this

Requires Authentication June 25, 2021

Drug-related problems of antibiotic use in gastroenteritis related to patient therapy outcomes at Universitas Gadjah Mada Hospital

Fivy Kurniawati, Nanang Munif Yasin, Farida Aulia, Gidfrie Vinanda Krisha

Page range: 761-766

More Cite this

Requires Authentication June 25, 2021

The impact of suitability of empirical antibiotics use on therapeutic outcome of respiratory tract infection patients at inpatient wards of Universitas Gadjah Mada Academic Hospital

Fivy Kurniawati, Nanang Munif Yasin, Safina Nur Azizah, Silvia Ayu Purbaningtyas

Page range: 767-771

More Cite this

Requires Authentication June 25, 2021

Genetic profile mutation *rpoB* in clinical isolate of rifampicin-resistant *Staphylococcus aureus*

Risa Zulfiana, Suharjono, Kuntaman

Page range: 773-776

More Cite this

Requires Authentication June 25, 2021

Hematological side effect analysis of linezolid in MDR-TB patients with individual therapy

Novan Yusuf Indra Pratama, Bambang Subakti Zulkarnain, Soedarsono, Umi Fatmawati

Page range: 777-781

More Cite this

Requires Authentication June 25, 2021

Adverse drug reaction and its management in tuberculosis patients with multidrug resistance: a retrospective study

Wenny Putri Nilamsari, Muhammad Fajar Rizqi, Natasya Olga Regina, Prastuti Asta Wulaningrum, Umi Fatmawati

Page range: 783-787

More Cite this

Requires Authentication June 25, 2021

Analysis of prophylactic antibiotic use and risk factor of postoperative infection in urological surgery patients

Ratri Rokhani, Suharjono, Kuntaman, Mohammad Akram

Page range: 789-794

More Cite this

Requires Authentication June 25, 2021

Molecular docking studies of *Nigella sativa* L and *Curcuma xanthorrhiza Roxb* secondary metabolites against histamine *N*-methyltransferase with their ADMET prediction

Ahmad Dzulfikri Nurhan, Maria Apriliani Gani, Aniek Setiya Budiatin, Siswandono Siswodihardjo, Junaidi Khotib

Page range: 795-802

More Cite this

Requires Authentication June 25, 2021

Prediction of compounds with antiosteoporosis activity in *Chrysophyllum cainito* L. leaves through *in silico* approach

Burhan Ma'arif, Hilwa Fitri, Nisfatul Lailatus Saidah, Luqman Alfani Najib, Achmad Hamdan Yuwafi, Ria Ramadhani Dwi Atmaja, Fidia Rizkiah Inayatillah, Meilina Ratna Dianti, Hening Laswati, Mangestuti Agil

Page range: 803-808

More Cite this

Requires Authentication June 25, 2021

Phyllanthin and hypophyllanthin, the isolated compounds of *Phyllanthus niruri* inhibit protein receptor of corona virus (COVID-19) through *in silico* approach

Honey Dzikri Marhaeny, Aty Widyawaruyanti, Tri Widiandani, Achmad Fuad Hafid, Tutik Sri Wahyuni

Page range: 809-815

More Cite this

Requires Authentication June 25, 2021

Cratoxylum sumatranum stem bark exhibited antimalarial activity by Lactate Dehydrogenase (LDH) assay

Lidya Tumewu, Fendi Yoga Wardana, Hilkatul Ilmi, Adita Ayu Permanasari, Achmad Fuad Hafid, Aty Widyawaruyanti

Page range: 817-822

More Cite this

Requires Authentication June 25, 2021

Endophytic fungi inhabiting *Physalis angulata* L. plant: diversity, antioxidant, and antibacterial activities of their ethyl acetate extracts

Kartika Dyah Palupi, Muhammad Ilyas, Andria Agusta

Page range: 823-829

More Cite this

Requires Authentication June 25, 2021

Exploration of several plants from Baung Forest on bone formation cell models

Retno Widyowati, Neny Purwitasari, Rice Disi Oktarina, Wiwied Ekasari, Saarah Khairunnisa, Hsin-I. Chang

Page range: 831-837

More Cite this

Requires Authentication June 25, 2021

In vitro antimalarial activity of *Garcinia parvifolia* Miq. Stem extracts and fractions on *Plasmodium falciparum* lactate dehydrogenase (LDH) assay

More Cite this

Case Report

Requires Authentication June 25, 2021

Effect of hydrocortisone on hypocorticolism caused by pituitary adenoma

Niswah N. Qonita, Hanik B. Hidayati

Page range: 895-898

More Cite this

Dewi Isadiartuti*, Noorma Rosita, Juni Ekowati, Achmad Syahrani, Toetik Ariyani and M. Ainur Rifqi

The thermodynamic study of *p*-methoxycinnamic acid inclusion complex formation, using β-cyclodextrin and hydroxypropyl-β-cyclodextrin

https://doi.org/10.1515/jbcpp-2021-0008
Received January 6, 2021; accepted March 4, 2021

Abstract

Objectives: Cyclodextrin's ability to form an inclusion complex with a guest molecule is a function of two factors. The first is steric and depends on the relative size of cyclodextrin cavity to the guest molecule, while the second is the thermodynamic interaction between the different system components. This study therefore aims to determine the effect of β-cyclodextrin and hydroxypropyl-β-cyclodextrin as complex formers, on thermodynamic parameter values (ΔH, ΔG, and ΔS) in the formation of inclusion complex with *p*-methoxycinnamic acid (*p*MCA).

Methods: The *p*MCA complex formation with β-cyclodextrin or hydroxypropyl-β-cyclodextrin was determined in 0.02 pH 4.0 M acetate buffer and 0.02 M pH 7.0 phosphate buffer, with a 0.2 μ value at 32, 37, and 42 ± 0.5 °C. This experiment was carried out in a waterbath shaker until a saturated solution was obtained. Subsequently, *p*MCA concentration was determined using UV spectrophotometer at the maximum *p*MCA wavelength, at each pH.

Results: The result showed *p*MCA formed inclusion complex with β-cyclodextrin or hydroxypropyl-β-cyclodextrin and exhibited increased solubility with increase in β-cyclodextrin or hydroxypropyl-β-cyclodextrin concentration. This temperature rise led to a decrease in the complex's constant stability (K). Furthermore, the interaction showed a negative enthalpy ($\Delta H<0$) and is a spontaneous processes ($\Delta G<0$). At pH 4.0, an increase in the system's entropy occurred ($\Delta S>0$), however, at pH 7.0, the system's entropy decreased ($\Delta S<0$).

Conclusions: The rise in *p*MCA solubility with increase in cyclodextrin solution concentration indicates an inclusion complex has been formed, and is supported by thermodynamic data.

Keywords: β-cyclodextrin; hydroxypropyl-β-cyclodextrin; inclusion complex; *p*-methoxycinnamic acid; solubility enhancement; thermodynamic.

Introduction

The compound *p*-methoxycinnamic acid (*p*MCA) is an ethyl *p*-methoxycinnamic derivative obtained from sand ginger plant (*Kaempferia galanga* Linn.), and known to inhibit the enzymes cyclooxygenase 1 and 2 [1], thus, functioning as an analgesic. According to Ekowati and Dyah [2], *p*MCA present in sand ginger exhibits an even greater painkiller activity, compared to acetosal [2]. However, *p*MCA has a low solubility of 0.712 mg/mL in water, at 25 °C [3]. This causes low *p*MCA dissolution rate, thus, leading to incomplete absorption and low bioavailability [4]. The formation of inclusion complexes is therefore a suitable effort to improve *p*MCA solubility.

An inclusion complex is a system between drug molecules (guest) trapped in the cavity of a complex forming material (host) [5, 6]. Factors influencing an inclusion complex's formation are the accordance between the host and guest cavities' sizes and polarities, as well as thermodynamic parameters [7, 8]. Cyclodextrin is a constituent of inclusions with the ability to the ingredients' solubility in water.

In addition, cyclodextrin (CD) is a cyclic oligosaccharide compound with at least 6 D-(+)-glucopyranose units bound to α-1,4 glycoside bonds, a toroidal shape, and a cavity with a hydrophobic interior and hydrophilic exterior. These compounds are able to increase an ingredient's solubility by forming an inclusion complex with the guest molecules and stabilizing with several interactions, including hydrogen bonds, Van der Waals bonds, hydrophobic interactions and

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electrostatic attraction. The cyclodextrin type most often used to form inclusion complexes is β-cyclodextrin, due to easy production, and relative inexpensiveness [8].

The cavity size of β-cyclodextrin and derivatives corresponds to the aromatic ring's size, thus the formation of inclusion complexes between these compounds and *p*MCA is highly likely [9].

Another commonly used cyclodextrin type for inclusion complex formation is hydroxypropyl-β-cyclodextrin (HPβCD). This is a derivative of β-cyclodextrin, with high solubility in water (above 50 g/100 mL, at 25 °C), due to the substitution of hydroxypropyl groups replacing the hydroxyl group in glucose. The compound is also less toxic and more environmentally friendly, compared to β-cyclodextrin [8, 10].

No previous studies have been conducted on the thermodynamics of inclusion complex formation between *p*MCA and cyclodextrin. This is a reversible reaction, and the complex compounds continuously formed undergo dissociation to produce free drugs and complex forming materials. However, the reaction is offset in a brief period of time, by the association of free drug molecules and complex forming materials. A molecule's ability to associate and dissociate to reach equilibrium is evident in the constant stability of complex formation (K) value [11]. This value is directly related to the complex formation free energy (ΔG) value [12], and is influenced by pH, because the drug compound's ionized or unionized form (guest) influences the inclusion complex formation [8].

Therefore, this study aims to determine the effect of β-cyclodextrin and hydroxypropyl-β-cyclodextrin as complex formers, on thermodynamic parameter values (ΔH, ΔG, and ΔS), in inclusion complex formation with *p*MCA, in a bid to better understand the interaction mechanism.

Materials and methods

The materials used in this study include *p*-methoxycinnamic acid (Sigma-Aldrich), β-cyclodextrin (Sigma-Aldrich), hydroxypropyl-β-cyclodextrin (Sigma-Aldrich), distilled water, disodium hydrogen phosphate (Sigma-Aldrich), sodium dihydrogen phosphate (Sigma-Aldrich), citric acid (Sigma-Aldrich), sodium citrate (Sigma-Aldrich), 96% analytical grade ethanol, and 96% technical grade ethanol.

Methods

Preparation buffer solutions

0.02 M pH 4.0 citrate buffer solution: This was prepared by mixing 0.02 M sodium citrate solution with 0.02 M citric acid solution and adding sodium chloride, to obtain a 0.20 ionic strength.

0.02 M pH 7.0 phosphate buffer solution: This was prepared by mixing 0.02 M disodium hydrogen phosphate solution with 0.02 M sodium dihydrogen phosphate solution, and adding sodium chloride, to obtain a 0.20 ionic strength.

Solubility testing

The *p*MCA's solubility in various β-cyclodextrin or hydroxypropyl-β-cyclodextrin concentrations (0.10^{-3}; 2.5×10^{-3}; 5.0×10^{-3}; 7.5×10^{-3}; and 10.0×10^{-3} M) was tested in pH 4.0 and 7.0 solutions, with 0.20 ionic strengths, at 32, 37, and 42 ± 0.5 °C (305, 310, and 315 K). About 5.0 mL of β-cyclodextrin or hydroxypropyl-β-cyclodextrin solutions with certain pHs and concentrations were placed into a 15-mL vial. The vials were then inserted in the waterbath shaker and the temperature was adjusted as required. After the trial temperature was obtained, about 15 mg of *p*MCA was added and the mixture was shaken at a frequency of 140/min, until a saturated solution was formed. An optimized determination of *p*MCA saturated solubility in distilled water was indicated by the absence of a rise in *p*MCA concentration, after shaking for 5 h, under the solubility test's conditions. Subsequently, the solution was collected with an injection syringe, filtered with a 0.22 μm Millipore paper and the concentration was determined using UV spectrophotometer, at each pH's maximum *p*MCA λ. The maximum *p*MCA λ were discovered to be 307.0 and 286.0 nm, pH 4.0 and 7.0, respectively.

Results

The solubility of *p*-methoxycinnamic acid in cyclodextrin solutions

Figures 1 and 2 show the *p*MCA solubility study in various β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) solution concentrations, carried out at different pHs and temperatures.

The complex stability constant and thermodynamic parameters

Table 1 shows the regression equation obtained from the curve of the relationship between the β-cyclodextrin (βCD) or hydroxypropyl-β-cyclodextrin (HPβCD) concentrations, and *p*MCA solubility.

The complex stability constant (K) is obtained using the formula below.

$$K_{(1:1)} = \frac{\text{slope}}{\text{intercept}\,(1 - \text{slope})} \quad (1)$$

Meanwhile, Table 2 shows the thermodynamic values of enthalpy (ΔH), free energy (ΔG), and entropy (ΔS) values, calculated using the formula below. The slope value obtained from each regression equation in Table 1 was then used to calculate the enthalpy value (ΔH cal/mol) using Eq. (2), where R represents the gas constant (1.987 calories/mol)

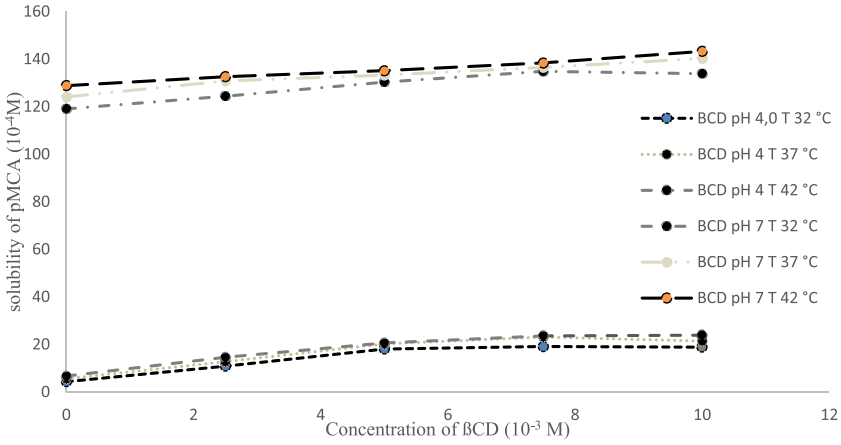

Figure 1: Solubility of *p*MCA in β-cyclodextrin (βCD) solutions at various pHs and temperatures.

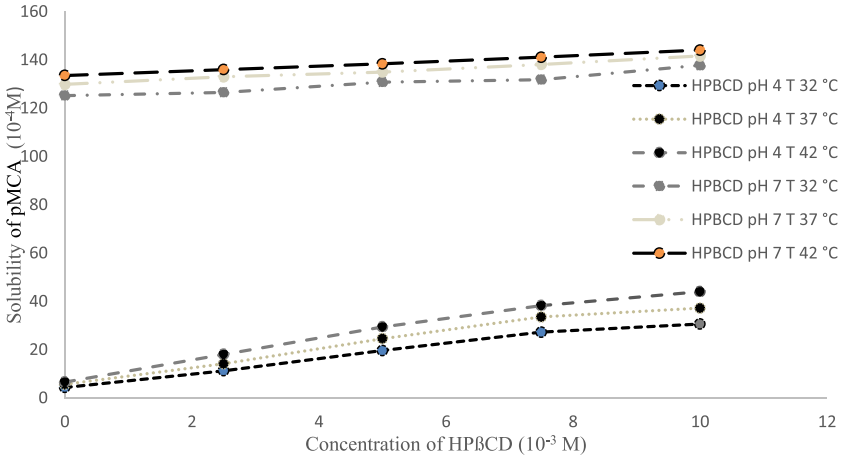

Figure 2: Solubility of *p*MCA in hydroxypropyl-β-cyclodextrin (HPβCD) solutions at various pHs and temperatures.

Table 1: Regression equation of the relationship between the pMCA solubility (M) and the concentration of cyclodextrin (M).

Cyclodextrin	pH	Temperature (°K)		
		305	310	315
β-cyclodextrin	4.0	$y = 0.14864\,x + 6.8120.10^{-4}$ r = 0.9045	$y = 0.16724\,x + 8.2880.10^{-4}$ r = 0.9072	$y = 0.17372\,x + 9.1680.10^{-4}$ r = 0.9418
	7.0	$y = 0.16040\,x + 120.50.10^{-4}$ r = 0.9491	$y = 0.15248\,x + 125.40.10^{-4}$ r = 0.9849	$y = 0.13824\,x + 128.73.10^{-4}$ r = 0.9938
Hydroxypropyl-β-cyclodextrin	4.0	$y = 0.27440\,x + 4.8700.10^{-4}$ r = 0.9923	$y = 0.33000\,x + 6.4753.10^{-4}$ r = 0.9899	$y = 0.38040\,x + 8.2140.10^{-4}$ r = 0.9918
	7.0	$y = 0.12140\,x + 124.33.10^{-4}$ r = 0.9699	$y = 0.11444\,x + 129.74.10^{-4}$ r = 0.9962	$y = 0.10452\,x + 133.36.10^{-4}$ r = 0.9949

$$\text{Slope} = -\Delta H\, 2,303\, R \tag{2}$$

Also, the free energy (Δ*G* cal/mol) was calculated using Eq. (3), where *R* represents the gas constant (1.987 calories/mol), *T* denotes absolute temperature (°K) and *K* indicates the complex stability constant value.

$$\Delta G = -2,303\, RT \log K \tag{3}$$

The value of entropy (Δ*S* cal/mol) was calculated using Eq. (4), by entering Δ*H* value as obtained from Eq. (2) and Δ*G* value as obtained from Eq. (3), where *T* denotes absolute temperature (°K).

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

Discussion

The solubility of *p*MCA in various β-cyclodextrin or hydroxypropyl-β-cyclodextrin concentrations (0×0.10^{-3}

Table 2: The complex stability constant (K) and the thermodynamic parameters (ΔH, ΔG, and ΔS) at various pHs and temperatures.

$1/T$ (°K)	pH	β-Cyclodextrin				Hydroxypropyl-β-cyclodextrin			
		K (M^{-1})	ΔH (cal/mol)	ΔG (cal/mol)	ΔS (cal/mol)	K (M^{-1})	ΔH (cal/mol)	ΔG (cal/mol)	ΔS (cal/mol)
305	4.0	256.30	−2,200.40	−3,361.89	3.81	776.53	-0.879.21	−4,033.57	10.34
310		242.31	−2,200.40	−3,382.42	3.81	760.64	-0.879.21	−4,085.51	10.34
315		229.32	−2,200.40	−3,402.48	3.82	747.44	-0.879.21	4,136.99	10.34
305	7.0	15.85	−4,391.34	−1,674.84	−8.91	11.11	−4,833.31	−1,465.48	−11.04
310		14.35	−4,391.34	−1,645.55	−8.86	9.96	−4,833.31	−1,418.58	−11.02
315		12.46	−4,391.34	−1,585.61	−8.91	8.75	−4,833.31	−1,354.97	−11.04

to 10.0×10^{-3} M) was tested in the medium of pH 4.0 and 7.0 buffer solutions, and the pH was selected based on the 4.04 *p*MCA pKa value. Based on the calculation using the Henderson–Hasselbalch equation, at pH 4.0 or pH equal to pKa, 50% of *p*MCA is in ionized form, while at pH 7.0 or 3 units above pKa, *p*MCA is about 99.9% ionized. The difference in the quantity of the guest molecule's unionized forms influences interaction with the hydrophobic cyclodextrin cavity.

The solubility study was conducted for a *p*MCA saturation time of 5 h. Figures 1 and 2 show the test results of *p*MCA solubility in β-cyclodextrin or hydroxypropyl-β-cyclodextrin. The *p*MCA solubility curve in β-cyclodextrin shows a BS type, meaning a rise in *p*MCA solubility occurred with increasing β-cyclodextrin concentration, but reached a plateau, and even tended to decrease at a certain point. This is because β-cyclodextrin's solubility in water is limited, thus, a complex deposition occurs at a certain concentration. Conversely, the *p*MCA solubility curve in hydroxypropyl-β-cyclodextrin shows an AL-type curve, meaning *p*MCA solubility rises with increasing hydroxypropyl-β-cyclodextrin concentration.

In the curves of *p*MCA solubility in β-cyclodextrin or hydroxypropyl-β-cyclodextrin, the calculated r value is greater, compared to the r table with a 95% confidence level of 0.754. This *p*MCA solubility curve's linearity shows the interactions occurring between guest molecules (*p*MCA) to form complexes with β-cyclodextrin or hydroxypropyl-β-cyclodextrin in a 1:1 M ratio. Table 1 shows the regression equation obtained from the *p*MCA solubility curve with β-cyclodextrin or hydroxypropyl-β-cyclodextrin concentration, while Table 2 shows the complex stability constant (K) value calculate using this equation.

According to Table 2, the increase in temperature causes a decrease in the complex stability constant (K). This shows the temperature rise causes the formed inclusion complex to become more unstable, meaning the interaction between *p*MCA and β-cyclodextrin or hydroxypropyl-β-cyclodextrin is released more easily. The cyclodextrin inclusion complex formation is a reversible reaction and temperature rise causes the guest *p*MCA molecule in the host cavity to dissociate out of the cyclodextrin cavity, as indicated by the lower K value. In addition, a rise in pH reduces the complex stability constant (K), and the amount of ionized *p*MCA influences the compound's ability to enter the β-cyclodextrin or hydroxypropyl-β-cyclodextrin cavity. Ionized *p*MCA tends to come out of the hydrophobic cyclodextrin cavity, making the complex less stable.

At pH 4.0, hydroxypropyl-β-cyclodextrin's stability constant complex (K) is greater, compared to β-cyclodextrin, indicating the complex bonds formed in the hydroxypropyl-β-cyclodextrin are stronger. This is probably because hydroxypropyl-β-cyclodextrin has a hydroxypropyl group with the ability to provide steric barrier for *p*MCA to escape from the HPβCD cavity.

Based on Table 2, a curve of the relationship between log K and $1/T$ is plotted, with β-cyclodextrin and hydroxypropyl-β-cyclodextrin inclusion complex formation at pH 4 as well as 7. Each curve provided a regression equation with a correlation coefficient value (r)>0.9, indicating a relationship between $1/T$ and log K. Subsequently, the slope obtained in each regression equation was used to calculate ΔH as in Eq. (2), as well as thermodynamic parameters (ΔG and ΔS), as in Eqs. (3) and (4).

The results of β-cyclodextrin or hydroxypropyl-β-cyclodextrin at various pH both produced a negative enthalpy value (ΔH), indicating an exothermic the dissolution process. Furthermore, the interaction between *p*MCA with β-cyclodextrin or hydroxypropyl-β-cyclodextrin is mainly hydrophobic. The free energy changes (ΔG) also had a negative sign, indicating a spontaneous the complex formation, while the entropy change (ΔS) in experiments with β-cyclodextrin or hydroxypropyl-β-cyclodextrin at pH 4.0 was positive, indicating an increase in system irregularities.

The regular water system surrounding the *p*MCA molecule becomes irregular because *p*MCA enters the cyclodextrin. This condition is not desirable because polar–nonpolar repulsion occurs between water molecules and the inner cyclodextrin cavity, thus, the condition is quickly replaced by the presence of guest molecules less polar than water molecules [13]. Meanwhile, in experiments with β-cyclodextrin or hydroxypropyl-β-cyclodextrin at pH 7.0, a negative value was obtained, indicating a lower system irregularity. This is possibly because the system irregularity caused by guest molecule entry and exit to and from the host molecule, no longer occurs. The cyclodextrin cavity is hydrophobic, thus, guest molecules in ionized form find it more difficult to enter.

Conclusions

Based on the results, *p*MCA was concluded to form an inclusion complex with both β-cyclodextrin and hydroxypropyl-β-cyclodextrin. The interaction between *p*MCA and hydroxypropyl-β-cyclodextrin is stronger, compared to β-cyclodextrin, as indicated by the higher complex stability constant (K) value. In addition, the temperature rise led to a constant decrease in stability, and the interaction showed a negative enthalpy ($\Delta H<0$) as well as a spontaneous process ($\Delta G<0$). In pH 4.0 a rise in the system's entropy occurred ($\Delta S>0$), while in pH 7.0 a reduction in the system's entropy was observed ($\Delta S<0$)

Acknowledgment: The authors are grateful to the Universitas Airlangga, for the provision of financial support towards this study, under the PTUPT 2017, and to Dr. Hanafi Tanojo, for rendering assistance in the discussions.
Research funding: This study was supported by an Applied Research on Excellence in Higher Education Institution (Penelitian Terapan Unggulan Perguruan Tinggi, PTUPT) with Grant Number: 004/ADD/SP2H/LT/DRPM/VIII/2017 provided by The Ministry of Research and Technology-National Research and inovation Agency of republic of Indonesia.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Informed consent: Not applicable.
Ethical approval: Not applicable.

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Biochemistry, Genetics and Molecular Biology
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Pharmacology, Toxicology and Pharmaceutics
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PUBLISHER

Walter de Gruyter GmbH

H-INDEX

33

PUBLICATION TYPE

Journals

ISSN

07926855, 21910286

COVERAGE

1985-1988, 1990-2020

INFORMATION

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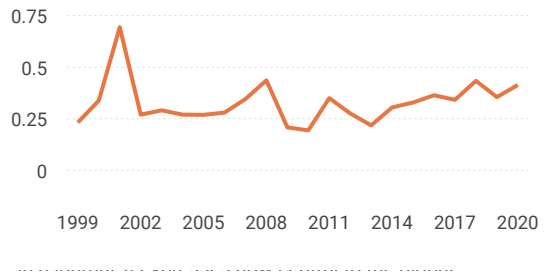

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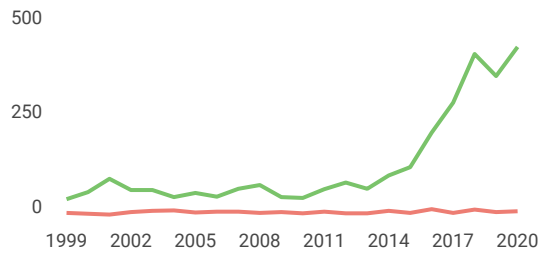

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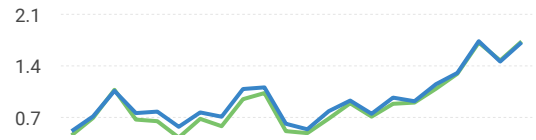

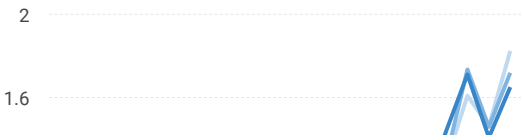

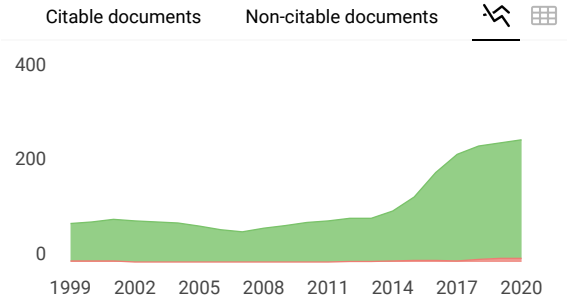

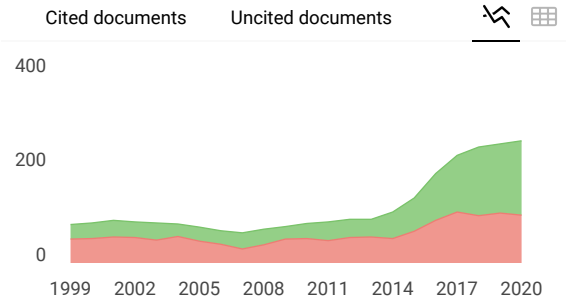

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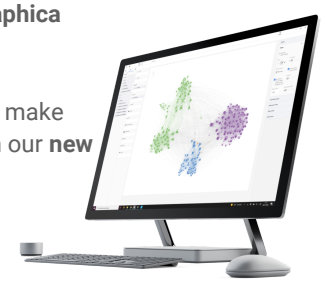

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